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(Deemed to be University)

Impact Analysis of Seed Money

DPU
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(DEEMED UNIVERSITY)

Seed money utilization and outcome analysis

College/Institute	Year 2015/2016	Year 2016/2017	Year 2017/2018	Year 2018/2019	Total
Medical					
Papers	3	6	4	2	15
Patents	0	0	0	0	0
conference	0	0	0	0	0
Project				01	ICMR RS 8,80,300
Biotech					
Papers	2	3	0	2	07
Patents	1	2	0	0	03
conference	11	7	9	4	31
Dental					
Papers	0	2	0	0	2
Patents	0	0	0	0	0
Conference	0	1	0	0	1
Extra	0	0	0	0	0
Project	0	0	0	1	BIG GRANT
	0	0	0	0	50,00,000/-
Physiotheraphy					
Papers	0	6	1	0	7
Patents	0	0	0	0	0
Conference	0	0	0	0	0
Nursing					
Papers	0	0	0	6	6
Patents	0	0	0	0	0

Dr Ramesh Bhonde

Director (Research)
Dr.D.Y. Patil Vidyapeeth, Pune

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Assessment of balance using bot-2 in age group 5-15 year school going children

Pooja Narendra Yengde*, Sanjivani Dhote, Tushar Palekar, Apoorva Dighe, Antara Pande

Dr. D. Y. Patil College of Physiotherapy, Pimpri, Pune, India

ABSTRACT

Introduction: Motor development is the gradual process by which child gain balance and coordination of the large muscle of legs trunk and arm and small muscle of the hand. The bruininks-Oseretsky test of motor proficiency Test that uses engaging, goal-directed activities to measure a wide array of motor skills in individuals ages 4 through 12.**Material and method:** In this study cross sectional analytical study design was used study setting was in Pimpri chinchwad municipality, Pune. Sample population was 5-15 year school going male and female, multistage stratified sampling method was used, sample size was 516, inclusion criteria was 5-15 year children male and female and exclusion criteria was neurological trauma or deficit, visual problem and other diagnosed medical condition. Required material was ruler, marker, measuring tap, stop watch and balance beam of bot kit. Outcome measure was balance total point score and descriptive category. **Conclusion:** The study concludes that there is slight difference in population male and female. But male performance is better than female in balance. According to age groups, 1, 3, 4, 5 is consistently increasing, age group 2 has more good performance.

Keywords: Balance, Bruiniks-Oseretsky**,** Motor development

Introduction

Motor development is the gradual process by which child gains balance and coordination of the large muscle of legs trunk and small muscle of the hand [1]. It includes age related changes in posture, movement and balance. Motor skill is a learned series of movement that combine to produce a smooth, efficient action. Neuromuscular development starts in embryonic stage and continues after birth.[1]Balance is an ability to maintain the line of gravity of body within the base of support with minimal postural sway.[2] A certain amount of gravity of a body with (e.g., breathing, shifting body weight from one foot to the other or from forefoot to rearfoot) or from external triggers (e.g., visual distortions, floor translation). An increase in sway is not necessarily an indicator of dysfunctional balance so much as it is an indicator of decreased sensorimotor control. [2] Maintaining balance requires
coordination of input from multiple sensory systems including the vestibular, somatosensory, and visual systems[3].

**Correspondence*

Pooja Narendra Yengde

Dr. D. Y. Patil College of Physiotherapy, Pimpri, Pune

E Mail: poojayengde@yahoo.com

Vestibular system: sense organs that regulate equilibrium; directional information as it relates to head position (internal gravitational, linear, and angular acceleration)[4]

Somatosensory system: senses of proprioception and kinaesthesia of joints; information from skin and joints (pressure and vibratory senses); spatial position and movement relative to the support surface; movement and position of different body parts relative to each other [4]

Visual system: Reference to verticality of body and head motion; spatial location relative to objects [4]

Balance is the ability to neutralize forces that would disturb equilibrium. Simply watching a young toddler take those first steps is evidence of this. Further evidence of balance can be seen in a variety of movement: from someone simply standing on one leg, to an intricate, dynamic movement during execution of a specific sports skill.[5]Balance deficit is observed in children with Hyperactive disorder,[6]autism spectrum disorder,[7] vestibular disorder,[8]developmental coordination disorder,[9] learning disability,[10] sensory integrative dysfunction,[11] and other motor impairment.[9]Functional tests of balance focus on maintenance of both static and dynamic balance, whether it involves a type of perturbation/change of canter ofmass or during quiet stance. Standardized tests of balance are available to allow allied health care

professionals to assess an individual's position control. Some functional balance tests that are available are: Romberg Tests functional reach test, performance-oriented mobility assessment (POMA) timed get up and go test, Balance efficacy scale[12],Berg balance scale[13], Star excursion test[14], Balance evolution systems test (BEST)[15],Balance error scoring system (BESS), bruininks-Oseretsky Test of motor proficiency (Bruininks, 1978) and also its second edition.[1]The Bruininks-Oseretsky test of motor proficiency, Second edition (BOT-2) is an individually administered test that uses engaging, goal-directed activities to measure a wild array of motor skills in individuals ages 4 through 21. The BOT-2 uses a subtest and composite structure that highlights motor performance in the broad functional areas of stability, mobility, strength, coordination, and object manipulation. This report will discuss four motor-area composite that is Fine Manual Control (FMC), Manual coordination (MC0, Body Coordination (BC) and Strength and Agility (SA). Each comprising two subtest and a Total Motor Composite.5 That total 8 subtests have 53 items. As BOT-2 testing involves game-like motor tasks which capture the child's interest and are not verbally complex, It is suitable for children of non-English speaking background. Also the authors report that it can identify motor deficits in individuals with 'mild to moderate' motor impairment and is validated and reliable for assessing subjects with 'mild to moderate' mental retardation. Furthermore, the motor activities incorporated in BOT-2 include gross motor (GM) tasks that assess hoping, jumping, running, ball skills, balance, strength and coordination and fine motor (FM) tasks that assess precision, integration and manual dexterity through drawing, writing and functional tasks such as threading blocks5. BOT-2 has been empirically validated for high-functioning persons diagnosed with autism, Asperger's, developmental coordination disorder, and mild/moderate intellectual disabilities.[16]The balance subtest of BOT-2is the fourth subtest, body coordination (BC), under gross motor composite and contain eight test-items, Balance subtest evaluates motor-control skills that are integral for maintaining posture when standing, walking or reaching. That is both static and dynamic balances. The number of performance trials for each item is 2 time. A raw score is recorded in best on them. Then converted to a numerical point score.[16]The skills that BOT-2 measures an important role in everyday tasks, including walking, running and participating in recreational and competitive sports. Learning about how an individual performs these task helps to identify special needs so that plans can be made to accommodate these need and develop programs to improve performance. [5]Aim of the study was to assess balance using Bruinink Oseretsky test of motor proficiency scale, 2nd edition in age group 5-15 year school going children and 1st objective of study to find out balance score using bruinink-oseretsky test-2 of motor proficiency scale, 2nd edition. 2nd objective was to find out balance descriptive category using Bruininks-oseretsky test-2 of motor proficiency, 2nd edition, 3 objective to find out balance point score and descriptive category among male and female using Bruininks-Oseretsky Test -2 Of Motor Proficiency, 2nd edition. And 4th objective was to find out balance point score and descriptive category according to age group using Bruininks Oseretsky Test of motor proficiency, 2nd edition.

Material and methods

Research committee of Dr. D. Y. Patil College of Physiotherapy approved this study. The tools used in this study were BOT-2 kit includes examiner manual, individual record from, student booklet, balance beam, target, ruler, marker, measuring tap, stopwatch.

Five hundred sixteen samples were assessed, in which 268 were female (mean age 10.69 years±) and 248 were male (mean age 10.66years±3.02). Information provided by the class teacher and school record were used to include the 5-15 year old children in five group (The age group 1 - 5.0-7.11, age group 2 - 8.0-9.11, age group 3 – 10.0-11.11, age group 4 – 12.0-13.11 and age group 5 – 14.0-15.11) according to the following criteria: no neurological trauma like spinal fracture, 6 month back, no visual and musculoskeletal problem, no neurological deficit or other diagnosed medical condition, The sample characteristic of the 516 is described in the table 1.

Table 1: Description of the study sample

AGE GRP	FEMALE	MALE
1(5,6,7)	59	52
2(8,9)	46	49
3(10,11)	61	47
4 (12,13)	51	49
5(14,15)	51	51

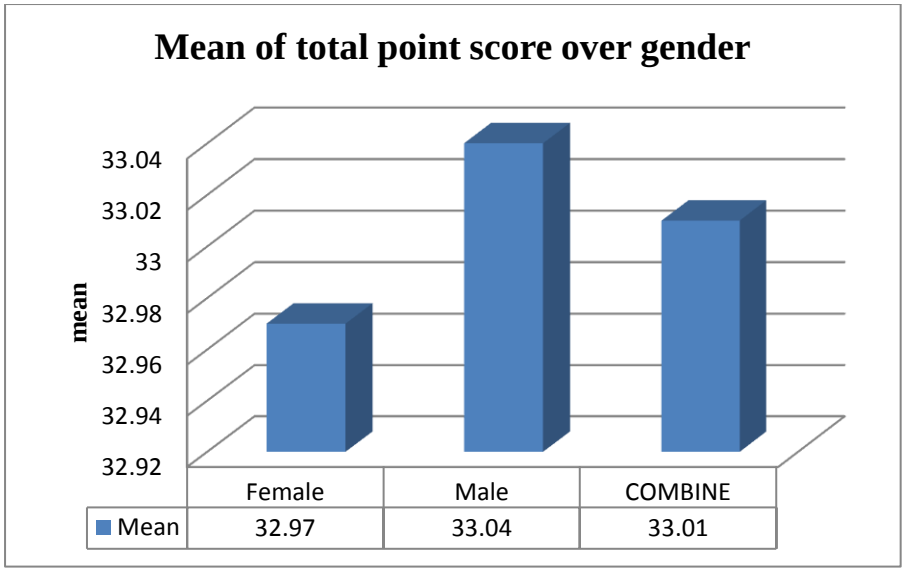
Result

Data analysis: Data analysis will consider age, gender and descriptive category according to BOT-2

Table 2: Data analysis

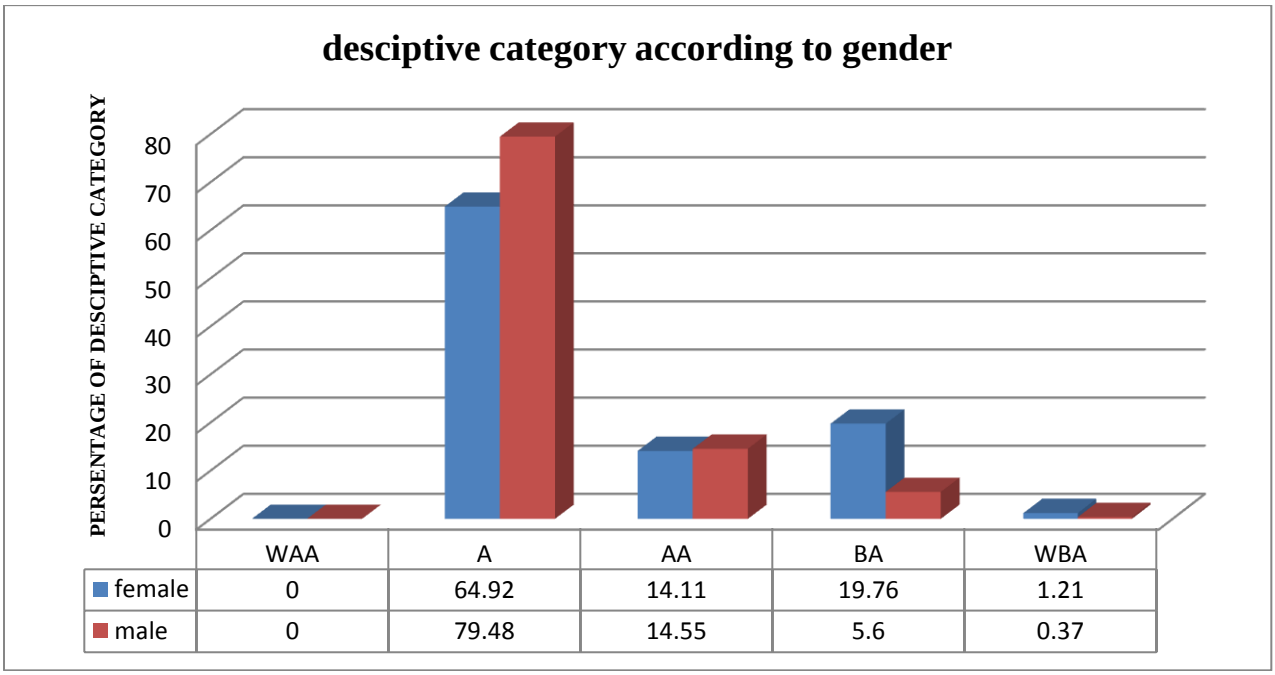
	Mean	Standard deviation
Female	32.97	3.02
Male	33.04	4.13
COMBINE	33.01	3.64

Graph 1: Mean of total point score over gender



Interpretation: graph no 2 represent that mean of balance total point score in female is 32.97 and mean of balance total point score in male is 33.04 and combine of female and male balance total point score is 33.01,

Graph 2: Represent mean of balance total point score in female

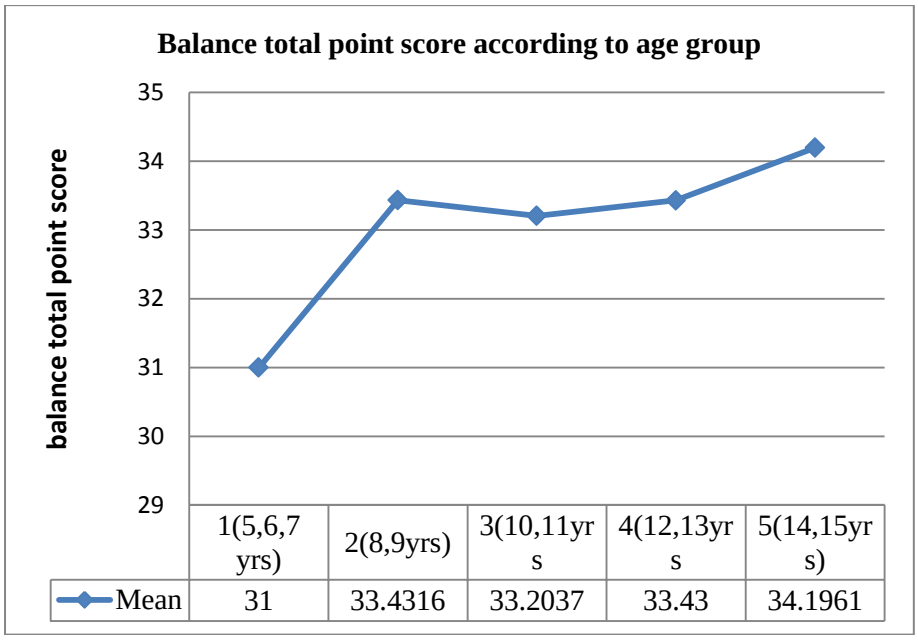


Interpretation: Graph 2 represent the population of female and male descriptive category in WAA is minimum that is 0%.In above average descriptive category 14.11% in female and 14.55% in male. In average descriptive category 64.92% in female and 79.48% in male. In below average descriptive category 19.76% was female and 5.6 % was male. In well below average descriptive category 1.21% was female and 0.37% was male.

Table 3: Age group versus sex and number

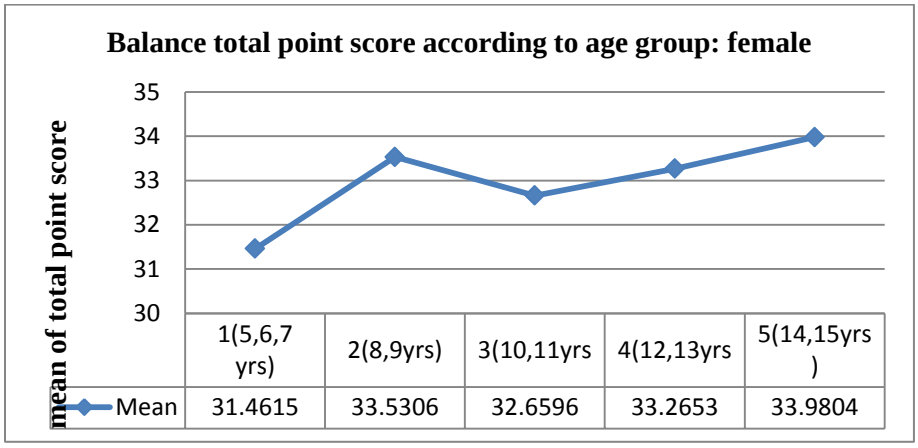
Age Gr.	Sex	n	B	
			Mean	SD
1	COM	111	31	4.94
	M	59	30.59	5.36
	F	48	31.46	3.46
2	COM	95	33.43	2.57
	M	46	33.33	2.49
	F	49	33.58	2.54
3	COM	108	33.2	4.17
	M	61	33.36	4.55
	F	47	32.66	4.17
4	COM	100	33.43	2.21
	M	51	33.59	2.2
	F	49	33.27	2.22
5	COM	101	34.2	2.32
	M	51	34.41	2.44
	F	51	33.98	2.39

Graph 3A: Balance total point score according to age group



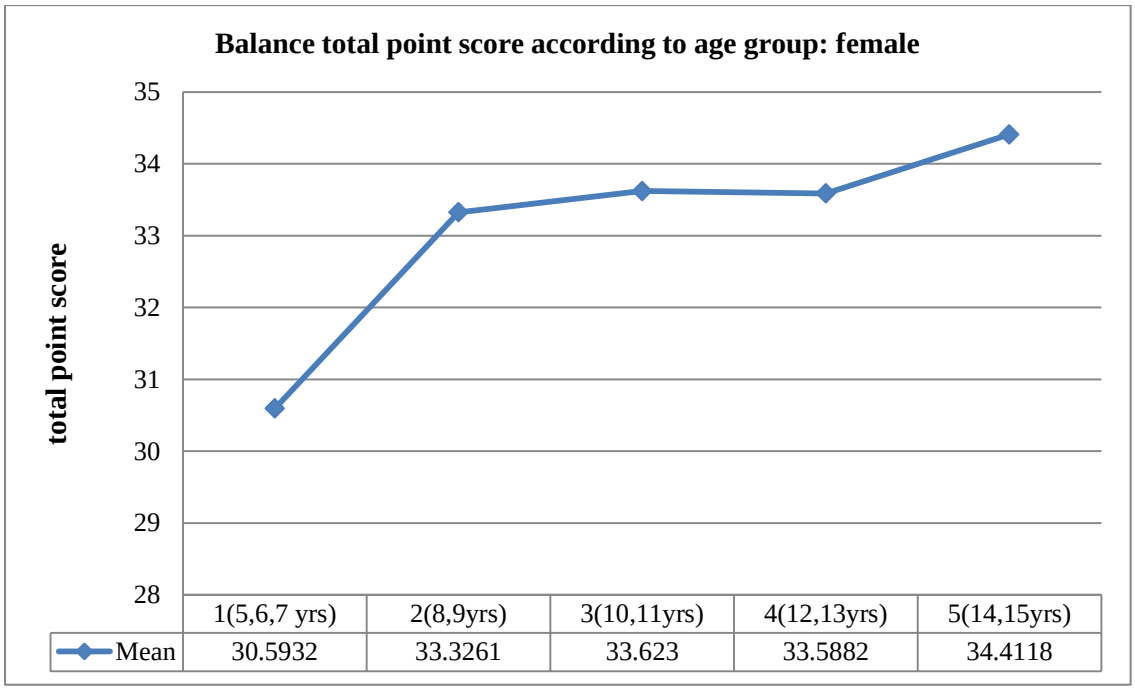
Interpretation:
Graph no 3A represent that in age group 1 there is mean of total point score is 31, and standard deviation is 4.94, In age group 2 there is mean of total point score 33.43 and standard deviation is 2.57, In age group 3 there is mean of total point score is 33.20 and standard deviation is 4.17, in age group 4 there is mean of total point score is 33.43 and standard deviation 2.21,in age group 5 there is mean of total point score is 34.19 and standard score 2.34.

Graph 3B: Balance total point score according to age group: female



Interpretation:
Graph no 3B represent that in age group 1of female there is mean of total point score is 31, and standard deviation is 3.46, In age group 2 female there is mean of total point score 33.53 and standard deviation is 2.54, In age group 3 female there is mean of total point score is 32.62 and standard deviation is 4.17, in age group 4 female there is mean of total point score is 33.26 and standard deviation 2.22,in age group 5 female there is mean of total point score is 33.98 and standard score 2.36.

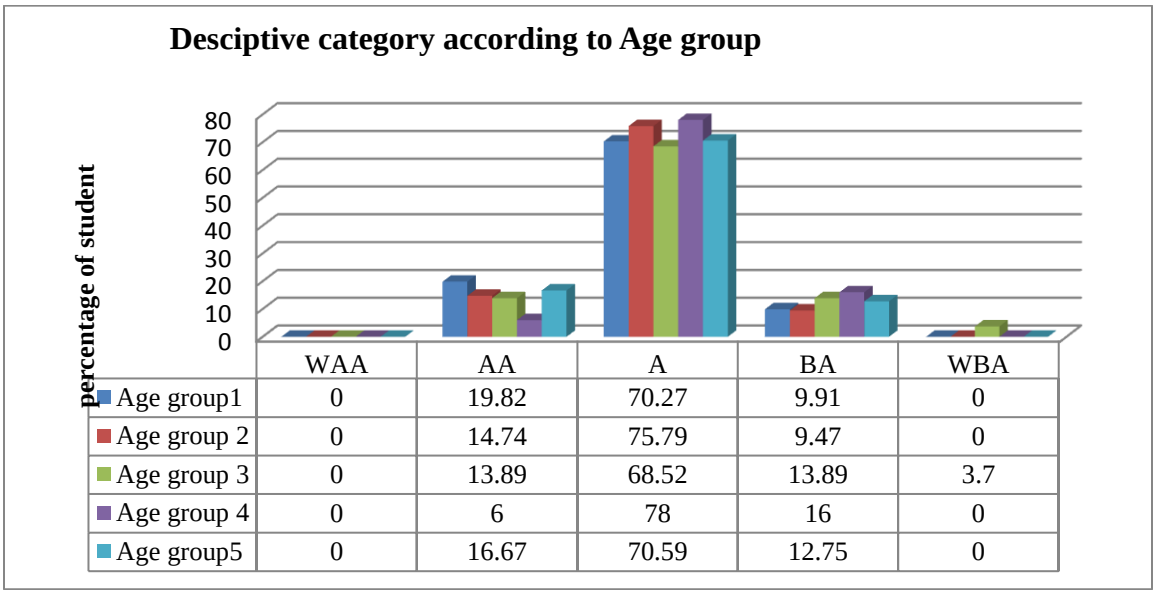
Graph 3 C: Balance total point score according to age group: female



Interpretation:

Graph no 3C represent that in age group 1of female there is mean of total point score is 30.59, and standard deviation is 3.46, In age group 2 female there is mean of total point score 33.33 and standard deviation is 2.49, In age group 3 female there is mean of total point score is 33.36 and standard deviation is 4.55, in age group 4 female there is mean of total point score is 33.59 and standard deviation 2.2,in age group 5 female there is mean of total point score is 34.41 and standard score 2.44.

Graph 4: Desciptive category according to age group



Interpretation

Graph no 4 represent descriptive category well above average in all age group is 0 %. In descriptive category above average in age group 1 is 19.82%, in age 2 is 14.74%, in age group 3 is 13.89%, in age group 4 is 6% and in age group5 is 16.67%. In Descriptive category average in age group 1 is 70.27%, in age 2 is 75.79%, in age group 3 is 68.52%, in age group 4 is 78% and in age group 5 is70.59 %. In Descriptive category below average in age group 1 is 9.91%, in age 2 is 9.47%, in age group 3 is 13.89%, in age group 4 is 16% and in age group 5 is 12.75 %. In Descriptive category well below average in age group 1 and 2 is 0%, in age group 3 is 3.7% and in age group 4 and 5 is 0 %.

Discussion

primary aim of the study was to find affection of balance in school going of 5-15 year of age group by using BOT-2.the balance subtest evaluates motor control skill that are integral for maintaining posture when standing, walking or reaching. Sample score is consistent with individuals who can maintain stability in a fixed position standing one leg on a balance beam when the eyes are open and when the eyes are closed. This study was conducted among 516 subject(mean age 10.67 year±3.2) in which 248 were male(mean age 10.69 year± 3.04) and 268 were female (mean age 10.66 year ± 3.02).According to data analysis of balance total motor score and gender graph there is slight difference in male and female mean of total point score which is slight more in male because male participate more in sports than female so males have more developed vestibular system, somatosensory system and visual system than female.[1]Descriptive category according to gender, According to the study done, in well below average descriptive category 1.21% were female and 0.37 % were male. In below average descriptive category, 19.76% were female and 5.6% were male. Maximum subject falls under average category that is 64.92% were female and 79.48% were male, In above average category 14.11 were female and 14.55 were male and in well above average category there was 0% population, these performance differences in males and female can be due to the nutritional status, the dietary intake of boys is more than girls. Nutrition status appears to be signification predictor for both fine and gross motor development.[1] Nutritional status may alter the learning process by influencing brain development and physical growth and accordingly modify the movement proficiency of the children by adjusting the strength, power, coordination and perception.[1] And it significantly related to physical growth and other parameters. Performance related fitness, is necessary for the execution of sports skill which is more in males than female, so that is the reason there is a great performance difference between the two.[1]Graph 3A, 3B and 4C shows total motor point score according to age group, in which the study reveals that as the age increases the mean values of point score also increases. Barnekow-Bergkvist et al. (1998) found that performance in physical test; height, weight and physical activity at the age of 13 contributed best of explain adult physical performance and physical activity. Therefore, it may be concluded that so far all the subject of coordination was concerned age factor was responsible for the higher mean value. Age group 4 and 5 having higher age, they had significantly performed better in comparison to age group 1, 2 &3 boys & girls. Balance is also related to limb length, general musculature and neuromuscular coordination, which are definitely influenced by the advancement of age. The remaining motor performance is related to lean body mass, general muscular, aerobic capacity and certain psychological state of mind (willingness to accept pain) and development of all of age. Therefore, it is obvious that age group 1, 2 and 3 will have less motor quality than that of age group 4 and 5 because of structural and functional differences with the higher age group.[20]Graph 4th shows descriptive category according to age groups ,in which the study revealed that in age group 1,2,3,4 and 5 descriptive category Average were more followed by Above Average ,Below Average than Well Below Average, study shows that difference between all age group is not linear because of descriptive category was according to the scale score and score that have undergone statistical transformation will be less exact in ability to detect real change that occurred because this standard score are age adjusted, progress will not be reflected in the test score unless the progress is faster than typical maturation.[21] difference can be due to socioeconomic status as we have taken homogenous sample from both public and private schools.[20] Children grow at different rates at different ages, and different children also develop at different rates, so there will be early and late developers. Not only are the rate of growth different, but also the changes in the body proportions can vary, and this will directly affect the ability to perform. Moreover, the motor performance is related to body stature, body weight, growth spurt, body composition, cardiovascular fitness and muscle strength.[20]

Acknowledgement

Having surmounted all the difficulties and after reaching the shore by completing the work of this study, I am realizing the limitations of language and words while acknowledging thanks to all those who helped me in this voyage.I thank my mother Mrs. Neena Yengde, my father Mr. Narendre Yengde & my sister miss manjari and shraddha for their moral support, prayers and encouragement that have been a pillar of strength throughout this work.Words are few and language seems feeble when the heart is full of gratitude, these few words cannot express my deep sense of gratitude to my esteemed **Guide Dr. Sanjivani Dhote**, who has been a constant source of inspiration to me since the very beginning of this work. Her unsurpassable teaching experience & scientific approach has increased my interest and knowledge in the subject. It is only because of her constructive supervision and overall encouraging sympathetic attitude that my work has acquired the present shape. It is with deep sense of gratitude and sincerity that I thank **Dr. TusharPalekar**, principal of Dr. D. Y. Patil College of Physiotherapy, Pimpri, Pune and **DR. D. Y. PATIL UNEVERSITY** for allowing to conduct project and helping me to successfully complete this study. From him I have tried to imbibe values, vast knowledge, experience and a high sense of professionalism. I am highly indebted to My Friends especially, **Prajkta K**, **Apoorva D, Antara P, Ashish G, Diksha G, Gurjit S, and Nargis H** for constantly supporting me and for bearing the brunt of this Herculaneum task.Finally, I express my sincere thanks to all my patients and the secretary of all schools, whose willingness to be a part of this study helped this work see the light of day**.** Lastly I would like to thank **God** for sending all these wonderful opportunities and giving me a chance to prove myself.

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Abbreviation

1. BOT-2 : Bruininks-Oseretsky Test Second Edition
2. F: Female
3. M: Male
4. StdDev: Standard Deviation
5. WAA: well Above Average
6. AA: Above Average
7. A: Average
8. BA: Below Average
9. WBA: Well Below Average

Source of Support: Nil
Conflict of Interest: None

Original Research Article

Age wise prevalence of developmental coordination disorder in school going children in west India

N Sanjivani Dhote[1*], J Palekar Tushar[2], Suvarna Ganvir[3]

[1]Associate Professor, Neuroscience Department of Dr. D.Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India
[2]Principal of Dr. D.Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India
[3]H.O.D. of Neuroscience, P.D.V.V.P.F'S, College of Physiotherapy, Ahmednagar, Maharashtra, India
[*]Corresponding author email: **dhotesanjivani@gmail.com**

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Abstract

Background: Children with movement skill difficulties that have not been diagnosed with a general medical condition. This difficulty in motor skill competence, observed in children who are developing well intellectually, is termed 'developmental coordination disorder' (DCD). DCD is a highly prevalent disorder (5-6% of school-aged children) so it is likely that there is at least one child with DCD in most classrooms. The early diagnosis of DCD can be helpful to prevent the future secondary complications. Aim: So purpose of this study is to find out age wise prevalence DCD in school going children in West India.
Materials and methods: It was a cross-sectional analytical study conducted in PCMC area schools Multistage stratified sampling done to assessing 516 children's by Using The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2). Statistical analysis used was Mean and Standard Deviation (SD) and Chi-square test was used to analysis.
Results: Age 8 and 9 year showed highest prevalence of DCD (3.16%). This difference in various age group was not statically significant as P=0.219 by chi-square test.
Conclusion: Age 8 and 9 year showed more prevalence of Developmental coordination disorder than other age.

Key words

DCD, Prevalence, Age group, BOT 2nd.

Introduction

Since the early 1900s, the scientific community has acknowledged a large group of children with movement skill difficulties who have not been diagnosed with a general medical condition [1]. This difficulty in motor skill competence, observed in children who are developing well intellectually, is termed 'developmental coordination disorder' (DCD). DCD is a recognized syndrome that was described by the World Health Organization in 1992 [2] and has been included in the diagnostic manuals of the American Psychiatric Association since 1989 [3].

"*DCD is defined, using the Diagnostic And Statistical Manual Of Mental Disorders, Fourth Edition (DSM-IV), as a condition marked by a significant impairment in the development of motor coordination, which interferes with academic achievement and/or activities of daily living (ADL). These difficulties are not due to a general medical condition (e.g., cerebral palsy) and are in excess of any learning difficulties is present* [4].

DCD is a highly prevalent disorder (5-6% of school-aged children) so it is likely that there is at least one child with DCD in most classrooms. One of the challenges of identifying children with DCD is the variety of ways in which it is revealed [5]. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) provides four criteria to classify a child as having DCD [3].

The difficulties may be considered to be mild, moderate or severe. Even though this condition is observed by many schoolteachers, as well as physical and occupational therapists, it is not an easy diagnosis to make due to multi-faceted diagnostic criteria and terminology problems [5]. Outcome measurements used to assess gross motor development in infants and children up to age 5, including the Peabody Developmental Motor Scale [6] (PDMS-2), second edition and the Alberta Infant Motor Scale [7] (AIMS). When children age out of either the PDMS-2 or the AIMS, one standardized assessment option physical therapists have is BOT-2nd [8-10]. The test-retest reliability and internal consistency of the total scale were excellent, with an ICC of 0.99 (95% confidence interval) and alpha of 0.92 The BOT-2nd can be used to evaluate a wide variety of fine and gross motor skills for children, teenagers and young adults 4-21 years of age. This is a test that can also be used by Physiotherapist, psychologists, adaptive physical education teachers, special education teachers and educational diagnosticians [8-11]. The prevalence of DCD in India is found to be 1.37%. The prevalence of DCD in other countries is estimated to be (5-8%) USA, (1.8%) UK, (5.7%) Greek, (5-9%) Canada, (1.7%) Belgium, and 6% worldwide [12-16]. As per the literature there are no studies found on the prevalence of DCD using BOT-2 in 5-15 years of age group in Indian scenario. Considering the importance of timely diagnosis of DCD and the child's performance on the BOT-2 will allow the physical therapist to identify areas of strength and areas of need in regards to the child's gross motor functioning, and can therefore help to guide treatment. The early diagnosis of DCD can be helpful to prevent the future secondary complications. So, purpose of study was to find out age wise prevalence of developmental coordination disorder in school going children.

Materials and methods

The Cross Sectional analytical study was conducted in Pimpri Chinchwad area of age group of 5 to 15 years. Total samples 516 were studied. The Subjects were divided according to age groups. Age Group 1 includes 5.0-7.11, age group 2 includes 8.0-9.11, age group 3 includes 10.0-11.11, age group 4 includes 12.0-13.11 and age group 5 includes 14.0-15.11. Inclusion criteria were normal and healthy school going

children. Exclusion criteria were neurological trauma like spinal fractures, any visul problem, or any congenital deficit. BOT$_{TM}$-2nd kit was used for assessment.

Procedure

The synopsis of the study was submitted to institutional ethical committee, after the clearance 516 subjects were selected who fulfilling the inclusion criteria. After explaining the purpose of the study to the subject/parent, they were informed that they can withdraw any time during the course of the study without giving reason for doing so. The parents/teacher was assured that their child's participation and non-participation would not affect their child's education. Subjects were selected on the basis of multistage sampling method. In the first stage, 3 English and 3 Marathi schools were selected randomly out of the total schools in Area. In 2nd stage, from each standard, any one division was selected randomly. In 3rd stage, from every division, boys and girls of same age were selected by random sampling method. A written informed consent was obtained from the subjects/parents one day prior to the assessment. Proper precautions were taken so that there was no harm to the child. Total children were divided into 5 age groups according to their chronological age. These age groups were divided for sampling convenience and for obtaining proper results. The age group 1 included age group ranging from 5.0-7.11 years , age group 2 included 8.0-9.11, age group 3 included 10.0-11.11, age group 4 included 12.0-13.11 and age group 5 included 14.0-15.11.

BOT-2nd was used to assess children's motor proficiency. BOT-2 is an individually-administered test that uses engaging, goal-directed activities to measure a wide array of motor skills in individuals aged 4 through 21 (Bruininks and Bruininks, 2005). The BOT-2 assesses motor proficiency in four motor-area composites; fine manual control (FMC), manual coordination (MC), body coordination (BC) and strength and agility (SA). BOT-2 has 8 subtests with 53 items and each motor-area composite has two subtests. The total motor composite score can be calculated by adding four composite scores together (53 items, 8 subtests and 4 four motor-area composites; score range = 0–320 points) (Bruininks and Bruininks, 2005). Subjects were assessed for these tasks and these raw score were converted to a numerical point score. Descriptive analysis done by using manual, point score converted in to five descriptive category that of WAA-Well above average, AA- Above Average, A – Average, BA-Below Average, WBA- Well Below Average.

Results

Demographic Data according Gender and Age Group was as per **Graph – 1**. Mean and standard deviation of subtest point score by Age group was as per **Table – 1**. Mean and standard deviation of Composite and Total Motor composite standard score by Age group and Gender was as per **Table – 2**. Descriptive category of Children on Total motor Proficiency According Age Group was as per **Graph – 2**. Prevalence of DCD among Age Groups was as per **Graph – 3**.

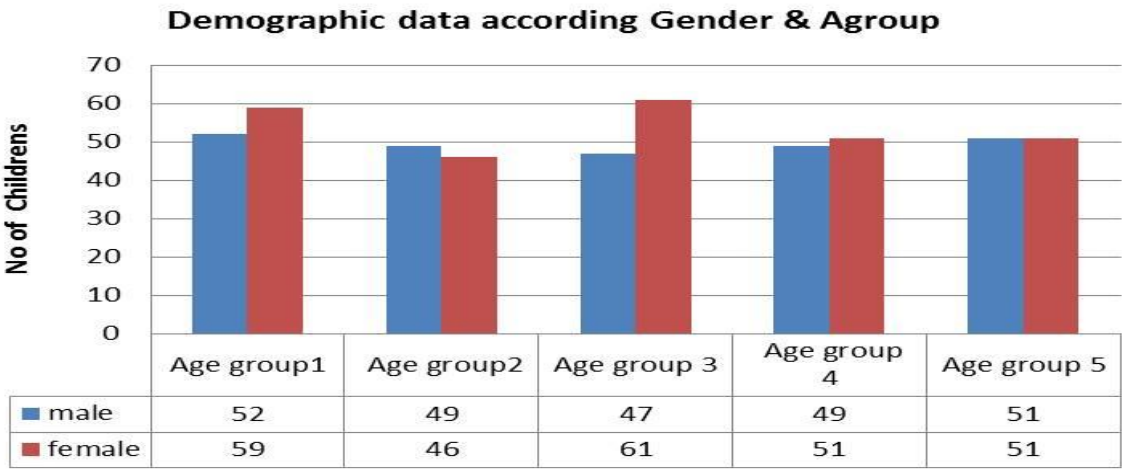
Discussion

The present study was carried out with the aim To find out Point score of all eight subtest, Descriptive category of four subtest Composite and Total Motor Composite component on BOT-2 in school going children of age group between 5 to 15 years according age groups.

Graph - 1 showed Five hundred and sixteen children (Mean age =10.67 years, SD = 3.03) participated in this study among that 248 and 268 were male and female respectively. Table 1a and 1b showed linear pattern subtest point score of Motor proficiency according to age group. The use of subtest point score will result in more precise measurement of function, because gain or deterioration will be related to specific area of motor control [17]. Barnekow-Bergkvist, et al. (1998) found that performance in physical tests; height, weight and physical activity at the age of 13 contributed best of explain adult physical

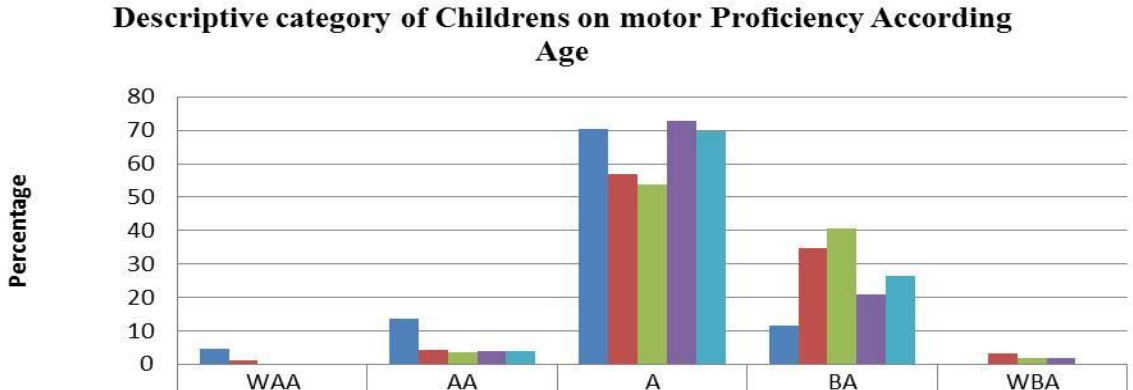
performance and physical activity. Therefore, it may be concluded that so far when all the subtest point score was concerned age factor was responsible for the higher mean value. Age group 4 and 5 having higher age, they had significantly performed better in comparison to Age group 1, 2 and3 boys and girls. Motor performance is related to lean body mass, general musculature, aerobic capacity and certain psychological state of mind (willingness to accept pain) and development of all of which are influenced by advancement of age. Therefore, it is obvious that Age group 1, 2 and 3 will have less motor quality than that of Age group 4 and 5 because of structural and functional differences with the higher age groups [18]. Magalhaes, et al. (1989), in their study on the development of bilateral coordination on certain jumping tasks observed improvement in the performance with age in their sample of 5 to 9 years of typical children [19]. Moreover, the motor performance is related to body stature, body weight, growth spurt, body composition, cardiovascular fitness and muscle strength [20] hence as age increases point score of motor proficiency also get increases.

Graph - 1: Demographic Data according Gender and Age Group.



	Age group1	Age group2	Age group 3	Age group 4	Age group 5
male	52	49	47	49	51
female	59	46	61	51	51

Graph - 2: Descriptive category of Children on Total motor Proficiency According Age Group.



	WAA	AA	A	BA	WBA
age group1	4.5	13.51	70.27	11.71	0
age group 2	1.05	4.21	56.84	34.74	3.16
age group 3	0	3.7	53.7	40.74	1.85
age group 4	0	4	73	21	2
age group 5	0	3.92	69.61	26.47	0

Graph - 3: Prevalence of DCD among Age Groups.

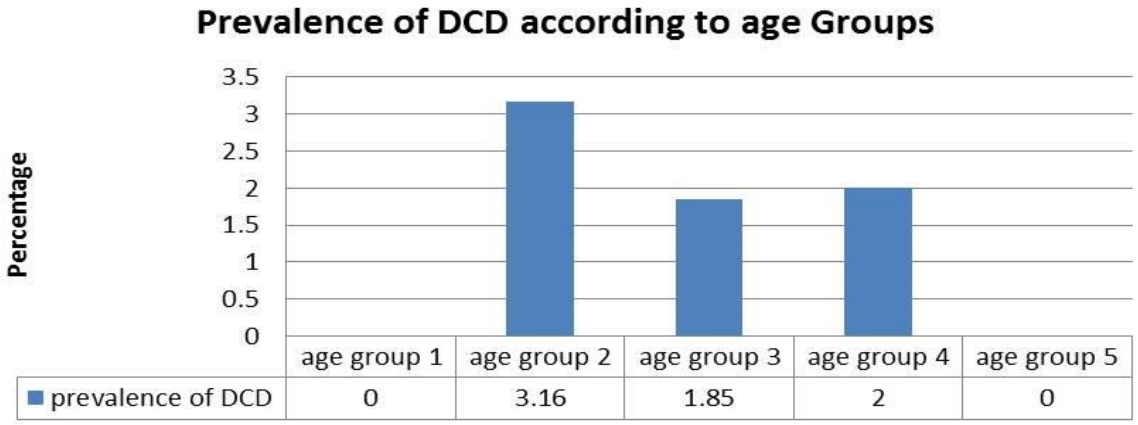


Table – 1: Mean and standard deviation of subtest point score by Age group.

Table - 1(a)

Age Group	Sex	N	FMP		FMI		MD		ULC	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (Age 5,6&7)	**COM**	111	24.47	5.89	24.97	7.53	19.19	5.73	16.91	9.08
2 (Age 8&9)	**COM**	95	30.56	6.40	30.61	6.72	25.08	4.56	27.47	8.32
3 (Age 10&11)	**COM**	108	32.08	5.82	33.06	5.04	29.16	4.51	32.23	6.04
4 (Age 12&13)	**COM**	100	35.33	5.79	34.10	5.71	32.62	4.50	34.02	4.25
5 (Age 14&15)	**COM**	101	36.36	5.45	35.24	4.88	33.78	4.05	35.39	3.82

Table - 1(b)

Age Group	Sex	N	BLC		B		RSA		S	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (Age 5,6&7)	**COM**	111	17.35	3.66	31.00	4.94	24.25	4.46	24.04	4.95
2 (Age 8&9)	**COM**	95	19.60	6.62	33.43	2.57	29.94	4.39	24.61	4.75
3 (Age 10&11)	**COM**	108	20.95	3.20	33.20	4.17	33.99	4.93	26.52	4.92
4 (Age 12&13)	**COM**	100	22.04	3.28	33.43	2.21	34.67	4.49	27.38	5.54
5 (Age 14&15)	**COM**	101	21.88	3.05	34.20	2.32	34.81	4.55	28.44	3.73

Abbreviation: COM: Combine (Male & female), N= Total number of sample, SD: Standard Deviation, FMP: Fine Motor Precision, FMI: Fine Motor Integration, MD: Manual Dexterity, ULD: Upper Limp Coordination, BLC: Bilateral Coordination, B: Balance, RSA: Running Speed And Agility

Table - 2a and **Table - 2b** showed Standard score, Descriptive category of all composite component and TMC did not showed any linear pattern of motor development with age growth because Brenda N. Wilson concluded Standard Score and Descriptive category that have undergone statistically transformation will be less exact in their ability to detect real changes that occurred. Because these standard score are age adjusted, progress will not be reflected in the score unless the progress is faster than typical maturation (which is not likely to occur with children who have motor problem). Therapist should consider using the subtest point score as accurate measure of change [17].

Graph - 2 showed Maximum Children were found in Average category of motor proficiency

followed by Below average category in all age groups. Age group 1 showed better performance than other group as maximum children from this group (88.28%) fall between average to well above average category. Graph 3 showed Developmental coordination disorder in various age group showed did not show statically significant difference as P=0.219. However age 8 and 9 years showed highest prevalence of DCD (3.16%) followed by Age 12 and 13 year (2%). The Indian children under-performed in the bilateral coordination subtest across all age group 7, 8 and 9 as compared to the USA normative sample. This observed developmental variation in the bilateral coordination patterns between Indian children and USA normative sample which may be attributed to the cultural and environmental (school) variations [20].

Limitation of the present study was socioeconomic status, Cardiorespiratory Fitness and Body Mass Index were not considered while finding out the prevalence of DCD. Further studies can be conducted to investigate Motor proficiency of school going children who were underweight at time of birth and preterm.

Table - 2: Mean and standard deviation of Composite & Total Motor composite standard score by Age group and Gender.

Table - 2(a)

Age Group	Sex	n	FMC		MC		BC	
			Mean	SD	Mean	SD	Mean	SD
1 (Age 5,6&7)	**COM**	111	25.46	7.90	25.94	8.83	31.81	7.23
2 (Age 8&9)	**COM**	95	23.65	9.75	25.91	8.55	28.73	6.55
3 (Age 10&11)	**COM**	108	21.05	8.54	27.95	21.09	28.69	9.83
4 (Age 12&13)	**COM**	100	23.08	8.81	27.71	7.53	28.66	6.31
5 (Age 14&15)	**COM**	101	23.40	9.24	28.05	7.49	28.77	7.56

Table - 2(b)

Age Group	Sex	n	S&A		TMC	
			Mean	SD	Mean	SD
1 (Age 5,6&7)	**COM**	111	34.58	6.83	49.17	9.45
2 (Age 8&9)	**COM**	95	30.09	5.62	45.06	9.10
3 (Age 10&11)	**COM**	108	30.31	5.67	42.87	9.04
4 (Age 12&13)	**COM**	100	28.77	4.84	43.55	8.40
5 (Age 14&15)	**COM**	101	27.15	4.19	44.94	9.17

Abbreviation: FMC: Fine Manual Control, MC: Manual Coordination, BC: Body Coordination, S and A**:** Strength and Agility and TMC: Total Motor Composite.

Conclusion

Age 8 and 9 years showed highest prevalence of Developmental coordination disorder followed by Age 12 and 13 years. This difference in various age group is not statically significant.

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Original article:

Assessment of upper limb coordination using bruininks-oseretsky test of motor proficiency, 2nd edition (bot-2), in 5-15 years school going children

***Apoorva D. Dighe, Dr.Sanjivani Dhote, Dr.Tushar Palekar, Antara Pande, Pooja Yengde, Gurjit Singh**

DR.D.Y.PATIL COLLEGE OF PHYSIOTHERAPY,PIMPRI PUNE-411018

Corresponding author*

ABSTRACT:

Background: The purpose of the study was to assess the upper limb coordination in school going children of 5-15 years using Bruininks- Oseretsky test of motor proficiency. Motor skill is a learned series of movements that combine to produce a smooth, efficient action. Coordination is achieved when subsequent parts of the same movement, or the movements of several limbs or body parts are combined in manner that is well timed, smooth, and efficient with respect to the intended goal.

Material and methods: A Cross sectional analytical study was conducted using BOT-2 Short Form. A multistage stratified sampling of children (n=516) aged 5-15 years was done that included 268 females and 248 males.

Results: 516 children were assessed (268 females, mean age 10.69 years±3.04 & 248 males, mean age 10.66 years ±3.02).8.87 females showed well below average score whereas 1.87 males showed below average score.

Conclusion: The study concluded that performance of females was poor as compared to males. Children of 12, 13, 14 & 15 years showed better results than 5-11 years children.

Keywords: Upper limb coordination (ULC), Bruininks-Oseretsky test (BOT), Developmental Coordination Disorder (DCD), Motor Coordination.

INTRODUCTION:

Motor development refers to the gradual process by which a child gains use and coordination of the large muscles of the legs, trunk and arms and the smaller muscles of the hands.[1]The neuromuscular development starts in embryonic stage which continues after birth. According to studies done in different parts of the world, development of motor skill is associated with physical activity like throwing or catching a ball, in both children and adolescent.[1]Physical activity has been an additional determinant of motor performance along with other factors.

When subsequent parts of same movement, or the movements of several limbs or body parts are combined in a manner that is well timed, smooth, and efficient with respect to the intended goal, coordination is achieved.[2] This involves the integration of proprioceptive information detailing the position and movement of musculoskeletal system with the neural processes in the brain and spinal cord that controls, plan and relay motor coordination. Age plays a key role in upper limb coordination. It allows a person to be involved in the participation of sport with a reasonable amount of success as it will aid fluid body movement for skill performance.[2] The participation in sport is helpful in developing a social network and achieving a sense of belonging in a community or social setting as well as in maintaining self regulation for daily tasks. Thus, children will be able to maintain appropriate and controlled body movement during task performance which reduces the energy required and thus minimises fatigue.

Upper limb coordination deficit is observed in children with Development coordination disorder(DCD), Learning Disabilities, Sensory Integrative Dysfunction and other motor impairments.[3]

In clinical practice, upper limb coordination deficits in children are identified by observations of poor coordination of two body sides, avoidance of crossing of midline, failure to develop a preferred hand and possibly right-left confusion.[4] For assessing this upper limb coordination many scales are available such as,

Movement Assessment Battery for children (Movement ABC-2)[5] Peabody Development Motor Scales (PDMS 2)[5]Maastrichtse Motoriek Test (MMT)[5]

Bruininks-oseretsky Test of motor proficiency, Second Edition (BOT-2)

It is an individually administered test that uses engaging, goal- directed activities to measure a wide array of motor skills in individuals of age 4-21.The BOT-2 uses a subset and composite structure that highlights motor performance in the broad functional areas of stability, mobility, strength, coordination and object manipulation. The Bruininks-oseretsky Test of motor proficiency-BOTMP,(Bruininks,1978) consists of 46 items grouped under eight different subtests of motor proficiency for children between 4 and 15 years of age.[4]

The upper limb coordination subset of BOTMP is the seventh subset under gross motor composite. The scoring system varies with each item, ranging from a 0- point (pass/ fail) to a 5-point scale. The number of performance trials for each item is specified. A raw score is recorded in the unit measured (e.g. number of catches, dribbles) and then converted to a numerical point score.[6] This motor –area composite measures control and coordination of the arms and hands, especially for object manipulation.[6]

The Upper Limb Coordination subtest consists of activities designed to measure visual tracking with coordinated arm and hand movement.

AIMS AND OBJECTIVES:

This study conducted with the aim:

To assess upper limb coordination using Bruininks-oseretsky Test of motor proficiency, Second Edition (BOT-2), in 5 to 15 years school going children.

The objectives of the study were, to find out Upper limb coordination point score and descriptive category using Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition, to find out upper limb coordination point score and descriptive category among males and females using Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition and find out upper limb coordination point score and descriptive category, according to age groups using Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition.

MATERIAL AND METHODS:

Research committee of Dr. D.Y. Patil College of Physiotherapy approved this study. The tools used in this study were BOT-2 kit includes examiners manual, individual record form, student booklet, tennis ball and target. A table and chair of appropriate to child's height and clipboard were additionally used.

Five hundred sixteen samples were assessed, in which 268 were females (mean age 10.69 years±3.04) and 248 were males (mean age 10.66 years±3.02). Information provided by the class teacher and school records were used to include the 5-15 years old children in five groups. (The age group 1-5.0-7.11,age group 2-8.0-9.11,age group 3-10.0-11.11,age group 4 -12.0-13.11 and age group 5-14.0-15.11), according to the following criteria: no neurological trauma like spinal fracture ,6

months back, no visual and musculoskeletal problems, no neurological deficit or other diagnosed medical condition. The sample characteristic of the 516 is described in the table 1.

TABLE 1

DESCRIPTION OF THE STUDY SAMPLE

AGE GRP	FEMALE	MALE
1(5,6,7)	59	52
2(8,9)	46	49
3(10,11)	61	47
4 (12,13)	51	49
5(14,15)	51	51

PROCEDURE

Subjects were selected on the basis of multistage sampling method. In the first stage, 3 English schools and 3 Marathi schools was selected randomly out of the total schools in Pimpri Chinchwad Area. In the second stage, from each standard, 1 division was selected. In third stage, from every division, boys and girls of same age were selected by stratified random sampling method.

A written informed consent was obtained from the parents one day prior to the assessment. A pre assessment was taken to record their socio demographic data and other parameters. Proper precautions was taken so that there was no harm to the child. Every child was asked to do 7 tasks- Dropping and catching the ball with both hands- The examinee was asked to drop the ball and after it bounces once, catches with both hands .A catch was counted incorrect if the examinee traps the ball against his or her body or catches with one hand. The number of correct catches out of 5 trials was recorded. second was Catching the ball with both hands from 10 feet distance – The examinee was standing just behind the line (i.e 10 feet from the examiner) and catch the ball with both hands which was carefully tossed underhanded and with a slight arc between the examinee's shoulders and waist. A catch was incorrect if, the examinee catches the ball against his or her body or if the ball was thrown above the shoulders, below the knees or outside the examinee's reach. The number of correct catches out of 5 trials was recorded. Thirdly, Dropping and catching the ball with one hand- The examinee was asked to drop the ball and after it bounces once, catch with preferred hand .A catch was counted incorrect if the examinee trapped the ball against his or her body or catches with the non-preferred hand. After that Catching the ball from 10 feet distance with one hand- The examinee was asked to stand just behind the line (i.e 10 feet from the examiner) and catches the ball with preferred hand which is carefully tossed underhanded and with a slight arc between the examinee's shoulders and waist. A catch was counted incorrect if the examinee catches the ball against his or her body or if the ball was thrown above the shoulders, below the knees or outside the examinee's reach. The number of correct catches out of 5 trials was recorded. Then dribbling the ball with one hand- The examinee was asked to drop the ball and then dribble the ball with preferred hand. Second trial was conducted if the examinee does not earn the maximum score of 10 dribbles on

the first trial. Correct number of dribbles was recorded up to 10. A Dribble was counted incorrect, if the examinee dribbles with non preferred hand, catches the ball or allows the ball to bounce more than once between dribbles. The sixth task was Dribbling the ball with alternating hand- The examinee was asked to drop the ball and then was asked to dribble the ball with alternating hands. Second trial was conducted if the examinee does not earn the maximum score of 10 dribbles on the first trial. Correct number of dribbles was recorded up to 10. A Dribble was counted incorrect, if the examinee does not alternate hands with each dribbles, catches the ball or allows the ball to bounce more than once between dribbles, and last task was Throwing a ball at a target- The examinee was asked to stand just behind the line (i.e.7 feet from the target) and is asked to throw the ball with preferred hand, over handed or with modified side arm motion. Number of correct throws was recorded out of 5. A Throw was counted incorrect if examinee misses the target, throwed underhand or stepped over the line while throwing.

Subjects were assessed for these tasks and these raw score were then converted to a numerical point score.

OBSERVATION & RESULTS:

All the subjects completed the upper limb coordination assessment and the results of the findings were converted on the percentage scale to get a better estimation of the upper limb coordination score in 5-15 years school going children.

The followings are the graphical representation of the findings of the study:-firstly, Mean and Standard deviaton of upper limb coordination point score, and DESCRIPTIVE ANALYSIS, which will consider Age, Gender & Descriptive category according to BOT-2.

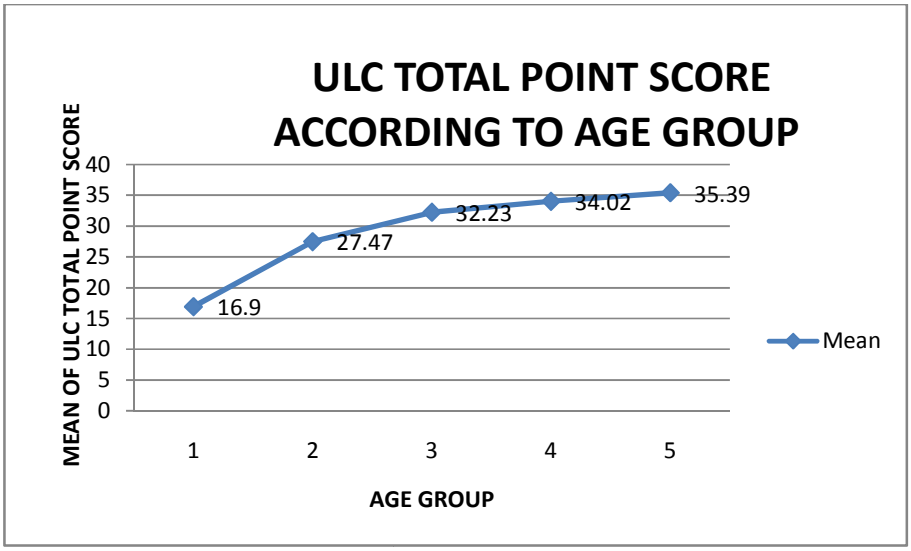
For interpretation of results, the values of mean & standard deviation was calculated by epi info 7.The mean score and standard deviation of the age group 1-5 according to male and female was interpreted.

TABLE 2; SUMMARTIVE VALUES OF UPPER LIMB COORDINATION MOTOR POINTSCORE ACCORDING TO AGE GROUPS

Age Gr.	Sex	N	ULC	
			Mean	SD
1	COM	111	16.9	9.07
	M	59	19.67	9.09
	F	48	13.76	8.05
2	COM	95	27.47	8.32
	M	46	30.89	7.25
	F	49	24.26	8.04
3	COM	108	32.23	6.03
	M	61	34.9	3.99
	F	47	28.76	6.5
4	COM	100	34.02	4.25
	M	51	35.62	2.74
	F	49	32.34	4.88

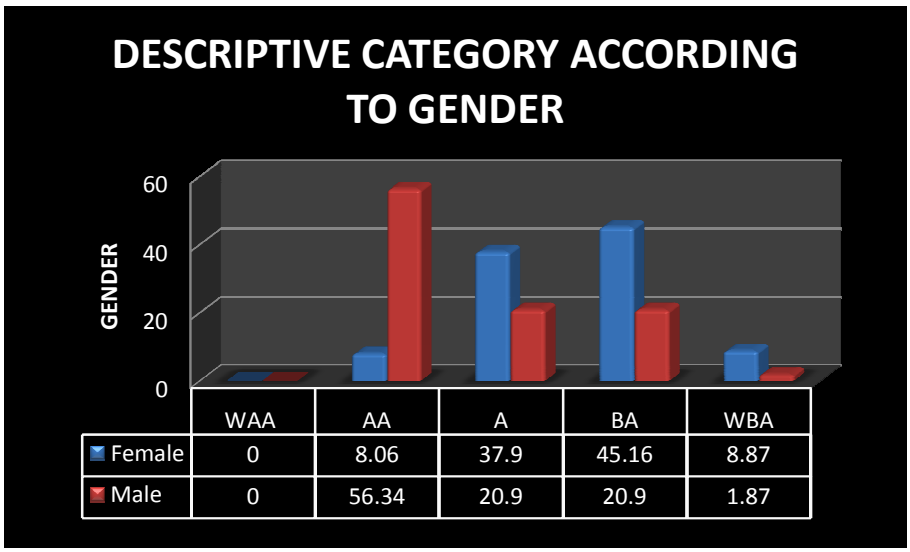
5	COM	101	35.39	3.82
	M	51	36.41	3.46
	F	51	34.37	3.91

GRAPH 1; UPPER LIMB COORDINATION TOTAL MOTOR POINT SCORE ACCORDING TO AGE GROUP



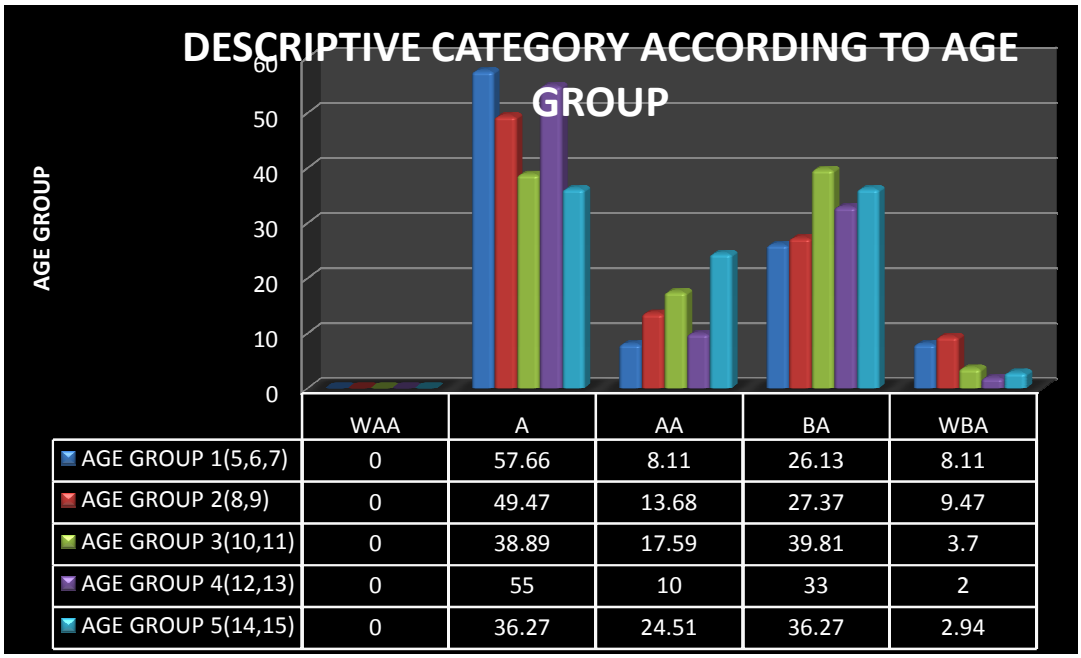
According to the graph, as the age group increases, the upper limb coordination total motor point score also increases.

Graph 2 - DESCRIPTIVE CATEGORY ACCORDING TO GENDER



The performance of the students was interpreted by the descriptive category and was compared among male & female. According to this, 45.16 % females showed Below average performance where as only 20.9% males showed Below average performance, where as 8.87% females showed Well Below average performance, where as only 1.87% males were under the category of Well Below average.

GRAPH 3: DESCRIPTIVE CATEGORY ACCORDING TO AGE GROUP



	WAA	A	AA	BA	WBA
AGE GROUP 1(5,6,7)	0	57.66	8.11	26.13	8.11
AGE GROUP 2(8,9)	0	49.47	13.68	27.37	9.47
AGE GROUP 3(10,11)	0	38.89	17.59	39.81	3.7
AGE GROUP 4(12,13)	0	55	10	33	2
AGE GROUP 5(14,15)	0	36.27	24.51	36.27	2.94

According to above graph, the scores of students were mostly higher in average category, except Age Group 3, where below average were more and Age Group 5 showed similar number in both average and below average category.

DISCUSSION:

The primary aim of the study was to find affection of upper limb coordination in the school going children of age 5-15 years using BOT 2 Scale. The upper limb coordination subtest of BOT 2,which is the seventh subtest under gross motor composite. Sample's score is consistent with individuals who generally can catch a tennis ball that is tossed from 10 feet away about 50% of the time, dribble a tennis ball two to five times, and hit a target with a tennis ball from 10 feet away about 25% of the time. The scoring system varies with each item, ranging from a 0-point (pass/fail) to a 5-point scale. The total number of samples were 516, (mean age 10.67 years ± 3.02)in which 268 females(mean age 10.69 years±3.04) & and 248 were males(mean age 10.66 years±3.02).

Descriptive category according to gender, showed a vast difference between males and females. According to the study done, 45.16 % females showed Below average performance where as only 20.9% males showed Below average performance, where as 8.87% females showed Well Below

average performance , where as only 1.87% males were under the category of Well Below average. These performance differences in males and females can be due to the dietary intake of boys is more than that of girls (satabdi ghosh et al 2013)[1]. Nutritional status appear to be significant predictor for both fine and gross motor development.[1] It also alters the learning process by influencing brain development and physical growth and accordingly modify the movement proficiency of the children by adjusting the strength, power, coordination and perception.[1]

Total motor point score according to age group, in which the study reveals that as the age increases the mean values of point score also increases. Barnekow- Bergkvist et al. (1998) found that performance in physical tests; height, weight and physical activity at the age of 13 contributed best to explain adult physical performance and physical activity. Therefore, it may be concluded that so far all the subtests of coordination was concerned age factor was responsible for higher mean value. Age group 4 &5 having higher age, they had significantly performed better in comparison to age group 1, 2 & 3 boys and girls.

Coordination was also related to limb length, general musculature and neuromuscular coordination, which are definitely influenced by the advancement of age. The remaining motor performance is related to lean body mass, general musculature, aerobic capacity and certain psychological state of mind (willingness to accept pain) and development of all of which are influenced by advancement of age. Therefore, it is obvious that age group 1, 2 &3 will have less motor quality than that of age group 4&5 because of structural and functional differences of higher age groups.

Descriptive category according to age groups, in which the study revealed that BA and WBA were more in the age group 1 & 2 as compared to age group 3, 4 & 5.[3] It is observed that children of age 8, 9, & 10 are less in the activities played with a tennis ball than the children of age 11, 12, &13.Environmental factors including the schedules of school's physical education activities may explain some of the remaining variability of scores. From the foregoing discussion of the leading researchers it has also been evident that the growth and development of body parts and functional capacity of the organs and systems improve rapidly during pre-pubertal stage and each year during this stage results significant improvement in stature. Chatterjee et al. (1992) has also reported that gradual increase in motor fitness measurements with the advancement of age on school going children of 5-15 years age. Therefore, it is expected that during pre- adolescent stage with advancement of age (12-15 years) that motor activity involving neuromuscular coordination will also increase, that is the reason age group 1&2 had WBA performances more than Age group 3,4 &5.

LIMITATIONS;

In our study, we were not able to take the socioeconomic status that can be probably a reason for the performance score difference amongst different age group students.

FURTHER SCOPE:

To establish normative data & to consider different medium schools and body mass index of the children.

CONCLUSION

The study concluded that, there is very slight score difference in males and females, although males showed better performance than females in coordination skills like catching and throwing of objects. Children of age 12,13,14,15 years showed better results than children of age 5, 6, 7, 8, 9, & 10 years i.e. as the age advanced performances of the students increased.

ACKNOWLEDGEMENT

Having surmounted all the difficulties and after reaching the shore by completing the work of this study, I am realizing the limitations of language and words while acknowledging thanks to all those who helped me in this voyage.

I thank my mother **Mrs.Dipti Dighe**, my father **Mr. Dilip Dighe** & my brother **Akshay Dighe** for their moral support, prayers and encouragement that have been a pillar of strength throughout this work. Words are few and language seems feeble when the heart is full of gratitude, these few words cannot express my deep sense of gratitude to my esteemed **Guide Dr. Mrs. Sanjivani Dhote**, who has been a constant source of inspiration to me since the very beginning of this work. Her unsurpassable teaching experience & scientific approach has increased my interest and knowledge in the subject. It is only because of her constructive supervision and overall encouraging sympathetic attitude that my work has acquired the present shape.

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Original Research Article **Allied Science**

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THE PREVALENCE OF DEVELOPMENTAL COORDINATION DISORDER IN SCHOOL GOING CHILDREN OF WEST INDIA

DR. SANJIVANI N DHOTE (PT)[1]*, DR. MANISHARATHI (PHD)[2] AND DR. TUSHARPALEKAR (PH.D)[3]

*[*1]Department of Neurosciences , Dr. D. Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune, India.*
[2]Department of community based rehabilitation , Dr. D. Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune, India.
[3]Department of Musculoskeletal , Dr. D. Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune, India.

ABSTRACT

Development specifies maturation of functions. It is related to the maturation and myelination of the nervous system and indicates acquisition of a variety of skills for optimal functioning of the individual.[1]Considering the importance of timely diagnosis of DCD andthe child's performance on the BOT-2 will allow the physical therapist to identify areas of strength and areas of need in regards to the child's gross motor functioning, and can therefore help to guide treatment.The early diagnosis of DCD can be helpful to prevent the future secondary complications. So purpose of this study is to find out the prevalence of DCD on BOT-2 in 5 to 15 years school going children. : It was a cross-sectional analytical study conducted in schools of Pimpri-Chinchwad area. This study included 516 students assessed by Using BOT-2nd edition. Prevalence of DCD was 1.16% (95%CI 0.43% to 2.51%). Where as female showed more prevalence of Developmental coordination disorder than Male

KEYWORDS: *Prevalence, Developmental coordination Disorder , School going childrens, motor proficiency.*

DR. SANJIVANI N DHOTE (PT)[1]*
Department of Neurosciences ,Dr.D.Y.Patil College of Physiotherapy, Pimpri, Pune, India.

***Corresponding Author**

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INTRODUCTION

Growth is an essential feature of life of a child that distinguishes him or her from an adult. The process of growth starts from the time of conception and continues until the child grows into fully mature adult. The terms growth and development are often used together. These are not interchangeable, because they represent two different fact of dynamics of change, i.e; those of quantity and quality. Growth and development usually proceed concurrently, but may not always be interrelated[1].Since the early 1900s, the scientific community has acknowledged a large group of children with movement skill difficulties who have not been diagnosed with a general medical condition [2]. This difficulty in motor skill competence, observed in children who are developing well intellectually, is termed 'developmental coordination disorder' (DCD). DCD is a recognized syndrome that was described by the World Health Organization in 1992 [3] and has been included in the diagnostic manuals of the American Psychiatric Association since 1989[4] . "Developmental coordination disorder (DCD) is defined, using the Diagnostic And Statistical Manual Of Mental Disorders, Fourth Edition (DSM-IV), as a condition marked by a significant impairment in the development of motor coordination, which interferes with academic achievement and/or activities of daily living (ADL). These difficulties are not due to a general medical condition (eg, cerebral palsy) and are in excess of any learning difficulties is present[5]. DCD is a highly prevalent disorder (5-6% of school-aged children) so it is likely that there is at least one child with DCD in most classrooms. One of the challenges of identifying children with DCD is the variety of ways in which it is revealed.[6] The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) provides four criteria to classify a child as having DCD[4]. The difficulties may be considered to be mild, moderate or severe. Even though this condition is observed by many school teachers, as well as physical and occupational therapists, it is not an easy diagnosis to make due to multi-faceted diagnostic criteria and terminology problems[6]. Outcome measurements used to assess gross motor development in infants and children up to age 5, including the Peabody Developmental Motor Scale[7], second edition and the Alberta Infant Motor Scale[8]. When children age out of either the PDMS-2 or the AIMS, one standardized assessment option physical therapists have is the Bruininks-Oseretsky Test of Motor Proficiency, second edition[9-11.] (BOT-2nd). The test-retest reliability and internal consistency of the total scale were excellent, with an Intraclass correlation Coefficient ICC of 0.99 (95% confidence interval) and alpha of 0.92 The BOT-2 can be used to evaluate a wide variety of fine and gross motor skills for children, teenagers and young adults 4-21 years of age. This is a test that can also be used by Physiotherapist , psychologists, adaptive physical education teachers, special education teachers and educational diagnosticians[9-13]The prevalence of DCD in India is found to be 1.37%. The prevalence of DCD in other countries is estimated to be (5-8%) usa, (1.8%) uk, (5.7%) greek, (5-9%) canada, (1.7%) belgium and 6% worldwide[13-17]. Considering the importance of timely diagnosis of DCD and the child's performance on the BOT-2 will allow the physical therapist to identify areas of strength and areas of need in regards to the child's gross motor functioning, and can therefore help to guide treatment.The early diagnosis of DCD can be helpful to prevent the future secondary complications. Aim of the study is to find out Point score of all subtest motor component, Descriptive category of Composite & Total Motor Composite component by using BOT_2nd in school going children Among Genders& according age group

MATERIALS & METHODOLOGY

The Cross Sectional analytical study was conducted in Pimpri C hinchwad area of age group 5 to 15 years. Total samples 516 were studied. The Subjects were divided according to age groups. Age Group 1 includes 5.0-7.11,age group 2 includes 8.0-9.11,age group3 includes 10.0-11.11,age group 4 includes 12.0-13.11 and age group 5 includes 14.0-15.11.Inclusion criteria were normal healthy school going children. Exclusion criteria were neurological trauma like spinal fractures, any visul problem, or any congenital deficit. BOT™-2nd kit used for assessment.

PROCEDURE

Institutional Ethical committee approval (reference No; DYPCPT/324/2016) was taken to conduct the study. .516 subjects were selected who fulfilling the inclusion criteria. After explaining the purpose of the study to the subject/parent, they were informed that they can withdraw any time during the course of the study without giving reason for doing so.Subjects were selected on the basis of multistage sampling method. In the first stage, 3 English & 3 Marathi schools were selected randomly out of total schools in Area. In 2nd stage, from each standard, any onedivision was selected Randomly . In 3rdstage, from every division, boys and girls of same age were selected by random sampling method. A written informed consent was obtained from the subjects/parents one day prior to the assessment. Proper precautions was taken so that there was no harm to the child.Total children were divided into 5 age groups according to their chronological age. These age groups were divided for sampling convenience and for obtaining proper results. BOT-2nd was used to assess children's motor proficiency. The BOT-2(53 items, 8 subtests and 4 four motor-area composites; score range = 0–320 points) fine manual control (FMC), manual coordination (MC), body coordination (BC) and strength and agility (SA).Subjects were assessed for these tasks and these raw score were converted to a numerical point score. Descriptive analysis done by using manual , they are categorized in to WAA-Well above average , AA- Above Average, A – Average, BA- Below Average , WBA- Well Below Average . Data from all subjects was entered in to computer database & analyzed with SPSS statistical Package (version14.0). Data analyzed by using percentage, mean & standard deviation from total number of sample.

RESULTS & OBSERVATION

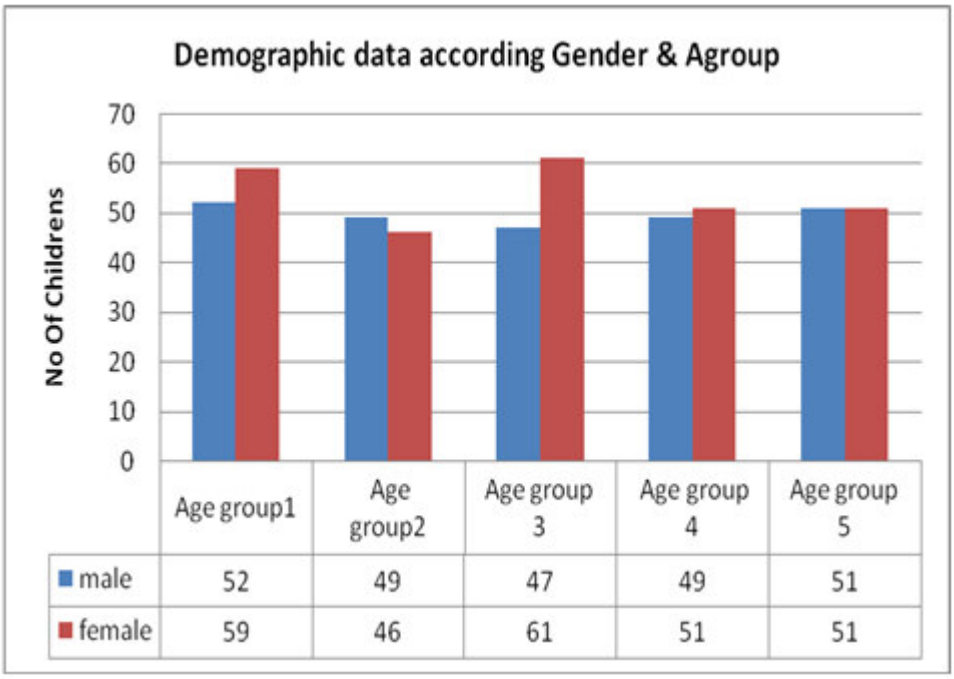


Figure 1
Demographic Data according Gender & Age Group

Table 1
Mean and standard deviation of subtest point score by Age group and Gender

Age Group.	Sex	N	FMP		FMI		MD		ULC		BLC		B		RSA		S	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (Age 5,6&7)	COM	111	24.47	5.89	24.97	7.53	19.19	5.73	16.91	9.08	17.35	3.66	31.00	4.94	24.25	4.46	24.04	4.95
	M	59	25.44	5.95	24.76	7.24	19.19	6.08	19.68	9.09	32.81	7.37	30.59	5.99	24.44	4.76	24.15	4.95
	F	48	23.37	5.66	25.21	7.91	19.19	5.36	13.77	8.05	30.67	6.97	31.46	3.38	24.04	4.12	23.90	4.98
2 (Age8&9)	COM	95	30.56	6.40	30.61	6.72	25.08	4.56	27.47	8.32	19.60	6.62	33.43	2.57	29.94	4.39	24.61	4.75
	M	46	30.24	6.21	31.33	6.45	24.59	5.13	30.89	7.25	29.96	5.72	33.33	2.46	31.04	4.81	25.85	3.31
	F	49	30.86	6.63	29.94	6.97	25.55	3.94	24.27	8.04	27.57	7.12	33.53	2.69	28.90	3.71	23.45	5.57
3 (Age 10&11)	COM	108	32.08	5.82	33.06	5.04	29.16	4.51	32.23	6.04	20.95	3.20	33.20	4.17	33.99	4.93	26.52	4.92
	M	61	31.92	6.63	33.61	5.51	29.64	3.91	34.90	3.99	30.97	7.55	33.62	4.60	35.00	4.76	27.46	4.77
	F	47	32.30	4.62	32.36	4.32	28.53	5.16	28.77	6.50	25.72	11.59	32.66	3.52	32.68	4.89	25.30	4.90
4 (Age 12&13)	COM	100	35.33	5.79	34.10	5.71	32.62	4.50	34.02	4.25	22.04	3.28	33.43	2.21	34.67	4.49	27.38	5.54
	M	51	34.78	5.04	35.55	4.34	33.63	4.25	35.63	2.75	28.90	6.14	33.59	2.03	36.51	3.56	28.57	5.24
	F	49	35.90	6.48	32.59	6.57	31.57	4.56	32.35	4.88	28.41	6.53	33.27	2.39	32.76	4.59	26.14	5.63
5 (Age 14&15)	COM	101	36.36	5.45	35.24	4.88	33.78	4.05	35.39	3.82	21.88	3.05	34.20	2.32	34.81	4.55	28.44	3.73
	M	51	37.51	4.96	34.92	5.18	33.49	4.80	36.41	3.47	29.57	6.17	34.41	2.18	36.84	3.96	29.25	3.42
	F	51	35.22	5.71	35.55	4.59	34.08	3.15	34.37	3.92	27.98	8.73	33.98	2.45	32.78	4.22	27.63	3.88

Abbreviation : COM: Combine (Male & female) , N= Total number of sample , SD: Standard Deviation , FMP : Fine Motor Precision , FMI: Fine Motor Integration , MD: Manual Dexterity , ULC: Upper Limp Coordination , BLC : Bilateral Coordination , B: Balance , RSA : Running Speed And Agility

Table2
Mean and standard deviation of Composite& Total Motor composite standard score by Age group and Gender

Age Group.	Sex	n	FMC		MC		BC		S&A		TMC	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 (Age 5,6&7)	COM	111	25.46	7.90	25.94	8.83	31.81	7.23	34.58	6.83	49.17	9.45
	M	59	27.25	7.92	28.27	7.15	32.81	7.37	35.24	5.71	51.88	9.68
	F	48	23.42	7.45	23.29	9.83	30.67	6.97	33.83	7.90	46.10	8.24
2 (Age 8&9)	COM	95	23.65	9.75	25.91	8.55	28.73	6.55	30.09	5.62	45.06	9.10
	M	46	25.46	9.28	28.96	8.88	29.96	5.72	31.83	5.47	48.39	8.87
	F	49	21.96	9.96	23.04	7.21	27.57	7.12	28.47	5.32	41.94	8.24
3 (Age 10&11)	COM	108	21.05	8.54	27.95	21.09	28.69	9.83	30.31	5.67	42.87	9.04
	M	61	22.97	9.28	29.41	8.65	30.97	7.55	31.49	6.05	46.54	9.42
	F	47	18.55	6.78	26.06	30.51	25.72	11.59	28.77	4.77	38.11	5.78
4 (Age 12&13)	COM	100	23.08	8.81	27.71	7.53	28.66	6.31	28.77	4.84	43.55	8.40
	M	51	23.92	8.24	30.63	7.22	28.90	6.14	30.22	4.22	45.69	7.33
	F	49	22.20	9.37	24.67	6.65	28.41	6.53	27.27	5.02	41.33	8.92
5 (Age 14&15)	COM	101	23.40	9.24	28.05	7.49	28.77	7.56	27.15	4.19	44.94	9.17
	M	51	24.16	9.07	29.84	8.08	29.57	6.17	27.25	4.68	45.45	7.47
	F	51	22.65	9.44	26.25	6.43	27.98	8.73	27.04	3.69	44.43	10.65

Abbreviation: FMC: Fine Manual Control, MC: Manual Coordination, BC: Body Coordination, S& A: Strength and Agility and TMC: Total Motor Composite

Table 3
Prevalence of Developmental coordination Disorder DCD (Motor Deficit)

Motor Deficit		Present	Absent
N=516	%	1.16	98.64

Figure 2
Prevalence of Developmental coordination Disorder DCD (Motor Deficit)

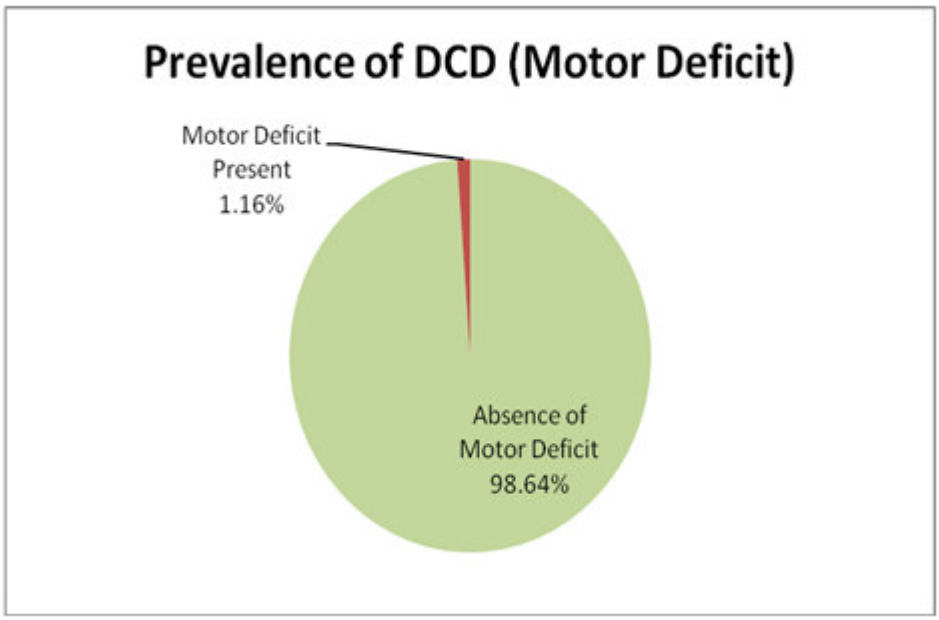
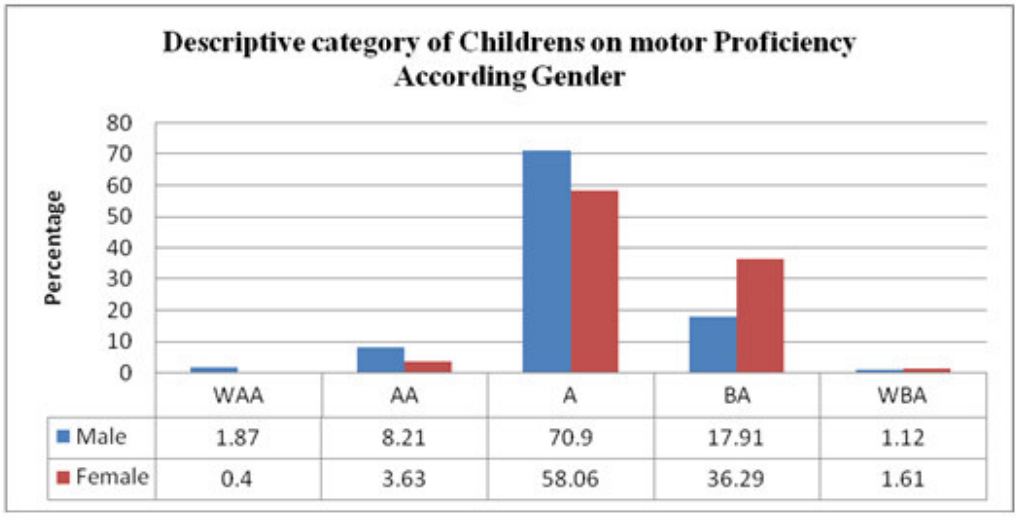


Figure 3
Descriptive category ofChildren on motor Proficiency According Gender



	WAA	AA	A	BA	WBA
Male	1.87	8.21	70.9	17.91	1.12
Female	0.4	3.63	58.06	36.29	1.61

Figure 4
Prevalence of DCD among Male And Female

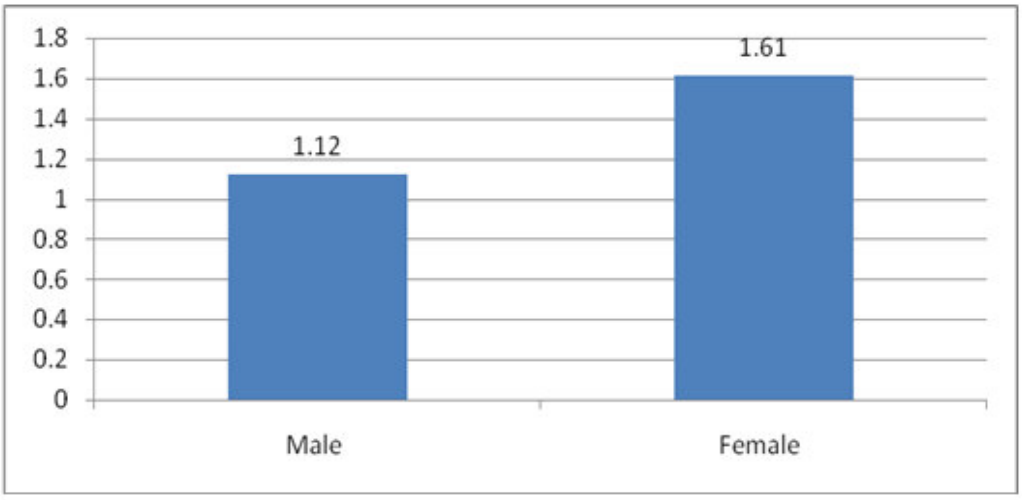
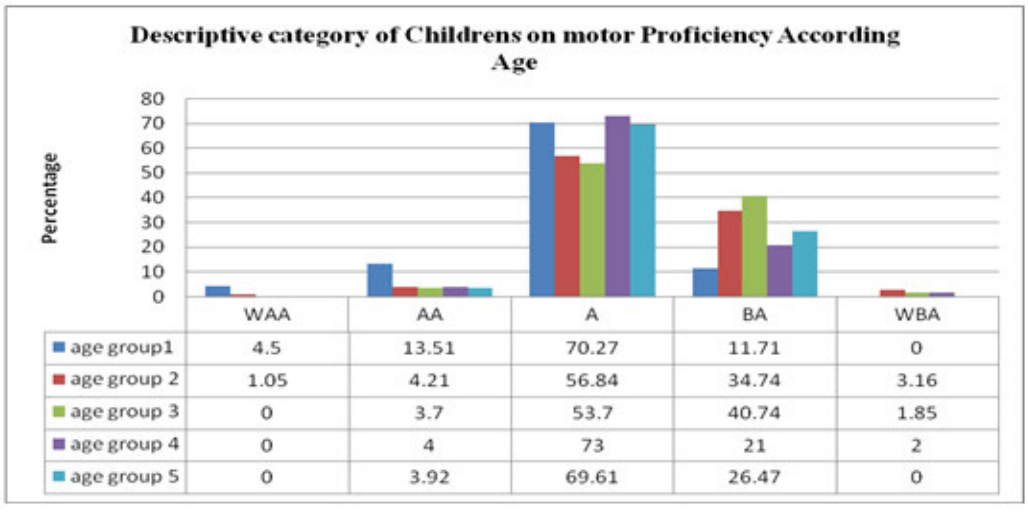
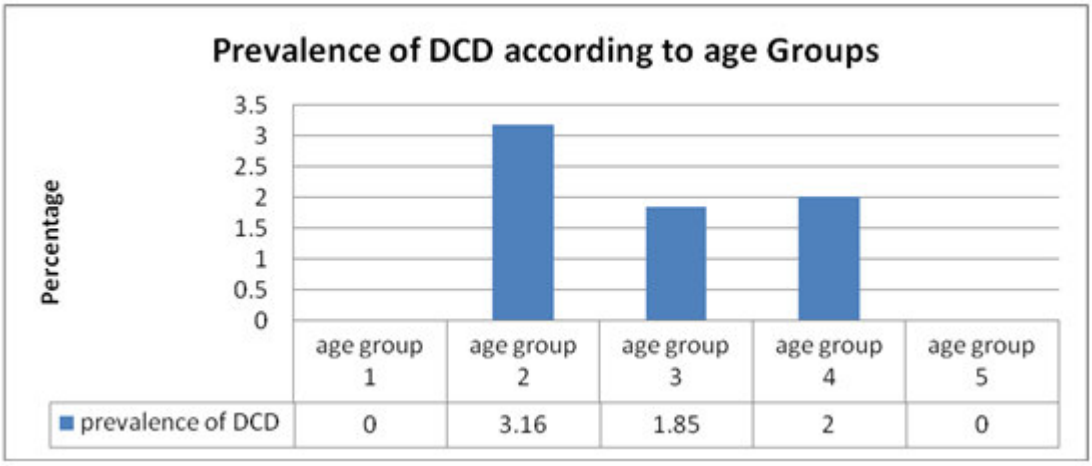


Figure 5
Descriptive category of Children on motor Proficiency According Age Group



	WAA	AA	A	BA	WBA
age group1	4.5	13.51	70.27	11.71	0
age group 2	1.05	4.21	56.84	34.74	3.16
age group 3	0	3.7	53.7	40.74	1.85
age group 4	0	4	73	21	2
age group 5	0	3.92	69.61	26.47	0

Figure 6
Prevalence of DCD among Age Groups



DISCUSSION

The present study was carried out with the aim 1) To find out point score & Descriptive category of all subtest motor component by using BOT-2nded. 2) To find out the prevalence of Developmental coordination disorder by using BOT-2nded in 5 to 15 yr of school going children, and 3) To find out prevalence of developmental coordination disorder according age group and gender. 1stgraph showed Five hundred & sixteen children (Mean age =10.67 years, SD = 3.03) participated in this study among that 248 & 268 were male & female respectively. Table 2 showed linear pattern subtest point score of Motor proficiency according to age group. The use of subtest point score will result in more precise measurement of function, because gain or deterioration will be related to specific area of motor control [18].Barnekow-Bergkvist et al. (1998) found that performance in physical tests; height, weight and physical activity at the age of 13 contributed best of explain adult physical performance and physical activity. Therefore, it may be concluded that so farwhen all thesubtest point score was concerned age factor was responsible for the higher mean value. Age group 4 & 5 having higher age, they had significantly performed better in comparison to Age group 1,2&3 boys & girls. Motor performance is related to lean body mass, general musculature, aerobic capacity and certain psychological state of mind (willingness to accept pain) and development of all of which are influenced by advancement of age. Therefore, it is obvious that Age group 1,2&3 will have less motor quality than that of Age group 4 & 5 because of structural and functional differences with the higher age groups [19]Magalhaes et al., (1989), in their study on the development of bilateral coordination on certain jumping tasks observed improvement in the performance with age in their sample of 5 to 9 years of typical children[20]. Moreover, the motor performance is related to bodystature, body weight, growth spurt, body composition, cardiovascular fitness and muscle strength [22]hence as age increases point score of motor proficiency also get increases. Standard score , Descriptive category of all compositecomponent & total motor composite did not showed any linear pattern of motor development with age growth becauseBrendaN.wilson concluded Standard Score & Descriptive category that have undergone statistically transformation will be less exact in their ability to detect real changes that occurred. Because these standard score are age adjusted , progress will not be reflected in the score unless the progress is faster than typical maturation (which is not likely to occur with children who have motor problem). Therapist should consider using the subtest point score as a accurate measure of change.[18] In this study, we found Prevalence of DCD by Using BOT-2nded. was 1.16% (95% CI 0.43% to 2.51%) from 516 children as they fall under Well below average descriptive category i.e motor deficit. This result showed the similar findings of the study done by Girish, Srilatha et al who showed prevalence of DCD in children between ages of 6-15 years attending mainstream schools in a school district in southern India using criteria of Diagnostic and Statistical Manual of Mental Disorder, Fifth Edition (DSM-5) was 0.8% in Southern India[22]. Another study conducted by Sankar U et.al.found out the prevalence rate of Developmental Coordination Disorder (DCD) at Kattankulathur among 5 - 10 years of age group by using The DevelopmentalCoordination Disorder Questionnaire (DCDQ) was 1.37%[15]. Another study conducted by Georgia D. Tsiotra et.al.investigated whether lifestyle differences between Canadian and Greek children are mirrored in DCD screening results. As compared with their Canadian peers (8%), Greek children exceeded expected DCD prevalence rates (19%) for pediatric populations[16,23]. Greek children demonstrated greater prevalence rates as they were relatively inactive compared with their peers from other countries[24-25]. Limited physical activity may result in a decline in selected fitness-related parameters and deterioration in motor skills acquisition[27]. Present study also showed that females are having more prevalence of DCD than males , however this difference is not statically significant as P=0.915 by Fisher's Exact test. These performance differences in males and females can be due to the nutritional status, as the dietary intake of boys is more than that of girls.(satabdighosh et al 2013)[27].Nutritional status appear to be significant predictor for both fine and gross motor development[27].Similar observations have been reported by other research workers in children of different countries (Bobbio et al., 2007; Chowdhury,Wrotniak, &Ghosh, 2010; Pollitt et al., 1994;)[28-30]. Nutritional status

may alter the learning process by influencing brain development and physical growth and accordingly modify the movement proficiency of the children by adjusting the strength, power, coordination and perception[26] Our study result are in accordance with Girish, SrilathaRaja et.al as in their study prevalence of DCD with girls (1.1%) affected more than boys (0.5%) at confidence interval of 95%. Girls were twice affected than boys[22]. Some researchers stated that girls with low socioeconomic status were less competent in locomotors skills compared with their high socioeconomic status peers (Hardy et al., 2012; Mészáros et al., 2008)[31]. However difference among gender in present study was not statistically significant. It is difficult to make exact comparisons between countries because the estimated prevalence is highly influenced by the means of assessment and the type of sample recruited Developmental coordination disorder in various age group did not show statically significant difference as P=0.219. However Age 8 &9 yrsshowed highest prevalence of DCD (3.16%) fallowed by Age 12 & 13yr (2%). The Indian children under-performed in the bilateral coordination subtest across all age group 7, 8 and 9 as compared to the USA normative sample.This observed developmental variation in the bilateral coordination patterns between Indian children and USA normative sample which may be attributed to the cultural and environmental (school) variations[32] In Bilateral Coordination component no children were found in well Above Average category (WAA) because scale score was not given for this category even though they scored maximum in Bilateral coordination point score. . So need to establish normative data for Indian population is suggested. Limitation of the present study was socioeconomic status, Cardiorespiratory Fitness& Body Mass Index were not considered while finding out the prevalence of DCD. Further studies can be conducted to investigate Motor proficiency of school going children whowere underweight at time of birth and preterm.

CONCLUSION

Prevalence of DCD by Using BOT-2nded. was 1.16% (95% CI 0.43% to 2.51%), where asfemale showed more prevalence of Developmental coordination disorder than Male .

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Research Article

Assessment of fine motor integration using bruininks oseretsky test of motor proficiency, 2nd edition, in 5 to 15 years of school going children

Diksha Gondkar*, Dhote Sanjivani,Tushar Palekar, Mohammed Zaid Tai

Dr. D.Y.Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pune, India

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ABSTRACT

Fine motor integration is the degree to which visual perception and finger hand movements are well coordinated. Through integration of visual input and motor output motor tasks are planned, monitored, adjusted and executed. The Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition, is a pediatric test of fine motor and gross motor skills. It is an individually administered test that uses goal directed activities to measure motor skills in individuals ages 4 through 21. A study was conducted among 516 number of students from English as well as Marathi medium schools. After assessing the fine motor integration in the study population it was found that as the age increases the fine motor integration also increases and it is more in male children than that of female children.

Introduction

Fine motor integration is the degree to which visual perception and finger hand movements are well coordinated. Through integration of visual input and motor output, motor tasks (e.g. writing) are planned, executed, monitored and adjusted [1].The age from 3 to 6 years is a sensitive period for development of fine movement skills (Gallahue and Donnely, 2003). Because most preschool children are naturally curious, love to play and explore, these fine motor skills are learned very easily especially when stimulation, opportunities to play and to be physically active or sports are offered. The mastery of certain movement skills is a prerequisite for daily life functioning and participation in later physical or sport-specific activities [2].At an early age, gross movement skills are necessary to move, stabilize and control body and objects while exploring the environment. Later in life, well developed gross movement skills help individuals to function more smoothly. Fine movement skills are necessary for development of basic self-help skills. During infancy, development is evaluated almost exclusively by motor development. Once a child can reach, grasp and walk, however, interest in further development of more complex movement skills is reduced and more attention is given to the development of cognitive, social and emotional aspects. Motor development is basically only taken into consideration when dysfunctions or efficient movement behavior appears (Davies, 2003). Research in the area of movement skill development mainly focuses on motor impairment and motor deficits. Hence, research on fine movement skill development and performance in developing children is fragmentary. The information which is available is mostly based on the sequences of developmental change in movement patterns and can be found in literature such as Gallahue and Ozmun (2006) and Haywood and Getchell (2005) [2].Different tools to assess movement performance in early childhood are available. The movement assessment can be norm- or criterion referenced. A norm referenced test compares the child's performance to hat of normative group and quantifies the child's movement skills competence. A criterion referenced test compares the child performance to pre determine criteria. A second form of movement skill assessment tool is also used with the scope on movement development and performance in typical preschool children, which includes Motoriktest fur Vier-bis Sechjarige Kinder (MOT4-6), Movement Assessment Battery for children (Movement- ABC), Paebody Development Scales (PDMS) koperkoordinationtest fur Kinder (KTK), Test of Gross Motor Development (TGMD), the Maastrichtse motoriek Test (MMT), the Bruininks-Oseretsky test of Motor proficiency (BOTMP). The second edition of Bruininks Oseretsky test of Motor proficiency is also used to evaluate the motor performance [2].The Bruininks-Oseretsky Test Of Motor

***Corresponding Author: Diksha Gondkar,** D Y Patil College of Physiotherapy, Pune, India **E-Mail:** dikshagondkar35@gmail.com

Proficiency, second edition (BOT-2) is preferred for this study as it is an individually administered test that uses engaging, goal-directed activities to measure motor skills in individuals aged 4 through 21 (Bruininks and Bruininks, 2005).it assesses four motor-area composites; Fine manual control (FMC), Manual Coordination (MC), Body Coordination (BC) and Strength and Agility (SA). BOT-2 has 4 subtests with 53 items and each motor composite has 2 subtests. As BOT-2 testing involves game-like motor tasks which capture the child's interest and are not verbally complex, it is suitable for children of non-english speaking background. Also the authors report that it can identify motor deficits in individuals with 'mild to moderate' motor impairment and is validated and reliable foe assessing subjects with 'mild to moderate' mental retardation. Furthermore, the motor activities incorporated in BOT-2 includes gross motor (GM) tasks that assess hopping, jumping, running, ball skills, balance, strength and coordination and fine motor (FM) tasks that assess precision, integration and manual dexterity through drawing, writing and functional tasks such as threading blocks [3].

The selection of the items is based on the following criteria:

- To provide a broad and general view on movement skill development status of a child;
- To represent significant aspects of motor behavior;
- To emphasize motor activity;
- To provide opportunity to discriminate between a broad range of motor abilities;
- To fall within the possibilities of mild and moderate mentally retarded children;
- To appeal to limited memory capacity and vocabulary of the child;
- Material has to be easily transported.

The scoring system varies according to the individual items; it ranges from a 2- point scale to a 13-point scale. The raw scores can be converted into a standard numerical score. Every child is asked to perform 8 tasks i.e. copying a circle, a square, overlapping circles, a wavy line, a triangle, a diamond, a star & overlapping pencils, given in fine motor integration subtest of BOT-2 scale. Subjects are assessed for these tasks and a raw score is recorded in the unit measured and then converted to a numerical point score. The use of test is recommended for motor impairment diagnosis, screening, placement decisions, development and evaluation of motor training program and supporting research goals [2].

Materials and methods

The study was to assess the fine motor integration in 5-15 years school going children. This study was a Cross sectional-Analytical Study design which was conducted in schools in Pimpri Chinchwad Municipal corporation 516 subjects of age group 5-15 years were selected in the study fulfilling the inclusion criteria. The inclusion criteria comprised of the students who were healthy and were willing to participate in this study whereas the exclusion criteria comprised of the students who had a neurological deficit or any upper limb fracture within 6 months or any diagnosed medical condition or any audio-visual defect. After explaining the purpose of the study to the parent, they were informed about their right to opt out of the study any time during the course of the study without giving reason for doing so. The parents were assured that their child's participation and non-participation would not affect their child's education.Subjects were selected on the basis of multistage sampling method. In the first stage, 3 English schools and 3 Marathi schools were selected randomly out of the total schools in Pimpri Chinchwad Municipal Corporation randomly. In the second stage, from each standard, 1 division was selected randomly. In third stage, from every division, boys and girls of same age was selected by stratified random sampling method.A written informed consent was obtained from the parents one day prior to the assessment. A assessment was taken to record their demographic details and other parameters. Every child was asked to perform 8 tasks i.e. copying a circle, a square, overlapping circles, a wavy line, a triangle, a diamond, a star & overlapping pencils, given in the fine motor integration subtest of BOT-2 scale.Table & chair for the examinee that was approximate for his or her height was given. Each page was torn from examinee booklet before placing it in front of the examinee. Erasing was not allowed. For each item, basic shape facet was scored, then all remaining facets and the total score for that item was also scored. The examinee used preferred drawing hand for all items in this subtest. Before administering each item, the task was taught to the examinee using verbal and nonverbal directions as necessary to ensure the examiner's understanding of the task. The overall size of the drawing was supposed to be least half the size of the stimulus.Subjects were assessed for those tasks and a raw score was recorded in the unit measured and then converted to a numerical point score. Further analysis was done with the help of BOT-2 manual. The data collected was analyzed using suitable statistical tests. The outcome measure of this study was 'Motor Point Score' of fine motor integration and 'Descriptive Category' of fine motor integration. The subjects were divided into 5 age groups. Age group 1 comprised of children of age 5.0-7.11 years, age group 2 comprised of age 8.0-9.11 years, age group 3 comprised of age 10.0-11.11 years, age group 4 comprised of 12.0-13.11 years and age group 5 comprised of 14.0-15.11 years.

Results

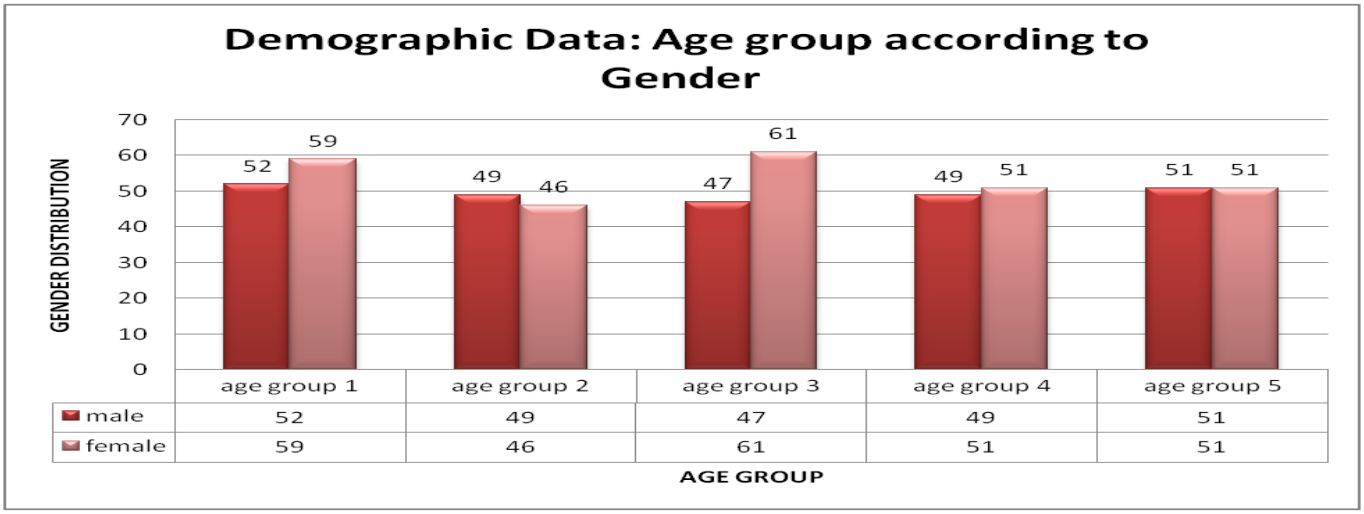

Fig 1: Graph 1 represents that, out of the total study population, 248 were male and 268 were female

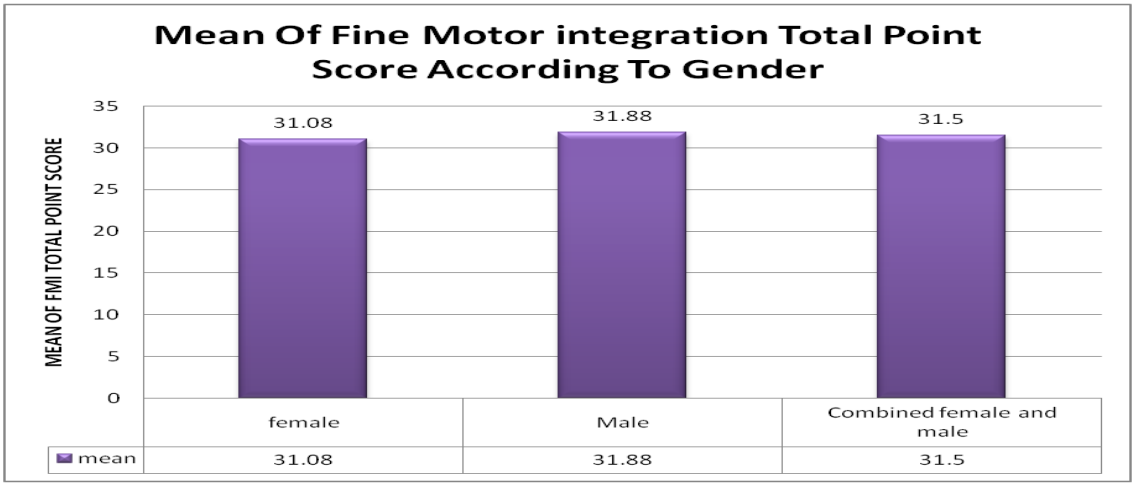

Fig 2: Mean and Standard deviation of FMI total point score among males and females

Interpretation: Graph 2 represents that, mean of fine motor integration point score is more in male i.e. 31.88 than female i.e.31.08.

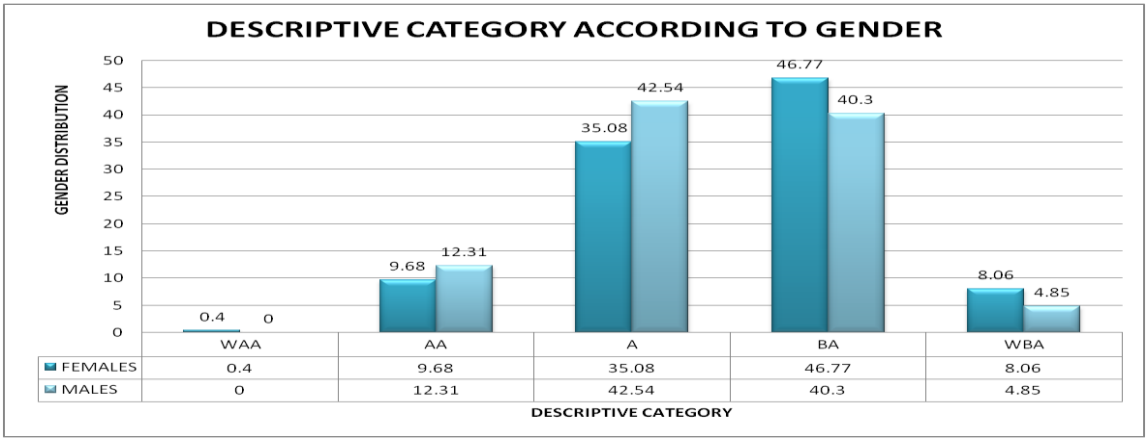

Fig 3: Descriptive category versus gender

Interpretation: Graph 3 represents that, more males fall in average and above average category than females and more females fall in below average and well below average category than males. 0.4% of the females fall in well above average category. Also number of females in well below average category is more than that of males.

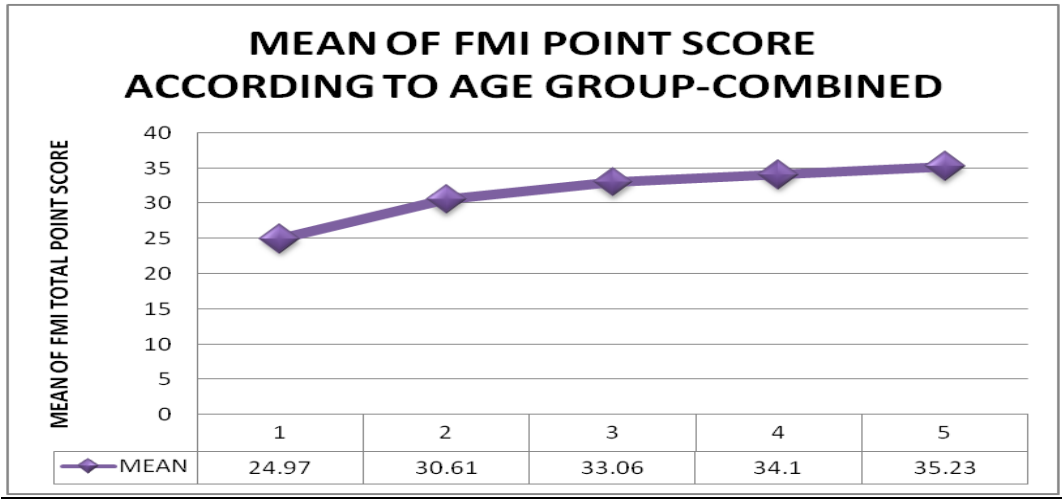

Fig 4: as the age group increases the mean of fine motor integration point score increases

Interpretation: The above graph represents that, as the age group increases the mean of fine motor integration point score increases.

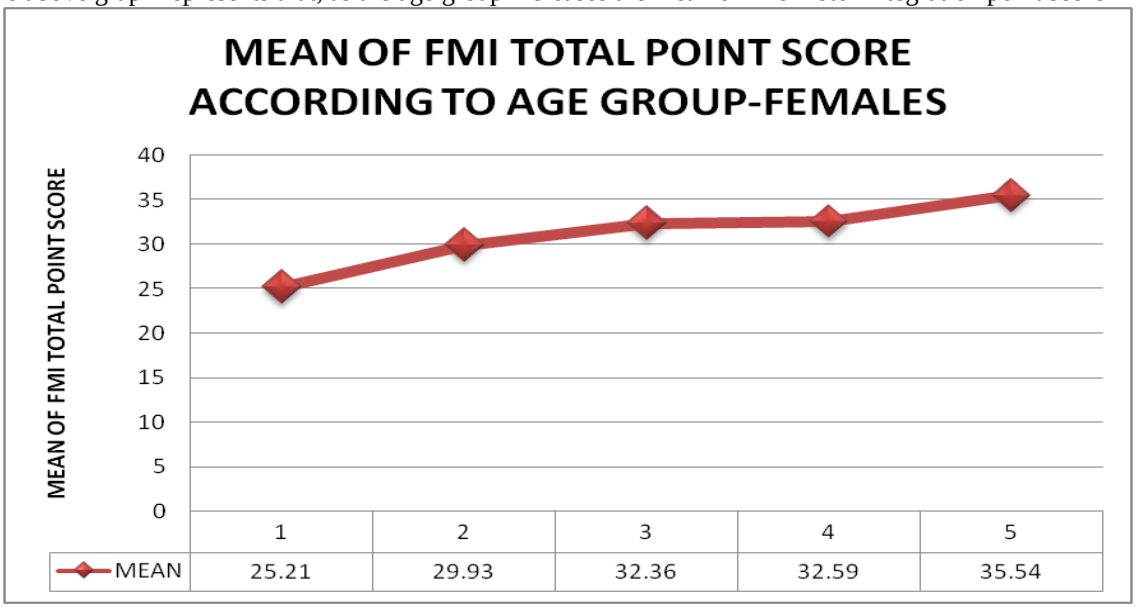

Fig 5: The above graph represents that, in female as age group increases, mean of fine motor integration point score increases

Interpretation: The above graph represents that, in female as age group increases, mean of fine motor integration point score increases.

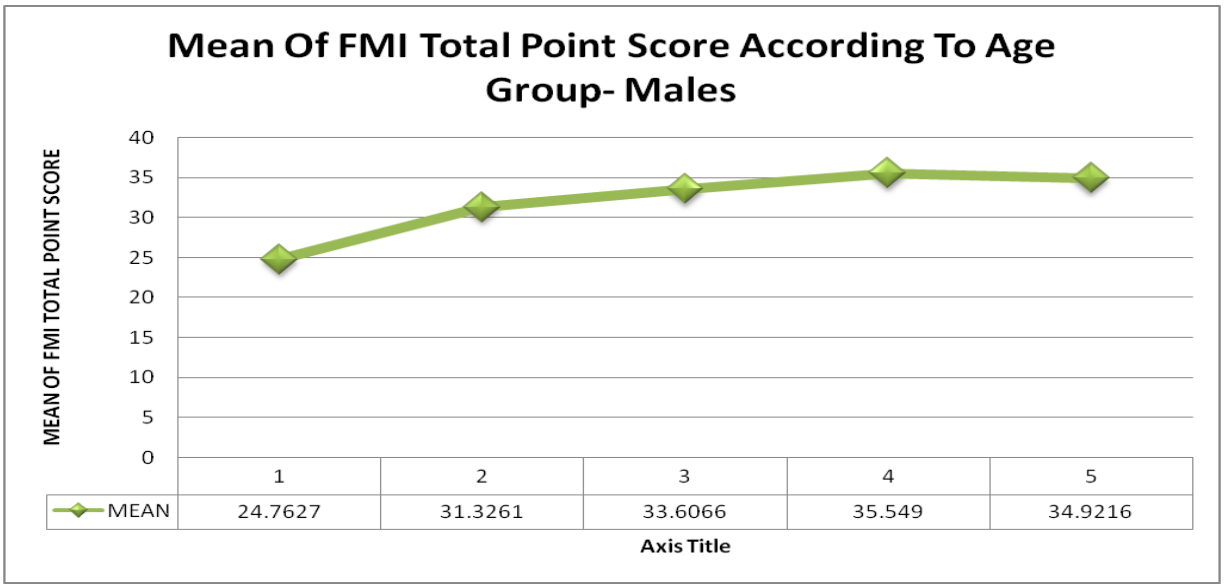

Fig 6: Graph 6 represents that as the age group increases the mean of point score of males also increases in linear pattern

Interpretation: Graph 6 represents that as the age group increases the mean of point score of males also increases in linear pattern.

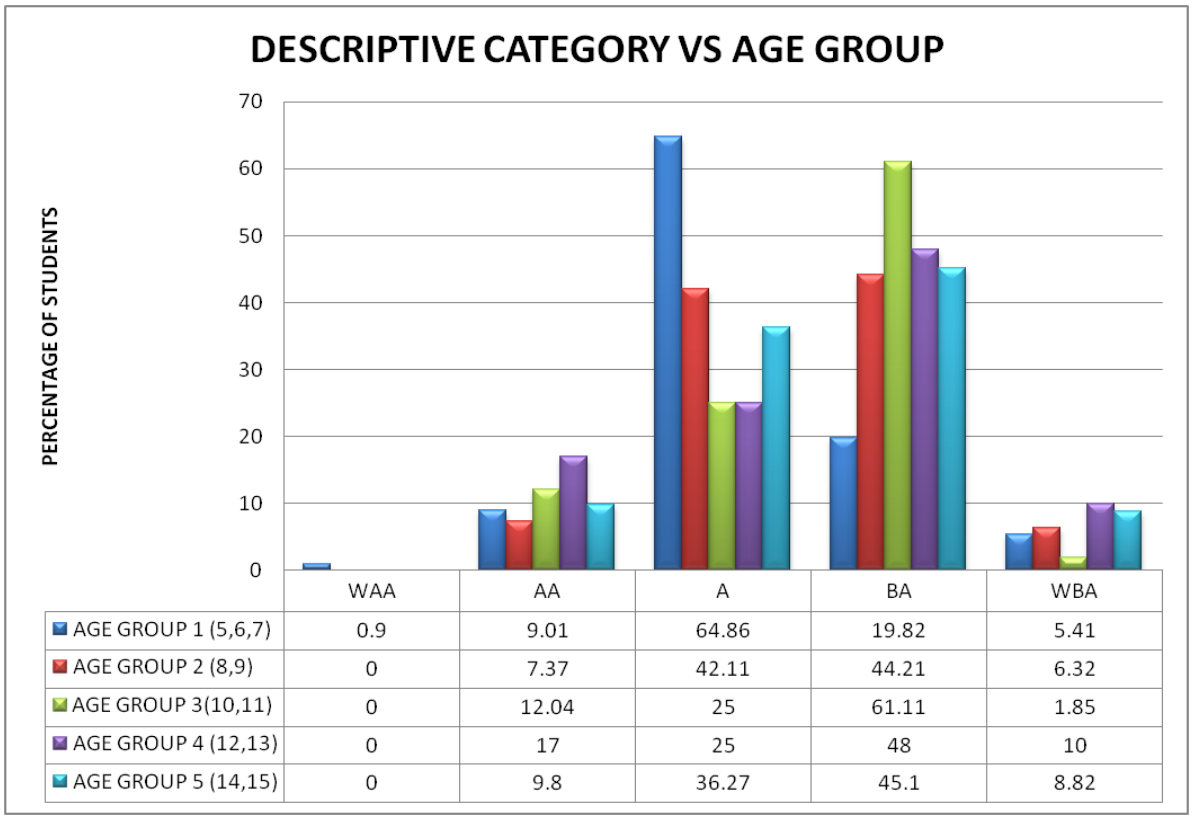

Fig 7: Descriptive category versus age group: Combined

Interpretation: The graph 7 represents 64.86% students of age group 1 lie in Average category, whereas 44.21%, 61.11%, 48% and 45.1% students of age group 2, 3, 4 and 5 respectively fall under Below Average category.

Discussion

The primary aim of the study was to assess the fine motor integration using BruininksOseretsky Test Of Motor Proficiency, 2nd edition in 5 to 15 years of school going children. It consisted of 8subtests for assessing fine motor integration. The total study population was 516 out of that 248 were males and 268 were females. The mean age of 5 years to 15 years 11 months was 10.67 years and standard deviation was 3.03. The mean age and standard deviation for males was 10.66 and 3.02 respectively and that for females was 10.69 and 3.04 respectively. In the study mean of point score was more in males i.e. 31.88 than females which was 31.08. Considering the descriptive category, more males fall under Above Average and average category and females fall under Well Below Average and Below Average category. Therefore, motor impairment is more in females than males as they fall more in below average and well below average category. Our result goes in accordance with the study done by Duger T, Bumin G, et al. in July 2009 which stated that there was significant difference in subtest 2 viz. fine motor integration; it was seen that fine motor skills in childhood showed variety between age, sex and academic learning. Another study done by SatabdiGhosh, SutanuChowdhury, et al. in 2013 stated that nutritional status appear to be significant predictors for fine motor development. It may alter the learning process by influencing brain development and physical growth, and accordingly modify the movement proficiency of children by adjusting the strength, power, coordination and perception [3,6].In this study we also found that, as age group increases total point score increases in males and females. This goes in accordance with the study done by Deurenberg et.al in 2005 which stated that motor development is the gradual process by which a child gains use and coordination of the large muscles of the legs, trunk and the smaller muscles of the hand. Neuromuscular development starts in embryonic stage and it continues after birth. Another study was done in 2009 by Wouter Cools et al. stated that at an early age, fine movement skills are necessary for the development of basic self-help skills. Drawing and writing are based on fine movement skill development.This is because as the age increases the neurological development occurs in the child and hand to eye coordination improves [2].Further result shows the performance between different age groups. It says that by considering the norms given in the BOT-2 manual, more subjects from age group 1 i.e. 64.89% fall in average category and the number deteriorates as the age increases. Only 0.9% from age group 1 falls in Well Above Average category. There is no age group from 2 to 5 that fall under well above average. Likely, more subjects from age group 5 i.e. 8.82% fall in well below average category and the number decreases sometimes and even increases with age. Thus, more children from age group 5 have motor impairment than other age groups. This result is in contrast with point score result as the descriptive categories are allotted according to the scale score. Brenda N. Wilson et al in their study said that, the use of subtest point score will result in a more precise measurement of function, because gains or deterioration will be related to specific areas of motor control. In addition, score that have undergone statistical transformations will be less exact in their ability to detect real changes that occurred. Because these standard scores are age adjusted, progress will not be reflected in the test scores unless the progress is faster than typical maturation. Therapists should consider using the subtest point scores as a more accurate measure of change [5] .The study has outlined the limitation of not taking the socioeconomic status and Body Mass Index for nutritional status. Because studies have shown that socioeconomic status alters the motor performance of child. According to ÖzgürMülazımoğlu-Ballı in his study stated that there were significant differences in the BOT-2 score and total score of different socioeconomic groups, in favor of high socioeconomic groups [7].

Conclusion

The conclusion to be drawn is fine motor integration is more in male children than female children. Also as the age increases the integration improves in both male children and female children.

Acknowledgement

Having surmounted all the difficulties and after reaching the shore by completing the work of this study, I am realizing the limitations of language and words while acknowledging thanks to all those who helped me in this voyage. I thank my mother Mrs. Meena Gondkar, my father Mr. Mahesh Gondkar & my sister Sakshi Gondkar for their moral support, prayers and encouragement that have been a pillar of strength throughout this work.I also thank Dr. D. Y. Patil University for giving us opportunity to carry this project work.Words are few and language seems feeble when the heart is full of gratitude, these few words cannot express my deep sense of gratitude to my esteemed Guide Dr. Mrs. Sanjivani Dhote, who has been a constant source of inspiration to me since the very beginning of this work. Her unsurpassable teaching experience & scientific approach has increased my interest and knowledge in the subject. It is only because of her constructive supervision and overall encouraging sympathetic attitude that my work has acquired the present shape.It is with deep sense of gratitude and sincerity that I thank Dr. TusharPalekar, principal of Dr. D. Y. Patil College of Physiotherapy, Pimpri, Pune for helping me to successfully complete this study.From him I have tried to imbibe values, vast knowledge, experience and a high sense of professionalism. I am highly indebted to My Friends especially Mohammed Zaid, PrajaktaKarambe, AntaraPande and Ashish Joseph for constantly supporting me and for bearing the brunt of this herculaneum task.
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Conflict of interest: We declare that we have no conflict of interest.

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Abbreviations, units, Etc

BOT-2 : Bruininks-Oseretsky Test Of Motor Proficiency

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Original Research Article

ASSESSMENT OF BILATERAL COORDINATION USING BRUININKS: OSERETSKY TEST OF MOTOR PROFICIENCY, 2ND EDITION (BOT-2), IN 5 TO 15 YEARS SCHOOL GOING CHILDREN

Prajakta Karambe *[1], Sanjivani N. Dhote [2], Tushar J. Palekar [3].

*[1,3] Dr. D. Y. Patil College of Physiotherapy, Dr. D. Y. Patil Vidyapeeth, Pune, Maharashtra, India.
[2] Department of Neuro Physiotherapy, MAEER'S College Of Physiotherapy, Dabhade, Pune, India.

ABSTRACT

Background: Bilateral coordination refers to the ability to coordinate both sides of the body at the same time in a controlled and organized manner. The development of bilateral coordination begins early in the life and is the basis for further motor development. Inadequate bilateral coordination can adversely affect overall motor coordination as well as cognitive development, thus negatively affecting academic performance. Bruininks-Oseretsky Test of Motor Proficiency is a standardized assessment tool to assess children's motor proficiency, bilateral coordination being the 4th subtest.

Materials and Methods: The materials used were Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition kit, table and a chair. The study was a cross sectional analytical study carried out in Pimpri Chinchwad Municipal Corporation schools. The subjects were selected by multistage sampling method and were asked to perform 7 tasks to assess bilateral coordination.

Discussion: The present study stated that, mean of bilateral coordination point score was more in males than females. The study also showed that, as a age increased, the mean of bilateral coordination point score increased both in male and female children.

Conclusion: Male children showed better performance than female and as the age increased, the performance got better.

KEY WORDS: Bilateral coordination, Bruininks-Oseretsky Test of Motor Proficiency, normal children.

Address for correspondence: Prajakta Karambe, 1/21 Manda House, Maruti Mandir Marg, Girgaum, Mumbai-400004, Maharashtra, India. Phone number: 7083889341
E-Mail: prajaktakarambe.1010@gmail.com

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INTRODUCTION

Bilateral coordination refers to the ability to coordinate both sides of the body at the same time in a controlled and organized manner, for example; stabilizing paper with one hand while writing/cutting with the other. It is the ability to use both sides of body in an integrated and skillful manner[1]. Neuromuscular development starts in embryonic stage and it continues after birth[2]. The development of bilateral coordination begins early in the life and is the basis for further motor development. Good bilateral coordination/integration is an indicator that both sides of the brain are communicating effectively and sharing information[3].

Inadequate bilateral coordination can adversely affect overall motor coordination as well as cognitive development, thus negatively affecting academic performance. Bilateral coordination deficit is observed in children with Developmental Coordination Disorder(DCD), Learning Disability, Sensory Integrative Dysfunction and

other motor impairments[1]. Bilateral coordination forms part of the general evaluation of the motor skills. In clinical practice, bilateral integration deficits in children are identified by observations of poor coordination of two body sides, avoidance of crossing of midline, failure to develop a preferred hand and possibly right-left confusion. Some standardized tests such as Sensory Integration Praxis Test (Ayres, 1989), DeGangi Berk Test of Sensory Integration (1983), Bruininks-Oseretsky Test Of Motor Proficiency (Bruininks, 1978) and also its second edition[1].

The earlier version, the Bruininks-Oseretsky Test of Motor Proficiency (BOTMP), is a widely used standardized assessment tool to assess children's motor proficiency with a long history of use in clinical practice and research. It is often used as the standard for criterion validation of other motor tests. It consists of 46 items grouped under 8 different subtests for children between 4.5 to 14.5 years of age [1]. The Bruininks-Oseretsky Test Of Motor Proficiency, Second Edition (BOT-2 preferred for this study as it is an individually-administered test that uses engaging, goal-directed activities) is to measure motor skills in individuals aged 4 through 21 (Bruininks and Bruininks, 2005). It assesses four motor-area composites; Fine Manual Control (FMC), Manual Coordination (MC), Body Coordination (BC) and Strength and Agility (SA). BOT-2 has 4 subtests with 53 items and each motor composite has 2 subtests [4]. As BOT-2 testing involves game-like motor tasks which capture the child's interest and are not verbally complex, it is suitable for children of non-English speaking background. Also the authors report that it can identify motor deficits in individuals with 'mild to moderate' motor impairment and is validated and reliable for assessing subjects with 'mild to moderate' mental retardation. Furthermore, the motor activities incorporated in BOT-2 include gross motor (GM) tasks that assess hoping, jumping, running, ball skills, balance, strength and coordination and fine motor (FM) tasks that assess precision, integration and manual dexterity through drawing, writing and functional tasks such as threading blocks [4].

BOT-2 has been empirically validated for high-functioning persons diagnosed with autism, Asperger's, Developmental Coordination Disorder, and mild/moderate intellectual disabilities [5].

The Bilateral Coordination subtest of BOT-2 is the fourth subtest, Body Coordiantion (BC), under gross motor composite and contains eight test-items. First and fifth item assess coordination of upper limb alone and remaining six items assess sequential and simultaneous coordination of upper limbs with lower limbs. The number of performance trials for each item is specified. A raw score is recorded in the unit measured (e.g. number of jumps, pivots, etc.) and then converted to a numerical point score [1].

The Bilateral Coordination subtest measures the motor skills involved in playing sports and many recreational games. The tasks require body control, and sequential and simultaneous coordination of the upper and lower limbs. Sample's score is consistent with individuals who can perform coordinated arm/hand and leg/foot movements when the limbs on the same sides of the body are synchronized, but have difficulty with coordinated arm/hand and leg/foot movements when the limbs on the opposite sides of the body are synchronized [6].

A child who has attained all the normal developmental milestones at the correct age may also have certain motor deficits which may be asymptomatic at early stage of life due to which the child slowly start adapting to these deficits. This adaptive behavior may show neuromotor disturbances in later stages. Parents or teachers are unaware of these deficits and neglect it. Without intervention difficulties persist into adulthood and are frequently accom- panied by other problems, both at home and at school, so assessment program should be done to evaluate children having coordination deficit and early intervention should be given to avoid the risk of any neuromotor disturbance later[7]. Thus, there is a need to assess bilateral coordi-nation deficit in children to rule out the most common age group having this problem. At the same time, it is also important to see which gender is more prone to these deficit.

MATERIALS AND METHODS

The study was to assess the bilateral coordina-

tion in 5-15 years school going children. The study was a cross-sectional analytical study which was conducted in schools from Pimpri-Chinchwad Municipal Corporation. 516 subjects of age group 5-15 years were selected in the study fulfilling the inclusion criteria. The subjects were divided into 5 age groups. Age group 1 comprised of children of age 5.0-7.11 years, age group 2 comprised of age 8.0-9.11 years, age group 3 comprised of age 10.0-11.11 years, age group 4 comprised of 12.0-13.11 years and age group 5 comprised of 14.0-15.11 years. The subjects included in the study were, healthy children both boys and girls of age 5 to 15 years. The exclusion criteria was children who had neurological trauma or spinal fracture 6 months back, any visual problem, any neurological deficit or diagnosed medical condition.

The objective of the study was, to find out bilateral coordination point score, to find out bilateral coordination descriptive category, to find out bilateral coordination point score and descriptive category among males and females and to find out bilateral coordination point score and descriptive category according to age groups using Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition. The outcome measure used for conducting the study was, 'Bilateral Coordination Motor Point Score' and 'Descriptive Category of Bilateral Coordination.

After explaining the purpose of the study to the subject/parent, they were informed about their right to opt out of the study any time during the course of the study without giving reason for doing so. The parents/teacher was assured that their child's participation and non-participation would not affect their child's education.

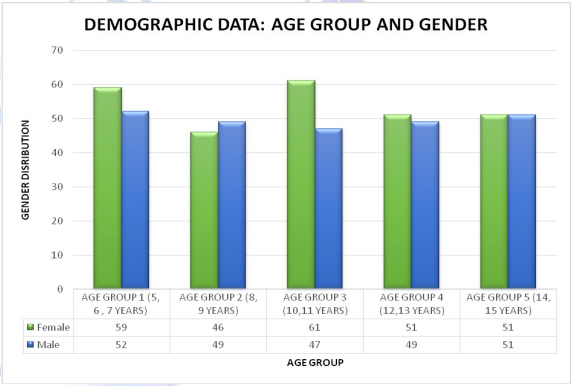
Subjects were selected on the basis of multistage sampling method. In the first stage, 3 English schools and 3 Marathi schools were selected randomly out of the total schools in Pimpri Chinchwad Municipal Corporation randomly. In the second stage, from each standard, 1 division was selected randomly. In third stage, from every division, equal number of boys and girls of same age were selected by stratified random sampling method. A written informed consent was obtained from the parents one day prior to the assessment. A pre assessment was taken to record their demographic details and other parameters.

Every child was asked to perform 7 tasks namely, touching nose with index fingers- eyes closed, jumping jacks, jumping in place- same side synchronized, jumping in place- opposite side synchronized, pivoting thumb and index finger, tapping feet and fingers- same side synchronized, tapping feet and fingers- opposite side synchronized.

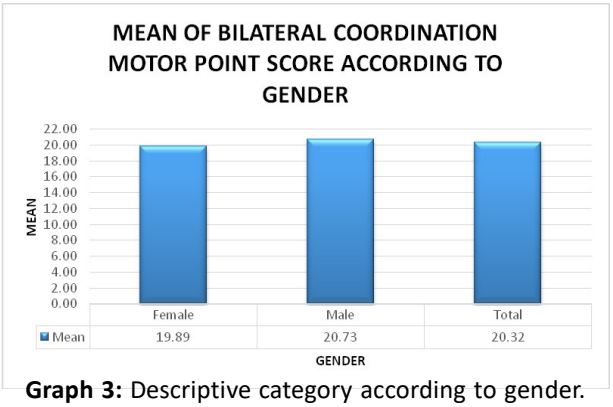
Subjects were assessed for these tasks and a raw score was recorded in the unit measured (e.g. number of jumps, pivots, etc.) and then converted to a numerical point score. Further analysis was done with the help of BOT-2 manual. The data collected was analyzed using suitable statistical tests.

RESULTS

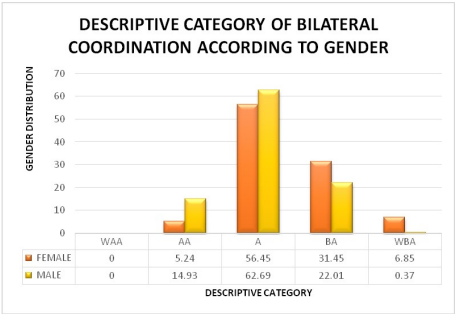
Graph 1: Demographic data: age group and gender.



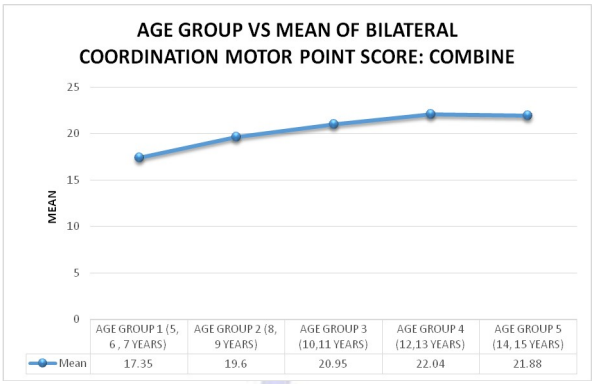
Graph 2: Mean of bilateral coordination motor point score according to gender.

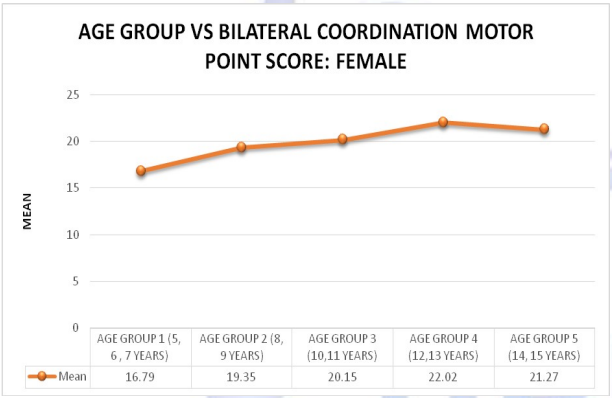


Graph 3: Descriptive category according to gender.

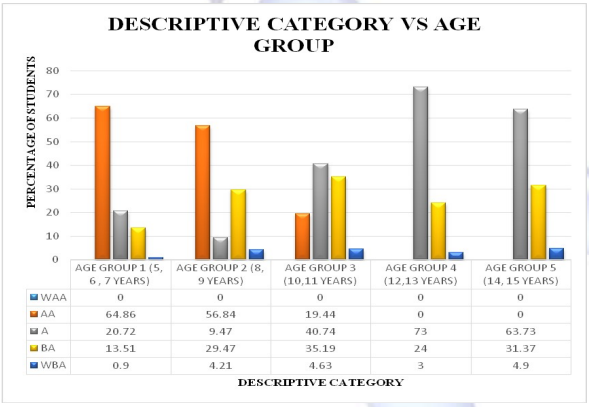


Graph 4: Mean and standard deviation of bilateral coordination motor point score according to age group.





Graph 5: Descriptive category of bilateral coordination according to age group.



The data analysis included mean and standard deviation of Bilateral Coordination Motor Point Score and age, gender and depicted categories according to BOT-2. The study showed the following result, Out of the total study population, 248 were male and 268 were female. The mean of bilateral coordination point score is more in male i.e. 20.72 than female i.e. 19.88. While considering the descriptive category, more males fall in average and above category than females and more females fall in below average and well below average category than males. None of the subject falls in well above average category. As the age group increases , the mean of bilateral coordination point score increases both in males as well as females. While considering above average category, there are more subjects in age group 1 and none in age group 5. Whereas, while considering well below average category, there are more subjects in age group 5 and least in age group 1. None of the subjects fall in well above average category.

DISCUSSION

The primary aim of the study was to assess bilateral coordination score using Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition. It consisted of 7 items for assessing bilateral coordination.

The study was conducted among 516 subjects out of which 248 were male and 268 were female of age 5 years to 15 years 11 months, with mean age 10.67 years and standard deviation 3.03. The mean age and standard deviation for males was 10.66 and 3.02 respectively and that for females was 10.69 and 3.04 respectively.

In this study we found out that, as the age group increases the bilateral coordination score increases both in male and female. This is consistent with a study by T. Balakrishnan, where he said that the differences in the motor performances are not obvious before puberty in the boys and girls. The performance of both the gender improved with age [1].

The study also says that, mean of bilateral coordination point score was more in males i.e. 20.72 than females i.e. 19.88. According to the descriptive category, more males fall under above average and average category and more females fall in below average and well below average category. Thus, females are more affected than males as they fall more in below average and well below average category. Our result goes in accordance with the article where T. Balakrishnan et al in his study says that, bilateral coordination subtest primarily examines the coordination between nervous and muscular system in the arms and legs or on both sides of the body. Moreover, the motor performance is related to body stature, body weight, growth spurt, body composition, cardiovascular fitness and muscle strength. Physiologically males have more body weight and better body composition and cardiovascular fitness than females so their score in better [1]. Also Satabdi

Ghosh et al states that, nutritional status appear to be significant predictors for both fine and gross motor development. Nutritional status may alter the learning process by influencing brain development and physical growth and accordingly modify the movement proficiency of the children by adjusting the strength, power, coordination and perception [2]. Another study by Robert H. Bruininks says that bilateral coordination subtest measures motor skills involved in playing sports and many recreational games [7]. Males are involved more in such activities hence performed well than females

Further result shows the performance between different age groups. It says that by considering the norms given in the BOT-2 manual, more subjects from age group 1 i.e. 64.86% fall in above average category and the number deteriorates as age increases and none of them fall in above average category from age group 5. Likely, more subjects from age group 5 i.e. 4.9% fall in well below average category and the number decreases with age and least in age group 1. Thus, more children from age group 5 have motor impairment than other age groups. This result is in contrast with point score result as the descriptive categories are allotted according to the scale score. Brenda N. Wilson et al in their study said that, the use of subtest point score will result in a more precise measurement of function, because gains or deterioration will be related to specific areas of motor control. In addition, score that have undergone statistical transformations will be less exact in their ability to detect real changes that occurred. Because these standard scores are age adjusted, progress will not be reflected in the test scores unless the progress is faster than typical maturation. Therapists should consider using the subtest point scores as a more accurate measure of change [8].

Studies have shown that socioeconomic status alters the motor performance of child. Özgür Mülazýmoðlu-Ballý in his study concluded that there were significant differences in the BOT-2 score and total score of different socioeconomic groups, in favour of high socioeconomic groups. Considering socioeconomic status is thus, important[9]. The study has outlined the limitation of not taking socioeconomic status and body mass index. We have also not considered the medium of the school of the children.

CONCLUSION

The study concludes that, male children showed better bilateral coordination score than female. Also, as the age group increases the bilateral coordination score increases.

ABBREVIATIONS

BOT-2 - BRUININKS – OSERETSKY TEST OF MOTOR PROFICIENCY, 2nd EDITION

Conflicts of interest: None

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Original article

Running Speed and Agility according to Bruininks Oseretsky Test of Motor Proficiency, 2nd edition

Antara Pande[1*], Manisha Rathi[2], Sanjivani Dhote[3], Tushar Palekar[4], Apoorva Dhige[5], pooja Yengde[6]

[1,5&6] Intern, [2]Professo, [4]Principal, Dr. D. Y. Patil College of Physiotherapy, Pimpri, Pune, [1]Intern, Dr. D. Y. Patil College of Physiotherapy, Pimpri, Pune ,

[3] Associate Professor, MAEER'S Physiotherapy College.

ABSTRACT

Introduction: Evaluation of running speed and agility in school going children of age 5 to 15 years. Running speed and agility (RSA) is essential in children like other factors like strength, endurance and power. The purpose of this study was to find out the total scores in children, to compare according to gender and to find the descriptive category according to age group. **Materials and methods:** It was a cross sectional analytical study conducted in schools of Pimpri Chinchwad area. This study included 248 males and 268 females which were assessed for by shuttle run test and hopping. **Result:** The total mean point score of female is 30.16 and male are 32.63. The performance of males was better than females. **Conclusion:** From the study, it is concluded that maximum number of students comes under average category and males showed better performance than females in Running speed and agility.

KEYWORDS: Running speed & agility, Bruininks Oseretsky Test of Motor Proficiency, 2nd edition.

INTRODUCTION

Running speed and agility is very essential component for children and athlete to develop. Agility refers to the ability of an individual to move or change the direction rapidly and easily. By working on agility, an athlete can be able to move quickly while maintaining control [1]. Speed is the maximal velocity at which a player can sprint [2]. In field sport athletes it is accepted that they have different running mechanics than sprint athletes [3]. The capacity to move swiftly both laterally and linearly is important if an athlete hopes to be competitive in any sport. Stride frequency, stride length and speed and endurance they all affect running speed [4].

By increasing the stride frequency the amount of time required in between the steps can be reduced easily. To improve stride frequency sprint assisted training is necessary as it enables the individual to run with increased speed e.g. Downhill running towing are two examples of sprint assisted training that will help in increasing the stride frequency. A speed or agility ladder is efficient for improving linear speed, agility and quickness [5]. As the performance of the individual improves, difficulty level should be increased by using more than one ladder as well as by including other different movement patterns.

Age plays key role in running speed and agility. It usually occurs between 18 to 24 months [6]. RSA allows a person to be involved in sports participation and it will aid body movements for physical skills [7]. RSA deficits will be seen in observations of poor coordination of body, failure to develop preferred leg and mostly right or left confusion. Impaired RSA is observed in children having Developmental Coordination Disorder (DCD), Learning Disabilities, Sensory Integrative Dysfunction and other motor impairments [8].

The *Bruininks-Oseretsky Test of Motor Proficiency*, Second Edition (BOT-2) is a test which includes engaging, goal-directed activities to measure the motor skills in children ages 4 through 21. The Bruininks-Oseretsky Test of Motor Proficiency – BOTMP, (Bruininks, 1978) contains 46 items

grouped under eight different subtests of motor proficiency mainly for children between 4.5 and 14.5 years of age.

The Running speed and agility subtest of BOTMP is the sixth subtest under gross motor composite. Activities in this subtest include a shuttle run test, hopping on one and both the feet and stepping over a balance beam. Performance on the shuttle run is measured in seconds; this item provides the opportunity to make clinical observation about gait pattern.

Therefore, the purpose of this study was (a) To find total point score and descriptive category according to gender in school children (b) To find Descriptive category according to age groups which will be helpful in finding well below average and below average children in running speed and agility.

MATERIALS AND METHODS

This cross sectional analytical study was conducted in Pimpri Chichwad area of age group 5 to 15 years. Total 516 samples were studied out of which 248 were males and 268 were females.

The subjects were divided according to gender and age groups. Age group 1 includes 5.0 to 7.11, age group 2 includes 8.0 to 9.11, age group 3 includes 10.0 to 11.11, age group 4 includes 12.0 to 13.11 and age group 5 includes 14.0 to 15.11. The samples were normal and healthy school going children. Inclusion criteria were normal and healthy school going children. Exclusion criteria were any neurological trauma like spinal fractures (6 months back), any visual problem, or any congenital defect.

Procedure: The study was approved by the institutional Ethical committee of Dr. D. Y. Patil College of Physiotherapy, Pune. The study was to assess the Running speed and agility in 5-15 years school going children. 516 Subjects of age group 5-15 years were selected in the study fulfilling the inclusion criteria. After explaining the purpose of the study to the subject, written informed consent was taken from the parents prior to the assessment. The subject was selected on the basis of multistage sampling method. In the first stage, 3 English schools and 3 Marathi schools were selected randomly out of total schools in Pimpri Chinchwad Area. In the second stage, from each standard, 1 division was selected randomly. In the third stage, from every division, boys and girls of the same age were selected by random sampling method.

A pre- assessment was taken to record their socio demographic data and other parameters. Every child was asked to perform 5 tasks given in Running speed and agility subtest of BOT-2 scale as follows:

1. Shuttle run test: In this task, 50 ft distance was marked and the shuttle block was placed on the end line. The examinee ran to the end line, picked up the block & runs back to the start line. The second trial was conducted if the examinee stumbles, falls, or drops the block before crossing the start line. The number of seconds that the examinee has taken was recorded.
2. Stepping sideways over a balance beam: In this task, the examinee had stood with feet together next to balance beam with hands on hips. The examinee has to step over the beam, one foot at a time & steps back to the original side. The second trial was conducted if the examinee stumbles or falls during the first trial. The number of correct steps performed in 15 seconds was recorded. A step was incorrect if the examinee fails to keep both the hands on hips. The task was stopped if the examinee stumbles or falls and the second trial were conducted.
3. One legged stationary hop: In this task, the examinee stood with feet together on the end line with hands on hips. The examinee has to raise non preferred leg behind him or her with knee bent 90 degrees & shin parallel to the floor. The examinee hopped up and down on the preferred leg. The second trial was conducted if the examinee stumbles or falls during the first trial. The number of correct hops performed in 15 seconds was recorded.
4. One legged side hop: In this task, the examinee stood with feet together next to the line with hands on hips. The examinee hopped back and forth over the line with knee bent 90 degrees. The second trial was conducted if the examinee stumbles or falls during the first trial. The number of correct hops performed in 15 seconds was recorded
5. Two legged side hop: In this task, the examinee stood with feet together next to the line with hands on hips. The examinee hopped back and forth over the line. The second trial was conducted if the examinee stumbles or falls during the first trial. The number of correct hops performed in 15 seconds was recorded. A hop was incorrect if the examinee fails to keep both the feet together, fails to keep hands on hips.

A trial was given to children before starting the tasks. Precautions were taken to avoid the children from falling. Subjects were assessed for these tasks and a raw score was recorded in the unit measured (e.g. a number of hopping) and then converted to a numerical point score. Further analysis was done with the help of the BOT-2 manual. The data collected were analyzed using suitable analysis.

RESULTS

516 samples were taken out of which 248 were males and 268 were females.The data summary of running speed and agility total point score according to gender is shown in table 1. The mean score of female were 30.15 $\pm$ 5.52 and males were 32.63 $\pm$ 6.48. The statistical results for score wise comparison of gender indicated a significant difference between males & females as $p \leq 0.001$. Male performance was higher than females. Percentage of the for descriptive category according to gender are shown in Table 2. It is indicated that participants in average category were more as compare to other groups amongst males and females which was statistically significant using chi square test ($p=0.03$).

Table 1. Running speed and agility total point score according to Gender

	Mean	Std Dev
Female	30.15	5.52
Male	32.63	6.48
Combined	31.44	6.16

p≤0.001 using Mann Whitney test.

Table 2. Descriptive Category according to Gender

	Descriptive category					
GENDER	Well above average	Above average	Average	Below average	Well below average	Total
Female	0.00%	3.63%	75.40%	20.97%	0.00%	100%
Male	0.00%	4.85%	82.46%	12.69%	0.00%	100%
TOTAL	0.00%	4.26%	79.07%	16.67%	0.00%	100%

Using chi square test, p=0.03.

Descriptive category according to age group is given in Table 3. In all groups, children coming in average category were maximum followed by below average category. This difference was statistically not significant when various age group was considered as p=0.88. Hence it is indicated that age group does not pursue according to descriptive category.

Table 3. Descriptive category according to age group

		Descriptive category				
	Well above average	**Above average**	**Average**	**Below average**	**Well below average**	**Total**
Age group 1	0	4.50%	80.18%	15.32%	0.00%	100.00%
Age group 2	0	4.21%	81.05%	14.74%	0.00%	100.00%
Age group 3	0	6.48%	75.93%	17.59%	0.00%	100.00%
Age group 4	0	4.00%	80.00%	16.00%	0.00%	100.00%
Age group 5	0	1.96%	78.43%	19.61%	0.00%	100.00%
TOTAL	0	4.26%	79.07%	16.67%	0.00%	100.00%

Using chi square test, p=0.88

DISCUSSION

The present work compared the running speed and agility in 5 to 15 school going children. When RSA total point score was analyzed, females have less mean score of 30.15 than that of males which have 32.63. This is mostly because males have greater muscle mass and a larger portion of it is fast twitch, which allows them to generate greater force, speed and energy. Also they have higher aerobic capacity (VO_2 max) as compare to females which is due to their typically having less body fat, more hemoglobin and muscle mass and larger lungs and heart as compare to women.

In case of descriptive category according to gender, more number of males comes under average category than females, in below average category number of females were more than males and in average category males were more than females. No participants come under well above average and well below average category. Overall it came to a result that males have better scores than females. It is given that the difference was greatest during the adolescent period. This is due to hormones as the primary male hormone is testosterone, which primarily stimulates muscle

mass development where as female primary hormone is estrogen, which stimulates fat accumulation. Testosterone also increases concentration of red blood cells and hemoglobin, both are important for transporting oxygen around the body [9]. Second, level of sport participation of females is significantly lower than that of boys. So, males are more active, fast and better in performing activities [10].

In case of descriptive category according to age group, as the age group increases, better scores are found in participants but there is slight drop in Age group 3. According to studies, body composition and body size are important factors that affect performance in motor related fitness. It also varies among performances and with age [9]. Other factors such as school physical education also play an important role in developing a child's physical fitness and education.

This result is in contrast with point score result as the descriptive categories are allotted according to the scale score. Brenda N. Wilson et al in their study said that, the use of subtest point score will result in a more precise measurement of functions, because gains or deterioration will be related to specific areas of motor control. In addition, score that have undergone statistical transformations will be less exact in their ability to detect real changes that occurred. Because these standard scores are age adjusted, progress will not be reflected in the test scores unless the progress is faster than typical maturation. Therapists should consider using the subtest point scores as a more accurate measure of change.

The study has outlined the limitation of not taking the socio-economic status and body mass index for nutritional status. Because studies have shown that socioeconomic status alters the motor performance of the child. According to Özgür Mülazımoğlu-Ballı in his study he stated that there were significant differences in the BOT-2 score and total score of different socioeconomic groups, in favor of high socioeconomic groups.

CONCLUSION

From the above study, it is concluded that maximum number of participants comes under average category. Mean point score of Running speed and agility was significantly more in males than females, maximum subjects were in average in males and females but when age was considered, descriptive category was not significant. The performance of male participants was better as compare to female participants.

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*Corresponding author: Antara Pande
E-Mail: antara077@gmail.com

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Assessment of Manual Dexterity According to Bruininks Oseretsky Test of Motor Proficiency, 2nd edition in 5-15 years school going children.

Paediatrics

KEYWORDS: Manual Dextrity, Bruininks Oseretsky Test of Motor Proficiency, 2nd edition.

Gurjit Singh	BPT Intern, Dr. D.Y.Patil College of physiotherapy, Pimpri, Pune
Dr. Sanjivani Dhote	MPT, Associate professor of MAEER'S Physiotherapy college, Talegone, Dhabahde.
Dr. Tushar Palekar	Ph.D, Principal Dr. D.Y.Patil College of physiotherapy, Pimpri, Pune
Apoorva Dighe	BPT Intern, Dr. D.Y.Patil College of physiotherapy, Pimpri, Pune.
Antara Pande	BPT Intern, Dr. D.Y.Patil College of physiotherapy, Pimpri, Pune.
Pooja Yengde	BPT Intern, Dr. D.Y.Patil College of physiotherapy, Pimpri, Pune.

ABSTRACT

INTRODUCTION : Evaluation of Manual Dexterity is important in school going children from 5 to 15 years. It is essential in children in like other factors like fine manual integration and fine manual precision. The Purpose of this study was to find out the total scores in children to compare according to gender and descriptive category according to age group.

OBJECTIVES. 1. To find out manual dexterity point score and descriptive category among genders . 2. To find out manual dexterity point score and descriptive category according to age group

MATERIALS & METHODS : It was a cross-sectional analytical study conducted i schools of Pimpri- Chinchwad area. This study included 248 males and 268 females which were assessed for making dots in circle, transferring pennies, sorting cards, stringing blocks and placing pegs in peg boards.

RESULT: The total mean point score of manual dexterity in females is 27.73 and in males is 27.96**.**

CONCLUSION : The study concludes that, male and female children showed similar performance on fine motor point score. also maximum numbers of children from all the age groups were in average category

INTRODUCTION :

Manual ability and performance of dexterity tasks require both gross and fine hand motions and coordination. Retarded children usually have difficulties performing manual activities such as grasping, releasing or manipulative objects, which is crucial in the performance of many activities of daily life. Manual activities require the cooperation of both hands, where the dominant hand performs both fine and gross manipulations, and the non dominant hand is used to stabilize objects. Retarded children develop their handedness on the less affected side. Manual dexterity is found to be a strong predictor of functional independence in activities of daily living.[3] Fine manual dexterity is related to the performance of more precise manipulations with the fingers. Reaching for object and grasping and manipulating them usually is effortless activity. Children need many years to learn how to use hands in increasingly sophisticated ways to feel, explore, grasp and manipulate objects.[6]

For assessing manual dexterity, many scales are available such as

Movement Assessment Battery for children (Movement ABC-2),[4]
Peabody Development Motor Scale (PDMS 2),[4]
Maastrichtse Motoriek Test (MMT)[4]

The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) is an individually administered test that uses engaging, goal-directed activities to measure a wide array of motor skills in individuals ages 4 through 21. The BOT-2 uses a subtest and composite structure that highlights motor performance in the broad functional areas of stability, mobility, strength, coordination, and object manipulation.[3] The Bruininks-Oseretsky Test of Motor Proficiency – BOTMP, consists of 46 items grouped under eight different subtests of motor proficiency for children between 4.5 and 14.5 years of age.[5]

MATERIALS AND METHODS : The Cross Sectional analytical study was conducted in pimprichinchwad area of age group 5 to 15 years. Total samples were studied out of which 248 were males and 268 were females.

The Subjects were divided according to gender and age groups. Age Group 1 includes 5.0-7.11,age group 2 includes 8.0-9.11,age group3 includes 10.0-11.11,age group 4 includes 12.0-13.11 and age group 5 includes 14.0-15.11.The Samples were normal and healthy school going children,Inclusion criteria were normal and healthy school going children. Exclusion criteria were neurological trauma like spinal fractures, any visul problem, or any congenital deficit.

PROCEDURE : The study was approved by the ethical committee of Dr. D.Y. Patil college of physiotherapy, Pune. The study was to asses the Manual dexterity in 5-15 years school going children. 516 Subjects of age group 5-15 years were selected in the study fulfilling the inclusion criteria. After explaining the purpose of the study to the subject, written informed consent as taken from the parents prior to the assessment.The subject was selected on the basis of multistage sampling method.In the first stage ,3 English schools and 3 Marathi schools were selected randomly out of total schools in Pimpri Chinchwad Area. In the second stage , from each standard 1 division was selected randomly. In the third stage, from every division , boys and girls of the same age were selected by random sampling method.

A Pre- assessment was taken to record their socio demographic data and other parameters. Every child was asked to perform 5 tasks given in Manual dexterity subtest of BOT-2 Scale as follows:

1. **Make Dots in circle :** The examinee holds the circle in preferred hand and makes one dot in each circle. The examinee may make dots in the circle in any order.
2. **Transferring Pennies** : Two piece of the penny cards was put together so that they can form a rectangle, with both the penny outline and box outline facing up. The penny pad was placed with the penny outline in side of the preferred hand of the examinee. The Pennies were placed infront of the penny outline and the box outline. The examinee picked up one penny at a time with preferred hand, transferred each penny to non-preferred hand, and then puts each penny back into the box. The examinee could pick up pennies in any order. The examinee's hand was over the box when dropping, not throwing, pennies into the box. If the examinee threw pennies, subjects were reminded to drop them into the box.

3. **Placing Pegs Into A Pegboard :** The peg and the pegboard was in front of the examinee while the box of the pegboard on the side of the examinee The examinee holded the pegboard with non preferred hand to avoid movement during the task.The examinee used one peg at a time in any order. If the examinee dropped any peg out of the pegboard they did not need to pick that up again,especially if it rolled out of the table. The examinee picked up a new peg from the pegboard.
4. **Sorting Cards:** One red squared and blue circled card was placed in front of the examinee**.** These were the reference cards showingthe examinee where to sort the cards.. The remaining cards were shuffled and placed between two reference cards.examinee used prefereed hand to pick one card at a time from the deck,sorting each other by colour .
5. **Stringing Blocks :** The blocks and strings were placed in front of the examineeThe examinee picked the string and one block at a time.The blocks did not need to be pushed to the other end of the string., the examinee aided by lightly holding the end of the string.

RESULT :

516 samples were taken out of which 248 were males and 268 were females.

Table 1. Manual dexterity point score according to Gender

	Mean	Std Dev
FEMALE	27.73	6.90
MALE	27.96	7.43
TOTAL	27.85	7.17

The data summary of **Manual dexterity** point score according to gender is shown in table 1. The mean score of female were 27.73 $\pm$ 6.90 and males were 27.96$\pm$ 7.43. The statistical difference between males & females were non Significant.

Table 2: DESCRIPTIVE CATEGORY ACCORDING TO GENDER

GENDER	WAA	AA	A	BA	WBA
FEMALE	0	5.24	58.87	33.47	2.42
MALE	0.37	18.66	62.31	16.79	1.87

This graph represents that more number of male subjects falls under average category followed by above average category followed by below average category and more number of female subjects falls under average category followed by below average category followed by above average category.

Table : 3 shows the data summary of Manual dexterity total point score according to gender, The mean score of female were 27.23 and males were 27.96.

AGE GROUP	WAA	AA	A	BA	WBA	Total
	0.90%	12.61%	56.76%	27.03%	2.70%	100.00%
Age group 1	0.00%	7.37%	58.95%	28.42%	5.26%	100.00%
Age group 2	0.00%	11.11%	59.26%	28.70%	0.93%	100.00%
Age group 3	0.00%	20.00%	62.00%	17.00%	1.00%	100.00%
Age group 4	0.00%	9.80%	66.67%	22.55%	0.98%	100.00%
Age group 5	1	63	313	128	11	516
TOTAL	0.19%	12.21%	60.66%	24.81%	2.13%	100.00%

Table : 3 shows that the statiscal results for score wise comparison of gender indicated a significant difference between males and femaless. Male Performance was higher than females Descriptive category according to age group , In all groups children coming under averae category we maximum followed by below average category. This difference was statistically not significant. Hence it indicated that age group does not pursue according to descriptive category.

Table 4: DESCRIPTIVE CATEGORY OF MANUAL DEXTERITY ACCORDING TO AGE GROUP

	WAA	AA	A	BA	WBA
AGE GROUP 1 (5,6,7 years)	0.9	12.61	56.76	27.03	2.7
AGE GROUP 2 (8,9 years)	0	7.37	58.95	28.42	5.26
AGE GROUP 3 (10,11 years)	0	11.11	59.26	28.7	0.93
AGE GROUP 4 (12,13 years)	0	20	62	17	1
AGE GROUP 5 (14,15 years)	0	9.8	66.67	22.55	0.98

Table 4 shows this graph represents that more number of children from all age groups fall under average category and students falling under age group 2(8,9 years), have more percentage that is 5.26 which comes under category of well below average that is more than other age groups.

DISCUSSION : The Primary aim of this study was to assess Manual dexterity using Bruininks-Oseretsky Test of Motor Proficiency, 2nd edition. Motor development is the gradual process by which a child gains use and coordination of the large muscles of the legs, trunk and the smaller muscles of the hand. Neuromuscular development starts in embryonic stage and it continues after birth. Nutritional and socio-economic factors play an important role in affection of motor development.

The manual dexterity sub-test was designed to assess hand-eye co-ordination, fine motor skills & fine motor activities. Samples' score is consistent with individuals who can complete all these tests.

The study was conducted among 516 subjects out of which 248 were male & 268 were females of age 5 years to 15 years 11 months (mean age – 10.67years & standard deviation – 3.03).

The study shows that both male and female subjects showed similar results. The mean of manual dexterity point score of female was 27.73 and of males was 27.96. There was a very slight difference between male and female in the average category in which 58.87% were females and 62.31% were males.

Our results goes in accordance with the article where Paula Aivazoglou Priosti et. al., says that as to gender, results showed that manual dexterity were similar for girls and boys in the two studied groups.[7] According to ÖzgürMülazımoğlu-Ballı, there were no significant difference in males and females among the manual dexterity scores.[5]

In this study we found that maximum children from all age group fall in average category. Our result is consistent with the study by Satabdi Ghosh et. al. in which they say that, fine motor performance score in school going children were better than that of other two occupational groups. This is because, the subjects are more likely to engage with fine motor tasks such as writing, drawing, grasping objects and reaching out to other objects, releasing the objects and turning the wrist in various directions the subjects were familiar in writing and drawing activities this gave them more advantage to score better in fine motor performances than other two occupational groups.[8]

According to the graph 4 (a,b,c) it shows the linear pattern that is as the age group increases the mean of manual dexterity point score also increases in both males and females. This is because, Manual dexterity of people at different age groups are influenced by various reasons like body mass, fat percentage and nutritional status.[7]

In this study we found out that students falling under age group 2 have more percentage which comes under well below average category that is more than other age groups. According to study done by T.Balakrishnan et al, It was found that Indian children with the U.S.A. normative sample revealed a significance difference in the performance when compared by age. The Indian children under-performed in the bilateral coordination subtest across all the age group 7, 8 & 9 as compared to the USA normative sample.[11]

The study has outlined the limitation of not taking the socio economic status. Because studies have shown that the socio economic status alters the fine motor skills of the child. Ozgur Mulazimoglu-Balli in his study concluded that there were significant

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differences in BOT-2 score and total score of different socio economic groups, in favor of high socio-economic group.

Limitation- It was seen that socio-economic status had not been mentioned in the study.

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RESEARCH ARTICLE

GENDER DIFFERENCE OF DEVELOPMENTAL COORDINATION DISORDER IN PRIMARY, SECONDARY & HIGHER SECONDARY SCHOOL

***,[1]Dr. N. Sanjivani Dhote and [2]Dr. J. Palekar Tushar**

[1]Associate Professor in Neuroscience Department of Dr. D.Y.Patil College of Physiotherapy, Pimpri, Pune
[2]Principal of Dr. D.Y.Patil College of Physiotherapy, Pimpri, Pune

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ABSTRACT

Context: Since the early 1900s, the scientific community has acknowledged a large group of children with movement skill difficulties who have not been diagnosed with a general medical condition. This difficulty in motor skill competence, observed in children who are developing well intellectually, is termed 'developmental coordination disorder' (DCD). Considering the importance of timely diagnosis of DCD and the child's performance on the BOT-2 will allow the physical therapist to identify areas of strength and areas of need in regards to the child's gross motor functioning, and can therefore help to guide treatment. The early diagnosis of DCD can be helpful to prevent the future secondary complications. So purpose of this study is to find out the prevalence of developmental coordination disorder on BOT-2 in 5 to 15 years school going children.
Settings and Design: It was a cross-sectional analytical study conducted in PCMC area schools.
Methods and Material: Multistage stratified sampling done to assessing 516 children's included 248 males and 268 females which were assessed by Using BOT-2nd edition.
Statistical analysis used: Mean and Standard Deviation (SD) & Fisher's test was used to analysis.
Result: Prevalence of DCD was among male 1.12% & female 1.61 from 516 children.
Conclusion: Female showed more prevalence of Developmental coordination disorder than Male.

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INTRODUCTION

Growth is an essential feature of life of a child that distinguishes him or her from an adult. The process of growth starts from the time of conception and continues until the child grows into fully mature adult. The terms growth and development are often used together. These are not interchangeable, because they represent two different facets of dynamics of change, i.e; those of quantity and quality. Growth and development usually proceed concurrently, but may not always be interrelated. Growth denotes a net increase in the size or mass of tissues. It is largely attributed to multiplication of cells and increase in the intracellular substance. Hypertrophy or expansion of cell size contributes to a lesser extent to the process of growth. Development specifies maturation of functions. It is related to the maturation and myelination of the nervous system and indicates acquisition of a variety of skills for optimal functioning of the individual. (Ghai *et al*., 2004) Since the early 1900s, the scientific community has acknowledged a large group of children with movement skill difficulties who have not been diagnosed with a general medical condition (Magalhaes *et al*., 2006). This difficulty in motor skill competence, observed in children who are developing well intellectually, is termed 'developmental coordination disorder' (DCD). DCD is a recognized syndrome that was described by the World Health Organization in 1992 (WHO 1992) and has been included in the diagnostic manuals of the American Psychiatric Association since 1989 (American Psychiatric Association, 2000). "Developmental coordination disorder (DCD) is defined, using the Diagnostic and Statistical Manual Of Mental Disorders, Fourth Edition (DSM-IV), as a condition marked by a significant impairment in the development of motor coordination, which interferes with academic achievement and/or activities of daily living (ADL). These difficulties are not due to a general medical condition (eg, cerebral palsy) and are in excess of any learning difficulties is present (Raghu Lingam *et al*., 2009). DCD is a highly prevalent disorder (5-6% of school-aged children) so it is likely that there is at least one child with DCD in most classrooms. One of the challenges of identifying children with DCD is the variety of ways in which it is revealed. (Prado *et*

******Corresponding author:* **Dr. N. Sanjivani Dhote,**
Associate Professor in Neuroscience Department of Dr. D.Y.Patil College of Physiotherapy, Pimpri, Pune

al., 2009) The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) provides four criteria to classify a child as having DCD (American Psychiatric Association, 2000).

A. Performance in daily activities that require motor coordination is substantially below given the person's chronologic age and measured intelligence. This may be manifested by marked delays in achieving motor milestones (e.g., walking, crawling, sitting) dropping things, "clumsiness," poor performance in sports, or poor handwriting.
B. The disturbance in criterion significantly interferes with academic achievement or activities of daily living.
C. The disturbance is not due to a general medical condition (e.g., cerebral palsy, hemiplegia or muscular dystrophy) and does not meet criteria for a Pervasive Developmental Disorder.
D. If mental retardation is present, the motor difficulties are in excess of those usually associated with it. (Wilson *et al*., 1995)

The difficulties may be considered to be mild, moderate or severe. Even though this condition is observed by many schoolteachers, as well as physical and occupational therapists, it is not an easy diagnosis to make due to multi-faceted diagnostic criteria and terminology problems (Prado *et al*., 2009). Outcome measurements used to assess gross motor development in infants and children up to age 5, including the Peabody Developmental Motor Scale (Folio and Fewell, 2000), second edition and the Alberta Infant Motor Scale (Johanna Darrah *et al*., 1998). When children age out of either the PDMS-2 or the AIMS, one standardized assessment option physical therapists have is the Bruininks-Oseretsky Test of Motor Proficiency, second edition (Bruininks and Bruininks, 2005; Bruininks, 1978; Burton and Miller, 1998) (BOT-2nd). The test-retest reliability and internal consistency of the total scale were excellent, with an ICC of 0.99 (95% confidence interval) and alpha of 0.92 The BOT-2 can be used to evaluate a wide variety of fine and gross motor skills for children, teenagers and young adults 4-21 years of age. This is a test that can also be used by Physiotherapist, psychologists, adaptive physical education teachers, special education teachers and educational diagnosticians (Bruininks and Bruininks, 2005; Bruininks, 1978; Burton and Miller, 1998; Wuang and Su, 2009).

Need of study: The prevalence of DCD in India is found to be 1.37%. The prevalence of DCD in other countries is estimated to be (5-8%) usa, (1.8%) uk, (5.7%) greek, (5-9%) canada, (1.7%) belgium and 6% worldwide (Judith M Peters and Ann Markee, 2007; Robert C Barnhort *et al*., 2003; Ganapathy Sankar and Saritha, 2011; Georgia D. Tsiotra *et al*., 2006; Nadia Cristina Valentini *et al*., 2012). As per the literature there are no studies found on the prevalence of DCD using BOT-2 in 5-15 years of age group in Indian scenario. Considering the importance of timely diagnosis of DCD and the child's performance on the BOT-2 will allow the physical therapist to identify areas of strength and areas of need in regards to the child's gross motor functioning, and can therefore help to guide treatment. The early diagnosis of DCD can be helpful to prevent the future secondary complications. So purpose of this study is to find out the prevalence of DCD on BOT-2 in 5 to 15 years school going children among gender.

Aim : To find out the prevalence of DCD on BOT-2 in 5 to 15 years of school going children.

Objective: 1. To find out various Descriptive category of Total Motor Composite component on BOT.2nd in school going children of age group between 5 to 15 years Gender. **2.** To find out the prevalence of Developmental coordination disorder by using BOT. 2nd in 5 to 15 yr of school going children Among Genders

MATERIALS AND METHODS

The Cross Sectional analytical study was conducted in pimprichinchwad area of age group 5 to 15 years. Total samples were studied out of which 248 were males and 268 were females. The Subjects were divided according to gender and age groups. Age Group 1 includes 5.0-7.11, age group 2 includes 8.0-9.11,age group3 includes 10.0-11.11, age group 4 includes 12.0-13.11 and age group 5 includes 14.0-15.11. The Samples were normal and healthy school going children, Inclusion criteria were normal and healthy school going children. Exclusion criteria were neurological trauma like spinal fractures, any visul problem, or any congenital deficit. By using BOT-2nd kit include examiners manual, individual record form, student booklet, two red pencils and a tennis ball. A table and chair of appropriate to the child's height, electronic timer and clipboard were additionally used.

Procedure

The synopsis of the study was submitted to institutional ethical committee, after the clearance from the institutional ethical committee. 500 subjects of age group 5-15 years were selected in the study fulfilling the inclusion criteria. After explaining the purpose of the study to the subject/parent, they were informed about their right to opt out of the study any time during the course of the study without giving reason for doing so. The parents/teacher were assured that their child's participation and non-participation would not affect their child's education. Subjects were selected on the basis of multistage sampling method. In the first stage, 3 English schools and 3 Marathi schools was selected randomly out of the total schools in PimpriChinchwad Area. In the second stage, from each standard, any one division was selected Ramdomly. In third stage, from every division, boys and girls of same age were selected by random sampling method. A written informed consent was obtained from the subjects/ parents one day prior to the assessment. Proper precautions was taken so that there was no harm to the child. Total children were divided into 5 age groups according to there chronological age. These age groups were divided for sampling convenience and for obtaining proper results. The age group 1 included age group ranging from 5.0-7.11 years, age group 2 included 8.0-9.11, age group 3 included 10.0-11.11, age group 4 included 12.0-13.11 and age group 5 included 14.0-15.11. The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) was used to assess children's motorproficiency. BOT-2 is an individually-administered test that uses engaging, goal-directed activities to measure a widearray of motor skills in individuals aged 4 through 21 (Bruininks and Bruininks, 2005). The BOT-2 assesses motorproficiency in four motor-area composites; fine manual control (FMC), manual coordination (MC), body coordination (BC) and strength and agility (SA). With 53 items and each motor-area composite has two subtests. The total motor composite score can be calculated by adding four

composite scores together (53 items, 8 subtests and 4four motor-area composites; score range = 0–320 points) (Bruininks and Bruininks, 2005). Subjects were assessed for these tasks and these raw score were converted to a numerical point score. Descriptive analysis done by using manual, point score converted in to five descriptive category that of WAA-Well above average, AA- Above Average, A – Average, BA- Below Average, WBA- Well Below Average

RESULTS

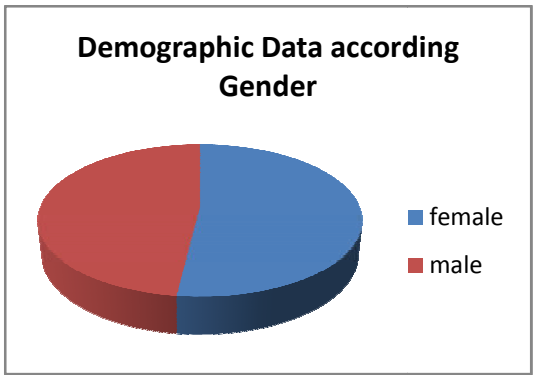
1st Table & Graph showed Five hundred & sixteen children (Mean age =10.67 years, SD = 3.03) participated in this study among that 248 & 268 were male & female respectively.

Graph 2nd showed Maximum Children were found in Average category of motor proficiency fallowed by Below average category. In the Average category male were more (70.9%) as compare to female (58.06%). Students who fall under the category of Well Below Average indicates that they have motor deficit and so they are considered as Developmental Coordination Disorders.

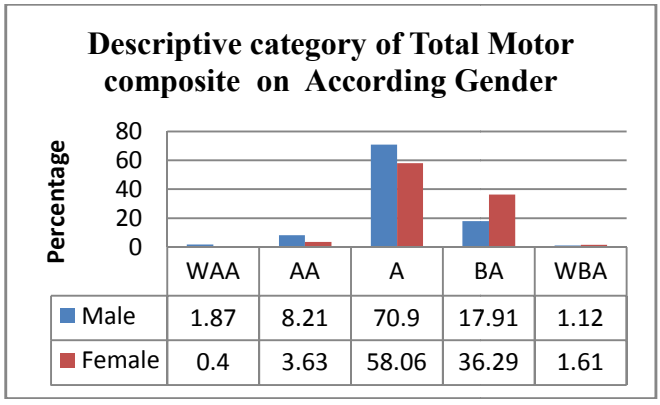
Graph 3rd shows prevalence of Developmental coordination disorder among males and females was 1.12% & 1.61% respectively, as they fall under the category of Well Below Average i.e motor impairment. Females are having more prevalence of DCD than males, however this difference is not statically significant as P=0.915 by Fisher's Exact test.

Table 1. Demographic Data according Gender

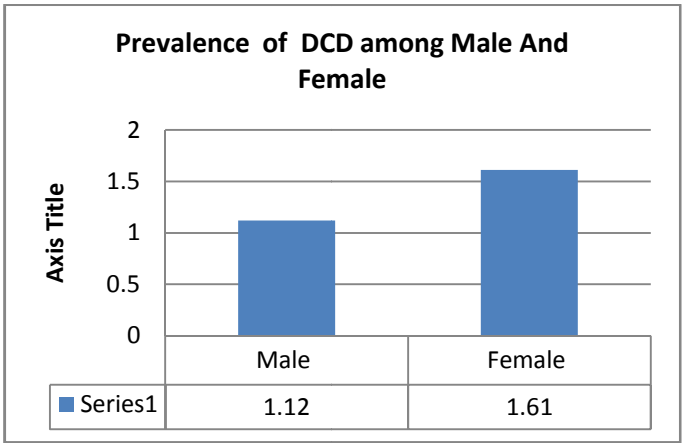
	n	mean age	SD
Female	268	10.69	3.02
Male	248	10.66	3.04



Graph 1. Demographic Data according Gender



Graph 2. Descriptive category of Total Motor composite on According Gender



Graph 3. Prevalence of DCD among Male And Female

DISCUSSION

The present study was carried out with the aim 1) To find out the prevalence of Developmental coordination disorder by using BOT-2nded in 5 to 15 yr of school going children among gender. Present study also showed that females are having more prevalence of DCD than males, however this difference is not statically significant as P=0.915 by Fisher's Exact test. These performance differences in males and females can be due to the nutritional status, as the dietary intake of boys is more than that of girls (Satabdighosh *et al*., 2013). Nutritional status appear to be significant predictor for both fine and gross motor development (Satabdighosh *et al*., 2013). Similar observations have been reported by other research workers in children of different countries (Bobbio *et al*., 2007; Chowdhury *et al*., 2010; Pollitt *et al*., 1994). Nutritional status may alter the learning process by influencing brain development and physical growth and accordingly modify the movement proficiency of the children by adjusting the strength, power, coordination and perception (Satabdighosh *et al*., 2013) Our study result are in accordance with Girish, SrilathaRaja *et al*. as in their study prevalence of DCD with girls (1.1%) affected more than boys (0.5%) at confidence interval of 95%. Girls were twice affected than boys (American Psychiatric Association, 1994). Some researchers stated that children's with low socioeconomic status were less competent in locomotors skills compared with their high socioeconomic status peers (Hardy *et al*., 2012; Mészáros *et al*., 2008). However difference among gender in present study was not statistically significant. It is difficult to make exact comparisons between countries because the estimated prevalence is highly influenced by the means of assessment and the type of sample recruited. In Bilateral Coordination component no children were found in well Above Average category (WAA) because scale score was not given for this category even though they scored maximum in Bilateral coordination point score. So need to establish normative data for Indian population is suggested. Chowdhury *et al*. (2010) reported that Indian children of higher socioeconomic status had a higher score for motor development than lower socioeconomic status counterpart. Almost all research's show that motor proficiency and socioeconomic status has a positive association. This indicates that low socioeconomic status children have low motor proficiency and vice versa (ÖzgürMülazımoğlu-Ballı *et al*., 2016). On the other hand, participating in different physical activities, in particular (e.g. gymnastics, swimming, dance etc.) outside of school, might

also determine these differences. High and middle socioeconomic status children might have more opportunity to participate in these kinds of activities. Motor skill practice is useful to improve the fine manual control competence of children (Logan *et al*., 2011). The children studied in the group were from public and private school. Limitation of the present study was socioeconomic status, Cardiorespiratory Fitness & Body Mass Index were not considered while finding out the prevalence of DCD. Further studies can be conducted to investigate Motor proficiency of school going children who were underweight at time of birth and preterm.

Conclusion

Females are having more prevalence of DCD than males. however this difference is not statically significant as P=0.915 by Fisher's Exact test.

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Standard Normative Values of Six Minute Walk Test in Healthy Children Aged 7–16 Years a Cross Sectional Study

Vardhan Vishnu*
Assistant Professor, Padmashree Dr. D.Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune

*Corresponding author: Vishnu Vardhan, Padmashree Dr. D.Y. Patil College of Physiotherapy, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune-411018. Mobile No: 08379917421, E-Mail: vishnudiwakarpt@yahoo.co.in (mailto:vishnudiwakarpt@yahoo.co.in?cc=gbehal@indianjournals.com)

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Abstract

Background and Objectives

Various studies had been done on six minute walk test to assess the functional capacity, but very few studies on six minute walk test were performed in children. The purpose of this study is to find out the normative values for six minute walk test in healthy children aged 7–16 years and correlate age, height, weight and body mass index with six minute walk distance

Method

460 children were selected of the age group of 7–16 years. Weight and height was measured by calibrated weighing scale and stadiometer by standard anthropometric methods before the test and six minute walk test was then performed. After completion of the test, total distance was measured. Descriptive analysis has been used to find out the normative values of six minute walk distance of the age group of 7–16 years in boys and girls respectively. Karl Pearson's correlation has been used to find out the correlation between age, height, weight, body mass index with six minute walk distance.

Results

In this study, normative values for six minute walk test in healthy children aged 7–16 years was established with mean distance in boys 469.09 ± 56.66 and girls 458.50 ± 55.44 respectively.

Interpretation and Conclusion

The results of this study show that normative values for six minute walk test in healthy subjects aged 7–16 years was established. Age and height have a positive correlation with six minute walk distance while weight and BMI have a negative correlation with six minute walk distance.

Top

Keywords

Six minute walk test, *Reference values*, *Age*, *Height*, *Weight*, *Body mass index*.

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Modelling approaches for tuberculosis: are they realistic?

The Article by Nicolas Menzies and colleagues (November, 2016),[1] based on a modelling approach, concludes that most tuberculosis control interventions in South Africa, China, and India are highly cost-effective. In this regard, I have a different viewpoint.

Such modelling approaches are useful in countries where the data are reliable and predictions are precise. However, when applied to low-income or high-burden countries they face a fundamental problem that the reality substantially differs from the basic assumptions. The models rarely account for political determinants such as investments in the health sector, political disruptions, migration, poverty, and poor regulation of the private sector including private pharmacies, which are at the root of existing tuberculosis and emerging anti-tuberculosis drug resistance in the world.

India, which carries the highest global burden of tuberculosis, only spends less than 1·5% of gross domestic product on health and a significant gap exists in the required and actual receipt of funding support.[2–4] The ground-level staff handling the tuberculosis programme work are contractual and frequently face salary issues, which has a negative influence on their work performance. Some high-burden countries still do not have a mandatory tuberculosis notification policy and its enforcement remains weak in others.[5] Many countries face temporary drug shortages and several behavioural factors are at the interface between the patient and the health system, which substantially influence tuberculosis diagnosis, treatment, and outcomes. Existing mathematical models rarely seem to capture these real-world scenarios, and one might see its reflection in the increased global burden of tuberculosis.[6]

I declare no competing interests.

Sachin Atre
atresachin2000@yahoo.com

Johns Hopkins University, Centre for Clinical Global Health Education (CCGHE), Baltimore, MD, USA and Dr D Y Patil Medical College, Hospital and Research Centre, Pimpri, Pune 411018, India

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How feasible is pharmaceutical regulation in India?

Patricia McGettigan and colleagues[1] (November 2017, e1075–76) draw attention to one of the most pressing pharmaceutical regulation issues in India, especially relevant as WHO adopts the Global Antimicrobial Resistance Action Plan.[2] Through a careful analysis, the authors showed that not only are overall sales of antibiotics rapidly increasing, but so too are the sales of Watch Group and Reserve Group antibiotics as well as unapproved fixed dose combination antibiotics. In my opinion, to understand the problem of overuse of antibiotics and emerging resistance we need to view it through an anthropological lens.

India has a population of 1·3 billion and a complex health-care system comprising the public sector and a vast, weakly regulated private sector that includes many formal and informal providers. There are several autonomous, private medical colleges across the country, which are widely known to charge huge capitation fees[3] from students who sometimes have no merit to become clinicians. Although the Government of India took a positive step in 2016 to remove huge capitation fees in private medical colleges and promote merit based admissions, the cost of regular tuition fees remains exorbitant. The clinicians who graduate from such expensive medical colleges might initially be motivated by trying to recover the money invested in their education. Furthermore, many practitioners from alternative systems of medicines such as Ayurveda, Unani, and homoeopathy, who prescribe antibiotics, unfortunately have little knowledge or training of antibiotic use. Whether from licensed clinicians or otherwise, the appropriateness and requirement of prescriptions remain questionable. If a pharmacist knows the patient in his or her locality, it is quite possible to get antibiotics even without prescriptions. Generally, there is one licensed pharmacist per pharmacy, but often in busy pharmacies, untrained supporting staff also attend patients.

In India prescription-based medicine dispensing has little meaning from a regulatory viewpoint because pharmacies do not have systems to monitor parameters such as the prescription's time validity, who has been prescribed medicines and for which health condition. Also, prescriptions are always returned to patients with hardly any evidence left with pharmacists except drug quantity record. Thus, tracking antibiotic use becomes practically impossible. The misuse of antibiotics is inevitable considering all the above circumstances.

Ironically, a country with a population as large as India's does not have adequate human resource for drug inspections nor to cater for many of the national disease control programmes—eg, tuberculosis.[4,5] One of the root causes for this state of affairs is the Indian Government's poor investment in the health sector.[6] When I asked pharmacists in rural India about visits by drug inspectors, the frequent answer was "once in a year or two", which might explain the grim regulatory control situation there. Moreover, India has wholesale drug markets, such as Bhagirath palace in Delhi, which are known for illegal drug sales.[7,8]

Unless these fundamental issues are dealt with, implementing WHO's Global Antimicrobial Resistance Action Plan remains unrealistic in India.

I declare no competing interests.

Sachin Atre
atresachin2000@yahoo.com

Johns Hopkins University, Centre for Clinical Global Health Education (CCGHE), Baltimore, MD, USA

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A tuberculosis-free world: is it a delusion?

I read with interest the *Lancet* Commission on tuberculosis.[1] Tuberculosis, the leading infectious cause of mortality in the world, resulting in nearly 10 million cases and 1·6 million annual deaths, has made the human race suffer for more than a century.[2] Despite the existence of cutting-edge technologies and treatments, we are not able to achieve control over the disease. I have some fundamental questions for the global community of tuberculosis researchers, policy makers, and programme planners, especially as we move towards the ambitious goal of making the world tuberculosis-free.

Do we not already have abundant policy reports and documents already available that outline the burden of tuberculosis and the situations in various countries around the world? Do we not already know that there is a gap of US$1·3 billion in allocated and actual funding for tuberculosis control?[1] Do we not already know that when programmes function properly, the private sector in countries with a high tuberculosis incidence follows standard guidelines, and regulatory systems act the way they are supposed to, emerging drug resistance can be controlled? Do we not already know why there are delays in tuberculosis diagnosis and starting treatment and what the barriers related to patients and health systems are in treatment compliance?[3] Do we not know that if investments are made in socioeconomic development, tuberculosis incidence can decline, which McKeown[4] showed before the start of the antibiotic era? Do we not already know that cutting-edge technology such as whole-genome sequencing can help not only in the early diagnosis of drug-resistant tuberculosis, but also in deciding on an individual-tailored treatment?[5]

If we have the knowledge and technology, we must understand the reasons for failure in translating them into practice. We must be able to identify and deal with the determinants of political will that are holding us back from taking necessary measures. We must ask why we are left only with jargon-filled discussions and high-level meetings producing recommendations that have, over the past many years, had little success in controlling tuberculosis. As an anthropologist, after carrying out field-based research on tuberculosis in India for nearly two decades, I feel that much of the research is repetitive, and we are just going round in circles. The time has come to accept the real-world scenario, deal with the problems with courage and openness, and have a realistic action plan if we really want to talk about the world being free from tuberculosis.

I declare no competing interests.

Sachin Atre
atresachin2000@yahoo.com

Center for Clinical Global Health Education, Johns Hopkins University, Baltimore, MD, USA; and Dr D Y Patil Medical College, Hospital and Research Centre, Dr D Y Patil Vidyapeeth, Pimpri, Pune 411018, India

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Authors' reply

We agree with Sachin Atre that building a tuberculosis-free world requires circumspect reflection on the failures of the past. Doing so also demands renewed ambition; only with sufficient political will and financial investments can we end the epidemic. As we underscore in the Commission,[1] failure to end the epidemic is morally inexcusable and economically indefensible. We must therefore move beyond the narrow view that tuberculosis is the problem of the world's poorest societies and recognise that the disease is a universal health problem for an interdependent global population.[2]

Atre rightly points out that there is insufficient discourse on how to effectively shape the political economy to secure substantive progress towards ending tuberculosis in countries such as India. Existing literature underemphasises the significance of political dynamics and processes within countries that can result in the expansion of tuberculosis programmes. However, we assert that the Commission does highlight some of the most important policy solutions, offering a roadmap to ensure evidence-based, cost-effective policies are implemented at scale.

Atre argues that much of the knowledge needed to end tuberculosis is already known, a point we agree with. Unfortunately, closing the so-called know–do gap[3] in many high-burden countries has been an especially stubborn challenge, exacerbated by a technical capacity gap. We believe that the Commission provides valuable insights into how to close this gap, including identifying the biggest programmes, policy, and implementation research (PPIR) priorities. The value proposition of PPIR for policy makers and national tuberculosis programmes is that such research can provide person-centered solutions for overcoming implementation bottlenecks that are country specific.

Finally, we agree with Atre that the key lever of change is a political one. Ending the epidemic will only happen when global leaders are held to account for their actions on tuberculosis. Hopefully, the Multisectoral Accountability Framework launched at the World Health


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Assembly on April 23, 2019,[4] can be a vehicle to secure real progress in the highest burden countries. As the Commission underscores, effective accountability processes, at national and subnational levels, are necessary to ensure a concerted, coordinated response. Efforts like *The Lancet* Tuberculosis Observatory to focus and sustain expectations on local political will, through engagement of parliamentarians, civil society, and local technical experts, will be crucial to ensuring the response is courageous and transparent.

We declare no competing interests.

*Michael J A Reid, Eric Goosby
michael.reid2@ucsf.edu

Division of HIV, Infectious Diseases, Global Medicine, University of California, San Francisco, CA 94158, USA

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Recurring acute encephalitis syndrome outbreaks in Bihar, India

See **Online** for appendix

The death of 154 undernourished children from acute encephalitis syndrome in a short period of 3 weeks[1] is deeply concerning. The World Report by Patralekha Chatterjee[1] is a timely update on a situation that recurs almost every year in Bihar, India. Chatterjee rightly pinpoints undernutrition, inadequate health facilities, and poor awareness among the local population as contributing factors to these deaths.

Although no consensus has been reached on the exact cause of the deaths in and around the lychee (*Litchi chinensis*) plantations of the Muzzafarpur district of Bihar, the most accepted cause is hypoglycaemic encephalopathy (known locally as Chamki fever). The children (aged 3–7 years) were from low-income families, had not eaten the previous evening, and had eaten unripe lychee in the morning.[2] Lychee contains hypoglycin A and methylenecyclopropylglycine (a toxic metabolite to mammals), which block the alternative pathway for glucose synthesis (fatty acid oxidation),[3,4] causing a drastic drop in blood glucose levels and metabolic hypoglycaemia.[5]

We urgently need to devise strategies to avoid similar deaths in future. Firstly, awareness programmes are essential in Bihar, involving public broadcasting media, primary schools, Anganwadi community health workers and government health-care centres. These programmes would provide education on undernutrition, hypoglycaemia induced by lychee consumption on an empty stomach, and the need for immediate intravenous glucose administration to the sick child if they show signs of hypoglycaemia. Secondly, improvements to hospital infrastructure and doctor-to-patient ratios would help to contain major outbreaks. Finally, addressing the challenge of malnutrition through appropriate implementation of India's National Food Security Act is crucial. Seeking support from international bodies, such as UNICEF and WHO, would be prudent to resolve this long-standing problem.

We declare no competing interests.

*Shampa Ghosh, Manchala Raghunath, *Jitendra Kumar Sinha
jksinha@amity.edu

National Institute of Nutrition, Hyderabad, India (SG, MR); and Amity Institute of Neuropsychology and Neurosciences, Amity University, Uttar Pradesh 201303, India (JKS)

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Parasitic encephalitis in immunocompetent individuals

Arun Venkatesan and colleagues[1] adeptly presented the infectious and autoimmune causes of acute encephalitis in immunocompetent adults. According to the authors, viruses are the largest group of acute encephalitis causative agents. The authors also listed bacterial, fungal, and parasitic causes of acute encephalitis. In the parasitic section, *Acanthoemeba* species, *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Baylisascaris procyonis* are listed. However, it seems that some of the important parasitic causes of acute encephalitis are missed (appendix). *Toxoplasma gondii* is a prevalent parasite worldwide that can cause toxoplasmic encephalitis. Although immunocompromised patients are more susceptible to acute infection and toxoplasmic encephalitis, several cases of encephalitis have been reported in immunocompetent individuals[2,3] with the symptoms of encephalitis, dementia, fever, headache, and mild weakness. Toxocariasis is another cause of encephalitis in immunocompetent adults. *Toxocara canis* and *Toxocara cati* are parasites of dogs and cats,

This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on Dec 14, 2016

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Author's reply

We thank Yang Yu and colleagues for their correspondence about our study assessing the diagnostic accuracy of Xpert MTB/RIF in the context of community-based active case finding for tuberculosis.[1] They raise concerns about sputum quality control and potential bias related to unblinded chest radiography reporting.

The ability to collect sputum samples of sufficient quality for tuberculosis testing is a common concern when testing predominately asymptomatic individuals in a community screening setting. We have shown, however, that sputum quality considerations might not be as important as expected. In our study, macroscopic sputum quality was determined with two parameters: sputum appearance and sputum volume. We chose to test sputum samples of of 0·5 mL or greater, the minimum volume recommended for testing of sputum sediments with Xpert MTB/RIF,[2] after having shown that 54 (32%) of the 168 specimens that were Xpert MTB/RIF-positive in the first year of the study were detected in samples that were 0·5–1·0 mL in volume. Our study also showed that neither sputum appearance nor sputum colour were predictive of the presence of *Mycobacterium tuberculosis* in sputum. In fact, 134 (84%) of 159 Xpert MTB/RIF-positive sputum samples were either salivary or mucoid in appearance.[3] This suggests that sputum quality parameters cannot be reliably used in pre-analytical procedures to exclude samples from testing.

We agree that a limitation of our study was the unblinded reporting of chest radiographs and that developments in computer-aided chest radiograph reporting hold some promise, particularly in resource-limited settings and those in which chest radiographs are used on a large scale, such as in tuberculosis screening. The unblinded chest radiograph reporting in our study might have overestimated the proportion of true positive tuberculosis cases. However, even when *M tuberculosis* culture alone (compared with a composite reference including chest radiograph) was used as the reference standard for Xpert MTB/RIF testing, the positive predictive value of Xpert MTB/RIF was 64·2%, and the estimated test specificity was 99·6%. Both these positive predictive value and specificity values are still substantially higher than those estimated in previous studies,[4,5] and suggest that Xpert MTB/RIF can be a valuable primary screening tool in the context of community-based active case finding for tuberculosis.

We declare no competing interests.

Jennifer Ho, Guy B Marks
jennifer.ho@sydney.edu.au

Woolcock Institute of Medical Research and Centre for Research Excellence in Tuberculosis (TB-CRE), University of Sydney, Sydney, NSW 2037, Australia; and South Western Sydney Clinical School, University of New South Wales, Sydney, NSW, Australia

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Tuberculosis burden in India's private sector

Nimalan Arinaminpathy and colleagues[1] attempted to provide a mathematical approach to estimate the burden of tuberculosis cases in India from drug sales data as an alternative to WHO's conventional approach. The authors already list several limitations of their approach in estimating the disease burden. On the basis of several years of first-hand operational research experience on tuberculosis in India, I wish to provide insights into further limitations of this approach.

The first paragraph of the Article gives the impression that the entire problem of tuberculosis in the country is due to the unregulated private health-care sector. Although the private sector is a problem, as evinced in earlier studies,[2,3] the fact that this sector manages more than 50% of tuberculosis cases cannot be completely ignored, especially in view of the inability of India's public sector to cater to the country's huge population. Several other factors influence tuberculosis diagnosis and treatment. These include patient behaviour—eg, shopping around, and treatment interruption resulting from either early or no symptomatic relief, side-effects of medicines, social stigma, financial factors, or migration. While making the estimates, the authors assumed that the average duration of tuberculosis treatment in the private sector would range from 2 to 6 months. Considering the extent of medical pluralism in India, this assumption could be strongly biased. Many patients continue tuberculosis treatment when they are sick and when they can afford to, and might discontinue treatment as a result of the reasons mentioned above. Treatment can range from a few days to months or even years. Moreover, it is usual practice for patients to obtain treatment from several places; however, there is no

documented evidence of the extent of such practices. In many areas, patients can get antituberculosis drugs without prescription. The regulatory enforcement for pharmacists remains weak, and many are unaware of rules. In the absence of an electronic database, it is hard to know the exact number of patients on treatment, and what quantities of different antituberculosis medicines they consume and from which places, especially in a country like India with the complex scenarios described here.

India's Government took a bold step of launching mandatory tuberculosis notification for all sector providers; however, enforcement of this policy is weak.[4] In my opinion, investing in a well designed electronic recording and reporting system that includes patient data, laboratory records, and drug sale data would be worthwhile, because it will help not only to assess the exact burden of tuberculosis in the country with a regulatory control, but also to plan resources for the future.

I declare no competing interests.

Sachin Atre
atresachin2000@yahoo.com

Johns Hopkins University, Centre for Clinical Global Health Education (CCGHE), Baltimore, MD, USA; and Department of Pulmonary Medicine, Dr DY Patil Medical College, Hospital and Research Centre, Pimpri, Pune 411018, India

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Authors' reply

We thank Sachin Atre for his interesting and thought-provoking comments on our Article.[1] We affirm that the findings of our study are not an alternative to the rigorous disease burden estimation published by WHO, but rather an alert about the potential tuberculosis burden being managed by India's private health-care sector. A crucial objective of our study was to highlight the difficulty in reconciling earlier estimates of the tuberculosis burden in India with the sheer amount of tuberculosis treatment that is taking place in the private sector. In fact, newly published interim WHO estimates are now substantially higher, reflecting in large part an increased appreciation of tuberculosis under-notification from the private sector.[2] We agree that there is tremendous uncertainty and probable heterogeneity in the duration of private antituberculosis treatment. In the absence of clarifying evidence, we can only present the plausible implications of the volume of antituberculosis drugs sold. We hope, however, that our findings show that there is an urgent need to strengthen disease surveillance in the private health sector and to undertake representative tuberculosis prevalence surveys in India.

We declare no competing interests.

**Nimalan Arinaminpathy, Puneet Dewan*
nim.pathy@imperial.ac.uk

MRC Centre for Outbreak Analysis and Modelling, School of Public Health, Imperial College London, London W21PG, UK (NA); and Bill & Melinda Gates Foundation, New Delhi, India (PD)

1 Arinaminpathy N, Batra D, Khaparde S, et al. The number of privately treated tuberculosis cases in India: an estimation from drug sales data. *Lancet Infect Dis* 2016; **16:** 1255–60.
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Motavizumab, RSV, and subsequent wheezing

Authors' reply

Eric Simões[1] hypothesises that the difference in impact on subsequent wheezing between our blinded randomised, double-blind, placebo-controlled trial of monoclonal antibody respiratory syncytial virus (RSV) prevention in healthy, term, Native American infants[2] and that of four other RSV prevention studies in preterm infants[3-6] might relate to differences in underlying wheezing susceptibility in the study populations. We wish to address three points and offer a revised version of Simões' table (correcting errors and filling omissions), comparing data across these studies (appendix).

See Online for appendix

The first point is the importance of doing studies across diverse populations. The impact of RSV prevention on long-term wheeze is likely to be influenced by complex host–environmental interactions, including early childhood infection with other respiratory pathogens and genetic susceptibility to wheezing. No single population can be considered as the referent, since rates of wheezing and atopy vary widely across the world with lower prevalence in low-income settings, perhaps reflecting differences in microbial exposure (the so-called hygiene hypothesis[7,8]). Early life exposures in the Native American populations in our study are more similar to those in low-income settings, where the greatest burden of RSV disease occurs. The cited comparator studies were all from high-income settings. Furthermore, at least two of the comparator studies have an extremely high proportion of parental atopy,[4,5] indicating a highly selected population and reducing the applicability of the wheezing prevention results to general populations.

Second, the wheezing burden in our Native American study population was substantial. Medically attended recurrent wheeze, observed in 3% of placebo recipients in our study, is only one measure of wheezing burden and is highly dependent on care seeking. Another outcome, serious early childhood wheeze between 1–3 years of age (appendix), was reported in 14% of placebo and 15% of motavizumab recipients in our study.[2]

Third, confounding occurs across studies. Simões compares wheezing rates in the control group across studies, infering that differences in

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SHORT COMMUNICATION

Ultrastructural Investigations in an Autosomal Recessively Inherited Case of Dyschromatosis Universalis Hereditaria

Aayush Gupta[1], Yugal Kishor Sharma[1], Kedar Nath Dash[1], Sampurna Verma[1], Vivek T. Natarajan[2] and Archana Singh[2]
[1]Department of Dermatology, Leprology and Venereology, Dr. D.Y. Patil Medical College, Pimpri, Pune, and [2]Systems Biology Group, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India. E-mail: archana@igib.in, aayushggupta@gmail.com
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The dyschromatoses are a group of rare genodermatoses characterised by the presence of asymptomatic mottled hyperpigmented macules admixed with variably sized hypopigmented macules (1). Dyschromatoses are divided into dyschromatosis symmetrica hereditaria (DSH) and dyschromatosis universalis hereditaria (DUH). DUH is clinically diagnosed on the basis of widely distributed small hypo- and hyperpigmented lesions with appearance in infancy or early childhood and progression until stagnation before adolescence (2). This division got highlighted recently by the detection of specific mutations in *ADAR1/DSRAD* and *ABCB6* genes in DSH and DUH, respectively (1, 3). First reported from Japan, DUH has subsequently been reported from several regions of the world as a generalised leucomelanoderma relatively sparing the face, palms and soles. It is usually transmitted in an autosomal dominant (AD) pattern with variable penetrance, with very few autosomal recessive (AR) and even sporadic cases reported (4, 5). In this study, we present ultrastructural findings in a case of DUH, with probable AR transmission.

CASE REPORT

A 23-year-old unmarried male, product of consanguineous marriage, presented to us with mottled dyspigmentary patches which appeared insidiously since the age of 15 years, initially over the trunk and slowly progressed over a period of 6 years onto the back, abdomen and proximal extremities sparing the palms and soles. He denied history of altered sensations, chronic drug intake, photosensitivity or any contact with chemicals. All, except the eldest of his siblings, were similarly affected. Neither their parents, nor any of the 9 children of his 4 siblings were affected. Pictures of 3 out of his 4 affected siblings are shown in Fig. S1[1]. Dermatological examination revealed innumerable irregular 1–10 mm sized hypopigmented macules interspersed with numerous minute hyperpigmented ones, over trunk, back and proximal extremities (Fig. 1). Hair, nail, mucosae, general physical and systemic examination revealed no abnormality. On the basis of these features, our case was diagnosed to have DUH. The family was counselled and the hypo- and hyperpigmented lesions of the proband were subjected to ultrastructural and molecular analysis.

Transmission electron microscopy of ultrathin sections of the skin biopsies revealed comparable number of melanocytes in the 2 skin lesions, though the number of melanosomes per melanocytes were significantly reduced in hypopigmented epidermis as compared to hyperpigmented lesion (Fig. 2). Ultrastructure of melanocytes from ethnicity- and skin colour-matched normal skin is shown in Fig. S2[1].

Melanocytes and keratinocytes exist in the skin as a functional 'epidermal melanin unit'. We therefore examined the distribu-

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2030

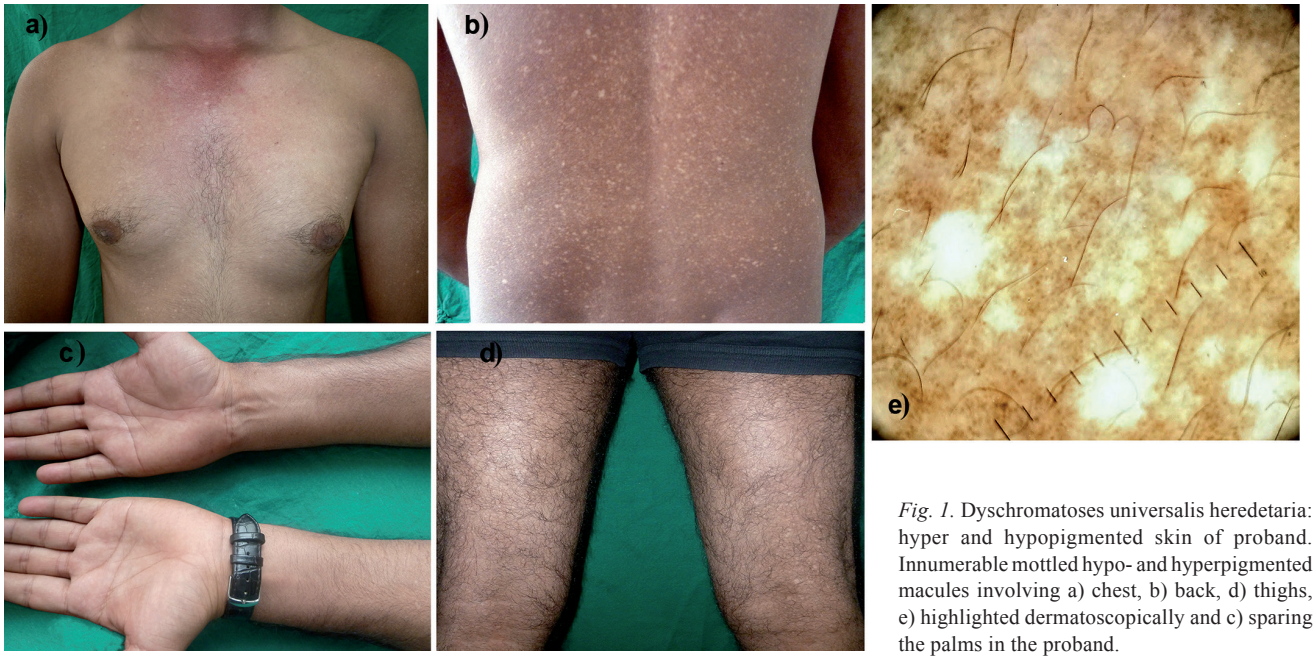

Fig. 1. Dyschromatoses universalis heredetaria: hyper and hypopigmented skin of proband. Innumerable mottled hypo- and hyperpigmented macules involving a) chest, b) back, d) thighs, e) highlighted dermatoscopically and c) sparing the palms in the proband.

tion of melanosomes in the basal and suprabasal keratinocytes. The keratinocytes in hyperpigmented lesion presented with a relatively higher number of melanosomes (Fig. S3 a–e, vertical panel 3 and 4[1]) compared to that of hypopigmented skin (Fig. S3 a–e, vertical panel 1 and 2[1]). The increase in melanosome numbers in hyperpigmented epidermis was more than 2-fold compared to hypopigmented lesions (Fig. S3f[1]). A large proportion of melanosomes were distributed in clusters and only a few of the single melanosomes were present in keratinocytes in both the lesions. Further analysis of melanosomes based on their distribution pattern in keratinocytes revealed significantly higher number of single as well as clusters of 2–4 melanosomes in hyperpigmented lesion compared to hypopigmented skin (Fig. S3g[1]). We did not observe significant difference in the sizes of the melanosomes between the keratinocytes of hypo- and hyperpigmented lesions (Fig. S4a[1]). Taken together, it suggests that in this form of DUH, melanocyte numbers are not affected. However, the melanosome synthesis and maturation are affected, accounting for lesser number of melanosomes in both melanocytes and keratinocytes of the hypopigmented lesions. Careful examination of the dermis showed presence of numerous melanophages in the hyperpigmented lesion (Fig. S4b–d[1]), which was rarely observed in the hypopigmented lesional dermis.

Clear differences in the melanosome numbers in the 2 types of skin lesions prompted us to investigate the regulation of melanogenesis pathway in DUH. We compared the transcript levels of 3 key melanogenic enzymes – DCT (dopachrome tautomerase), TRP1 (tyrosinase related protein 1) and Tyr (tyrosinase) – in the epidermis of the biopsies. Total epidermal RNA was isolated and converted to cDNA and quantitative real-time PCR analysis was carried out to examine the expression of DCT, TRP1 and TYR transcripts using TaqMan probes. Details of real-time PCR and ultrastructural studies are described in Appendix S1[1]. Interestingly, both DCT and TYR showed more than two-fold upregulation in the hyperpigmented lesion as compared to hypopigmented lesion, though no significant difference was observed in expression levels of TRP1 between the 2 skin lesions (Fig. S3h[1]).

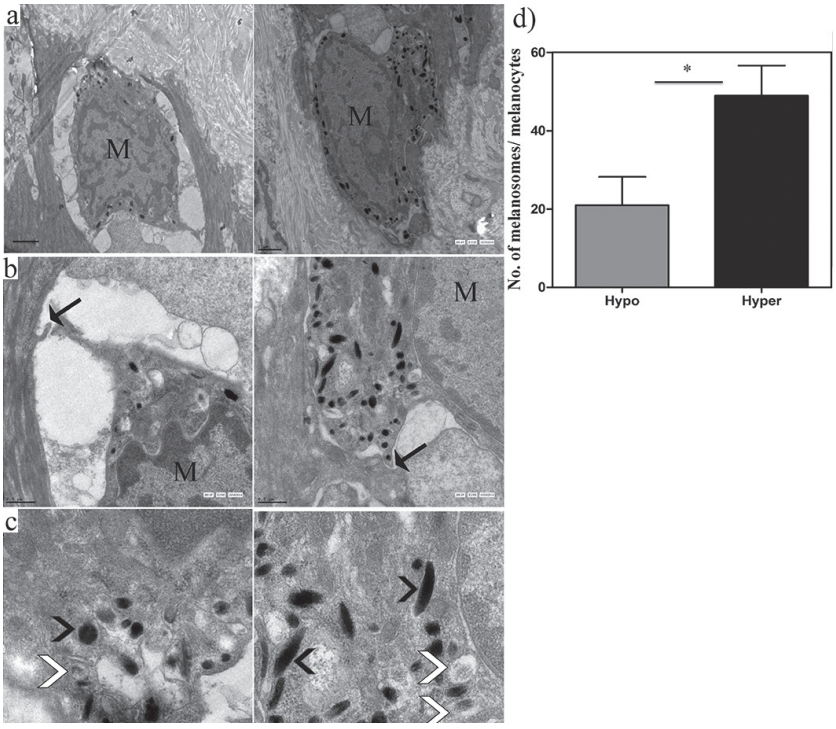

Fig. 2. Representative micrographs of melanocytes (M, at the dermal–epidermal junction) containing few melanosomes in the hypopigmented lesion (left panel) and relatively higher number of melanosomes in hyperpigmented lesion (right panel). Micrographs in a) show melanocytes at lower magnifications (scale bar 1 μm). Arrows in b) show absence of desmosomal connections in cell-cell junction between melanocytes and neighbouring keratinocytes (scale bar 0.5 μm). Magnified images in c) reveal presence of early (white arrowheads) and late stage melanosomes (black arrowheads) in both hypo- and hyperpigmented lesions (scale bar 0.2 μm). d) Melanocytes in hyperpigmented epidermis show significantly higher number of melanosomes ($n = 6$ melanocytes counted mean with SEM). *p-value < 0.05.

DISCUSSION

Although most commonly reported inheritance of DUH has been AD (1, 5–8), our patients' pedigree is highly suggestive of an AR transmission (consanguineous marriage of unaffected parents, etc.). However, in absence of detailed genetic analyses, the probability of low penetrant AD pattern of inheritance cannot be ruled out.

The hyperpigmented lesions in DUH reveal increased melanin in the basal layer and, rarely, melanin incontinence. The hypopigmented lesions show decreased melanin deposition in the basal layer (9). Electron microscopically, a number of studies of hypopigmented as well as the hyperpigmented skin (1, 2) have demonstrated morphologically normal melanocytes containing melanosomes of all stages, normal keratinocyte to melanocyte ratio and tyrosinase activity as indicated by a positive DOPA reaction. However, the number of melanosomes is higher in both melanocytes and keratinocytes in hyperpigmented lesions and this feature has been observed in other studies as well (1, 10). We speculate that the presence of heavily pigmented cells in dermis could add to the perception of skin colour of the hyperpigmented lesions. Interestingly, the number of melanocytes in hypopigmented lesions in leprosy are also known to be comparable to that of the uninvolved skin (11). In future studies, mechanisms that alter melanocyte functions in these 2 contrasting disorders (DUH vs leprosy) would provide an opportunity to understand regulatory circuits involved in skin pigmentation.

The *ABCB6* gene, located at 2q36, is the cause of AD transmitted form of DUH (2, 12). This gene belongs to a family of transporters that play a critical role in cellular transition metal homeostasis (13). Copper, a co-factor for tyrosinase in vertebrates, is a candidate ion that could utilise this transporter. In view of more than two-fold increase observed in TYR and DCT genes in hyperpigmented epidermis viz-a-viz hypopigmented one, it would

be interesting to investigate how mutations in copper transporter genes may result in transcriptional changes in tyrosinase and DCT.

Our cases have considerably later onset as well as stabilisation of the disease over time and an AR inheritance pattern. The latter has to the best of our knowledge been hitherto reported in 9 members of 2 families only, none of which were subjected to ultrastructural analysis. The identification of genetic loci for the AR form of DUH may soon lead to unravelling of its pathogenetic mechanisms and, if not identical to ABCB6, may shed further light on this extremely rare and genetic heterogenous condition.

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CASE REPORT

Case Report: Whole exome sequencing helps in accurate molecular diagnosis in siblings with a rare co-occurrence of paternally inherited 22q12 duplication and autosomal recessive non-syndromic ichthyosis. [version 1; peer review: 2 approved]

Aayush Gupta[1], Yugal Sharma[1], Kirti Deo[1], Shamsudheen Vellarikkal[2,4], Rijith Jayarajan[2], Vishal Dixit[2], Ankit Verma[2], Vinod Scaria[3,4], Sridhar Sivasubbu[2,4]

[1]Department of Dermatology, Dr. D.Y. Patil Medical College, Pune, India
[2]Genomics and Molecular Medicine, CSIR Institute of Genomics and Integrative Biology, Delhi, India
[3]GN Ramachandran Knowledge Center for Genome Informatics, CSIR Institute of Genomics and Integrative Biology, Delhi, India
[4]Academy of Scientific and Innovative Research, Center for Genome Informatics, CSIR Institute of Genomics and Integrative Biology, Delhi, India

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Abstract

Lamellar ichthyosis (LI), considered an autosomal recessive monogenic genodermatosis, has an incidence of approximately 1 in 250,000. Usually associated with mutations in the transglutaminase gene (*TGM1*), mutations in six other genes have, less frequently, been shown to be causative. Two siblings, born in a collodion membrane, presented with fish like scales all over the body. Karyotyping revealed duplication of the chromosome arm on 22q12+ in the father and two siblings. Whole exome sequencing revealed a homozygous p.Gly218Ser variation in *TGM1*; a variation reported earlier in an isolated Finnish population in association with autosomal recessive non-syndromic ichthyosis. This concurrence of a potentially benign 22q12+ duplication and LI, both rare individually, is reported here likely for the first time.

Keywords

Whole Exome Sequencing , Lamellar ichthyosis , chr22q12 , genodermatosis , TGM1

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 1 published 31 Jul 2015	✓ report	✓ report

1 **Mohamed Badawy Hassan Tawfik Abdel-Naser**, Ain Shams University , Cairo, Egypt

2 **Regina Fölster-Holst**, University of Kiel, Kiel, Germany

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding authors: Vinod Scaria (vinods@igib.in), Sridhar Sivasubbu (sridhar@igib.in)

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Introduction

Autosomal recessive congenital ichthyosis (ARCI), a heterogeneous disorder of cornification of skin, encompasses three clinical subtypes: lamellar ichthyosis (LI; OMIM 242300); congenital ichthyosiform erythroderma (CIE; OMIM 242100); and harlequin ichthyosis (HI; OMIM 242500)[1]. LI has an incidence of approximately 1 in 250,000. Over 115 mutations in *TGM1* and less frequent ones in six other genes (*NIPAL4z, ALOX12B, CGI-58, FLJ39501, ICHYN* and *ABCA12)* have been associated with the LI/CIE phenotypic spectrum worldwide[2,3]. Overlapping phenotypes and the non-specificity of the conventional histopathology, makes clinical diagnosis challenging in many cases and inaccurate in some[4]. Whole exome sequencing has become a useful diagnostic aid for genetic disorders including multigene dermatoses such as epidermolysis bullosa[5,6] and acrokeratosis verruciformis[7].

Case report

Two, 8 and 6-year-old, siblings born out of a non-consanguineous marriage (Figure 1a) presented with hyperpigmented fish-like scales all over the body including face and flexures ectropion, loss of lateral half of eyebrows and alopecia along the scalp margins (Figure 1b–1e). Both siblings were heat intolerant, photosensitive and hypohidrotic. Born uneventfully vaginally they were encased in a collodion membrane which was shed within a week of birth. There was no family history of any dermatoses. Slit lamp examination revealed bilateral keratitis. Karyotyping of their parents and the siblings performed previously revealed duplication of the chromosome arm on 22q12+ in the father and two siblings. The patients were put on daily oral (5 mg) isotretinoin after analysing their lipid profile.

Considering the diagnosis of LI, whole exome sequencing was attempted. Genomic DNA (gDNA) was isolated from 5 ml of blood[8] of each of the affected children after obtaining written informed consent conforming to the institutional ethical committee approvals (Dr. D.Y. Patil Vidyapeeth, Pune. Approval number DYPV/EC/178/14). The whole exome capture and library preparation (Nextera expanded exome, Illumina Inc., USA) were carried out according to the manufacturer's instructions and followed by high throughput sequence generation on Hiseq 2500 with default 101 paired end single index sequence by synthesis chemistry (Illumina Inc., USA). The raw sequence reads were trimmed at a Phred score of 30 leaving over 44.9 and 33.45 million reads respectively for the two siblings. The variations were called against the hg19 version of the human genome using standard GATK-Picard pipeline with Burrows-Wheeler Alignment according to GATK best practice[9]. The variants from the genomes of both siblings were further analysed using ANNOVAR[10] for coding region and also screened using the NCBI-Clinvar database (http://www.ncbi.nlm.nih.gov/clinvar/). Analysis revealed a homozygous p.Gly218Ser variation in *TGM1* previously reported to be associated with autosomal recessive non-syndromic ichthyosis in an isolated Finnish population[11]. The variant mapped to the transglutaminase domain in the protein (Figure 1g) and was also predicted to be pathogenic by both SIFT (Sorts Intolerant From Tolerant)[15] and PolyPhen2[16] (Polymorphism Phenotyping v2). This variation was further validated in parents and both siblings by site-specific PCR using a forward primer CTTCTCCTGGGGTCAGGCA and reverse primer GAGAAGTCCCAGGCTCCATC (Sigma Aldrich). The PCR was done using *taq* polymerase (Invitrogen, USA, Cat. No. 10342053) according to the manufacturer supplied protocol with a Tm of 60.5°C. The PCR products were size selected and gel purified (2% agarose) using qiaquick gel extraction kit (QIAGEN, NL) and performed capillary sequencing (Applied Biosystems) performed using manufacturer instruction. Analysis revealed the variant was heterozygous in parents, while homozygous in both affected siblings (Figure 1f).

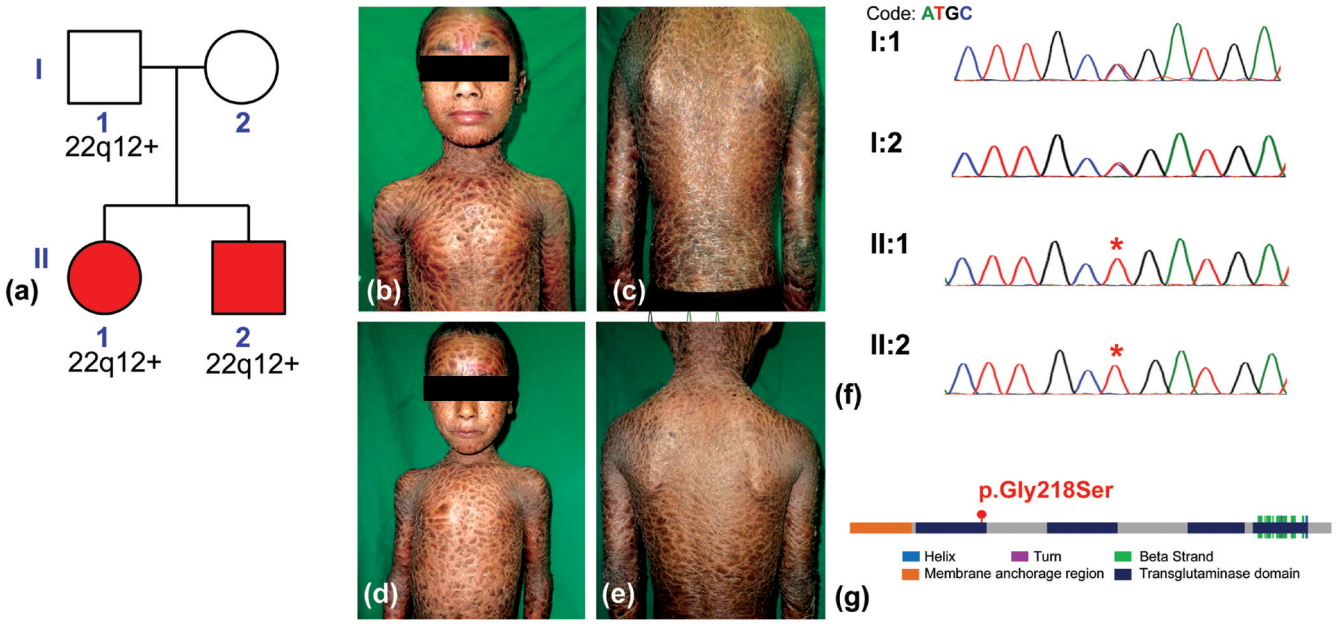

Figure 1. **a**) Pedigree of the family; (**b**), (**c**) and (**d**), (**e**) correspond to the ventral and dorsal views of siblings II:1, and II:2 respectively and shows hyperpigmented fish-like scales all over the body including face and flexures, ectropion, loss of lateral half of eyebrows and hair along scalp margins. Panel (**f**) shows the chromatogram from capillary sequencing for the parents and siblings, while panel (**g**) shows the domain organization of the protein and the location of the p.Gly218Ser variation with respect to the protein domains.

Follow up after two years of low dose isotretinoin, titrated intermittently, revealed complete subsidence of ectropion, eclabium and alopecia with residual fine scales.

Discussion

ARCI is a rare disorder with an estimated prevalence of 1 per 200,000 population in Europe and 1 per 200,000–300,000 population in the United States. Neonates with LI typically present with a collodion membrane which dries and peels away and is replaced by brown, plate-like scales over the entire body. Disease course ranges from very mild to severe, latter entailing ectropion, eclabium, scarring supraciliary and scalp alopecia, and palmoplantar hyperkeratosis[11].

DNA based molecular diagnosis is crucial in ichthyosis as it provides a firm basis for genetic counseling of affected individuals and families, and also permits prenatal diagnosis. In a cohort of 520 independent families with ARCI, mutations were identified by direct sequencing of the 6 ARCI genes identified to date in 78% of patients: 32% harbored mutations in *TGM1*, 16% in *NIPAL4*, 12% in *ALOX12B*, 8% in *CYP4F22*, 5% in *ABCA12*, and 5% in *ALOXE3*. Whole exome sequencing may fill in the diagnostic lacuna of at least 22% of the patients who failed in this study to exhibit mutations in any of the known ARCI genes, indicating the existence of additional loci, such as 2 loci on chromosome 12p11.2-q13[12]. The 22q12+ duplication is known to cause cat eye syndrome, which has a range of potential morbidities with the occurrence of characteristic triad of iris coloboma, aural tags and/or pits and anal atresia[14], though none of these features were present in the father or the children.

To the best of our knowledge, this is the first reported concurrence of a potentially benign 22q12+ duplication and LI, both of which are extremely rare individually. The mother of the siblings is now pregnant and the present finding will be used to help screen the foetus prenatally.

Consent

Written informed consent for publication of their clinical details and clinical images was obtained from the parent of the patients.

Data availability

The raw exome sequencing data are available at the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra), accession numbers SRX1096915 (II:1) and SRX1096920 (II:2).

Author contributions

AG, YKS and KD identified the patient, took the biopsies for histopathology and sent the blood for DNA analyses. They helped in writing the initial case report as well as editing and formatting the manuscript. SKV, RJ, VD and AV isolated the DNA, performed the quality checks, prepared the exome capture and sequencing library, performed the exome sequencing. SKV performed data quality checks on the reads, reference alignments, variant call and computational prioritisation of the variants, designed and performed the validation experiments. SS and VS conceptualised and oversaw the DNA isolation, quality checks, exome sequencing, exome sequence analysis and validation and contributed to writing the manuscript.

Competing interests

The authors declare that they have no competing interests.

Grant information

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I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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✔ **Regina Fölster-Holst**
Dermatologische Klinik, Universität Kiel, Kiel, Germany

Gupta *et al.* focused on two siblings with ARCI with confirmed TGM1 mutation. In addition they found 22q12 duplication in these siblings and in their father. The title and abstract are both appropriate for the article, and the abstract is a suitable summary. The diagnosis has been sufficiently described, however it is a little bit unclear whether the 22q12 duplication is associated with the ARCI. The authors mention the cat eye syndrome which is related to the duplication of the chromosome 22q12. What about the relevance of this finding? It should be worked out in more detail.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 10 August 2015

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✔ **Mohamed Badawy Hassan Tawfik Abdel-Naser**
Department of Dermatology, Andrology and STIs, Faculty of Medicine, Ain Shams University, Cairo, Egypt

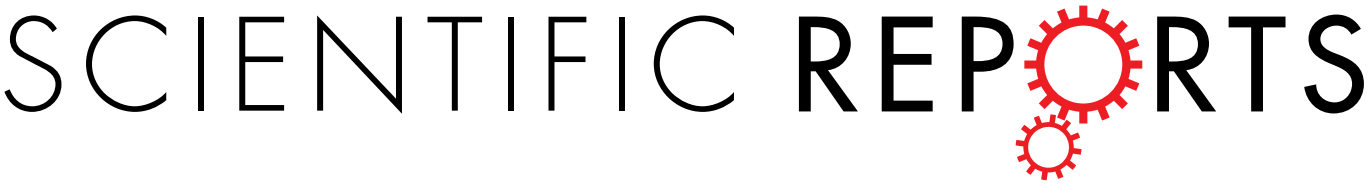
- The authors reported 2 siblings with lamellar ichthyosis (LI) and the family pedigree shows the autosomal pattern of inheritance. In addition, duplicate 22q12+ has been shown in the father and the 2 affected siblings. **It seems, however, that this chromosomal abnormality is unrelated to the LI** as the father is phenotypically normal and the 2 siblings do not show any of the

manifestations of this chromosomal abnormality. **The significance of this concurrence is not clear.**

- The authors may amend the manuscript by naming the site of the TGM1 mutation at the nucleotide level and the name of the gene transcript.
- It is not clear whether the authors examined the father and the affected siblings for manifestations of the duplicate 22q12+ (apart from the cat eye syndrome), such as learning difficulties, growth retardation, minor genital abnormalities of the boy etc.
- There are few typo errors. For instance, the "transglutaminase gene" is better given as "transglutaminase gene1" and "Ichyn" is "Ichthyin" etc.
- Ref.13 is not cited in the text and there seems to be a problem with enumeration of the references in the text.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.



OPEN

Mapping architectural and transcriptional alterations in non-lesional and lesional epidermis in vitiligo

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Archana Singh[1], Vishvabandhu Gotherwal[1,3], Päivi Junni[4], Vinaya Vijayan[5], Manisha Tiwari[1], Parul Ganju[2], Avinash Kumar[2], Pankaj Sharma[6], Tanveer Fatima[6], Aayush Gupta[7], Ananthaprasad Holla[8], Hemanta K. Kar[6], Sangeeta Khanna[1], Lipi Thukral[1], Garima Malik[1], Krishnamurthy Natarajan[9], Chetan J. Gadgil[5], Riitta Lahesmaa[4], Vivek T. Natarajan[1,3], Rajni Rani[2] & Rajesh S. Gokhale[1,2,5,10]

In vitiligo, chronic loss of melanocytes and consequent absence of melanin from the epidermis presents a challenge for long-term tissue maintenance. The stable vitiligo patches are known to attain an irreversible depigmented state. However, the molecular and cellular processes resulting in this remodeled tissue homeostasis is unclear. To investigate the complex interplay of inductive signals and cell intrinsic factors that support the new acquired state, we compared the matched lesional and non-lesional epidermis obtained from stable non-segmental vitiligo subjects. Hierarchical clustering of genome-wide expression of transcripts surprisingly segregated lesional and non-lesional samples in two distinct clades, despite the apparent heterogeneity in the lesions of different vitiligo subjects. Pathway enrichment showed the expected downregulation of melanogenic pathway and a significant downregulation of cornification and keratinocyte differentiation processes. These perturbations could indeed be recapitulated in the lesional epidermal tissue, including blunting of rete-ridges, thickening of stratum corneum and increase in the size of corneocytes. In addition, we identify marked increase in the putrescine levels due to the elevated expression of spermine/spermidine acetyl transferase. Our study provides insights into the intrinsic self-renewing ability of damaged lesional tissue to restore epidermal functionality in vitiligo.

Vitiligo is a multifactorial complex skin disorder characterized by patchy depigmented epidermal skin. The lesional skin from vitiligo subjects is characterized by the conspicuous absence of the pigment producing cells, melanocytes. Within these cells, melanin pigment is synthesized and deposited in specialized organelles called melanosomes, which are then transferred to the neighboring keratinocytes, constituting the minimal functional epidermal melanin unit in the human skin[1]. The presence of these pigment granules prevents ultraviolet radiation-mediated DNA damage and protects the skin from cutaneous malignancies[2,3]. Due to the selective loss of melanocytes during vitiligo, keratinocytes in these regions lack pigment and are vulnerable to environmental insults. Surprisingly contrary to this logical reasoning, vitiligo condition is increasingly considered to provide protection from melanoma and non-melanoma skin cancers[4,5].

[1]CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi, India. [2]National Institute of Immunology, ArunaAsaf Ali Marg, New Delhi, India. [3]Academy of Scientific and Innovative Research, New Delhi, India. [4]Turku Centre for Biotechnology, University of Turku and ÅboAkademi University, Turku, Finland. [5]CSIR-National Chemical Laboratory, Chemical Engineering Division, Pune, India. [6]Department of Dermatology, Post Graduate Institute for Medical Education and Research (PGIMER), Dr. Ram Manohar Lohia Hospital, New Delhi, India. [7]Department of Dermatology, Dr. D.Y. Patil Medical College, Pimpri, Pune, India. [8]MelanoSite, Center for Advanced Vitiligo Treatment and Collaborative Pigment Cell Research, New Delhi, India. [9]School of Life Sciences, Jawaharlal Nehru University, New Delhi, India. [10]Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, India. Correspondence and requests for materials should be addressed to V.T.N. (email: tn.vivek@igib.res.in) or R.R. (email: rajni@nii.ac.in) or R.S.G. (email: rsg@nii.ac.in)

Due to the apparent disappearance of melanocytes, the primary clinical management for vitiligo has focused on repopulating melanocyte cells as well as suppressing the immune response[6]. However, some of the recent studies propose a possible role of altered keratinocytes resulting in loss of homeostasis that may be contributing to the aetiopathogenesis of this disorder[7]. In addition to the conspicuous loss of melanocytes, reportedly the lesional skin also exhibits epidermal vacuolization, thickening of basement membrane, presence of T cell infiltrates and degenerating keratinocytes[8,9]. Interestingly, ultrastructural studies on the non-lesional skin of vitiligo subjects also report altered keratinocytes, including presence of extracellular granular material and vacuolar changes in basal keratinocytes, epidermal and dermal lymphocyte infiltrates and melanophages in the upper dermis[10,11]. Further, vitiligo patients are reported to exhibit systemic as well as localized oxidative stress[12,13], and millimolar levels of H_2O_2 are reported to be present in their epidermis[14]. This has prompted researchers to propose that even the apparently normal skin of patient may be contributing towards the disease pathogenesis and vitiligo therefore is treated as a systemic disorder[15]. Paradoxically, several studies report successful repigmentation of lesional skin using autologous transplantations of epidermal cells derived from gluteal non-lesional sites[16,17], suggesting that the non-lesional skin is 'competent' and is capable of restoring pigmentation of the transplanted lesional sites.

Previous genome-wide expression studies on intact biopsies from lesional and non-lesional skin compared to healthy controls indicated a functional role of natural killer cells and autoimmunity in melanocyte loss[18]. In another study, comparison of transcriptome between melanocyte cultures from the asymptomatic non-lesional vitiligo skin with healthy donors showed differential expression of genes involved in the regulation of melanosome maturation and antigen presentation, providing valuable insights into disease pathomechanisms[19]. Independent studies have suggested that lesional keratinocytes are prone to apoptosis and are unable to secrete melanocyte-sustaining factors such as the stem cell factor (SCF)[20,21]. While each of these studies highlights one component of disease manifestation, together these observations suggest the lesional vitiligo skin is likely to have several perturbations.

To comprehend interplay of inductive signals and cell intrinsic factors involved in vitiligo, we compared the matched lesional and non-lesional epidermis. Incidentally, skin not only shows extensive heterogeneity across individuals in a given population, but differences are also reported across various anatomical sites within an individual[22]. We therefore designed our study to first perform the pairwise comparison of the lesional and non-lesional skin of the same individual and then compare the data across various subjects. The lesional skin samples (L) were procured from different anatomical sites of vitiligo patients, while the non-lesional (NL) skin biopsies were obtained from hip/upper thigh. In this study, we have performed comparison between the lesional (L) and gluteal non-lesional (NL) epidermis at architectural, cellular and molecular levels to identify vitiligo-specific signatures. Pair-wise analysis reduced the heterogeneity, which enabled us to comprehensively delineate vitiligo-specific perturbations in the skin, as a consequence of the melanocyte loss.

Results

Transcriptome profiling reveals pervasive changes in lesional epidermis in vitiligo. To understand molecular changes in vitiligo, we performed genome-wide transcriptome analysis from matched lesional and non-lesional epidermis derived from punch biopsies obtained from 15 vitiligo subjects (details in Table 1). Total RNA was isolated and microarray hybridization carried out using human whole genome Illumina WG-6 microarray platform. Hierarchical clustering of the average normalized expression values for all the genes, to our surprise, clustered the lesional and non-lesional samples in discrete clades. This suggested a concordant enrichment of vitiligo-specific transcriptome signatures in the lesional skin (Fig. 1a). Paired t-test analysis ($p < 1 \times 10^{-5}$) yielded a total of 1705 genes including 786 upregulated and 919 downregulated genes that showed consistent differential regulation in all 15 subjects indicating a concerted footprint of the transcriptome in vitiligo epidermis.

Gene Set Enrichment Analysis using DAVID suite identified several distinct biological processes that were altered among the upregulated and downregulated genes (Fig. 1b,c). The biological processes that were upregulated included ribosome biogenesis and RNA processing, while cornification and melanin biosynthesis were amongst the major downregulated processes (Fig. 1b,c and Supplementary Table 1). Whereas the apparent down regulation of melanin biosynthetic pathway is consistent with the loss of melanocyte population from the lesional epidermis, alterations in the keratinocyte-specific functions such as cornification was unanticipated. Transcriptomic analyses thus suggest biological processes that were previously not connected to vitiligo.

Expression profile of genes involved in keratinocyte differentiation and cornification is altered in lesional epidermis. Microarray analyses of epidermal tissue suggested dysregulation of keratinocyte-specific processes in the lesional vitiligo skin. We therefore examined the keratinocyte-specific pathways in detail to understand implications of these gene expression changes. Keratinocytes undergo programmed differentiation to form terminally differentiated corneocytes, a process that is intricately linked to epidermal differentiation and cell-cell adhesion. Keratinocytes also express different adhesion proteins in various layers of the stratified epidermis. Indeed, the expression of genes involved in keratinization and epidermal differentiation were significantly altered in the lesional skin (Fig. 1d). While stratifin (SFN), corneodesmosin (CDSN), envoplakin (EVPL), transglutaminase-1 (TGM-1) and periplakin (PPL) mRNAs were significantly downregulated in the lesional epidermis, the expression of filaggrin (FLG) and involucrin (IVL) were not significantly altered (Fig. 1d). Desmosome and adherens junction components such as junctional protein (JUP) and plakophillins PKP1 and PKP3 were downregulated while PKP2 was upregulated in lesional samples. Desmoplakin (DSP), a component of desmosome complex, did not show a clear-cut pattern of regulation. The differential transcriptional regulation of adhesion components in the lesional epidermis suggested a possible aberration in the adhesion properties of keratinocytes in vitiligo (Fig. 1d). Two-way clustering of the genes involved in maintaining differentiation and adhesion is shown in Supplementary Fig. S1. Validation of some of the important genes involved in epidermal

S. No.	Patient ID	Age (years)	Sex	Type of Vitiligo	Age at onset (years)	Site of non-lesional punch	Site of lesional punch
Details of samples included in histology studies							
1.	VD107	59	M	Vulgaris	33	Gluteal	Lower leg
2.	VD108	24	M	Vulgaris	21	Gluteal	Wrist
3.	VD131	46	F	Vulgaris	45	Gluteal	Thigh
4.	VD135	23	F	Vulgaris	5	Gluteal	Upper back
5.	VD144	24	M	Vulgaris	18	Gluteal	Thigh
6.	VD155	16	M	Vulgaris	7	Gluteal	Lower leg
7.	DYP005	45	F	Vulgaris	44	Lateral thigh	Thigh
8.	RML25	40	F	Vulgaris	38	Gluteal	Face
9.	DYP003	27	F	Vulgaris	16	Lateral thigh	Thigh
10.	DYP004	23	M	Vulgaris	16	Lateral thigh	Upper back
11.	DYP007	32	F	Vulgaris	28	Lateral thigh	Lower leg
12.	VD128	18	M	Vulgaris	11	Gluteal	Thigh
13.	RML002	24	M	Vulgaris	23	Gluteal	Hand
14.	RML018	23	M	Vulgaris	22	Gluteal	Lower leg
15.	RML03	25	F	Vulgaris	24	Gluteal	Groin
Details of samples included in genome-wide transcriptome studies							
1.	VD39	28	F	Vulgaris	14	Gluteal	Near knee
2.	VD22	20	F	Vulgaris	9	Gluteal	Lower leg
3.	VD20	20	F	Vulgaris	7	Gluteal	Lower leg
4.	VD32	26	F	Vulgaris	14	Gluteal	Lower leg
5.	VD37	25	F	Vulgaris	11	Gluteal	Wrist
6.	VD40	20	M	Vulgaris	09	Gluteal	Lower leg
7.	VD49	10	F	Vulgaris	NA	Gluteal	Forearm
8.	VD23	13	F	Vulgaris	03	Gluteal	Forehead
9.	VD33	26	M	Vulgaris	NA	Gluteal	Lower leg
10.	VD24	45	F	Vulgaris	36	Gluteal	Neck
11.	VD38	16	F	Vulgaris	06	Gluteal	Forearm
12.	VD44	24	F	Vulgaris	12	Gluteal	Lower leg
13.	VD13	21	M	Vulgaris	18	Gluteal	Lower leg
14.	VD19	21	M	Vulgaris	NA	Gluteal	Forearm
15.	VD18	NA	F	Vulgaris	NA	Gluteal	Lower leg

Table 1. Details of samples included in histology & transcriptome studies.

differentiation including TGM1 and CDSN, by real time PCR confirmed consistent down regulation in most of the lesional samples (Supplementary Fig. S2). These data suggest substantial changes in the lesional keratinocytes.

Tissue architecture is perturbed in lesional vitiligo epidermis. To investigate whether the transcriptome changes in structural and adhesion genes could modulate process of differentiation in keratinocytes, we performed histopathological studies of lesional and non-lesional skin. We obtained punch biopsies from matched lesional and non-lesional skin from 15 patients with stable non-segmental vitiligo. The biopsy samples were fixed and cryo-sectioned for staining with hematoxylin-eosin (H&E) or with anti-S100 antibody. As anticipated, S100-positive cells were found only in the non-lesional skin but not in the lesional skin indicating specific absence of melanocytes (Fig. 2a). Transmission electron microscopy (TEM) also revealed presence of melanosomes in the non-lesional epidermal keratinocytes but not in the lesional epidermis (Fig. 2a). The H&E staining pattern revealed that the lesional samples have a thickened stratum corneum and also the complete epidermis region (Fig. 2a). The data from these stained cryo-sections ($n = 15$ matched pairs) were quantified using imageJ software suite[23] (Fig. 2b, Supplementary Fig. S3). We observed significant differences in the thickness of the stratified epithelia, from stratum basale to the top of stratum corneum in non-lesional and lesional skin samples (paired t-test, $p < 0.0015$). This thickening could be attributed to a more than two-fold increase (paired t test, $p < 0.0015$) in the thickness of stratum corneum in the lesional epidermis. Thickness of other viable cellular layers was comparable between the non-lesional and lesional samples (Fig. 2b). This is consistent with earlier studies that reported alterations in the thickness of stratum corneum and the whole epidermis in the lesional skin of vitiligo[24,25].

Careful examination of the H&E stained sections showed few additional architectural changes including majorly the blunting of the epidermis (Fig. 2a). We measured the epidermal thickness that extends from the epidermis, called rete ridges by calculating the ratio of the primary ridge (longest projection extending from stratum granulosum to stratum basale) to the secondary ridge (smallest projection extending from stratum granulosum to stratum basale). Interestingly, while the non-lesional epidermis showed secondary to primary ridge ratio far less than 1, this ratio in the lesional epidermis was closer to 1 confirming blunting of rete ridges in vitiligo lesions

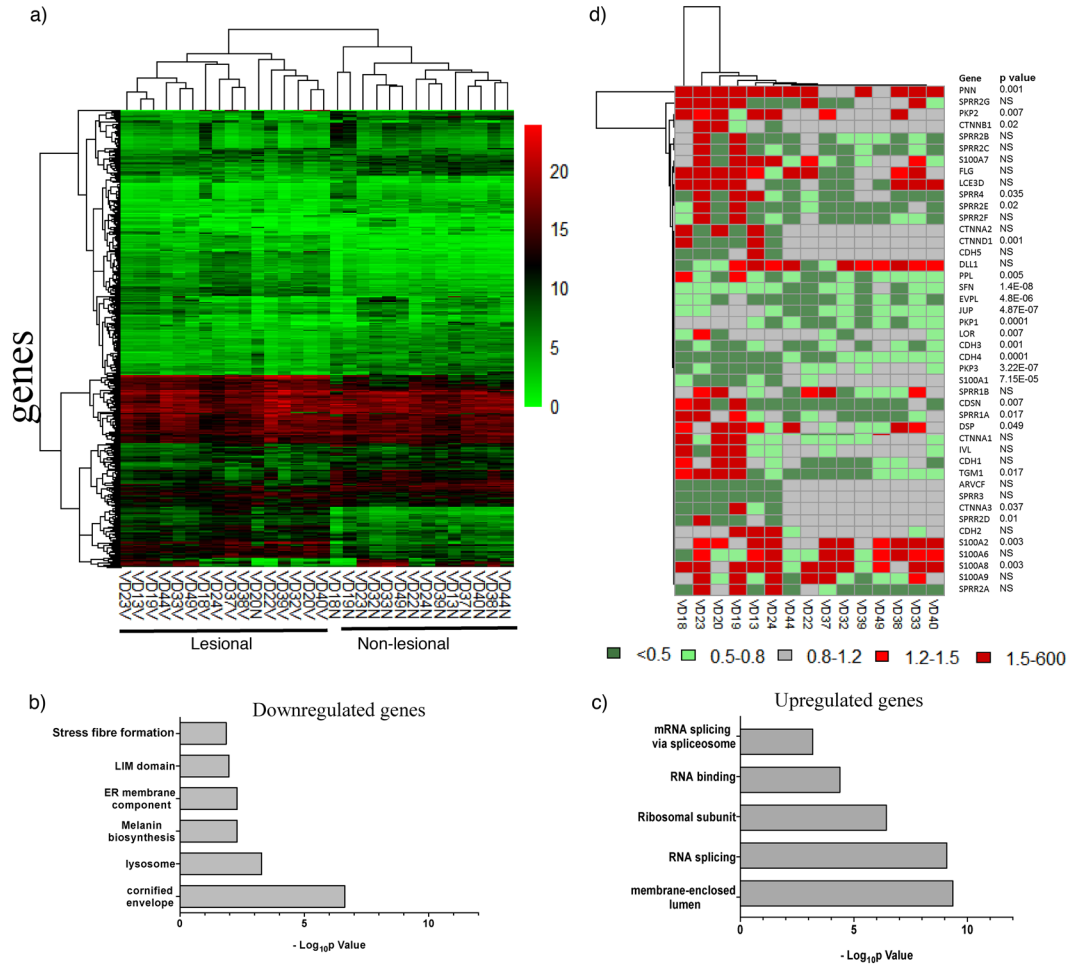


Figure 1. Dominant signature of keratinocyte pathology in the lesional vitiligo skin. (**a**) Clustering of samples based on average normalized expression values from microarray segregates NL and L skin (n = 15). Enrichment analysis of top 1% of (**b**) down regulated and (**c**) up regulated genes was performed by DAVID bioinformatics resource. Negative log transformed p-value of the enrichment of the pathways was calculated and represented as a bar graph. (**d**) Transcriptional regulation of genes involved in maintaining epidermal integrity and cell-cell adhesion in keratinocytes. Color scheme-Red- upregulated; green- downregulated; grey- not regulated in lesional as compared to matched non-lesional skin.

(Fig. 2b, paired t-test $p < 0.0009$). These changes seemed to be rather specific, since measurement of the cell packing density (cell numbers per unit area) did not reveal a significant difference between the non-lesional and lesional epidermis (Fig. 2b).

To rule out the possibility of anatomical differences contributing to the epidermal thickness in vitiligo (Table 1), we obtained seven pairs of matched lesional and non-lesional skin biopsies from anatomical sites proximal (P) to the lesional skin (within 5 cm distance from the margin of the lesions) and carried out histopathological evaluation. Statistical analyses revealed that the thickness of the stratified epidermis and that of the stratum corneum, as well as the rete ridge blunting were significantly different in the lesional skin compared to its neighboring non-lesional (proximal) skin (Supplementary Figs S4 and S5), providing further evidence that the thickened epidermis is indeed linked to vitiligo manifestation.

The increased thickness of the stratum corneum could be possibly due to an increase either in the total number of cornified layers or the size of the corneocytes that constitute the stratum corneum layer, or both. To address this, skin sections were subjected to TEM analysis. While taking ultrathin sections, we ensured that the stratum corneum layers were preserved. By measuring the corneocyte thickness in three consecutive corneum layers just above the stratum granulosum layer, our studies revealed larger corneocytes in the lesional skin ($p < 0.03$, $n = 4$) as compared to the non-lesional stratum corneum (Fig. 2c,d). Together with the expression studies, this data strongly suggests that larger corneocytes probably compensates for the decreased expression of cornification components and thereby provide a thicker stratum corneum in vitiligo lesions.

Putrescine levels are elevated in the lesional vitiligo epidermis. To delineate possible mechanisms underlying the formation of thickened stratum corneum in lesional epidermis, we examined pathways that can

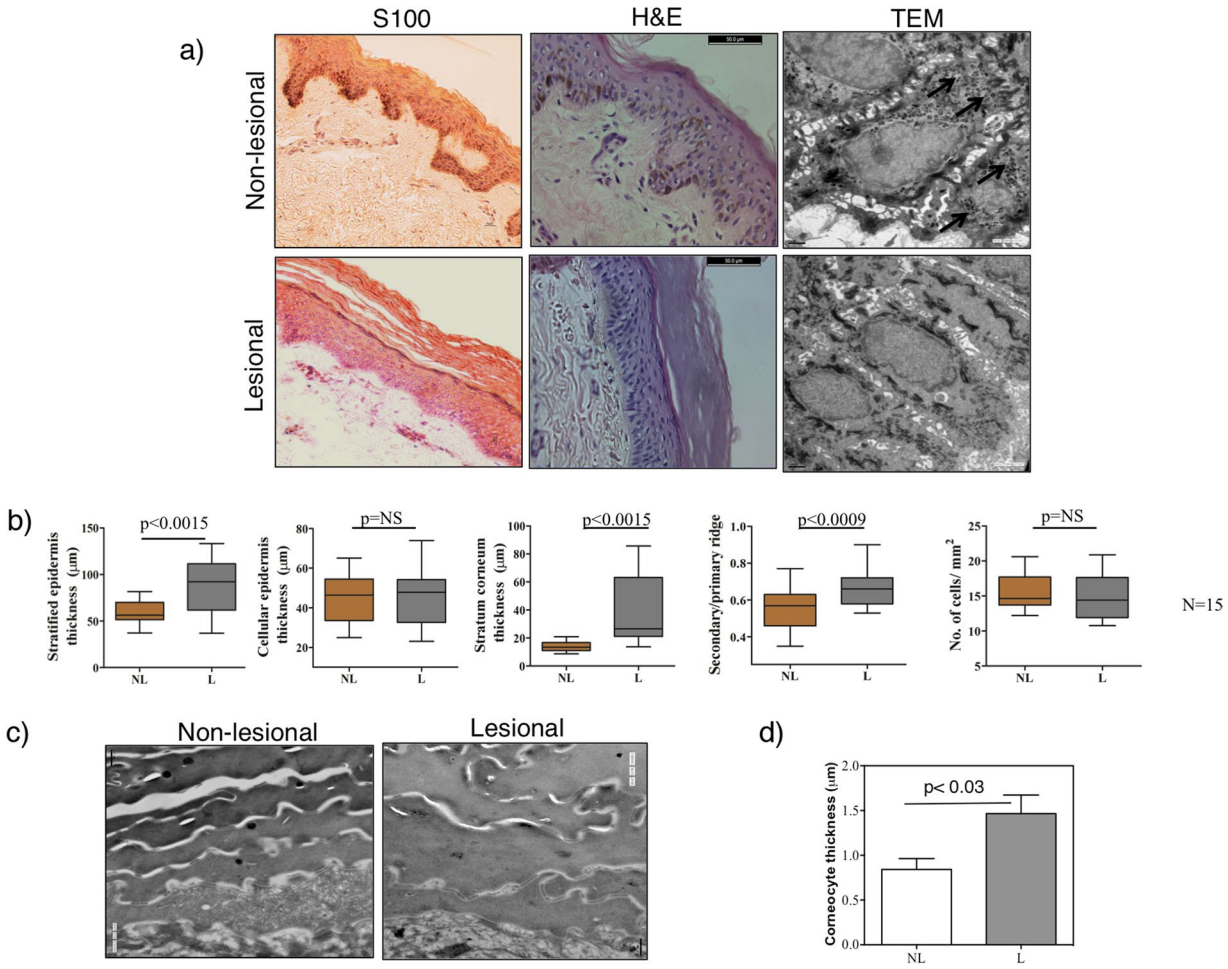
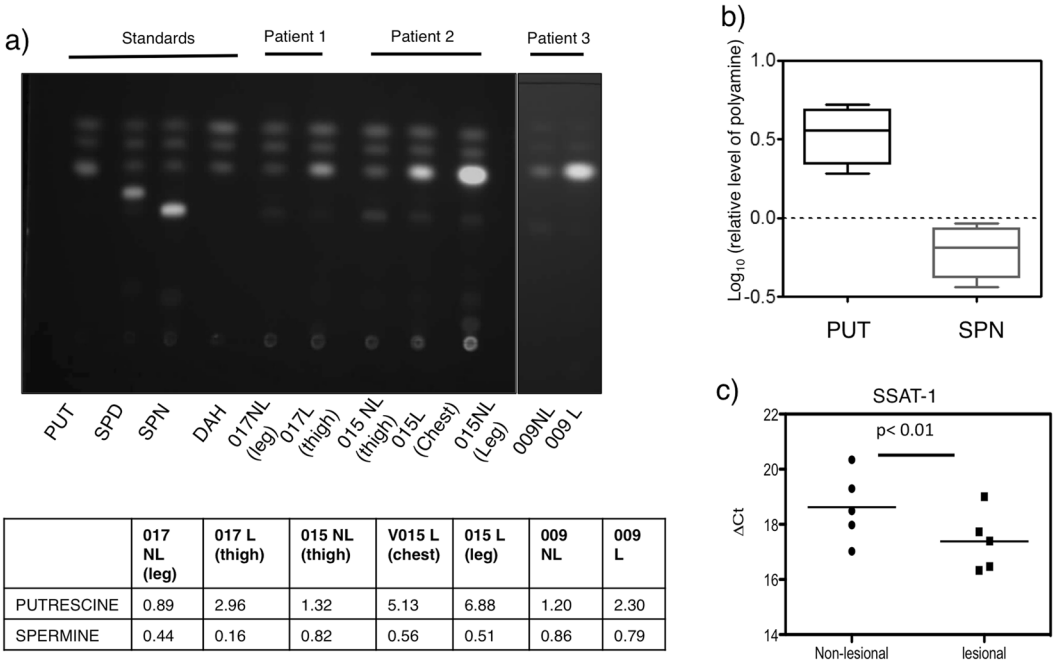


Figure 2. Architectural alterations in stable vitiligo lesions. (**a**) Immunohistochemical staining of melanocyte-specific S100 antigen, Hematoxylin and Eosin staining (H&E), and transmission electron micrographs (TEM) in paired non-lesional (NL) and lesional (L) skin sections. Arrows indicate melanosomes. Scale bar in S100 stained sections is 10 μm, 50 μm for H&E image and 1μm for TEM image. (**b**) Bar plots depicting architectural features quantitated in skin sections: thickness of stratified epithelia (from stratum basale to stratum corneum), cellular epidermis (from stratum basale to stratum granulosum) and thickness of stratum corneum in μm, ratio of length of secondary (μm) to primary ridge (μm) and the total number of cells per square mm in non-leisonal (NL) and lesional (L) samples (n = 15). The box plot represents the mean ± range of the data. Indicated p-values computed using a paired t-test involving n = 15 (NL vs L) pairs. (**c**) TEM images of stratum corneum (corneocytes) from non-lesional and lesional epidermis. Magnification is 1700X, scale bar is 0.5μm (**d**) Quantitation of thickness among three layers of corneocytes close to the viable epidermis across four pairs of matched NL and L skin sections, significance calculated using paired t test.

alter keratinocyte differentiation process. Intriguingly, transgenic mice overexpressing SSAT-1, which encodes spermine/spermidine acetyl transferase enzyme, was shown to have thicker stratum corneum with marked alterations in the keratinocyte differentiation process[26]. SSAT-1 catalyzes a key rate-limiting step in the degradation of spermidine (SPD) and spermine (SPN) to putrescine (PUT) and thus modulates polyamine levels. Polyamines are abundant metabolites present in all cell types and are known to participate in variety of cellular functions[27,28]. Interestingly, an early study suggested enhanced polyamine-mediated crosslinking in the psoriatic skin[29].

We therefore examined whether lesional vitiligo skin possess an abnormal polyamine metabolism. We extracted polyamines from the epidermis of vitiligo subjects using perchloric acid extraction method[30]. Thin layer chromatographic analysis of polyamines showed an elevated level of PUT and a reduced level of SPN in lesional epidermis compared to non-lesional samples (Fig. 3a,b). The increased steady state level of PUT could either be an outcome of increased biosynthesis or could result from the breakdown of SPN and SPD (Supplementary Fig. S6). The reciprocal relationship between the level of PUT and SPN could potentially be linked to catabolism of SPN and SPD. Therefore to test this possibility, we measured SSAT-1 mRNA levels by real time qPCR and found ~3-fold elevated SSAT-1 mRNA level in lesional samples (Fig. 3c, $p < 0.01$, $n = 5$) suggesting that an increased SSAT-1 enzyme levels could lead to an enhanced polyamine catabolism in vitiligo. In most mammalian cells, SPN is the abundant polyamine and the levels of PUT are comparatively lower. However, higher levels of PUT compared to SPN in the skin and its further elevation in vitiligo lesions prompted elucidation of the specific role of PUT.



	017 NL (leg)	017 L (thigh)	015 NL (thigh)	V015 L (chest)	015 L (leg)	009 NL	009 L
PUTRESCINE	0.89	2.96	1.32	5.13	6.88	1.20	2.30
SPERMINE	0.44	0.16	0.82	0.56	0.51	0.86	0.79

Figure 3. Altered polyamine metabolism in vitiligo epidermis. (**a**) Thin layer chromatographic analysis of polyamines in non-lesional and lesional epidermis after dansylation. Whole epidermis was extracted using perchloric acid and the extracts were dansylated along with standard polyamines. The reactions were extracted with toluene and spotted onto silica TLCs and developed on a cyclohexane: ethylacetate solvent system and detected under UV transilluminiscence. The amount of each of the polyamine per milligram of the epidermis is quantitated and represented across three patient samples (3 non-lesional and 4 lesional epidermis). (**b**) Log transformed relative levels of putrescine (PUT) and spermine (SPN) in three patient samples (3 non-lesional and 4 lesional epidermis) as detected by thin layer chromatography. Dotted line represents unchanged levels of polyamines with respect to the non-lesional skin (**c**) Real-time PCR analysis of Spermine/spermidine acetyl transferase-1 (SSAT-1) mRNA across five independent pairs of vitiligo lesional and non-lesional skin, represented as a scatter plot, horizontal line indicates mean. PUT- putriscine, SPD- spermidine, SPN- spermine, DAH- 1,7-diaminoheptane. NL- non-lesional, L- lesional skin.

Corneocyte formation is an unusual differentiation process, wherein the cells undergo non-classical apoptosis[31]. The cells progressively lose the organelles but remain biochemically active. Structural proteins such as filaggrin and late cornified envelope proteins (LCEs) are extensively crosslinked by transglutaminase enzyme, giving rise to the corneocyte. As this layer showed extensive changes in lesional epidermis histologically and microarray analysis also demonstrated alterations in cornification process, we investigated whether the PUT was localized to the stratum corneum. Towards this, we specifically isolated the stratum corneum from normal epidermis using urea extraction protocol that dissolves the cellular epidermis. The isolated cornified layer was divided into two equal fractions: one was treated with perchloric acid for extracting non-covalently bound polyamines and the other was subjected to total acid hydrolysis (HCl) to obtain covalently conjugated polyamines from this tissue. We observed higher levels of PUT upon total acid hydrolysis, while other polyamines including SPN could not be detected from the stratum corneum, indicating the presence of covalently conjugated PUT in the cornified envelope (Supplementary Fig. S7). As the levels of PUT are elevated in the total epidermis of vitiligo lesions, we propose that these are localized to the stratum corneum layer and contribute to the alterations observed in the histological sections.

Functional gene networks are altered in vitiligo. Having studied the architectural changes and their underlying molecular basis, we decided to address the implications of global transcriptional alterations observed in vitiligo. Only a small number of regulated genes belonged to recognizable categories in the enrichment analysis. However, the pathological manifestations observed in the vitiligo epidermis would be contributed by many of the regulated genes that form the vitiligo transcriptome. Since genetic predisposition is known in vitiligo and variants mapping to genes involved in different aspects of the disease are involved in the pathogenesis, we set out to address whether the altered gene expression profile in vitiligo lesions had any association with implicated genes. Towards this systems-level understanding, we constructed functional interactomes of genes that have been previously reported to genetically predispose individuals to vitiligo, in the genome-wide association studies (GWAS)[32]. A set of 45 genes associated with vitiligo served as the input for this analysis (Supplementary Table 2). The catalogue of physical/binding interactions for each of the 45 genes was extracted from the BIOGRID database[33]. This generated a correlated collection of global networks and sub-networks from the genes and their corresponding functional linkages i.e., interacting genes (Supplementary Fig. S8).

Further, genes showing altered expressions in vitiligo microarray data were mapped onto the aforesaid networks. This second layer enabled us to understand "regulatory profiles" to reveal key perturbations in vitiligo. We analyzed the data by comparing changes in the number of subjects in whom a particular gene was differentially expressed in vitiligo lesions and was coded red or green to depict upregulation or downregulation respectively in at least 66% of the subjects analyzed (i.e. $\geq$11 out of 15 subjects). The network analysis implicated major perturbations in cellular processes such as SCF-KIT signaling, oxidative stress, apoptosis, stress response, vitamin D receptor and immune response (Fig. 4, Supplementary Fig. S9). Through this analysis we observed that the dynamic changes in RNA map to the same gene networks that are implicated in genetic predisposition of vitiligo. These networks link the functional state of skin in vitiligo to the known genetic predispositions in this complex disorder and provide an opportunity to comprehend the disease pathogenesis from the static genetic polymorphisms to dynamic gene regulatory networks.

Discussion

Our study reports widespread alterations in the lesional epidermis at architectural, cellular, and transcriptomic levels and suggests an altered state of the epidermis in vitiligo. Despite heterogeneity in the appearance of clinical features between each vitiligo subject, an important observation that emerged from the genome-wide transcriptome analysis was the large-scale concerted changes in the cornification process in all 15 subjects with stable vitiligo. While the apparent downregulation of genes involved in melanogenesis is anticipated owing to the loss of melanocytes, alteration in keratinocyte-specific process is non-intuitive. We speculate that the absence of melanocytes from the vitiligo skin patch induces localized constraints for the maintenance of tissue homeostasis resulting in an altered state of the skin in vitiligo lesions.

Earlier transcriptomic studies on vitiligo have evaluated changes in intact biopsies containing both epidermis and the underlying dermis[18]. In that study, the authors also compared the lesions with the skin of healthy controls and provided important insights into the role of innate immune system and Natural Killer (NK) cells in causing melanocyte death. Another study on cultured human melanocytes from non-lesional skin of vitiligo subjects compared to healthy donors demonstrated abnormalities in melanocyte functions that could lead to antigen presentation by these cells[19]. In a recent genome-wide association study, altered Wnt signaling pathway was linked to melanocyte loss in vitiligo[34]. These studies have provided insights into different aspects of the disease biology. However, it is interesting to note that we still do not understand how the lesional skin maintains its functions and adapts to the absence of melanocytes. By analyzing matched lesional and non-lesional epidermal samples, we have not only minimized inter-individual differences but also provide a better understanding of alterations present in lesional epidermis, over and above the systemic alterations in vitiligo.

During our investigation, we observed widespread architectural differences in the lesional skin, as compared to the non-lesional skin. While changes in the thickness of epidermis were previously reported, we indicate that the thickening of epidermis is primarily due to the stratum corneum layer. We also highlight the blunting of rete-ridges at the junction of epidermis and dermis, which would decrease surface area of interaction in the lesional skin. Further, our studies demonstrate enlarged corneocytes in the lesions, which contribute to the thickening of the stratum corneum. The lesional skin is seemingly functional as there are no apparent changes in the skin barrier reported clinically in vitiligo subjects.

On a mechanistic basis, the molecular change contributed by polyamine metabolism is a new perspective unraveled in this study that could potentially explain several changes observed in the lesional vitiligo skin. Polyamines contribute to various cellular functions and their role in skin is addressed mainly in the context of cancers[35]. Our study demonstrates upregulation of SSAT-1, the key rate-limiting enzyme involved in polyamine catabolism with a concomitant increase in PUT and a decrease in SPN. While in most cells polyamines have a conventional role, skin is different in having abundance of PUT, which we could trace to the stratum corneum layers in the normal healthy skin. It is tempting to speculate that the lesional epidermal PUT in vitiligo contributes to the thicker stratum corneum. In addition to changes in keratinocyte differentiation brought about by altered polyamine levels, PUT could be involved in structural changes by participating in covalent couplings as we observe far greater levels of PUT upon hydrolysis compared to perchloric acid extraction. We hypothesize that the thicker stratum corneum in vitiligo skin is a likely adaptation that probably protects vitiligo keratinocytes from ultraviolet radiations. Associated changes in skin architecture such as the epidermal blunting could be an outcome of alterations in the proliferative capacity of the stem cell niche, which needs to be further evaluated.

Mapping of vitiligo transcriptomic changes onto the interactome network generated from the known genetic associations enabled us to visualize the dynamic changes in the genome that could culminate in vitiligo. It is interesting to note that in most categories the key implicated node is not affected but the associated genes show concerted changes, which indicates alteration in the functioning of that pathway. c-KIT mediated signaling is critical in maintaining the melanocyte survival and it is interesting that the entire network is down-regulated. Though SCF (KITLG) is upregulated, a decrease in downstream components would render this pathway dysfunctional. In the immune system module, proteosomal components are upregulated supporting the role of antigen presentation and autoimmunity in vitiligo. Further studies on these pathways would help to delineate their role in maintaining the diseased state and altered homeostasis in vitiligo and offer interventions for correction.

Besides the apparent depigmentation, the altered lesional epidermis is competent to perform its biological functions and is also suggested to be protective against skin cancers[4,5]. We propose that architectural, cellular and molecular changes observed in this study may be playing a protective role against carcinogenic UV-induced damage. Such adaptive changes in physiological conditions have been defined as 'enantiostatic' adaptations[36,37]. While biological systems have a tendency to revert back to homeostatic state, vitiligo skin is a challenge in terms of restoring tissue function due to the permanent loss of melanocytes. The adaptations described in our study whereby the system adapts itself to maintain the essential function is an example of tissue 'enantiostasis'. Such modifications while on one hand may be protective response to maintain the integrity of the tissue, but on the

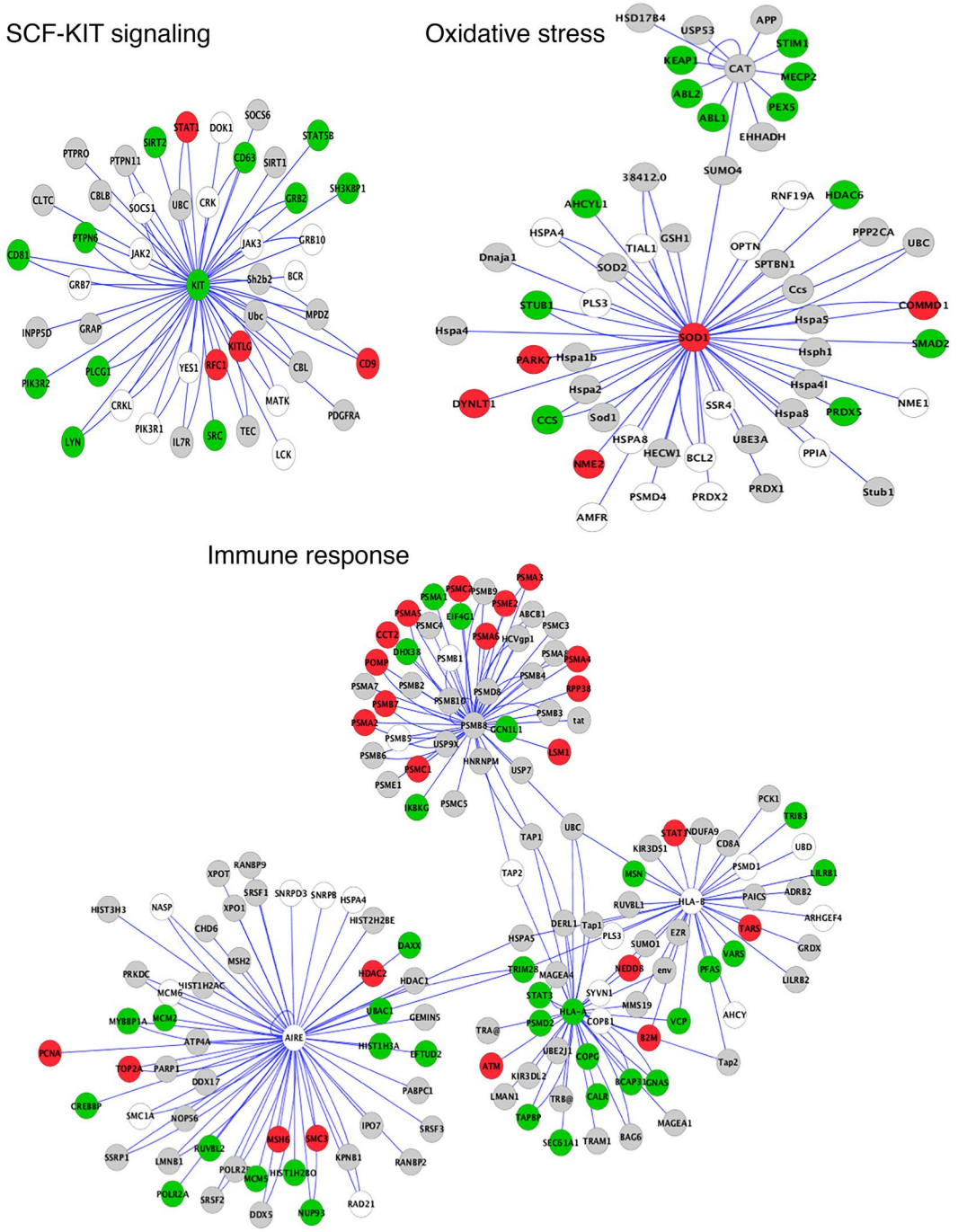


Figure 4. Altered functional networks in vitiligo. Network map of clusters of genes associated with vitiligo predisposition along with their interacting partners were mapped to the differential expression of genes observed in vitiligo. Three networks are shown: SCF-KIT signaling, oxidative stress and immune response. Each node (gene) is colored according to upregulation (red), downregulation (green), or no regulation (grey). White denotes proteins for which corresponding probes were not found in the microarray. The regulation score was calculated from microarray experiments performed on 15 vitiligo samples, where only agreement between $>=11$ samples was included as a significant regulation.

other hand could contribute to the maintenance of disease states. Such changes in tissue modification may be a contributory factor in variety of other pathological conditions. Future studies would focus on finding ways to reverse these enantiostatic states that could provide means to treat stable vitiligo patches.

In summary, our study provides important insights into the pathophysiology of vitiligo skin and delineates gross tissue level perturbations of the epidermis in vitiligo. Many of these changes could be adaptive in nature and may be involved in protecting the skin from environmental insults in the absence of melanin. We propose that these adaptive changes also preclude remigration and sustenance of melanocytes from neighboring uninvolved

skin. Currently stable vitiligo is treated by enriched stem cell pool of melanocytes with limited success. Strategies that can incorporate restoration of architectural niche of vitiligo skin may greatly improve autologous transplantation therapy.

Materials and Methods

Patient recruitment and sampling. Skin punch biopsies (3–4 mm) from lesional and non-lesional sites from vitiligo patients were obtained after taking informed consent. Institutional Human ethics committees of Ram Manohar Lohia Hospital, New Delhi and National institute of Immunology, New Delhi, approved the study and it is in agreement with Declaration of Helsinki principles. Non-lesional biopsies were obtained from gluteal/lateral thigh region and the lesional samples were taken from affected parts of the body (Table 1). All the lesional and non-lesional skin biopsies were obtained from stable vitiligo vulgaris patients scheduled to undergo punch grafting or melanocyte/epidermal cell transplantation. The biopsies were obtained from the patients prior to therapeutic surgical intervention. The proximal non-lesional biopsies were obtained from non-lesional sites within a distance of 5 cm from the margins of the lesions. All the patients included in the present study were Indians, had non-segmental vitiligo (vitiligo vulgaris), and the disease was stable for at least 6 months at the time of taking the biopsy.

Quantitation of architectural changes in vitiligo. Punch biopsies were immediately fixed in buffered formalin solution and four-micron thick cryo-sections were stained with hematoxylin and eosin. Morphometric evaluation of the images was carried out using ImageJ software and the data was analyzed using paired t-test using Graphpad Prism suite and represented as mean $\pm$ range of the measurement. For detecting melanocytes, sections were stained with S100 antibody (dilution 1:100; Dako Cytomation, Glostrup, Denmark) for 45 minutes at room temperature. Slides were washed and incubated with labeled polymer alkaline phosphatase (Dako Cytomation) for 30 minutes at room temperature. Reaction was developed with fuchsin counterstained with hematoxylin.

RNA isolation and microarray. Total RNA was isolated using Trizol method from the whole epidermal cells from 15 pairs of non-lesional and lesional skin samples after separation of epidermis from the dermis using Dispase II. RNA samples were cleaned using RNEasy columns form Qiagen. RNA integrity and quality was measured using Bioanalyzer and samples with RIN score ≥ 8 were taken ahead for labelling and hybridization. Whole genome microarray was carried out using Illumina WG-6 array using manufacturer's guidelines. Preliminary data normalization and analysis was carried out using Bead Studio software. Further analysis was carried out using Genome Studio version 2011.1. High throughput data of 48,803 probes specific for 37804 genes from non-lesional and vitiligo epidermis of 15 subjects was thus obtained.

Vitiligo data analysis. The probes with background subtracted expression values of genes in negative were eliminated from the analysis and the expression values were average normalized. This was done by taking the mean of the entire data for each of the 15 NL and L samples, dividing every expression value by this mean and then multiplying it by 500. Paired t-test was carried out using Matlab (Mathworks, Inc, Natick, MA).

Transmission electron microscopy. Transmission electron microscopy was performed on non-lesional and lesional vitiligo skin samples using standard methods. Briefly, 3 mm skin pieces were fixed in 2.5% gluteraldehyde and 4% paraformaldehyde, osmicated in 1% osmium tetroxide, dehydrated in graded series of alcohol and infiltrated with Epon 812 resin. Ultrathin sections were cut on RMC ultramicrotome, collected on copper grids and stained with uranyl acetate and lead citrate. Samples were visualized on Tecnai G2 20 twin (FEI) transmission electron microscope.

TLC for Polyamine detection. Whole epidermis was dissolved in 2% perchloric acid and dansylated using 400 µl dansyl chloride (5 mg/ml) at pH 2.0. 200 µl of saturated sodium carbonate was added and incubated at 70 °C for 10 min and the reaction was quenched using 100 µl of 150 mg/ml proline at room temperature for 30 min. Dansylated polyamine was extracted using 500 µl Toluene and spotted on silica thin layer chromatographic column along with standard dansylated polyamines. The mixture was separated using cyclohexane ethyl acetate solvent system and visualized under UV transilluminence (500 millisec exposure). PUT, SPD, SPN in first 3 lanes were used as standards. DAH was used as internal standard to calculate the relative amount of specific polyamines present in the epidermis with respect to corresponding standards. Image Quant 5.2 software was used for densitometry quantification.

Functional networks generation. An initial dataset of 45 genes showing predisposition to vitiligo was obtained from previously characterized GWAS studies[32]. Protein interactions for each gene was prepared using BIOGRID database[33]. This database stores human protein interactions that have been experimentally characterized and therefore, served as a good repository for in-house generated vitiligo protein network. In total, we obtained 1416 interactions from input set of 45 genes (or proteins). We prepared a network map using Cytoscape, a commonly used visualization tool to construct linkages between proteins. The global network resulted in six major sub-networks (hubs with densely connected proteins), namely, KIT, XBP1-MSH6, SOD-CAT, PSMB8-AIRE-HLA, FAS-CASP, and VDR as shown in Supplementary Fig. S8. The physical interactions of the proteins depicted in the 6 sub-networks are shown in Supplementary Table 3. These sub-networks were then overlaid with vitiligo microarray expression information with each mRNA (protein) showing upregulation (red), downregulation (green), or no regulation (grey). The regulation score was calculated from microarray experiments performed on 15 vitiligo samples, where similar regulation in ≥ 11 samples was considered as a significant regulation. Thus, Fig. 4 and Supplementary Fig. S9 shows a comprehensive functional interactome of protein-interaction and their regulation patterns of the 6 sub-networks in vitiligo subjects.

Data and materials availability. Microarray data has been deposited in NCBI Gene Expression Omnibus portal accession number GSE75819.

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Author Contributions

R.S.G., R.R. conceived the study. R.S.G., R.R., V.T.N. and A.S. designed the experiments. A.S., V.G., M.T., P.G., A.K., L.T., G.M., V.T.N. conducted the experiments. P.J. and R.L. planned, designed and performed the vitiligo microarray studies and V.V., S.K., C.J.G. were involved in microarray data analysis. P.S., T.F., A.G., A.H. and H.K.K. were involved in patient selection, recruitment and clinical handling of subjects, collection of biological material and interpretations. A.S., V.G., L.T., K.N., C.J.G., V.T.N., R.R. and R.S.G. analyzed the data and interpreted the results. A.S., V.T.N., R.R. and R.S.G. wrote the manuscript.

Additional Information

Supplementary information accompanies this paper at doi:10.1038/s41598-017-10253-w

Competing Interests: R.S.G. is the co-founder director on the board of Vyome Biosciences, a biopharmaceutical company in the area of dermatology. K.N. is on the board of Ahammune Biosciences, a biopharmaceutical company in the area of dermatology.

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Original Article

Quality of life in vitiligo: Relationship to clinical severity and demographic data

ABSTRACT

Background: Vitiligo, a common, acquired, idiopathic, depigmenting disorder of the skin and/or mucosae has a profound effect on the patient's quality of life (QoL). However, its relationship with clinical severity remains equivocal.

Aim: To measure the impairment in QoL of patients having vitiligo and correlate it with the severity of the disease.

Materials and Methods: A cross-sectional, questionnaire-based study was conducted on a cohort of 100 consecutive vitiligo patients attending the outpatient department of our tertiary care hospital over a period of 1 year. A physician utilized the vitiligo area severity index to measure the severity of the disease, whereas the patient's QoL was assessed by using the vitiligo impact scale-22. Demographic data and clinical characteristics were also documented. Spearman's correlation coefficient, chi-squared, and independent '*t*' tests were used as appropriate.

Results: Our study revealed a highly significant correlation between the extent of vitiligo and impairment in QoL. Patients with early onset of disease, those having previously taken any kind of treatment, unmarried individuals or those with vitiligo involving the face and/or upper extremities had a significantly greater impact on their QoL.

Conclusion: The severity of vitiligo, among other factors, is significantly correlated with the impairment in the QoL of its sufferers.

Keywords: Quality of life, severity, VASI, VIS-22, vitiligo

INTRODUCTION

Vitiligo, a common acquired depigmenting disorder, affects approximately 1% of the population worldwide and is clinically characterized by well-demarcated areas of depigmentation as a result of loss of melanocytes.[1,2] Although generally considered to be a cosmetic issue, vitiligo is usually psychologically devastating, especially in darker individuals.[3] Patients with vitiligo often suffer from poor body image along with low self-esteem, and experience discomfort, inferiority, and discrimination in social and societal relationships,[4] leading ultimately to an impaired quality of life (QoL).[5]

Though many studies have been successful in quantifying the negative impact of vitiligo on QoL, its direct correlation with disease severity remains equivocal.[6] Hence, we undertook this study to delineate demographic factors contributing to the impairment of QoL in patients suffering from vitiligo and to ascertain their correlation, if any, with its clinical severity.

MATERIALS AND METHODS

This cross-sectional, questionnaire-based study was conducted after receiving ethical clearance from our Institute and comprised a cohort of 100 patients of vitiligo (of any type) attending the outpatient department of our tertiary care hospital over a period of 1 year. After obtaining their informed consent and demographic details, each patient was asked to fill in, without any time limit, the vitiligo impact scale-22 (VIS-22) questionnaire.[7]

Milind A. Patvekar, Kirti S. Deo, Sampurna Verma, Preeti Kothari, Aayush Gupta

Department of Dermatology, Dr. D.Y. Patil Medical College and Hospital, Pimpri, Pune, India

Address for correspondence: Dr. Aayush Gupta, M.B.B.S, M.D, Sanjeevani Nursing Home, Salar Gunj Gate, Panipat 132103, Haryana, India.
E-mail: aayushggupta@gmail.com

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VIS-22, a disease-specific, modified version of VIS questionnaire,[8] validated in the Indian population, consists of 22 easily comprehensible questions: 19, common to all patients and one each for patients who are married, unmarried, working, or studying. Individual responses are scored from 0 to 3; a higher score indicating a worse QoL.[7]

The clinical severity of vitiligo was assessed using the vitiligo area severity index (VASI),[9] which is a standardized, sensitive method to measure the extent and percentage of de- and/or repigmentation. This index divides the patient's body into five mutually exclusive regions: the hands, upper extremities (includes axillae), trunk, lower extremities (includes buttocks and inguinals), and the feet. The face and neck are assessed separately. For each region, the VASI is determined by measuring in 'hand units' (1% per unit), the area of vitiligo, and multiplying this with the extent of depigmentation within each 'hand unit' (possible values being 0, 10, 25, 50, 75, 90 or 100%: 10%, only specks of depigmentation; 25%, pigmented area exceeds depigmented one; 50%, pigmented area is equal to the depigmented area; 75%, depigmented area exceeds the pigmented area; 90%, only specks of pigmentation and 100%, complete depigmentation). The total VASI, with a score ranging from 0 to 100, is then calculated using the formula: VASI = Σ (all body sites) (hand units) $\times$ (residual depigmentation).

Statistical analysis

Quantitative variables were described using percentages, ranges, means, and standard deviations. The independent sample *t*-test, Chi-squared test, and Spearman's correlation analysis were performed using the Statistical Package for the Social Sciences version 22 (SPSS Inc., Chicago, IL, USA for Windows) software as appropriate. A two-tailed probability value of less than 0.05 was considered significant.

RESULTS

Demographic data

One hundred patients, 45 males and 55 females [Figure 1], with age ranging from 18 to 69 (mean: 34.3± 13.38) years, consisted our study population; students (31%), housewives (29%), and professionals (20%) forming the majority. Of them, 90 were Hindus, eight Muslims, one Christian, and one Sikh. A total of 59 individuals were married; 37, single and 4, divorcees. The majority (30%) first noticed vitiligo between 15–24 years of age; 28%, between 25 and 34 years and 17%, between 35 and 44 years; 30%, presented to our department within 5–10 years of onset of vitiligo, whereas 24% did so after a period of 10 years [Table 1]. A first-degree family history was present in 24 (11 males, 13 females) patients. Nonsegmental vitiligo was the most common (96) type encountered by us (vulgaris, 63%; focal, 17%; acral, 5%; acrofacial, 5%; mucosal, 4%; and universal, 2%). Segmental vitiligo was seen in only four patients [Figure 2]. Lower extremities were the most common body part to be involved (74%), followed by upper extremities (58%), the scalp being involved in only 13% of our patients. The majority of our study population (72%) had already taken some kind of therapy prior to visiting our institute, the most common being alternative (Ayurveda/Homeopathy) medicine by 53 (73.61%) [Table 1].

Vitiligo impact scale-22

The mean VIS-22 in our study was 32.57 ± 18.44, higher in females (34.07 ± 17.19) than males (30.73 ± 19.12), though not significantly so. There was a significant negative correlation ($\rho = -0.197$, $P = 0.049$) between the patients'

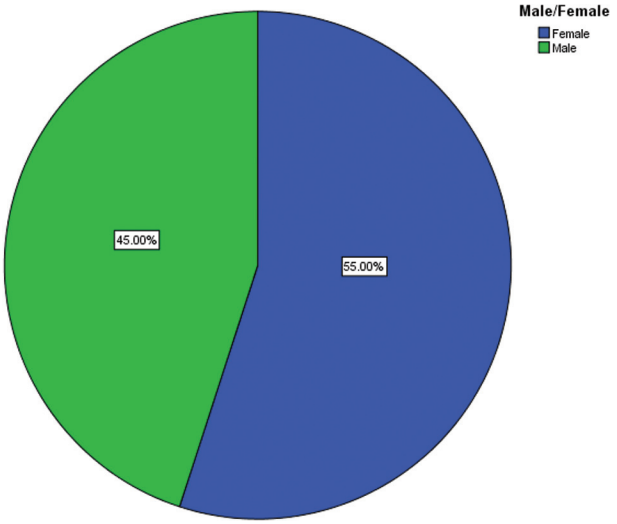

Figure 1: Sex-wise distribution

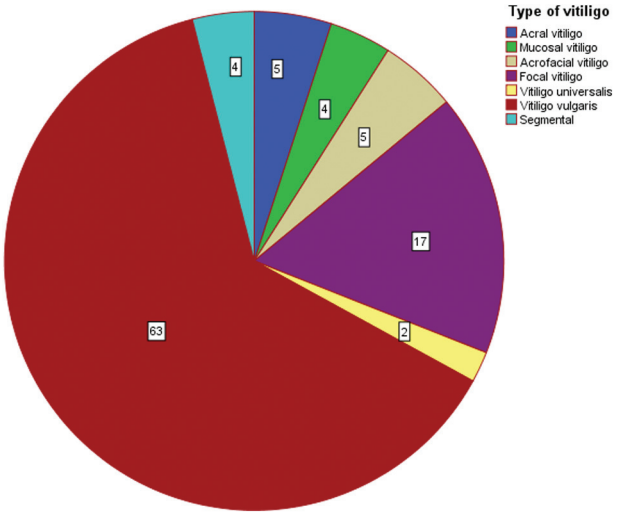

Figure 2: Type of vitiligo

Table 1: Demographical data

		Male	Female	Total
Patients occupation	Student	14	17	31
	Manual laborer	4	1	5
	Clerical	4	3	7
	Farmer	2	1	3
	Professional	16	4	20
	Unemployed	5	0	5
	Household work	0	29	29
Marital status	Single	19	18	37
	Married	24	35	59
	Divorcee	2	2	4
Age at onset (years)	5–14	4	10	14
	15–24	15	15	30
	25–34	13	15	28
	35–44	8	9	17
	45–54	3	2	5
	55–64	2	4	6
Time since onset [year(s)]	<½	7	5	12
	½–1	5	3	8
	1–3	6	10	16
	3–5	3	2	5
	5–10	14	16	30
	10–20	8	16	24
	>20	2	3	5
Duration of disease (months)	<6	20	20	40
	6–11	4	1	5
	11–17	4	6	10
	18–23	3	0	3
	24–35	9	2	11
	>36	23	8	31
Location of lesions	Scalp	3	10	13
	Face	20	27	47
	Chest and abdomen	17	33	50
	Back	10	25	35
	Upper extremities	22	36	58
	Lower extremities	32	46	78
	Genitalia	7	7	14
Treatment taken	NBUVB	3	7	10
	PUVA	5	21	26
	Topical steroids	10	23	33
	Topical tacrolimus	3	5	8
	Systemic steroids	8	20	28
	Alternative medicine	21	32	53
	None	17	11	28
Family history of vitiligo	Yes	11	13	24
Vitiligo impact scale-22		30.73	34.07	32.57
Vitiligo area severity index		8.57	18.36	13.95

age and the VIS-22. Gender, occupation, religion, family history, and duration of disease had no correlation with the VIS-22. Single patients had a significantly higher VIS-22 (38.51 ± 19.21) as compared to married ones (29.42 ± 17.16, $P = 0.047$). Patients with vitiligo involving the face (41.23 ± 18.18 vs 24.89 ± 15.06, $P < 0.000$) and upper extremities (38.07 ± 16.51 vs 24.98 ± 18.01, $P < 0.000$) had a significantly higher VIS-22 than those whose disease did not involve these areas. Patients with vitiligo vulgaris had a significantly higher VIS-22 (37.60 ± 17.12) than those with focal vitiligo (22.59 ± 16.12, $P = 0.026$). Surprisingly, patients having taken any kind of treatment in the past had a significantly higher VIS-22 (37.68 ± 16.53) than those who had taken none (20.68 ± 17.04, $P < 0.000$); this impairment being significantly more pronounced in patients who had taken psoralen and ultraviolet A (PUVA) therapy (45.19 ± 13.48 vs 28.14 ± 17.95, $P < 0.000$), topical steroids (41.97 ± 15.09 vs 27.94 ± 18.26, $P < 0.000$), and/or systemic steroids (41.54 ± 16.92 vs 29.08 ± 17.92, $P = 0.002$).

Vitiligo area severity index

The overall mean VASI in our study was 13.95 ± 21.42 (range: 1–99), significantly higher in females (18.36 ± 23.92) than in males (8.57 ± 16.63, $P = 0.018$). Patients with a positive family history had a significantly higher VASI score (24.82 ± 29.14) than those without (10.52 ± 17.2, $P = 0.03$). There was no association of VASI with the duration of disease, though it had a significant, positive correlation with increasing age ($\rho = 0.265$, $P = 0.008$). Patients who had undergone prior treatment had a significantly higher VASI (18.57 ± 23.92) than those who had not taken any treatment (2.54 ± 2.32, $P < 0.000$). Farmers had a significantly higher VASI at presentation (50 ± 49.00) as compared to students (11.69 ± 18.39, $P = 0.045$) and those employed in clerical jobs (5.77 ± 6.63, $P = 0.041$). Divorcees were found to have a significantly higher VASI (39.25 ± 45.35) than patients who were single (12.84 ± 18.60, $P = 0.049$) as well as married (12.94 ± 20.40, $P = 0.045$).

Correlation of vitiligo impact scale-22 scores with vitiligo area severity index

There was a significant, positive correlation between VIS-22 and VASI ($\rho = 0.422$, $P < 0.000$).

DISCUSSION

Although vitiligo equally affects people across all races and ethnicities, a greater impact on QoL is generally seen in individuals with darker skin types. This is especially true in India where vitiligo was once considered to be one of the top 3 major medical problems.[10] Our study reiterates the significant impairment of QoL in patients of vitiligo reported in earlier studies. However, in contrast to several earlier studies reporting females to have a worse QoL, our study revealed no such significant difference in the QoL of females vis-a-vis males.[11]

There was a significant negative correlation between age and impaired QoL, that is, the impairment of QoL decreased progressively with age in our study. A previous study, too, demonstrated that negative experiences due to vitiligo in childhood continue to be associated with impairment of QoL in young adults, probably as childhood, particularly adolescence, is characterized by rapid psychological and social development, which when coupled with emotional vulnerability and negative experiences due to disfiguring diseases such as vitiligo may permanently affect their psychological and emotional development.[12]

Single patients had a significantly higher impairment of QoL than married ones in our study, similar to another study conducted in Saudi Arabia, probably because of the inherent stability and security provided by marriage. In India, the inability to find suitors for marriage by sufferers of vitiligo forms one of the major reasons of their significantly higher VIS-22.[13] In contrast, an Iranian study revealed a greater impact on QoL of patients who were married due to a unique law that supports Iranian men to file for divorce without any alimony if, before marriage, the presence of vitiligo was not disclosed by their wives.

Our study reiterated the finding of most previous studies which demonstrated that vitiligo involving the face and hands had the greatest impact on QoL, probably as a result of the highly visible nature of these sites and the inability to cover them with clothes.[11]

Surprisingly, in contrast to earlier studies, patients treated previously for vitiligo had a significantly greater impairment of their QoL than that in those untreated.[11] This suggests that there is a 'paradoxical' increase in impairment of QoL due to vitiligo with attempted treatment, probably due to increased expectations. This impairment was significantly more pronounced in patients having previously taken PUVA therapy, topical steroids, and/or systemic steroids as compared to Narrow band ultraviolet B, topical tacrolimus, and Ayurveda, suggesting a higher level of satisfaction with the latter.

Increasing age, female sex, and/or positive family history were associated with significantly increased severity of vitiligo probably due to underlying genetic factors and specific HLA haplotypes.[14] A significantly higher VASI of farmers could be explained by lesser social and societal pressures leading to a delay in their presentation to a hospital. A significantly greater clinical severity of vitiligo in divorcees could probably be explained by stress-induced exacerbation of their disease.

QoL based on VIS-22 demonstrated a significant, positive correlation with the extent of vitiligo as assessed by VASI in our study, findings consistent with multiple prior studies.[15-17] However, this is in contrast to other dermatological conditions such as acne vulgaris and melasma, where the correlation between disease severity and impaired QoL remains equivocal.[18,19]

Small sample size, a lack of guaranteed reliability of the self-reported QoL, and inability to get patients investigated for biochemical and/or serological abnormalities formed the limitations of this study.

In conclusion, an increased extent of vitiligo, younger age, and vitiligo involving the face and/or upper extremities is associated with a worse QoL, which may in turn lead to exacerbation of disease, forming a vicious circle. Thus, the impairment of QoL in vitiligo patients should be routinely assessed via objective scoring systems such as the VIS-22, and the findings incorporated into therapeutic decision-making, as patients having a greater impairment of their QoL may benefit from more aggressive intervention, such as phototherapy and systemic therapy including surgery and/or psychological intervention.

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Conflicts of interest
There are no conflicts of interest.

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Correlating Impairment of Quality of Life and Severity of Melasma: A Cross-sectional Study of 141 Patients

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Correlating Impairment of Quality of Life and Severity of Melasma: A Cross-sectional Study of 141 Patients

Preeti Kothari, Yugal Kishor Sharma, Milind A. Patvekar, Aayush Gupta

From the Department of Dermatology, Dr. D. Y. Patil Medical College and Hospital, Pune, Maharashtra, India

Address for correspondence:
Dr. Preeti Kothari, "Uttam", Bungalow No 165, Jankidevi Public School Road, Near Versova Telephone Exchange, Andheri West, Mumbai - 400 053, Maharashtra, India.
E-mail: kotharipreeti112@gmail.com

Abstract

Background: The relationship between impaired quality of life (QoL) due to melasma and its clinical severity remains equivocal despite several studies. **Aim:** The aim was to study the correlation, if any, between the clinical severity and the impairment in QOL due to melasma. **Methods:** This cross-sectional, questionnaire-based study was conducted on a cohort of 141 patients of melasma attending the outpatient department of our referral hospital. A physician measured the severity of melasma using the melasma area and severity index (MASI), while melasma-related QoL (MELASQOL) score was calculated utilizing the validated Hindi version of the MELASQOL questionnaire filled by the patients. Correlations of these two scores with each other and with components of the demographic data were attempted using the Statistical Package for the Social Sciences, version 20. **Results:** Significantly greater impairment in QoL was found in patients with a history of prior use of triple combination therapy and in patients with hirsutism and/or polycystic ovarian disease. The severity of melasma was found to be significantly higher in patients with a history of recurrence and tobacco chewing. **Limitations:** The sample size could have been larger. Ultrasonography could have been carried out in all cases of hirsutism. **Conclusion:** The severity of melasma does not correlate with the impairment in QoL.

Key Words: *Melasma area and severity index, melasma, melasma-related quality of life, quality of life*

What was known?
- Melasma leads to impairment of quality of life
- The correlation of melasma related quality of life vis-à-vis its clinical severity remains equivocal.

Introduction

Melasma, a common disorder of acquired hyperpigmentation, is characterized by tan or brown macules/patches localized to photo-exposed areas of the face, particularly the malar areas, forehead, and chin.[1]

Although the impairment in quality of life (QoL) of patients with melasma is well established, its relationship with clinical severity remains equivocal; measures of the latter – such as the commonly used melasma area severity index (MASI)[2] – do not reflect the psychosocial distress experienced by the patients.[1,3] Recognition of this distress is necessary in reassuring patients about their social and emotional problems, thereby contributing to the effective management of melasma. Hence, we undertook this study to find the correlation between severity and impairment in QoL, if any, and with components of demographic data in patients with melasma.

Methods

This cross-sectional questionnaire-based study was carried out after receiving ethical clearance from our institute and comprised of a cohort of 141 patients of melasma attending the outpatient department of our tertiary care hospital. After obtaining their informed consent and demographic details, each patient was asked to fill in without any time limit the recently validated Hindi version[4] of the 10-item melasma QoL (MELASQOL) questionnaire which can usually be completed in 1 min. It incorporates a Likert scale of 1–7 (1, signifying not

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bothered at all and 7, bothered all the time); higher the score, worse is the QoL, ranging from 10 to 70.[5]

The clinical severity was assessed using MASI which is calculated by the subjective assessment of three factors: area (A) of involvement, darkness (D), and homogeneity (H). The face is divided into four regions: forehead (f), right malar (rm), left malar (lm), and chin (c); the first three weighted 30% each and chin, 10%. The area of involvement in each of these 4 facial regions is given a numeric value of 0–6 (0 = no involvement; 1 = <10%; 2 = 10%–29%; 3 = 30%–49%; 4 = 50%–69%; 5 = 70%–89%; and 6 = 90%–100%). Darkness and homogeneity are rated on a scale from 0 to 4 (0 = absent; 1 = slight; 2 = mild; 3 = marked; and 4 = maximum). The MASI score = 0.3A(f) (D[f] + H[f]) + 0.3A(lm) (D[lm] + H[lm]) + 0.3A(rm) (D[rm] + H[rm]) + 0.1(c) (D[c] + H[c]), and ranges from 0 to 48.

Quantitative variables were described using percentages, ranges, means, and standard deviations. Student's independent *t*-test, analysis of variance test, and Spearmen correlation analysis were carried out using the Statistical Package for the Social Sciences, SPSS version 20 (SPSS Inc., Chicago, IL, USA) as appropriate. A two-tailed probability value of less than 0.05 was considered statistically significant.

Results

Demographic data

One hundred and forty-one patients (males, 36; females 105), with age ranging from 20 to 76 (mean: 32.35±7.427) years constituted our study population; their main occupations being, housewives (59; 41.8%), professionals (38; 27%), and laborers (35; 24.8%). One hundred and eighteen patients (83.7%) were married; 23 (16.3%), single and none, divorced. Melasma lesions were first noticed between the ages of 31 and 35 years by 41 (29.1%); 26–30 years by 37 (26.2%) and 21–25 years by 30 (21.3%) patients. The duration of melasma ranged from 1 month to 25 (mean, 3.1 ± 3.39) years. Majority (88; 62.4%) of the patients presented during their first spell of melasma; remaining (53; 37.6%), after having had treatment for a mean duration of 5.92 (±6.028) months: 22 (41.45%) with the triple combination; 10 (18.86%) with other skin lightening agents and 8 (15.1%) with topical steroids [Table 1]. Of the seventy-one (50.35%) patients who admitted using over-the-counter treatment for >6 months, 44 (61.9%) used fairness cream; 13 (18.3%), powder; 9 (12.7%), ayurvedic cream and 5 (7.1%), other applications. Sun exposure was the principal aggravating factor in 60 (42.6%) of our study population. The onset of melasma was associated with pregnancy in 34 (32.4%) and with intake of oral contraceptives in 17 (16.2%) cases. Eleven (7.8%) patients gave a history of chewing tobacco; 1 (0.7%) each of smoking cigarettes and drinking

Table 1: Clinico-epidemiological details of patients

Patient details	*n* (%)
Gender	
Male	36 (25.53)
Female	105 (74.47)
Occupation	
Housewive	59 (41.8)
Professional	38 (27)
Laborer	35 (24.8)
Business	5 (3.5)
Student	4 (2.8)
Marital status	
Married	118 (83.7)
Single	23 (16.3)
Age at onset	
15-20	13 (9.2)
21-25	30 (21.3)
26-30	37 (26.2)
31-35	41 (29.1)
36-40	17 (12.1)
41-45	2 (1.42)
46-50	1 (0.71)
Prior treatment taken	
Yes/no	53 (62.4)/88 (37.6)
Triple combination	22 (41.45)
Other skin lightening agent	10 (18.86)
Topical steroid	8 (15.1)
Sunscreen	6 (11.32)
Others/no documentation	7 (13.21)
Over-the-counter products	
Fairness cream	44 (61.9)
Powder	13 (18.3)
Ayurvedic cream	9 (12.7)
Others	5 (7.1)
Aggravating factors/associations	
Sun exposure	60 (42.6)
Pregnancy	34 (32.4)
Oral contraceptives	17 (16.2)
Thyroid disease	7 (4.9)
Polycystic ovarian disease	5 (3.5)
Diet	
Vegetarian	41 (21.07)
Mixed	100 (70.92)
Menstrual history	
Regular	78 (74.28)
Irregular	22 (20.95)
Menopausal	3 (2.85)
Hysterectomy	2 (1.90)
Family history	
Yes	39 (27.7)
No	102 (72.34)
Location of lesions	
Left malar	139 (98.6)

Contd...

Table 1: Contd...

Patient details	*n* (%)
Right malar	137 (97.2)
Forehead	29 (20.6)
Chin	9 (6.4)
Patterns of melasma	
Malar	98 (69.5)
Centrofacial	43 (30.5)
Fitzpatrick skin type	
Type V	128 (90.8)
Type IV	11 (7.8)
Type III	2 (1.4)

alcohol and another one who smoked cigarettes as well as drank alcohol. Of the seven (4.9%) cases with a history of thyroid disease, 6 (85.71%) were female. History of melasma in first-degree relatives was given by 39 (27.7%) cases. Menses were regular in 78 (74.28%) and irregular in 22 (20.95%) while three (2.85%) females were menopausal and two (1.90%) had undergone hysterectomy. The lesions were present on left malar region in 139 (98.6%); right malar, 137 (97.2%); forehead, 29 (20.6%) and chin, 9 (6.4%). The most common pattern of melasma observed in our study was malar (98;69.5%) followed by centrofacial (43;30.5%). Body mass index (BMI) ranged between 15.43 and 33.87 (mean; 21.75 ± 3.07); 9 (6.38%) having a BMI between 25 and 30 and 4 (2.83%) having a BMI >30.

Melasma-related quality of life score

The overall mean MELASQOL score was 28.61 (±12.92; range, 10–64), higher in males (29.25±12.44) than in females (28.39±13.13). Question 8, i.e., "skin discoloration making you feel unattractive to others," had the highest mean score (4.16±2.215); while question 7, i.e., "skin condition making it hard to show affection," the lowest (2.12±1.742). While MELASQOL score was found to have a significant negative correlation (ρ=-0.170; *P*=0.044) with the duration of disease, no correlation was found with gender, occupation, marital status, age at onset, positive family history, menstrual history, alcohol consumption, cigarette smoking, history of thyroid disease, BMI, skin type, location of lesions, and the pattern of melasma [Table 2]. The patients who had used triple combination before presenting to our hospital (22; 15.60%) had a significantly greater MELAQOL score (33.90±14.10) than those who had not used any treatment (27.63±12.51; *P*=0.036). MELASQOL score of patients who had taken treatment other than with the triple combination was not affected significantly. Three female patients with polycystic ovarian disease (PCOD) had significantly higher MELASQOL score (44±15.52; *P*=0.041) than those without (27.91±13.2). Five cases (4.8%) with hirsutism (three, known PCOD patients; two, neither having symptoms of PCOD nor ultrasonic evidence thereof), too, had a significantly higher MELASQOL score (40.4±13.01; *P*=0.040) than those who did not (27.7±13.26). Surprisingly, females who took oral contraceptives (17, 16.2%) had a significantly lower MELASQOL score (21.23±10.81; *P*=0.013) than those who did not (29.97±13.45). Vegetarians (41; 29.07%), too, had a significantly lower MELASQOL score (24.85±11.22; *P*=0.027) than those who consumed a mixed diet (30.15±13.3).

Melasma area and severity index score

The overall mean MASI score in our study was 9.07 (±6.12; range, 1.20–32.40); the highest individual scores being for the right (3.97) and left (3.86) malar regions. The MASI score was found to be higher in female (9.32±6.46) than in male (8.33±5.03), though not significantly so. MASI correlated significantly with the age of the patient (ρ=0.178; *P*=0.035) but not with the duration of their melasma [Table 2]. Patients who had melasma in the past (22; 15.6%) had a significantly higher MASI (12.01±7.96) than those who presented with the first episode (8.52±5.60; *P*=0.014).

Patients who chewed tobacco (11; 7.8%) had a significantly higher MASI (13.33±6.68) than those who did not (8.71±5.96; *P*=0.016). Positive family history, menstrual history, diet, BMI, skin type, location of lesions, pattern of melasma, history of previous treatment, sun exposure, PCOD, stress, intake of oral contraceptives revealed no correlation with the MASI score.

QoL vis-a-vis clinical severity

There was no correlation between the MELASQOL-and MASI scores (ρ=0.151; *P*=0.074).

Discussion

Our study, like previous Indian studies, revealed a marked female preponderance (F:M=3:1).[6] However, this preponderance was found to be lower than various South East Asian (Malaysia, 6:1; Indonesia, 24:1; Singapore, 21:1) and South American (Brazil, 39:1) studies.[7,8]

The mean age of our cases (32.35±7.427 years) as well as of most studies from India[7] was found to be lower than studies conducted in Singapore[9] (42.3 years) and Brazil[10] (38.43 years).

Only the malar (69.5%) and centrofacial (30.5%) patterns were found in our study, similar (malar, 65.9%; centrofacial, 34.1%) to an Iranian study of 400 cases.[11] The absence of the mandibular pattern was also reported by a Korean study, 52% and 48% of whose participants had the centrofacial and malar patterns, respectively.[12] However, in contrast to our study, centrofacial preponderance has been reported by most Indian studies, the largest with 312 cases

Table 2: Statistically significant correlations of Melasma Quality of Life and Melasma Area and Severity Index

Factor	MELASQOL	*P*
History of using triple combination		
Yes	33.90±14.10	0.036
No	27.63±12.51	
PCOD		
Present	40.4±13.01	0.040
Absent	27.7±13.26	
History of using oral contraceptives		
Yes	21.23±10.81	0.013
No	29.97±13.45	
Diet		
Vegetarian	24.85±11.22	0.027
Mixed	30.15±13.3	
Factor	**MASI**	***P***
Past history of melasma		
Yes	12.01±7.96	0.014
No	8.52±5.60	

PCOD: Polycystic ovarian disease, MASI: Melasma Area and Severity Index, MELASQOL: Melasma quality of life

revealing centrofacial (54.44%), malar (43.26%) and mandibular (1.6%) patterns.[9]

A history of aggravation by the sun, given by 42.6% of our cases was also reported by 55.5% cases of another Indian study.[9] A positive family history of melasma that was seen in 27.7% of our cases was reported by ~ 33.33% participants in the same Indian study.[9]

Though not statistically significant, the MELASQOL score in our study was surprisingly found to be higher among males than in females. However, the total mean MELASQOL score (28.61) in our study was lower than most other Indian and foreign studies,[1] being higher than only a single French study (20.9),[13,14] suggesting lesser concern about cosmesis among patients of our study.

Unlike the significant positive correlation between the duration of melasma and MELASQOL score in Mexican and French studies.[13,14] our study had a significantly negative correlation between these parameters; the same could possibly be a result from a tendency of our people to come to terms with their condition with the passage of time. However, our study along with some others[5,13,14] revealed that cases treated previously for melasma had a significantly higher MELASQOL than those who had received no treatment, suggesting there to be a "paradoxical" increase in impairment of QOL with attempted treatment.

The significantly higher MELASQOL score of our patients having PCOD and/or hirsutism could probably result from the cumulative impact of manifestations of their underlying disorders while the significantly lesser impairment of QoL in cases taking oral contraceptives from their, somewhat, lower MASI. The "confounding" finding of significantly lesser MELASQOL score in our study patients on a vegetarian diet versus — those on mixed — needs unraveling by future larger studies.

The lower mean MASI score (9.07) in our study — as compared to that of Brazilian (10.6), American (14.68), Singaporean (12.1), Spanish (10), and north Indian (20) studies[1,4,15] could be due to the preponderance of the malar (69.5%) pattern in our study along with other racial and/or environmental factors.

The significant correlation of MASI score with the age at presentation but not the duration of the disease or the age of onset suggests the possible contribution toward the severity of melasma by multiple unknown factors. The lack of correlation of MASI score with factors such as pregnancy, stress, PCOD, or intake of oral contraceptives in our study was surprising and could probably be due to their episodic nature. Lack of ultrasonography in all cases of hirsutism was one of the limitations of our study. The surprising significant higher MASI score in cases who chewed tobacco in our study also necessitates validation by larger studies.

As compared to cases presenting with their first spell of melasma, those with past history had a significantly higher MASI score; the majority of the latter having gone through pregnancy i.e. could have got primed for a more severe disease course.

QoL based on Hindi version of MELASQOL questionnaire did not correlate with the severity of melasma as assessed by MASI in our study population. A similar lack of correlation in several recent studies – including the original by Balkrishnan *et al.*,[5] and two studies from South America[4] – suggests the clinical severity to be one among many criteria (known and unknown) with which the patients assess the burden of their disease. Nonetheless, positive correlation between QoL based on MELASQOL/generic questionnaires and the clinical severity of melasma has also been reported in some, including an Indian, studies.[4]

Conclusion

The lack of correlation between the QoL and clinical severity in the present study most likely resulted due to the "paradoxical" decrease in impairment of QoL with the increasing duration of melasma despite its greater severity with age.

A significantly greater impairment of QoL identified with shorter duration of melasma and the presence of PCOD and/or hirsutism in our study is possibly indicative of the requirement of extra sensitivity on the part of the physicians for these patient groups. In general,

the patients of melasma may well be treated as per the severity of their lesions, while the revival of their impaired QoL may necessitate in-depth counseling and psychotherapy, as appropriate.

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Nil.

Conflicts of interest

There are no conflicts of interest.

What is new?

- There was no correlation between impairment in QoL and clinical severity of melasma in our study probably due to the "paradox" of decrease in impairment of QoL with increase in duration and/or age.
- Our study identified two groups – those with shorter duration of melasma and those having symptoms of PCOD – of patients with a significantly greater impairment of QoL
- Patients treated previously with "triple combination" therapy had a greater impairment in their QoL as compared to those who had taken any other/no treatment.

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Research Article

COMPARATIVE STUDY BETWEEN BENZALKONIUM CHLORIDE TRI-SODIUM PHOSPHATE AND NALC-NAOH DECONTAMINATION METHODS FOR RECOVERY OF *MYCOBACTERIUM TUBERCULOSIS* FROM PULMONARY SAMPLE

BHIRANGE S., MISRA R.N., HATOLKAR S., GUPTA N. AND JADHAV S.*
Department of Microbiology, Dr D.Y. Patil Medical College Hospital and Research Centre, Dr D. Y. Patil University, Pimpri, Pune, 411018, India
*Corresponding Author: Email - patilsv78@gmail.com

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Abstract- Introduction: Tuberculosis [TB] is a major public health concern worldwide over the last decades. About 80% of the global TB burden is in low income countries. Globally Multi drug resistant Tuberculosis caused an estimated 6 lack new TB cases and 2.40000 deaths in 2016.MDR TB accounts for 4.1% of all new TB and 19% of previously treated cases. Most of them occur in South America, Southern Africa, India, China and the former Soviet Union. Microbial diagnosis of TB consists of conventional and molecular methods but decontamination plays major role for perfect staining and cultures. **Material and methods:** 60 pulmonary samples were treated with benzalkonium chloride Tri-sodium phosphate and NALC-NaOH methods. All processed for ZN staining and LJ cultures were incubated at 37°C for 8 weeks. **Results:** Out of 60 respiratory clinical samples, 36 (60%) clinical samples were Z-N smear positive and 24(40%) were Z-N smear negative. Culture positivity was observed as 33 (55%) by NALC-NAOH method and 35 (58.3%) by Benzalkonium chloride method.
Conclusion: Benalkonium chloride trisodium phosphate appears to be a reasonable incorporation in decontamination procedure and is fairly non-toxic to *mycobacterium* to improve growth in culture and a reasonably better mucolytic reagent.

Keywords- *Tuberculosis, Benzalkonium chloride method*

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Introduction

Tuberculosis (TB) is a major public health concern worldwide: despite a regular, although slow, decline in incidence over the last decade, as many as 8.6 million new cases and 1.3 million deaths were estimated to have occurred in 2012. About 80% of the global TB burden is in low-income countries, where pulmonary disease and transmission are serious public health problems [1,2]. Multi-drug-resistant tuberculosis (MDR-TB) is a form of tuberculosis (TB) infection caused by bacteria that are resistant to treatment with at least two of the most powerful first-line anti-TB medications (drugs), isoniazid and rifampin. Some forms of TB are also resistant to second-line medications, and are called extensively drug-resistant TB (XDR-TB) [3,4]. MDR-TB caused an estimated 600,000 new TB cases and 240,000 deaths in 2016 and MDR-TB accounts for 4.1% of all new TB cases and 19% of previously treated cases worldwide. Globally, most MDR-TB cases occur in South America, Southern Africa, India, China, and the former Soviet Union [5,6]. The microbiological diagnosis of TB is an important tool for disease control. It consists of both conventional methods (acid-fast microscopy, culture, biochemical identification, anti-tuberculosis drug-susceptibility testing; DST) and modern molecular techniques. The targets of microbiological testing include the detection and isolation of mycobacteria, species identification, detection of drug resistance, monitoring patient responses to therapy and epidemiological typing of *Mycobacterium* strains [7,8]. A culture is performed on either solid media, for example Lowenstein-Jensen (L-J) or Middlebrook 7H10/11 (Petroff's method, modified Petroff's, NAOH 2% N-acetyl-cystein), or liquid media. There are several types of decontamination methods for digestion and homogenization of pulmonary specimens for TB. In that we are selecting NALC–NAOH method and Benzalkonium Chloride tri-sodium phosphate method to evaluate for their independent efficacies and for culture recovery of *Mycobacterium tuberculosis*.

In developing countries, culture on Lowenstein-Jensen solid medium is the gold standard for microbiological diagnosis of TB and requires about 10 bacilli/ml of specimen for recovery of mycobacteria. The slow growth rate of the pathogen leads to a delay of 4-6 weeks in obtaining a definitive diagnosis [3,4, 9,10].

Material and Methods

The study was conducted at the Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital and Research Center, Pimpri, Pune. 60 clinical samples were diagnosed for pulmonary tuberculosis. Each sample was processed with following procedures: Sample Preparation About 5 ml of BAL and sputum samples were collected. These were divided into two equal aliquots and processed for ZN Staining and decontamination. The amount of the samples was 2-2.5 ml approximately for both methods. BAL samples were centrifuged and sediment was processed for decontamination procedure. Work was done in Biosafety cabinet level II Ziehl-Neelsen (Spot and early morning samples of sputum were collected in 2 sterile wide mouth containers were processed and graded on the same day as per Revised National Tuberculosis Control Program (RNTCP) guidelines) (ZN) staining for each sample was done before and after decontamination [5].

Decontamination of sputum sample

1) NALC-NAOH method
2) Benzalkonium Chloride phosphate method

Culture: After decontamination with both methods. The samples were inoculated directly on Lowenstein Jensen (LJ) medium prepared in house.

Table-1 *Distribution of direct smear examination and culture results*

Results	Microscopy			Culture		
	Direct (%)	NALC-NAOH (%)	BCTSP (%)	Direct (%)	NALC-NaOH (%)	BCTSP (%)
Positive	36	36 (60 %)	36(60 %)	36	33 (55%)	35 (58.3%)
Negative	24	24 (40%)	24(40%)	24	24 (40%)	23 (38.3%)
contamination	NA	00	00	NA	3 (5%)	2 (3.3%)
Total		60			60	60

Table-2 *Week wise (Wk) growth observations on LJ Medium*

Methods	Total Samples	1st Wk	2nd Wk	3rd Wk	4th Wk	5th Wk	6th Wk	7th Wk	8th Wk	Total%
Nalc-NaOH method	60	0	4(6.7%)	9(15%)	10(16.7%)	3(5%)	2(3.3%)	4(6.7%)	1(1.6%)	33%
Benzalkonium trisodium phosphate Method	60	0	0	25(41.7%)	5(8.3%)	2(3.3%)	2(3.3%)	1(1.6%)	0	35%
Contamination NALC-NaOH method	0	0	0	0	1	2	0	0	0	
Contamination Benzalkonium chloride Tri-sodium Phosphate method	0	0	0	0	1	1	0	0	0	

Table-3 *Comparison of two decontamination methods for rate of contamination, negative culture and culture positives*

Concentration method	No. of contaminated slopes	Negative cultures (No of slopes with no growth upto 8 weeks	Positive cultures (No.of slopes with growth upto 8 weeks)
Nalc-NaOH method	3 (5%)	24 (40%)	33 (55%)
Benzalkonium tri-sodium phosphate Method	2 (3.3%)	23(38.3%)	35 (58.3%)

Table-4 *Sensitivity and specificity for culture of NALC-NaOH and BCTSP method*

NALC-NaOH/ BCTSP r	NALC-NaOH Method +ve	NALC-NaOH Method -ve
BCTSP Method +ve	32	3
BCTSP Method -ve	1	24

Note: NALC-NaOH method is Gold Standard and BCTSP method is a Test method

NALC-NAOH Method

The sample was treated with an equal volume of N-acetyl-L-cysteine (NALC) plus 2 percent NaOH, the mixture was vortexed for 20 sec and kept at room temperature for 15 min. To this was added phosphate buffer (pH- 6.8-7) and centrifuged at 3000 g for 15 min. The deposit was re-suspended in 1 ml of buffer, and 0.1 ml from this was used as an inoculum to LJ culture media [6].

Benzalkonium Chloride –Trisodium Phosphate Method

5 g analar tri-sodium phosphate was dissolved in 20 ml hot sterile distilled water, to which 0.35 ml of 17 per cent benzalkonium was added. Equal parts of sample and prepared solution were mixed in a mechanical shaker, and then allow it to stand for 30 min at room temperature; this solution was neutralized by adding phosphate buffer (pH 6.8-7) and centrifuged at 3000 g for 15 min [7, 8].The concentrated deposit was re-suspended in 1 ml of sterile normal saline, and then LJ culture media was then inoculated by 0.1 ml of sediment solution. The culture slants were incubated at 37ºC up to 8 weeks. All Slopes were observed for occurrence of growth daily for first week and then at weekly intervals for 8 weeks. Absence of growth at the end of 8th week was reported as negative culture. Contamination, if any, was recorded separately.

Results

Out of 60 samples, 36 (60%) were ZN smear positive and 24(40%) were smear negative. Culture positivity was observed as 33 (55%) by NALC-NAOH method and 35 (58.3%) by Benzalkonium chloride method [Table-1]. On LJ typically rough, tough and buff colored growth of *Mycobacteria* appeared frequently after 3 weeks; however, some specimens took a longer period for exhibiting typical growth (6-8 wk).

Discussion

In this study, we used the NALC-NAOH decontamination method of Kent and Kubica *el al*. as it is widely used and recommended by standard laboratory manuals by WHO, BCTSP concentration method resulted better than NALC-NAOH method [1]. Microscopy and culture are the two important tools for diagnosis of TB but as microscopy requires as few as 10-100 bacilli/ml to be detected and it also detects dead bacilli, culture is considered as Gold standard [9]. When researcher processed for Microscopy before and after decontamination with both methods did not show any difference in smear readings. Negative culture and contamination rate were minimum with BCTSP method. Study done by Kent and Kubica et. al. and Pathak, Deshmukh and Menon (1973) suggested to use NALC-NaOH method over modified Petroff's Method (NaOH-4%)[1,16]. We kept our concentration as suggested. Diagnosis cannot be established only with smear and it should be correlated with culture and also provides confirmation in smear doubtful cases. One sample which did not show growth after decontamination with NALC-NAOH method showed growth after decontamination with BCTSP method which increases its sensitivity. When growth rate was concern it has been observed that average growth rate is good *i.e.* generally in 3rd week with BCTSP method. Comparison of two methods after smear microscopy shown following results for sensitivity and specificities. In Smear positive samples culture of BCTSP Method showed 96.9% and 88.89% Sensitivity and specificity over culture of NALC-NaOH method while in smear negative cases it showed 100% Sensitivity and specificity over 95.65% method. In this study more positive culture were achieved by BCTSP method. BCTSP method is more efficient method shown by these results. Our results were also comparable by Chattergee M. and Zabel L. in their studies. [14-16] .It has been reported that decontamination rate of BCTSP is lesser due to its chemical combination. NaOH method may treat the sample harshly causing killing of Mycobacteria. Average growth time to be positive is considerably less *i.e.* two weeks for smear positive cases with BCTSP method is added advantage for early diagnosis however BCTSP processed sediments cannot be used for further processing like Molecular diagnostics could be one of the limitations.

Conclusion

The modified Petroff's method is applied in combination with NALC-NaOH method and is now widely used for decontamination of pulmonary samples from tuberculosis patients. Addition of benzalkonium to trisodium phosphate appears to be a reasonable digestion procedure since these are fairly non-toxic to mycobacteria and a reasonably better mucolytic reagent. As sample size is less in this study it could be a limitation to comment.

Application of research: Benzalkonium chloride trisodium phosphate appears to

be a reasonable incorporation in decontamination procedure and is fairly non-toxic to *mycobacterium* to improve growth in culture and a reasonably better mucolytic reagent.

Research Category: Medical Microbiology

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***Principal Investigator or Chairperson of research: Dr Savita Jadhav**
University: Dr D. Y. Patil University, Pimpri, Pune, 411018
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Study area / Sample Collection: Department of Microbiology, Dr D.Y. Patil Medical College Hospital and Research Centre, Pimpri, Pune, 411018, India

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Original Article

Rural prevalence of type 2 diabetes mellitus: A cross sectional study

Hetal K Rathod, Sanjay S Darade[1], Uday B Chitnis, Jitendra S Bhawalkar, Sudhir L Jadhav, Amitav Banerjee

Department of Community Medicine, Padmashree Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D.Y. Patil Vidyapeeth, [1]Medical Officer, Maharashtra State Health Services, Primary Health Centre, Walha, District Pune, Maharashtra, India

ABSTRACT

Background: Recent studies in India indicate rising trends of diabetes even in rural areas. Continuous monitoring of the diabetes situation is required by repeated cross sectional studies in different parts of the country both urban rural to plan control measures. **Aim:** To estimate the prevalence of Type 2 diabetes in a sample of rural population and explore associations between diabetes and known risk factors. **Materials and Methods:** A cross sectional study was carried out in 3 villages in the rural field practice area of a medical college in Pune, India. All eligible adults of both genders were included and screened for diabetes by house to house survey. A total of 1000 subjects were examined. Physical examination included measuring height, weight, and waist hip ratio. Blood glucose was estimated using glucometer. Family history of diabetes was also elicited. Data was analysed by descriptive statistics using proportions with 95% confidence intervals. Various associations were explored using using Odds Ratio with 95% confidence intervals as applicable. **Results:** The prevalence of diabetes mellitus was 9.1% (91/1000; 95% CI 7.4, 11). Most cases of newly detected diabetics were in the age group 36 – 40 years. There was no association between gender and diabetes (OR = 1.38, 95% CI 0.88, 2.17). Overweight status was associated with diabetes: 38.5% (35/91) of diabetics were overweight compared to 18.6% (169/909) of non-diabetics (OR = 2.74, 95% CI 1.69, 4.41). Similarly abnormal waist hip ratio was associated with diabetes: 47.25% (43/91) of diabetics had high waist hip ratio compared with 29.59% (269/909) of non-diabetics (OR = 2.13, 95% CI 1.35, 3.37). Also family history was strongly associated with diabetes: 27.5% (25/91) of diabetics gave positive family history compared with 9.4% (85/909) of non-diabetics (OR = 3.67, 95% CI = 2.13, 6.30). **Conclusion:** The burden of diabetes was present in the rural population studied. The associated known risk factors were also prevalent and showed strong relationship with diabetes. Diabetes mellitus erstwhile thought to be a disease of urban life appears to be equally prevalent in the rural setting.

Key words: India, Type 2 diabetes, risk factors, rural population

INTRODUCTION

The diabetes situation in India has worsened in the last two decades. Estimates from studies in the decade 1990 to 2000 show prevalence ranging from 6.3% to 11.6%.[1-5] Majority of studies in the subsequent decade indicate a rising trend. The National Urban Diabetes Survey (NUDS) a population based survey carried out in six large cities in India among 11, 216 subjects aged over 20 years showed the age standardized prevalence of 12.1%.[6] The Prevalence of Diabetes in India Study (PODIS) done in 108 centres reported a prevalence of 5.9% in the urban and 2.7% in rural areas.[7] A house to house survey in rural Mysore reported a prevalence of 3.8%[8] The Chennai Urban Rural Epidemiology Study (CURES) showed a prevalence of 15.5% in Chennai in 2006[9] The Amrita Diabetes and Endocrine Population Survey (ADEPS), a community based cross-sectional survey done in urban areas of Ernakulam district in Kerala has revealed a very high prevalence of 19.5%[10] This study has reported the highest prevalence of diabetes in a population in India. A recent study from Maharashtra showed a high prevalence of diabetes in a population in India.[11] A very high prevalence of 13.2% was also reported in a rural

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Corresponding Author: Dr. Hetal K. Rathod, Associate Professor, Department of Community Medicine, Padmashree Dr. D. Y. Patil Medical College, Pune - 411 018, Maharashtra, India. E-mail: hetalwaghela@rediffmail.com

population of Andhra Pradesh by Chow *et al* 2006.[12] A multicentric study on industrial populations in different parts of the country reported prevalence of 10.1%.[13]

There are indications that Indians have a younger age of onset of diabetes compared to other ethnic groups.[14] Whereas a study in 1986[15] at Delhi reported that none of the diabetics were aged less than 30 years, the National Urban Diabetes Survey (NUDS) done in 2001 showed that the prevalence of diabetes in those aged below 30 was 5.4%.[6,16] A further shift towards younger age groups was demonstrated by the Chennai Urban Rural Epidemiology Study (CURES).[9] These temporal trends towards lower age groups are disturbing and have long term impact on health and economy of the country.

Gender distribution from community studies in India show conflicting results. While some studies show female predisposition[4,16-18] others have reported higher prevalence in males.[17,19] Still others, have found no gender difference in prevalence.[6,7,16]

The association of socio-economic status and diabetes also shows interesting changes. What was earlier considered a disease of the rich and affluent is now also prevalent among the blue collar workers. The Chennai Urban Population Study (CUPS)[19,17] was done to assess the effect of socio-economic status on the prevalence of Type 2 diabetes and related abnormalities. The prevalence was 12.4% in the middle income group and 6.4% in the lower income group. Similarly, a study from New Delhi showed that even the slum dwellers had high prevalence of obesity, glucose intolerance and dyslipidemia.[18,20] Moreover, studies show that poor diabetic subjects are more prone to complications as they have inadequate access to health care.[19,21]

Though a number of studies on diabetes have been carried out in the country, and the risk factors are well known, we require continuous monitoring of the diabetic situation in the country which is in a state of rapid socioeconomic transition with attendant lifestyle changes. In view of this, the present study was carried out to get an estimate of diabetes problem and associated risk factors in a sample of a rural population in Maharashtra India.

Material and Methods

Study design

A cross-sectional population-based study.

Study area

The study was conducted in the rural field practice area of a medical college in Pune, India. The rural field practice area is located about 30 km from the city of Pune and comprises a group of seven villages around the holy pilgrimage Alandi on the banks of the river Indrayani, totalling a population of 40,000.

Sampling

Three villages in the rural field practice area were selected. These three villages were selected due to their proximity to the rural health center to facilitate treatment and follow up of the detected cases. In these villages, all eligible adults of both gender were screened for diabetes mellitus by house to house survey.

Interview and data collection

On visiting the house, members in the household, who were 25 years old and above of either gender, were invited to participate in the study and were offered to sign a written consent form. All those who agreed were included in the study. Data were collected from the study participants by face to face interview and physical examination on a pretested structured instrument. Physical examination included anthropometry (height, weight, waist circumference, and hip circumference), blood pressure measurements in sitting position, and general examination. Blood glucose estimation was done using Glucometer (Bayer Corporation — Principal Sensor, calibrated for plasma glucose)

Ethical issues

Besides consent from each study participant, ethical clearance for the study was obtained from the Institutional Ethical Committee. All diabetics both old cases and newly diagnosed cases were provided treatment and referral at the medical college hospital.

Case definition of diabetes mellitus

The World Health Organization criterion for laboratory diagnosis and monitoring of diabetes mellitus was followed for the case definition of diabetes mellitus.[20, 22] A person was labelled as diabetic if he was diagnosed and on treatment for diabetes; or if random blood sugar by screening at the time of house to house survey was equal to or more than 200 mg/dl. These subjects were further investigated at the rural health training centre, Alandi for fasting blood sugar and two hours postprandial blood sugar. If fasting blood sugar was equal to or more than 126 mg/dl and postprandial equal to or more than 200 mg/dl than labelled as new patient of diabetes mellitus.Persons with raised blood sugar were also tested ketone bodies in urine.

Height

This was measured with tape to the nearest centimetre. Subjects were requested to stand upright without shoes

with their back against the wall, heels together and eyes directed forward.

Weight

It was measured with traditional spring balance (bathroom scale) that was kept on a firm horizontal surface. Subjects were asked to wear light clothing and weight was recorded to the nearest 0.5 kg.

Waist circumference

Waist was measured using steel measuring tape with measurement half way between the lower border of the ribs and iliac crest in a horizontal plane.[21,23]

Hip circumference

Hip circumference was measured at the widest point over buttock.[21,23]

Waist-hip ratio (WHR)

Waist hip ratio considered abnormal > 0.95 for males and >0.85 for females.[21,23]

Body mass index

It was calculated using the formula:

BMI = Weight (Kg)/height (m^2)

Obesity was defined as anyone having BMI equal and above 25 kg/m^2 according to the recommended guidelines for Indians

Results

Non response rate: Out of 1020 eligible subjects approached, 1000 agreed to participate giving a non-response rate of less than 2%.

Age and gender of the study population. The mean age of the study population was 41.67 years ± 14.4 years; 44.4% (444/1000) were males and 55.6% (556/1000) were females.

Religion. Majority98.5% (985/1000) were Hindus, followed by Muslims 0.9% (9/1000), Buddhists 0.2% (2/1000), Jain 0.1% (1/1000), Sikh 0.2% (2/1000), others 1% (1/1000).

Marital status. Most 94.5% (945/1000) were married, 3.5% (35/1000) were unmarried, 1.8% (18/1000) were widowed, 0.1% (1/1000) were divorced and 0.1% (1/1000) were separated.

Educational status. Of the total sample surveyed, 32.5% (325/1000) were illiterate, 45.0% (450/1000) had completed schooling and 22.5% (225/1000) had college education.

Prevalence of diabetes mellitus in the study population. Out of the 1000 adult subjects surveyed 9.1% (91/1000) were found to be diabetics (95% CI 7.4, 11) by house to house survey. Out of these 4.5% (45/1000) were known diabetics and 46/1000 (4.6%) were newly detected cases during the present survey.

Age wise distribution of diabetes mellitus. This is shown in Figure 1. The point to note is that while known diabetics were confined to older age groups, the largest proportion of newly detected diabetics, 28.3% (13/130) was contributed by the age group 36-40 years.

Gender and diabetes. Out of the 444 males in the study, 21 were known diabetics and 26 were newly detected cases. Out of the 556 females in the study, 24 were known diabetics and 20 were newly detected cases. There was no significant gender difference in the prevalence of diabetic mellitus (OR = 1.38 with 95% CI 0.88 to 2.17)

Obesity and diabetes mellitus. There was a positive association between obesity and diabetes mellitus as shown in Table 1.

Association of waist hip ratio and diabetes mellitus. There was also positive association between waist hip ratio and diabetes mellitus [Table 1]

Positive family history and diabetes mellitus. As expected there was an association of diabetes mellitus with a positive family history of diabetes as shown in Table 1.

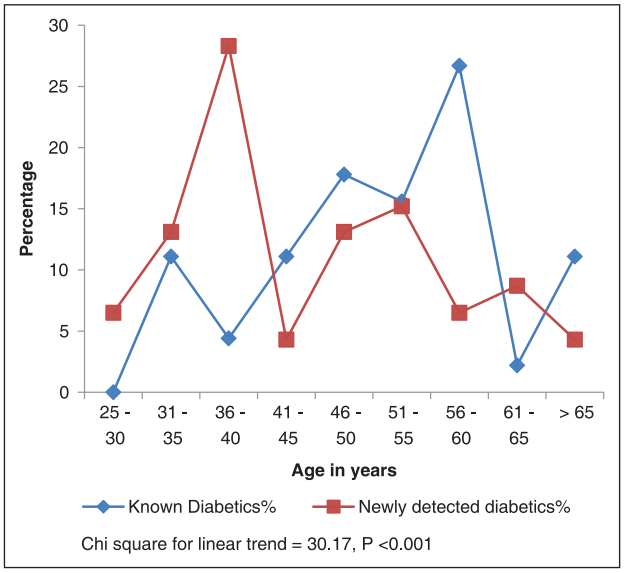

Figure 1: Association between age and diabetes mellitus

Table 1: Association of various risk factors with diabetes mellitus

Risk Factor	Diabetic (%)	Non Diabetic (%)	Odds Ratio	95% Confidence interval	*P* value
Obesity					
Obese	35 (38.5)	169 (18.6)	2.7	1.68-4.41	<0.001
Non-Obese	56 (61.5)	740 (81.4)			
Waist Hip Ratio					
Normal WHR	48 (52.75)	640 (70.41)	2.13	1.35-3.37	<0.001
Above normal WHR	43 (47.25)	269 (29.59)			
Family History					
Present	25 (27.5)	85 (9.4)	3.67	2.13-6.30	<0.001
Absent	66 (72.5)	824 (90.6)			

Discussion

This study finding highlights the fact that diabetes which was erstwhile associated with urban lifestyle is also prevalent in the rural population. This may be because urban ways of living and sedentary lifestyles are gradually being adopted by the rural masses as well.

Another important finding of the present study was that the largest proportion of newly detected cases 28.3% (13/130) were in the relatively younger persons in the age group of 36 to 40 years, which was highly significant.

Prevalence of diabetes in the present study, i.e. 9.1% (95% CI 7.4, 11) is similar to a study from rural Maharashtra carried out some years back which found prevalence of 9.3%.[11] Around the same period, a study in rural area of Andhra Pradesh found a higher prevalence of 13.2%.[12] Similar prevalence has been reported from central Kerala (9%).[9] A study from Gujarat reported a higher prevalence at 13.8%,[22,24] while a study from the northern state of Kashmir around the same period found a much lower prevalence of 6.05%.[23,25]

The other associations found in the present study such as associations of diabetes with obesity, abnormal waist hip ratio (indicator or central obesity), and family history of diabetes, re-emphasises that diabetes mellitus is a combination of several metabolic abnormalities. Most of the cardiovascular risk factors such as dyslipidemia, hypertension, central obesity, and glucose intolerance have been shown to be associated with glucose intolerance and combination of these can lead to coronary heart disease. The combinations of these factors was first coined as "syndromeX" by Reaven[24,26]

Indians have insulin resistance and adiposity even at birth as compared to Europeans.[25,27] Barker *et al* suggested that this consequence of inadequate intrauterine nutrition.[26,28] Another view is that CAD and low birth weight may have common genetic determinants. In India, according to the National Health Survey, the prevalence of low birth weight among neonates is 28%.[27,29] A strong association for low birth weight with insulin resistance has been shown in Indian children. A study of a cohort of 1492 subjects followed starting in 1969 revealed that the prevalence of diabetes was highest among subjects with lowest weight at age 2 and highest weight at age 12.[28]

In view of above factors which are now emerging and are intimately associated with rural poverty, in times to come diabetes may emerge as a disease of poverty rather than a disease of affluence which it was in the developed world decades earlier.

Poor people in rural India have a tendency to go barefoot and even when wearing footwear rarely use socks, making them vulnerable to diabetic foot, an important complication.

In view of the emerging problem of diabetes catching up in the rural areas, the traditional strategy of control i.e. the high risk strategy and population strategy should also be implemented in rural populations.

While the present sample may not represent all rural areas of the country as India is a multiethnic country with vast regional differences, they indicate that in coming decades India will have to cope with a high rural burden of diabetes care. The challenge seems more daunting because of the fact that India has a poor track record of equitable distribution of health services with the rural people having poor access to quality health services[29] which is essential for management of lifelong condition such as diabetes. As diabetes is also one of the major risk factors for coronary heart disease, the incidence of this condition also may show a rising trend in rural India because of undetected and uncontrolled diabetes.

Conclusion

The burden of diabetes seems to assuming public health importance in rural areas due to dissemination of urban lifestyle and multiple risk factors.

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Research Article

INCIDENCE OF *M. mucogenicum* INFECTION IN TERTIARY CARE HOSPITAL INDIA: RECENT INCREASE IN NUMBER OF NTM CASES

VYAWAHARE CHANDA R., JADHAV SAVITA V.*, MISRA RABINDRA NATH, GANDHAM NAGESWARI R. AND HATOLKAR SWARUPA

Department of Microbiology, Dr. D Y Patil Medical College, Pune
*Corresponding Author: Email-patilsv78@gmail.com

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Abstract- Nontuberculous mycobacteria (NTM) are emerging pathogens that affect both immune-compromised and immune-competent patients. Recently *Mycobacterium mucogenicum* (*M. mucogenicum*) has been identified as significant cause of post surgical wound infection, soft tissue infection, catheter related sepsis, peritonitis, following peritoneal dialysis, bacterimia in patients undergoing haemodialysis, and central venous line associated sepsis, meningitis, pneumonia and lymphadenitis. **Aims and objectives**: The present study was designed for retrospective analysis to identify incidence of *M. mucogenicum* from various clinical samples and to do comparative analysis with reference to clinical syndrome, predisposing factors and its demographic information. **Materials and Methods**: Various clinical samples were received from suspected NTM infection with symptomatic and compatible radiographic findings. Isolation, identification of NTM and *M. mucogenicum* were done by standard conventional methods and liquid culture in automated MB BacT culture system. Molecular genotyping were done by Line probe assay (LiPA) for identification of NTM and *M. mucogenicum*. **Results:** A total of 13 strains of *M. mucogenicum* were identified out of 30 NTM strains. Of the total *M. mucogenicum*; 11 [85%] of the strains were isolated from extra pulmonary origin and 2 [15%] strains were from lung infections; of which 1 was of the paediatric patient having tuberculous lymphadenitis and 1 case was from geriatric age group having tuberculous appendicitis only one case was immune-compromised while remaining 12 cases were immune-competent. Isolation of *M. mucogenicum* from case of endometrium and sub ovarian cyst were rare findings from present study. **Conclusion:** Attention should be given to *M. mucogenicum* isolates as a possible etiology of infection. Clinicians should be alert to those unique aspects of NTM disease concerning diagnosis with advanced molecular methods and successful treatment with limited options.

Keywords- NTM, *M. mucogenicum*, LiPA.

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Introduction

Nontuberculous mycobacteria (NTM) are emerging pathogens that affect both immune-compromised and immune-competent patients. The incidence and prevalence of NTM lung disease are increasing worldwide and rapidly becoming a major public health problem.The American Thoracic society (ATS) and Infectious Diseases Society of American (IDSA) published clinical guidelines for NTM in 2007.[1-3] Because of the difficulty in distinguishing between NTM isolation and diseases, clinical and microbiological criteria are needed for the diagnosis of NTM lung disease. Currently recommended treatment regimens, drug resistance patterns, and treatment outcomes differ according to the NTM species, and management is a lengthy complicated process with limited therapeutic options. [4,5] In NTM *Mycobacterium mucogenicum (M.mucogenicum)* has emerged as a frequent cause of infection in healthy as well as in immune-compromised patients. *M.mucogenicum* was frequently isolated from tap water, drinking water, aquatic environments like cooling towers, pools and shower water. *M. mucogenicum* was first recognised as a human pathogen in late 1970s, when it was isolated from several patients who were undergoing chronic peritoneal dialysis. [6-8] *M.mucogenicum* has been identified as water contaminant in hospital settings and could be the source of nosocomial infections such as post surgical wound infection, soft tissue infection, catheter related sepsis, peritonitis, following peritoneal dialysis, bacteraemia in patients undergoing haemodialysis, and central venous line associated sepsis, meningitis, pneumonia and lymphadenitis. *M. mucogenicum* was also reported as principal cause of infection involved in bacteraemia patients after bone marrow transplantation at a hospital in Minnesota. [9-11] However, some cases have occurred in immune-competent patients. Increasing incidence of rapidly growing Mycobacteria (RGM) infections in various clinical infections and rising incidence of *M. mucogenicum* infections in hospital settings drew attention to the prevalence of *M. mucogenicum* in our set-up.Since treatments and outcomes differ depending on the NTM species, its identification is clinically important. Traditional biochemical tests or high performance liquid chromatography for NTM identification have been replaced by molecular methods such as line probe hybridization, polymerase chain reaction (PCR), restriction fragment length polymorphism analysis, real-time PCR, and DNA sequencing. Some commercial kits are available, including the AccuProbe system (Hologic Inc.), INNO-LiPA Mycobacteria system (Fujirebio Europe, Ghent, Belgium), and GenoType Mycobacterium system (Hain Lifescience, Nehren, Germany) [4,12-13] The present study was designed for retrospective analysis to identify *M. mucogenicum* from various clinical samples and to do comparative analysis with reference to clinical syndrome, predisposing factors and its demographic information.

Material and Methods

Study was conducted in tertiary care hospital From January 2013 to December 2013 in department of Microbiology. Various clinical samples were received from

suspected NTM infection with symptomatic and suggestive radiographic findings. Related sociodemographic details and clinical findings of the patient were also documented.

Clinical samples: Various pulmonary and extra-pulmonry samples like sputum, bronchoalveolar levage (BAL), plural fluid, ascetic fluid, pus, FNAC aspirate, Lymph Node tissue, endometrial tissue, appendix tissue, CSF etc. received in the Department of Microbiology.

Ethical approval was waived for this retrospective laboratory database study

Microbiological investigation:

All the samples except samples from the sterile body sites were decontaminated by using N-acetyl L-cysteine and sodium hydroxide (NALC-NaOH) method[14]and followed as per the routine protocol for mycobacterial identification which includes: Direct microscopic examination of samples by Ziehl-Neelsen (Z-N) staining technique, culture on solid Lowenstein Jensen (L.J.) [10]media as well as liquid culture in automated MB BacT culture (Biomeuriex USA), Line probe assay (LiPA): DNA extraction and PCR- based DNA amplification and reverse hybridization. Those samples showing negative results for mycobacterium tuberculosis complex by LiPA were considered as non tuberculous mycobacteria (NTM) and then subjected for further speciation using Genotype mycobacterium CM and AS kit. [15-18]

Direct Microscopic examination of samples by Z-N staining technique:

All the samples were examined microscopically after Z-N staining for the presence of acid fast bacilli in the direct samples.

Culture on solid Lowenstein Jensen media as well as liquid culture in automated MB BacT culture (Biomeuriex USA): The MB/BacT system consists of a bottle containing 10 ml of modified Middlebrook 7H9 broth enriched with casein, bovine serum albumin, and catalase. Before inoculating specimen, bottles were supplemented with 0.5 ml of MB/BacT MAS supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin) which was reconstituted with 10 ml of MB reconstituting fluid according to the manufacturer's instructions. Bottles were placed inside the BacT Alert 3D instrument (Bio Merieux Durham, USA) and incubated at 37°C for 6 weeks. Any bottle which displayed as positive was taken out of the instrument.

Similarly all the samples were also inoculated on LJ medium and incubated at 37°C for 8 weeks. Bottles were examined for growth every week and L.J. bottles failing to show any growth after 8 weeks were discarded as negative.

Microscopy: Any growths obtained in the bottle were stained by Z-N staining for detection of acid fast bacilli (AFB).

Line probe assay (LiPA): DNA extraction and PCR- based DNA amplification and reverse hybridization were performed with a TwinCubator (Hain Lifescience GmbH, Nehren, Germany).(9) Results were read by lining strips code provided with the kit. In order for results to be valid, CC (Conjugate control) and AC (Amplification control) bands appeared for every sample.

GenoType® Mycobacterium CM and **GenoType® Mycobacterium AS:**

All isolates suggestive of NTM were then subjected to two commercial kits for further speciation, Genotype mycobacterium CM for detection of common NTMs. Isolates not identified by this were further tested with the Genotype AS assay for additional species of NTM. This test is based on the DNA-STRIP® technology and permits the identification of various Mycobacterial species such as *M. simiae, M. mucogenicum, M. goodie, M. celatum, M. smegmatis, M. genavense, M. lentiflavum, M. heckshornense, M. szulgai, M. phlei, M. haemophilum, M. kansasii, M. ulcerans, M. gastri, M. asiaticum and M. shimoidei*

DNA extraction: DNA extraction was performed by sonication. Identification of Mycobacterium tuberculosis complex (MTBC) and NTM species were carried out by using specific sets of primers designed to amplify a species specific 23S rRNA gene sequence of *Mycobacterium* species.

Amplification: Amplification mixture was prepared & amplification carried out in thermal cycler which involved 01 cycles of denaturation solution (DEN) at 95°C for 15 min, annealing of primers at 95°C for 30 s, 2 min at 58°C for 10 cycles, then 20 cycles at 95°C for 25 s, 53°C for 40 s and 70°C for 40 s and final primer extension at 70°C, 8 min for 01 cycle.

Hybridization: Hybridization was done on TwinCubator. 20 µl of amplified product was mixed with 20 µl of DEN (blue) added and incubated for 5 min at room temperature. Then 1 ml of pre-warmed hybridization buffer (HYB, Green) was added, followed by gentle shaking. Now strip was placed in a manner to make sure complete flooding of solution over strips. Then tray was placed in TwinCubator and was incubated for 30 min at 45°C, followed by complete aspiration of HYB. Washing was done by 1 ml of stringent (STR) wash solution followed by ringer solution wash to each strips and incubated. Then 1 ml of diluted conjugate was added to each strips and incubated for 30 min on TwinCubator. Strips were washed again with 1 ml of ringer solution for 1 min, after that 1 ml of diluted substrate were added to each strips and incubated for 3-20 min in the absence of light without shaking. Rinsing was done twice with distilled water to stop the reaction. Strips were removed and dried between two layers of absorbent paper.

Interpretation: Evaluation and interpretation of results were done based on the presence and absence of different bands and compared with reference band as provided in the kit.

Result:

A total of 13 strains of *M. mucogenicum* were identified out of 30 NTM species from various samples received in the department of Microbiology from the suspected cases of NTM infection. The absence of any other pathogenic agent supports the potential clinical significance of *M. mucogenicum*. Of the total 13 *M. mucogenicum* strains 10(76.92%) strains were showed acid-fast bacilli in direct Z-N staining [Fig-1], 11 were also grown on Mac-Conkey's agar and were stained with Gram's staining showed gram positive bacilli and were catalase test positive. All these strains were grown on L-J medium in 3-7 days of incubation at 37°C [Fig-2]. Growths of all 13 strains were also detected in liquid culture in automated MB BacT 2-5 days.[Fig-3]

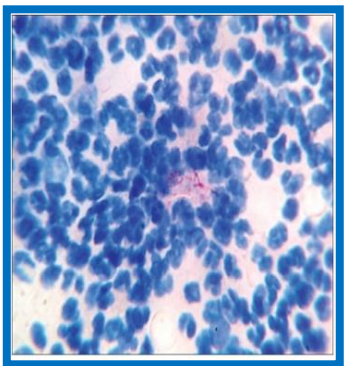

Fig-1 Acid-fast Bacilli from clinical specimen

Fig-2 Growth on L.J medium

Fig-3 Growth in liquid medium: BacT/ALERT

Table-1 *Details of the isolated M.mucogenicum strains*.

Sr.no	Age (yrs)	Sex	Diagnosis	Sample
1	45	M	Lung infection	Bronchoalveolarlevage (BAL)
2	52	M	Tubercular empyema	Pus
3	43	F	Right axillary swelling	Lymph Node tissue
4	28	F	Breast cancer	FNAC fluid
5	51	M	Pleural effusion	Pleural fluid
6	28	M	Osteomyelitis (Left femur)	Drain tip
7	6	M	TB lymphadenitis	Lymph Node tissue
8	28	M	Miliary Tuberculosis	BAL
9	65	F	Tuberculous appendicitis	Excised appendix tissue
10	48	M	Pleural effusion	Pleural fluid
11	30	F	Tuberculous appendicitis	Excised appendix tissue
12	20	F	Endometritis	Endometrial tissue
13	30	F	Right subovarian cyst	Endometrial tissue

Of the 13 samples 7(53.84%) samples were from male and 6(46.15%) samples were from female. 11 [85%] of the samples were from extra pulmonary origin and 2 [15%] ceases were from lung infections [Table-1]

Among the 13 cases only 1 was of the paediatric patient having tuberculous lymphadenitis and 1 case was from geriatric age group having tuberculous appendicitis, rest all were from young adults.

MB BacT liquid cultures were positive in all the 13 cases.

Following the Genotype assay for mycobacterium speciation, all the 13 strains were identified only as Mycobacterium spp. by using CM assay, when further followed with AS assay they identified as *M.mucogenicum*. [Fig-4]

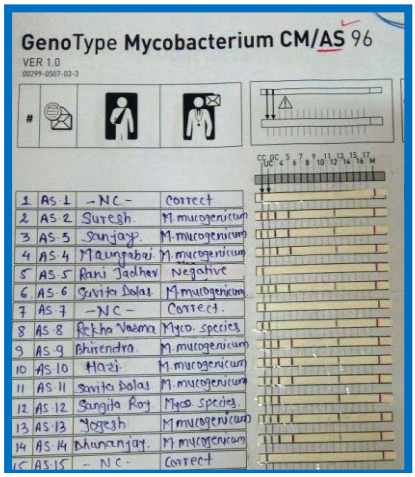

Fig-4 Genotype Mycobacterium CM/AS

Discussion

For the diagnosis of NTM disease, patients suspected to have NTM infection are required to meet all clinical and microbiologic criteria as per the American Thoracic Society (ATS) 2007[3]. Conventional biochemical tests used to identify different mycobacterial species are complex and time consuming. The development of molecular methods allows the characterization of new species and NTM identification at a subspecies level. Even after the identification of NTM species from clinical specimens, clinicians should consider the clinical significance of such findings. Besides the limited options, treatment is lengthy and varies by species, and therefore a challenge. Treatment may be complicated by potential toxicity with discouraging outcomes.

In the study mentioned period we isolated 30 NTM species; on further evaluation with Gernotype Mycobacterium CM/AS assay for speciation, 13 were *M. mucogenicum*. We could also able to isolate *M.fortuitum* in 7 samples. However, 10 isolates could not be speciated by either kit

M. mucogenicum is considered as clinically significant species of NTM. It was described in 1982 as *M. chelonae* like organism and in 1995 was delineated as a unique species *M. mucogenicum*; because of mucoid appearance of colonies.[7]

In the present study we have isolated 13 *M. mucogenicum* from various clinical samples were received from suspected NTM infection with symptomatic and suggestive radiographic findings.

Of the total 13 NTM isolates; 85% clinical specimens were from extra pulmonary site while 2 [15%] clinical samples were from lung disease and both are young immune-competent male patients.

With emergence of case reports and series from diverse countries and regions, it has become clear that the distribution of NTM species that are isolated from clinical samples differs greatly by region.

Catheter related infections are the most clinically significant cases of *M. mucogenicum* infection. From the present study we have isolated only one *M. mucogenicum* in drain tip of osteomyelitis infection and patient was immune-competent. Han XY *et al.,* (2007)[1] reported 52% *M. mucogenicum* of total NTM from blood stream and catheter related infections while in contrast Gaviria *et al.,* (2000)[19] reported 9% *M. mucogenicum* from catheter related infections which shows that frequency of isolation of clinically significant *M. mucogenicum* is increasing.

Skin and soft tissue *M. mucogenicum* infections by Shehan JM *et al.,* (2008)[20], reported skin infection associated with etanercept and Gomez-Moyano *et al.,* (2009) [21] reported furuncle-like lesions in immuno-competent patient. In the present study we have isolated *M. mucogenicum* from two endometrial tissues specimen from endometritis and right sub ovarian cyst of sexually active females and both are immuno-competent. So far there are no documented reports of isolation of *M. mucogenicum* from endometrial tissue. *M. mucogenicum* was also isolated from fine needle aspiration cytology (FNAC) fluid of breast cancer patient. There are not well documented reports of *M. mucogenicum* infection in breast cancer patients.

In present study, *M. mucogenicum* were isolated from 2 cases of disseminated infection *i e.* Tuberculous lymphanditis of six year old male patient and right axillary swelling of 43 year old female patient. Chetchotisakd P *et al*., (2000) reported *M. abscessus* infection in 16 Thai patients manifested as lymphadenopathy and multiple-organ involvement. Toidi A et al., (2006)[22] reported two fatal cases of *M. mucogenicum* central Nervous System Infection in immuno-competent patients from France.

Our study did not perform the drug susceptibility of these isolate, howeverall *M.mucogenicum* infections showed successful treatment outcome when treated by amikacin, cefoxitin, claritromycin, imipenem, trimethoprim-sulfamethoxazole, amoxicillin, amoxicillin-clavulanate, erythromycin, azithromycin, ofloxacin, gatifloxacin, levofloxacin and linezolid according to type of infection. Han *et al*.,[1] reported 100% of *M. mucogenicum* isolates were susceptible to amikacin, cefoxitin, claritromycin, imipenem and trimethoprim-sulfamethoxazole Despite availability of this in vitro data, the management of *M.mucogenicum* infection is largely based on clinical experience.

Conclusion

Attention should be given to *M. mucogenicum* isolates as a possible etiology of infection. As it is estimated that the most common origin of this bacterium is the water supply from hospital settings, consequently standard regular monitoring of hospital water and updating the infection control measures are obligatory preventive measures for patient care. High index of suspicion can detect NTM which would provide correct treatment modalities.Clinicians should be alert to those unique aspects of NTM disease concerning diagnosis with advanced molecular methods and successful treatment with limited options.

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Author Contributions: All author equally contributed

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Trends in HbA1c levels and implications for diabetes screening in tuberculosis cases undergoing treatment in India

A. N. Gupte,*† V. Mave,* S. Meshram,‡ R. Lokhande,‡ D. Kadam,‡ S. Dharmshale,‡ R. Bharadwaj,‡ A. Kagal,‡ N. Pradhan,§ S. Deshmukh,§ S. Atre,¶ T. Sahasrabudhe,¶ M. Barthwal,¶ S. Meshram,¶ A. Kakrani,¶ V. Kulkarni,§ S. Raskar,§ N. Suryavanshi,§ R. Shivakoti,* S. Chon,* E. Selvin,* N. Gupte,* A. Gupta,*† J. E. Golub*†

*Johns Hopkins University School of Medicine, and †Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; ‡Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospitals, and §Byramjee Jeejeebhoy Government Medical College-Johns Hopkins University Clinical Research Site, Pune, ¶Dr D Y Patil Medical College, Hospital and Research Centre, Dr D Y Patil Vidyapeeth, Pimpri, Pune, India

SUMMARY

SETTING: The optimal timing of screening for diabetes mellitus (DM) among tuberculosis (TB) cases is unclear due to the possibility of stress hyperglycemia.

DESIGN: We evaluated adult (⩾18 years) pulmonary TB cases at treatment initiation as well as at 3 months, 6 months and 12 months. DM was identified by self-report (known DM) or glycated hemoglobin (HbA1c) ⩾ 6.5% (new DM). Trends in HbA1c levels during treatment were assessed using non-parametric tests.

RESULTS: Of the 392 participants enrolled, 75 (19%) had DM, 30 (40%) of whom had new DM. Of the 45 participants with known DM, respectively 37 (82%) and 40 (89%) received medication to lower glucose levels at treatment initiation and completion; one participant with new DM initiated glucose-lowering medication during follow-up. The median HbA1c level in participants with known, new and no DM was respectively 10.1% (interquartile range [IQR] 8.3–11.6), 8.5% (IQR 6.7–11.5) and 5.6% (IQR 5.3–5.9) at treatment initiation, and 8.7% (IQR 6.8–11.3), 7.1% (IQR 5.8–9.5) and 5.3% (IQR 5.1–5.6) at treatment completion ($P < 0.001$). Overall, 5 (12%) with known and 13 (43%) with new DM at treatment initiation had reverted to HbA1c < 6.5% by treatment completion ($P = 0.003$); the majority of reversions occurred during the first 3 months, with no significant reversions beyond 6 months.

CONCLUSION: HbA1c levels declined with anti-tuberculosis treatment. Repeat HbA1c testing at treatment completion could reduce the risk of misdiagnosis of DM.

KEY WORDS: TB; DM; transient hyperglycemia; glycated hemoglobin; India

THE TUBERCULOSIS (TB) and diabetes mellitus (DM) co-epidemic has received considerable attention in recent years. DM is likely to increase the risk of TB nearly three-fold and result in unfavorable treatment outcomes.[1,2] TB cases are also more likely to have DM than the general population, and the prevalence of DM in adults with newly diagnosed pulmonary TB (PTB) in endemic settings is 5% to 35%.[3–7] Approximately 415 million adults worldwide have DM, nearly 80% of whom live in low- and middle-income countries.[8] Furthermore, nearly half of all DM cases worldwide remains undiagnosed.[8] India is home to nearly one third of the global TB burden, with 2.7 million incident cases and over 400 000 deaths in 2016.[9] India also hosts the world's largest burden of DM, with over 69 million adults living with the disease in 2015.[8] A recent study from southern India reported a strikingly high prevalence of DM, nearing 55% among adults with PTB,[10] and DM may account for approximately 15% of the disease burden in high TB burden countries.[11]

Given the high prevalence of DM in TB cases, a disproportionately high burden of undiagnosed DM in many low- and middle-income countries with ongoing TB epidemics, and the deleterious association between the two diseases, the World Health Organization (WHO) recently recommended bi-directional screening in people with TB and DM in endemic settings.[12,13] However, the optimal implementation of this strategy is hampered by numerous challenges, a critical one being stress hyperglycemia.[14,15] Chronic infections, including TB, can induce transient hyperglycemia, and early studies have shown a decrease in the proportion of TB cases with hyperglycemia diagnosed by oral glucose tolerance testing (OGTT) or fasting blood glucose (FBG) assessments after initiation of anti-tuberculosis treatment.[3,16] Since then, glycated hemoglobin (HbA1c)

Correspondence to: Akshay N Gupte, Phipps 540, 600 N Wolfe Street, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21287, USA. e-mail: agupte1@jhmi.edu

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testing has been widely used for DM screening worldwide. While OGTT and FBG assessments measure the glycemic status of an individual during the few hours before testing, HbA1c levels correlate with average ambient blood glucose levels during the preceding 2–3 months,[17] potentially serving as a stable test for DM screening during the acute phase of TB.

The primary objective of the present analysis was to characterize trends in HbA1c levels during anti-tuberculosis treatment and its potential impact on DM screening from an ongoing cohort study of new adult PTB cases with and without DM in Western India.

MATERIALS AND METHODS

Study population

We consecutively enrolled newly diagnosed adult ($\geqslant$18 years) PTB cases at the Byramjee-Jeejeebhoy Government Medical College-Sassoon General Hospitals (BJGMC-SGH) and Dr D Y Patil Medical College (DYPMC) in Pune, India, from December 2013. Individuals with rifampicin-resistant TB, human immunodeficiency virus coinfection, anti-tuberculosis treatment exceeding 7 days or previous history of TB were excluded. PTB cases were diagnosed by the presence of acid-fast bacilli (AFB) on smear microscopy, *Mycobacterium tuberculosis* DNA on Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) assay or *M. tuberculosis* growth on liquid culture. Sputum specimens were decontaminated using the sodium hydroxide-sodium chloride method after direct AFB staining. Centrifuged sputum specimens were inoculated on a BACTEC™ MGIT™ (Mycobacteria Growth Indicator Tube) (BD, Sparks, MD, USA) before incubation.

All study participants provided written informed consent in their native language. The study protocol was approved by the Institutional Review Boards of Johns Hopkins Medicine, Baltimore, MD, USA; BJGMC-SGH, Pune; and DYPMC, Pune, India.

Definitions of diabetes

HbA1c testing was performed using high-performance liquid chromatography (BioRad Laboratories, Hercules, CA, USA)[18] at enrollment (TB treatment initiation) as well as at 3 months, 6 months (treatment completion) and 12 months. Participants reporting current use of medication to lower glucose levels or a self-reported physician diagnosis at enrollment were classified as having known diabetes (KDM), irrespective of their HbA1c level. Newly diagnosed diabetes (NDM) was classified as HbA1c $\geqslant$ 6.5% in participants without KDM at enrollment. Pre-DM was classified as having a HbA1c level between 5.7% and 6.5% in participants without KDM at enrollment.

Participants received either an FBG or random blood glucose (RBG) test (Cobas c111; Roche Diagnostics, Rotkreuz, Switzerland) on venous blood at enrollment. As the primary objective of our study was to measure trends in HbA1c during anti-tuberculosis treatment, we did not use blood glucose results for classifying DM in our cohort.

Statistical analysis

Participant characteristics were compared by glycemic status (euglycemia, pre-DM or DM) at enrollment using the Kruskal-Wallis test for non-normally distributed continuous data and Fisher's exact test for categorical data. The median and interquartile range (IQR) of Hba1c levels and the proportion of participants who changed their glycemic status were compared between follow-up visits using the Wilcoxon sign-rank and McNemar's χ^2 tests, respectively. Change in glycemic status was defined as reversion (change in diagnostic classification towards the euglycemic end of the glycemic spectrum) or progression (change in diagnostic classification towards the diabetic end of the glycemic spectrum) (Appendix Figure A.1).* Logistic regression was used to measure the odds ratio (OR) for reversion in participants with NDM compared with those with KDM. Receiver-operator characteristic curve (ROC) analysis was performed to identify a baseline HbA1c threshold to classify participants with NDM who retained an HbA1c level of $\geqslant$6.5% following anti-tuberculosis treatment. Statistical significance was determined at $P < 0.05$. Analyses were performed using Stata v13.0 (StataCorp, College Station, TX, USA).

RESULTS

We enrolled 690 adult PTB cases; 438 (63%) had completed at least 6 months of follow-up at the time of this report and were included in the analysis. We excluded 46 (10%) participants in whom HbA1c data were unavailable at any visit during the first 6 months of follow-up (Appendix Figure A.2). Participants with unavailable HbA1c results were similar to those with HbA1c results in terms of their enrollment characteristics. The median (IQR) age and body mass index (BMI) of our cohort at enrollment was respectively 31 years (IQR 23–44) and 17 kg/m^2 (IQR 15–20).

HbA1c levels at TB treatment initiation

Of the 392 participants included in our analysis, 201 (51%) had hyperglycemia (pre-DM or DM) and 75 (19%) had DM at enrollment; 40% (30 of 75) had NDM (Figure 1). The median Hba1c level in

*The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2018/00000022/00000007/art00016

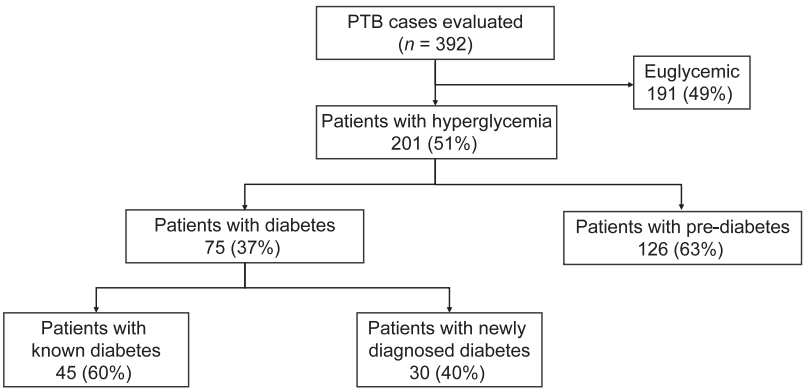

Figure 1 Glycemic status assessed by HbA1c at enrollment. Consort diagram depicting participant enrollment and glycemic status by HbA1c assessments at enrollment. PTB = pulmonary tuberculosis, HbA1c = glycated hemoglobin.

participants with KDM and NDM was respectively 10.1% (IQR 8.3–11.6) and 8.5% (IQR 6.7–11.5) ($P = 0.10$). Median self-reported duration of DM among those with KDM was 2 years (IQR 1–5). Of 45 participants with KDM, 37 (82%) were on glucose-lowering medications. Three participants with KDM and one with NDM initiated glucose-lowering medications by 6 months of anti-tuberculosis treatment. Participants with NDM were younger ($P = 0.05$), had lower BMI ($P = 0.02$), shorter time to *M. tuberculosis* growth on liquid culture ($P = 0.04$), tended to have higher smear grade ($P = 0.08$) and were less likely to have a first-degree relative with DM ($P = 0.06$) than those with KDM (Table 1).

HbA1c levels during TB treatment

The median HbA1c level in participants with KDM decreased from 10.1% (IQR 8.3–11.6) at enrollment to 9.0% (IQR 7.3–11.1) by 3 months ($P = 0.04$), with no significant change by 6 months (8.7%, IQR 6.8–11.3, $P = 0.85$) of anti-tuberculosis treatment. Six-month percentage change in the HbA1c level among participants with KDM was –7% (95% confidence interval [CI] –13 to 0, $P = 0.05$). Similarly, the median HbA1c level in participants with NDM decreased from 8.5% (IQR 6.7–11.5) at enrollment to 7.3% (IQR 5.8–9.5) by 3 months ($P < 0.001$), with no significant change by 6 months (7.1%, IQR 5.9–8.7, $P = 0.80$) of anti-tuberculosis treatment. The 6-month percentage change in the HbA1c level among participants with NDM was –14% (95%CI –19 to –8, $P < 0.001$). Similar trends in the median HbA1c level were observed in participants with pre-DM and euglycemia at enrollment, falling from 6.0% (IQR 5.8–6.1) at enrollment to 5.6% (IQR 5.4–5.8) by 6 months of anti-tuberculosis treatment among those with pre-DM ($P < 0.001$; 6-month percentage change of –7% [95%CI –8 to –6]), and from 5.4% (IQR 5.2–5.6) at enrollment to 5.2% (IQR 5.0–5.4) by 6 months of anti-tuberculosis treatment among those with euglycemia ($P < 0.001$; 6-month percentage change of –2% [95%CI –3 to –1]) (Figures 2A and 2B, Table 2).

A subset of 194 (49%) participants completed 12 months of follow-up at the time of this report: 83 (43%) with euglycemia, 65 (34%) with pre-DM and 46 (24%) with DM at enrollment. The median HbA1c level dropped further to respectively 5.4% (IQR 5.2–5.7, $P < 0.001$) and 5.1% (IQR 4.9–5.3, $P = 0.05$) by 12 months among participants with pre-DM and euglycemia at enrollment. We did not find a significant change in HbA1c levels beyond 6 months of anti-tuberculosis treatment among participants with KDM or NDM.

HbA1c reversion during anti-tuberculosis treatment

The proportion of participants with DM declined from 19% at enrollment to 15% by 6 months of anti-tuberculosis treatment ($P < 0.001$). Two participants with KDM had HbA1c < 6.5% at enrollment and were excluded from the reversion analysis. An HbA1c level below 6.5% by 6 months of anti-tuberculosis treatment was seen in 18 (25%) participants with DM at enrollment: 13 (72%) had NDM and 5 (28%) had KDM ($P = 0.003$). Logistic regression analysis adjusted for age, sex, bacterial load, smoking, alcohol consumption and BMI at enrollment found that participants with NDM were nearly five times more likely to revert their HbA1c level to a non-diabetic range by 6 months of anti-tuberculosis treatment (adjusted OR [aOR] 4.51, 95%CI 1.06–19.12, $P = 0.04$) compared with KDM. The proportion of participants with pre-DM decreased from 32% to 15% by 6 months of anti-tuberculosis treatment ($P < 0.001$); 87 (69%) participants with pre-DM at enrollment had an HbA1c level in the euglycemic range at 6 months (Figure 3). Of the 18 and 87 participants with DM and pre-DM who reverted by 6 months of TB treatment, respectively 12 (67%) and 75 (88%) did so within the first 3 months ($P < 0.01$) (Table 2). We did not find significant diabetic reversions beyond 6 months of anti-tuberculosis

Table 1 Participant characteristics according to glycemic status at enrollment

Characteristics	Euglycemia ($n = 191$) median [IQR]	Pre-diabetes ($n = 126$) median [IQR]	New DM ($n = 30$) median [IQR]	Known DM ($n = 45$) median [IQR]	*P* value*
HbA1c, %	5.4 [5.2–5.6]	6.0 [5.8–6.1]	8.5 [6.7–11.5]	10.1 [8.3–11.6]	<0.001†
Duration of diabetes, years	—	—	—	2 [1–5]	—
Glucose-lowering medications, *n* (%)	—	—	—	37 (82)	—
Age, years	27 [22–35]	29 [23–40]	46 [38–50]	50 [43–55]	<0.001†
Male, *n* (%)	106 (56)	78 (62)	27 (90)	35 (78)	<0.001†
BMI, kg/m^2	17 [15–19]	17 [15–19]	19 [16–22]	21 [18–24]	<0.001†
Waist circumference, cm‡	69 [63–75]	71 [64–78]	78 [71–85]	81 [73–89]	<0.001†
Ever smoker, *n* (%)	34 (18)	20 (16)	6 (20)	10 (22)	0.79
Regular alcohol consumption, *n* (%)	44 (23)	29 (23)	9 (30)	15 (33)	0.43
Diabetes in first-degree relatives, *n* (%)§	5 (3)	13 (11)	2 (7)	11 (26)	<0.001†
Hemoglobin, g/dl	11.9 [10.7–13.2]	11.6 [10.5–13]	12.3 [10.6–13.2]	12.8 [11.8–13.9]	0.01†
AFB smear, *n* (%)					
Negative	67 (35)	37 (30)	3 (10)	14 (31)	0.05†
1+	67 (35)	52 (42)	19 (63)	21 (47)	
2+	36 (19)	24 (19)	4 (13)	8 (18)	
3+	21 (11)	11 (9)	4 (13)	2 (4)	
MGIT TTD, days	10 [7–13]	10 [7–12]	8 [6–11]	10 [8–12]	0.87

* *P* values reported are by Fisher's exact test or for trends across groups defined by glycemic status using non-parametric tests.
† Statistically significant.
‡ Measured at the upper margin of the lateral iliac crest.
§ Defined as parents, siblings or children.
IQR = interquartile range; DM = diabetes mellitus; HbA1c = glycated hemoglobin; BMI = body mass index; AFB = acid fast bacilli; MGIT = Mycobacterial Growth Indicator Tube; TTD = time to (*M. tuberculosis*) detection.

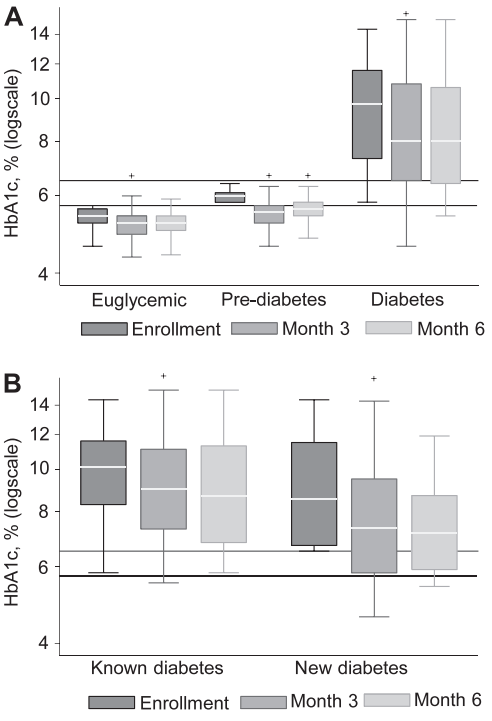

Figure 2 **A)** Change in HbA1c levels during anti-tuberculosis treatment by glycemic status at enrollment. Figure shows median (IQR) HbA1c levels during anti-tuberculosis treatment by glycemic status at enrollment. **B)** Change in HbA1c levels during anti-tuberculosis treatment by diabetes status at enrollment. Figure gives median (IQR) HbA1c levels during anti-tuberculosis treatment by type of diabetes (known and new) at enrollment. HbA1c = glycated hemoglobin; solid line = 6.5% threshold for diagnosing diabetes; dashed line = 5.7% threshold for diagnosing pre-diabetes; + = significant change since previous visit.

treatment in the subset of participants completing 12 months of follow-up.

HbA1c reversion and blood glucose

Nearly 70% of our cohort received RBG testing at enrollment. Overall, 27 (73%) participants with KDM had blood glucose in the diabetic range ($\geqslant$126 mg/dl for FBG and $\geqslant$200 mg/dl for RBG) compared with 16 (59%) participants with NDM ($P = 0.01$); none had HbA1c < 6.5% or blood glucose in the diabetic range (Appendix Table A). Interestingly, 14 (67%) participants had HbA1c $\geqslant$ 6.5% and blood glucose in the non-diabetic range, 71% of whom had NDM ($P = 0.03$) and reverted their HbA1c to the non-diabetic range by 6 months compared with only 4 (9%) participants with HbA1c $\geqslant$ 6.5% and blood glucose in the diabetic range at enrollment ($P = 0.01$). Area under the curve analysis in a subset of 23 participants with NDM at TB treatment initiation and who completed 12 months of follow-up identified a HbA1c cut-off point of 7.5% at TB treatment initiation for correctly classifying all participants with an HbA1c level in the diabetic range at both 6 months and 12 months of follow-up; 91% of participants with NDM at TB initiation and with HbA1c < 6.5% at both 6 and 12 months had blood glucose in the non-diabetic range at enrollment.

DISCUSSION

Our study is among the first to describe longitudinal trends in HbA1c levels and their potential impact on DM screening in a well-characterized cohort of PTB

Table 2 Change in glycemic status during anti-tuberculosis treatment*

	Euglycemia ($n = 191$)		Pre-diabetes ($n = 126$)		New diabetes ($n = 30$)		Known diabetes ($n = 45$)	
Glycemic status at enrollment	3 months *n* (%)	6 months *n* (%)	3 months *n* (%)	6 months *n* (%)	3 months *n* (%)	6 months *n* (%)	3 months *n* (%)	6 months *n* (%)
Glycemic status during follow-up								
Euglycemia, *n* (%)	180 (94)	185 (97)	98 (78)	85 (67)	6 (20)	7 (23)	3 (7)	0
P value†	—	—	<0.001‡	<0.001‡	0.03‡	0.31	0.25	—
Pre-diabetes, *n* (%)	11 (6)	6 (3)	28 (22)	39 (31)	5 (17)	6 (20)	2 (4)	7 (16)
P value†	<0.001‡	0.04‡	—	—	0.06	0.31	0.50	0.25
Diabetes, *n* (%)	0	0	0	2 (2)	19 (63)	17 (57)	40 (89)	38 (84)
P value†	—	—	—	0.31	—	—	—	—
Percentage change in HbA1c since previous visit								
% (95%CI)	−3 (−4 to −2)	1 (−1 to 2)	−9 (−10 to −8)	3 (1 to 5)	−12 (−18 to −7)	0 (−6 to 6)	−6 (−12 to 1)	0 (−5 to 6)
P value	<0.001‡	0.10	<0.001‡	0.01‡	<0.001‡	0.98	0.06	0.91

* Progression and reversion was defined as change in diagnostic classification towards the diabetic and euglycemic end of the glycemic spectrum, respectively.
† McNemar's χ^2 test for the proportion of participants progressing/reverting since the previous visit.
‡ Statistically significant.
TB = tuberculosis, CI = confidence interval; HbA1c = glycated hemoglobin.

cases receiving anti-tuberculosis treatment in a high TB and DM burden setting.

HbA1c levels declined significantly during anti-tuberculosis treatment, irrespective of glycemic status at treatment initiation; the greatest decline was seen in participants with NDM. Transient hyperglycemia was common, with nearly 25% of participants with DM and 70% with pre-DM reverting their HbA1c levels to the non-diabetic and euglycemic range during TB treatment, respectively. Furthermore, changes in glycemic status were common during the first 3 months of anti-tuberculosis treatment, especially in PTB cases with NDM. Our data suggest the need for repeat HbA1c testing at least 3 months after TB treatment initiation, and ideally at treatment completion, to reduce the risk of misdiagnosis of DM and pre-DM in individuals with TB.

Despite a high proportion of TB cases with hyperglycemia at TB treatment initiation, 46% reverted their HbA1c levels to the euglycemic range by treatment completion. Transient hyperglycemia on OGTT and FBG has been reported during the acute phase of TB disease,[3,5,16] and two recent studies from low DM and/or TB burden settings found similar reductions in HbA1c levels following TB treatment initiation.[19,20] Our study extends these findings to a high TB and DM burden setting. HbA1c levels decreased during anti-tuberculosis treatment in all participants, irrespective of their glycemic status at treatment initiation. The greatest reduction in HbA1c levels was observed during the first 3 months of anti-tuberculosis treatment, with no significant change beyond 6 months. While the precise mechanisms of transient hyperglycemia in TB are unclear, insulin resistance due to inflammatory stress and a long duration of illness before HbA1c testing may have played a role.[15]

Among participants with DM at TB treatment initiation, nearly 25% reverted their HbA1c levels to the non-diabetic range by 6 months; no significant reversions occurred beyond this time. Participants with NDM were at greatest risk of reverting their HbA1c to the non-diabetic range by TB treatment completion. Our results are consistent with studies

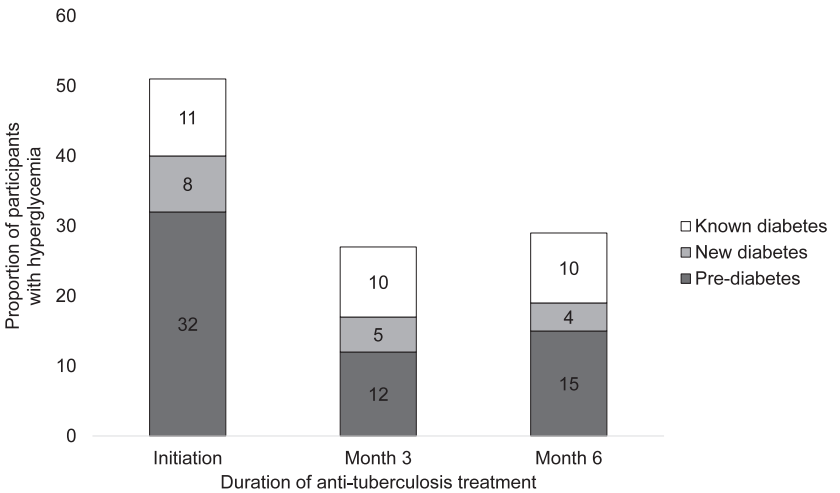

Figure 3 Proportion of participants with hyperglycemia during anti-tuberculosis treatment by glycemic status at enrollment.

Table A Glycemic status by random or fasting blood glucose testing at enrollment ($n = 366$)

Characteristics	No diabetes ($n = 323$) *n* (%)	Diabetes ($n = 43$) *n* (%)	*P* value*
Fasting blood glucose test	111 (34)	13 (30)	0.73
HbA1c, %, median [IQR]	5.6 [5.3–6.0]	10.3 [8.9–12.3]	<0.001†
HbA1c ⩾ 6.5%	19 (6)	43 (100)	<0.001†
Anti-diabetes medications	8 (3)	23 (53)	<0.001†
Age, years, median [IQR]	29 [22–38]	48 [40–55]	<0.001†
Male	194 (60)	33 (77)	0.04†
BMI, kg/m^2, median [IQR]	17 [15–19]	20 [18–23]	<0.001†
Waist circumference, cm, median [IQR]‡	69 [63–77]	80 [73–90]	<0.001†
Ever smoking	54 (17)	9 (21)	0.52
Regular alcohol consumption	76 (24)	12 (28)	0.57
Diabetes in first-degree relatives§	15 (5)	9 (23)	<0.001†
Hemoglobin, g/dl, median [IQR]	12 [11–13]	13 [12–14]	0.003†
AFB smear			
Negative	104 (32)	10 (23)	0.08
1+	121 (38)	22 (51)	
2+	59 (18)	10 (23)	
3+	37 (12)	1 (2)	
MGIT TTD, days, median [IQR]	10 [7–13]	10 [8–14]	0.46

* *P* values reported are by Fisher's exact test or for trends across groups defined by glycemic status using non-parametric tests.
† Statistically significant.
‡ Measured at the upper margin of the lateral iliac crest.
§ Defined as parents, siblings or children.
HbA1c = glycated hemoglobin; IQR = interquartile range; BMI = body mass index; AFB = acid fast bacilli; MGIT = mycobacterial growth indicator tube; TTD = time to (*M. tuberculosis*) detection.

describing transient hyperglycemia in comparable populations.[10,20,21] Compared with participants with KDM, those with NDM were less likely to have characteristics typical of conventional diabetic phenotypes (e.g., older age, higher BMI and family history of DM), more likely to have higher bacterial burden at TB treatment initiation, exhibit greater percentage declines in HbA1c levels during anti-tuberculosis treatment, and more likely to have blood glucose in the non-diabetic range and lower HbA1c levels at TB treatment initiation. These individuals might represent a distinct phenotype of 'transient DM' due to increased susceptibility to TB-induced hyperglycemia and may be misdiagnosed during screening. Importantly, our findings may also reflect unmasking of subclinical insulin resistance among individuals with an epigenetic predisposition to DM. Similar to gestational DM and stress hyperglycemia in hospitalized patients, whether transient DM in TB is associated with an increased risk of subsequent DM and microvascular disease needs further study.[22,23]

Our study had two main limitations. First, we did not perform OGTT to confirm DM diagnosis; however, stress-induced transient hyperglycemia has been reported with OGTT.[5,15,16] Second, declines in Hba1c levels during follow-up may be explained, in part, by regression to the mean (RTM). However, Hba1c is a stable test with minimal within-individual variability,[24] and we found consistent declines in absolute as well as percentage HbA1c levels during anti-tuberculosis treatment irrespective of glycemic status at treatment initiation. Given the biological plausibility and previous evidence of transient hyperglycemia in TB, and the pragmatic nature of our study, RTM is unlikely to diminish the implications of our findings in real-world screening activities, reinforcing our conclusion that repeat HbA1c testing following anti-tuberculosis treatment should be undertaken to reduce the risk of misdiagnosis of DM in TB.

Despite these limitations, results from our study have several key implications. The WHO and the American Diabetes Association (Arlington, VA, USA) recommend confirming a HbA1c test result of ⩾6.5% within 2 weeks of the initial test.[25,26] In our study, transient hyperglycemia was common despite the long half-life of HbA1c, and HbA1c levels declined significantly during anti-tuberculosis treatment in all participants, irrespective of glycemic status at treatment initiation. Our data suggest that repeat HbA1c testing should be delayed for at least 3 months from TB treatment initiation to confirm a diagnosis of DM. Second, reversion of HbA1c levels to a non-diabetic range was common in participants with NDM, highlighting the need to confirm a DM diagnosis following treatment, particularly in participants with NDM and an HbA1c level between 6.5% and 7.5%. Importantly, longitudinal studies investigating the risk of DM and microvascular disease among individuals with transient hyperglycemia during TB should be undertaken. Finally, emerging evidence suggests that medications such as metformin and statins, commonly prescribed in DM, may have antimycobacterial activity.[27,28] The impact of tran-

sient hyperglycemia on the optimal timing of DM and pre-DM screening and glucose monitoring in TB is likely to gain importance in the near future if evidence from randomized clinical trials support adjuvant therapy and tighter glycemic control to improve clinical outcomes in TB and DM.

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Conflicts of interest: none declared.

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APPENDIX

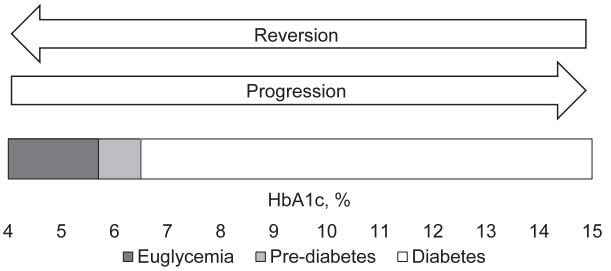

Figure A.1 Pictorial representation of reversion and progression of glycemic status. Reversion was defined as individuals with an HbA1c level in the diabetic (or pre-diabetic) range at enrollment who then had an HbA1c level in the pre-diabetic or euglycemic range (only euglycemic range for individuals with pre-diabetes at enrollment) during follow-up. Progression was defined as individuals with an HbA1c level in the euglycemic (or pre-diabetic) range at enrollment who then had an HbA1c level in the pre-diabetic or diabetic range (only diabetic range for individuals with pre-diabetes at enrollment) during follow-up. HbA1c = glycated hemoglobin.

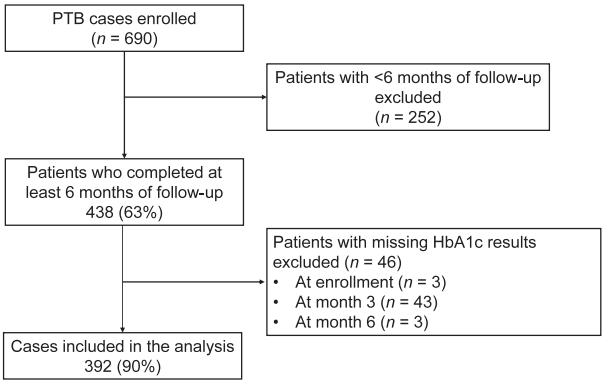

Figure A.2 Enrollment schema. Consort diagram depicting analytic sample size and participant exclusion. PTB = pulmonary tuberculosis; HbA1c = glycated hemoglobin.

RÉSUMÉ

CONTEXTE : Le moment optimal du dépistage du diabète (DM) parmi les cas de tuberculose (TB) n'est pas clair en raison de la possibilité d'hyperglycémie de stress.

SCHÉMA : Nous avons évalué des cas de TB pulmonaire adulte (⩾18 ans) lors de la mise en route du traitement, à 3 mois, à 6 mois et à 12 mois. Le DM a été identifié par autodéclaration (DM connu) ou par une HbA1c ⩾ 6,5% (DM nouveau). Les tendances de l'HbA1c pendant le traitement ont été évaluées grâce à des tests non paramétriques.

RÉSULTATS : Sur les 392 participants enrôlés, 75 (19%) avaient un DM; 30 (40%) d'entre eux avaient un DM nouveau. Sur les 45 participants ayant un DM connu, 37 (82%) et 40 (89%) ont reçu un médicament hypoglycémiant, respectivement, lors de l'initiation du traitement et de son achèvement ; un participant atteint d'un DM nouveau a débuté un traitement hypoglycémiant pendant le suivi. L'HbA1c médiane des participants ayant un DM connu, nouveau et pas de DM a été respectivement de 10,1% (intervalle interquartile [IQR] 8,3–11,6), 8,5% (IQR 6,7–11,5) et 5,6% (IQR 5,3–5,9) à la mise en route du traitement et de 8,7% (IQR 6,8–11,3), 7,1% (IQR 5,8–9,5) et de 5,3% (IQR 5,1–5,6) à l'achèvement du traitement ($P <$ 0,001). Au total, 5 (12%) patients ayant un DM connu et 13 (43%) ayant un DM nouveau lors de la mise en route du traitement ont ramené leur HbA1c à moins de 6,5% lors de l'achèvement du traitement ($P = 0,003$) ; la majorité de ces réversions est survenue pendant les 3 premiers mois, sans réversion significative au-delà de 6 mois.

CONCLUSION : Les niveaux d'Hba1c ont baissé avec le traitement de la TB. Le dosage répété de l'Hba1c lors de l'achèvement du traitement pourrait réduire les diagnostics abusifs de DM.

RESUMEN

MARCO DE REFERENCIA: No es claro cuál es el calendario óptimo para la detección de la diabetes (DM) en los casos de tuberculosis (TB), debido a una posible hiperglucemia de estrés.

MÉTODO: Se evaluaron casos de TB pulmonar en adultos (⩾18 años) al comienzo del tratamiento y a los 3 meses, 6 meses y 12 meses. La DM se definió por autorreferencia (DM conocida) o por una glucohemoglobina (HbA1c) ⩾6,5% (caso nuevo de DM). Se examinó la evolución de la concentración de HbA1c durante el tratamiento mediante pruebas no paramétricas.

RESULTADOS: De los 392 participantes inscritos, 75 sufrían DM (19%) y de ellos 30 fueron casos nuevos (40%). De los 45 participantes con DM conocida, 37 recibían fármacos hipoglucemiantes al comienzo del tratamiento antituberculoso (82%) y 40 pacientes al completarlo (89%); un participante con diagnóstico nuevo de DM inició el tratamiento hipoglucemiante durante el seguimiento. La mediana de la HbA1c al comienzo del tratamiento antituberculoso en los pacientes con DM conocida fue 10,1% (amplitud intercuartílica [IQR] 8,3–11,6), en los casos nuevos de DM fue 8,5% (IQR 6,7–11,5) y en los pacientes sin DM fue 5,6% (IQR 5,3–5,9) y al final del tratamiento este valor fue 8,7% (IQR 6,8–11,3), 7,1% (IQR 5,8–9,5) y 5,3% (IQR 5,1–5,6), respectivamente ($P <$ 0,001). En general, cinco pacientes con DM conocida (12%) y 13 casos nuevos de DM (43%) al comienzo del tratamiento antituberculoso revirtieron la HbA1c a $<$ 6,5% al final del tratamiento ($P = 0,003$); la mayoría de las reversiones ocurrió durante los primeros 3 meses y después de los 6 meses no se observaron reversiones significativas.

CONCLUSIÓN: Los valores de la HbA1c disminuyen con el tratamiento antituberculoso. El hecho de repetir la determinación de la HbA1c al finalizar el tratamiento podría disminuir los diagnósticos incorrectos de DM.

Original Paper

Use of Smartphone-Based Video Directly Observed Therapy (vDOT) in Tuberculosis Care: Single-Arm, Prospective Feasibility Study

Samuel B Holzman[1*], MD; Sachin Atre[2*], PhD; Tushar Sahasrabudhe[2], MD; Sunil Ambike[2], MSW; Deepak Jagtap[2], MSW; Yakub Sayyad[2], BHMS; Arjun Lal Kakrani[2], MD; Amita Gupta[1], MD; Vidya Mave[1], MD; Maunank Shah[1], MD, PhD

[1]Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, United States

[2]Dr D Y Patil Medical College, Hospital, and Research Center, Dr D Y Patil Vidyapeeth, Pune, India

[*]these authors contributed equally

Corresponding Author:
Maunank Shah, MD, PhD
Division of Infectious Diseases
Johns Hopkins University School of Medicine
1550 Orleans St, Cancer Research Building, Room 1M-10
Baltimore, MD,
United States
Phone: 1 443 287 0401
Fax: 1 410 502 7029
Email: mshah28@jhmi.edu

Abstract

Background: India accounts for nearly one-quarter of the global tuberculosis (TB) burden. Directly observed treatment (DOT) through in-person observation is recommended in India, although implementation has been heterogeneous due largely to resource limitations. Video DOT (vDOT) is a novel, smartphone-based approach that allows for remote treatment monitoring through patient-recorded videos. Prior studies in high-income, low disease burden settings, such as the United States, have shown vDOT to be feasible, although little is known about the role it may play in resource-limited, high-burden settings.

Objective: The goal of the research was to assess the feasibility and acceptability of vDOT for adherence monitoring within a resource-limited, high TB burden setting of India.

Methods: We conducted a prospective, single-arm, pilot implementation of vDOT in Pune, India. Outcome measures included adherence (proportion of prescribed doses observed by video) and verifiable fraction (proportion of prescribed doses observed by video or verbally confirmed with the patient following an incomplete/unverifiable video submission). vDOT acceptability among patients was assessed using a posttreatment survey.

Results: A total of 25 patients enrolled. The median number of weeks on vDOT was 13 (interquartile range [IQR] 11-16). Median adherence was 74% (IQR 62%-84%), and median verifiable fraction was 86% (IQR 74%-98%). More than 90% of patients reported recording and uploading videos without difficulty.

Conclusions: We have demonstrated that vDOT may be a feasible and acceptable approach to TB treatment monitoring in India. Our work expands the evidence base around vDOT by being one of the first efforts to evaluate vDOT within a resource-limited, high TB burden setting. To our knowledge, this is the first reported use of vDOT in India.

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KEYWORDS

Video DOT; mHealth; tuberculosis; medication adherence; telemedicine; India; mobile phone; smartphone

Introduction

Globally, tuberculosis (TB) is the leading cause of infectious disease-related mortality, responsible for 1.6 million deaths annually [1]. The incidence of TB is higher in India than anywhere in the world, with roughly 2.8 million cases reported in 2017, nearly 27% of the global TB burden [1]. To achieve positive treatment outcomes, adherence to TB therapy is critical [2,3]. However, socioeconomic and health system barriers in India are common and negatively impact adherence [4-6]. Failure to complete treatment can lead to relapse and the emergence of multidrug-resistant TB (MDR-TB), resulting in further disease transmission.

The World Health Organization (WHO) encourages the tailored use of multidimensional adherence interventions, including social, material, and psychological support, and emphasizes monitoring through directly observed treatment (DOT) [7]. Compared with self-administered therapy, those managed with DOT have demonstrated an improved rate of treatment completion [7,8]. Completion of therapy is vital not only for the patient but also the community, as public health efforts to mitigate disease spread require treatment success.

Unfortunately, DOT is often burdensome for patients and, paradoxically, can have a negative impact on adherence for some [9]. In India, DOT has historically been largely clinic-based (although there are differences in the public and private sector), wherein patients are required to bear the financial and logistical burden of frequent travel to and from the clinic for treatment monitoring. In doing so, patients risk lost wages due to time away from work. Additionally, providers must record and dispense daily treatments, a process that can be onerous and prohibitive in resource-constrained settings. While DOT is formally recommended under the current TB treatment guidelines set forth by India's Revised National Tuberculosis Control Program (RNTCP), in practice, DOT implementation (ie, observing and documenting each prescribed dose) in the community is inconsistent, and associated barriers can lead to treatment default [10-15].

More recently, video directly observed therapy (vDOT) has been introduced as a patient-centered alternative to in-person DOT, with pill ingestion monitored remotely via digital video capture. vDOT has been implemented using synchronous technologies [16-19] such as Skype and FaceTime as well as asynchronous technologies [20,21], where recorded videos are uploaded and digitally stored for future review. This latter method allows for video capture to occur at times convenient for the patient and eliminates the need for vDOT to be scheduled around staff availability. Recent work has shown asynchronous vDOT to be feasible, well received by patients and providers, and associated with high rates of treatment adherence [20-27]. Further, two economic evaluations in the United States have suggested vDOT to be cost effective over in-person DOT [20,27]. These encouraging findings have led both the US Centers for Disease Control and Prevention and WHO to suggest vDOT as a viable alternative to in-person DOT [28-30].

While data on vDOT are becoming increasingly robust, vDOT has yet to be rigorously evaluated within low- and middle-income countries of high disease burden such as India. Despite resource constraints, cellular technology has spread rapidly through India. As of 2017, there were a recorded 1.2 billion cellular connections and 291.6 million smartphone users within the country, suggesting that vDOT may have a role in this setting [31,32]. Additionally, recent changes to RNTCP guidelines have prioritized daily therapy (ie, 7 days per week) over three-times-per-week therapy, a change that further questions the feasibility of in-person DOT within a system already stretched thin and underscores the need for alternative approaches to adherence monitoring and support [14,33,34].

To address this critical knowledge gap, we conducted a prospective pilot of vDOT in Pune, India. Specifically, we addressed the feasibility and acceptability of vDOT within this resource-limited setting of high disease burden.

Methods

Overview

We conducted a prospective, single-arm, pilot implementation of vDOT in Pune, India. The mobile app emocha vDOT (emocha Mobile Health Inc) was used for treatment monitoring and adherence support (Figure 1). The patient-facing portion of the platform (ie, the mobile app) allows patients to record and transmit treatment videos. The interface also prompts patients to report any medication-related side effects (by checking off relevant symptoms from a prepopulated list). Through a calendar function, patients are able to review treatment progress and track adherence. Use of the software requires a camera-enabled tablet or smartphone device with at least intermittent access to Wi-Fi or cellular data. The app supports both Android and iOS operating systems. The provider portion of the platform can be accessed on a desktop, laptop, tablet, or smartphone (using a mobile browser) and is used by medical staff to review treatment videos. Providers are notified of any patient-reported treatment side effects. Given the system's asynchronous nature, submitted videos can be reviewed at any time following digital capture and transmission.

The emocha app is compliant with US Health Insurance Portability and Accountability Act (HIPAA) regulations and allows for asynchronous vDOT (Figure 2). Video capture occurs via the app. In the event that the device loses internet service or does not have access to internet service during video capture or upload, the videos (or any untransmitted component) remain encrypted on the device; all videos are uploaded automatically to secure servers when connection is restored (Wi-Fi or cellular data). Following transmission, videos are automatically wiped from the smartphone memory. Encrypted patient data, therefore, remain within the device only for the period between video capture and Web upload. Providers are able to access uploaded data via a secure Web interface through which they review submitted videos and track treatment progress.

The study was conducted at the Dr DY Patil Medical College Center and took place between January 2017 and June 2018. Study procedures were approved by the local institutional ethics committee and the institutional review board at Johns Hopkins University in Baltimore, Maryland.

Figure 1. The patient-facing portion of the emocha video directly observed therapy mobile app allows patients to record and transmit treatment videos, report any medication-related side effects, and review treatment progress and track adherence. The provider portion of the platform can be used by medical staff to review treatment videos and accessed from multiple devices.

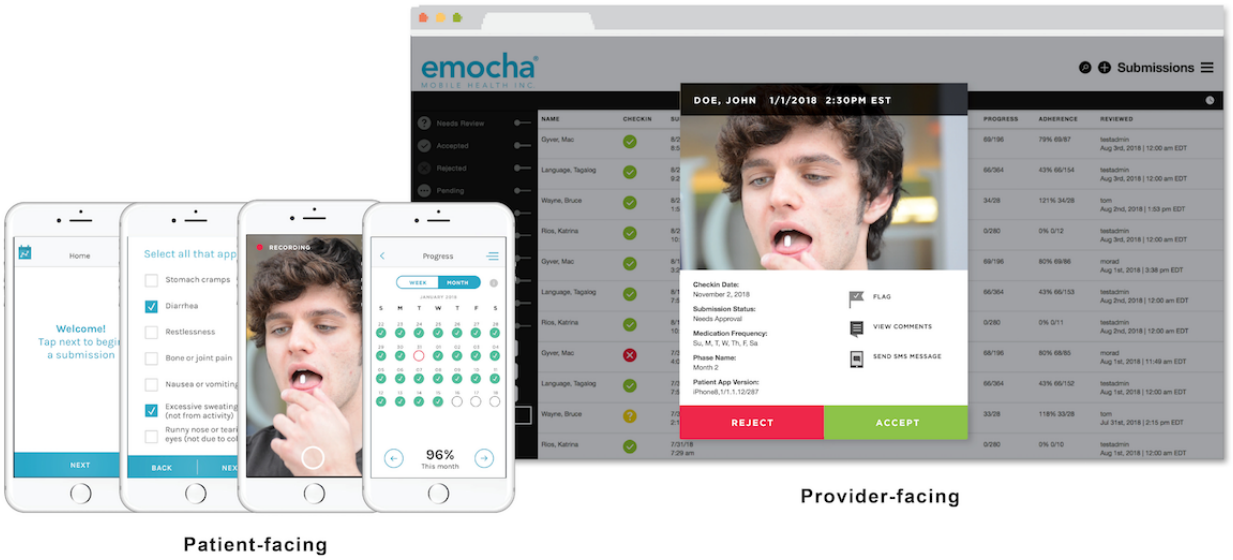
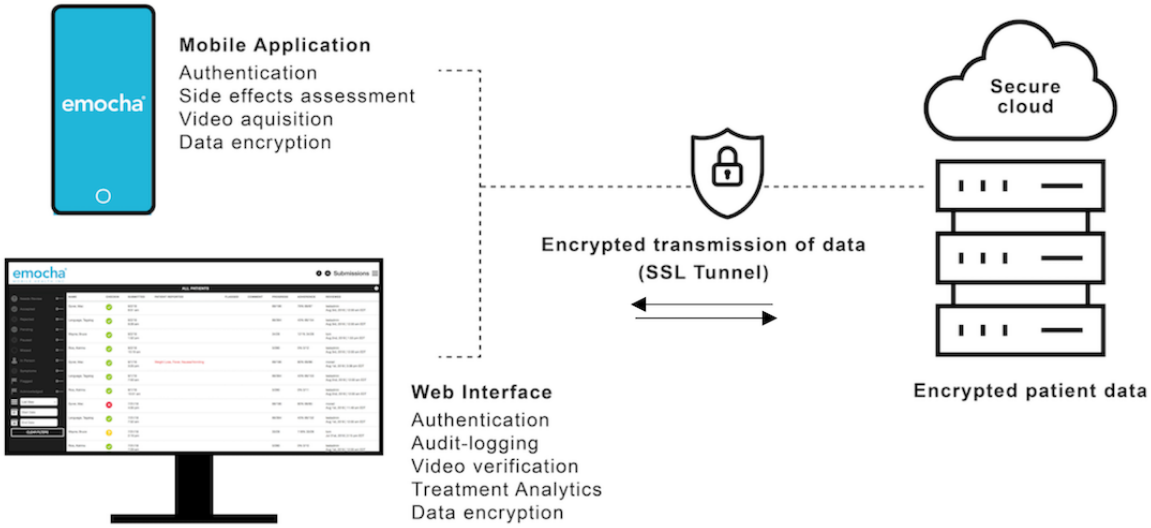


Figure 2. Data flow and security with the emocha video directly observed therapy mobile app.



Participants

Dr DY Patil Medical College Hospital is a private hospital that contains a government (public) TB treatment center (directly observed treatment, short-course, or DOTS center) as a public-private mix initiative. Patients diagnosed with or treated for TB at either Dr DY Patil or local DOTS centers were eligible for the study. Inclusion required age >18 years, signed informed consent, and >2 remaining months of TB therapy. Patients with MDR disease and HIV were excluded. Given this was a pilot study, we enrolled a convenience sample. Some patients were approached at the time of diagnosis, although many were assessed for eligibility midtreatment. Those not participating in the study received treatment and observation as per the local standard of care. Local guidelines recommend DOT for all intensive phase doses and for at least one dose per week during the continuation phase [14], although implementation is heterogenous and largely determined by local resources and patient preference (oral communication, T Sahasrabudhe, MD, November 2018).

Prior to enrollment, patients were required to establish basic smartphone proficiency and demonstrate the ability to successfully navigate the emocha app. A version of emocha translated into Marathi (the primary local language) was available to those with limited English. Patients without access to a smartphone were provided one by the study. Regardless of the device used, each participant was provided Rs 200 (US $3) each month to cover the cost of video submissions and a one-time incentive payment of Rs 100 (US $1.50) to cover travel expenses.

Study Procedures

A total of 35 patients were selected for this study based on a convenience sampling method. All patients provided written informed consent and were permitted to withdraw from the study at any time. Demographic information including

participant medical history and TB diagnosis were collected using a standardized case report form. Data were subsequently entered into a digital database by study staff. During their first study visit, participants were introduced to vDOT by a study staff member who provided each with a unique username and password and conducted a step-by-step tutorial outlining the process for how to create and submit a treatment video. Patients were then observed as they attempted to submit a dummy video independently. Additional training was provided on an as-needed basis.

Prior to formal enrollment, patients underwent a conditional 1-week run-in period, during which they were closely monitored for their continued ability to successfully record and submit videos. Any technical or logistical barriers arising during this period were addressed prior to formal study enrollment, which was only able to occur following successful completion of this trial period. For those enrolled, vDOT continued through treatment completion or until consent was withdrawn. Text message reminders via the emocha app were automatically sent to patients in the absence of expected video submissions. All incomplete or unverifiable videos (eg, medication could not be seen or video did not transmit due to network issue) were followed up with a staff phone call to verbally verify whether the dose was taken.

Feasibility

Feasibility was assessed by two primary outcomes. The first was treatment adherence, or the proportion of all prescribed treatment doses directly observed by video. As noted above, incomplete or unverifiable videos were followed up with a phone call for verbal verification. As such, a second metric, verifiable fraction, was used to describe the proportion of all prescribed doses that were either directly observed (by video) or verbally confirmed (following incomplete/unverifiable videos). All data analysis was completed in Stata 14 (StataCorp LLC).

Acceptability

To assess vDOT acceptability among patients, a posttreatment survey was administered comprising a series of categorical and Likert scale questions addressing issues such as mobile phone and internet access, emocha ease of use, convenience, and privacy. To increase our understanding of potential implementation barriers, patients were also informally asked to comment on their experiences and highlight any challenges or concerns they had related to the use of vDOT. Patient responses were noted by study staff at the time of survey administration. Staff were also asked to comment on patient-level barriers observed during the study.

Results

Study Participants

Of 35 patients who were consented and initiated the run-in phase (Figure 3), 10 did not complete the run-in and left the study. Reasons for run-in failure were related to technological (eg, inability to effectively use platform or poor cellular/Wi-Fi connectivity) and psychosocial (eg, concerns regarding privacy) barriers. Twenty-five patients were ultimately enrolled and formally initiated on vDOT with emocha. There was no study drop out, and all 25 patients completed therapy on vDOT.

Patient characteristics are described in Table 1. The median age was 27 (interquartile range [IQR] 24-42) years, 40% (10/25) were female, and 72% (18/25) reported their local language as Marathi. Most patients were low income with a monthly income less than Rs 16,000 (US $225). The majority of patients (22/25, 88%) had access to a smartphone and the internet. Three patients (3/25, 12%) required the use of a study phone. Almost three-quarters (18/25, 72%) of patients had pulmonary TB, and the remainder (07/25, 28%) had extrapulmonary disease.

Figure 3. Study flow diagram. vDOT: video directly observed therapy.

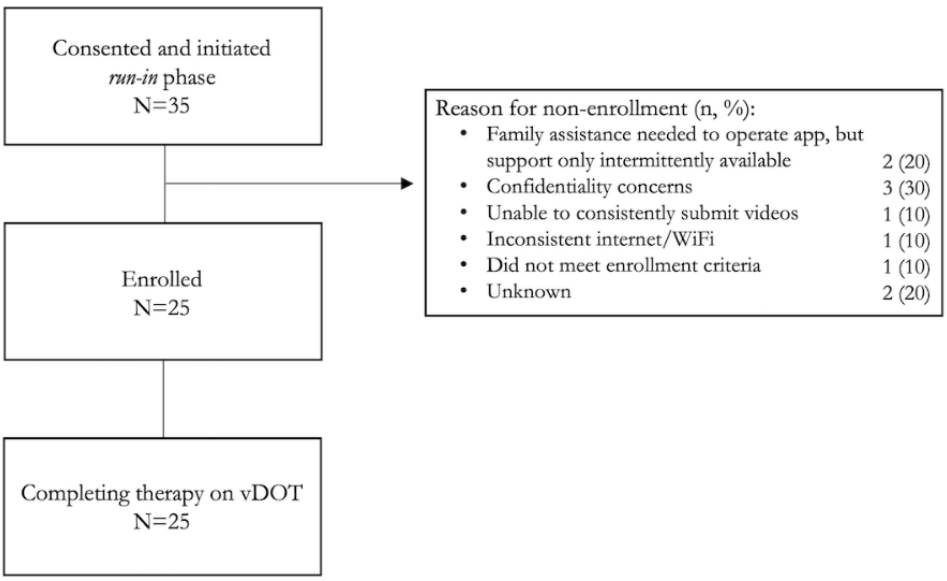


Table 1. Patient and disease characteristics (n=25).

Variable	Value
Age, year (median, IQR[a])	27 (24-42)
Female, n (%)	10 (40)
Indian state of origin, n (%)	
Maharashtra	18 (72)
Haryana	2 (8)
Karnataka	1 (4)
Tamil Nadu	1 (4)
Other	3 (12)
Primary language, n (%)	
Marathi	18 (72)
Hindi	6 (24)
Kannada	1 (4)
Employed, n (%)	10 (40)
Average monthly income (Rs), n (%)	
<2000	6 (24)
2000-4000	0 (0)
4000-8000	6 (24)
8000-16,000	13 (52)
>16,000	0 (0)
Homeless, n (%)	1 (4)
Residence, n (%)	
Urban	21 (84)
Rural	4 (16)
Married, n (%)	13 (52)
Primary mode of transportation, n (%)	
Private vehicle	0 (0)
Bus/train	0 (0)
Auto-rickshaw	8 (32)
Other private transportation	17 (68)
Substance use, n (%)[b]	
Alcohol	1 (4)
Tobacco use	0 (0)
Illicit drug use	0 (0)
Medical comorbidities, n (%)[b]	
Diabetes	3 (12)
Hypertension	1 (4)
Cancer	0 (0)
Technology, n (%)	
Regular access to a smartphone	22 (88)
Daily access to Wi-Fi or cellular data	22 (88)
Used personal device for study	22 (88)

Variable	Value
Tuberculosis category, n (%)	
Pulmonary[c]	
Smear positive	14 (56)
Smear negative	4 (16)
Exclusively extrapulmonary	7 (28)

[a]IQR: interquartile range.

[b]Categories not mutually exclusive, each out of 25 total participants.

[c]Pulmonary disease with or without extrapulmonary involvement.

The majority of patients were initiated on vDOT during the continuation phase (20/25, 80%), with 20% (5/25) beginning during the intensive phase. The median number of weeks on vDOT was 13 (IQR 11-16), with a range of 9 to 23 weeks (Table 2). A total of 80% (20/25) of patients received daily (7 times per week) therapy, while 20% (5/25) received an intermittent (3 times per week) regimen. No in-person DOT was documented either before or after implementation of vDOT. Overall, 60% (15/25) of patients reported at least one treatment-related side effect. The most commonly reported symptoms were nausea/vomiting (8/15), abdominal pain (3/15), and itching (2/15).

Table 2. Video directly observed therapy outcomes and data utilization (n=25).

Variable	Value
Adherence[a] (%), median (IQR[b])	74 (62-84)
Verifiable fraction[c] (%), median (IQR)	86 (74-98)
Dosing frequency, n (%)	
3 times per week DOT[d]	5 (20)
7 times per week DOT	20 (80)
Treatment phase at enrollment, n (%)	
Intensive	5 (20)
Continuation	20 (80)
Number of weeks on vDOT[e], median (IQR)	13 (11-16)
Total uploaded videos[f] (n)	1722
Mean uploads per patient, mean (SD)	91 (53)
Number of rejected videos per patient	
Mean (SD)	1.6 (2.4)
Range	0-8
Video length (seconds), median (IQR)	44 (31-52)
Video size (MB), median (IQR)	1.5 (1.1-1.7)

[a]Proportion of total prescribed doses completed under video observation. Of note, no in-person directly observed therapy was noted either before or after the implementation of video directly observed therapy.

[b]IQR: interquartile range.

[c]Proportion of total prescribed doses verified by any means, including successful observation by video upload and verbal dose confirmation (by phone or in person) following the submission of an incomplete or poor quality video.

[d]DOT: directly observed therapy.

[e]vDOT: video directly observed therapy.

[f]Total video (accepted + rejected + run-in phase) uploads across all patients over the length of the study.

Feasibility

Median adherence on vDOT was 74% (IQR 62%-84%, Table 2). After including verbally verified doses (following unverifiable or incomplete videos), the median verifiable fraction was 86% (IQR 74%-98%). An average of 91 (SD 53) videos were submitted per patient. The average number of rejected videos per patient was 1.6 (SD 2.4), with 56% (14/25) having no rejected videos at all. The most common reasons for video rejection were poor quality of video and medication not

fully seen. The median video length was 44 (IQR 31-52) seconds and associated with a median file size of 1.5 (IQR 1.1-1.7) MB.

Acceptability

A total of 22 posttreatment surveys were completed; 3 patients declined participation. Study outcomes for those declining involvement were similar to those of the general study population; each patient completed >14 weeks on vDOT with an adherence >70%.

A total of 91% (20/22) of surveyed patients described emocha as easy to use (Table 3). All patients (22/22, 100%) reported being able to record videos without difficulty, 95% (21/22) were able to upload without difficulty, and 91% (20/22) found text message reminders helpful. Further, all found they were able to communicate concerns and medication side effects effectively through the emocha platform. The majority felt vDOT would be more convenient (20/22, 91%) and preferred (20/22, 91%) over in-person DOT (Table 4). While 82% (18/22) felt vDOT would preserve patient privacy over in-person DOT, 18% (4/22) disagreed and felt in-person DOT would be more private.

Table 3. Responses from patient agreeability survey (n=22).

Survey statements (rated on a 5-point Likert scale)	Agree[a] n (%)	Disagree[b] n (%)
emocha was easy to use	20 (91)	2 (9)
I was able to record videos without difficulty	22 (100)	0 (0)
I was able to upload videos without difficulty	21 (95)	1 (5)
emocha text message reminders were helpful	20 (91)	2 (9)
I was able to communicate concerns and side effects using emocha effectively	22 (100)	0 (0)

[a]Agree/strongly agree were grouped.

[b]Neutral/disagree/strongly disagree were grouped.

Table 4. Responses from patient preference survey (n=22).

Survey statements (categorical)	Value, n (%)
Videos were most often uploaded using	
Wi-Fi at the clinic	0 (0)
Wi-Fi at home or other location	0 (0)
Cellular data (3G/4G)	22 (100)
Which better preserves patient privacy?[a]	
vDOT[b]	18 (82)
In-person DOT[c]	4 (18)
No preference	0 (0)
Which is more convenient?[a]	
vDOT	20 (91)
In-person DOT	2 (9)
No preference	0 (0)
Preference for therapeutic monitoring[a]	
vDOT	20 (91)
In-person DOT	2 (9)
No preference	0 (0)

[a]In-person directly observed therapy (DOT), either prior to enrollment or while on video directly observed therapy (vDOT), was inconsistently performed and/or documented based on chart reviews. Answers referring to in-person DOT are therefore based on patient perceptions of what in-person DOT would be like.

[b]vDOT: video directly observed therapy.

[c]DOT: directly observed therapy.

Study coordinator notes were reviewed and summarized in Table 5. Broadly, these notes revealed patient-level barriers impacting the successful implementation and use of vDOT. Included were psychosocial factors, such as the privacy concerns and stigma,

and mental health barriers. Despite survey data suggesting that most were able to record and upload videos without issue, poor connectivity and cellphone-related challenges (eg, subscriber identity module [SIM] card malfunction) were noted in a few cases.

Table 5. Patient-level barriers to successful video directly observed therapy use as identified by study staff.

Barrier to vDOT[a] use	Representative patient quotes and/or problem details
Psychosocial	
Stigma	"Recently one of my close relatives expired. As you know, we need to be at home to complete all the rituals up to 15 days after death. All the relatives are there, around all the time, and it became difficult to go out as well. So I could not take videos. Otherwise they would have started asking. Due to that, sometimes I missed my medicines."
Hospital admission	One patient suffered from severe alcohol dependence. The patient was successful on vDOT for a period but later admitted for detoxification. The patient's phone was confiscated at the time of admission, leaving him unable to upload videos during his hospital stay.
Stress	"My 1-year-old son fell from the bed and his hand got fractured. He was unwell, so we were under stress. I took tablets but during that time, I did not record videos."
Technology-related	
Connectivity	"I went to my village for 8 days for some work. As we do not have range and connectivity to the internet, I could not send videos."
vDOT-related challenges	"The registration process is a bit complicated and time-consuming. Can it be simplified?"
	"The [vDOT] app got hanged in my mobile. I did not know how to reinstall it. So I could not send videos."
	"When [recording a] video, if I get a call, the application used to suddenly shut down. So the video [would get lost]."
SIM[b] card	"I did not submit Know Your Customer documents required for SIM verification. Hence my SIM card was deactivated for some time...I was not able to send videos."

[a]vDOT: video directly observed therapy.

[b]SIM: subscriber identity module.

Discussion

Principal Findings

Our pilot study suggests that vDOT may be a feasible option for verification of medication adherence for TB patients in India. Among enrolled participants who completed a short run-in period to assess technological literacy, we found that a median 74% of all prescribed doses were observed. Further, when including doses verbally confirmed (following incomplete video submissions), the proportion of verified doses (verifiable fraction) increased to 86% (based on 1722 submitted and reviewed videos), exceeding the adherence goal of >80% set forth by current treatment guidelines [28]. This degree of adherence is comparable to that described using vDOT in other settings, such as the United States, and advances current evidence supporting vDOT, as prior work has largely focused on implementation within resource-rich settings [16,20,27,35]. To the best of our knowledge, this is the first reported use of vDOT in India.

Our demonstration of vDOT feasibility within the Indian context is both timely and critical given the recent RNTCP guideline changes emphasizing the need for daily over intermittent (3 times per week) therapy [14,33,34]. While a DOTS strategy, based on the principle of direct treatment observation, has been in place in India for over two decades, in practice, DOT implementation has been inconsistent.

In Pune, our experience has been that patients are often provided medication weekly or biweekly, with adherence monitoring largely based on self-report. At best, clinic services, including in-person DOT, are generally available 6 days per week, permitting a maximum of only 85% of prescribed (daily) doses to be observed. In contrast, by decoupling video capture from provider review, asynchronous vDOT potentially allows for all (100%) doses to be observed and obviates the need to coordinate DOT around staff availability.

To successfully and sustainably implement DOT in India, alternatives to in-person DOT are clearly needed. vDOT has the potential to be this alternative and to fill the needed gap. Our study is among the first in a resource-limited setting to demonstrate that daily therapy can be confirmed through the use of innovative mobile technologies. vDOT saves health care worker time and obviates the need for in-person visits to observe treatment [22]. For settings where home visits are employed solely for DOT, vDOT may reduce costs and save time even further [18,20,27,36,37]. vDOT may also have other previously unrecognized benefits related to infection control. Provisions for personal protective equipment (ie, masks for health care workers) or environmental controls (isolation rooms) are limited in India; vDOT offers a mechanism to closely monitor patients while reducing potential transmission opportunities. Additionally, we observed that patients derived benefit from avoiding frequent clinic visits, for which associated travel leads to lost time and, often, wages. Most importantly, vDOT provides

solid evidence of treatment adherence. Our study also highlights a need for patient training (eg, run-in period with onboarding to the technology), counseling, and follow up in cases of missed doses to assure successful treatment completion.

Of note, India has already endorsed another electronic form of treatment monitoring, 99DOTS: when a patient removes a pill from a blister pack, a number is revealed that completes a toll-free phone number printed on the pack, which the patient then calls to report having taken daily medication [12,33]. While 99DOTS may be a feasible means for basic adherence monitoring [38], vDOT has the distinct advantage of providing video confirmation of pill ingestion. It is also important to consider that the use of vDOT allows for adherence support in addition to adherence tracking. The platform used in this study captures side effects and TB symptoms, and videos can also be used to notify providers of treatment concerns, such as rashes, which can be preliminarily evaluated from afar through submitted videos. Moreover, the current platform allows automated messaging reminders, which patients reported to be a benefit. Newer versions of the software offer secure chat functionality (with health care providers) and case management tools that may further support treatment adherence. India recently rolled out a direct benefits transfer scheme that encourages treatment adherence through the use of financial incentives (Rs 500 per month while on therapy) [39,40]. 99DOTS is currently being used as a mechanism to monitor treatment adherence, but it is limited. For the reasons noted above, a more reliable tamper-proof means of adherence monitoring would be beneficial.

Limitations and Strengths

While our work supports further evaluation of vDOT within India, we acknowledge several study limitations. First, our sample size was small and, while we have shown vDOT to be feasible in one location, its acceptability and feasibility in other parts of India remain unknown. Second, we were unable to compare adherence on vDOT to that under the existing standard of care, which at our site was primarily self-administration (thus precluding documentation of prestudy adherence). Our findings, however, suggest that vDOT implementation could substantially improve adherence documentation compared with current practice. Through broader implementation, vDOT has the potential to enable enhanced accountability among TB clinics with regard to treatment adherence. Improvements in documentation would also increase the availability of high-quality data on TB treatment completion for public health reporting practices. Whether vDOT is associated with improved patient outcomes compared with standard of care is still unknown and was not assessed within the scope of this pilot study.

We also acknowledge a significant attrition over the course of our run-in period. One-third of those who consented did not ultimately participate in the study. Drop out during this period was largely driven by technological barriers related to infrastructure (eg, inconsistent cellular coverage) or inability/unease with smartphone operation. Further, despite the fact that we used a HIPAA-compliant app (emocha) with stringent security controls, several participants withdrew consent over privacy concerns. Some patients noted a fear that their treatment videos might end up publicly viewable on the internet. While cellphone technology has spread rapidly across India, cellular coverage remains incomplete and not all have become immediately facile with the technology. With time, these barriers may diminish. A strength of our study was the use of a run-in period, which was advantageous in that it allowed for rapid identification of those with sufficient mobile phone literacy to be candidates for vDOT. In our study, all those who completed the run-in period and enrolled in the study successfully finished therapy on vDOT.

Conclusions

Despite its promise, there remain questions regarding vDOT that must be addressed. Larger controlled and comparative trials will be needed to better evaluate the effectiveness of vDOT against the current standard of care or alternative technologies in resource-limited, high disease burden settings. Future studies addressing cost and cost effectiveness are also needed. Last, in other settings such as the United States, vDOT has successfully been coupled with individualized case management to allow real-time intervention after missed doses; the role of this approach in India is unknown [20]. Overall, our work has shown that despite socioeconomic and structural barriers, vDOT may be a feasible approach for treatment monitoring in India.

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Authors' Contributions

MS created the study concept and design. SA, SBH, SA, DJ, TS, SM, MB, and AK were responsible for the acquisition of data and SA and SBH for statistical analyses. SBH, MS, SA, and TS performed data interpretation. SA and SBH drafted the initial manuscript, and all authors participated in manuscript revision.

Conflicts of Interest

MS is one of the inventors of the miDOT technology. Under a license agreement between emocha Mobile Health Inc and Johns Hopkins University, MS and the university are entitled to royalties on technology described in this article. This arrangement has

been reviewed and approved by Johns Hopkins University in accordance with its conflict of interest policies. To mitigate any potential conflicts of interest, all clinical decision making regarding use of miDOT or enrollment in the study was made by nonconflicted department of health clinicians and staff; MS recused himself from all data analysis but assisted with results interpretation.

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Abbreviations

DOT: directly observed treatment
DOTS: directly observed treatment, short-course
HIPAA: Health Insurance Portability and Accountability Act
IQR: interquartile range
MDR-TB: multidrug-resistant tuberculosis
RNTCP: Revised National Tuberculosis Control Program
SIM: subscriber identity module
TB: tuberculosis
vDOT: video directly observed therapy

WHO: World Health Organization

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Original Article

Correlation of NAFLD fibrosis score and BARD score with ultrasonographic evidence of nonalcoholic fatty liver disease in overweight patients: A prospective study

Arjun Lal Kakrani, Zubin Dev Sharma, Sarabjeet Singh Thind[1], Vijayashree Shrirang Gokhale

Departments of Medicine, and [1]Radiodiagnosis, Pad. Dr. D.Y. Patil Medical College and Research Centre, Sant Tukaram Nagar, Pimpri, Pune, India

Address for the Correspondence:
Dr. Zubin Sharma, Department of Medicine, Pad Dr. D. Y. Patil Medical College and Research Hospital, Sant Tukaram Nagar, Pimpri, Pune - 18, India.
E-mail: drzubinsharma@gmail.com

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) fibrosis score and BARD score are two of the many noninvasive scoring systems used in the evaluation of the fibrosis in patients with NAFLD biochemically. Ultrasound (USG) is the most common imaging modality for detection of hepatic fibrosis, as it is inexpensive and easily available. **Aims:** This study attempts to correlate the biochemical and ultrasonographic evidence of fibrosis. It tries to correlate two noninvasive tools for assessing fibrosis in overweight population with NAFLD. **Materials and Methods:** Prospective study was conducted in which 106 patients participated with BMI; more than 25 underwent ultrasonography for evidence of fatty liver, which was then categorized in three grades, and also scored using biochemical parameters to obtain the NAFLD fibrosis score and BARD score. The scores were then compared with the grades of fatty liver on USG to see for correlation between the two. **Results:** No statistically significant correlation was found between biochemical evidence of fibrosis and USG evidence of fibrosis in overweight patients of NAFLD. **Conclusion:** Thus, the biochemical evidence of fibrosis or NAFLD in the form of NAFLD fibrosis score did not correlate with USG evidence of fatty liver. The USG findings of fatty liver may not directly correlate with actual fibrosis in these patients. **Context:** NAFLD is also an emerging disease in developing countries, which remains silent for years. Noninvasive methods are required for early diagnosis. This study attempts to correlate two noninvasive tools for assessing fibrosis in overweight population of NAFLD. Most of the patients of NAFLD are asymptomatic thus invasive methods are not routinely recommended in them. Thus, it becomes pertinent to study the noninvasive tools extensively for their possible additive use.

Key words: BARD score, imaging fatty liver, nonalcoholic fatty liver disease fibrosis score, nonalcoholic steatohepatitis

INTRODUCTION

Nonalcoholic fatty liver disease (NALFD) and nonalcoholic steatohepatitis (NASH) are common often "silent" liver diseases. They resemble alcoholic liver disease, but occur in people who drink little or no alcohol. The major feature in NASH is fatty infiltration of the liver, along with inflammation. Most people with NASH feel well and are not aware that they have a problem. It is commonly an accidental diagnosis when patient is evaluated for some unrelated illness. Asymptomatic transaminase raise is the most common finding in patients of NAFLD.

Nevertheless, NASH can be severe and can lead to cirrhosis, in which the liver is permanently damaged and scarred and can progress to hepatocellular carcinoma.

Currently no non-invasive modality is validated enough to be used for staging of patients with NAFLD and estimating the fibrosis especially in developing countries where advanced imaging studies such as elastography and MRI may not be routinely available.

Ultrasonography (USG) is the most common imaging modality for detection of hepatic fibrosis, being relatively inexpensive and easily available. On USG, fatty liver is seen as a bright liver with echogenicity

of liver more than the right kidney. Overall, USG has a sensitivity of 60-94% and a specificity of 84-95% for detecting fat,[1] NAFLD fibrosis score and BARD score are two of the many noninvasive scoring systems used in the evaluation of the fibrosis in patients of NALFD biochemically.

Angulo *et al.*,[2] developed and validated a simple noninvasive scoring system consisting of routinely measured and easily available clinical and laboratory variables to discriminate between the presence or absence of advanced fibrosis in patients with NAFLD. In a multicenter trial consisting of 480 patients in the derivation cohort and 253 patients in the validation cohort, a low cutoff (≤1.455) signified the absence of advanced fibrosis and a high cutoff (>0.676) indicated the presence of advanced fibrosis.[2]

The NAFLD fibrosis score consists of six variables, namely age, BMI, AST/ALT ratio, hyperglycemia, platelet count, and serum albumin.

The BARD score was calculated using three easily available variables. These include BMI >28 kg/m (1 point), AST/ALT ≥0.8 (2 points), and diabetes (1 point). Harrison *et al.*,[3] using this score, showed that a score of 2 to 4 was associated with an odds ratio of 17 (95% CI, 9.2-31.9) for predicting advanced fibrosis.

NAFLD is an emerging disease, even in developing countries, which remains silent for years. Most of the patients suffering from NAFLD are asymptomatic thus invasive methods are not routinely recommended in them. Thus, it becomes pertinent to study the noninvasive tools extensively for their possible additive use.

Till date no study has directly compared the NAFLD fibrosis score with imaging changes of the fatty liver on USG. This study attempts to correlate the biochemical and ultrasonographic evidence of fibrosis. It tries to correlate two noninvasive tools for assessing fibrosis in overweight population of NAFLD.

MATERIALS AND METHODS

After clearance from institutional ethics committee and consent, all patients aged 18 and above with body mass index (BMI) of more than 25 and evidence of at least grade 1 fatty liver on ultrasonography (USG) were included in this study.

Patients with common (HBV, HCV) and less common (autoimmune, Wilson's disease, alpha-1-antitrypsin deficiency) liver diseases, hepatic malignancies, infections of biliary tract, alcohol intake of more than 40 g/week in men and 20 g/week in women were excluded. Also, patients with a history of systemic illnesses known to cause fatty liver disease, and those who are receiving or have recently received drugs (including herbal medicines) known to raise ALT, AST to cause fatty liver disease were excluded.

Total 106 patients fulfilling the inclusion–exclusion criteria were included in the study. It was a prospective study done at a tertiary care hospital between July 2010 and September 2012 and data were generated as per the performa and patient's records.

Definitions

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) was defined as (a) there is evidence of hepatic steatosis, either by imaging or by histology and (b) there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders

Overweight

Patients with body mass index (BMI) of more than 25 calculated as mass (in kilogram) per height (in meters). It was taken as per the World Health Organization (WHO) international classification.

Fatty liver

Fatty liver was defined by the presence of at least two of three abnormal findings on abdominal ultrasonography. The USG of all patients was done on the same USG machine and in similar lighting conditions.

Diffusely increased echogenicity (bright) liver with liver echogenicity greater than kidney or spleen, hepatic vascular blurring, and deep attenuation of ultrasound signal in posterior liver and diaphragm.

Fatty liver was graded by following means:

Grade 1 (Mild) – minimal diffuse increase in hepatic echogenicity with normal visualization of diaphragm and intrahepatic vessel borders

Grade 2 (Moderate) – moderate diffuse increase in hepatic echogenicity with slightly impaired visualization of intrahepatic vessels and diaphragm

Grade 3 (Severe) – marked increase in echogenicity with poor penetration of posterior segment of right lobe of liver and poor or no visualization of hepatic vessels and diaphragm.

Metabolic syndrome – metabolic syndrome was defined as per the International Diabetes Federation Guidelines (IDF).[4]

On inclusion in the study, the patient were assessed by anthropometric, clinical, and biochemical parameters. The data generated were then used to score the patients using the NAFLD fibrosis score and the BARD score. Data were also analyzed to categorize patients with metabolic syndrome using the above said IDF guidelines.

The various variables assessed were as follows:

1. Anthropometry: Height, weight, BMI, waist circumference, hip circumference, waist to hip ratio
2. Blood pressure measurement
3. Biochemical: S. Bilirubin, Serum ALT, AST and ALP, S. Proteins including albumin, globulin, S. GGT, S. Ferritin, and fasting serum lipids
4. Complete hemogram.

NAFLD fibrosis score[2]

The NAFLD fibrosis score was calculated according to the following formula:

$-1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{IFG/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/A LT ratio} - 0.013 \times \text{platelet (}\times 109/\text{L)} - 0.66 \times \text{albumin (g/dL)}.$

NAFLD score ≤1.455 = less probability of fibrosis
NAFLD score −1.455-0.675 = indeterminate score
NAFLD score >0.675 = high probability of fibrosis.

BARD score[3]

BARD score was calculated as weighted sum: BMI ≥28 = 1 point + AAR of ≥0.8 = 2 points + DM = 1 point. BARD score of more the 2 indicates fibrosis.

SPSS 16.0 was used for statistical analysis. Data were analyzed using statistical analysis tools like ANOVA for multivariate analysis. *F* and *P* values were obtained for estimating relation between the scores and increasing grade of fatty liver on imaging.

RESULTS

Majority of the study participants (n = 35) were in the age group of 41-50. There were 14 participants between 18 and 30 years of age. There were seven between the age group of 71-80. Thus, the study group was well represented from all age groups. Sixty nine out of 106 study participants were females and 37 were males. There were twenty seven patients with grade 1 fatty liver in BMI between 25-29.9, 32 in BMI range of 30-34.5, five in BMI range in 35-39.9 and none in more than 40. There were ten patients with fatty liver grade 2 in the BMI range of 25-29.9, 15 in the BMI range of 30-34.9, six in the BMI range of 35-39.9 and three in the BMI range more than 40. There were total five cases of grade 3 fatty liver, one each in the BMI range of 30-34.9 and 35-39.9 and three in BMI range of more than 40. Thus, in total, 63% study participants had grade 1 fatty liver on USG and 32% had grade 2 fatty liver.

The mean NAFLD fibrosis score with grade 1 fatty liver was −0.44, grade 2 fatty liver had mean score of −0.13, and grade 3 fatty liver had mean score of 0.15. The *F* value was 0.99 for the relation between Imaging grades and NAFLD fibrosis score. The *P* value was 0.12 [Figure 1].

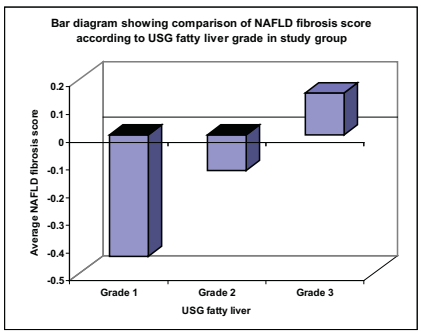
The mean BARD score with grade 1 fatty liver was 2.81 ± 0.74, grade 2 fatty liver had mean score of 2.94 ± 0.95, and grade 3 fatty liver had mean score of 2.60 ± 0.89. The *F* value obtained was 0.53, thus, rendering the relation not significant statistically. The *P* value was 0.18 [Figure 2].

DISCUSSION

This study was conducted to correlate biochemical and imaging in patients with NAFLD who were overweight with BMI of more than 25. One hundred and six patients were included in this study and they were assessed in terms of imaging evidence of fatty liver, biochemical parameters, and NAFLD fibrosis score and BARD score. The imaging changes were subsequently compared with the aforementioned noninvasive scores of NAFLD to correlate whether imaging changes match with biochemical changes.

No study has till date directly compared the NAFLD fibrosis score with imaging changes of the fatty liver on USG.

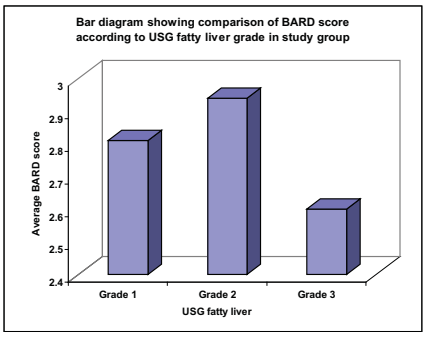
USG fatty liver	n	NAFLD fibrosis score	F Value	P Value
		Mean ± SD		
Grade 1	67	-0.44 ± 1.25	0.99	>0.05
Grade 2	34	-0.13 ± 1.35		
Grade 3	5	0.15 ± 1.06		



The above figure compares the average NAFLD fibrosis scores with imaging grades of fatty liver. The relation between the two is not statistically significant. Hence, the imaging evidence of liver damage in NAFLD does the correlate with non-invasive scores of NAFLD like the NAFLD Fibrosis Score

Figure 1: Comparison of NAFLD Fibrosis score according to USG fatty liver grade in study group

USG fatty liver	n	BARD score	F Value	P Value
		Mean ± SD		
Grade 1	67	2.81 ± 0.74	0.53	>0.05
Grade 2	34	2.94 ± 0.95		
Grade 3	5	2.60 ± 0.89		



The above figure compares the BARD score with imaging grades of fatty liver. The relation is also not statistically significant. Thus reinforcing that the imaging evidence of fatty liver does not correlate with the biochemical evidence of fatty liver

Figure 2: Comparison of BARD score according to USG fatty liver grade in study group

In this study, the mean NAFLD fibrosis score with grade 1 fatty liver was −0.44, grade 2 fatty liver had mean score of −0.13, and grade 3 fatty liver had mean score of 0.15. The *F* value was 0.99 for the relation between imaging grades and NAFLD fibrosis score.

This relation was not found to be statistically significant. Thus, the biochemical evidence of fibrosis or NAFLD in the form of the NAFLD fibrosis score did not correlate with imaging evidence of fatty liver. Thus, imaging findings of fatty liver may not directly correlate with actual fibrosis in these patients.

It was also attempted to correlate the BARD score with the imaging

studies. The *F* value obtained was 0.53, thus, rendering the relation not significant statistically. Thus, the BARD score did not correlate with imaging evidence of fibrosis like the NAFLD fibrosis score.

Out of two noninvasive scores, though both statistically non significant, NAFLD fibrosis score correlated better with the imaging changes as compared to the BARD score.

This result is to be taken into account in respect to other studies that have validated the ultrasonography as a modality of screening of fatty liver. Overall, USG has a reported sensitivity of 60-94% and a specificity of 84-95% for detecting fat,[1] but combined fat and fibrosis can show up a hyperechoic liver in 98.7% of patients known as 'fatty fibrotic pattern'. Sensitivity depends on the amount of fat in liver, however, both sensitivity and specificity are poor in morbid obesity.[5]

Patients with severe steatosis have marked increase in echogenicity and poor posterior penetration and poor or nonvisualization of the diaphragm and the intrahepatic vessels.[5] However, ultrasound has the disadvantage of being subjective and less sensitive and specific in patients with obesity. Hence, in patients, like in this study, who have higher BMI levels, USG may not correctly reflect the amount of fibrosis.

Also, USG being operator dependent may have contributed to this result.

Another point raised with this study is the possible use of biochemical and imaging study together as additive modality for monitoring and estimation of fibrosis in NAFLD patients. Though this study was not designed to look into this aspect, but additive role of these two noninvasive modalities should be looked into by carefully designed studies for possible use.

CONCLUSION

No statistically significant correlation was found between biochemical evidence of fibrosis and USG evidence of fibrosis in overweight patients of NAFLD.

Thus, the biochemical evidence of fibrosis or NAFLD in the form of NAFLD fibrosis score did not correlate with imaging evidence of fatty liver. The USG findings of fatty liver may not directly correlate with actual fibrosis in these patients. The study raises question of estimation of fibrosis in patients of NAFLD using USG and biochemical parameters alone and possible use of both modalities together as additive evidence. Future studies planned to look into the noninvasive diagnosis, staging and monitoring of patients of NAFLD using various modalities together could yield better results.

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Prevalence of dysglycemia and clinical presentation of pulmonary tuberculosis in Western India

V. Mave,*† S. Meshram,*‡ R. Lokhande,*‡ D. Kadam,*‡ S. Dharmshale,*‡ R. Bharadwaj,*‡ A. Kagal,*‡ N. Pradhan,* S. Deshmukh,* S. Atre,§ T. Sahasrabudhe,§ M. Barthwal,§ S. Meshram,§ A. Kakrani,§ V. Kulkarni,* S. Raskar,* N. Suryavanshi,* R. Shivakoti,† S. Chon,† E. Selvin,¶ A. Gupte,¶ A. Gupta,*† N. Gupte,*† J. E. Golub†¶

*Byramjee-Jeejeebhoy Medical College-Johns Hopkins University Clinical Research Site, Pune, India; †Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; ‡Byramjee-Jeejeebhoy Government Medical College, Pune, §Dr D Y Patil Medical College, Pune, India; ¶Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

SUMMARY

SETTING: Pune, India.

OBJECTIVES: To estimate the prevalence and risk factors of pre-diabetes mellitus (DM) and DM, and its associations with the clinical presentation of tuberculosis (TB).

DESIGN: Screening for DM was conducted among adults (age ⩾ 18 years) with confirmed TB between December 2013 and January 2017. We used multinomial regression to evaluate the risk factors for pre-DM (glycated hemoglobin [HbA1c] ⩾ 5.7–6.5% or fasting glucose 100–125 mg/dl) and DM (HbA1c ⩾ 6.5% or fasting glucose ⩾ 126 mg/dl or random blood glucose > 200 mg/dl or self-reported DM history/treatment) and the association of dysglycemia with the severity of TB disease.

RESULTS: Among 1793 participants screened, 890 (50%) had microbiologically confirmed TB. Of these, 33% had pre-DM and 18% had DM; 41% were newly diagnosed. The median HbA1c level among newly diagnosed DM was 7.0% vs. 10.3% among known DM ($P < 0.001$). DM (adjusted OR [aOR] 4.94, 95%CI 2.33–10.48) and each per cent increase in HbA1c (aOR 1.42, 95%CI 1.01–2.01) was associated with >1+smear grade or ⩽9 days to TB detection.

CONCLUSION: Over half of newly diagnosed TB patients had DM or pre-DM. DM and increasing dysglycemia was associated with higher bacterial burden at TB diagnosis, potentially indicating a higher risk of TB transmission to close contacts.

KEY WORDS: TB; pre-diabetes mellitus; diabetes mellitus; risk factors; clinical presentation; India

THE INTERSECTION OF TUBERCULOSIS (TB) and diabetes mellitus (DM) has recently received global attention. DM increases the risk of TB by nearly three-fold, and persons with both TB and DM may have worse TB treatment outcomes.[1–5] Globally, TB continues to be the leading cause of morbidity and mortality from any single infectious pathogen, with approximately 10.4 million incident cases and 1.8 million deaths in 2015.[1] With diets rich in fat and refined carbohydrates, combined with decreasing daily physical activity, the burden of DM is reaching epidemic proportions, even in resource-limited settings.[6,7] The TB epidemic in India is the largest in the world, with over 2.8 million cases occurring in 2015.[1,8] India also has a staggering 69 million adults living with DM, the largest burden in the world.[7] The convergence of these two enormous epidemics in India thus has major implications for global TB control.

Recent estimates suggest that DM may account for approximately 15% of all pulmonary TB (PTB) cases in high TB and DM burden countries.[9] Data from India indicate a wide range of DM prevalence (6.5–33%) among TB cases; all of these estimates are much higher than for the general population.[9–14] More recently, a report from South India showed that over 75% of TB cases had dysglycemia, with 54% meeting the criteria for DM.[15] As the diet and genetics of the people and the epidemiology of dysglycemia are likely to vary in different parts of India, region-specific data are needed, particularly for Western India, where data are limited.

Previous research has shown that TB in people with DM may have a different clinical presentation than in those without DM.[16] These differences include increased symptoms of TB, increased involvement of the lungs, and higher bacterial burdens. However, these findings have been inconsistently reported.[16,17]

Correspondence to: Vidya Mave, 1st Floor, Pathology Museum, BJ Medical College Clinical Trials Unit, Jai Prakash Narayan Road, Pune, Maharashtra 411001, India. e-mail: vidyamave@gmail.com
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We hypothesize that the clinical presentation of TB among people with DM will be more severe than in TB patients without DM. Furthermore, we hypothesize that glycemic status may alter the clinical severity of TB at disease presentation.

An understanding of the prevalence, risk factors and clinical presentation of TB according to pre-DM and DM status may be particularly informative for the early recognition of individuals at an increased risk for adverse TB treatment outcomes. Within a cohort study investigating the impact of DM on TB treatment outcomes, we present the prevalence and risk factors and the clinical presentation of TB patients with and without pre-DM and DM.

METHODS

Study design and study sites

Between 23 December 2013 and 4 January 2017, eligible individuals with suspected TB at the Revised National TB Control Programme (RNTCP) centers in Pune and Pimpri-Chinchwad Municipal Corporations (PCMCs) of Maharashtra, India, were approached by the study counsellors. They were referred to the clinical research site of Byramjee-Jeejeebhoy Medical College-Sassoon General Hospital (BJMC-SGH) in Pune. BJMC-SGH is a large, public-sector, tertiary-care teaching institution that serves approximately 7 million population in the surrounding urban, semi-urban and rural low-income populations. A second site, Dr D Y Patil Medical College, Pune, a private affiliated medical college with a large hospital catering to low- and lower-middle-income populations, initiated the study in July 2016, and obtained referrals from RNTCP centers in the PCMC region.

The Institutional Review Board of Johns Hopkins School of Medicine, Balitimore, MD, USA, and the ethics committees at BJMC-SGH and Dr D Y Patil Medical College, Pune, approved the project.

Study procedures

The eligibility criterion was adults aged $\geqslant$18 years with suspicion of PTB. Those with a previous history of TB, current anti-tuberculosis treatment for at least 7 days and those diagnosed with rifampin-resistant TB were excluded.

Consenting participants were administered a questionnaire to collect information on demographics and medical history, including TB history and TB risk factors. All enrolled participants underwent anthropometric assessments and clinical evaluations. Chest radiography was performed on patients confirmed to have TB.

Laboratory investigations comprised a baseline fasting or random blood glucose test (Cobas c111; Roche Diagnostics, Rotkreuz, Switzerland), glycated hemoglobin (HbA1c) test (BioRad Laboratories, Hercules, CA, USA) and sputum evaluations. Spontaneously expectorated sputum was collected on two occasions—on the spot and early morning. All sputum specimens collected underwent direct smear for acid-fast bacilli (AFB) staining, Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) assays and culture (in Mycobacterial Growth Indicator Tube [MGIT] liquid culture and Löwenstein Jensen media). *Mycobacterium tuberculosis* growth on liquid culture was confirmed using *p*-nitrobenzoic acid, and cultures with confirmed growth of *M. tuberculosis* were subjected to phenotypic drug susceptibility testing.

Study definitions

Suspected TB was defined according to RNTCP criteria and included a cough for at least 2 weeks and any other symptoms suggestive of TB, such as fever, night sweats, loss of weight or loss of appetite. Confirmed TB was defined as positive AFB smear, Xpert results or *M. tuberculosis* complex in sputum culture. DM was defined as having either HbA1c $\geqslant$6.5%, fasting blood glucose (FBG) level of $\geqslant$126 mg/dl, random blood sugar $>$ 200 mg/dl or DM diagnosis by self-reporting, or currently taking DM medication. Pre-DM was defined as HbA1c of $\geqslant$5.7% and $<$6.5% or FBG of $\geqslant$110 and $<$126 mg/dl. Body mass index (in kg/m^2) was calculated using World Health Organization definitions for underweight ($<$18 kg/m^2), normal (18–24.9 kg/m^2), or overweight ($\geqslant$25 kg/m^2).

Assessments of TB severity

Clinical presentation of TB among patients with pre-DM, DM and no DM was assessed using clinical, radiographic and laboratory findings collected at baseline. Using a published clinical score[17] that gives one point to each major TB-related symptom—cough, fever, weight loss, night sweats, anorexia, hemoptysis and malaise—TB disease was classified as severe ($\geqslant$4) and non-severe TB ($\leqslant$3). Radiologic findings of cavitary lung lesions and/or the involvement of $\geqslant$1 lung lobe, and microbiologic findings of $>$1+ sputum AFB and/or shorter time to TB detection (TTD) in culture, were classified as severe TB disease. Shorter TTD by culture was defined as less than the median number of days by MGIT culture.

Statistical analysis

DM prevalence and binomial exact 95% confidence intervals (CIs) were calculated as the number of DM cases divided by the total number of microbiologically confirmed TB cases. Baseline categorical and continuous variables for sociodemographic, clinical characteristics and clinical presentation among confirmed PTB cases by DM were summarized using proportions and median values with interquartile range (IQR), respectively, and compared using either Fisher's exact test or Wilcoxon rank-sum test; $P <$

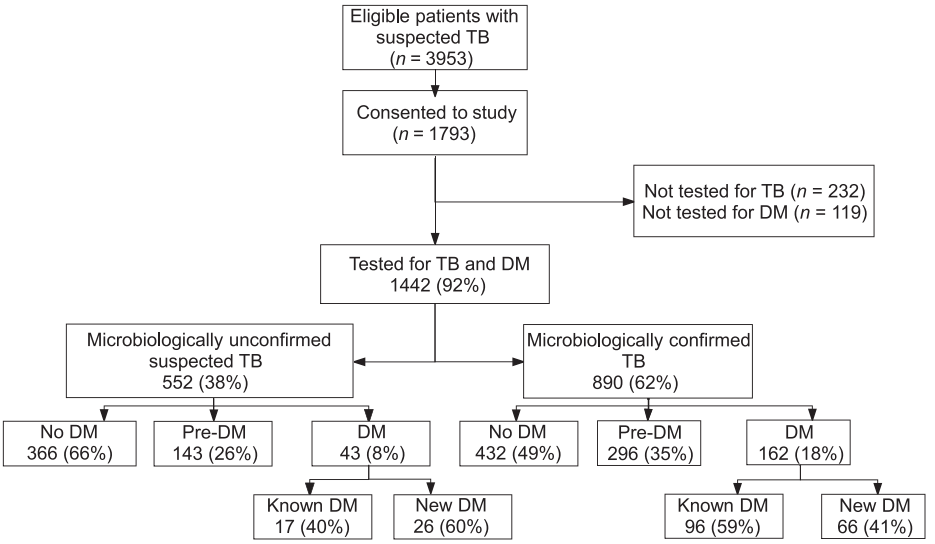

Figure Flowchart of study enrollments and prevalence of pre-DM and DM among patients with suspected and confirmed TB in Pune, India. TB = tuberculosis; DM = diabetes mellitus.

0.05 was deemed statistically significant. Univariable and multivariable multinomial logistic regression was performed to assess the factors associated with pre-DM and DM among cases with confirmed TB in comparison with those without DM. All variables that had $P < 0.1$ in the univariable analysis and known risk factors for DM were used in the multivariable analysis to estimate adjusted odds ratios (aORs). In addition, univariable and multivariable logistic regressions were performed to determine the association of pre-DM and DM with TB disease severity. Data were analyzed using Stata v13.1 (StataCorp, College Station, TX, USA).

RESULTS

Prevalence of and factors associated with pre-diabetes mellitus and diabetes mellitus among pulmonary tuberculosis cases

Of 3953 eligible participants approached by the study team, 1793 (45%) consented to participate; 1442 (80%) underwent investigations for both TB and DM (Figure). Of these, 890 (62%) patients were diagnosed with microbiologically confirmed TB (Figure). The median age was 32 years (IQR 22–44); 589 (66%) were males (Table 1). The prevalence of pre-DM and DM was respectively 33% (95%CI 30–36) and 18% (95%CI 16–21). Of the 162 with DM, 96 (59%) were known to have DM and 66 (41%) were newly diagnosed. Prevalence of DM among TB patients aged <25 years was 2% (95%CI 0–8) compared with respectively 12% (95%CI 6–17) and 45% (95%CI 38–51) among TB patients aged 25–40 years and those aged >40 years. Overall, 42 (6%) TB patients had human immunodeficiency virus (HIV) infection, and 6 (6%) TB patients with DM had HIV. The median level of HbA1c for pre-DM was 6.0% (IQR 5.9–6.1) and for DM it was 9.1% (IQR 6.9–11.6). The median level of HbA1c among cases with newly diagnosed DM was 7.0% (IQR 6.6–10.2) compared with 10.3% (IQR 8.6–12.1) among patients with known DM ($P < 0.001$).

In multivariate analyses, older age (aOR 1.97, 95%CI 1.17–3.30) and being overweight (aOR 4.49, 95%CI 1.36–14.83) were associated with pre-DM (Table 2). Furthermore, males (aOR 1.81, 95%CI 1.01–3.24) were more likely to be diagnosed with DM. Compared with TB patients aged <25 years, patients aged 25–40 years (aOR 10.05, 95%CI 2.99–33.77) and those aged >40 years (aOR 56.43, 95%CI 16.57–192.1) were more likely to have DM at TB diagnosis and being underweight was protective against DM (aOR 0.27, 95%CI 0.16–0.46).

Tuberculosis symptoms, radiologic features and microbiologic findings

The median duration of TB-related symptoms was 45 days for all patients (Table 3). Overall, 712 (84%) TB patients reported ⩾4 symptoms; of these, 280 (39%) had pre-DM and 140 (20%) had DM. In multivariable analysis, patients with DM were more than four-fold as likely to have ⩾4 symptoms (aOR 4.24, 95%CI 1.24–14.43) than those without DM (Table 4). Patients with pre-DM were nearly twice as likely to have ⩾4 symptoms (aOR 1.72, 95%CI 0.85–3.49); however, this association did not reach statistical significance.

On radiography, patients with pre-DM and DM had similar presentations according to pre-DM and DM status ($P = 0.08$) (Table 3). Cavitary TB disease, the involvement of more than one lung lobe, or miliary infiltrates suggestive of extensive lung in-

Table 1 Sociodemographic and clinical characteristics of TB patients by pre-DM and DM status, Pune, India

Characteristics	Confirmed TB				
	Overall ($n = 890$) *n* (%)	No DM ($n = 432$) *n* (%)	Pre-DM ($n = 296$) *n* (%)	DM ($n = 162$) *n* (%)	*P* value
Sex					
Male	589 (66)	259 (60)	205 (69)	125 (77)	<0.001
Female	301 (34)	173 (40)	91 (31)	37 (23)	
Age, years, median [IQR]	32 [24–44]	27 [23–35]	30 [24–43]	47 [40–55]	<0.001
Age group, years					<0.001
<25	281 (32)	180 (42)	96 (32)	5 (3)	
25–40	347 (39)	187 (43)	120 (41)	0 (25)	
>40	262 (29)	65 (15)	80 (27)	4117 (72)	
Location					
Urban	820 (92)	403 (93)	270 (91)	147 (91)	0.42
Rural	70 (8)	29 (7)	26 (9)	15 (9)	
Smoked tobacco products	890	432	296	162	0.30
Non-smoker	723 (81)	356 (82)	232 (78)	135 (83)	
Smoker	167 (19)	76 (18)	64 (22)	27 (17)	
Alcohol					
No	625 (70)	298 (69)	209 (71)	118 (72)	0.66
Yes	265 (30)	134 (31)	87 (29)	44 (27)	
CAGE*					0.84
<2	117 (44)	59 (44)	37 (43)	21 (48)	
⩾2	148 (56)	75 (56)	50 (57)	23 (52)	
Household income, INR/month†					0.27
<5000	208 (25)	107 (27)	70 (25)	31 (20)	
>5000	625 (75)	296 (73)	205 (75)	124 (80)	
Education					0.003
More than primary	644 (72)	329 (76)	215 (73)	100 (62)	
None or primary	246 (28)	103 (24)	81 (27)	62 (38)	
Religion					0.04
Non-Hindu	214 (24)	88 (20)	82 (28)	44 (27)	
Hindu	676 (76)	344 (80)	214 (72)	118 (73)	
Anemia‡					0.05
No	59 (86)	364 (84)	247 (85)	148 (92)	
Yes	7123 (14)	66 (15)	44 (15)	13 (8)	
HIV					0.56
Negative	529 (93)	255 (93)	173 (91)	101 (94)	
Positive	42 (7)	19 (7)	17 (9)	6 (6)	
Body mass index§					<0.001
Normal	250 (28)	102 (23)	65 (22)	83 (51)	
Underweight	603 (68)	326 (75)	213 (72)	64 (40)	
Overweight	37 (4)	4 (1)	18 (6)	15 (9)	

* CAGE score determines high alcohol dependence.
† US$1 = 64 INR.
‡ Defined as hemoglobin < 8 g/dl.
§ Underweight: <18.5 kg/m^2; normal: 18.5–24.9 kg/m^2; overweight: >25 kg/m^2.
TB = tuberculosis; DM = diabetes mellitus; IQR = interquartile range; CAGE = 1) Have you ever felt you needed to cut down on your drinking? 2) Have people annoyed you by criticizing your drinking? 3) Have you ever felt guilty about drinking? 4) Have you ever felt you needed a drink first thing in the morning (eye-opener) to steady your nerves or to get rid of a hangover? INR = Indian rupee; HIV = human immunodeficiency virus.

volvement was seen in 374 (42%) TB patients; 134 (36%) had pre-DM and 79 (21%) had DM. In multivariable analysis, patients with DM and those with pre-DM were more likely to have extensive lung involvement, although neither association reached statistical significance (Table 4).

The median TTD was 8 days (IQR 6–11). Higher smear grade > 1+ or TTD < 9 days—suggestive of higher bacterial burden—was seen among 712 (84%) TB patients, 238 (84%) patients with pre-DM and 140 (93%) DM patients. In multivariate analysis, patients with DM had a nearly five-fold higher risk for higher smear grade and shorter TTD (aOR 4.94, 95%CI 2.33–10.48) (Table 4).

Among those with DM, with each unit increase in the HbA1c, the odds of having ⩾4 symptoms (aOR 1.15, 95%CI 0.73–1.81) was higher; however, this did not meet statistical significance. No association was seen with lung involvement and a unit increase in HbA1c level (aOR 1.04, 95%CI 0.77–1.41). Importantly, each unit increase in HbA1c level had 42%

Table 2 Multinomial logistic regression analysis for risk factors for pre-DM and DM among confirmed TB patients, Pune, India

Characteristics	Confirmed TB ($n = 890$) Univariable analysis OR (95%CI)	Multivariable analysis aOR (95%CI)*
Sex		
Pre-DM		
Male	1.50 (1.10–2.06)	1.44 (0.97–2.13)
Female	1	1
DM		
Male	2.26 (1.49–3.41)	1.81 (1.01–3.24)
Female	1	1
Age group, years		
Pre-DM		
<25	1	1
25–40	1.20 (0.86–1.69)	1.17 (0.78–1.75)
>40	2.31 (1.53–3.48)	1.97 (1.17–3.30)
DM		
<25	1	1
25–40	7.70 (2.97–19.95)	10.05 (2.99–33.77)
>40	64.8 (25.34–165.7)	56.43 (16.57–192.1)
Location		
Pre-DM		
Urban	1	—
Rural	1.34 (0.77–2.32)	
DM		
Urban	1	—
Rural	1.42 (0.74–2.72)	
Smoking tobacco products		
Pre-DM		
Non-smokers	1	—
Smokers	1.29 (0.89–1.87)	
DM		
Non-smokers	1	—
Smokers	0.94 (0.58–1.52)	
Alcohol		
Pre-DM		
No	1	—
Yes	0.93 (0.67–1.28)	
DM		
No	1	—
Yes	0.83 (0.55–1.24)	
CAGE†		
Pre-DM		
CAGE <2	1	—
CAGE ⩾2	1.06 (0.61–1.83)	
DM		
CAGE <2	1	—
CAGE ⩾2	0.86 (0.43–1.7)	
Religion		
Pre-DM		
Non-Hindu	1	1
Hindu	0.67 (0.47–0.94)	0.64 (0.42–0.97)
DM		
Non-Hindu	1	1
Hindu	0.69 (0.45–1.04)	0.71 (0.40–1.28)
Anemia‡		
Pre-DM		
No	1	1
Yes	0.98 (0.65–1.49)	1.31 (0.80–2.16)
DM		
No	1	1
Yes	0.48 (0.26–0.90)	0.92 (0.41–2.07)
HIV status		
Pre-DM		
Negative	1	—
Positive	1.32 (0.67– 2.61)	
DM		
Negative	1	—
Positive	0.79 (0.31– 2.05)	
Body mass index§		
Pre-DM		
Normal	1	1
Underweight	1.02 (0.72–1.46)	1.02 (0.66–1.56)
Overweight/obese	7.06 (2.29–21.79)	4.49 (1.36–14.83)
DM		
Normal	1	1
Underweight	0.24 (0.16–0.36)	0.27 (0.16–0.46)
Overweight/obese	4.61 (1.47–14.41)	1.98 (0.55–7.12)

* Adjusted for covariates with $P < 0.1$ in univariate analysis and covariates known to be associated with a risk of DM.
† CAGE score determines high alcohol dependence.
‡ Defined as hemoglobin < 8 g/dl.
§ Underweight: <18.5 kg/m^2; normal: 18.5–24.9 kg/m^2; overweight: >25 kg/m^2.
TB = tuberculosis; DM = diabetes mellitus; OR = odds ratio; CI = confidence interval; aOR = adjusted OR; CAGE = 1) Have you ever felt you needed to cut down on your drinking? 2) Have people annoyed you by criticizing your drinking? 3) Have you ever felt guilty about drinking? 4) Have you ever felt you needed a drink first thing in the morning (eye-opener) to steady your nerves or to get rid of a hangover? HIV = human immunodeficiency virus.

higher odds of higher smear grade and shorter TTD (aOR 1.42, 95%CI 1.01–2.01).

DISCUSSION

We found that over half of the newly diagnosed PTB patients in our study cohort had DM or pre-DM. It is not surprising to note that males and older TB patients had a higher risk for DM, whereas being underweight was protective against DM and pre-DM. These findings corroborated previously reported predictors of dysglycemia among patients with TB in India.[12] We observed that the median HbA1c level of TB patients among newly diagnosed DM was considerably lower than that among known DM patients, and closer to the standard pre-DM glycemic range. Finally, TB patients with DM, specifically those with high HbA1c levels, had higher bacterial burdens at presentation.

The DM prevalence of 18% among TB cases in our study is representative of the study area.[9–15] Specifically, the estimated prevalence of DM in the general population of Maharashtra state would be 6% based on our study results, assuming three-fold higher DM prevalence among TB cases,[4] which is similar to reports for western India.[18] Furthermore, the higher prevalence of DM reported by Kornfeld et al. (54%) is likely due to the higher median age (>45 years) of TB patients in their South Indian population.[15] We also observed a 44% DM prevalence among TB patients aged >40 years.

It should be noted that lower HbA1c levels were observed among those newly diagnosed with DM compared with those with known DM.[12,15] The median HbA1c level for newly diagnosed DM was marginally higher than the HbA1c-defined pre-DM

Table 3 Clinical presentation of TB by pre-DM and DM status, Pune, India

Characteristics	No DM ($n = 432$) *n* (%)	Pre-DM ($n = 296$) *n* (%)	DM ($n = 162$) *n* (%)	*P* value
Symptoms				
Duration of illness, days, median [IQR]	45 [30–60]	45 [30–90]	45 [30–75]	0.30
Cough	426 (99)	296 (100)	162 (100)	0.05
Duration, days, median [IQR]	30 [15–60]	30 [20–60]	30 [20–60]	0.25
Fever	370 (86)	263 (89)	145 (90)	0.32
Duration, days, median [IQR]	20 [15–40])	30 [15–45]	20 [12–45]	0.14
Night sweats	250 (58)	178 (60)	95 (58)	0.83
Duration, days, median [IQR]	15 [10–30]	25 [15–45]	21 [15–45]	0.03
Loss of appetite	353 (82)	250 (84)	138 (85)	0.50
Duration, days, median [IQR]	30 [15–60]	30 [20–60]	30 [15–60]	0.42
Weight loss	343 (79)	223 (75)	127 (78)	0.42
Duration, days, median [IQR]	30 [21–60]	30 [30–60]	45 [30–60]	0.17
Shortness of breath	268 (62)	192 (65)	102 (63)	0.74
Duration, days, median [IQR]	30 [15–60]	30 [15–60]	30 [15–60]	0.75
Physical examination				
Waist circumference, cm, median [IQR]	69 [63–75]	70 [65–76]	78 [72–88]	<0.001
Hip circumference, cm, median [IQR]	82 [78–86]	82 [78–86]	86 [83–93]	<0.001
Mid-upper arm circumference, cm, median [IQR]	22 [20–23]	21 [19–23]	24 [21–25]	<0.001
Body mass index, kg/m^2, median [IQR]	17 [15–18]	17 [15–19]	20 [17–23]	<0.001
Hemoglobin, g/dl, median [IQR]	11.8 [10.1–13.1]	11.3 [10.3–12.6]	12.3 [11.1–13.6]	0.003
Systolic blood pressure, mmHg, median [IQR]	112 [110–114]	112 [108–114]	114 [110–120]	<0.001
Diastolic blood pressure, mmHg, median [IQR]	72 [70–76]	72 [70–76]	76 [70–80]	<0.001
Chest radiography				
Normal	0	2 (1)	0	0.28
Abnormal: not TB	1 (0.6)	1 (0.7)	0	
Abnormal: compatible with TB	72 (40)	45 (31)	35 (40)	
Abnormal: highly suggestive of TB	109 (60)	99 (67)	52 (60)	
Infiltrate	117 (97)	139 (96)	87 (100)	0.18
Unilateral	39 (21)	25 (17)	18 (21)	0.22
Bilateral	139 (76)	115 (78)	69 (79)	
Unknown	4 (2)	7 (5)	0	
Number of lobes				
1	28 (16)	14 (10)	12 (14)	0.08
2	72 (41)	64 (46)	49 (56)	
>2	77 (44)	62 (44)	26 (30)	
Miliary infiltrates	13 (7)	6 (4)	7 (8)	0.37
Cavitary	84 (46)	69 (47)	39 (45)	0.95
Mycobacterial findings				
Direct smear result				
Negative	135 (32)	79 (27)	34 (22)	0.17
Scanty	66 (16)	46 (16)	25 (16)	
1+	102 (24)	77 (26)	55 (35)	
2+	67 (16)	54 (19)	30 (19)	
3+	49 (12)	35 (12)	14 (9)	
Concentrated smear result				
Positive	313 (75)	227 (78)	132 (84)	0.06
Time to MGIT result, days, median [IQR]	8 [6–12]	8 [5–11]	8 [6–10]	0.14

TB = tuberculosis; DM = diabetes mellitus; IQR = interquartile range; MGIT = Mycobacterial Growth Indicator Tube.

range. While this finding has been observed in previous studies, the precise mechanism for this is unclear. While transient stress or inflammation-induced hyperglycemia is common in sepsis and chronic infections including TB,[19–21] whether TB disease uncovers subclinical DM among those with epigenetic predisposition to DM has yet to be explored. Furthermore, whether new DM is transient with a similar pathophysiology to that of gestational DM (a transient DM that affects women with a genetic predisposition to DM)[22] should also be considered and explored further in longitudinal studies.

Consistent with our study findings, previous research has shown that the number of symptoms may be higher among TB patients with DM.[16,17,23] Furthermore, we found that patients with DM had over four-fold higher odds of higher mycobacterial burden, as demonstrated by higher smear grade and shorter TTD by culture. Moreover, each per cent increase in HbA1c level among those with DM was associated with higher mycobacterial burden, suggesting that uncontrolled glycemia could lead to severe TB disease at baseline, which has potential implications for managing DM in high TB burden countries. Our results also suggest that the risk of TB transmission to close contacts may be higher from TB patients with DM; however, this needs to be explored in community settings. Individuals with TB and DM have altered inflammation, T-helper type-1 immune

Table 4 Diabetes as a risk factor for severe TB disease* using multinomial logistic regression analyses

	Univariable analysis		Multivariable analysis†	
TB disease markers	RRR (95%CI)	*P* value	RRR	*P* value
TB symptoms				
Pre-DM				
<4	Reference	—	Reference	—
⩾4	1.49 (0.81–2.76)	0.20	1.72 (0.85–3.49)	0.14
DM				
<4	Reference	—	Reference	—
⩾4	1.89 (0.82–4.36)	0.13	4.24 (1.24–14.43)	0.02
Cavitary TB disease or ⩾1 lobe affected or miliary infiltrates				
Pre-DM				
Absent	Reference	—	Reference	—
Present	1.34 (0.65–2.79)	0.43	1.44 (0.64–3.20)	0.38
DM				
Absent	Reference	—	Reference	—
Present	1.29 (0.55–3.04)	0.56	1.99 (0.66–6.00)	0.22
Smear grade >1+ or TTD <9 days				
Pre-DM				
No	Reference	—	Reference	—
Yes	1.24 (0.84–1.85)	0.28	1.36 (0.89–2.08)	0.15
DM				
No	Reference	—	Reference	—
Yes	3.12 (1.62–6.04)	0.001	4.94 (2.33–10.48)	<0.001

* Giving one point to each major TB-related symptom (cough, fever, weight loss, night sweats, anorexia, hemoptysis and malaise), TB disease was classified as severe (⩾4) and non-severe TB (<3). Radiologic findings of cavitary lung lesions and/or involvement of ⩾1 lung lobe, and microbiologic findings of >1+ sputum AFB and/or shorter TTD in culture was classified as severe TB disease. Shorter TTD by culture was defined as less than the median number of days required for MGIT culture.

† Adjusted for age, sex, religion, education, body mass index and anemia.

TB = tuberculosis; RRR = relative risk reduction; CI = confidence interval; DM = diabetes mellitus; AFB = acid-fast bacilli; TTD = time to TB detection; MGIT = Mycobacterial Growth Indicator Tube.

responses, as well as defective neutrophil and macrophage function.[24] In addition, TB-infected alveolar macrophages among DM patients may alter host cell recognition and delay innate immune responses.[25–27] Whether these immune changes result in more severe TB disease among DM patients should be explored further.

Our study was conducted primarily at one state-run, public-sector facility and a second privately run facility in the last 6 months of the study. Selection bias may have occurred as we had to obtain participant consent for both TB and DM screening. However, the populations that were screened for DM in confirmed TB cases were from the national program, and were representative of India's population. As our study was cross-sectional, we could not assess the temporality of the association between HbA1c level and disease severity. Despite these limitations, our results are generalizable to other TB populations in India, particularly those with a similar age range.[11,13,14]

The slower-than-expected decline in global TB incidence despite massive global TB control efforts could, in part, be attributed to the huge, unfolding epidemic of DM in resource-limited settings with high TB burdens.[7,28] With the DM burden set to exceed 500 million people by 2030, and with India being the global frontrunner of this burden,[7] a better understanding of TB and DM interactions is critical to inform optimal TB control efforts. Bidirectional screening for TB and DM has been recommended, but with very high TB and DM burdens, the resources required to screen all TB patients for DM and vice versa may limit the feasibility of these strategies.[29,30] Our study adds to the growing body of literature supporting the view that screening for DM among TB cases can be tailored to specific regions and to specific subgroups within a target population. Furthermore, our results indicate that patients with DM may present with more severe TB disease. Future studies should investigate whether HbA1c levels among newly diagnosed DM patients revert to a normoglycemic range following successful anti-tuberculosis treatment and assess the role of host-directed therapies with metformin/statins and/or aggressive glycemic control in TB patients with DM to reduce disease severity and improve outcomes.

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RÉSUMÉ

CONTEXTE : Pune, Inde.
OBJECTIF : Estimer la prévalence et les facteurs de risque de pré-diabète (DM) et de DM, et son association avec la présentation clinique de la tuberculose (TB)
SCHÉMA : Une recherche de DM a été réalisée parmi des adultes (⩾18 ans) atteints de TB confirmée entre décembre 2013 et janvier 2017. Nous avons utilisé une régression multinomiale pour évaluer les facteurs de risque de pré-DM (HbA1c ⩾ 5,7 et jusqu'à 6,5% ou glycémie à jeun de 100–125 mg/dl) et DM (HbA1c ⩾ 6,5% ou glycémie à jeun ⩾ 126 mg/dl ou glycémie à toute heure > 200 mg/dl ou antécédents de DM/de traitement déclaré par les patients) et association de dysglycémie avec la gravité de la TB maladie.
RÉSULTATS : Sur 1793 participants dépistés, 890 (50%) avaient une TB confirmée par microbiologie. Parmi eux, 33% avaient un pré-DM et 18%, un DM ; 41% ont été des diagnostics nouveaux. L'HbA1c médiane parmi les nouveaux cas de DM a été de 7,0% contre 10,3% parmi les DM connus ($P < 0,001$). Le DM (OR ajusté [ORa] 4,94 ; IC95% 2,33–10,48) et chaque augmentation de pourcentage de l'HbA1c (ORa 1,42 ; IC95% 1,01–2,01) ont été associés avec une augmentation de >1+ du grade du frottis ou moins de 9 jours jusqu'à la détection de la TB.
CONCLUSION : Plus de la moitié des nouveaux patients TB diagnostiqués avaient un DM ou un pré-DM. Le DM et une dysglycémie croissante ont été associés avec une charge bactérienne plus élevée lors du diagnostic de TB, indiquant un risque potentiel plus élevé de transmission de la TB à des contacts proches.

RESUMEN

MARCO DE REFERENCIA: La ciudad de Pune, en la India.
OBJETIVOS: Calcular la prevalencia de pre-diabetes (DM) y DM y su asociación con el cuadro clínico inicial de la tuberculosis (TB).
MÉTODO: Se llevó a cabo una detección sistemática de la DM en los adultos (a partir de los 18 años) con diagnóstico confirmado de TB, de diciembre del 2013 a enero del 2017. Mediante un modelo de regresión polinómica se evaluaron los factores de riesgo de padecer pre-DM (HbA1c de 5,7% hasta 6,5% o glucemia en ayunas de 100 a 125 mg/dl) y DM (HbA1c ⩾6,5%, glucemia en ayunas ⩾126 mg/dl, una glucemia aleatoria > 200 mg/dl o un antecedente autorreferido de diagnóstico o tratamiento de la DM) y la asociación de la disglucemia con la gravedad de la enfermedad tuberculosa.
RESULTADOS: De los 1793 participantes examinados, 890 presentaron TB con confirmación microbiológica (50%). De estos casos, el 33% tenía pre-DM y el 18% DM; en el 41% de los casos se trató de un diagnóstico nuevo. La mediana de la HbA1c en los casos recién diagnosticados fue 7,0% contra 10,3% en los pacientes con diagnóstico conocido ($P < 0,001$). Se asociaron con una gradación de la baciloscopia superior a 1+ o con ⩽9 días hasta la detección de la TB, la DM (OR ajustado [aOR] 4,94; IC95% 2,33–10,48) y cada unidad de aumento de la HbA1c (aOR 1,42; IC95% 1,01–2,01).
CONCLUSIÓN: Más de la mitad de los pacientes con diagnóstico reciente de TB presentó DM o pre-DM. La DM y un aumento de la disglucemia se asociaron con una mayor carga bacilar en el momento del diagnóstico de TB, lo cual puede indicar un mayor riesgo de transmisión de la enfermedad a los contactos cercanos.

Original Research Article

Partial restoration of the haematological profile in 4-week-old pups after six generations of improved nutrient supply

M M Karandikar[1], A R Joshi[2], M S Karandikar[3*]

[1]Assistant Professor, [2]Professor and HOD, Department of Physiology, Bharati Vidyapeeth Medical College, Dhankawadi, Pune, INDIA.
[3]Professor, Department of Physiology, D.Y. Patil Medical College, D.Y. Patil University, Pimpri, Pune, INDIA.
Email: mskarandikar@yahoo.com

Abstract

David Barker's epidemiological studies in England and Wales found that impaired growth during intra-uterine life leads to increased risk for cardio-vascular disease, diabetes and cancers in the developing foetus. He attributed this to the poor health of the pregnant mothers causing foetal undernutrition. The developing foetus shows "adaptations" to the sub-optimal environment called as "programming" that primarily include endocrine and metabolic adaptations as well as epigenetic and apoptotic changes along with decreased cell numbers in various tissues. A majority of these studies in both animal and human populations have primarily focused on cardio-vascular, renal and endocrine adaptations to undernutrition. We have previously shown that 50 generations of undernutrition in a Thrifty Jerry Wistar Rat colony causes hypoplasia of the bone marrow. The present study has evaluated the effects of diet restoration for 6 generations (recuperation) on the haematological profile in 28-day pups in this multigenerationally undernourished colony. This Transition (Recuperation) colony after 6 generations of the normalised diet showed a higher Mean Corpuscular Volume (55.20 vs 50.87 µm3) as compared to the control colony and a high RBC distribution width (30.26 vs 13.75). Platelet count (724.6 vs 496.2 x 103/microliter) and Plateletcrit (0.4498 vs 0.3203) was also higher in the recuperation colony. We have previously shown that 2 generations of the recuperation cannot completely the reverse the changes that result due to 50 generations of undernutrition. However,6 generations of the recuperation diet leads to the correction of the anaemia but worsens the platelet count. This study has important consequences in a developing country like India

Key Words: Foetal Programming, Multigenerational Undernutrition, Complete Blood Count, Pups, Recuperation Diet

***Address for Correspondence:**
Dr. Dr. M.S. Karandikar, Professor, Department of Physiology, D.Y. Patil Medical College, D.Y. Patil University, Pimpri, Pune 411018.
Email: mskarandikar@yahoo.com
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INTRODUCTION

Worldwide studies have shown an increasing burden of Type 2 Diabetes Mellitus and cardiovascular disease in developing countries like India. (Echouffo-Tcheugui 2012). Evidence from a variety of studies have shown that this occurs due to poor nutrition during foetal life leading to intrauterine growth retardation (IUGR) and development (Godfrey and Barker 2000). Such an insult during the intrauterine life leads to adaptations during foetal life called as "programming". These adaptations involve hormonal, metabolic, chemical and structural changes during foetal life (McMillen and Robinson 2005). The Indian phenotype shown by Yajnik *et al* provide such evidence of foetal programming during the gestational period. The Pune Maternal Nutrition Study has described the "Thin-Fat" Indian phenotype. This phenotype, present at birth is centrally obese, insulin resistant but has a lower soft tissue weight. This probably is a consequence of multigenerational programming due to undernutrition (Yajnik, Fall *et al* 2003).We have previously shown that 50 generations of multigenerational undernutrition in a Wistar rat model

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(Thrifty Jerry) shows similar insulin resistance, central obesity along with higher homocysteine, vitamin B_{12} and folate levels. These animals show increased susceptibility to diabetes and ischemic heart disease (Hardikar *et al*). The diet restriction is also known to lead to alterations causing necrosis, hypocellularity and extracellular modifications of the matrix. (Travlos GS (2006), Fried W *et al* (1978), Vituri CL (2000), Prestes-Carneiro *et al* (2006) and Borelli P *et al* (1995). We have previously evaluated the role of multigenerational undernutrition on the haematological profile of 4-week-old Wistar pups. Bone marrow hypoplasia was a characteristic feature along with an increase in inflammatory response. (M M Karandikar *et al*). The present study evaluated the role of six generations of a standard (recuperation) diet in the multigenerationally undernourished colony pups.

MATERIALS and METHODS

Wistar rats that were undernourished for 50 generations received a standard rat diet (transition rat colony) for 6 generations (Hardikar *et al* 2015).The control group received a standard rat feed.28 day pups were bled retro-orbitally and the Complete Blood Count (CBC) was measured on an auto-analyser (Mindray BC2800).The study was ethically approved by the institutional ethics committee.

Statistical Analysis

All estimations were carried out in triplicates and values are expressed as +/- Std Error of Mean (SEM). The statistical significance was evaluated by the unpaired t test using GraphPad Prism 6 version software.

RESULTS

Table 1: Complete Blood Counts in Control and Transition Wistar Rat Pups (28 Day)

	Control Mean ± SEM	Recuperation Mean ± SEM	P value
WBC(x10³/µL)	7.700 ± 0.7514 n= 6	9.680 ± 0.6909 n= 5	0.0890
Lymphocytes%	65.05 ± 5.812 n= 6	60.52 ± 1.582 n= 5	0.5087
Monocytes%	3.600 ± 0.3055 n= 6	3.700 ± 0.2387 n= 5	0.8085
Granulocytes%	31.28 ± 5.552 n=6	35.78 ± 1.459 n= 5	0.4920
RBCs (x10⁶/µL)	7.597 ± 0.5254 n= 6	7.352 ± 0.1947 n=5	0.6962
Hb (g/dl)	11.92 ± 0.9548 n=6	12.28 ± 0.2818 n= 5	0.7456
Platelets (x 10³/µL) **	496.2 ± 46.97 n= 6	724.6 ± 27.05 n= 5	0.0032
HCT	38.70 ± 3.007 n= 6	40.52 ± 0.9085 n= 5	0.6079
MCV (x 10³/µL) **	50.87 ± 0.8969 n= 6	55.20 ± 0.5070 n= 5	0.0033
MCHC(g/dl)	30.70 ± 0.5825 n= 6	30.26 ± 0.1077 n= 5	0.5163
RDW****	13.75 ± 0.6571 n= 6	30.26 ± 0.1077 n= 5	< 0.0001
MPV(FL)	6.400 ± 0.2875 n=6	6.160 ± 0.09798 n= 5	0.4855
PDW	14.98 ± 0.1662 n= 6	14.86 ± 0.05099 n=5	0.5312
PCT*	0.3203 ± 0.04095 n= 6	0.4498 ± 0.01539 n= 5	0.0265

* p< 0.01, ** p<0.001, ***/****P<0.0001

DISCUSSION

David Barker and his colleagues in Southampton, England were analysing the cause of early mortality and morbidity in England and Wales. They found out that the poor health of the mothers was responsible for the many changes or “adaptations” in the developing foetus, called as programming. These adaptations were responsible for the increased risk of cardio-vascular disease, diabetes mellitus and cancers. He thus formulated the concept of “foetal origins of adult disease” which stated that “intrauterine factors, particularly nutrition, act in early life to program the risks for adverse health outcomes in adult life” (Godfrey and Barker 2000). The programming is a result of the adaptations of the various organ systems to under-nutrition during key stages of development. A variety of epidemiological observations and animal studies have confirmed such an association. (McMillen and Robinson 2005). Yajnik *et al*, in the Pune Maternal Nutrition Study (PMNS) have described the “Thin-Fat Indian phenotype”, a result of undernutrition during foetal life (Yajnik, Fall *et al* (2003), Yajnik and Deshmukh, (2012).The animal and human studies carried out so far have primarily focused on the cardiovascular, renal and metabolic adaptations. Very few studies have evaluated the role of chronic undernutrition and its effect on the haemopoietic environment. We have previously described the role of multigenerational undernutrition (50 generations) on the hematopoietic environment in a Wistar rat colony (The Thrifty Jerry Colony) that showed hypoplasia of the bone marrow along with granulocytosis. (M M Karandikar *et al*). The present study evaluated the role of providing a standard diet for 6 generations to the multigenerationally undernourished rat colony on the hemopoietic environment (The Transition Colony).The undernourished colony had shown a hypoplastic bone marrow as seen by decreased Total Leukocyte Count, Red

blood Cell Count, Platelet count and Haemoglobin concentration (M M Karandikar *et al*). Restoration of the diet for 6 generations could restore the total leukocyte count, Red blood Count and haemoglobin concentration to normal. Mean Corpuscular Volume showed an increase in the transition colony (55.20 vs 50.87 x 10^3/µL). A previous study carried out by Hardikar *et al* have shown partial restoration of the blood indices after 2 generations of the recuperation diet. Restoration of the diet for 6 generations in the transition colony thus showed complete reversal of the macrocytic anaemia. It required 6 generations to restore normality after 50 generations of undernutrition. The study also found out an increased RBC distribution width (RDW) in the transition colony indicative of anisocytosis and poikilocytosis. The exact cause for this needs to be evaluated. A normalization of the White Blood Cell counts was also seen in these pups after 6 generations of a normalised diet. However, an increased platelet count (724.6 vs 496.2 x 10^3/µL) and plateletcrit (0.44 vs 0.32) in the transition colony was also observed. The cause of the increased platelet count needs further investigation.6 generations of the recuperation thus could not completely reverse the effects of 50 generations of undernutrition. We have not carried out epigenetic studies to evaluate the role of a recuperation diet on restoration of the hypoplastic bone marrow. This study would further enable us to provide an understanding into the role of the recuperation diet on changes in the hemopoietic environment.

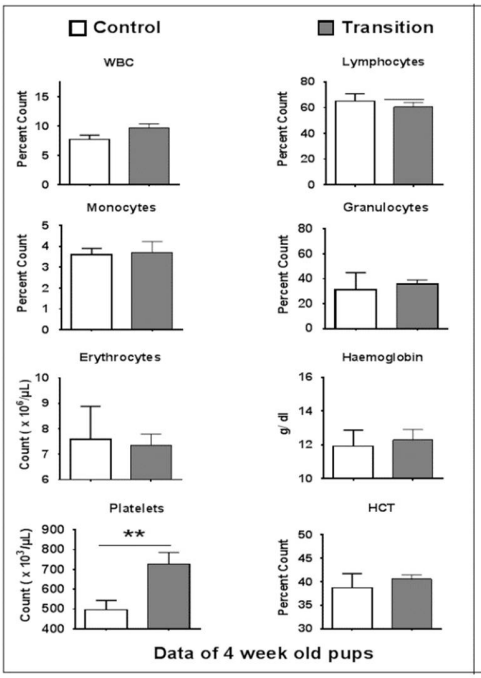

Figure 1: Blood Cell Count in Control and Transition Wistar Rat pups (28 days)

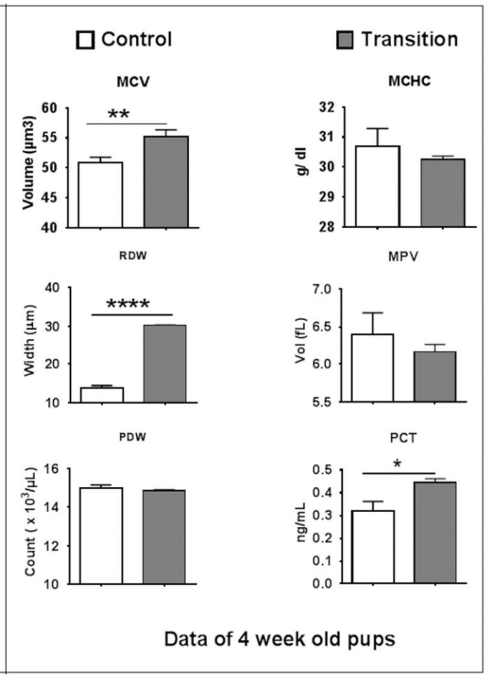

Figure 2: Blood Cell Count in Control and Transition Wistar Rat pups (28 days)

CONCLUSION

This study has shown that 6 generations of providing a standard or recuperation diet could not completely reverse the effects of 50 generations of undernourishment. Future studies that would involve animals that have been provided with a recuperation diet over many more generations along with epigenetic studies may be able to throw light on restoration of the hemopoietic environment in the undernourished rats.

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COMPARATIVE ASSESSMENT OF THE CONVENTIONAL PROCEDURE AND RAPID MOLECULAR LINE PROBE ASSAY (LPA) FOR DIAGNOSIS OF MULTIDRUG RESISTANCE *Mycobacterium tuberculosis* CLINICAL ISOLATES FROM WESTERN PART OF INDIA

JADHAV S.V.*, MISRA R.N., GANDHAM N., ANGADI K., VYAWAHARE C. AND GUPTA N.

Department of Microbiology, Dr. D.Y. Patil Medical College, Hospital and Research Centre (Dr. D.Y. Patil University) Pune - 411 018, MS, India.
*Corresponding Author: Email- patilsv78@gmail.com

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Abstract- *Background*: India has the uppermost TB burden in the world and about one fifth of occurrence of TB cases occurs in India. Since conventional diagnostic procedures have limitations, definitive and rapid diagnosis of tuberculosis particularly extrapulmonary tuberculosis is demanding to provide better treatment outcomes and reduces the transmission of MDR-TB. *Methods*: 100 clinical specimens from suspected tuberculosis were received in microbiology laboratory during 1st June 2012 to 30th June 2013. Line probe assay (LPA GenoTypeMTBDR*plus* VER 2.0 was compared to the "Gold Standard" of combined culture and clinical diagnosis. Bact/Alert 3D MB-Bact (BioMerieux Durham, North Carolina,USA) rapid automated system and L.J. media were used for culture. Positive growths in either media were identified using standard conventional methods and subjected to susceptibility testing. *Results*: 43 specimens were Lowenstein Jenesen culture positive for *M.tb* and 47 specimens were LPA positive for *M.tb* complex. Two specimen were smear positive and culture negative and but positive by LPA. 19 samples were culture positive for non tuberculous mycobacteria (NTM) and further analyzed as possible NTM by LPA. For LPA, overall sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) were 95.74%, 100, 100, and 96.36% respectively. Detection of the mutations in the *rpoB*gene of *M. tuberculosis* has been reported to be an accurate predictor of rifampicin resistance. The most frequently observed mutation was Ser-513-leu in *rpoB* gene. *Conclusion*: LPA performs uniformly well, provided results approximately within 48 hrs. in direct detection and provides susceptibility results along with mutations in nine genes which will be significant in understanding the genetic makeup of diverse *M.tb* strains and in boosting the development of new diagnostics and vaccines. In the early stages, detection of MDR-TB provides better treatment outcome and reduces the transmission of MDR-TB.

Keywords- MDR tuberculosis, rapid diagnosis, Line probe assay

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Introduction

Tuberculosis (TB) is one of the leading infectious diseases in the world and is responsible for more than 9 million new cases and 2 million deaths annually. Tuberculosis control in the South-East Asia Region Annual Report published in 2012 by World Health Organization (WHO) estimated that India is the highest TB- burden country in the world in terms of absolute numbers of incident cases that emerge each year and it contributed one fourth of the estimated global incident TB cases in 2010 [1-3]. A combination of rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) with or without streptomycin, is recommended for the standard or first-line treatment of TB. MDR-TB (multidrug-resistant tuberculosis) is defined as TB that is resistant at least to RMP and INH, the two more important first-line anti-TB drugs [3]. Resistance to these drugs has been linked to mutations in at least nine genes; *ropB* for RMP, *katG, inhA, aphC, ksaA* for INH resistance *rpsL* and *rrs* for streptomycin resistance, *embB* for EMB resistance and *pncA* for PZA resistance [4,5]. Emergence and spread of MDR- TB is the foremost medical and public problem. A preventive measure to control spread of MDR- TB in community is imperative. Since the slow growth rate of the causative agent *Mycobacterium tuberculosis (M.tb)*, isolation, identification and drug susceptibility testing of this organism can take several weeks or longer. In the last few years, there have been considerable technological advances in the area of diagnosis of TB and MDR- TB. In the early phase of clinical infection of TB, detection of TB and MDR-TB provides better treatment outcome and reduces the transmission of MDR-TB. Several molecular methods have been developed for direct detection, species identification, and drug susceptibility testing of Mycobacteria. These methods can potentially reduce time from weeks to days or hours.

Molecular line probe assay (LPA) is available *i.e.* GenoType MTBDR*plus* VER 2.0 (HainLifesciences GmbH, Nehren, Germany) [6,7].

In the present study, we compared "Gold Standard" diagnostics methods with rapid molecular diagnostic test. We used LPA which is targeting *rpoB, katG, inhA* gene mutaion and its detection is

based on nucleic acid amplification technology which allows for rapid detection of *M.tb* Complex. This is followed by reverse hybridization along with rifampicin (RMP) resistance and/or isoniazid(INH) resistance in smear –positive and smear-negative sputum samples or in culture isolates. Species included in TB causing *M.tb* Complex are *M. tuberculosis, M. africanum, M. bovis subsp. Bovis, M. bovis subsp. Caprae, M. bovis BCG, M. microti, M. canettii, and M. pinnipedii.* The identification of RMP resistance was facilitating by the detection of the most significant associated mutation of the *rpoB* gene (coding for β-subunit RNA polymerase). Detection of *katG*gene (coding for the catalase and peroxidase) and *inhA*gene (coding for NADH enoyl ACP reductase) identified INH resistance. With the purpose of that the test is indicated as an aid for the diagnosis and intended for use in clinical laboratories for rapid detection of MDR-TB.

Materials and Methods

The study was carried on 100 clinical specimens from clinically suspected cases of pulmonary and extrapulmonary tuberculosis from Dr. D. Y. Patil Medical College, Hospital and Research Centre Pimpri Pune-a tertiary care hospital in India.

Specimens were collected in sterile container according to the revised National Guidelines [8] and received in microbiology laboratory during 1st June 2012 to 30th June 2013.

Both acid fast smear positive and smear negative samples from these specimens were analyzed. LPA was compared to the "Gold Standard" of combined culture and clinical diagnosis. GenoType MTBDR*plus* VER 2.0 (HainLifesciences GmbH, nehren, Germany) was used targeting *rpoB, katG, inhA* genes. Sputum samples were processed in class II biosafety cabinet in a biosafety level (BSL)-3 laboratory. Samples were decontaminated by N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) method [5]. After decontamination they were neutralized with phosphate-buffered saline (0.067 M, pH 6.8) and centrifuged at 3,500 × *g* for 20 min. The pellet was suspended in 1 ml of phosphate-buffered saline out of which 0.5 ml of the processed specimen was inoculated into MB/BacT bottles and L.J. (Lowenstein-Jenson) medium each. Specimens collected from sterile sites were concentrated by centrifugation without prior decontamination. 500µl of the processed sample was used for DNA isolation in a screw capped tube.

Media and Culturing Methods

The MB/BacT system consists of a bottle containing 10 ml of modified Middlebrook 7H9 broth enriched with casein, bovine serum albumin, and catalase. Before inoculating specimen, bottles were supplemented with 0.5 ml of MB/BacT MAS supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin) which was reconstituted with 10 ml of MB reconstituting fluid according to the manufacturer's instructions. Bottles were placed inside the BacT Alert 3D instrument (Bio Merieux Durham, USA) and incubated at 37°C for 6 weeks. Any bottle which displayed as positive was taken out of the instrument. The L.J. bottles were incubated at 37°C for 8 weeks and were read weekly and identified as soon as sufficient growth was visible. L.J. bottles failing to show any growth after 6-8 weeks were discarded as negative [9-11].

Microscopy

Any growth obtained on the bottle was stained by ZN (Ziehl - Neelsen) for detection of acid fast bacilli.

Conventional Drug Susceptibility Testing

Proportion Method

The proportion method is currently the method of choice in the majority of laboratories in the world. DST (Drug susceptibility testing) was performed on LJ media containing anti tubercular drugs with streptomycin (4µg/mL), isoniazid (0.2µg/mL), rifampicin (40µg/mL), ethambutol (4µg/mL). Standardization of inoculum: Inoculum was matched to 1 Mc.Farland solution and inoculated on drug containing L.J. media [8].

Incubation and Reading

The inoculated slopes were incubated at 37°C and were examined every week. The contaminated bottles were discarded and the sensitivity testing repeated using the original purified growth. The reading was recorded at 28th and 42nd day of incubation as per RNTCP (Revised National Tuberculosis Control Programme) guidelines [8].

Quality Control

Standard strain of *M. tuberculosis* H37Rv was tested from time to time for quality assurance.

Line Probe Assay

Molecular LPA is based on nucleic acid amplification technology which allows for rapid detection of *M.tb* Complex along with resistance to RMP and INH.

A total of 100 specimens were assessed, of that 61 specimens were from extrapulmonay infections and 39 were from pulmonary infections. All specimens were screened by staining with ZN technique. Bact/Alert 3D (BioMerieux Durham, North Carolina,USA) rapid automated system and L.J. media were used for culture. Positive growths in either media were identified using standard conventional methods and subjected to susceptibility testing. The performance of line (LPA) was assessed.

Procedure

DNA from the decontaminated specimen was extracted using the chemical cell lysis method. DNA extraction kit named GenoLyse® HAIN Lifesciences was used for the same. The extracted DNA was then subjected to PCR (Polymerase Chain Reaction) followed by LPA by reverse hybridization method for the analysis and diagnosis of TB. PCR was carried out using primers specific for *M.tb* complex gene loci and genes associated with resistance to RMP and INH. An initial denaturation at 95°C for 15min was carried out to allow complete separation of the two templates. Further the amplification profile was as follows; denaturation at 95°C for 25 sec, annealing at 50°C for 40 sec and extension at 70°C for 40 sec. A hold of 70°C for 8 min was done, in order to complete the final extension. A total of 30 cycles with the above profile were performed. The amplified product was then analyzed by "Reverse Hybridization" technique using the DNA strip technology. The strips provided are pre-attached with 27 different probes (bands) including six controls (conjugate, amplification) [12]. *M. tb complex* (TUB), *rpoB, katG and inhA* controls), eight *rpoB* wild-type (*WT1–WT8*) and four mutant probes (*rpoB MUT D516V, rpoB MUT H526Y, rpoB MUT H526D*, and *rpoB MUT S531L*), one *katG* wild-type and two mutant probes (*katG MUT S315T1* and *katGMUT S315T2*), and two inhA wild type and four mutant probes (*inhA MUT1 C15T, inhA MUT2A16G, inhA MUT3A T8C, inhA MUT3B T8A).* Conjugate control **(CC)** line must develop in this zone, documenting the efficiency of conjugate bind-

ing and substrate reaction. Amplification control **(AC):** When the test is performed correctly, a control amplicons will bind to Amplification Control zone. When only CC and AC bands developed, this represents valid negative result. *M. tuberculosis* complex **(TUB)**, this zone hybridizes, with amplicons generated from all members of *M. tb complex*. Locus control (*rpoB, kat G, ingA*), this zones detect a gene region specific for the representative locus. Wild type probes comprise the most important resistance regions of the respective genes. When wild type probes of a gene stain positive, there is no detectable mutation within the examined regions. This indicates that the strain tested is sensitive for the representative antibiotic. Mutation probes detect some of the most common resistance-mediating mutations [Fig-1].

Complementary to MTB complex gene loci and the mutant products of the *rpoB, katG* and *inhA* genes that are involved in imparting resistance to RMP and INH drugs. The banding profile seen was indicative for the presence of *M. tb* complex and 1st line drug resistance. Either missing of wild-type band or the presence of mutant band was taken as an indication of a resistant strain. Incomplete amplification of RIF and/or INH genes was considered as an inv*al*id result.

Quality Control for LPA

In order to validate the correct performance and implementation of kit constituents, each strip includes 5 control zones i.e. CC- conjugate control zone to check the binding of the strip and a correct chromogenic reaction, AC- amplification control zone to check successful amplification reaction and three locus control zones (*rpoB, katG and inhA*) to check optimal sensitivity of the reaction for each of the tested gene loci.

Results

The culture and LPA were evaluated for their abilities to detect *M. tb* complex in 100 patients those were suspected of having pulmonary and extra pulmonary mycobacterial infections. 62 specimens were culture positive for mycobacterial infections of which, in 43 specimens *M.tb* were isolated and while in 19 specimens NTM were isolated by conventional method. Total 66 specimens were LPA positive of which 47 were *M.tb* complex and 19 were NTM. Two specimens were smear positive and culture negative and positive by LPA [Fig-1].

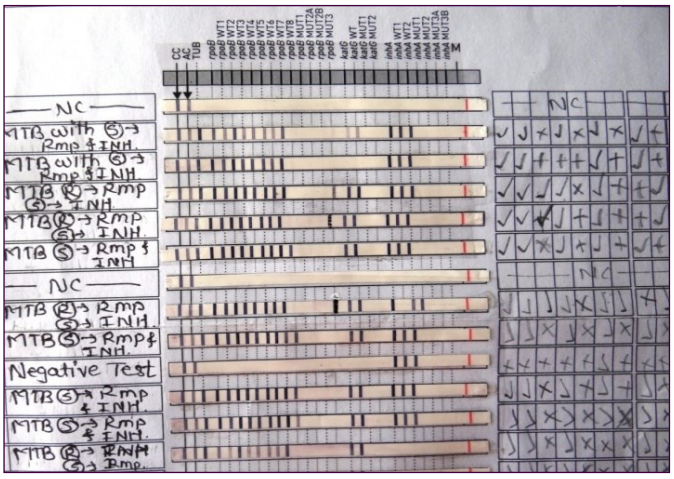

Fig. 1- Banding pattern of DNA strip with respect to RMP and/or INH resistance.

NTM were analyzed as possible NTM by LPA as well as by "Gold Standard" conventional method. For LPA, overall sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs) were 95.74%, 100, 100, 96.36% respectively. For culture, overall sensitivity, specificity, PPVs, NPVs were 91.48%, 100, 100, 92.98% respectively.

Table 1- Drug Profiling Of Tot*al* Suspected Specimens By MTBDR plus V2 assay (n=47)

Sr. No	Drug profile	Frequency	Percentage(%)
1	MTB with RIF & INH sensitivity	30	63.82
2	MTB with RIF monoresistance	10	21.27
3	MTB with INH monoresistance	2	4.25
4	MDR-TB	5	10.63
	Total 19 NTM were detected by PLA- MTBDR plus V2		

Of the 47 LPA positive *M.tb* strains, 30(63.82%) strains were RMP and INH sensitive. 10(21.27%) strains were RMP monoresistant and 2(4.25%) strains were INH monoresistant. 5(10.63%) strains were MDR-TB. Of the 5 MDR-TB results by conventional DST method, LPA detected 5 as a MDR-TB. Conventional DST methods also detected similar results in 7 strains with RMP monoresistance and 2 were resistant to INH, while 30 strains were RMP and INH sensitive [Table-1]. Overall concordance between genotypic LPA test and phenotypic conventional DST was 98% (47/45). NTM were detected in 19 strains which were also confirmed by GenoType®Mycobacterium CM VER1.0 (HAIN LIFE SCIENCES) assay and these results were in agreement of conventional culture method.

Mutation Patterns in LPA

7 RIF monoresistant strains had a mutation in *rpo B* S531L (MUT 3 band). Other mutations associated with RIF resistance in MDR-TB strains included *rpo B* H526D (MUT2B band), *rpo B* D516V (MUT 1 band) and H526Y (MUT 2Aband) however these mutations were not seen in RIF monoresistant strains. The most frequent mutation found in INH monoresistant strains was *kat G* S315 T2 (MUT 2 band). Mutation in MDR-TB strains were *inh*AT8C (MUT 3A) and *inh*AT8A (MUT 3B) [Table-2].

The turnaround time of LPA assay was 48 hours whereas; it was 70 -75 days for phenotypic DST *i.e.*25-30 days for conventional culture growth and another 30-40 days for DST.

Discussion

In the recent times major importance has been given for precise and early diagnosis of the MDR-TB, which is extremely advantageous to disrupt further transmission of the disease. In view of the fact that there are many report globally regarding rapid diagnosis on *M.tb* infections. In the present study, first time we have evaluated performance of LPA test for detection of *M. tb* Complex along with RIF and INH resistance in direct sputum samples or in culture isolates of clinically suspected tuberculosis patients. Subsequently, genotypic LPA and phenotypic L.J proportion DST results were compared. We observed that LPA test results had a good concordance with the conventional DST with a added advantage of a shorter turnaround time. In the present study, overall sensitivity, specificity, PPVs, NPVs for LPA were 95.74%, 100, 100, and 96.36% respectively. For culture, overall sensitivity, specificity, PPVs, NPVs were 91.48%, 100, 100, 92.98% respectively are in agreement with results of meta-analysis done by Ling *et.al* [13]. Sensitivity (97%)

and specificity (100%) for detection of MDR-TB in the present study corroborated with a previously reported study by Anek-vorapong *et.al* [14] and Raveendran R. *et.al* [15] from Thiland and India respectively and these findings suggest that performance of LPA is similar to conventional DST in a quality assured TB laboratory.

In the present study, MDR-TB were detected in 10.63% strains while MTB with RIF monoresistance were detected in 14.89% strains and MTB with INH monoresistance were detected in 4.25% strains. Joel Bazira *et.al* (2010) study from Uganda- showed 4.8% resistant to INH, 3.2% resistant to RIF while 1.6% to both INH and RMP (MDR) [16]. Maurya *et.al* [17] reported 4.7% INH resistant and RIF is 4.2% which was similar to other responds from Germany, Italy, Finland, France, Denmark, Turkey, Vietnam and Taiwan. The sensitivity of Genotype ® MTBDR plus assay for detection of MDR-TB was 97.7%. Umubyeyi AN *et.al* [18] reported 6.2% INH resistant and 3.9% MDR from East African countries. Kibiki *et.al* [19] reported 9.9% resistant to INH & 2.7% resistant to RIF while 2.7% MDR from Northern Tonzania. MDR-TB prevalence is estimated to be 2.3% among new cases and 12-17% among re-treatment cases. However, due to the size of population and number of TB cases reported annually, India ranks second among the 27 MDR-TB high burden countries worldwide after China.

Table 2- Mutational Patterns Associated With RIF & INH Resistant Genes In MTB Strains

Gene analyzed	Mutational pattern	Codons analyzed	Frequency	Percentage
rpo B gene-wild type banding	rpo B WT -1	505-509	40	90.9
	rpo B WT -2	510-513	44	100
	rpo B WT -3	510-517	42	95.45
	rpo B WT -4/wts	516-522	43	97.72
	rpo B WT -6	522-526	41	93.18
	rpo B WT -7	526-529	42	95.45
	rpo B WT -8	530-533	22	50
rpo B gene –mutational banding	rpo B MOT 1/	D 516V/D516 Y /de/515 /del 518/N518/S522L/S522Q	0	0
	rpo B MOT 2A	H526 Y	0	0
	rpo B MOT 2B	H 556D/ H526R/ H526N/ H526L/ H526S/ H526C	0	0
	rpo B MOT 3	S531L/S531W/L533P	6	13.63
kat G gene- Wild type banding	Kat G WT	351	40	90.9
	Kat G MUT 1	S315T 1	4	9.09
	Kat G MUT 2	S315T 2	0	0
Inh A gene- Wild type banding	Inh A WT -1	0.9375	39	88.63
	Inh A WT -2	-8	43	97.72
Inh A gene- Mutational banding	Inh A MUT 1	CIST	4	9.09
	Inh A MUT 2	A169	0	0
	Inh A MUT 3A	T8C	0	0
	Inh A MUT 3B	T8A	0	0

Among molecular tests, LPA provides a better DST profile as compared to Gene-Xpert, and offers additional advantage of deciding the drug regimen in patients with INH monoresistance. WHO recommends addition of ethambutol as a third drug in the continuation phase in settings where the level of isoniazid resistance among new TB cases is high [5]. Additionally, this test can also be useful for systematic surveillance of INH monoresistance in countries with high isoniazid resistance. The genetic basis of the resistance against the anti-tubercular drugs has been unraveled.

RMP resistance is most common due to point mutations and small insertions and deletions in the *rpoB* gene which code for B-subunit of RNA polymerase in *M.tb.* RMP is key drug in treatment of TB and is also a useful surrogate marker for MDR-TB. RMP resistance is known to be associated with mutations in 81 base pair region (codon 527 to 533) of the *rpoB* gene [13].

In the present study, the finding of dominant mutation for RMP resistance is *rpoB* S531L, similar to a previously published report by Mani C *et.al* and Mitto P *et.al* from India and Italy respectively [20,21]. One false RMP resistant strain with missing WT8 band was observed with the LPA test. The nature and frequency of mutation in *rpo B* gene of RMP resistance in clinical isolates of *M.tb* vary considerably according to geographical locations. Distribution of mutations of *katG* and *inhA* genes is known to vary in different geographical regions. Frequencies of *katG* gene, *inhA* gene and combined *katG* and *inhA* gene mutations in the present study 2.73% and 6.84% respectively are within the range of previously reported studies [18-19]. Finding of frequency of combined mutations of *KatG* and *InhA* in the present study is comparable to a recent study from Uttar Pradesh India [17]. Diagnosis of extrapulmonary tuberculosis is a challenge as clinical manifestations are indistinct and typical radiograph finding may not evident till late in the disease, as a result, rapid detection plays very important role in diagnosis and in the early hour's treatment. Hence development of rapid diagnosis such as LPA has become priority [22-23]. In the present study, of 47 LPA positive strains, 20 were from cases of extrapulmonary tuberculosis which were diagnosed early as compared to conventional results come out. Positive treatment outcome were seen in such cases.

The present study highlights the facts that conventional culture methods however "Gold Standard" but obligatory for time utilization as compare to LPA results. LPA detected *M.tb* in two samples which failed to grow on conventional cultures. LPA test minimizes chances of contamination which demonstrate superiority of LPA test. Limitations of Genotype MTBDR*plus* VER 2 assay include need for an appropriate infrastructure, adequately trained and trained laboratory workforce.

Conclusion

Our results reveal that LPA performs uniformly well with pulmonary and extrapulmonary tuberculosis samples and provided results approximately within 48 hrs. in direct detection as compared to conventional DST method. Present study established the finding that LPA test is highly sensitive and specific for rapid diagnosis of MDR-TB. It represents an important key aspect in smear negative samples as it is gravely important that any tuberculosis patient should not be overlooked. Additionally, the test also detects nonresistance to INH and RMP. More studies with large number of samples are essential to corroborate these preliminary findings, and describe the accurate place of this test in the diagnostic algorithm for MDR-TB under programmatic settings in higher TB burden countries like India.

Ethics Statement: Study protocols were approved by the institutional ethics committee.

Conflicts of Interest: None declared.

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Research Article

FREQUENCY OF MUTATION IN ISONIAZID RESISTANT ISOLATES OF *Mycobacterium tuberculosis* COMPLEX FROM WESTERN MAHARASHTRA INDIA

HATOLKAR SWARUPA M., MISRA RABINDRA NATH, GANDHAM NAGESWARI R., ANGADI KALPANA, JADHAV SAVITA V. AND GUPTA NEETU

Dr D. Y. Patil Medical College, Hospital and Research Centre, Dr D. Y. Patil Vidyapeeth, Pimpri, Pune 411018
*Corresponding Author: Email- rn.misra@dpu.edu.in, patilsv78@gmail.com

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Abstract- Introduction: The alarmingly worsening scenario of Multi drug-resistant tuberculosis (MDR-TB) call for urgent need for a simple method for the rapid detection of drug-resistant TB. In MTB katG mutations are major cause of INH resistance. The usefulness of INH, a key component of short-course chemotherapy of tuberculosis, is threatened by the emergence of drug-resistant strains of MTB with mutations in the katGgene. Objective: This study is an effort to study the frequency of mutations in INH in Western Maharashtra, India. Methods: Samples were processed for two molecular methods, GenoTypeMTBDRplus (LiPA) and Dideoxy Sanger Sequencing. Samples processed for DNA extraction, nested PCR reaction was done by annealing at 55^0c with specific primers. After confirmation of band on Gel Doc, Sequencing was done with one primer. Sequencing was also done for inhA and inhA promoter region. Result: Major mutation found was S315T i.e. ser is replaced by thr at 315 positions. We could find other mutation at different positions. Conclusion: Molecular tests are rapid and accurate. S315T can be potential genetic marker for isoniazid resistance.

Keywords- MTB, katG, Isoniazide mutations, Genetic marker S315T.

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Academic Editor / Reviewer: Dr Jalal Ali Bilal, Dr Sourav Sharma, Dr Abheepsa Mishra

Introduction

The alarmingly worsening scenario of Multi drug-resistant tuberculosis (MDR-TB) call for urgent need for a simple method for the rapid detection of drug-resistant TB in clinical settings. Testing of mycobacterial culture and drug susceptibility testing (DST) capacity are limited in resource-scares countries; leading to inadequate treatment and development of favoring resistance [1].

Isoniazid (INH) is an effective first-line anti-tuberculosis drug. KatG, a catalase–peroxidase enzyme, converts INH to an active form in Mycobacterium tuberculosis, and katG mutations are major causes of INH resistance [1,2]. The usefulness of INH, a key component of short-course chemotherapy of tuberculosis, is threatened by the emergence of drug-resistant strains of MTB with mutations in the katG gene and is associated with clinically significant levels of INH resistance [3].

INH has a simple chemical structure consisting of hydrazide group attached to a pyridine ring, but its mode of action is very complex. One of the proposed mechanisms is that it enters the M. tuberculosis as a prodrug by passive diffusion and is activated by catalase-peroxidase enzyme, encoded by katG [4]. Some studies shown that is involved in biosynthesis of mycolic acid [5].

Objective: This is an effort to study the frequency of mutations in INH mono resistant isolates and in MDR strains of MTB complex with two different molecular methods, LiPA and Dideoxy sanger sequencing for single nucleotide polymorphism from Western Maharashtra.

Material and Methods

Study was conducted in tertiary care hospital from Dr. D.Y. Patil Medical College, Hospital & Research Centre, Pune India over a period of two years i.e. January 2014- December 2016.

Samples were processed for ZN staining before and after Decontamination of the sample with NALC-NaOH method. [6]

Samples were processed for two molecular methods Line Probe Assay (LiPA), [7] (Hain Life sciences GmbH, Nerhren, Germany) and Sanger Sequencing (3130 genetic analyzer, Applied Biosystems) [8]. Study was done on monodrug and multidrug resistant strains of MTB complex.

Two different methods were chosen as LiPA was available at institute and it gives information about MTBC complex & drug resistance in shorter time but could not provide information about exact Single nucleotide polymorphism (SNP). To confirm the exact SNP mutation and validation of LiPA results Sanger sequencing was the best method available in collaboration.

Inclusion criteria

Monodrug and Multidrug resistant isolates (growth cultures or excess amount availability of decontaminated sediment).

LiPA test was criteria along with clinical correlation from suspected cases of tuberculosis; clinical samples; Sputum, BAL Pleural Fluid, Tissue, Pus, CSF.

Extracted DNA required for both molecular testing could be possible only with grown cultures or excess amount of decontaminated sediment

LiPA was performed as per manufacture's instruction.

Dideoxy Sanger Sequencing

Isolation of genomic DNA

DNA was isolated using QIAGEN kit DNAeasy (CAT.NO. 56404) as per

manufacturer's instructions.

kat-G genotyping

PCR Primers and Amplicon size

KatG gene amplification using nested PCR primers targeting for catalase-peroxidase gene of M. tuberculosis was performed. Nested primers included one outer set of primers and one inner setoff primers. Upstream outer primer [9, 10] and downstream outer primer 5'TAAGCGGGATCTGGAGAA3', and inner primer set for the second round consisting of upstream primer 5'GTCCTTGGCG GTGTATT3' and downstream primer: 5'CATGAACGACGTCGAAAC 3'. Outer primer set codes for 547 bp region from KatG gene, while the inner primer set codes for a region 304 bp within the 547 bp. Analysis of the results on agarose gel electrophoresis and visualization of the amplified products over the GelDoc - XR (BioRad) was done for 2nd round amplification products.

PCR Reaction setup

PCR mix typically consisted of 50 uL of final reaction volume containing 10mm Tris-HCl (pH 8.3), 50mm KCl, 2.5mm $MgCl_2$, 0.01% (w/v) gelatin, 50 pmol of respective primers (mentioned above), 2.5 nmol of each of the four deoxy nucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 1U of Taq DNA polymerase (Invitrogen, USA). The PCR cycle conditions were: Initial denaturation at 95°C for 5 minutes, 95°C for 1 minute to denature the DNA, then cooled to 55°C for 45 sec, heating to 72°C for 1 min for extension, cycle repeated 30 times with final incubation at 72°C for 10 min, for nested PCR 94°C for 30 s to denature the DNA, then cooled to 52°C for 30s, heating to 72°C for 30 sec for extension, cycle repeated 35 times with final incubation at 72°C for 10 min. Amplicons from first PCR were used as template for second round nested PCR.

Table-1 *PCR REACTION /PRIMERS*

Mutation	Primer	Amplicon size	Annealing Temp	Mutation position
katG (1)	Fw: 5´ CGTGATCCGCTCATAGAT3´ Rv: 5´ TAAGCGGGATCTGGAGAA 3´	547 bp	55 °C	S315T
katG (nested)	Fw: 5´ GTCCTTGGCGGTGTATT 3´ Rv: 5´ CATGAACGACGTCGAAAC 3´	304 bp	52 °C	S315T
InhA promoter	Fw: 5´ GGCACGTACACGTCTTTATGTA 3´ Rv: 5´ GGTGCTCTTCTACCGCCGTGAA 3´	479 bp	55 °C	C15T / A16G
inhA	Fw: 5´ AAACGGATTCTGGTTAGCGG 3´ Rv: 5´ CGGGTTGATGCCCATCCCGG 3´	300 bp	55 °C	T8C / T8A

Table-2 *Association of mutation pattern in LiPA and Sequencing*

Gene	LiPA failing Wild type band	Codon analyzed	Developing mutation band	Mutation
katG	katG WT	315	KatG MUT 1	S315T 1
			KatG MUT 2	S315T 2
inhA	inhA WT1	-15	inhA MUT 1	C15T
		-16	inhA MUT 2	A16G
	inhA WT2	-8	inhA MUT 3A	T8C
			inhA MUT 3B	T8A

Results

Polymerase Chain reaction result:

Each sample show PCR amplicons of desired size on agarose gel

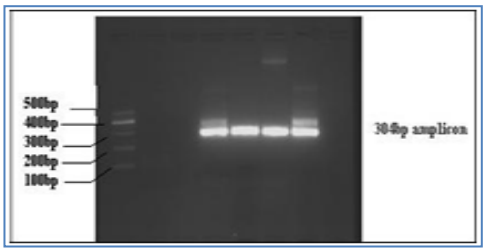

Fig-1 Kat-G gene PCR IMAGE: 1% (W/V) Agarose Gel electrophoresis: Lane 1: 500 bp DNA marker; Lane 2: NTC; Lane 4 and 8: sample PCR Products

DNA sequencing

Sequences were aligned with katG REF or wild type sequence gene using- http://www.genome.jp/tools-bin/clustalw(Kyoto University Bioinformatics Center) [11]

Following are the genes sequenced and are analyzed for drug resistance using different online available tools and databases.

https://umr5558-bibiserv.univ-lyon1.fr/mubii/mubii-select.cgi [12], https://tbdreamdb.ki.se/Info/Default.aspx [13], http://tuberculist.epfl.ch/index.html [14],

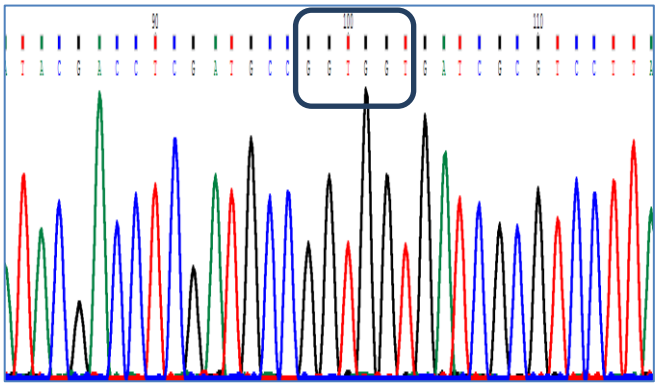

Fig-2 Mutation site for S315T Mutation S315T/GCT-GGT

Total 50 samples processed for Line Probe assay. Male to Female ratio was 26:24.Out of 50 samples,38 were Pulmonary samples and 12 Extra-Pulmonary samples. ZN Staining was positive in 34 samples and 16 were Negative. From these 50 samples 30 samples were further processed for sequencing which were observed to be resistant for isoniazid by LiPA. From 21 MDR sequenced samples 19 showed only S315T mutation and 2 samples showed more than one mutation in addition to S315T. One sample showed N218K, V230A and T108C at promoter site. Another sample showed only G99T promoter mutation. From 9 mono resistant sequenced samples 5 showed S315T mutation, one sample showed only C101T promoter site mutation and rest did not show any mutations.

Sequences were credited to NCBI data base with following

NCBI Accession numbers

Bankit 2051477 MG019932 - MG019937
Bankit 2051485 MG019938 - MG019947
Bankit 2051500 MG019948 - MG019955

Discussion

The distribution and frequency of drug resistance mutations are variable across regions and countries. The frequency of katG mutation ranged from58.5% to 93.7% and for inhA is 4.7%to 79.4%.The mostly commonly mutated katG locus was katG315 and for inhA was 15 [15]. Genotype MTBDRplus were relatively rapid and simple method for detection of drug resistant TB but the disadvantage of this test is resistance detection is available for limited number of mutations. LiPA is also very much useful in Extra pulmonary TB also Other rapid molecular method as GeneXpert cannot detect the INH resistance. GenProbe can detect MTB complex for extra pulmonary samples but cannot detect drug resistance [16,17]. Phenotypic studies were not possible if there was contamination or less colony number for preparation of inoculum. Challenge is to diagnose extra–pulmonary tuberculosis even for the most practiced clinicians as clinical manifestations are vague, non-specific and typical chest radiograph findings may not be evident till late in the disease. Phenotypic methods for mycobacteriological culture and drug susceptibility testing are slow and cumbersome. Newer techniques for rapid detection of MTB and its anti–TB drug resistance have therefore become a priority hence with the development of molecular tests *e.g.*, LiPA are most advanced [18]. S315T mutations are the common katG mutations observed in this study correlates with the other studies and commonly associated with high level INH resistance [19]. Studies have revealed that mutation in katG gene is responsible for 60-70% of isoniazid resistant strains. A study by Negi *et. al.*, in India reported 74.19% of S315T katG mutation in MTB strains from Delhi [20]. But in contrast the

Ser315Thr mutations accounted for 52-64% of strains in Central Asia. There is need for development of kits targeting more resistant gene mutations, possibly targeted for each specific geographic region. Two different mutations of katG observed in this study were N218K,V230A and different promoter mutations were also observed as C101T,T108C,G99T.This insists for more mutation studies to be done [21]. A study done by Hazbon et al indicated that most studies examined relatively small numbers of isolates or failed to include sufficient number of drug susceptible controls to demonstrate statistically significant associations [22].

Conclusion: S315T can be potential genetic marker for isoniazid resistance. These mutations can be rapidly evaluated by rapid molecular diagnostic methods as LiPA.

Application of the research: Early detection of resistant strains of Mycobacterium tuberculosis infection will help in the institution of suitable therapy and also helps to reduce treatment failure and increase of resistant strains.

Research category: MDR *Mycobacterium tuberculosis*

Abbreviation:
MDR: Multidrug resistant

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***Research Guide or Chairperson of research: Dr R. N. Misra**
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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Original Research Article

The haematological profile with fifty generations of undernutrition cannot be reversed with six generations of a recuperation diet in a wistar rat colony

M M Karandikar[1], A R Joshi[2], M S Karandikar[3*]

[1]Assistant Professor, [2]Professor and HOD, Department of Physiology, Bharati Vidyapeeth Medical College, Dhankawadi, Pune, INDIA.
[3]Professor, Department of Physiology, D.Y. Patil Medical College, D.Y. Patil University, Pimpri, Pune, INDIA.
Email: mskarandikar@yahoo.com

Abstract

David Barker and colleagues in Southampton, England were trying to identify all-cause mortality and morbidity in the English population. They found out that undernutrition during foetal life was primarily responsible for the adult morbidity and mortality. These adaptations or "Programming" were necessary for survival of the foetus in an unfavourable environment. Poor health of the mother during pregnancy was responsible for the increased risk of cardiovascular disease, stroke and diabetes in these offspring. This led to the articulation of "The Foetal Origins of Adult Disease" hypothesis which states that, "Undernutrition in foetal life acts to program the risk for early onset of disease in the adult life". Similar studies carried out in India have revealed the "Thin-Fat" Indian phenotype. These initial observations have been confirmed later by many human and animal studies. We have previously reported that Wistar Rats (Thrifty Jerry) that have been undernourished for fifty generations show leukopenia, macrocytic anaemia and increased platelet count. The present study tried to evaluate if a standard rat diet (Recuperation diet) provided could reverse the harmful effects of 50 generations of undernutrition. The blood indices and the platelet count were restored after 6 generations of a recuperation diet. However, the white blood cell count was higher along with the presence of lymphocytosis and granulocytosis .6 generations of the recuperation diet could not completely reverse the effects of multigenerational (50 generations) undernutrition.

Key Words: Foetal Programming, Multigenerational Undernutrition, Complete Blood Count, Recuperation Diet

***Address for Correspondence:**
Dr. M.S. Karandikar, Professor, Department of Physiology, D.Y. Patil Medical College, D.Y. Patil University, Pimpri, Pune 411018, INDIA.
Email:mskarandikar@yahoo.com
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INTRODUCTION

David Barker's epidemiological studies in Southampton, England showed that under-nutrition in foetal life leads to adaptations that can be detrimental in adult life. His thus pioneered the concept of "Foetal Origins of Adult Disease" (Godfrey and Barker 2000). This work has been substantiated and expanded by both animal as well as many human studies. These adaptations or "programming" are a result of the changes that are at a hormonal, cellular as well as metabolic level (McMillan and Robinson 2005). Yajnik *et al* have studied these adaptations in the Indian population and have found that Indians at birth are centrally obese, insulin resistant but have a lower soft tissue mass, "The Thin-Fat Phenotype" (Yajnik, Fall *et al* 2003).As a result of these adaptations, coronary heart disease, hypertension and Diabetes Mellitus in developing countries like India is very common and has assumed epidemic proportions

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(Echouffo-Tcheugui 2012).We have studied the effects of multigenerational undernutrition in a Wistar rat model (Thrifty Jerry) and have shown that these rats mimic the Indian phenotype. These animals are centrally obese, show insulin resistance, deficiency of Vitamin B_{12} and folate levels along with lower soft tissue growth (Hardikar *et al* 2015).Studies carried out on the hematopoietic environment in undernourished animals have shown the presence of hypocellularity, necrosis, and extracellular matrix modifications (Travlos GS (2006), Fried W *et al* (1978), Vituri CL(2000), Prestes-Carneiro *et al* (2006) and Borelli P *et al*(1995).The Thrifty Jerry rats showed the presence of leukopenia, macrocytic anaemia and increased platelet count with fifty generations of undernutrition (M.M.Karandikar *et al*).The present study evaluated the role of providing a standard rat diet (recuperation diet) to the undernourished animals (transition colony) for 6 generations.

MATERIALS AND METHODS

The undernourished wistar rat colony was given a standard rat pellet diet for 6 generations- "Transition Colony".10-week-old adult female rats from the 6th generation of the transition colony were studied. The control group received a standard rat feed. Blood was collected by retro-orbital bleeding and serum was immediately separated and analysed for Complete Blood Count (CBC) on an auto analyser (Mindray BC2800). The study was ethically approved by the institutional ethics committee.

Statistical Analysis

All estimations were carried out in triplicates and values are expressed as +/- Std Error of Mean (SEM). The statistical significance was evaluated by the unpaired t test using Graph Pad Prism 6 version software.

RESULTS

Table 1: Complete Blood Counts in Control and Transition Adult Wistar Rats

Estimations	Control Mean ± SEM	Recuperation Mean ± SEM	P value
HCT	30.57 ± 5.535, n= 6	38.80 ± 1.011, n= 5	0.2160
MCV	53.68 ± 0.6635, n= 6	53.56 ± 0.6875, n= 5	0.9007
MCHC	29.58 ± 0.3458, n= 6	30.40 ± 0.1000, n= 5	0.0674
RDW****	14.97 ± 0.3685, n= 6	11.34 ± 0.4045, n= 5	< 0.0001
PLT	812.3 ± 40.98, n= 6	706.4 ± 46.48, n= 5	0.1204
MPV	7.067 ± 0.5506, n= 6	5.067 ± 1.018, n= 5	0.1148
PDW	15.53 ± 0.2092, n= 6	14.90 ± 0.08944, n= 5	0.0295
PCT	0.4960 ± 0.05083, n= 6	0.3694 ± 0.07310, n= 5	0.1781
WBC****	5.700 ± 0.7933, n= 6	8.940 ± 0.4589, n= 5	0.0087
Lymphocytes****	81.38 ± 2.022, n= 6	56.02 ± 0.8261, n= 5	< 0.0001
Monocytes**	2.783 ± 0.2926, n= 6	3.900 ± 0.2121, n= 5	0.0157
Granulocytes****	15.87 ± 1.808, n= 6	40.08 ± 0.9661, n= 5	< 0.0001
RBCs	5.648 ± 1.001, n= 6	7.258 ± 0.1753, n= 5	0.1838
Hb	9.017 ± 1.656. n= 6	11.68 ± 0.3338, n= 5	0.1851

Significance: * p< 0.01, ** p<0.001, ***/****P<0.0001

DISCUSSION

The foetal origins of adult hypothesis proposed by David Barker and his colleagues analysed the role of foetal undernutrition and diseases in adult life. His studies have shown that foetal undernutrition cause hypertension, coronary heart disease and type 2 diabetes in adult life. (Godfrey and Barker 2000). These adaptations labelled as "programming" are due to adjustments in a variety of systems that occur during fetal life and occur at the structural, metabolic and hormonal level. Since David Barker's studies, human and animal studies have confirmed and modified these initial observations and is now called as Developmental Origins of Adult Disease (DoHAD) (McMillan and Robinson 2005). The Pune Maternal Nutrition Studies (PMNS) by Yajnik *et al* have studied the Indian scenario that have led to the understanding of "Thin-Fat" Indian phenotype [Yajnik, Fall *et al* (2003), Yajnik and Deshmukh, (2012)]. We have carried out studies in a Wistar rat model (Thrifty Jerry) that has been undernourished for 50 generations. The animals were insulin resistant, centrally obese and had smaller visceral organs (Hardikar *et al*).Studies carried out so far have primarily focused on the endocrine, metabolic, cardiovascular and renal adaptations to undernutrition. A few studies have evaluated the role of undernutrition on the haemopoietic environment and have shown the presence of Hypocellularity, necrosis and and extracellular matrix modifications (Travlos GS (2006), Fried W *et al* (1978), Vituri CL (2000), Prestes-Carneiro *et al* (2006) and

Borelli P *et al* (1995). However very few studies have evaluated the role of multigenerational undernutrition (50 generations). We have previously reported the role of multigenerational undernutrition on the blood cell counts and have shown leukopenia, macrocytic anaemia and increased platelet count in these animals (M.M. Karandikar *et al*). This study has evaluated the role of providing a standard rat diet (Recuperation diet) to the undernourished animals for 6 generations. The transition colony showed the presence of leucocytosis (5.70 in Control vs 8.94 in Transition $x10^3/\mu L$). This was associated with granulocytosis (15.87 vs 40.08 %) and lymphopenia (81.38 vs 56.02%). This was suggestive of a state of acute infection. This exact cause is unknown and would require further evaluation. The macrocytic anaemia as seen in the undernourished colony was completely corrected with 6 generations of a recuperation diet. MCV (53.68 vs 53.56 μm^3) and MCHC (29.58 vs 30.40 g/dl) was normalized. Red cell count, hematocrit and hemoglobin levels were also normal in the transition colony. This showed that 6 generations of a recuperation diet could completely reverse the macrocytic anemia. Hardikar *et al* have evaluated the same effect in 2 generations of a recuperation diet. They could show partial improvement but the changes were not completely reversed. 6 generations of the recuperation diet were required to reverse the changes of macrocytic anemia. Interestingly red cell distribution width showed a significantly lower value (14.97 vs 11.34) showing cells that had no anisocytosis or poikilocytosis. This needs further evaluation. This study also showed that platelet levels were normalized in the transition colony after 6 generations of the recuperation diet. We have not carried out bone marrow studies nor have we looked the epigenetic changes that could be involved in this transition colony. Further studies that would involve both bone marrow evaluation and epigenetic studies could throw light on the adaptations and reversal of the macrocytic anaemia and restoration of the platelet count. The presence of granulocytosis is indicative of an acute state of infection in these animals. This study has thus shown that 6 generations of a normalised diet could not completely reverse the effects of 50 generations of undernutrition in the Thrifty Jerry Colony.

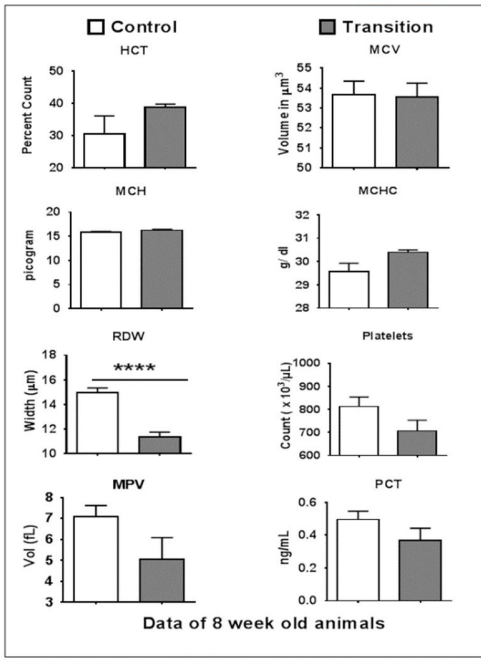

Figure 1: Blood Cell Count in Control and Transition Adult Female Wistar Rat

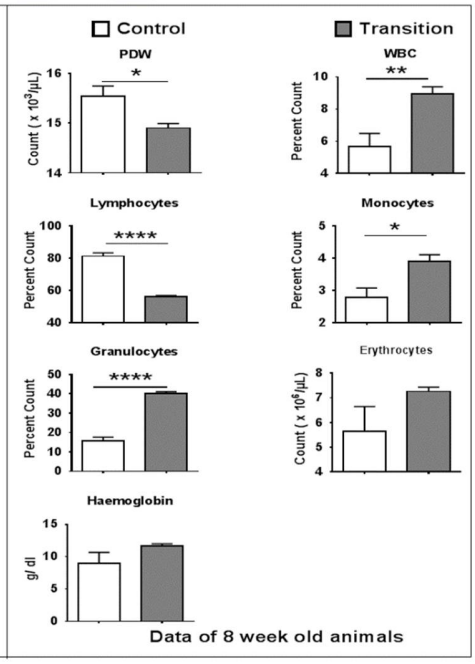

Figure 2: Blood Cell Count in Control and Transition Adult Female Wistar Rat

CONCLUSIONS

Multigenerational undernutrition for more than 50 generations that causes macrocytic anaemia can be reversed with 6 generations of a recuperation diet. This is however associated with leucopoenia, granulocytosis and lymphopenia indicative of an inflammatory state.

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PROKARYOTES

Whole-Genome Sequencing and Annotation of a Drug-Resistant Extrapulmonary Clinical Isolate of Beijing Genotype *Mycobacterium tuberculosis* from Pune, India

Swarupa M. Hatolkar,[a] Rabindra N. Misra,[a] Rajesh Mahato,[b] Savita Jadhav[a]

[a]Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital & Research Centre, Dr. D. Y. Patil Vidyapeeth (DPU), Pune, India

[b]ArrayGen Technologies Pvt. Ltd., Pune, India

ABSTRACT Whole-genome sequencing has emerged as a powerful tool to map genetic diversity among *Mycobacterium tuberculosis* isolates and identify the genomic signatures associated with drug resistance, pathogenesis, and disease transmission. Isolate LJ319 of the *Mycobacterium tuberculosis* complex (MTC)-Beijing genotype circulating in Maharashtra, India, which was obtained from the cerebrospinal fluid (CSF) of an immunocompetent patient, was subjected to whole-genome sequencing.

Tuberculosis (TB) remains one of the leading causes of morbidity and mortality worldwide. Extrapulmonary tuberculosis (EPTB) constitutes around 15 to 20% of TB cases in immunocompetent individuals (1). Whole-genome sequencing has emerged as a powerful tool to map genetic diversity among *Mycobacterium tuberculosis* isolates and identify the genomic signatures associated with drug resistance, pathogenesis, and disease transmission. Several pulmonary isolates of *M. tuberculosis* have been sequenced over the years. However, availability of whole-genome sequences of *M. tuberculosis* isolates from extrapulmonary sites is limited. Whole-genome sequencing in conjunction with comprehensive drug susceptibility testing can reveal clinically relevant mutations associated with drug resistance (2, 3).

In the present research, *Mycobacterium tuberculosis* LJ319 of the *M. tuberculosis* complex (MTC)-Beijing genotype circulating in the population of the Maharashtra state, which was isolated from cerebrospinal fluid (CSF) from an immunocompetent patient, was subjected to whole-genome sequencing (4). Isolation was done on conventional Lowenstein Jensen (LJ) solid culture. The isolate was found to be resistant to rifampin and isoniazid, and multidrug resistance (MDR) was confirmed with a line probe assay and Sanger sequencing methods (5, 6).

The paired-end sequencing was performed on an Illumina MiSeq platform. High-quality reads were mapped to the genome of reference strain *M. tuberculosis* H37Rv (GenBank accession no. NC_000962) using SPAdes v. 3.11, generating a reference assembly with an average read-mapping coverage of 100×.

The National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline was used for the annotation of the reference assembly.

The total numbers of genes and coding sequences (CDSs) identified are 4,285 and 4,074, respectively. Three types of rRNA, namely, 5S, 16S, and 23S, have been annotated. There are 44 tRNAs and 71 genes that exhibited frameshift mutations.

We have performed spoligotyping of the same strain and after analysis found that it is an East Asian Beijing genotype with the 43-spacer binary code (00000000000000000000000000000000000000111111111).

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Address correspondence to Swarupa M. Hatolkar, swarupa.hatolkar2007@gmail.com.

Accession number(s). The annotated whole-genome sequence has been deposited in the NCBI GenBank database with the accession no. CP026742.

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(71)**Name of Applicant :**
1)Dr. D. Y. Patil Vidyapeeth
Address of Applicant :Sant Tukaram Nagar Pimpri, Pune-411018 India Maharashtra India
(72)**Name of Inventor :**
1)PALEKAR, Dr.Tushar J.
2)VARDHAN, Dr.G.D.Vishnu

(57) Abstract :
The present invention provides a real-time chest expansion measuring system (100) comprising a sensor unit (102) and a display unit (104). The sensor unit (102) is having a belt (105) with a sensing means (106), a first processor (108), a first radio module (110), a battery (112) and a charger (114). The display unit (104) is having a second processor (116), a second radio module (118), a user interface and a memory means (122). The sensing means (106) is configured to sense chest expansion of a person and provide corresponding data to the first processor (108). The first processor (108) is configured to analyze and process the data in real-time. Further, the first radio module (110) transmits the processed data to the display unit (104). The second processor (116) receives the processed data through the second radio module (118) and the user interface displays the processed data in real-time. FIG. 1

No. of Pages : 31 No. of Claims : 10

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Sir/Madam,

The Director General of the Council sanctions the above mentioned research scheme initially for a period of one year from **16.09.2019** subject to extension upto the total duration specified in para 3 below.

The Director General of the Council also sanctions the budget allotment of **Rs. 8,80,300/- (Rupees eight lakhs eighty thousand three hundred only)** as detailed in the attached statement for the period ending the **15.09.2020.**

The grant-in-aid will be given subject to the following conditions:-

1. The payment of the grant will be made in lump-sum to the head of the Institution. The first installment of the grant will be paid generally as soon as a report regarding the commencement of the project and appointment of the staff is received by the Council. The demand for payment of the subsequent installment of the grant should be placed with the Council in the prescribed proforma attached.

2. The staff appointed on the project should be paid as indicated in the budget statement attached.

3. The approved duration of the scheme is **2 ½** year. The annual extension will be given after review of the work done on the scheme during the previous year.

4. A report on the progress made will be submitted to the Council as and when called for.

5. The institute will maintain a separate account of the receipts and the expenditure incurred on the scheme and will furnish a utilization certificate and an audited statement of account pertaining to the grant.

6. The Host Institute shall utilize the grant after following the provisions laid down in the GFRs 2017 and T.A. Rules.

7. The PI is advised to keep the fund in a separate Saving Bank Account opened for research funds received from ICMR so as to ensure that interest earned thereon is also credited in to the Fund Account

O/C

P.T.O.

8. The other terms and conditions are indicated in Annexure-1

The receipt of this letter may please be acknowledged.

Yours faithfully,

(Ishwar Likhar)
Admn. Officer
For Director-General

This issues with the concurrence of Finance Section vide RFC No. (P.N.27) NCD/Adhoc/88/2019-20 dated 03.09.2019
No. 5/4/7-16/2019/NCD-II

1. Copy together with a copy of the budget statement forwarded for information to Dr. Varsha S Dabadghao, Professor, Department of Medicine, Dr. D.Y. Patil Medical College, Pimpri, Pune-411018
2. Copy together with a copy of the budget statement forwarded to the **Accounts Section** for information and necessary action.
3. A. O.
4. Mr. Hemant, Sr. T.O., ICMR
5. IRIS Cell (2014-1283)
6. Dr. Chanchal Goyal, Scientist 'E', Online Cell, ICMR

Budget Statement
16.09.2019 to 15.09.2020

No.5/4/7-16/2019-NCD-II

Subject: - "Prevalence of microalbuminuria as a marker of early nephropathy in obese non diabetic and non hypertensive adults with reference to adiopokine levels" Under Dr. Varsha S Dabadghao, Pune

Staff	**1st Year**
Lab Technician@ Rs. 18,000/- pm PB 5200+GP 2800=8000x10% = 800 @Rs. 18,800/-PM- (2nd year)	2,16,000/-
Data Entry Operator-A @ Rs. 17,000/- pm PB 5200+GP 2400=7600x10% = 760 @Rs. 17,760/-PM- (2nd year) @Rs. 18,520PM-(3rd year)	2,04,000/-
Statistician @ Rs. 32,000/- pm	
Total (A)	**4,20,000/-**
Contingencies (Recurring (kits and other items)	
Eliza kits	2,50,000/-
Microalbuminuria and lab tests	65,000/-
Stationary, Printing, Writing	26,000/-
Misc.	
Overall cost escalations	5000
Total (B)	**3,46,000/-**
Total (A+B)	**7,66,000/-**
5 % overhead Charges	38,300/-
Travel	6000/-
Non-recurring (Equipment)	
Cryovials, micropipettes, beakers, mic equipment etc)	**70,000/-**
Grand total	**8,80,300/-**

(Rupees eight lakhs eighty thousand three hundred only)

ANNEXURE-I

TERMS AND CONDITION OF THE GRANT

i) Approval of the research proposal and the grant being released is for the specific project sanctioned and should be exclusively spent on this project within the stipulated time.

ii) Expenditure should be on no account exceed the budget sanctioned for the enquiry. Expenditure incurred over the above the sanctioned amounts against one or more sub-heads of expenditure such as pay, allowances, contingencies etc, shall be met without reference to the ICMR, by re-appropriation of savings under remaining sub-heads provided by re-appropriation of incurred during the financial year is within the over all sanctioned ceiling of that year.

iii) No expenditure shall be incurred on items not sanctioned by the Council. Savings should also not be re-appropriated for meeting or incurring expenditure on staff that has not been sanctioned by the council.

iv) The grant paid by the Council shall be refunded in full by the Institution if and when the grantee concerned discontinues a scheme midway or does not follow the detailed technical programme laid down and approved.

v) Receipts realised by the project officer and the sale proceeds, if any, will be remitted to the Council as miscellaneous receipts and should not be utilized for meeting expenditure on the scheme.

vi) All facilities for the conduct of the research scheme basic equipment and other ordinary laboratory chemicals, glass ware, furniture and other help as may be required for the smooth working of the scheme shall be provided by the institute.

Staff :-

vii) The staff employed on the research scheme will not be treated as employees of the Council and the deployment of such staff at the time of completion or termination of the project will not be the concern/responsibility of the Council. They will be subjected to administrative control of the Institution and will be appointed generally in accordance with the normal recruitment rules and procedure of the Institute.

viii) The Council will not be liable to bear any expenditure on pension/provident fund contribution and/or leave salary contribution incurred and committed by the grantee Institution for persons appointed on deputation from another organizations.

viii (A) An undertaking on part-I (specimen attached) (Appendix 'A') to be obtained from the Head of the Institute where extra-mural project funded by ICMR are being sanctioned, may be sent to Council. The second part of the U.K. to be obtained from each employees, by the Principle Investigator.

No grant will be released unless the undertaking is receive by us sufficiently in advance to consider any release.

* undertaking

Release of funds

ix) The first installment of the grant will be paid as soon as a report regarding the commencement of the project and appointment of staff is received by the Council. The Demand for payment of subsequent installments of the grant should be placed with the Council in the prescribed form (Appendix 'B').

x) The institute will maintain separate audited account for this project. If it is found expedient. Keep a part of whole of the grant in a back account earning interest, the interest thus earned should be reported to the Council. The interest thus earned will be treated as a credit to be adjusted towards further installment of the grant.

xi) The accounts will be subject to audit by the authorized auditor of the Institutions. In case, facilities are not available for such auditing, the account will be audited by the Council's own internal auditors. Latest by the end of December, following the financial year for which the grand is paid, an audit certification from, the auditors to the effect that "the accounts have been audited and that the money was actually spent on the objects for which it was sanctioned" shall be submitted to the Council.

xii) Further grants will be stopped unless audited statements of accounts, utilization certificates are received within a period of one year after the end of the financial year for which grant was sanctioned.

Stores:

xiii) All expendable and non-expendable articles required for work of the enquiry should be purchased in accordance with the procedure in vogue in the institution. For permanent and semi-permanent assets acquired solely or mainly out of the grant, a separate audited record in the form of register in the prescribed Performa enclosed shall be maintained by the Institute. The term "assets means (1) immovable property and (ii) movable property of capital nature where the value exceeds Rs. 1,000/-. Separate assets registers for items costing Rs. 20,000/- or more and less than Rs. 20,000/- each item may be maintained. (Appendix "C").

For other stores purchased from the Council's grant, the Performa will be the same as is being used by the Institute.

All the assets acquired from the grant will be property of the Council and should, not without the prior sanction of the Council, be disposed of or encumbered or utilized for purpose other than those for which the grant has been sanctioned.

Publications

The financial assistance rendered by the council should be acknowledged in any published account of work for which the grant is given.

The council publishes own journal " Indian Journal of ('B') Medial research", In case, it is proposed to publish the papers based on the work done under the auspices of the Council in Journals other than the IJMR, the name of the journal in which it is proposed to publish the paper may please be intimated. A reprint of paper when published may please be sent to the Council for information and record.

Prior permission of the Council should be obtained before publication of any such papers in a foreign journal.

Patents

The Council shall have the right to make out patents in respect of inventions/discoveries make under a scheme/project financed by the council. The officer-in-charge or the staff employed on ICMR Schemes shall not apply or obtain patents for any invention/discovery made by them without prior approval of the council.

All patents will be registered with NDRC in the name of the Indian Council of Medical Research.

Termination of Enquiry:

Prior permission of the Council should be obtained if the investigator desires to discontinue the enquiry. The reasons for discontinuing the scheme should invariably be stated. The investigator should submit a complete and detailed report of the work done by him on the project till the date of relief.

Any unspent balance out of the funds given to the institute shall be refunded to the ICMR on termination of the scheme.

A final report is required to be submitted within one month from the date of termination of the enquiry.

A list (in duplicate) of non-expendable and expendable articles together with property registers and suggestions for disposal of the articles should be sent to the Council within a month from the date of termination of the enquiry.

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