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Details of papers published in the journals notified on UGC – CARE list in the UGC website 2017

S.N	UGC website/ Scopus/ Web of Science/ PubMed	Publication type	Publication title	Author name	Journal name	Year
1.	Scopus	Original article	Assessment of handedness and footedness by lateral preference inventory	Susie Jeyalyn David, S. Rajasankar	Research Journal of Pharmacy and Technology	2017
2.	Scopus	Original article	The effect of Oxalis corniculata extract against the behavioral changes induced by 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice	K. Aruna, P. Devi Raja Rajeswari, S. Raja Sankar	Journal of Applied Pharmaceutical Science	2017
3.	28420370	Original article	Neuroprotective effect of Demethoxycurcumin, a natural derivative of Curcumin on rotenone induced neurotoxicity in SH-SY 5Y Neuroblastoma cells	Ramkumar M, Rajasankar S, Gobi VV, Dhanalakshmi C, Manivasagam T, Justin Thenmozhi A, Essa MM, Kalandar A, Chidambaram R.	BMC complementary and alternative medicine	2017
4.	28384869	Original article	Does Iron Deficiency Anemia and its Severity Influence HbA1C Level in Non-Diabetics? An Analysis of 150 Cases	Rajagopal L, Ganapathy S, Arunachalam S, Raja V, Ramraj B	Journal of Clinical and Diagnostic Research	2017

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5.	27512228	Original article	Lumbar epidural varices : An unusal cause of lumbar claudication	Subbiah M, Yegumuthu K.	Indian journal of Orthopedics	2016
6.	28776523	Original article	Screening of health-care workers for latent tuberculosis infection in a Tertiary Care Hospital.	Janagond AB, Ganesan V, Vijay Kumar GS, Ramesh A, Anand P, Mariappan M.	Int J Mycobacteriol	2017
7.	28857727	Review article	Fungal endocarditis in paediatrics: a review of 192 cases (1971-2016).	Ganesan V, Ponnusamy SS, Sundaramurthy R	Cardiol Young	2017
8.	28776523	Original article	Screening of health-care workers for latent tuberculosis infection in a Tertiary Care Hospital	Janagond AB, Ganesan V, Vijay Kumar GS, Ramesh A, Anand P, Mariappan M	International journal of mycobacteriology	2017
9.	28784374	Original article	Pre-treatment Factor Structures of the Montgomery and Åsberg Depression Rating Scale as Predictors of Response to Escitalopram in Indian Patients With Non-Psychotic Major Depressive Disorder	Basu A, Chadda R, Sood M, Rizwan SA.	Asian J Psychiatr	2017
10.	29916423	Original article	Inequity in access to inpatient healthcare services for non-communicable diseases in India and the role of	Jeyashree, K., Prinja, S., Kumar, M. I., & Thakur, J. S.	The National medical journal of India	2017

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11.	28928319	Original article	Effect of a brief smoking cessation intervention on adult tobacco smokers with pulmonary tuberculosis: A cluster randomized controlled trial from North India.	Goel S, Kathiresan J, Singh P, Singh RJ.	Indian J Public Health	2017
12.	29059214	Original article	Effect of glycemic control and type of diabetes treatment on unsuccessful TB treatment outcomes among people with TB-Diabetes: A systematic review	Shewade, H.D., Jeyashree, K., Mahajan, P., Shah, A.N., Kirubakaran, R., Rao, R. and Kumar, A.M.,	PloS one	2017
13.	28928348	Original article	Cross-cultural adaptation of Jefferson Scale of Empathy-health professions students version: An experience with developing the Tamil translation	Jeyashree K, Kathirvel S, Prathibha MK	Educ Health.	2017
14.	28690162	Original article	National guidelines on screening for Diabetes among patients with Tuberculosis in India: Need for clarity and change in screening cut off?	Hemant Deepak Shewade, Kathiresan Jeyashree, Preetam Mahajan, Ajay MV Kumar.	Diabetes & Metabolic Syndrome: Clinical Research & Reviews.	2017

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15.	28594824	Original article	Comparing yield and relative costs of WHO TB screening algorithms in selected risk groups among people aged 65 years and over in China, 2013	Zhang C, Ruan Y, Cheng J, Zhao F, Xia Y, Zhang H, Wilkinson E, Das M, Li J, Chen W, Hu D, Jeyashree K, Wang L.	PLoS ONE	2017
16.	28442941	Original article	Competency-based tool for evaluation of community-based training in undergraduate medical education in India – a Delphi approach.	Shewade HD, Jeyashree K, Kalaiselvi S, Palanivel C, Panigrahi KC.	Advances in Medical Education and Practice.	2017
17.	28884034	Case Report	Snakebite induced thrombotic microangiopathy leading to renal cortical necrosis	Ying Mao Gn, Arvind Ponnusamy, and Vikram Thimma	Case reports in nephrology	2017

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RESEARCH ARTICLE

Assessment of handedness and footedness by lateral preference inventory

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ABSTRACT:
Handedness is considered as the most obvious type of behavioral and cognitive asymmetry reported in human beings. Also we have footedness in the motor domain. This study investigated the percentage distribution and their significance for handedness and footedness in both sexes of adolescent age group in both left and right handers based on laterality preference inventory. Handedness got a significant result whereas footedness score has no significance based on the laterality preference inventory.

KEYWORDS: Laterality, handedness, footedness.

INTRODUCTION:

Handedness is the most obvious type of cognitive and behavioural asymmetry reported in human beings. It refers to the preference to perform several tasks with one hand rather than the other. Also, the brain mechanism underlying handedness is such that handedness is contralaterally related to the two hemispheres, left hand to the RH and right hand to the LH. There is evidence that left- and right-handers differ when compared on a variety of behavioural measures[1]. Generally, right-handers are more homogeneous than left-handers on behavioural measures, as they are more likely to prefer the righthand for manual activities, to be more dexterous and powerful with the right-hand, and to have language lateralized to the contralateral left cerebral hemisphere. Thus, in right-handers the neural systems that contribute to these behaviours are lateralized predominantly to the LH, and the hand used to write is contralateral to the hemisphere mediating language functions. In contrast, in most lefthanders language is ipsilateral to the preferred hand. About 60% of left-handers have LH dominant for language.

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Also it is quite often to see that the left handers are dissociated from the society due to the cultural skill habits which predominantly visions right handed behavior as a token of discipline, mannerism and moral attitude[2]. Early research activities have documented that in many non-western cultures the prevalence of right handedness is greater than that found in the Western cultures[3]. Functional asymmetries in healthy individuals are often inferred from the performance asymmetries on central (direct) and peripheral (indirect) measures. Further, these measures vary in the degree of involvement of responses at perceptual and motor levels. The central measures operating at the perceptual level mainly tap the biases in visual, auditory, and tactual modalities. The central measures operating at the motor level include physiological measures such as Electromyography and Galvanic Skin Response. The peripheral measures of asymmetry in the perceptual domain involve preference measures of eyedness, earedness, and conjugate lateral eye movement (CLEM), and in the motor domain the preference and performance measures of handedness and footedness. These diverse measures assess laterality in disparate ways. Among these various measures of understanding the implications of hemispheric asymmetry, hand preference analysis has proved to be prominent and has gained importance amongst the researchers[4].

Handedness has several concerns with allocation of activity at the work places in lines of performance ability. This is important because the environment and various gadgets and instruments are often organized in favour of right-handed people. Handedness, however, has been approached from the perspective of preference as well as performance. While performance measures are behavioural and objective, the preference measures involve self reports and are primarily based on memory.

It has been commented that the incidence of left-handedness is more in males than in females [5]. It was also stated that the left-handedness was more in the younger age group than in the older adults. A study on foot overlapping (leg crossing) in German samples [6] concluded that 62% of the population were right leg crossers, 26% were left leg crossers and that 12% were ambilateral. Foot overlapping is correlated to some extent with footedness and handedness. Digital interlocking, arm folding and handedness have a place in human biology, far beyond their possible uses in genetics and in population comparisons as they are directly related to the cerebral voluntary control actions and hence are most important in the investigation of brain disorders.

MATERIALS AND METHODS:

A sample of 210 student volunteers were selected from various schools for the study. They were selected in such a way that the sample for our study consisted of equal number of right handed and left handed volunteers. Sample selection was based on Systematic random sampling method.

Inclusion criteria:

1. Consenting individuals both male and female between 11- 17 years.
2. Consenting right handers matching to left handers were rolled in.

Exclusion criteria:

1. Individuals having any gross deformity were excluded
2. Individuals who cannot give consent to participate in the study.

The parents of these volunteers were informed about the intended study, its procedures and consent was also obtained from the parents of each volunteer before inclusion in this protocol, which received the approval of the Institutional Human Ethics Committee.

Handedness and footedness were assessed by Lateral Preference Inventory[7]. The Lateral Preference Inventory is a brief, 16-item questionnaire, which validly measures hand, foot, eye, and ear preference

RESULTS AND DISCUSSION:

Table-1: Percentage distribution for Handedness score between Left and Right handers (LPI)

Left Handedness					Right Handedness				
Score	Male		Female		Score	Male		Female	
	No	%	No	%		No	%	No	%
-4	27	40.9	15	38.5	+4	25	37.8	15	38.5
-3	16	24.3	11	28.2	+3	19	28.8	11	28.2
-2	9	13.6	5	12.8	+2	11	16.7	8	20.5
-1	14	21.2	8	20.5	+1	11	16.7	5	12.8
Total	66	100.0	39	100.0	Total	66	100.0	39	100.0
Mean±SD	2.2±1.2		2.2±1.2			2.1±1.1		2.1±1.1	
Significance	t =.010, df=103, P>0.05					t =.202, df=103, P>0.05			
Mean±SD	2.2±1.2					2.1±1.1			
Significance	t =0.307 df=208, P<0.001								

40.9% of male left handers had the maximum score -4.
38.5% of female left handers had the maximum score -4.
37.8% of male right handers had the maximum score +4.
38.5% of female right handers had the maximum score +4.
In both left and right handers male students showed a higher percentage for handedness score.

Handedness score for left handers was not significant P>0.05
Handedness score for right handers was not significant P>0.05
Handedness score when considered for both left and right handers was highly significant P<0.001

Table-2 Percentage distribution for Footedness score in Left handers (LPI)

Left Handedness						
Footedness score	Male			Female		
	Frequency	Percent	Cumulative Percent	Frequency	Percent	Cumulative Percent
-4	17	25.7	25.8	10	25.6	25.6
-3	13	19.7	45.5	8	20.5	46.2
-2	7	10.6	56.1	4	10.3	56.4
-1	10	15.2	71.2	7	17.9	74.4
Total	47	71.2		29	74.3	
1	4	6.1	77.3	3	7.7	82.1
2	3	4.5	81.8	1	2.6	84.6
3	5	7.6	89.4	3	7.7	92.3
4	7	10.6		3	7.7	100.0
Total	19	28.8		10	25.7	
Total	66	100.0	100.0	39	100.0	
Mean±SD	1.2 ± 2.8			1.4 ± 2.6		
Significance	t =0.32, df=103 , P>0.05					
Mean±SD	1.3±2.7					

Table-3 Percentage distribution for Footedness score in right handers (LPI)

Right Handedness						
Footedness score	Male			Female		
	Frequency	Percent	Cumulative Percent	Frequency	Percent	Cumulative Percent
-4	1	1.5	1.5	2	5.1	5.1
-3	1	1.5	3.0	Nil	Nil	-
-2	Nil	Nil	-	1	2.6	7.7
-1	1	1.5	4.5	1	2.6	10.3
Total	3	4.5		4	10.3	
1	12	18.2	22.7	7	17.9	28.2
2	9	13.6	36.4	6	15.4	43.6
3	17	25.8	62.1	9	23.1	66.7
4	25	37.9	100.0	13	33.3	100.0
Total	63	95.5		35	89.7	
Total	66	100.0	100.0	39	100.0	66
Mean±SD	2.6 ± 1.6			2.2 ± 2.1		
Significance	t =1.1, df=103, P>0.05					
Mean±SD	2.4 ± 1.9					

Among the left handers, 71.2% of males used their left foot whereas 28.8% used their left foot; In females 74.3% were left footed and 25.7% were left footed. In both sexes footedness scores were not significant P>0.05 Among the right handers, 95.5% of males used their right foot whereas only 4.5% used their left foot; In females 89.7% were right footed and 10.3% were left footed. In both sexes footedness scores were not significant P>0.05

While there are a number of self-report inventories for the measurement of handedness[8], very few questionnaires have been developed that can provide a quick, valid measure of all four indexes of lateral preference: Handedness, footedness, eyedness and earedness. A series of behaviorally validated items have been gathered together to form the Lateral Preference Inventory (LPI), which is a brief but valid and reliable measure of hand, foot, eye, and ear preference. Data are simply scored for each four item scale as (R—L), where R is the number of "right" responses and L is the number of "left". This means that we have scales that go from -4 to 4, with -4 meaning consistent left-sidedness and 4 meaning consistent right-sidedness for any index. A score of zero would indicate ambilaterality. Using the simple left-right dichotomy (cutting at 0, with LPI = 0, included with the lefts), females are more likely to be right-handed [90.8% vs. 88.2%; x 2 (l) = 5.99, p < .05]. Females are also more often right-footed (88.9% vs. 83.9%; x 2 (l) = 17.63, p < .001) [7].

During the onset of hemispherical asymmetry analysis, researchers postulated that there is a direct correspondence between all the features: handedness, footedness, eyedness[9]. But in fact studies show that there is an ipsilateral correlation among these features. Also it showed the correlation of handedness with the different parameters. Left handed females showed a 20% tendency of using the right foot. Right footed females had a 98.8% chance of using the right hand. Left footed females had a 100% chance of using the left hand. Left handed males had a 50% chance of using the right foot. Left footed males had a 50% chance of using the right hand. Both males and females showed a significant correlation between handedness and foot preference (P<0.001). A difference of opinion on this matter was indicated in the study on the foot and eye preference in adults, their relationship with the hand, sex and age [10].

There is a higher proportion of crossed hand-foot preference in men than in women. Most importantly cross dominance favours sports persons lending them with additional skill sets which is of prime importance to the sports flavour [11]. Cultural moralization too forces the behavior related to handedness with the feminine gender affected the most, to follow right handedness.

Results from the tapping, pegboard events using monozygotic twins reports that there is strong deviation in performing activities with right and left hands, the right-handers being more strongly lateralized than left-handers[12].

It is also found that there is a direct correlation between the preference and its performance which is quite significant with the young adults[13]. Peters argued that preference induces asymmetry in a given skill as a result of increased use of the preferred hand[14]. However, hemispheric specialization has also been implicated. Thus, rapid finger tapping has shown right hand advantage which is attributed to LH specialization for the organization and control of sequential movement[15]. Right hand advantage in performance has been attributed to the fact that LH is superior in timing the forces involved in accelerating and decelerating the movement. This view goes well with the work on aiming as well as the studies showing right hand advantage in rapid finger tapping[16]; finger sequencing[17] and throwing [18]. Few researchers have found that aiming asymmetries in favour of right hand became more pronounced as the task demands have increased. It reflects LH's precision in the timing of muscle contraction and information processing proficiencies [19].

CONCLUSION:

The extent of hand-preference is likely to influence performance asymmetry of both hand and foot. The consistent left- and right handers would show strong lateralization and consequently more pronounced motor asymmetries in performance. The peripheral preference measures include self-reported endorsement of the use of long limbs like hand and foot, and sensory receptors like eye and ear. It is hoped that the normative data provided here may prove to be a useful indication of the distribution of lateral preference in adolescents. We need multitask framework to explore the hemispheric asymmetries

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The effect of *Oxalis corniculata* extract against the behavioral changes induced by 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the development of rigidity, resting tremors and postural instability. Recently, the focus of PD's treatment has shifted towards herbal medicines. The aim of the study was to evaluate neuroprotective effect of alcoholic extract of *Oxalis corniculata* (Oxalidaceae), via the analysis of behavioral features in MPTP (1-methyl,4- phenyl-1,2,3,6-tetra hydro pyridine) mouse model. Behavioral studies were performed by the actophotometer**,** elevated plus maze**,** rotarod, hole board, step down and step through tests. Treatment with *O. corniculata* reversed the alterations in locomotor and muscle coordination in MPTP induced Parkinsonic mouse. The results achieved in this study reveal that different doses of *O. corniculata* increased memory retention and retrieval significantly. Memory retention and retrieval enhancement by *O. corniculata* extract could be due to the presence of antioxidants such as flavonoids, coumarins, tocopherols and phenolic acids and their power in scavenge reactive oxygen species.

INTRODUCTION

Parkinson's disease (PD) is an age-related progressive neurodegenerative disease, which is characterized by resting tremors, rigidity, postural abnormalities, particularly stooped posture, and difficulty or failure to execute willed movements, i.e. bradykinesia, akinesia and festinating gait (Tillerson *et al.*, 2003; Oida *et al.*, 2006). Parkinson's disease was recorded in ancient India. It was called as *Kampavatha*. Many traditional medicinal plants were used for the healing of many diseases including stress, insomnia, anxiety, arthritis and other disorders related to the central nervous system (CNS) such as PD and Alzheimer's disease. The extracts of herbal medicine have been reported for its defensive effects against neurotoxicity *in vitro and in vivo* models of Parkinson's disease (Sankar *et al.*, 2007; Babita *et al.*, 2014; Rajasankar *et al.*, 2009; Srinivasagam Raja Sankar *et al.*, 2007). MPTP (1-methyl -4 –phenyl-1, 2, 3, 6-tetrahydropyridine) is a potent neurotoxin which results in selective degeneration of dopaminergic neurons projecting from substantia nigra pars compacta (SNpc) into striatum and mimics PD-like symptoms in experimental models (Smeyne and Jackson-Lewis, 2005). *Oxalis corniculata* is a medicinally important plant indigenous to tropical and subtropical regions of the world. *Oxalis corniculata* exhibits wound healing activity (Taranalli *et al.*, 2004), cardio relaxant activity (Achola *et al.*, 1995), nematocidal activity (Silamar and Leandro, 2005), anticancer activity (Kathiriya *et al.*, 2010), antimicrobial activity (Raghvendra *et al.*, 2006), antifungal activity (Iqbal *et al.*, 2001), antiamoebic activity (Manna *et al.*, 2010), antiimplantation and abortifacient (Sharangouda and Patil, 2007), allelopathic activity (Itani *et al.*, 1999), antioxidant activity (Reddy *et al.*, 2010) and steroidogenic activity (Seraphim and Sinha, 2010). Recently, *O. corniculata* was also reported to exhibit potent anxiolytic potential, nephroprotective, anti-stress and memory enhancing properties (Sai Sampath *et al.*, 2011).

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But so far their protective effect in neurodegenerative disorders such as PD has not been studied. In the present study, the protective effect of *O. corniculata* against MPTP-induced behavioral deficits in mouse model of PD was investigated.

EXPERIMENTAL

Chemicals and reagents

MPTP hydrochloride was purchased from Sigma Chemical Co. Analytical grade chemicals were used for the experimental analysis. Stock solutions of all chemicals were prepared in double distilled water and the dilutions were made fresh on the day of the experiment.

Plant extract

The medicinal plant *Oxalis corniculata* was collected from Tirunelveli District, Tamil Nadu, India. Mature and healthy plants were collected naturally from different locations after the rainy season (February, March and April). The collected specimens were identified referring to the Flora of Presidency of Madras (Gamble, 1915-1921) and Flora of Tamil Nadu Carnatic (Mathew, 1983 -1988). The collected plant materials were shade-dried at room temperature (20-25^{o}C) for a period of 3 weeks to 8 weeks. For extraction, the completely dried materials were made into coarse powder by mechanical grinder. The powder was passed through a 40-mesh sieve, to get a uniform particle size. A weighed quantity of powder was subjected to continuous hot percolation in soxhlet apparatus with ethanol at 65-70^{o}C. All the solvent had been removed by evaporating under reduced pressure using rotaflash evaporator. The concentration of the extract was 12% w/w. when compared to the dried starting material. It was then stored at -20°C until required.

Animals

C57 Black male mice, weighing 25-30 gm were used. All animals were obtained from the Animal house, KMCH College of Pharmacy, Coimbatore, Tamil Nadu. They were allowed food and water *ad libitum* upto the experimentation period. Prior to use, the mice were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. Each animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room and were made between 9.00 to 17.00 h in the experimental room. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (KMCRET/PhD/ /2014-15), New Delhi, India.

Experimental protocol

The following experimental procedure was followed to evaluate the locomotor behavioral effect of *O. carniculata* (OC) on MPTP induced mice. 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP): 15 mg/kg of MPTP as a neurotoxin i.p. twice, 4 h apart *in vivo* and 20 µM *in vitro*. MPTP was purchased from Sigma, India. MPTP was dissolved in 0.9 % saline and administered i.p. Intraperitonial injection of MPTP was given to Groups II,III, IV and V. Oral dosage of Carbidopa + Levadopa (Standard drug for Parkinson's disease treatment) was given to Groups III.

The animals were divided into six groups, each consisting of six mice.

1. Group I served as vehicle control (Distilled water)
2. Group II received MPTP (20 mg/kg, i.p) (Sigma-Aldrich, Bangalore, India) four consecutive days,
3. Group III received MPTP + carbidopa + levodopa (100 mg/kg, p.o)
4. Group IV received MPTP + crude extract (250 mg/kg, p.o)
5. Group V received MPTP + crude extract (500 mg/kg, p.o)
6. Group VI received only crude extract (500 mg/kg, p.o)

Experimental analysis

The actions of plant extract on spontaneous locomotor activity were measured automatically using Actophotometer (Medicraft photoactometer, model No: 600-40, S. No: PA-0149, India) (Rabbani *et al.,* 1995). Motor Co-ordination test was conducted using a Rota Rod apparatus (Inco Ambala, India) (Kulkarni, 1987). The elevated plus maze was carried out as described by Pellow *et al*. (1985). Hole board test was done by the standard procedure (Nade and Yadav, 2008). Step down inhibitory avoidance was measured by the procedure given by Dhingra *et al.* 2006. Passive avoidance task (Step Through Latency - STL) was studied by the Tamburella's procedure (Tamburella *et al.,* 2012).

Statistical analysis

Values are expressed as the mean ± S.D. Statistical implication (p) calculated by one way ANOVA followed by Dunnett's. ns- not significant $^{*}P< 0.001$ $^{**}P< 0.01$ $^{***}P< 0.05$ calculated by comparing treated group with control group.

RESULTS

Actophotometer

The animals induced with MPTP shows sluggish movement in the Actophotometer. But, when the animals are treated with MPTP and 500 mg of *Oxalis* extract the movements are increased. When the animals are treated only with the alcoholic extract of *Oxalis* show significant increase in movement ($p<0.001$) when compared to MPTP + standard treated group as shown in Table 1. MPTP + 500 mg/kg *Oxalis* extract shows improvement in movement of the animal when compared with the movement of MPTP induced groups. This may be due to the CNS stimulant property of the drug.

Table 1: Effects of *O. corniculata* ethanol extract in the Actophotometer test and Elevated plus maze test.

Group	Actophotometer (A.F)	Elevated plus maze (A.F) open entries	Elevated plus maze (A.F) Dark entries
Control	79.60 ±12.012	12.00 ±10.040	157.00 ±20.861
Only MPTP	48.80 ±29.201	2.17 ±1.169	140.67 ±15.175
MPTP + Standard	63.00 ±9.772	8.17 ±7.167	156.50 ±12.046
MPTP + Oxalis 250mg/kg	56.20 ±14.516	5.33 ±5.279	142.83 ±13.393
MPTP + Oxalis 500mg/kg	57.80 ±20.813	4.83 ±8.035	146.00 ±14.642
Only Oxalis 500mg/kg	67.33 ±14.306	5.80 ±3.033	158.50 ± 8.871

Table 2: Effects of *O. corniculata* ethanol extract in the Hole Board test and Rota rod test.

Group	Hole board (A.F)	Rota rod apparatus (A.F)
Control	16.43 ±24.186	133.67 ±30.898
Only MPTP	7.29 ±10.935	40.17 ±12.090
MPTP + Standard	12.14 ±16.466	33.00 ±10.119
MPTP + Oxalis 250mg/kg	8.71 ±3.251	43.00 ±13.100
MPTP + Oxalis 500mg/kg	13.50 ±4.416	35.33 ±16.525
Only Oxalis 500mg/kg	14.57 ±18.564	36.50 ±11.502

Table 3: Effects of *O. corniculata* ethanol extract in the Step down test and step through test

Group	Step down (A.F)	Step through (A.F)
Control	1.50 ±0.548	1.67 ±0.516
Only MPTP	1.17 ±0.408	1.17 ±0.408
MPTP + Standard	1.33 ±0.516	1.33 ±0.516
MPTP + Oxalis 250mg/kg	1.17 ±0.408	1.17 ±0.408
MPTP + Oxalis 500mg/kg	1.33 ±0.516	1.33 ±0.516
Only Oxalis 500mg/kg	1.17 ±0.408	1.50 ±0.548

Elevated plus maze

The entries of C57 mice into dark are the normal character. The control group of mice shows more number of dark entries. The MPTP induced mice shows reduction of number of dark entries. But the group treated with MPTP and 500 mg of *Oxalis* extract shows increase in dark entries (146.00 ±14.642). This proves that the nature of normal activity (i.e. entering into the dark area) of the animals is induced when the extract of *Oxalis* is administered. The effects of group and maze experience on plus-maze behavior in male mice are summarized in Table 1.

Hole Board

In the hole board test, the normal, control group of animals show more number of dips into the hole board as an escaping mechanism. The group induced by MPTP shows less number of dips compared to control group. But, when the animals are treated with *Oxalis* 500 mg extract, show great improvement and exhibit more number of dips more than that of MPTP + standard groups. The number of head poking after administration of MPTP + *Oxalis* 500 mg/kg was comparable with the standard drug carbidopa + levodopa (100 mg/kg) and the values are significantly higher compared to the control group (Table: 2). Selected plant extract exhibits significant activity as compared to MPTP + standard.

Rota rod apparatus

A formal test related to beam walking is balancing on a rotating rod, the **"**rotorod*"* test. The test consisted of a rotating rod upon which the animal balances. As the animals learn to balance, the rod is turned increasingly faster. The measure of motor skill is the time the animal spends on the rotorod as a function of speed with which the rod rotates (Le Marec *et al.*, 1997).

The capacity of withstanding in the rotarod apparatus is more in the control group. But, when the animals are induced with MPTP, they fall very easily from the rotatod and the time taken for each fall becomes very minimal. The group treated with MPTP and 250 mg of *Oxalis* extract shows slight improvement. The result from the Rotarod test showed that the extract significantly increased the motor coordination of the tested animals. The data shows that an average the mice treated with 250 and 500 mg/kg of the ethanolic extract of *Oxalis corniculata* were able to maintain equilibrium on the rotating rod and stayed on longer without falling (Table: 2) as compare with MPTP + standard.

Step down

Effect of ethanol extract of *Oxalis corniculata* on memory was observed using step down model in mice. Almost all the groups show similar results only. There is no much difference between the control group or induced group or treated group with *Oxalis*. The results obtained for the MPTP + 500 mg/kg *Oxalis* treated group can improve the long-term retention of a step-down inhibitory avoidance task in mice (Table: 3). MPTP + 500 mg/kg *Oxalis* treated and MPTP + Standard drug exhibits similar activity.

Step through

Step through is a passive avoidance test. It is a simple and rapid test method for memory assessment (Das *et al.*, 2000). In the test-performed animals treated with MPTP exhibited a sharp decline in memory retention and treatment with *O. corniculata* improved the condition and was comparable to control animals.

On the 7th day MPTP administered mice showed significant difference and remarkable decrease in step through transfer latency (1.17 ±0.408), when compared to control (1.67 ±0.516) and *O. corniculata* (500 mg/kg) ethanol extract treated mice (1.50 ±0.548) (Table: 3). Group V and Group VI animals were treated with alcoholic extracts of *O. corniculata* shows restoration of locomotion compared to control group at the significant level.

DISCUSSION

Alcoholic extract of *O. corniculata* was assessed for the neuropharmacological evaluation in MPTP treated mice. Spontaneous motor activity, exploratory behaviour and motor synchronization were decreased in MPTP treated group, it could be due to motor mutilation and muscle relaxant effect. On treatment with alcoholic extract of *O. corniculata* reversed the behavior alterations induced by MPTP on 3rd day, but maximum effect of extract was seen on 6th day of treatment. It might be due to presence of phytoconstituents like L-dopa, polyphenols and flavonoids (Mohanasundari *et al.,* 2006).

Elevated plus maze (EPM) is one of the most important animal behavioral experiments used in evaluation of anxyolitic effect of drugs (Pellow *et al.,* 1985; Pellow and File, 1986). Moreover, it is known that anxiolytic agents increase the frequency of entries and the time spent in open arms of the EPM (Pellow *et al.,* 1985). Administration of *O. corniculata* extract significantly increased ($p < 0.05$) the percentage of entries and permanence time into open arms, compared with control group. The results of experiment confirmed that on first exposure to the plus maze, male mice exhibit a clear preference for the closed/protected parts of the apparatus. The transfer latency measured and the *Oxalis* treated mice was drastically shorter than on the first day, indicating the ability of the rats to recall the learned aspect in a lesser period of time. The effects of *O. corniculata* seem to be more potent than MPTP group in comparison to experimental groups. The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Antianxiety agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms. The spontaneous decrease in basal activity score implicates the reduced anxiety recorded using Actophotometer. The antianxiety and antidepressant effects may be due to the flavonoid (Vikas *et al.,* 2012) content present in the alcoholic extract of *Oxalis corniculata*. Hole board test indicates that head dipping behaviour is sensitive to changes in the emotional state of the animal and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head poking behaviour (Takeda *et al.,* 1998). *O. corniculata* extract increased head-dip counts without changing locomotion in the hole-board test. Additionally, *O. corniculata* increased visits numbers to board compared with control group. These results imply that *O. corniculata* has a considerable anxiolytic effect in this paradigm. The ethanolic extract of *O. corniculata* contains flavanoids, phenolic content as well as terpenoid compounds like β – sitosterol, caemphesterol, etc, which are probably responsible for the actions (Bajracharya, 1979).

The hole board test is useful for modeling anxiety in animals, in this test an anxiolytic-like state may be reflected by an increase in head –dipping behaviors (Takeda *et al.,* 1998). In the present study, results showed that ethanolic extract (500 mg/kg) of *Oxalis corniculata* increased the head dipping corroborating the anxiolytic-like effect previously shown in the light- dark test. The anxiolytic effect of alkaloids (Martinez-Vazquez *et al.,* 2012), flavonoids (Li *et al.,* 2011) and saponins (Wei *et al.,* 2007) has been previously reported and, therefore, suggest that these anxiolytic effects may be due to the above constituents. Most of the anxiolytic agents exert their action by opening of activated GABAchloride channel. It is also reported that many flavonoids were found to be ligands for the GABA-A receptors in the central nervous system (Mohammed, 2014). The above results reveals that the ethanolic extract of *Oxalis corniculata* have satisfactory anxiolytic effect.

Rota rod test a classical animal behaviour test used to evaluate peripheral neuromuscular blockade and the motor coordination (Amos *et al.,* 2001), a deficit in motor coordination would very likely affect performance in the behavioral tests. Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation. Plant extract showed significant increase in the locomotory score and fall of time of the mice from the rotating rod.

The investigation of the pharmacology of memory between the 1960s and 1980s involved the analysis of the modulation of memory, namely the drugs, hormones, neurotransmitters and neuromodulators that affect memory consolidation. The studies showed that modulatory substances could influence the basic mechanisms of memory processing (Bär *et al.,* 1982). Memory improving drugs generally work by altering the neurotransmitters in the brain that are involved in the initial learning of a memory or its subsequent reinforcement (Ingole *et al.,* 2008). The present study investigated the effect of *Oxalis corniculata* on memory retention and retrieval using the passive avoidance step down model. The results achieved in this study reveals that different doses of *O. corniculata* improved memory retention and retrieval significantly ($p<0.05$). Previous studies on memory retention and retrieval indicated that plants contain large amounts of antioxidants which improve memory, for example, various species of *Ocimum* (Dokania *et al.,* 2011; Sarahroodi *et al.,* 2012), Saffron (Abe and Saito, 2000), *Ginkgo biloba* (Rigney *et al,* 1999), green tea (Wu *et al,* 2012), *Magnolia officinalis* (Lee *et al,* 2012), Curcumin (Bishnoi *et al,* 2008) and *Anacyclus pyrethrwn* (Sujith *et al,* 2012). Memory retention and retrieval enhancement by *O. corniculata* extract could be due to the presence of antioxidants such as flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids and their power in scavenge reactive oxygen species.

In view of the above results, it concludes the *O. corniculata* extract in different experimental series enhanced learning and memory performance in mice, probably a result of reduces of oxidative stress by antioxidant defense mechanisms. This effect of *O. corniculata* concerned modulation of cholinergic neurotransmission, and that it may be utilize as a potential agent in treatment of neurodegenerative diseases such as Alzheimer's disease, Parkinsonism and other type of dementia and memory deterioration. There were some significant improvements in motor performance, coordination and spontaneous activity with no significant change in stride length or grooming tendencies. It can be interpreted that these parameters are correlated with dopaminergic markers in the striatum (Tillerson *et al.,* 2002; Tillerson and Miller, 2003).

CONCLUSION

The findings of this study indicate that OC extract attenuates MPTP-induced cognitive and behavioural impairments in mouse model of PD. Regulation of antioxidant defense mechanisms by OC may partly be responsible for its neuroprotective effect in MPTP-induced PD mice.

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RESEARCH ARTICLE Open Access

Neuroprotective effect of Demethoxycurcumin, a natural derivative of Curcumin on rotenone induced neurotoxicity in SH-SY 5Y Neuroblastoma cells

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Abstract

Background: Mitochondrial dysfunction and oxidative stress are the main toxic events leading to dopaminergic neuronal death in Parkinson's disease (PD) and identified as vital objective for therapeutic intercession. This study investigated the neuro-protective effects of the demethoxycurcumin (DMC), a derivative of curcumin against rotenone induced neurotoxicity.

Methods: SH-SY5Y neuroblastoma cells are divided into four experimental groups: untreated cells, cells incubated with rotenone (100 nM), cells treated with DMC (50 nM) + rotenone (100 nM) and DMC alone treated. 24 h after treatment with rotenone and 28 h after treatment with DMC, cell viability was assessed using the MTT assay, and levels of ROS and MMP, plus expression of apoptotic protein were analysed.

Results: Rotenone induced cell death in SH-SY5Y cells was significantly reduced by DMC pretreatment in a dose-dependent manner, indicating the potent neuroprotective effects of DMC. Rotenone treatment significantly increases the levels of ROS, loss of MMP, release of Cyt-c and expression of pro-apoptotic markers and decreases the expression of anti-apoptotic markers.

Conclusions: Even though the results of the present study indicated that the DMC may serve as a potent therapeutic agent particularly for the treatment of neurodegenerative diseases like PD, further pre-clinical and clinical studies are required.

Keywords: Demethoxycurcumin, Rotenone, Oxidative stress, Apoptosis, Neurodegenerative diseases

Background

Parkinson's disease (PD) is one of the most common and progressive neurodegenerative disease, which affects the movement of aged population and is characterized by the selective loss of dopaminergic neurons in the substantia nigra of pars compacta (SNpc) [1] and depletion of dopamine (DA), a neurotransmitter responsible for movement. Though the cause of PD is unknown, abnormal processes such as Lewy body formation, calcium homeostasis, glutamate toxicity, inflammation, proteasome dysfunction and apoptosis are reported to be involved in induction and PD progression. Enhanced mitochondrial dysfunction and its mediated oxidative stress also play a key role in the pathophysiology of PD [2].

Animal and epidemiological studies have indicated that the exposure of pesticide can also increase the risk of PD [3, 4]. Rotenone, a naturally occurring plant flavonoid and well known neurotoxic pesticide, readily crosses

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the blood-brain barrier due to its high lipophilic nature, transverse the cellular membrane without the need of dopamine transporter. Further, it accumulates and impairs mitochondrial function, mediates oxidative stress and ultimately leads to neurodegeneration [5–7]. Accordingly, a rotenone induced PD model might have various advantages than several other PD models [8].

Levodopa (L-DOPA) remains the most effective treatment for PD. Prolonged treatment of L-DOPA is associated with various side effects and resistance. Although several strategies are developed to manage the disease, the mechanism still remains enigmatic. In recent years, considerable research has been carried out on identifying naturally occurring neuroprotective substances, aimed to prevent or delay the neurodegenerative processes. Turmeric has long been known as a spice, dye and home medicine for jaundice, menstrual difficulties, bloody urine, hemorrhage, toothache, bruises and chest pain [9, 10]. Epidemiological studies by Ganguli et al., [11] have suggested that the consumption of turmeric by the Indian populations is correlated with the low incidence of Alzheimer's disease (AD)/PD as compared to the Caucasians. The neuroprotective effect of turmeric can be attributed due to the presence of active polyphenol, curcumin which imparts the characteristic color and properties [12]. Both the in vitro and in vivo experiments indicated that the anti-parkinsonic effect of curcumin attributed due to its anti-oxidant [13], mitochondrial protective [14], signal modulating [15], anti-inflammatory [16, 17] and anti-apoptotic properties [18].

Other than curcumin, demethoxycurcumin (DMC) (4-hydroxycinnamoyl-(feruloyl) methane) (Fig. 1) and bisdemethoxycurcumin (BMC) (bis(4-hydroxycinnamoyl)-methane), are the other curcuminoids mainly present in turmeric. 95% of curcumin as a mixture of three curcuminoids, typically comprising around 77% curcumin, 17% DMC, and 6% BMC [19, 20] were available commercially. Curcumin analogs such as DMC and BMC, have also been reported to possess considerable antioxidant, anti-inflammatory and anti-proliferative activities [21, 22]. Predominantly DMC is reported to have better anticancer and anti-inflammatory activity compared to curcumin [23, 24]. The curcuminoids, curcumin, DMC and BMC strongly inhibited βA-fibril formation in AD [25]. So in the present study, the neuroprotective effect of DMC against rotenone induced in vitro model of PD is analyzed by measuring the levels of reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and the expressions of pro-apoptotic and anti-apoptotic indices in SH-SY5Y neuroblastoma cells.

Methods

Chemicals

Rotenone, DMC, thiobarbituric acid (TBA), MTT, 2–7-diacetyldichlorofluorescein (DCFH-DA), rhodamine 123 (Rh-123), heat-inactivated fetal calf serum (FCS), Dulbecco's modified Eagle's medium (DMEM), glutamine, penicillin–streptomycin, EDTA, and trypsin were acquired from Sigma-Aldrich (Bangalore, Karnataka, India. Anti- Bax, Bad, Bcl-xL, Bcl-2, caspase-3, caspase-6, and caspase-8, caspase-9, cyt-c (cytosol and mitochondria) anti-bodies were obtained from Cell Signaling Technology, Inc. (Beverly, MA, USA) and β-actin antibody were purchased from Santa Cruz Biotechnology, Inc., (Dallas, TX, USA). Anti-mouse and anti-rabbit secondary anti-bodies were purchased from Bangalore Genei Pvt. Ltd., (Bangalore, Karnataka, India). All other chemicals and biochemical used were of analytical grade.

Neuronal cell culture

Human SH-SY5Y neuroblastoma cell lines were procured from National Center for Cell Science, Pune, India. The cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 1% antibiotic/antimycotic and 10% FBS solution. Cultures were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO_2 and 95% air. Cell culture medium was changed for every 2 days. DMC and rotenone were freshly made in dimethylsulfoxide (0.05%) for each experiment. DMC was added 2 h prior to rotenone treatment.

Assessment of neuronal viability

About 3×10^3 SH-SY5Y cells were seeded per well in a 96-well culture plate. To determine the toxicity of rotenone and DMC, cells in the medium were incubated with different concentrations of rotenone (5, 10, 50, 100, and

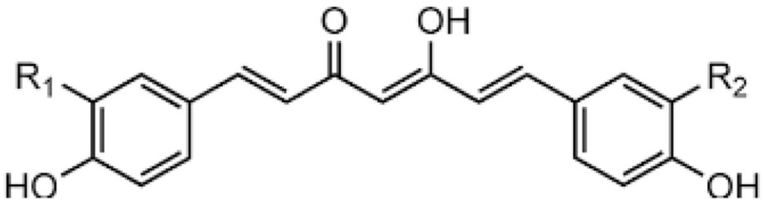

Fig. 1 Structure of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin: $R_1 = R_2 = OCH_3$. Demethoxycurcumin: $R_1 = H$; $R_2 = OCH_3$. Bisdemethoxycurcumin: $R_1 = R_2 = H$

200 nM) and DMC (5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM and 1 μM) for 24 h. To determine the neuroprotective effect of DMC, SH-SY5Y cells were pretreated with various concentrations of DMC (5 nM, 10 nM, 20 nM and 50 nM) for 2 h and then incubated with rotenone (effective dose) for 24 h. The cells were incubated with 5 mg/mL MTT for 4 h at 37 °C, after treatment with different testing agents. The medium was removed carefully after the incubation and the formazan crystals were dissolved in 150 μL of DMSO and absorbance of formazan reduction product was measured by spectrophotometer at 570 nm using a microplate reader. Four independent experiments were performed from each group [26]. Based on the results obtained from cell viability assay, the effective dose of DMC against rotenone toxicity was utilized to study the effect of DMC by assessing ROS, MMP, apoptosis, and apoptotic protein markers expression.

Measurement of intracellular reactive oxygen species

A non-fluorescent probe, 2,7-diacetyl dichlorofluorescein (DCFH-DA), can penetrate into the intracellular matrix of cells, is oxidized by ROS to form fluorecent dichlorofluorescein (DCF). This method is used to estimate the levels of endogeneous ROS formation in control and experimental cells. After the Pre-treatment with DMC (50 nM) for 2 h, the cells (1×10^5 cells/well in 6-well plates) were incubated with rotenone (100 nM) for 24 h and then incubated with 100 μL DCFHDA for 30 min at 37 °C and washed twice with PBS to remove the excess probe; Glucose enriched PBS is used for the suspension of cells and then transferred to a fluoroslide and visualized using a fluorescent microscope. Fluorescent measurements were done with excitation and emission filters set at 485 ± 10 nm and 530 ± 12.5 nm, respectively (Shimadzu RF-5301PC spectrofluorimeter) and the images were captured using fluorescence microscope [6].

Measurement of mitochondrial membrane potential (MMP)

MMP changes were determined by the mitochondrial-specific, incorporation of a cationic fluorescent dye Rhodamine-123 (Rh-123). After treatment with DMC for 2 h and rotenone for 24 h as previously described, the cells (1×10^5 cells/well in 6-well plates) were changed to fresh medium (100 μL) containing 1 μL of fluorescent dye Rh-123 (5 mmol/L) and kept for 30 min at room temperature (37 °C). The test cells were then collected, washed twice with PBS, and estimated by using blue filter (450–490 nm). Orange–red fluorescence is emited by polarized mitochondrion, and depolarized mitochondrion emits green fluorescence. Spectrofluorometer is used to measure the fluorescent intensity at 535 nm [27].

Dual staining

Acridine orange (AO) and ethidium bromide (EB) fluorescent probes were used to analyze apoptosis by fluorescence microscopy. After treatment schedule as described in previous experiments, medium was removed from the plates; cells (1×10^5) were washed with PBS twice and stained with 100 μg/mL of AO and EB stain. To remove excess dye, cells were incubated for about 20 min at room temperature and then washed with warm PBS. Fluorescence microscopy was used for the morphological studies and photographed. Further spectrofluorometer was used to measure fluorescence intensity at 535 nm [5].

Immuno blotting

After the treatment schedule, SH-SY5Y cells (1×10^5) in 6-well plates were harvested, washed with PBS, and lysed in 100 μL lysis buffer (20 mM Tris-HCl, pH 7.4150 mM NaCl,1 mM EDTA, 30 μg/mL apoprotein, and 1 mM phenyl methyl sulfonyl fluoride) followed by centrifugation (1000 x *g* for 5 min at 4 °C). The cytosolic fractions were saved and the pellets were solubilized in the mitochondrial lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 0.2% Triton X-100, 0.3% NP-40, 100 μM PMSF, 10 μg/mL leupeptin, and 2 μg/mL apoprotein) kept on ice and vertex for 20 min followed by pelleting at 1000 x *g* for 10 min at 4 °C in order to remove insoluble material. Protein concentration was quantified using Lowry et al. [28] and subjected to 10% polyacrylamide gel electrophoresis. The separated proteins were blotted onto a PVDF membrane using semidry transfer (BIORAD). 5% non-fat milk is used for blocking in TBS at 25 °C for 1 h, blots were probed with various antibodies: caspase-3, caspase-6, caspase-8, and caspase-9, cytochrome-c (Cyt-c) (cytosol and mitochondria), Bax, Bcl-2, BAD and Bcl-xL (1:1000) and μ-actin (1:2000). Horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG were employed as the secondary antibodies (1: 2000). Protein bands were visualized by enhanced chemiluminescence method with ECL-kit (GenScript ECL kit, USA) [29].

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by the Duncan's multiple range test (DMRT) using Statistical Package for the Social Science software package version 12.0. Results were expressed as mean ± SEM for four experiments in each group. $P < 0.05$ was considered significant.

Results

Effect of DMC and rotenone on the survival of SH-SY5Y cells

We first evaluated, whether DMC alone or rotenone alone treatment were toxic to SH-SY5Y dopaminergic cell line. Cells were treated with various concentrations

of DMC (5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM and 1 μM) and rotenone (5 nM, 10 nM, 50 nM, 100 nM and 200 nM) for 24 h and cell survival was determined by MTT assay (Fig. 2a and b). As shown in Fig. 2a, when exposed to DMC concentrations of 50 nM or lower, the viability of SH-SY5Y cells was the same as untreated control cells. However, a slight decrease of cell viability was observed with 100 nM and more significant toxicity was seen in 200 nM, 500 nM and 1 μM DMC treatment. Rotenone treatment (5 nM, 10 nM, 50 nM, 100 nM and 200 nM for 24 h) induced a dose-dependent reduction in cell viability with approximately LD50 observed at 100 nM (Fig. 2b). Consequently, cytotoxic induction with 100 nM rotenone for 24 h was used in the subsequent experiments.

DMC protects against rotenone-induced cytotoxicity

The protective effect of DMC against rotenone induced toxicity with cell viability increasing to 86 ± 3.97% of control in the presence of 50 nM of DMC. So based on the dose-response data, the treatments of 50 nM DMC and 100 nM rotenone were chosen for further experiments (Fig. 3).

DMC ameliorated rotenone-induced oxidative stress

To determine the changes in intracellular ROS in human dopaminergic cells during rotenone-induced cell death and DMC mediated protection, ROS production in SH-SY5Y cells was measured using fluorescent dye DCF-DA. The levels of intracellular ROS markedly increased after treatment with rotenone. However, pretreatment with DMC (50 nM) significantly decreased rotenone induced ROS production (Fig. 4a and b).

DMC prevents rotenone-induced reduction of the MMP

The MMP was rapidly reduced when SH-SY5Y cells were exposed to 100 nM of rotenone for 24 h, which was detected by the weakening of the fluorescence intensity of a mitochondrial specific probe, Rh-123. As compared to control cells, rotenone treatment increased the Rh-123 negative cells. Pretreatment with 50 nM of DMC protected cells

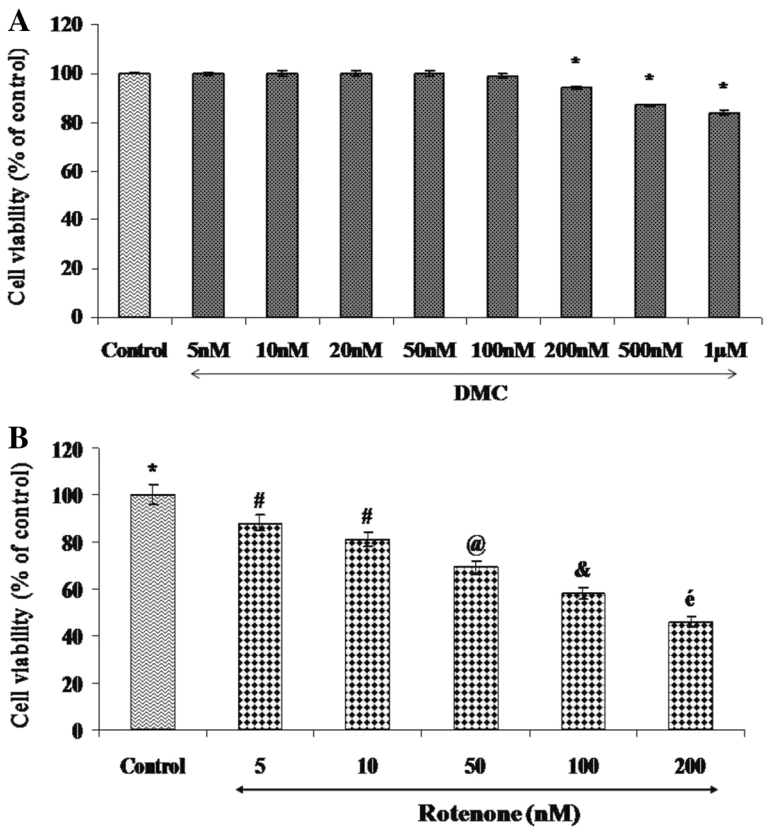

Fig. 2 Effect of DMC on rotenone induced cytotoxicity in SH-SY5Y cells. **a** shows the dose dependent effect of DMC at various concentrations (5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM and 1 μM). Up to 50 nM did not induce any toxicity after 24 h treatment, whereas slight toxicity was induced at 1 μM concentration. **b** shows the dose-dependent effect of rotenone (5, 10, 50, 100, and 200 nM) induced cell toxicity after 24 h. An approximately half-maximal inhibition of cell viability was obtained at 100 nM rotenone concentration. Values are expressed as the percentage of the untreated control and represented as mean ± SEM of four independent experiments in each group. Values not sharing a common superscript are significant with each other ($p<0.05$)

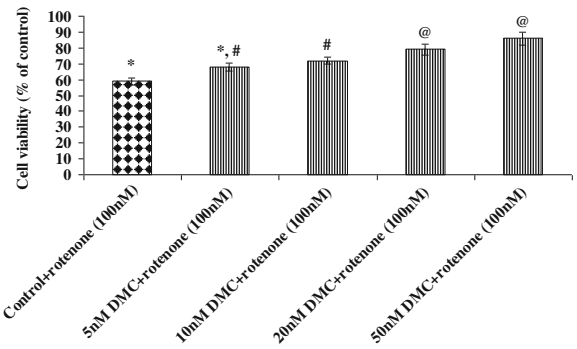

Fig. 3 The protective effect of DMC (5, 10, 20 and 50 nM) against rotenone induced cell death was determined by MTT assay. Values are expressed as the percentage of the untreated control and represented as mean ± SEM of four independent experiments in each group. Values not sharing a common superscript are significant with each other ($p<0.05$)

against the rotenone induced lowering of MMP, decreasing Rh-123 negative cells (Fig. 5a and b).

DMC ameliorated rotenone-induced apoptosis

The rate of apoptosis was determined by double staining of rotenone and DMC treated SH-SY5Y cells, through AO and EB. Rotenone exposed cells revealed orange luminescent apoptotic body formation, when compared to control and treatment with DMC increased cell viability and decreased apoptotic cell death. Control cells which fluoresced brightly with green nuclei and normal morphology are showed in Fig. 6a and b. In Hoechst staining, treatment with 100 nM rotenone resulted in nuclear condensation and fragmentation. DMC pretreatment significantly protected the rotenone-induced nuclear damage (Fig. 7) due to its anti-apoptotic properties.

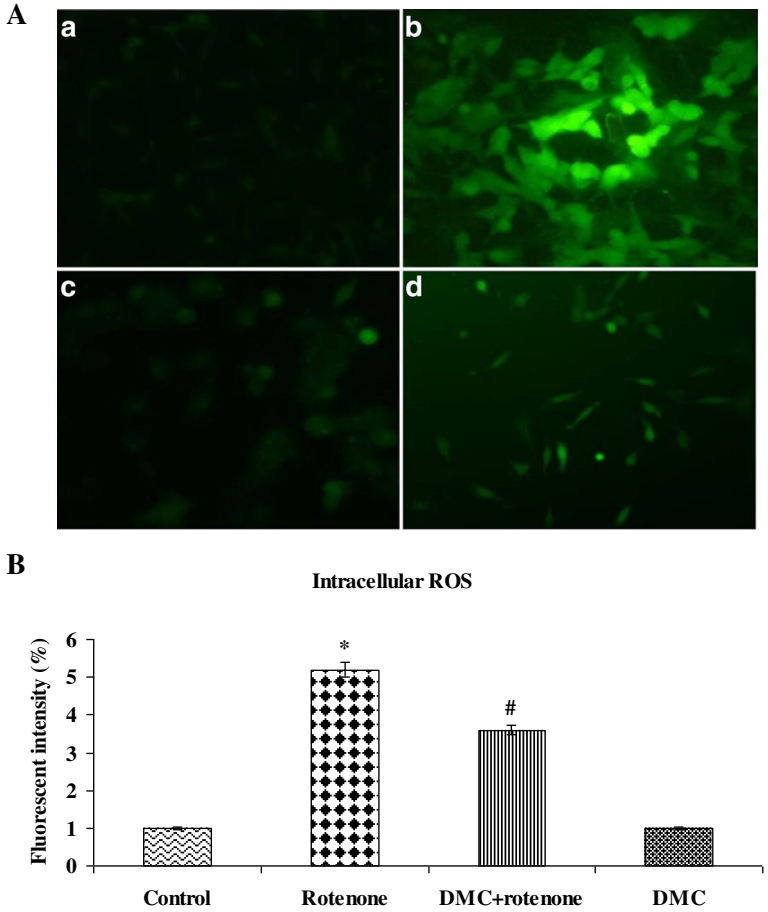

Fig. 4 a DMC reduced ROS formation as stained by 1μM CM-H2 DCFDA. **a** Photomicrograph showing the preventive effect of DMC (50 nM) against rotenone induced ROS generation. *a* Control, *b* rotenone, *c* DMC + rotenone and *d* DMC. **b** ROS levels were significantly increased in rotenone (100 nM) treated cells as compared to control cells, while DMC (50 nM) pretreatment significantly decreased the levels of ROS as compared to rotenone alone treated cells. Values are given as mean ± SEM of four independent experiments in each group. *$p<0.05$ compared to control and #$p<0.05$ compared to rotenone group (DMRT)

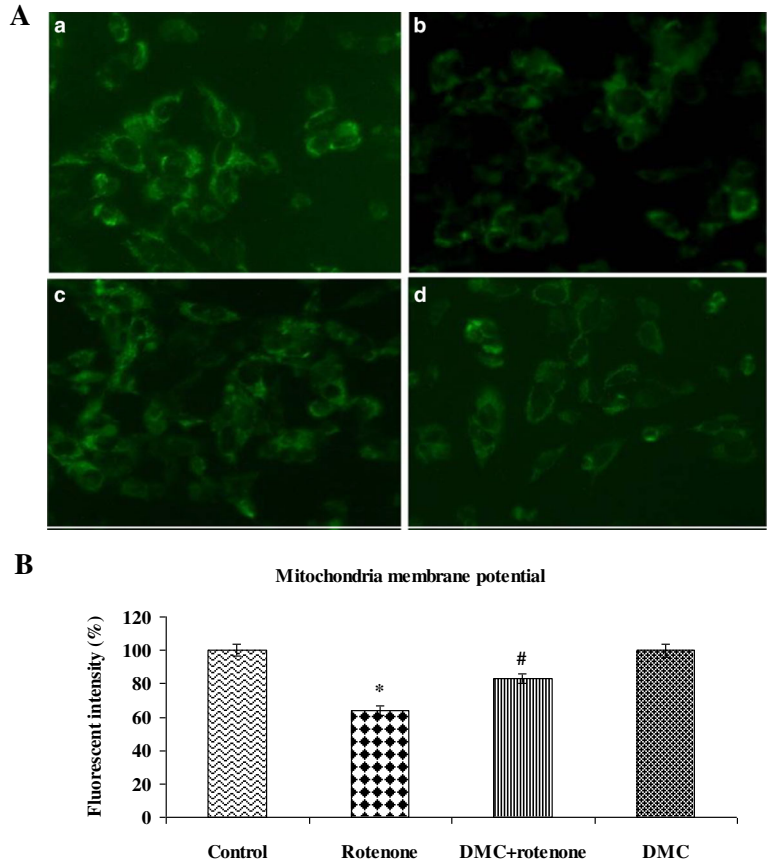

Fig. 5 DMC stabilizes MMP as stained by Rh-123. **a** Photomicrograph showing the preventive effect of DMC (50 nM) against rotenone induced mitochondria membrane potential. *a* Control, *b* rotenone, *c* DMC + rotenone and *d* DMC. **b** Rotenone (100 nM) significantly decreased MMP, while cells that were pretreated with DMC (50 nM) significantly increased MMP. Values are given as mean ± SEM of four independent experiments in each group. *$p<0.05$ compared to control; #$p<0.05$ compared to rotenone groups (DMRT)

DMC effect on rotenone induced proapoptotic and antiapoptotic gene expressions

To analyze the protective effect of DMC on rotenone-induced apoptosis, we assessed the expression of pro- and anti-apoptotic markers and Cyt-c release from the mitochondria in to the cytosol of cells. The expression of Bax, BAD, caspase-3, caspase-6, caspase-8, caspase-9 in mitochondria and Cyt-c in cytosol was increased whereas the distribution of Bcl-2, Bcl-xL and Cyt-c in mitochondria was significantly decreased by the rotenone treated group as compared with control. Pretreatment of cells with DMC gradually restored the excessive expression of these proteins (Fig. 8a and b).

Discussion

At the time of initial diagnosis of PD, approximately 50% dopaminergic neurons in the nigro-striatal pathway have degenerated [3], and a large population of the remaining nigral neurons are affected by stress [30, 31]. So even in the in vitro model, it was important to employ concentrations of toxins that caused about 50% cell death, as the scenario resembles the situation at the time of initial diagnosis of PD. The data obtained from the MTT assay indicated that the rotenone (100 nM) treatment induced about 50% cell death, which is in consistent with previous studies demonstrating that exposure of cells to high concentrations of rotenone for a relatively short period of time (24 h) results in a mixed population of cells, in which some are healthy, some are no longer viable, and some are dead [5–7, 32]. Although DMC exerts its neuro-protective effect in a dose dependent manner, the maximum cell viability (86%) was obtained at (50 nM) concentration in MTT assay. This is chosen as an effective dose and used for further studies.

Normally the apoptotic features were measured in in vitro models using Dual, TUNEL, Hoechst 33,342 and DAPI staining. To confirm the anti-apoptotic effect of DMC against rotenone induced neurotoxicity on SH-SY5Y cells, we further performed the apoptosis assessment by dual staining. In dual staining, apoptotic cells uptake EB and emit red orange fluorescence, whereas AO

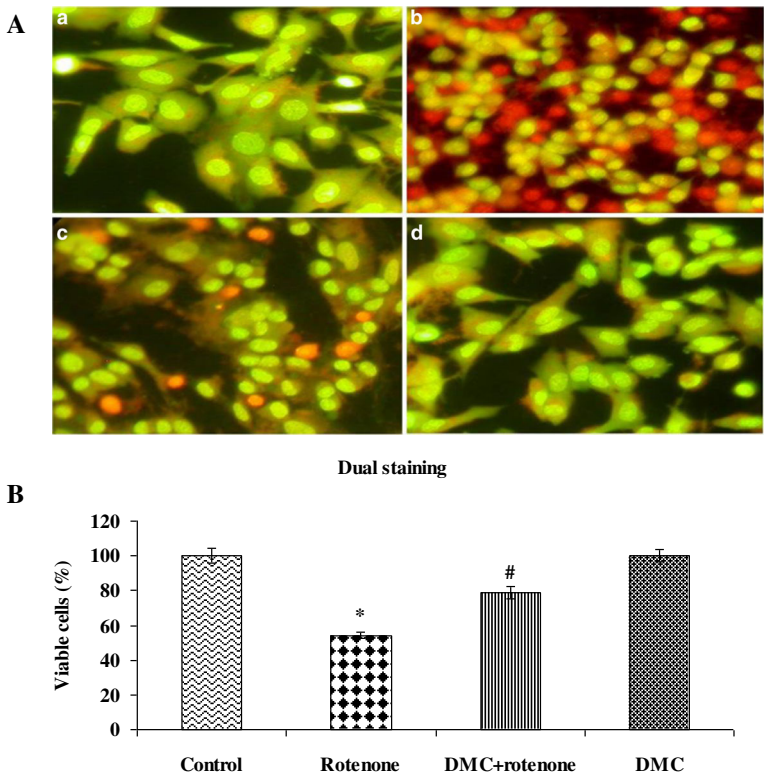

Fig. 6 DMC protects SH-SY5Y cells against rotenone induced apoptosis. **a** Photomicrograph showing the antiapoptotic effect of DMC (50 nM) against rotenone at a concentration of 100 nM effective dose. *a* Control, *b* rotenone, *c* DMC + rotenone, and *d* DMC. **b** Rotenone (100 nM) treatment induced cell apoptosis compared to control cells; pretreatment with DMC (50 nM) suppresses these apoptotic features. Values are given as mean ± SEM of four independent experiments in each group. *$p<0.05$ compared to control and #$p<0.05$ compared to rotenone group (DMRT)

is a DNA selective cationic dye that freely enters normal cell nuclei and emits green fluorescence. SH-SY5Y cells treated with rotenone emits more orange fluorescence than the control, while DMC pretreated cells emit more green fluorescence as compared to rotenone alone treated cells during dual staining. Results obtained from the MTT assay and dual staining technique, indicated that DMC inhibited the apoptosis induced by rotenone. Sirisidthi et al. [17] reported that the treatment of all the three curcuminoids (curcumin, DMC and BMC) increased the survival of rat PC12 and normal human umbilical vein endothelial cells (HUVEC) from amyloid β (1–42) insult, which corroborates our results.

Rotenone mimics the pathological features of PD in both in vitro and in vivo models by enhancing the overproduction of ROS [33], imbalance of cellular antioxidant systems [34], mitochondrial membrane depolarization [35], the formation and opening of the mitochondrial permeability transition pore [36], redistribution of Cyt-c [37], and eventually leading to cell death. The inhibition of complex I (also known as NADH:ubiquinone oxidoreductase; catalyzing the first step of electron transfer in the mitochondrial electron transport chain) by rotenone is accompanied with excess ROS formation and even a small level inhibition is sufficient to increase ROS production. Evidence from animal models suggests that rotenone induces oxidative effects that are responsible for some of the toxicity, and that these effects can be blocked by antioxidant therapy [38, 39]. Sirisidthi et al. [17] demonstrated that curcuminoids including DMC not only protects amyloid β (1–42) toxicity but also exhibit stronger antioxidative activity than vitamin-E. Dairam et al. [40] found that DMC is more potent in reducing lipid peroxidation than BMC. DMC has one methoxy group, while BMC has no methoxy groups. The presence of methoxy group on the phenyl ring might be responsible for this compound's potent antioxidant property [41].

Oxidative stress is reported to be a primary mechanism of rotenone-induced degeneration of dopaminergic neurons [42] by inducing loss of MMP. It leads to the opening of permeability transition pores, or mega channels that has been identified as the first steps in the apoptotic process [43]. Apoptotic cell death, resulting from oxidative stress and diminished MPP, typically involves release of Cyt-c from the mitochondria to the cytoplasm. Rotenone treatment reduced membrane potential resulting in increased mitochondrial permeability and enhanced release of Cyt-c to cytosol, which leads to a decrease in its level in

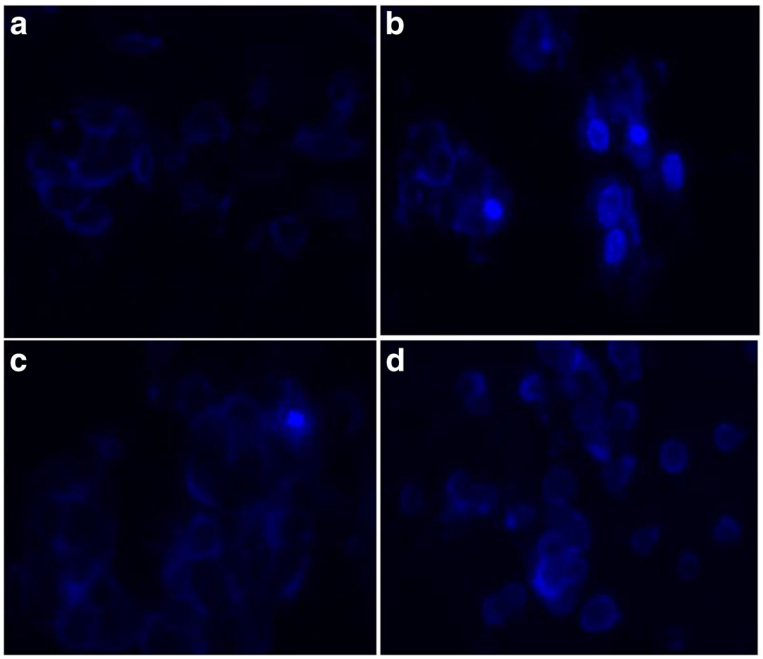

Fig. 7 Nuclear morphology of SH-SY5Y cells stained with DAPI. Neuronal cells stained with DAPI showing the antiapoptotic effect of DMC (50 nM) against rotenone (100 nM). Nuclear condensation and/or fragmentation are indicator of apoptosis. **a** Control, **b** rotenone, **c** DMC + rotenone and **d** DMC. It is possible to observe some apoptotic cells in B, but not in the others groups

mitochondria fraction and a concomitant increase in cytosol [44]. Cyt-c is an important electron carrier in the mitochondrial respiratory chain and a death messenger in the cytosol to form apoptosome complexes with Apaf-1, dATP, and caspases 3 and 9 [45]. We found that DMC prevented rotenone induced MMP loss and cytosolic accumulation of Cyt-c, suggesting that neuro-protective effects of DMC are mediated in part by the preservation of mitochondrial function.

Anti-apoptotic (Bcl-2, Bcl-xL, Bcl-w, A1, and Mcl-1) and pro-apoptotic Bcl-2 family proteins (BAD, Bax, Bak, and Bok) are mainly responsible for the fine balance of apoptotic mitochondrial pathway regulation. The pro-apoptotic Bcl-2 family members Bax and Bak serve to permeabilize the mitochondrial outer membrane, allowing for release of Cyt-c, whereas the anti-apoptotic Bcl-2 family members including Bcl-2 and Bcl-xL function to inhibit these pro-apoptotic proteins [46]. Moreover BAD promotes apoptosis by forming heterodimers with Bcl-xL or Bcl-2 [47]. Akt phosphorylates BAD at serine 136 [48], whereas p9RSK, a downstream target of ERK1/2, phosphorylates BAD at serine 112 [49, 50]. Dephosphorylated BAD is localized to the mitochondria along with Bcl-2 and Bcl-xL, where it can induce apoptosis. When S112 and S136 of BAD are phosphorylated, BAD remain in the cytosol, as it is bound to 14–3-3 proteins rather than Bcl-2 or Bcl-xL [44]. This phosphorylated form of BAD does not promote apoptosis. We found that rotenone exposure increased the expressions of Bax and dephosphoryrated BAD and diminished the expressions of Bcl-2 and Bcl-xL, suggesting that neurotoxin would induce apoptosis. DMC showed its anti-apoptotic effect predominantly through the down regulation of Bax and BAD, and upregulation of Bcl-2 and Bcl-xL. Ahmed and Gilani, [51] indicated that DMC and curcumin protects neuronal cells from Aβ insult by enhancing the expressions of Bcl-xL and Bcl-2, where DMC was found to be more effective when compared with other constituents in the curcuminoid mixture. Moreover Liao et al. [52] demonstrated that all three curcuminoids including DMC activated extracellular signal-regulated protein kinase 1/2 (ERK1/2) in PC12 cells, thereby promoting the phosphorylation of BAD and inhibiting apoptosis.

Both the extrinsic (death receptor) and intrinsic (mitochondrial) apoptotic pathways are involved in the pathogenesis of PD. In intrinsic pathway, Cyt-c could form apoptosomes along with procaspase 9 and apoptosis-activating factor-1(Apaf-1), leading to the activation of caspase 9 and successive activation of caspase 3 [53]. Procaspase 9 binds to Cyt-c and Apaf to form an apoptosome by activating caspases 9, resulting in the subsequent proteolytic activation of the executioner caspases 3, 6 and 7, eventually resulting in apoptosis. In the extrinsic pathway, activation of caspases 8 results in proteolytic activation of the executioner caspases 3, 6, and caspases 7 resulting in apoptosis [54]. In the present study,

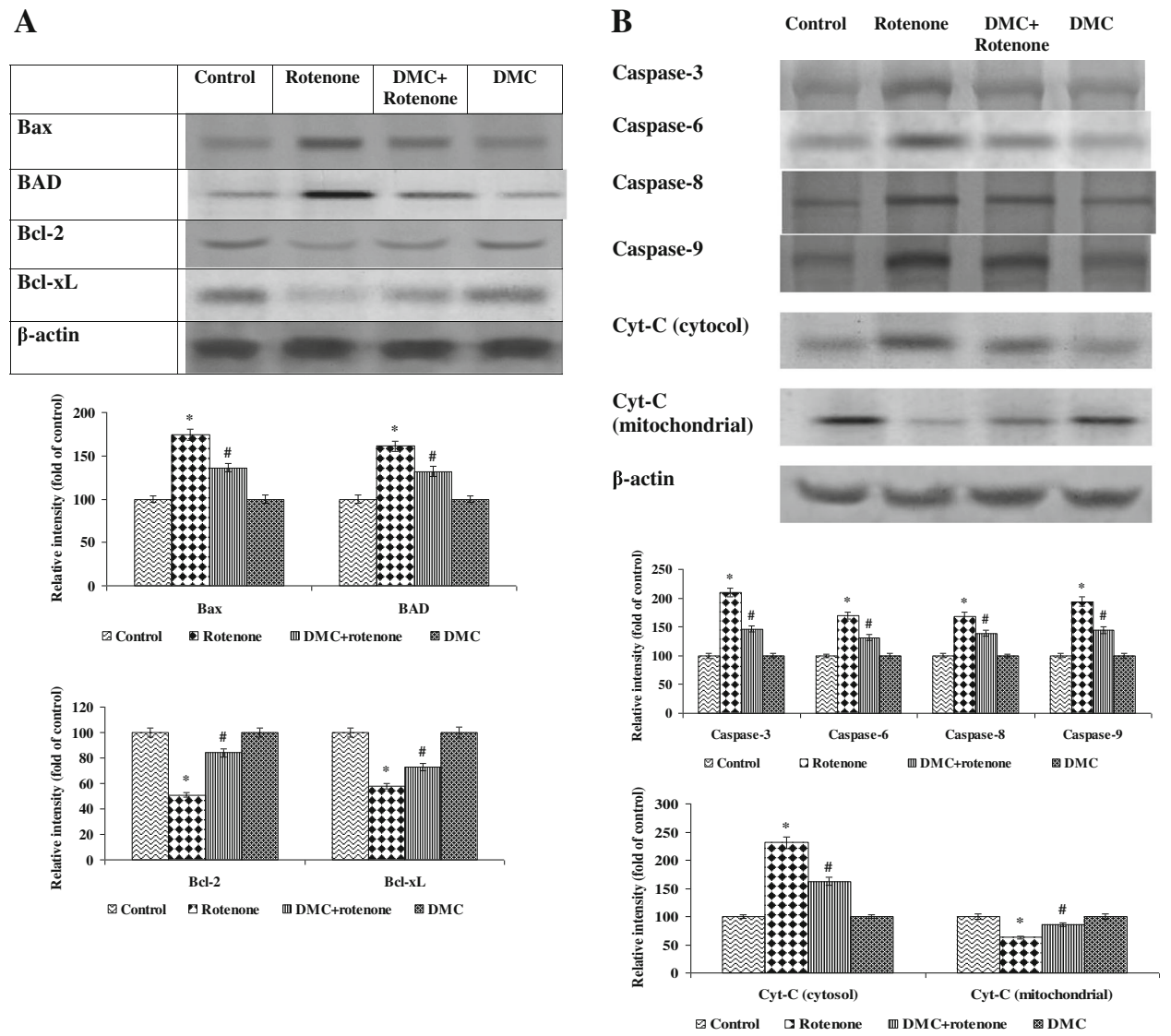

Fig. 8 The effect of DMC on the expressions of apoptotic proteins. **a** and **b** show the expression of Bax, BAD, caspase-3, caspase-6, caspase-8, caspase-9 in mitochondria and Cyt-c in cytosol was increased while the expressions of Bcl-2, Bcl-xL and Cyt-c in mitochondria was significantly decreased by the rotenone treatment as compared with control. Pretreatment with DMC gradually restored the imbalanced expression profile of these proteins. Immunoblots are representative of at least four independent experiments. Values are given as mean±SEM in each group. *$p<0.05$ compared to control and #$p<0.05$ compared to rotenone group (DMRT)

expressions of caspases 3, 6, 8, and 9 significantly increased in rotenone exposed cells, which indicate that both the intrinsic and extrinsic pathways of apoptosis were activated. Animal studies led by Ahmed and Gilani, [51] showed that the oral administration of DMC decreased the levels of caspase-3 (the main executioner of both the pathways of apoptosis) in the hippocampus of Aβ (1–42) injected rats. Findings of our current study demonstrate that DMC pretreatment may suppress apoptosis, not only by regulating pro and anti-apoptotic indices but, by attenuating oxidative stress and mitochondrial dysfunction.

Conclusions

This study demonstrates that curcumin is not the sole active pharmaceutical ingredient in turmeric, but that other constituents are also responsible for the neuroprotective effect of turmeric.

Additional file

Additional file 1: The datasets supporting the conclusions of this article are included within the article. (DOCX 23 kb)

Abbreviations
BAD: Bcl-2-associated death promoter; BCl-2: B-cell lymphoma 2; BCl-xL: B-cell lymphoma-extra large; Cyt c: Cytochrome-c; DA: Dopamine; DCFH-DA: 2,7-diacetyl dichlorofluorescein; DMC: Demethoxycurcumin; EDTA: Ethylenediaminetetraacetic acid; EGTA: (Ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid); MMP: Mitochondrial membrane potential; MTT: Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS: Phosphate-buffered saline; PD: Parkinson's disease; Rh-123: Rhodamine-123; ROS: Reactive oxygen species

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Availability of data and materials
The datasets supporting the conclusions of this article are included within this article (Additional file 1).

Authors' contributions
MR, VVG and CD performed laboratory work. SR, TM, AJT and RC initiated, designed the study and supervised and assisted in writing the paper. MME and AK assisted in writing the paper and interpretation of results and statistical analysis. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable. The study does not include any animal work, human participants, human data or human tissue.

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Original Article

Pathology Section

Does Iron Deficiency Anaemia and its Severity Influence HbA1C Level in Non Diabetics? An Analysis of 150 Cases

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ABSTRACT

Introduction: Anaemia has a high prevalence having great impact worldwide and potentially contributing to the pathogenesis of various chronic diseases. Approximately 1/3rd of patients with anaemia have iron deficiency. American Diabetes Association (ADA) has affirmed Glycated Haemoglobin (HbA1C) ≥ 6.5% as a diagnostic criterion for Diabetes Mellitus (DM). Variation of HbA1C in Iron Deficiency Anaemia (IDA) has clashing results.

Aim: To decide the impact of IDA on HbA1C levels among non diabetics. To assess and analyse the variation of HbA1C according to the degree of anaemia (mild, moderate and severe).

Materials and Methods: This cross-sectional study was carried out in SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu from February 2016 to October 2016 and approved by our Institutional Ethical Committee. Totally 150 non diabetics (75 with IDA and 75 without IDA) were included in this study. Medical history was recorded and HbA1C, Haemoglobin (Hb), Haematocrit (Hct), red cell indices, serum iron, ferritin and Fasting Plasma Glucose (FPG) were tested.

Results: The IDA patients in this study had a mean HbA1C (6.84±0.07%) which was higher than the non anaemic group (5.12±0.04%) and this difference was statistically significant (p< 0.05). HbA1C level was increased when severity of anaemia worsened. Also, noteworthy statistical significance was observed between no anaemia, mild, moderate and severe anaemia (p< 0.05).

Conclusion: In this study, we observed a positive correlation between IDA and elevated HbA1C level in non-diabetic population.

Keywords: Degree of anemia, Glycated hemoglobin, Iron status

INTRODUCTION

Anaemia is a major health issue that has a wide global impact. Approximately 1/3rd of patients with anaemia have iron deficiency [1]. HbA1C has been broadly utilized as a marker of glycaemic status overpast three months [2]. According to ADA guidelines, HbA1C levels ought to be kept underneath 6.5% in every diabetic patient to prevent the progression of micro vascular complications [3].

HbA1C levels are not influenced by blood glucose levels alone. Studies suggest that conditions like IDA, haemolytic anaemia, alcohol ingestion, pregnancy, blood loss, uraemia may alter HbA1C levels independent of glycaemic status [4].

The relationship between iron status and HbA1C level has long been a topic of debate in the literature [5]. Few studies report that iron deficiency increases HbA1C level and intend to explain on the basis of both modification of the quaternary structure of haemoglobin and HbA1C levels in old and new red blood cells [4-7]. According to studies conducted by Kalasker V et al., there were no variation between HbA1C levels in patients with IDA and controls [5].

English E et al., reported a recent review in 2015 about the controversies, in this issue and highlighted the need for further studies in this field to confirm and elucidate the role of anaemia on HbA1C results [8].

Since only limited number of studies has been carried out in Indian population, we were prompted to conduct the current study to determine the impact of IDA on HbA1C in non diabetic population to annul the effect of glucose on HbA1C. We also analysed the variation of HbA1C according to the degree of anaemia (mild, moderate and severe).

MATERIALS AND METHODS

This was a descriptive analytical cross-sectional study carried out in SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu between February 2016 to October 2016 and approved by our Institutional Ethical Committee. Totally 150 non diabetics aged >18 years (75 patients with IDA and 75 patients without IDA) were included in our study. An informed consent was obtained from all the subjects. Those having Hb <13 gm/dl in males and <12 gm/dl in females, Hct < 40% in males and <36% in females, mean corpuscular volume (MCV) <80 fl, Mean Corpuscular Haemoglobin (MCH) <26 pg/cell, Mean Corpuscular Haemoglobin Concentration (MCHC) <32 gm/dl and peripheral smear showing microcytic hypochromic picture were considered to have IDA and confirmed by their serum iron (<60 µg/dl) and ferritin levels (<15 µg/l) [9].

A total of 75 non diabetics without IDA were enrolled to serve as controls. All the laboratory parameters analysed for study group and for the control group as well.

Medical history was recorded in order to exclude DM, the patients had to have no history of DM and two FPG<126 mg/dl, performed close to the date of the complete blood count. Patients with history of chronic alcohol ingestion, kidney diseases, blood loss, haemolytic anaemia and pregnant patients were excluded from both the study and control group.

On the basis of Hb level, anaemic patients were categorized as mild anaemia (12-12.9 gm/dl for males and 11-11.9 gm/dl for females), moderate anaemia (9-11.9 gm/dl for males and 8-10.9 gm/dl for females), and severe anaemia (<9 gm/dl for males and <8 gm/dl for females).

Measurements

Hb, Hct, MCV, MCH, MCHC were measured by SYSMEX XT-1800i analyser. HbA1C measured by HPLC method using Bio-Rad D10 analyser. Plasma glucose estimated by glucose oxidase/peroxidase method, serum iron (TPTZ) and serum ferritin (Bio-Rad Quanimune Ferrin IRMA, Biorad lab).

Absolute HbA1C levels were calculated from the measured HbA1C levels by using the formula [5].

$$\text{Absolute HbA1C (gm/dl)} = \frac{\text{HbA1C (\%) x Hb (gm/dl)}}{100}$$

STATISTICAL ANALYSIS

The data were analysed using SPSS version 20 and presented as mean±S.D for continuous variables. Unpaired t-test was applied for comparison of group means. Pearson's coefficient was calculated to determine correlation between two variables. A p-value <0.05 was considered statistically significant.

RESULTS

In this study, non diabetics with IDA had a mean HbA1C % (6.84±0.07) which was significantly higher ($p < 0.05$) than the non-anaemic group (5.12±0.04). Absolute HbA1C (gm/dl) in those patients with IDA and non anaemic group were 0.78±0.01 and 0.73±0.01 respectively which was also statistically significant ($p < 0.05$) [Table/Fig-1].

The difference in mean Hb, Hct, MCV, MCH and MCHC between patients with IDA and non anaemic was statistically significant ($p < 0.05$) [Table/Fig-2].

Mean serum iron and ferritin levels in IDA patients were 42±0.61 µg/dl and12.09±1.21 µg/l and in non anaemic patients were 74±0.32 µg/dl and 41.06±0.43 µg/l respectively which was statistically significant ($p < 0.05$).

Parameters	IDA	Non anaemic	t-test	p-value
HbA1C %	6.84±0.07	5.12±0.04	22.219	0.0001
Absolute HbA1C (gm/dl)	0.78±0.01	0.73±0.01	5.029	0.0001

[Table/Fig-1]: Comparison of HbA1C% between anaemic and non anaemic group. Statistical analysis: Unpaired t-test

Parameters	IDA	Non anaemic	t-test	p-value
Haemoglobin (g/dl)	11.46±0.08	14.31±0.16	-16.078	0.0001
Haematocrit (%)	37.07±0.29	42.20±0.47	-9.328	0.0001
Mean corpuscular volume (fl)	78.56±0.22	86.19±0.59	-3.425	0.001
Mean corpuscular haemoglobin (pg/cell)	27.17±0.33	29.75±0.21	-6.511	0.0001
Mean corpuscular haemoglobin concentration (%)	31.11±0.24	33.51±0.14	-8.767	0.0001

[Table/Fig-2]: Comparison of red cell indices.

A total of 75 non-diabetics with IDA were further categorized according to degree of anaemia as shown in [Table/Fig-3]. We also analysed HbA1C level in various degree of anaemia and observed that HbA1C increases as severity of anaemia worsen which was statistically significant ($p < 0.05$) [Table/Fig-4].

ANOVA =229.815, p =0.0001.

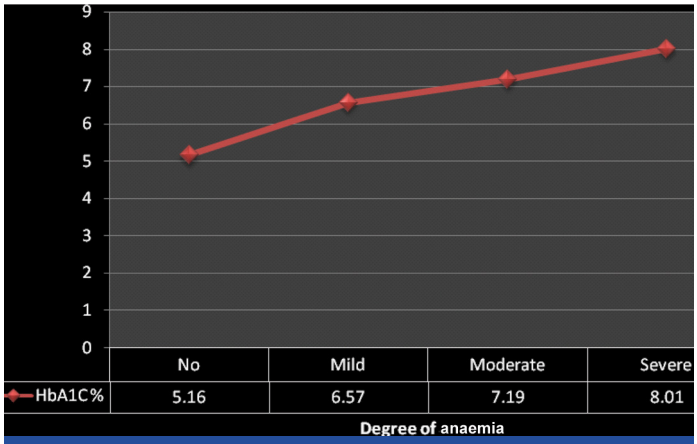
Degree of Anaemia	Mild	Moderate	Severe
Total number	40	30	5

[Table/Fig-3]: HbA1C variation according to the degree of anaemia.

No anaemia to mild anaemia, t-test: -14.323, p = 0.0001

Mild anaemia to Moderate anaemia, t-test:-4.483, p = 0.0001

Moderate anaemia to Severe anaemia, t-test: -3.397, p= 0.0001



[Table/Fig-4]: Variation of HbA1C according to degree of anaemia.

DISCUSSION

HbA1C is the most frequently occurring fraction of haemoglobin A_1. HbA1C reflects glycaemic status for the previous three months. ADA guidelines have not only considered it as the primary target for glycaemic control but also included it as a diagnostic criterion [3]. Besides blood glucose, HbA1C levels can be affected by conditions unrelated to diabetes like anaemia [7]. Approximately 1/3rd of patients with anaemia have iron deficiency [1]. We observed that Hb, Hct, and red cell indices were lower in anaemic patients, along with a microcytic hypochromic blood picture confirming IDA. This is in accordance with results of Barbieri et al., [10].

In this study, we observed higher HbA1C level in non diabetics with IDA than those without IDA which was statistically significant. This is after excluding patients with other confounding factors known to interfere with HbA1C level. This result coincides with the study results of Christy et al., who also observed that HbA1C levels were significantly higher in IDA patients and decreased after treatment with iron. The mechanisms leading to increased HbA1C levels in IDA were not clear [4].

The proposed theories for increased HbA1C levels in IDA were: a) Alteration of quaternary structure of haemoglobin leading to more rapid glycation of globin chain [5,11]; b) Decrease in the Hb concentration might lead to an increase in the glycated fraction at a constant glucose level, because HbA1C is measured as a percentage of total Haemoglobin A [6]; c) In IDA, reduced red cell production leads to a higher average age of circulating erythrocytes and therefore, increased HbA1C levels [5].

Our study results are also consistent with the study done by El-Agouza I et al., who reported that a decline in the Hb level might lead to increase in the glycated fraction at a fixed glucose level, because HbA1C is measured as a percentage of total Hb [6]. Similar results were also reported by Coban E et al., and Kim C et al., [12,13].

Coban et al., showed a very large difference between HbA1C levels in non diabetic patients with and without IDA [12].

Several other studies done before also showed similar results as our present study [6,11,12,14] [Table/Fig-5].

Studies by Ford ES et al., reported no significant difference in mean HbA1C concentration according to the IDA status as well as before

Study	Year	Number screened	IDA	Non anaemic	Significance
El-Agouza I et al., [6]	2002	81	6.1±0.6	5.2±0.4	p < 0.05
Coban E et al., [12]	2004	100	7.4±0.8	5.9±0.5	p < 0.05
Shanthi B et al., [11]	2013	100	7.6±0.5	5.5±0.8	p < 0.05
Silva JF et al., [14]	2016	122	5.6±0.4	5.3±0.4	p < 0.05
Present study	2017	150	6.8±0.07	5.1±0.04	p < 0.05

[Table/Fig-5]: Comparison of present study HbA1C levels with previous studies having similar results.

and after iron treatment [15]. Also, Saudek CD et al., suggested that red cell age was unlikely to be a significant factor in explaining the changes in HbA1C levels during the treatment of IDA and believed that the reported differences in HbA1C concentrations before and after iron supplementation were due to differences in the laboratory methods used for measuring HbA1C [16].

Sinha et al., and few other studies contradicts with our results reporting that HbA1C levels are lowered in IDA [5,7,15] [Table/Fig-6].

Study	Year	Number screened	IDA	Non anaemic	Signifi-cance
Ford ES et al., [15]	2011	8296	5.5±0.1	5.4±0.2	p > 0.05
Sinha N et al., [7]	2012	100	4.6±0.6	5.5±0.6	p > 0.05
Kalasker V et al., [5]	2014	80	5.9±0.4	6.5±0.3	p > 0.05

[Table/Fig-6]: Previous studies with contradicting HbA1C results from the present study.

Ferritin is a storage form of iron, and it reflects the true iron status [1]. In our study, serum ferritin as well as serum iron level was indirectly proportional to HbA1C. As explained previously, in IDA, ferritin is decreased with increase in the red cell life span which is associated with increased HbA1C. This goes in hand with other study results of Shanthi B et al., and Raj S et al., [11,17].

We also analysed HbA1C results in different degrees of anaemia and found that HbA1C level increases as severity of anaemia worsens. This result of ours was in accordance with the results of Silva JF et al., [14] [Table/Fig-7].

Study	Year	Number screened	Degree of Anaemia				Signifi-cance
			No	Mild	Moderate	Severe	
Silva JF et al., [14]	2016	122	5.3±0.40	5.5±0.40	5.6±0.40	5.7±0.40	p < 0.05
Present study	2017	150	5.1±0.04	6.5±0.09	7.1±0.11	8.0±0.01	p < 0.05

[Table/Fig-7]: Comparison of study results showing HbA1C variation according to the degree of anaemia.

LIMITATION

Few limitations of this study were small sample size and with this cross-sectional design, we couldn't follow up after iron therapy and the mechanism by which anaemia affects HbA1C was not evaluated. Also, it should be mentioned that it was performed in a group of patients without DM and the impact of anaemia on HbA1C levels may be greater and clinically relevant in patients with DM, especially in the presence of poor metabolic control.

CONCLUSION

In this study, we observed a positive correlation between IDA and elevated HbA1C level in non diabetic population. HbA1C increases with severity of anaemia. This spurious elevation of HbA1C in iron depletion leads to over or under diagnosis of diabetes at the cut off point (6.5%). Thereby, this study insists on the utter importance to exclude IDA by iron studies and to correct it before any diagnostic or therapeutic decision is made based solely on HbA1C level.

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Lumbar epidural varices: An unusual cause of lumbar claudication

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Abstract

Lumbar epidural varices can also present with radiculopathy similar to acute intervertebral disc prolapse (IVDP). However as the magnetic resonance imaging (MRI) in these patients are usually normal without signifcant compressive lesions of the nerve roots, the diagnosis is commonly missed or delayed leading to persistent symptoms. We present a rare case of acute severe unilateral claudication with a normal MRI unresponsive to conservative management who was treated surgically. The nerve root on the symptomatic side was found to be compressed by large anterior epidural varices secondary to an abnormal cranial attachment of ligamentum favum. Decompression of the root and coagulation of the varices resulted in complete pain relief. To conclude, lumbar epidural varices should be considered in the differential diagnosis of acute onset radiculopathy and claudication in the

Original Article

Screening of Health-care Workers for Latent Tuberculosis Infection in a Tertiary Care Hospital

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Abstract

Background: Health-care workers (HCWs) are at increased risk of acquiring tuberculosis (TB) than the general population. While national-level data on the burden of TB in general population is available from reliable sources, nationally representative data on latent tuberculosis infection (LTBI) burden in HCWs in the high burden countries is lacking. **Methods:** A prospective study was carried out to assess the risk of TB infection among HCWs who directly engage in medical duties. HCWs were recruited between January 2014 and December 2015. A structured questionnaire was used for risk assessment of TB infection among HCWs, including sociodemographic characteristics (e.g., age, gender, period of professional work, and employed position), knowledge of TB prevention and control, and history of professional work. A single-step tuberculin skin test (TST) using 5 international units (IU; 0.1 ml) of tuberculin (purified protein derivative from *Mycobacterium bovis* Bacillus Calmette–Guérin [BCG]). TB infection was determined using a TST induration ≥10 mm as a cutoff point for TST positivity. TST-positive participants were further subjected to detailed clinical evaluation and chest radiography to rule out active TB. The associations between TB infection and the sociodemographic characteristics, duration of possible exposure to TB while on medical duties, BCG vaccination, and knowledge about TB were estimated using Chi-square test. A two-sided $P < 0.05$ indicated statistical significance. **Results:** A total of 206 eligible HCWs signed the informed consent and completed the questionnaires between January 2014 and December 2015. The age of the participants ranged from 18 to 71 years, with a mean age of 27.13 years. TST induration size (mean 6.37 mm) the TST results suggested that 36.8% (76/206) were infected with TB using a TST induration ≥10 mm as a cut-off point. All 76 TST-positive HCWs showed no evidence of active TB in clinical evaluation and chest radiography. However, during the study, two HCWs developed pulmonary TB (both TST baseline test negative). Statistical analysis suggested that age, duration of employment as a health-care professional, literacy status, and working in medical wards/OP/Intensive Care Unit were significantly associated with TB infection. **Conclusions:** Many studies propose serial tests of LTBI as effective occupational protection strategies. However, practically, it is not feasible because it has to be done at frequent intervals, but how frequently to be done is not clear. Another concern is even if found to have LTBI, there are no clear consensus guidelines about the treatment in high prevalence settings. The prevalence of LTBI is so high in countries like India that affected HCWs could not be exempted from working in high-risk areas. The depth of knowledge of TB prevention and control among HCWs should be improved by regular infection control training.

Keywords: Latent tuberculosis, Mantoux test, tuberculin

Introduction

Health-care workers (HCWs) are at increased risk of acquiring tuberculosis (TB) than the general population. While national-level data on the burden of TB in general population is available from sources such as World Health Organization (WHO), nationally representative data on LTBI burden in HCWs in the high burden countries is lacking. Latent tuberculosis infection (LTBI) does not produce disease manifestations and is not infectious albeit it results in persistent immune response against *Mycobacterium tuberculosis* antigens.[1]

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However, there remains a 10-15% lifetime risk of developing active TB. There is no gold standard test for diagnosis of LTBI. Tuberculin skin test (TST) and blood interferon-gamma release assay (IGRA) tests are performed to diagnose LTBI. The WHO recommends that Bacillus Calmette–Guérin (BCG) vaccine be

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administered during infancy in TB endemic countries. Center for disease control (CDC) guidelines state that TST reactivity caused by BCG vaccine generally wanes with the passage of time. A person with a history of BCG vaccination can be tested and treated for LTBI if they react to the TST. TST reactions should be interpreted based on risk stratification regardless of BCG vaccination history. Furthermore, CDC states that considering comparable performance between TST and IGRA but increased cost, replacing TST with IGRAs in low-income and other middle-income countries is not recommended.[2]

The present study was conducted in a tertiary hospital with 350 beds. Suspect cases of pulmonary TB are referred to the department of pulmonary medicine which acts as Revised National Tuberculosis Control Programme (RNTCP) cell. Under RNTCP, two sputum samples are checked by the microbiologist and treatment of positive cases monitored by a dedicated health-care worker. Annually, around 300 new cases are detected in the hospital. Patients were treated on ambulatory basis as well as inpatients. During the early months of the study, all positive cases were admitted to the medical wards and allotted corner beds. There were no separate wards for pulmonary TB patients. There is no provision of negative pressure room for TB cases. N95 respirators were not available regularly to the health-care workers. Given the high prevalence of TB cases in the hospital and lack of stringent infection control practices, this study was proposed. During the later period of the study, separate ward for sputum positive cases was allotted. Patients who remain positive after the intensive phase of treatment were checked for rifampicin resistance by gene expert TB under RNTCP. However, no separate ward for MDR TB cases has been provided.

Methods

Study design and settings

A prospective study was carried out between January 2014 and December 2015 to assess the risk of TB infection among HCWs who directly engage in medical duties. Participants included nurses, laboratory technicians, paramedical technicians, and housekeeping workers. All the HCWs who were engaged in medical duties for more than 6 months were eligible. Each HCW was recruited by their department supervisor and encouraged to complete a self-administrated and standard structured questionnaire. TST was performed on all potential participants unless they declined to take or were not available during the study. The study was approved by the Ethics Committees of the Institute. Out of 321 health-care workers, 206 consented to participate in the study. This includes 130 nurses, 5 technical staff, 28 laboratory technicians, and 43 housekeeping staff. Elven workers with history of TB were not included. The study was conducted after obtaining institutional ethics committee approval, and all the participants provided written informed consent before recruitment.

Data collection and tuberculin skin test

A structured questionnaire was used for risk assessment of TB infection among HCWs, including sociodemographic characteristics (e.g., age, gender, period of professional work, and employed position), knowledge of TB prevention and control, history of professional work, and clinical work. A single-step TST using 10 international units (IU; 0.1 ml) of tuberculin (purified protein derivative from *Mycobacterium bovis* BCG, (Span diagnostics). The TST was administered using the Mantoux method by experienced staff, and participants returned 48–72 h after TST inoculation to obtain results, which were confirmed independently by two microbiologists. The horizontal diameter of induration size was measured using a standardized ruler, and the results obtained by the two microbiologists were averaged. LTBI was determined using a TST induration ≥10 mm as a cutoff point for TST positivity. However, BCG vaccination history was recorded for all the participants recorded, and its effect on the results was evaluated.

TST-positive participants were further subjected to detailed clinical evaluation and chest X-ray examination to rule out active TB. The associations between TB infection and the sociodemographic characteristics, experiences of medical duties, BCG vaccination, and knowledge about TB were estimated using Chi-square test. A two-sided $P < 0.05$ indicated statistical significance.

Results

A total of 206 eligible HCWs signed the informed consent and completed the questionnaires between January 2014 and December 2015. The age of the participants ranged from 18 to 71 years, with a mean age of 27.13 years. Eight were males. Of the participants, 78.6% (162/206) reported having received a BCG vaccination at birth. Mean TST induration size was 6.37 mm. The TST results showed that 36.8% (76/206) were infected with TB using a TST induration ≥10 mm as a cutoff point. All 76 TST-positive HCWs revealed no evidence of active TB on clinical and radiological evaluation. Statistical analysis suggested that age, duration of employment as a health-care professional, literacy status, and working in medical wards/OP/Intensive Care Unit were significantly associated with TB infection [Tables 1 and 2]. Other factors such as sex, body mass index, contact with TB patient in hostel/house/neighborhood, BCG vaccination, and knowledge about TB were not significantly associated with TB infection [Tables 1, 3 and 4]. Out of 206 health-care workers, 15 had generalized/systemic (more than one anatomical surface apart from test site) urticaria. We identified one participant with bulla, one with a bleb, and one with blister [Figures 1-3]. They were treated with skin emollients and antihistamines. There were no major adverse reactions.

Discussion

The results suggest that more than one-third of the HCWs had LTBI. This is likely due to high exposure to TB patients in the absence of optimum TB control measures in the high background TB prevalence in India in general and our study

Table 1: Association between demographic characteristics and latent tuberculosis infection

Factors	LTBI (%)	*P*
Sex		
Female	64/198 (32.3)	0.71
Male	3/8 (37.5)	
Age		
<30	33/145 (22.7)	0.0071
30-39	12/39 (30.7)	
>40	12/22 (54.5)	
Education		
Illiterate	19/42 (45.2)	0.000162
Primary school	0/26 (0)	
Bachelor degree	34/138 (24.6)	
BMI		
≤18.5	13/61 (21.3)	0.903
18.5-25 (94)	29/121 (23.9)	
>25 (18)	6/24 (25)	
BCG vaccination		
Yes	52/162 (32)	0.2738
No	18/44 (40.9)	

LTBI: Latent tuberculosis infection, BMI: Body mass index, BCG: Bacillus Calmette-Guérin

Table 2: Association between medical work and latent tuberculosis infection

Factors	LTBI (%)	*P*
Duration of professional exposure to TB patients (years)		
<1	5/74 (6.7)	0.0021
1-5	28/110 (25.4)	
>5	7/22 (31.8)	
Area of work		
Medical	32/117 (27.3)	0.04703
Surgical	9/59 (15.2)	
Laboratory	3/30 (10)	

LTBI: Latent tuberculosis infection, TB: Tuberculosis

setting in particular. Studies on latent TB among HCWs have only been conducted in dispartate regions of the country.

In 2005, Pai *et al*.[3] estimated the prevalence of LTBI among 720 health-care workers with one step tuberculin test and found the prevalence of 41%. In 2006, Pai *et al*.[4] performed serial testing of tuberculin test and IGRA among 216 medical and nursing students, 22% were TST-positive, and 18% were QFT-positive at baseline. Among 147 participants with concordant baseline negative results, TST conversions occurred in 13.6%, and QFT conversions occurred in 11.6% participants. In 2010, Christopher *et al*.[5] estimated the prevalence of 50.2% with serial tuberculin testing among 468 nursing students. In the multivariate analysis, TST positivity was strongly associated with time spent in health care. This is in concordance with the present study. Vijaykumar and Gopalakrishnan[6] studied the prevalence of LTBI in 85 nursing students with serial testing of TST and IGRA and concluded

Table 3: Association between habit and latent tuberculosis infection

Factors	LTBI	*P*
Immunocompromised status		
Diabetes		
Yes	1/4 (25)	0.597
No	31/202 (15.3)	
Steroid use		
Yes	1/4 (25)	0.2969
No	19/202 (9.4)	
Known TB patient in house/hostel		
Yes	2/12 (16.66)	0.8716
No	29/194 (14.9)	
Known TB patient in neighborhood		
Yes	0/4 (0)	0.5578
No	16/202 (7.9)	

LTBI: Latent tuberculosis infection, TB: Tuberculosis

Table 4: Association between knowledge of tuberculosis and latent tuberculosis infection

Score	LTBI	*P*
0-5	6/21 (28.5)	0.6664
6-10	22/62 (35.48)	
>10	36/123 (29.2)	

LTBI: Latent tuberculosis infection

that combination of TST and IGRA is ideal for screening to detect LTBI and modification of IGR A result interpretation is needed to be of significance in TB endemic countries.

There is also a growing recognition that LTBI is a spectrum, and accumulating evidence suggests that none of the existing LTBI tests can resolve this spectrum,[7,8] particularly with onetime testing.[9] In 2011, Joshi *et al*.[10] performed a cross-sectional comparison of TST and QFT in a cohort of 726 HCWs with young trainees making up half the cohort. A total of 360 (50%) HCWs were found to be positive using either the TST or QFT assay at baseline, and 226 (31%) were found to be positive using both tests. Six years after the baseline survey, HCWs were followed up. Of the 674 HCWs followed, 14 had developed active TB disease. Incidence rates of TB disease in the TST and QFT positive and negative subgroups were similar. In the present study, one of the participants negative for latent TB by Mantoux test went on to develop sputum positive pulmonary TB (Grade 3+) in the next 4 months. Current LTBI tests may not be able to identify the subset that is at highest risk of future disease, as confirmed by a new meta-analysis.[11] Therefore, the search for more predictive biomarkers or combinations of biomarkers and risk factors must continue.

TST has evolved over 100 years; despite all the stern scrutiny and standardizing measures, this test is still not devoid of side effects. Although adverse reactions to TST are uncommon, local allergic reactions to tuberculin or its components can occur in 2%–3% of those tested.[12] Studies have authenticated

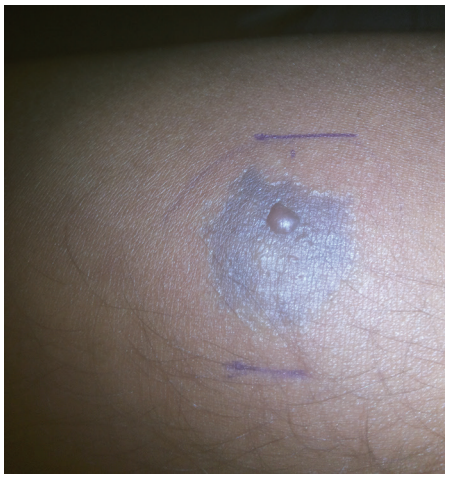

Figure 1: Vesicle following tuberculin skin test

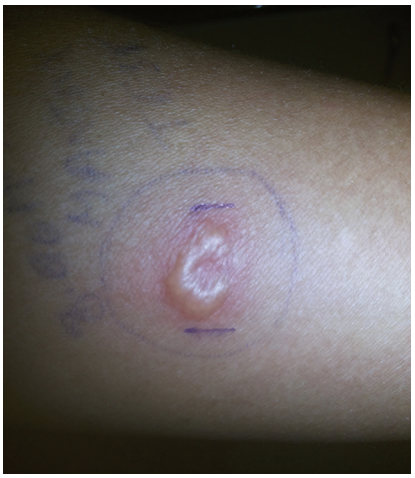

Figure 2: Bulla following tuberculin skin test

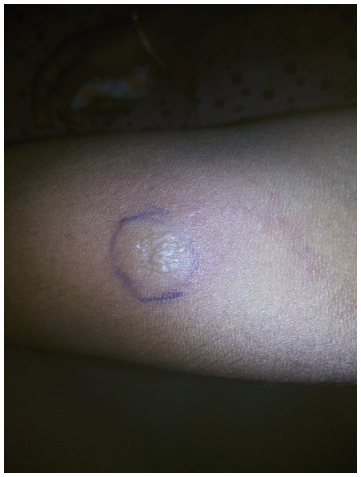

Figure 3: Blister following tuberculin skin test

the fact that there is no linear relation between tuberculin dose and the skin reaction observed[13] and hence, our results cannot be ascribed to using higher strengths of TU. In this study, only minor side effects were observed. In 2014, Christopher *et al*.[14] screened 755 nursing students for baseline two-step TST. In 623 individuals, adverse events were recorded when reported during the TST reading and 132 individuals answered an investigator administered questionnaire assessing all likely side-effects. In cohort A only 1.3% reported adverse events. In cohort B, as per the investigator administered questionnaire; 25% reported minor side effects. Itching and local pain were the most common side effects encountered. There were no major adverse events reported. In particular, the adverse events were similar in the second step of the test and not more severe.

Important concern overuse of screening tests in health-care workers is about the treatment. In the present study, participants who were found to have latent TB infection were not offered treatment in accordance with WHO guidelines.[1] Resource-limited countries and other middle-income countries should implement existing WHO guidelines for latent TB treatment on people living with HIV and child contacts below 5 years of age as a priority. But the infected individuals were explained about the chances of developing active TB and educated about the infection control measures. Despite, two participants (both TST baseline negative) developed pulmonary TB during the study.

Limitation of the study

Only one step TST was done. A negative test was not followed by another test to check booster phenomenon and testing at regular intervals not done to detect seroconversion. HIV testing was not included in the study which has definite implications. The risk of progression from LTBI to TB disease is 7% to 10% each year for those with both LTBI and untreated HIV infection. Those with LTBI who are not HIV infected have a 10% risk over their lifetime. The prevalence of latent TB among the general population in India is not known.

Conclusion

Most TB control programs in low- and middle-income countries have focused on case detection and treatment using the DOTS strategy. However, occupational infection control measures are not implemented as regular tasks in the TB infection control programs because of the high TB burden and limited resources. Many studies propose serial tests of LTBI as effective occupational protection strategies. However, practically it is not feasible because it has to be done at frequent intervals but how frequently to be done is not clear. The prevalence of latent TB is so high that the affected HCWs could not be exempted from working in high-risk areas. The depth of knowledge of TB prevention and control among HCWs should be improved by regular infection control training. There should be uniformity in the policy of TB treatment and care. Patients should be treated on ambulatory basis as much as possible. Consistent N95 respirator use by HCWs while attending suspected or diagnosed TB patients and by confirmed sputum positive TB patients themselves must be emphasized. Future research is needed to identify and test the effectiveness of feasible and affordable environmental control

and respiratory protection measures in resource-constrained settings.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Cardiology in the Young 2017; Page 1 of 7
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Original Article

Fungal endocarditis in paediatrics: a review of 192 cases (1971–2016)

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Abstract *Background:* The aims of this article were to review the published literature on fungal endocarditis in children and to discuss the aetiology and diagnosis, with emphasis on non-invasive methods and various treatment regimes. *Methods:* We systematically reviewed published cases and case series of fungal endocarditis in children. We searched the literature, including PubMed and individual references for publications of original articles, single cases, or case series of paediatric fungal endocarditis, with the following keywords: "fungal endocarditis", "neonates", "infants", "child", and "cardiac vegetation". *Results:* There have been 192 documented cases of fungal endocarditis in paediatrics. The highest number of cases was reported in infants (93/192, 48%) including 60 in neonates. Of the neonatal cases, 57 were premature with a median gestational age of 27 weeks and median birth weight of 860 g. Overall, 120 yeast – fungus that grows as a single cell – infections and 43 mould – fungus that grows in multicellular filaments, hyphae – infections were reported. With increasing age, there was an increased infection rate with moulds. All the yeast infections were detected by blood culture. In cases with mould infection, diagnosis was mainly established by culture or histology of emboli or infected valves after invasive surgical procedures. There have been a few recent cases of successful early diagnosis by non-invasive methods such as blood polymerase chain reaction (PCR) for moulds. The overall mortality for paediatric fungal endocarditis was 56.25%. The most important cause of death was cardiac complications due to heart failure. Among the various treatment regimens used, none of them was significantly associated with better outcome. *Conclusions:* Non-invasive methods such as PCR tests can be used to improve the chances of detecting and identifying the aetiological agent in a timely manner. Delays in the diagnosis of these infections may result in high mortality and morbidity. No significant difference was noted between combined surgical and medical therapy over exclusively combined medical therapy.

Keywords: Fungal; endocarditis; neonates; vegetation; paediatrics

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THE AIMS OF THIS ARTICLE WERE TO REVIEW THE published literature on fungal endocarditis in children and to discuss the aetiology and diagnosis, with emphasis on non-invasive methods and various treatment regimes. Invasive fungal infections have evolved into important causes of morbidity and mortality in children with severe underlying illnesses. Irrespective of age and the underlying condition, fungal endocarditis remains difficult to diagnose, and responses to treatment depend on early diagnosis and restoration of host defences. For more than three decades, options for antifungal chemotherapy have been limited to amphotericin B with or without flucytosine. Recent years, however, have witnessed an expanded clinical experience with antifungal triazoles and the development of less-toxic lipid amphotericin B formulations and echinocandins; moreover, new diagnostic modalities have been developed to aid earlier diagnosis.

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Children, particularly neonates and young infants, represent a unique patient population with regard to both patterns of fungal infections and disposition to antifungal agents.

Biological characteristics that may be unique to paediatric age groups include specific anatomical, physiological, and immunological aspects. The comparably small diameter of blood vessels provides a nidus for catheter-associated *Candida* thrombophlebitis, thrombosis, and endocarditis. In neonates, physiological differences such as the larger fractional water content, the smaller plasma protein fraction, relatively larger organ volumes, and the functional immaturity of hepatic metabolism and renal excretion may all lead to profound differences in drug distribution, metabolism, and elimination. Specific immunological characteristics in neonates include a functional immaturity of mononuclear and polymorphonuclear phagocytes and T lymphocytes as well as a possibly increased susceptibility to the immunosuppressive effects of corticosteroids. These deficiencies may render neonates susceptible to nosocomially acquired opportunistic fungal infections.[1]

Methods

We systematically reviewed published cases and case series of fungal endocarditis in children. We searched the literature, including PubMed and individual references for publications of original articles, single cases, or case series of paediatric fungal endocarditis, with the following keywords: "fungal endocarditis", "neonates", "infants", "child", and "cardiac vegetation". In addition, the reference list of each article was searched manually to verify that all published cases were collected for this review. Care was taken to exclude cases likely to reflect duplicate reporting. Criteria for a case or a case series to be included were the following: detection of fungal pathogens in blood plus echocardiographic findings and/or detection of fungal pathogens in cardiac sites after surgery or postmortem. Statistical evaluation of differences in proportions and calculation of odds ratios and 95% confidence intervals were performed using Fisher's exact test for categorical variables. A two-sided p value of <0.05 indicated statistical significance.

Results

Aetiology

A total of 98 articles were analysed with 192 documented cases of fungal endocarditis in children. Most of the case data were retrieved from previous review articles, case reports, or multi-institutional case series; 144 cases were documented from 63 articles in a single meta-analysis study in 2005.[2] The time distribution of cases with outcome is presented in Table 1. Overall, 120 yeast infections and 43 mould infections were reported. Identity of the fungus was not known in other cases. The majority of infections were due to yeast with *Candida albicans* (72 cases) being the most common, followed by *Candida tropicalis* (13 cases) and *Candida parapsilosis* (13 cases). Other yeasts were reported in a minority of cases, including *Candida dublinensis*,[3] *Candida krusei*,[4] *Saccharomyces cerevisiae*,[5] *Hansenula anomala*,[6] and *Kodamaea ohmeri*.[7] Moulds were reported in only seven infants with *Aspergillus fumigatus* being the most common.

Table 1. Time distribution of cases with outcome.

Years	Number of cases reported	Number of cases that survived	% Survival rate per decade
1971–1980	11	1	9
1981–1990	25	5	9.6
1991–2000	59	33	55.9
2001–2010	43	28	65.1
2011–2016	24	17	70.8

Out of 93 infant cases, 60 were in neonates ($\leqslant$28 days of birth), of which 57 were born prematurely – that is, $<$37 weeks of gestation – with a median gestational age of 27 weeks and median birth weight of 860 g. Among the 33 infants aged 28 days, 16 were born prematurely with a median gestational age of 31 weeks and median birth weight of 1050 g.

Among the 46 cases aged 1–10 years, 27 infections were due to yeast. The most common species included *C. albicans* (14 cases), followed by *C. tropicalis* (7 cases), *Histoplasma capsulatum*,[8] *Rhodotorula*,[9] and *C. haemulonii*[10] in a minority of cases. Of the 19 mould infections, *Aspergillus* was the most common pathogen. Zygomycetes such as *Lichtheimia corymbifera*[11] and dematacious fungi such as *Neoscytalidium dimidiatum*[12] were also documented.

In 25 children aged 11–19 years, mould infections were reported at a higher rate. Of 25 infections, eight were due to yeast and 17 due to moulds. In 28 documented cases, age was not specified. The summary of the incidence of fungal aetiology with reference to age group and survival rate is presented in Table 2.

Mode of diagnosis

In all the yeast infection cases, blood culture was positive, except in one case where the aetiology was established via postmortem diagnosis.[13] Of 43 cases of mould infections, blood culture was positive in eight

Table 2. Summary of the incidence of fungal agents and survival rates.

Age	Pathogen isolated	Number of cases	Number of cases survived
Infants	Yeasts		
	Candida albicans	54	31
	Candida parapsilosis	12	6
	Candida spp	10	3
	Candida tropicalis	5	5
	C. dublinensis	1	1
	Candida krusei	1	0
	Kodamaea ohmeri	1	0
	Saccharomyces cerevisiae	4	UN
	Moulds		
	Aspergillus flavus	1	0
	Aspergillus fumigatus	3	1
	Fusarium spp	2	1
	Phialemonium obovatum	1	0
1–10 years	Yeasts		
	Candida albicans	14	11
	Candida tropicalis	7	5
	Candida parapsilosis	1	1
	Candida haemolounii	1	1
	Rhodotorula pilimanae	1	1
	Histopasma capsulatum	2	UN
	Saccharomyces spp	1	0
	Moulds		
	Aspergillus fumigatus	4	1
	Aspergillus flavus	2	2
	Zygomycete	1	0
	Lichtheimia corymbifera	1	1
	Aspergillus niger	1	0
	Aspergillus nidulans	1	0
	Aspergillus spp	7	3
	Neosartorya fischeri	1	0
	Neoscytalidium dimidiatum	1	0
11–19 years	Yeasts		
	Candida albicans	4	3
	Candida tropicalis	1	0
	Candida guilliermondi	1	UN
	Candida spp	1	0
	Hansenula anomola	1	1
	Moulds		
	Aspergillus fumigates	4	1
	Aspergillus niger	2	1
	Aspergillus flavus	5	2
	Aspergillus terreus	1	0
	Aspergillus spp	2	1
	Fusarium solani	1	0
	Scedosporium Arnium leporinum	1	0

UN = unknown.

cases (Table 3). Fungal cultures were obtained from eight cases, and tissue histology studies were carried out. In 13 cases, diagnoses were made postmortem. Polymerase chain reaction (PCR) was carried out in only a few cases. Of the four cases of mould infection with negative blood culture, PCR blood was positive for mould, which was then supported by positive histology (one *Aspergillus niger*, two *Aspergillus flavus*, one *A. fumigatus*).[14,15] In a few

Table 3. Incidence of blood culture-positive moulds.

Blood culture-positive moulds	Number of cases positive by blood culture/total number of cases
Fusarium solani	3/3
Phialemonium oblovatum	1/1
Scedosporium apiospermum	1/1
Lichtheimia corymbifera	1/1
Aspergillus flavus	1/7
Aspergillus fumigates	1/11

cases of mould infection, there were other accessible sites that were concurrently fungal culture positive along with blood culture. In one case, a lesion over the head and a sternotomy wound yielded *Aspergillus nidulans*.[16] In another case, sputum cultures were positive for *A. fumigatus*.[17] In the above-mentioned cases, blood cultures were negative, and fungal vegetations were confirmed after histological findings.

Risk factors

Of 93 infants, 73 (78.4%) were born premature; furthermore, the majority of fungal endocarditis cases had an association with central venous catheters (65 of 93, 69.8%). Regardless of confirmed bacteraemia, antimicrobial agents were administered in 76 of 93 (81.7%) infants. Previous or concurrent confirmed bacteraemia was recorded in 19 of 93 (20.4%) infants. Other predisposing factors included open-heart surgeries for heart disease in 43 of 192 (22.3%) cases and chemotherapy for malignancies in 16 of 192 (8.3%) cases. *Aspergillus* species was found in 24 of 43 (55.8%) cases who had previously undergone open-heart surgery.

Site of vegetation

From pathological aspects, infective endocarditis IE was classified on the basis of site of vegetation. The most common site was the right side of the heart (86 cases), followed by the left side (21 cases), and combined (13 cases). Both valvular (57 cases) and mural vegetations (50cases) were documented.

Treatment

The heterogeneity of the antifungal regimens recorded in this systematic review reflects partially the lack of treatment recommendations for this age group. There was a trend for using certain regimens as they were being developed over time. Between 1983 and 1995, the majority of cases were treated with a combination of amphotericin B and lluorocytosine. From 1999 to 2005, the combination of amphoteracin B and fluconazole was most commonly

used. Caspofungin and voriconazole combination treatment was reported only after 2005 and in 2011. The outcome of different treatment regimens in yeast and mould infections is depicted in Tables 4 and 5.

Recombinant tissue plasminogen activator (rtPA) was tried in patients who had persistent candidaemia and thrombocytopaenia after 3 weeks of conventional antifungal therapy.[18–20] In all cases, the vegetations resolved with one to three doses of rtPA without major complications or need for surgery (Table 6).

Outcome

The overall fatality rate was 56.25%. Significant differences in outcome were reported before and after 2000 (56 cases with 59% case fatality before 2000 versus 22 cases with 33% case fatality after 2000, $p = 0.0013$). Infection with yeast was associated with significantly better outcomes when compared with moulds (35 cases of yeasts with 42% case fatality versus 28 cases of moulds with 65% case fatality, $p \leqslant 0.05$). Among the various antifungal regimens used, none was associated with a significantly better outcome. Furthermore, when the combination treatment of antifungals with surgery was compared with therapy without surgery, we observed that 35 of 53 (66%) reported cases treated with both antifungal drugs and surgery were alive when compared with 42 of 70 (60%) who received only antifungal drugs ($p = 0.57$). Initial antifungal combination therapy was not significantly associated with better outcome when compared with monotherapy. Among the 40 cases who received initial combination therapy, 14 (35%) succumbed to the disease, compared with 15 (48.3%) out of 31 cases who received monotherapy ($p = 0.33$). The most important cause of death was cardiac complication in the form of failure. Extracardiac complications such as embolisation, septic shock, multiorgan failure, disseminated fungal infection, acute renal failure also significantly contributed to mortality in many cases.

Table 4. Summary of different treatment regimens for yeast infections with outcome.

Treatment regimen	Number of cases treated	Number of cases survived
AMB monotherapy	29	15
AMB and 5-FC	15	7
AMB and FLU	16	11
AMB and VOR	2	2
AMB and CAS	4	3
Combined surgical and medical treatment	33	26

AMP = amphoteicin B; FLU = fluconazole; VOR = voriconazole; CAS = caspofungin; 5-FC = 5-fluorocytosine

Table 5. Summary of different treatment regimens for mould infections with outcome.

Treatment regimen	Number of cases treated	Number of cases survived
AMB monotherapy	2	1
AMB and VOR	3	3
Combined surgical and medical	20	9

Discussion

Fungal endocarditis remains a rare, yet serious entity. There has been an increase in fungal endocarditis due to various medical interventions to improve survival of life, especially those related to premature infants and repair for children with CHD. *Candida* spp are the most frequently encountered pathogens. Filamentous fungi including *Aspergillus* spp also contribute to a significant number of cases. The diagnosis is extremely difficult, primarily because of the fact that the indications for fungal endocarditis are not clearly evident.

Table 6. Details of neonatal Candida endocarditis treated by thrombolytic therapy.

Gestational age/birth weight	Site of vegetation	Fungus	Initial antifungal duration	Thrombolytic therapy	Outcome
24 weeks/566 g[5]	D12: foramen ovale, 7 mm	*Candida tropicalis*	AMB 1 mg/kg /day – 5 days	rtPA 0.3 mg/kg/hour over 6 hours – 4 days	After 4th dose, suspicious right parietal haemorrhage. Next 3 days, complete lysis of vegetation
27 weeks/1000 g	D56: tricuspid valve	*Candida albicans*	AMB and FLU for 4 weeks	Intravenous urokinase for 6 days	
26 weeks/800 g[6]	D8: interatrial septum and RA	*Candida albicans*	Liposomal AMB 5 mg/kg/day for 3 weeks	rtPA 0.2 mg/kg/hour over 6 hours – 3 days	Complete lysis in 3 days
33 weeks, 1380 g[4]	D22: RV posterior wall	*Candida albicans*	Liposomal AMB and FLU/CAS for 2 weeks	rtPA 0.3 mg/kg/hour over 6 hours – single dose	Complete lysis in 4 days

rtPA = recombinant tissue plasminogen activator; RA = right atrium; RV = right ventricle

In microbiological terms, reliable laboratory diagnosis is of particular concern. Obtaining sufficient volumes of blood and mistaken identity of contaminants as pathogen are some of the limitations. The use of special medium formulations for the recovery of yeasts is generally not necessary, because most of them grow well in conventional aerobic, blood culture broths within 2–3 days. Exceptions to this rule include *Candida glabrata* and *Cryptococcus neoformans*, which typically require 3–5 days of incubation. In this review, all but one species of yeast grew in blood culture medium. Reliable growth of filamentous fungi in automated blood culture systems is difficult to obtain. Of the moulds, *Fusarium* can be recovered in blood culture broth, but most other filamentous fungi could not be detected.[21] All the three cases of *Fusarium solani*[22–24] in paediatric endocarditis were positive in blood culture. A few other moulds such as *Aspergillus*, *Scedosporium*,[25] *Lichtheimia*,[11] and *Phialemonium*[26] were also recovered.

Traditionally, laboratory diagnosis is based on two techniques, culturing the causative organism from blood and serological tests for the detection of organisms that are difficult to isolate. Although serological diagnostic methods such as the detection of mannan and galactomannan can be useful for detecting fungal infections, they are not reliable. In the above-documented cases, only a few cases have used this diagnostic modality. In a case of endocarditis due to *A. fumigatus* where the blood culture was negative, mannan and galactomannan assay also provided negative results, and fungal endocarditis was confirmed only by positive histology and blood PCR.[14] The gold standard for the diagnosis of fungal endocarditis is culturing and isolation of aetiological agents from emboli, infected valves, or other materials collected by invasive methods such as surgery. In cases with documented mould endocarditis, diagnosis was established only after tissue histology and tissue culture in 16 cases. When one examines surgical materials such as vegetations, valves, and grafts for potential fungal pathogens, it is important to note that conventional culture techniques yield very high rates of false-positive results (13–55%) compared with previous blood cultures or nucleic acid amplification test NAAT-based testing of surgical materials.[27] It is best that culture-based methods be supplemented with molecular methods or histological examination of tissues for greater specificity. In this review, four cases of blood PCR-positive moulds were supported by positive histology.

Specimens that are easily accessible, such as peripheral emboli, sternotomy wounds after open cardiac surgery, and distal necrotic lesions, should also be screened for aetiology. Pulmonary involvement is very common with fungal endocarditis. Less-invasive specimens such as bronchoalveolar lavage and protected tracheal specimens can also be used.

Delay in diagnosis was a major determining factor of mortality in many of the mould infection cases – 17 cases were diagnosed postmortem. In two cases, *Aspergillus* mural endocarditis was documented.[28] The classic manifestations of endocarditis and abrupt embolic occlusion of large peripheral arteries characteristic of fungal valvular endocarditis were not seen in patients with mural endocarditis. In another case, where Zygomycetes infection was reported, transoesophageal echocardiogram was carried out only at a later stage, which detected a large intra-atrial mass. The patient died on the same day, and the postmortem examination revealed the aetiology.[29] A total of 13 cases were reported before 1980s. Some of them underwent CHD repair, which predisposed them to fungal endocarditis; two-dimensional echocardiogram was performed, which could not detect the vegetations as they were in the patch area.[30]

Although early diagnosis and therapy can lead to good clinical outcomes, the use of blood culture to diagnose fungal endocarditis is not reliable, and use of cultures of materials from the infected site remains the "gold standard"; however, this method is invasive, requires major surgery for sample collection, and is not possible in patients with poor condition. The time between sampling and obtaining results of cultures may be as long as 7–10 days, which may be too long for the introduction of effective treatment. Although there is only limited evidence, molecular methods using blood may be more accurate and reliable for the diagnosis of these infections.

As there are no consensus guidelines for the treatment of fungal endocarditis, different combinations of antifungal drugs were used as they were being developed over time. Amphotericin B has been the first-line antifungal agent for medical therapy, although it does not penetrate vegetations well. Although imidazoles such as fluconazole do not have proven efficacy in human fungal IE, long-term suppressive therapy with these agents has been recommended by experts for patients with infections caused by susceptible organisms who cannot undergo curative surgery. In this review, some cases were on prophylactic antifungals for 6 months to 1 year. Oral fluconazole was used for *Candida* endocarditis, and oral voriconazole/itraconazole was used for *Aspergillus* endocarditis.

The addition of 5-fluorocytosine (100–150 mg/kg per day, divided every 6 hours) to amphotericin B administered orally for *Candida* endocarditis may provide additional benefit. The rationale is that the two drugs may act synergistically and potentiate fungal killing. The use of liposomal forms of amphotericin B is an alternative for patients with moderate-to-severe renal impairment or those with unacceptable infusion-related toxicities.

Combination therapies with new triazoles such as voriconazole show promise in the treatment of candidiasis refractory to conventional therapy. In this review, three cases – *C. parapsilosis* in a preterm infant,[31] *A. fumigatus*,[17] and *Fusarium*[22] – were successfully treated with initial combination therapy of Amphotericin B and voriconazole. Although it cannot be recommended as a standard treatment for neonatal patients on the basis of the results of case reports with limited samples sizes, these drugs appear to be safe antifungal agents for use in critically ill, preterm infants with persistent fungaemia despite AMB treatment.

Echinocandins are in comparison relatively new agents, having been approved for candidaemia only within the past decade. Similar to amphotericin B, echinocandins are fungicidal, and similar to the lipid formulations of amphotericin B they have good activity against candidal biofilms. In five neonates with *C. albicans* endocarditis, caspofungin was used in combination with other antifungals. In one case, there was no resolution of vegetation with combined amphotericin B and caspofungin treatment, and the baby died of renal failure.[32] In two other neonates, fluconazole resulted in successful treatment after failure of the initial combination treatment with amphotericin B and caspofungin.[33] In a preterm neonate, rtPA caused complete resolution after failure with 6 weeks of AMB and CAS.[18] A neonate with Eustachian valve endocarditis was successfully managed with VOR and CAS.[34] Although the combination therapy of echinocandins with other antifungals has been compared with the treatment of invasive candidiasis, no studies were available regarding treatment of paediatric fungal endocarditis.

Interestingly, there have been a few cases of neonatal fungal endocarditis treated successfully with recombinant tissue plasminogen activator in combination with antifungal therapy. Tissue plasminogen activators are thrombolytic agents that act directly on plasminogen and convert it to plasmin. Plasmin, in turn, digests fibrin. tPA can indiscriminately dissolve not only pathological thrombi but also haemostatic clots, and thereby lead to serious haemorrhagic complications. Therefore, close monitoring of the coagulation profile is extremely important. Even with older vegetations the therapy can be helpful. Neonates need a higher dose and increased number of doses for infective endocarditis resolution.[35] This could possibly be explained by differences in the clotting factor cascade that is seen in neonates compared with older children. Recombinant tissue plasminogen activator will help breakdown the vegetation, allowing better penetration of the antimicrobials, and therefore resulting in faster resolution of vegetations.

Conclusion

Fungal endocarditis remains a rare infection. The rarity of the disease prevents defining specific guidelines regarding the therapeutic management; however, with the extensive use of intravenous antibiotics and many interventional procedures, the incidence of fungal infections is on the rise. To complicate it further, least common and less-pathogenic organisms are also involved in serious infections. Non-invasive methods such as PCR tests can be used to improve the chances of detecting and identifying the aetiological agent in a timely manner. Delays in diagnosis of these infections may result in high mortality and morbidity.

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Original Article

Screening of Health-care Workers for Latent Tuberculosis Infection in a Tertiary Care Hospital

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Abstract

Background: Health-care workers (HCWs) are at increased risk of acquiring tuberculosis (TB) than the general population. While national-level data on the burden of TB in general population is available from reliable sources, nationally representative data on latent tuberculosis infection (LTBI) burden in HCWs in the high burden countries is lacking. **Methods:** A prospective study was carried out to assess the risk of TB infection among HCWs who directly engage in medical duties. HCWs were recruited between January 2014 and December 2015. A structured questionnaire was used for risk assessment of TB infection among HCWs, including sociodemographic characteristics (e.g., age, gender, period of professional work, and employed position), knowledge of TB prevention and control, and history of professional work. A single-step tuberculin skin test (TST) using 5 international units (IU; 0.1 ml) of tuberculin (purified protein derivative from *Mycobacterium bovis* Bacillus Calmette–Guérin [BCG]). TB infection was determined using a TST induration ≥10 mm as a cutoff point for TST positivity. TST-positive participants were further subjected to detailed clinical evaluation and chest radiography to rule out active TB. The associations between TB infection and the sociodemographic characteristics, duration of possible exposure to TB while on medical duties, BCG vaccination, and knowledge about TB were estimated using Chi-square test. A two-sided $P < 0.05$ indicated statistical significance. **Results:** A total of 206 eligible HCWs signed the informed consent and completed the questionnaires between January 2014 and December 2015. The age of the participants ranged from 18 to 71 years, with a mean age of 27.13 years. TST induration size (mean 6.37 mm) the TST results suggested that 36.8% (76/206) were infected with TB using a TST induration ≥10 mm as a cut-off point. All 76 TST-positive HCWs showed no evidence of active TB in clinical evaluation and chest radiography. However, during the study, two HCWs developed pulmonary TB (both TST baseline test negative). Statistical analysis suggested that age, duration of employment as a health-care professional, literacy status, and working in medical wards/OP/Intensive Care Unit were significantly associated with TB infection. **Conclusions:** Many studies propose serial tests of LTBI as effective occupational protection strategies. However, practically, it is not feasible because it has to be done at frequent intervals, but how frequently to be done is not clear. Another concern is even if found to have LTBI, there are no clear consensus guidelines about the treatment in high prevalence settings. The prevalence of LTBI is so high in countries like India that affected HCWs could not be exempted from working in high-risk areas. The depth of knowledge of TB prevention and control among HCWs should be improved by regular infection control training.

Keywords: Latent tuberculosis, Mantoux test, tuberculin

Introduction

Health-care workers (HCWs) are at increased risk of acquiring tuberculosis (TB) than the general population. While national-level data on the burden of TB in general population is available from sources such as World Health Organization (WHO), nationally representative data on LTBI burden in HCWs in the high burden countries is lacking. Latent tuberculosis infection (LTBI) does not produce disease manifestations and is not infectious albeit it results in persistent immune response against *Mycobacterium tuberculosis* antigens.[1]

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However, there remains a 10-15% lifetime risk of developing active TB. There is no gold standard test for diagnosis of LTBI. Tuberculin skin test (TST) and blood interferon-gamma release assay (IGRA) tests are performed to diagnose LTBI. The WHO recommends that Bacillus Calmette–Guérin (BCG) vaccine be

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administered during infancy in TB endemic countries. Center for disease control (CDC) guidelines state that TST reactivity caused by BCG vaccine generally wanes with the passage of time. A person with a history of BCG vaccination can be tested and treated for LTBI if they react to the TST. TST reactions should be interpreted based on risk stratification regardless of BCG vaccination history. Furthermore, CDC states that considering comparable performance between TST and IGRA but increased cost, replacing TST with IGRAs in low-income and other middle-income countries is not recommended.[2]

The present study was conducted in a tertiary hospital with 350 beds. Suspect cases of pulmonary TB are referred to the department of pulmonary medicine which acts as Revised National Tuberculosis Control Programme (RNTCP) cell. Under RNTCP, two sputum samples are checked by the microbiologist and treatment of positive cases monitored by a dedicated health-care worker. Annually, around 300 new cases are detected in the hospital. Patients were treated on ambulatory basis as well as inpatients. During the early months of the study, all positive cases were admitted to the medical wards and allotted corner beds. There were no separate wards for pulmonary TB patients. There is no provision of negative pressure room for TB cases. N95 respirators were not available regularly to the health-care workers. Given the high prevalence of TB cases in the hospital and lack of stringent infection control practices, this study was proposed. During the later period of the study, separate ward for sputum positive cases was allotted. Patients who remain positive after the intensive phase of treatment were checked for rifampicin resistance by gene expert TB under RNTCP. However, no separate ward for MDR TB cases has been provided.

Methods

Study design and settings

A prospective study was carried out between January 2014 and December 2015 to assess the risk of TB infection among HCWs who directly engage in medical duties. Participants included nurses, laboratory technicians, paramedical technicians, and housekeeping workers. All the HCWs who were engaged in medical duties for more than 6 months were eligible. Each HCW was recruited by their department supervisor and encouraged to complete a self-administrated and standard structured questionnaire. TST was performed on all potential participants unless they declined to take or were not available during the study. The study was approved by the Ethics Committees of the Institute. Out of 321 health-care workers, 206 consented to participate in the study. This includes 130 nurses, 5 technical staff, 28 laboratory technicians, and 43 housekeeping staff. Elven workers with history of TB were not included. The study was conducted after obtaining institutional ethics committee approval, and all the participants provided written informed consent before recruitment.

Data collection and tuberculin skin test

A structured questionnaire was used for risk assessment of TB infection among HCWs, including sociodemographic characteristics (e.g., age, gender, period of professional work, and employed position), knowledge of TB prevention and control, history of professional work, and clinical work. A single-step TST using 10 international units (IU; 0.1 ml) of tuberculin (purified protein derivative from *Mycobacterium bovis* BCG, (Span diagnostics). The TST was administered using the Mantoux method by experienced staff, and participants returned 48–72 h after TST inoculation to obtain results, which were confirmed independently by two microbiologists. The horizontal diameter of induration size was measured using a standardized ruler, and the results obtained by the two microbiologists were averaged. LTBI was determined using a TST induration ≥10 mm as a cutoff point for TST positivity. However, BCG vaccination history was recorded for all the participants recorded, and its effect on the results was evaluated.

TST-positive participants were further subjected to detailed clinical evaluation and chest X-ray examination to rule out active TB. The associations between TB infection and the sociodemographic characteristics, experiences of medical duties, BCG vaccination, and knowledge about TB were estimated using Chi-square test. A two-sided $P < 0.05$ indicated statistical significance.

Results

A total of 206 eligible HCWs signed the informed consent and completed the questionnaires between January 2014 and December 2015. The age of the participants ranged from 18 to 71 years, with a mean age of 27.13 years. Eight were males. Of the participants, 78.6% (162/206) reported having received a BCG vaccination at birth. Mean TST induration size was 6.37 mm. The TST results showed that 36.8% (76/206) were infected with TB using a TST induration ≥10 mm as a cutoff point. All 76 TST-positive HCWs revealed no evidence of active TB on clinical and radiological evaluation. Statistical analysis suggested that age, duration of employment as a health-care professional, literacy status, and working in medical wards/OP/Intensive Care Unit were significantly associated with TB infection [Tables 1 and 2]. Other factors such as sex, body mass index, contact with TB patient in hostel/house/neighborhood, BCG vaccination, and knowledge about TB were not significantly associated with TB infection [Tables 1, 3 and 4]. Out of 206 health-care workers, 15 had generalized/systemic (more than one anatomical surface apart from test site) urticaria. We identified one participant with bulla, one with a bleb, and one with blister [Figures 1-3]. They were treated with skin emollients and antihistamines. There were no major adverse reactions.

Discussion

The results suggest that more than one-third of the HCWs had LTBI. This is likely due to high exposure to TB patients in the absence of optimum TB control measures in the high background TB prevalence in India in general and our study

Table 1: Association between demographic characteristics and latent tuberculosis infection

Factors	LTBI (%)	*P*
Sex		
Female	64/198 (32.3)	0.71
Male	3/8 (37.5)	
Age		
<30	33/145 (22.7)	0.0071
30-39	12/39 (30.7)	
>40	12/22 (54.5)	
Education		
Illiterate	19/42 (45.2)	0.000162
Primary school	0/26 (0)	
Bachelor degree	34/138 (24.6)	
BMI		
≤18.5	13/61 (21.3)	0.903
18.5-25 (94)	29/121 (23.9)	
>25 (18)	6/24 (25)	
BCG vaccination		
Yes	52/162 (32)	0.2738
No	18/44 (40.9)	

LTBI: Latent tuberculosis infection, BMI: Body mass index, BCG: Bacillus Calmette-Guérin

Table 2: Association between medical work and latent tuberculosis infection

Factors	LTBI (%)	*P*
Duration of professional exposure to TB patients (years)		
<1	5/74 (6.7)	0.0021
1-5	28/110 (25.4)	
>5	7/22 (31.8)	
Area of work		
Medical	32/117 (27.3)	0.04703
Surgical	9/59 (15.2)	
Laboratory	3/30 (10)	

LTBI: Latent tuberculosis infection, TB: Tuberculosis

setting in particular. Studies on latent TB among HCWs have only been conducted in dispartate regions of the country.

In 2005, Pai *et al*.[3] estimated the prevalence of LTBI among 720 health-care workers with one step tuberculin test and found the prevalence of 41%. In 2006, Pai *et al*.[4] performed serial testing of tuberculin test and IGRA among 216 medical and nursing students, 22% were TST-positive, and 18% were QFT-positive at baseline. Among 147 participants with concordant baseline negative results, TST conversions occurred in 13.6%, and QFT conversions occurred in 11.6% participants. In 2010, Christopher *et al*.[5] estimated the prevalence of 50.2% with serial tuberculin testing among 468 nursing students. In the multivariate analysis, TST positivity was strongly associated with time spent in health care. This is in concordance with the present study. Vijaykumar and Gopalakrishnan[6] studied the prevalence of LTBI in 85 nursing students with serial testing of TST and IGRA and concluded

Table 3: Association between habit and latent tuberculosis infection

Factors	LTBI	*P*
Immunocompromised status		
Diabetes		
Yes	1/4 (25)	0.597
No	31/202 (15.3)	
Steroid use		
Yes	1/4 (25)	0.2969
No	19/202 (9.4)	
Known TB patient in house/hostel		
Yes	2/12 (16.66)	0.8716
No	29/194 (14.9)	
Known TB patient in neighborhood		
Yes	0/4 (0)	0.5578
No	16/202 (7.9)	

LTBI: Latent tuberculosis infection, TB: Tuberculosis

Table 4: Association between knowledge of tuberculosis and latent tuberculosis infection

Score	LTBI	*P*
0-5	6/21 (28.5)	0.6664
6-10	22/62 (35.48)	
>10	36/123 (29.2)	

LTBI: Latent tuberculosis infection

that combination of TST and IGRA is ideal for screening to detect LTBI and modification of IGR A result interpretation is needed to be of significance in TB endemic countries.

There is also a growing recognition that LTBI is a spectrum, and accumulating evidence suggests that none of the existing LTBI tests can resolve this spectrum,[7,8] particularly with onetime testing.[9] In 2011, Joshi *et al*.[10] performed a cross-sectional comparison of TST and QFT in a cohort of 726 HCWs with young trainees making up half the cohort. A total of 360 (50%) HCWs were found to be positive using either the TST or QFT assay at baseline, and 226 (31%) were found to be positive using both tests. Six years after the baseline survey, HCWs were followed up. Of the 674 HCWs followed, 14 had developed active TB disease. Incidence rates of TB disease in the TST and QFT positive and negative subgroups were similar. In the present study, one of the participants negative for latent TB by Mantoux test went on to develop sputum positive pulmonary TB (Grade 3+) in the next 4 months. Current LTBI tests may not be able to identify the subset that is at highest risk of future disease, as confirmed by a new meta-analysis.[11] Therefore, the search for more predictive biomarkers or combinations of biomarkers and risk factors must continue.

TST has evolved over 100 years; despite all the stern scrutiny and standardizing measures, this test is still not devoid of side effects. Although adverse reactions to TST are uncommon, local allergic reactions to tuberculin or its components can occur in 2%–3% of those tested.[12] Studies have authenticated

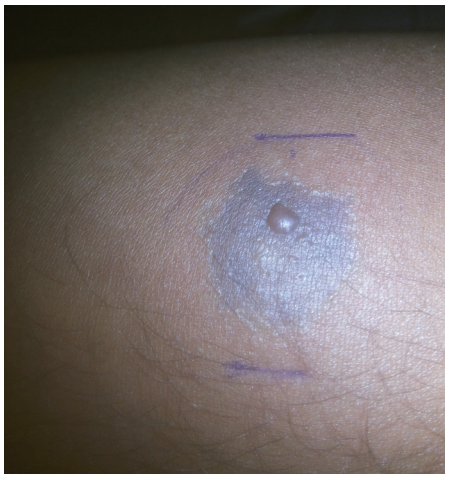

Figure 1: Vesicle following tuberculin skin test

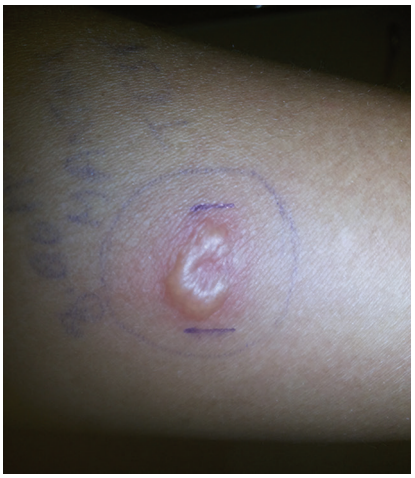

Figure 2: Bulla following tuberculin skin test

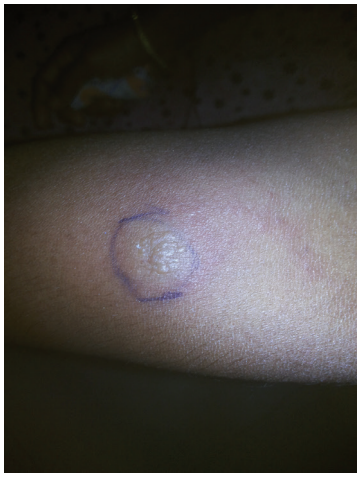

Figure 3: Blister following tuberculin skin test

the fact that there is no linear relation between tuberculin dose and the skin reaction observed[13] and hence, our results cannot be ascribed to using higher strengths of TU. In this study, only minor side effects were observed. In 2014, Christopher *et al*.[14] screened 755 nursing students for baseline two-step TST. In 623 individuals, adverse events were recorded when reported during the TST reading and 132 individuals answered an investigator administered questionnaire assessing all likely side-effects. In cohort A only 1.3% reported adverse events. In cohort B, as per the investigator administered questionnaire; 25% reported minor side effects. Itching and local pain were the most common side effects encountered. There were no major adverse events reported. In particular, the adverse events were similar in the second step of the test and not more severe.

Important concern overuse of screening tests in health-care workers is about the treatment. In the present study, participants who were found to have latent TB infection were not offered treatment in accordance with WHO guidelines.[1] Resource-limited countries and other middle-income countries should implement existing WHO guidelines for latent TB treatment on people living with HIV and child contacts below 5 years of age as a priority. But the infected individuals were explained about the chances of developing active TB and educated about the infection control measures. Despite, two participants (both TST baseline negative) developed pulmonary TB during the study.

Limitation of the study

Only one step TST was done. A negative test was not followed by another test to check booster phenomenon and testing at regular intervals not done to detect seroconversion. HIV testing was not included in the study which has definite implications. The risk of progression from LTBI to TB disease is 7% to 10% each year for those with both LTBI and untreated HIV infection. Those with LTBI who are not HIV infected have a 10% risk over their lifetime. The prevalence of latent TB among the general population in India is not known.

Conclusion

Most TB control programs in low- and middle-income countries have focused on case detection and treatment using the DOTS strategy. However, occupational infection control measures are not implemented as regular tasks in the TB infection control programs because of the high TB burden and limited resources. Many studies propose serial tests of LTBI as effective occupational protection strategies. However, practically it is not feasible because it has to be done at frequent intervals but how frequently to be done is not clear. The prevalence of latent TB is so high that the affected HCWs could not be exempted from working in high-risk areas. The depth of knowledge of TB prevention and control among HCWs should be improved by regular infection control training. There should be uniformity in the policy of TB treatment and care. Patients should be treated on ambulatory basis as much as possible. Consistent N95 respirator use by HCWs while attending suspected or diagnosed TB patients and by confirmed sputum positive TB patients themselves must be emphasized. Future research is needed to identify and test the effectiveness of feasible and affordable environmental control

and respiratory protection measures in resource-constrained settings.

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Conflicts of interest

There are no conflicts of interest.

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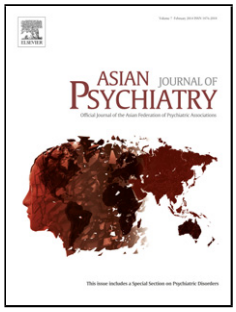

Title: Pre-treatment factor structures of the Montgomery and Åsberg Depression Rating scale as predictors of response to escitalopram in Indian patients with non-psychotic major depressive disorder

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Title Page

Full Title: Pre-treatment factor structures of the Montgomery and Åsberg Depression Rating scale as predictors of response to escitalopram in Indian patients with non-psychotic major depressive disorder

Running Title: Factor structures of depression rating scale as predictors of escitalopram response

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Abstract:

Background: Major Depressive Disorder (MDD) is a broad heterogeneous construct resolving into several symptom-clusters by factor analysis. The aim was to find the factor structures of MDD as per Montgomery and Asberg Depression Rating Scale (MADRS) and whether they predict escitalopram response.

Methods: In a longitudinal study at a tertiary institute in north India, 116 adult out-patients with non-psychotic unipolar MDD were assessed with MADRS before and after treatment with escitalopram (10-20mg) over 6-8 weeks for drug response.

Results: For total 116 patients pre-treatment four factor structures of MADRS extracted by principal component analysis with varimax rotation altogether explained a variance of 57%: first factor 'detachment' (concentration difficulty, lassitude, inability to feel); second factor 'psychic anxiety' (suicidal thoughts and inner tension); third 'mood-pessimism' (apparent sadness, reported sadness, pessimistic thoughts) and fourth 'vegetative' (decreased sleep, appetite). Eighty patients (68.9%) who completed the study had mean age 35.37±10.9 yrs, majority were male (57.5%), with mean pre-treatment MADRS score 28.77±5.18 and majority (65%) having moderate severity (MADRS <30). Among them 56 (70%) responded to escitalopram. At the end of the treatment there were significant changes in all the 4 factor structures ($p<0.01$). Vegetative function was an important predictor of response ($p<0.01$, odd's ratio: 1.3[1.1-1.6]95% CI). Melancholia significantly predicted non-response ($p=0.04$).

Conclusions: Non-psychotic unipolar major depression having moderate severity in north Indian patients as per MADRS resolved into four factor-structures all significantly improved

with adequate escitalopram treatment. Understanding the factor structure is important as they can be important predictor of escitalopram response.

Key-words:

Antidepressants

Unipolar depression

Factor analysis

Mood Disorder

Predictor

Main Text

Introduction: Major depressive disorder (MDD) is one of the most common psychiatric disorders; in most countries about 8-12% of the general population suffer from at least one major depressive episode during their life-time (1)(2). In the famous Global Burden of Disease study of 1990s depression has been recognized as the fourth largest cause of disability, and by 2020 it is expected to occupy the second position (3). Inspite of the disease burden our understanding of the very nature of the construct of 'depression' is still inadequate and currently it is understood to be a spectrum ranging from neurotic to melancholic depression(4). Tracking the changes in its nature and severity during treatment is a challenge and may be facilitated using symptom-based depression instruments. One such tool useful in the assessment is the Montgomery and Asberg Depression Rating Scale (MADRS) which is a 10 item clinical rating scale derived from the Comprehensive Psychopathology Rating Scale. Nine items are based upon patient report and one is rater's observation of patients. MADRS has a high inter - rater reliability. Scores on the scale correlate significantly with scores on a standard rating scale for depression, the Hamilton Rating Scale (HRS) - thereby indicating its validity (5). Since the items cover a range of signs and symptoms, subscales may exist within the MADRS corresponding to common cluster of depressive symptoms. Several prior studies (see table 1) have identified possible MADRS subscales and symptom clusters by applying factor analysis (extraction of latent variables from a larger set of observable variables) to the set of ten items of MADRS (6)(7)(8)(9)(10)(11)(12)(13)(14)(15)(16). We thereby saw a large variability in the factor structure of depression in MADRS from binary to quaternary.

An important reason of studying the factor structure of depression is that they may be differentially affected by treatment thereby acting as predictors of treatment response. In one of the previous studies Higuchi et al. found that among Japanese patients the factor relating to vegetative functions is a predictor of fluvoxamine response (17). Also it has been shown in treatment resistant depression that the factor related to 'dysphoria' is a good predictor of response to ECT(18). Such findings should lead to similar research in our population where (19) it has been shown that the clinical manifestations of depression are different from other cultures. But none of the studies to our knowledge studied the MADRS factor structure of Indian depressive patients. So, in this study the aim was to find the different factor structures of MDD as per MADRS and whether they predict the response to one of the most commonly used antidepressants namely escitalopram in north Indian patients.

Methods:

Study setting and participants:

The sample of the study comprised of patients following up in the psychiatry outpatient department (OPD) of All India Institute of Medical Sciences, a tertiary care hospital in north India between August, 2010 and June, 2011. To be included for the study, the patient needed to be in the age group 18-65 years, suffering from depressive symptoms and off all psychotropic drugs including antidepressants for a minimum period of one week (for fluoxetine the minimum period of five weeks). Patients with severe medical diseases, on medications known to produce mood symptoms, pregnant and breast-feeding women, patients with recent suicide attempt (i.e. suicidal attempt in the index episode) or history of hypersensitivity to escitalopram were excluded. Past history of manic episodes in self or first degree biological relative, psychotic disorder, severe personality disorder and any substance dependence except nicotine were the other exclusion criteria for the study.

Procedure:

Patients were assessed with Mini International Neuropsychiatric Interview (M.I.N.I.) to diagnose major depressive disorder as per the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 4th edition-Text Revision (DSM-IV-TR). Patients with melancholic features as per DSM-IV were also noted. After the diagnosis, written informed consent was taken. Socio-demographic and basic clinical information was collected on a semi structured proforma designed for the study. Severity of depression was assessed on Montgomery Asberg Depression Rating Scale (MADRS) and Clinical Global Impressions scale (CGI). Patients having a minimum score of 22 on the MADRS scale and at least moderately ill in the clinical severity item on CGI were recruited for the study to rule out sub-syndromal states.

After the first assessment, patients were started on tablet escitalopram (10 mg) to be taken once daily in the morning, as per the standard treatment practices. The subjects continued their follow up in the out-patient services. Dose could be increased to 15-20 mg per day, if clinically indicated by the treating psychiatrist as per standard treatment guidelines and researchers had no role in this decision. During the period of treatment, no other psychotropic drugs except low dose anxiolytics, sedatives and hypnotics for a brief period of initial 2-3 weeks were allowed (mostly low dose benzodiazepines as per our standard clinical practice). However, medications for general medical condition, if any, were allowed. The patients did not receive any form of psychotherapy during this period. If the patients needed any other laboratory investigations during this period they were performed (e.g. thyroid profile, complete haemogram etc.) as per clinical decision of the treating psychiatrist (either senior resident or consultant psychiatrist).

Patients were followed-up after six to eight weeks of initiation of therapy, which is a reasonable duration for study of anti-depressant side-effects. At the time of second assessment, patients were considered compliant if drugs were taken on at least 80% of days. Compliance to daily treatment was checked by empty strips of tablets, self-report by the patient and any family member. Patients who did not come for follow-up were given two telephonic reminders and only then they were considered drop-outs. On follow up, the subjects were assessed on MADRS, CGI and adverse events on medications were noted if any. Patients were classified as responder if there was at least 50% reduction in initial MADRS score; otherwise taken as non-responder. On CGI scale, to be defined as responder, the subject needed to have at least a score of three (mildly ill) or less in the severity of illness item and two (much improved) or less in the global Improvement item, and at least moderate improvement in the efficacy index. The study was approved by the institute ethics committee.

Statistical analysis:

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 17.0. To generate factors (symptom dimensions), principal component analysis was performed with varimax rotation on the 10 symptom categories of the MADRS scale and an eigen-value of greater than 1 (Kaiser criteria) was used to select the number of factors from the initial. Factor loading of greater than 0.40 was considered robust. Initially to understand the factor-structure of MADRS, factor analysis was done for all the recruited patients. Thereafter the values of the individual factors were calculated for the compliant patients across the study. Also, the association between socio-demographic variables, clinical characteristics, factor structures and drug response (responder/non-responder) was detected using chi-square for categorical variables and t test for continuous variables. Pre-treatment and post-treatment factor structures were compared across the responder and non-responder groups by means of paired t test. Logistic regression was performed by incorporating those independent predictor variables clinically considered significant ($p<0.1$) in univariate analysis to construct a logistic model. The odd's ratios were calculated in the logistic model with 95% confidence interval. All were 2-tailed tests and the results were considered significant at $p<0.05$.

Results: In total we selected 120 patients – however two patients refused consent, another two were excluded for developing manic/psychotic symptoms in the follow-up as shown in figure 1A. Finally 116 patients were recruited with the following socio-demographic and clinical features - mean current age 35.87±10.6yrs, 71(61.2%) patients were male, mean age of onset of depression was 32.75 ± 10.62yrs, 64(55.2%) having depressive episode of duration less than 6 months, 80 (69%) having less severity (MADRS<30) and the mean MADRS score being 28.32±4.86. Other socio-demographic and clinical features for the total patients (n=116) is illustrated in table 2A and 2B respectively. As per Table 3, principal component analysis of the different items on MADRS with varimax rotation for the 116 patients led to the derivation of four factors: factor I (detachment) with high loading on 'concentration difficulty'(0.70); 'inability to feel' (0.64) and 'lassitude item'(0.55); factor 2 (psychic anxiety) with robust loadings on 'suicidal thoughts'(0.82), 'inner tension' (0.68) ; factor 3(mood-pessimism) had high loadings on items like 'reported sadness'(0.83), 'apparent sadness' (0.45) and 'pessimistic thoughts'(0.45) whereas factor 4(vegetative functions) had similar high loadings on 'reduced appetite' (0.72) and reduced sleep (0.70). We also performed a oblique rotation (direct oblimin) and found a similar factor structure (table not shown).

In the follow-up visit after 6-8 weeks, 80 patients (68.96%) were complaint and the rest 36(31.04%) were non-complaint. No patient reported of any significant adverse event leading to non-compliance. Among the compliant patients (n=80), 56 (70%) were responder and 24(30%) were non-responders - details of their socio-demographic and clinical characteristics is showed in Table 4A and B - the average age was 35.37±10.9 years and 46(57.5%) were male. Majority of the patients were married 64(80.0%) - 63(78.8) had at least 8 years of schooling and were employed (50%) in either unskilled (25.0%) or skilled work (26.8%). The mean age of onset of depressive episode was 32.42±10.41 yrs. Forty-eight (60%) patients had

duration of depressive episode less than 6 months. Thirty-one patients (38.8%) had melancholic features and 42(52.5%) had suicidal ideation in the current episode of depression. Whereas the number of patients having a past history of depression and a positive family history was 18(22.5%) and 13(16.3%) respectively. The mean pre-treatment MADRS score was 28.77±5.18. Only 28(35%) patients had considerably severe depression (MADRS>30). When the socio-demographic and clinical features were compared across the responder and the non-responder groups it was found that there was no statistically significant association. The quaternary factor structures of MADRS were calculated and compared across the responder and non-responder groups – only worsened vegetative function was significantly associated with non-response (0.04) – for others there were no significant association (see table 4C). Also when the pre-treatment and post-treatment factor structures were compared across responder and non-responder groups it was found that all the four factor structures post-treatment had significant decrease when compared to the pre-treatment condition ($p<0.01$). Finally in the logistic model (Table 5) adjusting for other variables we found that factor 4 (vegetative functions) was statistically significant predictor of drug response ($p<0.01$, odd's ratio=1.3[1.1-1.6]95% CI). Similarly the statistical significance for melancholic features was observed after adjusting for other variables ($p=0.04$) and it was found to be a strong predictor of non-response.

Discussion:

To our knowledge this is one of the first studies where in an Indian population the factor structure of MADRS has been studied. The symptom cluster derived out of factor analysis in this study highlights the variable factor structure of depression as found in previous studies (Table 1). Our quaternary factor structures bear similarity to some previous studies by Craighead et al., Williamson et al. or Quilty et al(7)(14)(16) though important differences exist with other studies. Our factor 3 'mood-pessimism' where mood symptoms have loaded together with 'pessimistic thoughts' was similar to the 'cognitive pessimism' by Craighead et al. There may be some controversy in this regard as in most of the previous studies 'pessimistic thoughts' had aggregated with 'suicidal ideation' in the same factor unlike our study where they have loaded on two different factors namely 'mood pessimism' and 'psychic anxiety' respectively. As it can be seen in table 3, the 'pessimistic thoughts' item of MADRS loaded heavily on both the factors namely 'psychic anxiety' and 'mood-pessimism'. On performing correlation analysis between these two factors (table not shown) the Pearson correlation was low ($r=0.2$, $p=0.02$) – hence probably they are two mutually exclusive factors. Moreover, 'pessimistic thoughts' is more related to depressed mood than suicidality which is currently believed to have stress-diatheses as an independent aetiological basis apart from sole mood symptoms (20) (21). So, 'pessimistic thoughts' was allowed to cluster with mood symptoms in 'mood-pessimism'. In this factor 2(psychic anxiety) containing the two items 'inner tension' and 'suicidal thoughts' is very much related to a similar factor by Parker et al. which had an addition of 'pessimistic thoughts'(11). The difference emerging because probably the study was done exclusively on geriatric patients with the mean age of 70±7.7 years while mean age in our study is 35.37±10.90 years.

The reason for this variable factor structures may be manifold. Firstly from table 1 it is quite apparent that all these studies were very heterogeneous in terms of the patient selected –

some were with brain damage, whereas others were geriatric or bipolar patients. Secondly, sample size and the statistical procedures were variable like use of orthrogonal, oblique rotaion or Kaiser/ Catell criteria were used. Finally, from table 1 we can see that the studies have drawn patients from different ethnic and cultural background leading to differential symptomatology in depression. Since transcultural studies show that Indian patients are pre-occupied with somatic symptoms and less of guilt, probably the 'neurovegetative' factor of Williamson et al. or Quilty et al was reduced to 'vegetative' factor of our study(19).

When we tried to find the factor structures as predictors of drug response we found that vegetative functions were predictors of response to escitalopram. This has been similarly predicted in previous studies though some contradictions exist (22). Among the socio-demographic profile none were significant predictor of drug response. In Sequential Treatment Alternatives to Relieve Depression (STAR*D) study it has been shown that less education is a significant predictor (23). In this study probably because of the small sample size, such a conclusion cannot be drawn. Among other clinical features presence of melancholia and longer duration of depressive episodes have shown significant association with drug non-response as expected from previous literature (24). Proportion of responder and non-responders in intention to treat analysis (50% responder) in this study is similar to previous studies (25). However, the proportion in per protocol analysis (70%) is higher than previous studies probably because our sample is free of all major comorbidities or complications (26). Hence, this relatively homogeneous sample is one of the strengths of this study. Patients with any psychotic symptoms, bipolarity (even family history of mania), recent suicidal attempt or other significant psychiatric and medical co-morbidities were excluded. As far as the factor structure is concerned both oblique and orthogonal structures predicted similar factor structures in this study thereby adding to its robustness. Literature has emphasized the absence of clinically useful predictors of anti-depressant response (24). In

this context the study can stimulate further research to examine factor structures as predictors of response.

One of the limitations in this study was due to MADRS not being validated in the Indian population. However, it has been used widely in previous research in India. Also the patient profile in this study comprising of mostly urban, educated and belonging to higher socio-economic status may make this population more suitable for assessment by MADRS as per Kuruvilla et al.(27). But, on the contrary, this socio-demographic nature of our patients derived by purposive sampling may limit the generalizability. In this study sample size was also limited for factor analysis. Other weaknesses in our methodology are regarding compliance and lack of blinding. Compliance was checked by means of self-report, family report and verifying the empty strips only and no biochemical corroboration was done. Our study had a attrition rate of 30%. However, on comparison of the socio-demographic and clinical profile of patients who dropped out and patients who completed the study no significant differences were found (table not shown). This study was not a fixed dosage study and the dosage was mostly determined by the best practice guidelines with a minimum dosage of 10mg - only 41% received the full dosage of escitalopram. There was only one follow-up and more frequent follow-ups would have been desirable. Another limitation of the factor structure of MADRS found in this study is that their stability across gender or across treatment duration or their reliability was not studied. However, such estimates had no direct relationship with the aims and objectives of this study and were beyond our scope. Also the definition of 'treatment–naive' might not be sufficient to rule out all treatment influences given the short drug wash-out period. A large number of other biological, psychological and social parameters known to influence anti-depressant response were not considered.

Conclusion:

So, it can be concluded that in north Indian patients presenting to a tertiary care mental health set-up with moderate to severe non-psychotic major depressive disorder the Montgomery Asberg Depression Rating Scale can be resolved into four factor structures namely detachment, psychic anxiety, mood-pessimism, vegetative functions with the latter being a good predictor of response to escitalopram treatment after 6-8 weeks of follow-up. Melancholic features came out to be a predictor of non-response to escitalopram in our study. However, the validity and reliability of the factor structures across treatment duration and other parameters like gender and age needs to be studied in large Indian patient populations preferably from the community for a wider generalizability.

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Tables

Table 1. Factor structure of MADRS in patients with depressive disorder - review of literature:

Author	Study participants	**Extraction method/ Rotational**	Factor-Structure

		techniques	
Galinowsky and Lehert(1995)	N=137,French, male=64%, major depression out-patients	**Principal Component Analysis (PCA)/ unrotated**	Factor1(1,2,3,6,7,8,9,10)factor2(4,5)factor3(3,6**)**
Craighead and Evans(1996)	N=340, English, male=33%, depressive disorder inpatients	**Maximum Likelihood Estimation/Promax**	Cognitive-pessimism(item1,2,9,10), anxious(item 1,7,8), cognitive-anxious(item 3,6), vegetative(item 4,5)
Hammond (1998)	N=100, English, male=26%, geriatric depressives	**PCA/ oblimin and varimax**	Anhedonia (item7,8,1,6), dysphoria(item 3,2,10)
Andersson et al. (1999)	N=72, Norwegian, male= 79.16%, in-patients with brain damage,	**PCA/oblimin**	Depressed mood (item2,9), somatic symptoms(item 3,4,5), negative symptoms(item 6,7,8)
Benazzi(2001)	N=306, Italian, male=37%,	**Not Reported/squared**	Factor1(item1,2,8,9), Factor 2 (item 3,4),

	MDD outpatients	**multiple correlation**	Factor3 (item 6,7,8,9)
Parker(2003)	N=225, English, male, geriatric depressives	**Exploratory Factor Analysis/promax**	Dysphoria/apathy/retardation(item1,2,6,7,8), psychic anxiety (item 3,9,10), vegetative(item 4,5)
Gabriwelewicz et al. (2004)	N=102, Polish, geriatric depressives with mild cognitive impairment,male=29.4%	**PCA/varimax**	Anhedonia-pessimism(Item1,2,8,9), Anxiety-vegetative(Item 3,4,5,10), Cognitive-Inhibition(Item6,7)
Suzuki et al. (2005)	N=132, Japanese, male=38.63%, MDD	**PCA/varimax and promax**	Dysphoria (items 9, 10,2), retardation(items 7, 8, 1,6), vegetative symptoms(items 4,5)
Williamson et al. (2006)	N=788,English ,male=37%, bipolar I depression	**PCA/ uquimax**	Sadness(item1,2), negative thoughts (item9,10),detachment(item6,7,8) ,neurovegetative sympoms(Item3,4,5)
Farner et	N=163,	**PCA/oblimin**	Anhedonia(items7,8,10,5

al.(2009)	Norwegian,post-stroke depressive inpatients, male=50.3%		) sadness(item1,2,3) agitation(item3,6,4)
Quilty et al.(2013)	N=621,French, depressive out-patients	**Confirmatory factor analysis/ not reported**	Sadness(item1,2), negative thoughts (item9,10),detachment(item6,7,8) ,neurovegetative sympoms(Item3,4,5)

MADRS items 1 –apparent sadness, 2-reported sadness, 3-inner tension, 4 – reduced sleep, 5 – reduced sleep, 6-concentration difficulties, 7-lassitude, 8-inability to feel, 9-pessimistic thoughts, 10-suicidal thoughts.

Table 2. Socio-demographic and clinical profile of the total patients (n=116)

Table 2A. Socio-demographic profile of the total patients(n=116)

Socio-demographic parameters		Total patients (n=116)
Current age (yrs)		35.87±10.68

Gender	male	71 (61.2)
	female	45 (38.8)
Education	Less than 8th std	17(14.65)
	Above 8th std	99 (85.34)
Occupation	Unemployed	59(50.86)
	Employed	57(49.14)
Marital status	Married	94(81.0)
	unmarried	22(19.0)
Income(rupees)	Upto 10,000	73(62.93)
	More than 10,000	43(37.06)

Table 2B Clinical profile of the total patients (n=116)

Clinical parameters		Total patients (n=80)
Age of onset of depressive episode		32.75 ± 10.62yrs
Duration of depressive episode	Less than equal to 6 months	64(55.18)
	More than 6 months	52(44.82)
Melancholia	present	44(37.9)
	Absent	49(61.3)

Suicidal ideation	Present	60(51.7)
	Absent	56(48.3)
Severity(MADRS>30)	Present	36(31)
	Absent	80(69)
Previous episode of depression	Present	23(19.8)
	Absent	93(80.2)
Family history of depression	Present	19(16.4)
	Absent	97(83.6)
Mean pre-treatment MADRS score		28.32±4.86

Table 3. Factor loadings of the MADRS after varimax rotation

Item	Factor1	Factor 2	Factor 3	Factor 4
Concentration difficulty	0.70			
Inability to feel	0.64		(0.47)	
Lassitude	0.55			
Suicidal Thoughts		0.82		
Inner Tension		0.68		
Reported sadness			0.83	
Apparent sadness			0.52	
Pessimistic thoughts		(0.44)	0.45	
Reduced appetite				0.72
Reduced sleep				0.70
Percentage of total variance explained(57)	15.22	14.95	13.92	12.88

Factor loadings <0.40 are deleted. The values in parentheses are the second highest loading values

Kaiser-Meyer-Olkin Measure of sampling adequacy =0.51

Bartlett Test of Sphericity<0.01

Table 4. Socio-demographic and clinical profile of the compliant patients(n=80)

Table 4A. Socio-demographic features:

Socio-demographic parameters		Response (n=56)	Non-reponse (n=24)	Total patients (n=80)	P (Chi-square)
Current age (yrs)		34.21±9.82	38.08±12.91	35.37±10.90	0.14(-1.5)
Gender	male	33 (58.9)	13 (54.2)	46 (57.5)	0.69 (0.15)
	female	23 (41.1)	11 (45.8)	34 (42.5)	
Education	Less than 8^{th} std	14(25)	3 (12.5)	17(21.3)	0.21(1.5)
	Above 8^{th} std	42(75)	21 (87.5)	63(78.8)	
Occupation	Unemployed	28(50)	12(50)	40(50)	1.00 (0.00)
	Employed	28(50)	12(50)	40(50)	
Marital status	Married	46(82.1)	18(75.0)	64(80.0)	0.46(0.53)

	unmarried	10(17.9)	6(25.0)	16(20.0)	
Income (rupees)	Upto 10,000	38(67.9%)	14(58.3)	52(65.0)	0.41(0.67)
	More than 10,000	18(32.1%)	10(41.7%)	28(35.0)	

Table 4B. Clinical features related to responder and non-responder:

Clinical parameters		Response (n=56)	Non-response (n=24)	Total patients (n=80)	P (χ^2/t)

Age of onset of first depressive episode(yrs)		34.21±9.82	38.08±12.91	32.42±10.41	0.39 (-0.87)
Duration of depressive episode	Less than 6 months	37(66.1)	11(45.8)	48(60.0)	0.09(2.8)
	More than 6 months	19(33.9)	13(54.2)	32(40)	
Melancholia	present	18(32.1)	13(54.2)	31(38.8)	0.06(3.43)
	Absent	38(67.9)	11(45.8)	49(61.3)	
Suicidal ideation	Present	28(50.0)	14(58.3)	42(52.5)	0.49(0.47)
	Absent	28(50.0)	10(41.7)	38(47.5)	
Severity(MADRS>30)	Present	20(25)	8(10)	28(35)	1.00(0.04)
	Absent	36(45)	16(20)	52(65)	
Initial total MADRS score		28.82±4.74	28.67±6.20	28.77±5.18	0.39 (-0.24)
Previous episode of depression	Present	15(26.8)	3(12.5)	18(22.5)	0.16(1.9)
	Absent	41(73.2)	21(87.5)	62(77.5)	
Family history of depression	Present	8(14.3)	5(20.8)	13(16.3)	0.46(0.53)
	Absent	48(85.7)	19(79.2)	67(83.8)	

Table 4C. **Pre-treatment** factor structure in relation to responder and non-responder:

Factor-structures		Response (n=56)	Non-response (n=24)	Total patients (n=80)	P (t)
Factors derived out of varimax rotation	Factor 1(detachment)	8.28±2.65	9.50±2.30	8.28±2.65	0.06(-1.9)
	Factor 2(psychic anxiety)	3.71±2.14	3.83±1.95	3.75±2.07	0.82(-0.23)
	Factor 3(mood-pessimism)	10.93±1.37	11.00±1.86	10.9±1.52	0.85(-0.19)
	Factor 4(vegetative functions)	5.75±2.38	4.41±3.01	5.35±2.63	**0.04(2.11)**

Table 5: Logistic Regression Analysis to show the predictors of escitalopram response:

			95% CI

Variables	β(S.E.)	p	Lower	Odd's Ratio	Upper
melancholia	-1.2(0.60)	**0.04**	0.09	0.29	0.95
Duration of depressive episode	-0.85(0.55)	0.19	0.14	0.42	1.2
Factor 1(detachment)	-0.20(0.12)	0.08	0.65	0.82	1.02
Factor 4(vegetative functions)	0.31(0.11)	**0.01**	1.09	1.3	1.6

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	impairment,male= 29.4%		Inhibition(Item6,7)
Suzuki et al. (2005)	N=132, Japanese, male=38.63%, MDD	**PCA/varimax and promax**	Dysphoria (items 9, 10,2), retardation(items 7, 8, 1,6), vegetative symptoms(items 4,5)
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	Above 8th std	99 (85.34)
Occupation	Unemployed	59(50.86)
	Employed	57(49.14)
Marital status	Married	94(81.0)
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Income(rupees)	Upto 10,000	73(62.93)
	More than 10,000	43(37.06)

Table 2B Clinical profile of the total patients (n=116)

Clinical parameters		Total patients (n=80)
Age of onset of depressive episode		32.75 ± 10.62yrs
Duration of depressive episode	Less than equal to 6 months	64(55.18)
	More than 6 months	52(44.82)
Melancholia	present	44(37.9)
	Absent	49(61.3)
Suicidal ideation	Present	60(51.7)
	Absent	56(48.3)
Severity(MADRS>30)	Present	36(31)
	Absent	80(69)
	Present	23(19.8)

Previous episode of depression	Present	23(19.8)
Previous episode of depression	Present Absent	19(80.2) 97(83.6)
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Inner Tension		0.68		
Reported sadness			0.83	
Apparent sadness			0.52	
Pessimistic thoughts		(0.44)	0.45	
Reduced appetite				0.72
Reduced sleep				0.70
Percentage of total variance explained(57)	15.22	14.95	13.92	12.88

Factor loadings <0.40 are deleted. The values in parentheses are the second highest loading values

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Occupation	Unemployed	28(50)	12(50)	40(50)	1.00 (0.00)
	Employed	28(50)	12(50)	40(50)	
Marital status	Married	46(82.1)	18(75.0)	64(80.0)	0.46(0.53)
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Income (rupees)	Upto 10,000	38(67.9%)	14(58.3)	52(65.0)	0.41(0.67)
	More than 10,000	18(32.1%)	10(41.7%)	28(35.0)	

Table 4B. Clinical features related to responder and non-responder:

Clinical parameters		Response (n=56)	Non-response (n=24)	Total patients (n=80)	P (χ^2/t)
Age of onset of		34.21±9.82	38.08±12.91	32.42±10.41	0.39

first depressive episode(yrs)					(-0.87)
Duration of depressive episode	Less than 6 months	37(66.1)	11(45.8)	48(60.0)	0.09(2.8)
	More than 6 months	19(33.9)	13(54.2)	32(40)	
Melancholia	present	18(32.1)	13(54.2)	31(38.8)	0.06(3.43)
	Absent	38(67.9)	11(45.8)	49(61.3)	
Suicidal ideation	Present	28(50.0)	14(58.3)	42(52.5)	0.49(0.47)
	Absent	28(50.0)	10(41.7)	38(47.5)	
Severity(MADRS> 30)	Present	20(25)	8(10)	28(35)	1.00(0.04)
	Absent	36(45)	16(20)	52(65)	
Initial total MADRS score		28.82±4.74	28.67±6.20	28.77±5.18	0.39 (-0.24)
Previous episode of depression	Present	15(26.8)	3(12.5)	18(22.5)	0.16(1.9)
	Absent	41(73.2)	21(87.5)	62(77.5)	
Family history of depression	Present	8(14.3)	5(20.8)	13(16.3)	0.46(0.53)
	Absent	48(85.7)	19(79.2)	67(83.8)	

Table 4C. **Pre-treatment** factor structure in relation to responder and non-responder:

Factor-structures		Response (n=56)	Non-response (n=24)	Total patients (n=80)	P (t)

Factors derived out of varimax rotation	Factor 1(detachment)	8.28±2.65	9.50±2.30	8.28±2.65	0.06(-1.9)
	Factor 2(psychic anxiety)	3.71±2.14	3.83±1.95	3.75±2.07	0.82(-0.23)
	Factor 3(mood-pessimism)	10.93±1.37	11.00±1.86	10.9±1.52	0.85(-0.19)
	Factor 4(vegetative functions)	5.75±2.38	4.41±3.01	5.35±2.63	**0.04(2.11)**

Table 5: Logistic Regression Analysis to show the predictors of escitalopram response:

			95% CI		
Variables	β(S.E.)	p	Lower	Odd's Ratio	Upper
melancholia	-1.2(0.60)	**0.04**	0.09	0.29	0.95
Duration of depressive episode	-0.85(0.55)	0.19	0.14	0.42	1.2
Factor 1(detachment)	-0.20(0.12)	0.08	0.65	0.82	1.02
Factor 4(vegetative functions)	0.31(0.11)	**0.01**	1.09	1.3	1.6

Highlights:

- Factor analysis by varimax rotation resolved MADRS into four factor-structures.
- All factors significantly affected by escitalopram treatment.
- 'Vegetative functions' is a significant predictor of response to escitalopram.
- Melancholia is a predictor of non-response to escitalopram.

Original Articles

Inequity in access to inpatient healthcare services for non-communicable diseases in India and the role of out-of-pocket payments

KATHIRESAN JEYASHREE, SHANKAR PRINJA, MAN INDER KUMAR, JARNAIL SINGH THAKUR

ABSTRACT

Background. Growing evidence suggests that non-communicable diseases (NCDs) result in considerable economic burden for individuals and households. With the poor facing a greater burden of NCDs than the rich in India, we undertook this study to analyse the horizontal equity in utilization and vertical equity in out-of-pocket expenditure for NCD care.

Methods. We used data of 14 large Indian states from the National Sample Survey 60th round to compute hospitalization rates for NCDs. Mean per capita consumption expenditure (MPCE) was computed and used as a proxy measure for socioeconomic status. Out-of-pocket payment as a proportion of MPCE was estimated by wealth quintile (Q) to assess the vertical equity in payments. Concentration index (CoI) was computed to measure the extent of equity, and its 95% confidence interval was estimated to assess statistical significance.

Results. Overall, NCD hospitalizations in public facilities in India were used more by the poor (CoI –0.041), while the rich used proportionately more services in the private sector (CoI 0.174). Out-of-pocket expenditure in public facilities was consistently lower than that in private facilities in urban and rural areas. The mean out-of-pocket expenditure for inpatient services for NCDs was found to be more among the rich in both public (Q5 ₹13 016, Q1 ₹4197) and private (Q5 ₹22 974, Q1 ₹8225) facilities.

Conclusion. Public facilities are utilized more by poorer individuals. Strengthening the capacity of the public sector to deliver NCD care is required to meet equitable outcomes.

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INTRODUCTION

In India, non-communicable diseases (NCDs) are the leading cause of mortality, and responsible for 60% of all deaths and 62% of all disability-adjusted life years (DALYs) lost.[1] NCDs accounted for nearly 40% of all hospital stays and 35% of all outpatient visits in 2004. This figure is a marked rise compared to 32% hospital stays and 22% outpatient consultations in 1995–96, and is projected to rise even further. Thus, NCDs put a major burden on the health system, besides posing a challenge for households to finance treatment.[2] NCDs are projected to cause a cumulative loss of US$ 237 billion by 2030 in India.[3]

A reversal of social gradient in NCDs is seen in India, where lower educational, occupational and socioeconomic status (SES) have been found to be associated with the risk of chronic diseases, their risk factors[4,5] and mortality.[6,7] Further, the poorer population groups are most likely to be unable to cope with the costs of treatment for NCDs.[8] The proportion of out-of-pocket (OOP) expenditures for NCDs rose from 31.6% in 1995 to 47.3% in 2004.[2] The odds of incurring catastrophic hospitalization expenditures are nearly 160% higher with cancer and 30% higher for cardiovascular diseases and injuries than for a communicable condition.[9]

An earlier analysis found that the rich have greater access to hospital services than the poor, particularly for more technically complex services.[10] It has been reported that the poorest 20% population use only 10% of public subsidy while 33% is used by the richest quintile.[11] A study by Xavier *et al.* in 2008 indicates that hospital services, particularly in the public sector, are utilized more by the poor in India. However, wide state-specific differences exist. In terms of care for NCDs, the authors found that economically weaker sections had poor treatment outcomes of acute coronary syndrome, not because of the difference in risk factors but because of differences in treatments received.[12] The poor were less likely to receive evidence-based treatments due to issues of availability, accessibility and affordability; addressing these issues would go a long way in reducing mortality due to these diseases among the poor. However, this hospital-based study was unable to capture the unmet need for those who never accessed services. No study so far has specifically analysed hospital admissions for NCDs in India from an equity perspective.

We aimed to determine horizontal equity in access to inpatient

services for NCDs in India. Horizontal equity is examined in terms of utilization rates of NCD-related hospitalizations among different wealth quintiles. We also explore the question of vertical equity in OOP expenditure for NCD hospitalizations. Given the diversity in healthcare infrastructure across India, we also examined the inter-state variation. Finally, we analysed the determinants of utilization and OOP expenditure for NCD hospitalizations.

METHODS

Data source and description

We used data from the National Sample Survey 60th round on 'Morbidity and health care'. The survey covered data on self-reported morbidity, its types, utilization of healthcare services and expenditure of households for availing healthcare services. A total of 47 302 households in rural and 26 566 households in urban area were surveyed. Detailed household consumption expenditure was recorded, along with other sociodemographic information including caste, occupation, gender and education. Information on hospitalization was collected for every event of hospitalization of a member, whether living or deceased at the time of the survey over a period of 365 days preceding the date of enquiry. Hospitals included public facilities such as district hospitals, community health centres, primary health centres and urban dispensaries and those in private sector including private hospitals, nursing homes, etc. A total of 13 310 hospitalization cases were identified in public and private facilities situated in rural and urban areas.

We specifically analysed hospitalizations where the cause of admission was for heart disease, hypertension, bronchial asthma, neurological and psychiatric disorders, diabetes mellitus, fractures, poisoning and cancers/tumours. A total of 4392 NCD-related hospitalizations were analysed. The percentage of NCD hospitalizations varied from 0.5% in Odisha to 4.9% in Andhra Pradesh and Karnataka. We restricted our analysis to 14 large states so that we had adequate statistical power to comment on the state-level figures.

Total expenditure incurred for medical treatment received during the reference period (365 days) included expenditure on items such as admission fees, medications, oxygen, transfusions, materials for bandage and plasters, diagnostic imaging and investigations, and hotel charges. It also included all personnel charges (medical and paramedical staff), surgical operations and charges of ambulances and transportation.

Data analysis

Households were ranked according to their SES and grouped into five wealth quintiles (Q1 to Q5), ranging from the poorest to the richest. Monthly per capita consumption expenditure (MPCE) was computed to assess the SES. The MPCE was adjusted for age composition and household size according to the Organization for Economic Cooperation and Development (OECD) equivalence scale.[13] Prevalence rate of NCD hospitalizations in respective wealth quintiles was computed. Similarly, economic burden due to hospitalization was calculated as average expenditure per episode, and OOP expenditure as a proportion of annual consumption expenditure. For both hospitalizations and OOP payments, analysis was done separately for rural and urban areas, and for public and private facilities, among respective wealth quintiles.

Concentration index (CoI) was calculated to assess the extent of equity in the distribution of service utilization. The values of CoI ranged from +1 to –1; with a positive value suggesting pro-rich and a negative value suggesting a pro-poor distribution. Pro-rich distribution implies that the outcome was found more in the richer sections of society and vice versa. CoI was estimated for NCD-related hospitalizations in public and private health facilities in urban and rural areas.[14]

Mean OOP expenditure incurred for hospitalization as a result of NCD was calculated across the wealth quintiles and statistical significance of this difference was analysed using one-way Anova. Association of SES with NCD hospitalization was analysed using logistic regression controlling for possible confounders such as age, sex, proportion of elderly population, doctor–population ratio, percentage of public expenditure on health, gross state domestic product per capita and literacy rate. Similarly, association of SES with total expenditure during hospitalization was analysed using linear regression. Apart from the confounders controlled for association of SES with hospitalization, other factors such as proportion of public expenditure on health in the state and state gross domestic product were also included in the linear regression model.

RESULTS

National level

At the national level, the overall NCD hospitalizations showed a pro-rich pattern (CoI 0.089; 0.072–0.105) suggesting that the rich utilize inpatient NCD care services more than the poor (Table I, Fig. 1). Private facilities were also found to have a pro-rich utilization pattern (CoI 0.174; 0.141–0.207). A similar pattern was observed when stratified into urban or rural private facilities, though the CoI was not significant ($p>0.05$) for the rural private sector. Overall, in public facilities, the utilization was found to be pro-poor (CoI –0.041; –0.050 to –0.031). The pattern was consistent across rural and urban public facilities.

At the state level, overall NCD hospitalizations followed a pro-rich pattern in all the states. In Gujarat, Maharashtra and the southern states, the poor were found to use inpatient services for NCDs in public facilities to a greater extent than the wealthy, reflecting the pattern at the national level. This overall trend was also observed in rural and urban public facilities of these states (Figs 2 and 3, Table II). On the contrary, northern states included in the analysis showed a pro-rich pattern of utilization of public facilities for NCD care. CoIs for utilization of public facilities ranged from –0.024 (Maharashtra) to 0.165 (Odisha). On the other hand, the wealthy utilized inpatient NCD care in private facilities more compared with the poor across all the states studied. This pattern persisted across urban and rural strata

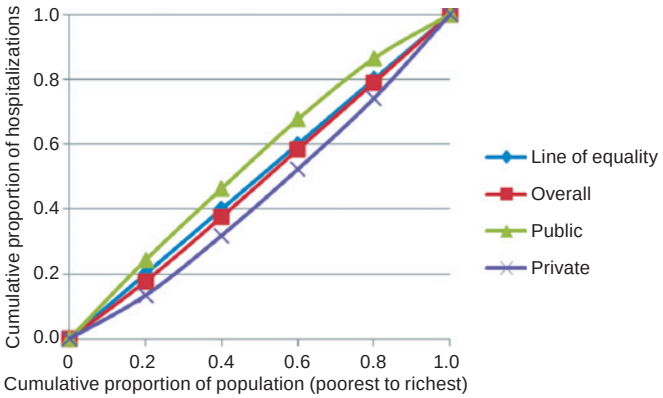

FIG 1. Overall, public and private sector inpatient care utilization for non-communicable diseases

TABLE I. Horizontal equity in inpatient service utilization for non-communicable diseases in hospitals, India, 2004–05

States	Concentration index								
	Overall	Lower limit	Upper limit	Public	Lower limit	Upper limit	Private	Lower limit	Upper limit
Punjab	0.095*	0.072	0.118	0.095*	0.073	0.117	0.095*	0.069	0.121
Haryana	0.119*	0.087	0.152	0.018*	0.008	0.028	0.173*	0.128	0.217
Rajasthan	0.121*	0.101	0.140	0.110*	0.092	0.128	0.139*	0.113	0.165
Uttar Pradesh	0.157*	0.129	0.185	0.086*	0.059	0.114	0.192*	0.160	0.223
Bihar	0.142*	0.117	0.167	0.115*	0.088	0.142	0.150*	0.123	0.178
West Bengal	0.146*	0.114	0.178	0.053*	0.038	0.068	0.368*	0.264	0.473
Odisha	0.185*	0.156	0.214	0.165*	0.132	0.199	0.252*	0.209	0.294
Madhya Pradesh	0.175*	0.141	0.209	0.028*	0.016	0.039	0.311*	0.253	0.369
Gujarat	0.036*	0.020	0.051	–0.153*	–0.206	–0.100	0.128*	0.086	0.170
Maharashtra	0.100*	0.074	0.125	–0.024*	–0.040	–0.007	0.147*	0.107	0.187
Andhra Pradesh	0.142*	0.113	0.170	–0.043*	–0.067	–0.019	0.224*	0.174	0.274
Karnataka	0.117*	0.088	0.147	–0.072*	–0.092	–0.052	0.200*	0.153	0.247
Kerala	0.030*	0.022	0.039	–0.118*	–0.172	–0.063	0.106*	0.066	0.146
Tamil Nadu	0.075*	0.057	0.092	–0.144*	–0.176	–0.112	0.232*	0.179	0.285
Overall (14 states)	0.089*	0.072	0.105	–0.041*	–0.050	–0.031	0.174*	0.141	0.207

* Significant at 5% level of significance

(Figs 2 and 3). CoIs for utilization of private facilities ranged from 0.066 (Kerala) to 0.473 (West Bengal).

OOP expenditure for NCD hospitalizations

Overall, the OOP costs for NCD hospitalization in India was ₹11 327.82 in the public sector and ₹21 917.91 in private facilities. The mean OOP expenditure for inpatient services for NCDs was higher for the rich in both public and private facilities (Table III). Overall, the richer quintiles spent 3.1 times and 2.79 times higher than the poorer quintiles in the public and private sectors, respectively.

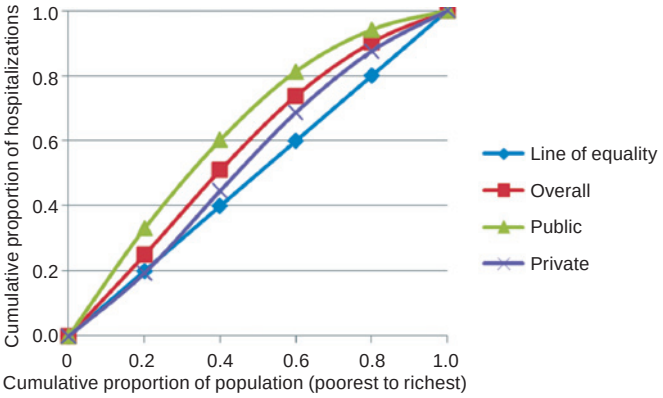

FIG 2. Overall, public and private sector inpatient care utilization for non-communicable diseases—rural sector

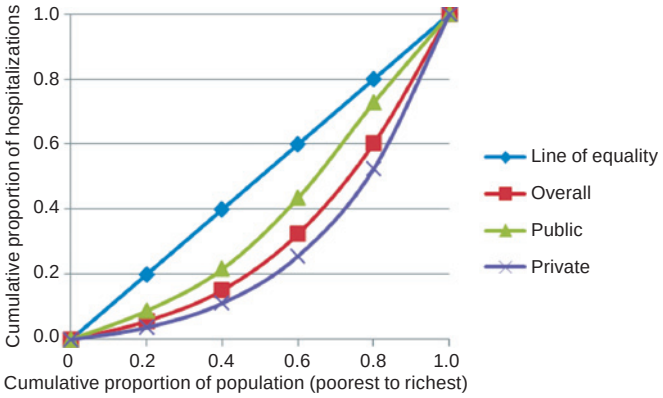

FIG 3. Overall public and private sector out-of-pocket expenditure on inpatient care for non-communicable diseases—urban sector

Table II. Horizontal equity in inpatient service utilization for non-communicable diseases in rural and urban sector hospitals, India, 2004–05

States	Concentration index											
	Rural public sector			Urban public sector			Rural private sector			Urban private sector		
	Overall	LL	UL	Overall	LL	UL	Overall	LL	UL	Overall	LL	UL
Punjab	0.00	–0.01	0.02	0.22*	0.16	0.28	0.15	–0.15	0.46	0.03*	0.01	0.04
Haryana	–0.04*	–0.05	–0.04	0.16*	0.12	0.20	0.14	–0.14	0.42	0.18*	0.14	0.22
Rajasthan	0.11*	0.09	0.14	–0.07*	–0.09	–0.04	0.15	–0.15	0.45	0.07*	0.04	0.09
Uttar Pradesh	0.08*	0.06	0.10	0.09*	0.05	0.13	0.19	–0.18	0.56	0.17*	0.13	0.21
Bihar	0.10*	0.09	0.12	–0.02	–0.06	0.03	0.17	–0.16	0.51	0.16*	0.10	0.22
West Bengal	0.06*	0.04	0.07	0.01	–0.01	0.03	0.32	–0.30	0.94	0.35*	0.23	0.48
Odisha	0.14*	0.12	0.16	0.18*	0.10	0.26	0.28	–0.26	0.81	0.26*	0.21	0.31
Madhya Pradesh	0.04*	0.03	0.04	–0.03	–0.05	0.00	0.27	–0.26	0.80	0.24*	0.17	0.31
Gujarat	–0.28*	–0.32	–0.24	–0.168	–0.24	–0.07	0.13	–0.13	0.39	0.12*	0.08	0.16
Maharashtra	–0.10*	–0.11	–0.08	–0.13*	–0.18	–0.08	0.16	–0.16	0.48	0.16*	0.10	0.21
Andhra Pradesh	0.02*	0.00	0.05	–0.16*	–0.20	–0.13	0.23	–0.22	0.68	0.25*	0.18	0.31
Karnataka	0.00	–0.04	0.03	–0.11*	–0.15	–0.07	0.17	–0.16	0.50	0.20*	0.14	0.26
Kerala	–0.08*	–0.12	–0.04	–0.18*	–0.25	–0.10	0.06	–0.05	0.16	0.17*	0.12	0.22
Tamil Nadu	–0.09*	–0.10	–0.08	–0.19*	–0.25	–0.14	0.23	–0.22	0.67	0.19*	0.14	0.25
Overall (14 states)	–0.08*	–0.09	–0.07	–0.10*	–0.13	–0.06	0.14	–0.13	0.40	0.15*	0.11	0.20

* Significant at 5% level of significance LL lower limit UL upper limit

Logistic regression revealed that belonging to a richer quintile, older age and male sex of the individual in addition to higher proportion of elderly population, higher doctor–population ratio, and lower literacy rate were significant predictors of NCD hospitalization (Table IV). Linear regression showed that, overall, belonging to a richer quintile, increasing age, high doctor–population ratio and low proportion of public expenditure on health were significant predictors of the total expenditure during an NCD hospitalization (Table V).

DISCUSSION

The rising burden of NCDs in developing countries has adverse effects at the household, healthcare system and macroeconomic level.[15] India spends 48% of its total health expenditure on NCDs.[16] Not only are the poor more afflicted than the rich by NCDs, but the outcome of NCDs is also unfavourable for the poor due to differential access to and affordability of treatment. Hence, the course of disease in the poor is different and they experience higher mortality compared to their richer counterparts.[12,17] Thus, NCDs are exacerbating health inequities between the rich and the poor.

Our study analysed a nationally representative sample of households to understand utilization patterns and expenditure incurred on inpatient services for NCDs through an equity lens. The utilization of inpatient services for NCDs was found to be equitable in the public sector in both rural and urban areas. In the

TABLE III. State-wise mean expenditure on hospitalization for non-communicable diseases across quintiles in public and private sectors

States	Public					Private				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Punjab	6916 (3773.2)	15 445 (17 692.5)	12453 (13021.7)	12420 (12663.9)	21736 (50846.8)	9862 (8971.9)	8746 (10926.3)	14588 (27943.3)	17381 (30419.5)	37755 (103762.4)
Haryana	5453 (5830.1)*	4417 (4262.3)	7955 (12619.9)	19455 (41326)	38503 (88515)	5524 (3134.1)*	10869 (8771.1)	10501 (9359.5)	12928 (13833.1)	22032 (33084.5)
Rajasthan	7439 (9972.3)*	7168 (8523.3)	9074 (14395.1)	10438 (19571.1)	12788 (21740.6)	13581 (11846.9)	14784 (17474)	13894 (14992.1)	17118 (31470.1)	16352 (21636)
Uttar Pradesh	8055 (11845.4)*	8380 (14604.9)	10655 (21148.3)	12987 (23426.6)	21879 (47771.6)	9505 (12443.5)*	12919 (21681.6)	12743 (17188.4)	14777 (19390.4)	20623 (36883.3)
Bihar	6097 (8600)*	8363 (14119.3)	12160 (37755.2)	15663 (38992)	24005 (30134.5)	7309 (10510.7)*	8434 (22290.2)	14738 (37341.3)	14839 (21102.3)	22684 (34579.7)
West Bengal	3371 (5895.2)*	3057 (4066.1)	4816 (10063.7)	9867 (33543.3)	9877 (19985.4)	10582 (14681.7)*	10155 (12325.2)	13532 (13374)	17723 (31163.8)	28921 (55655.3)
Odisha	4458 (7644.5)*	5487 (9923.3)	8118 (11056)	10142 (15718.7)	9830 (19744.5)	10639 (14556.3)*	12006 (14505.4)	18283 (18928.3)	19079 (29212)	22157 (27974.6)
Madhya Pradesh	4165 (5849.7)*	6306 (10884)	6082 (18222.4)	10084 (21080.1)	5743 (6755.9)	7181 (9521.7)*	8528 (11365.2)	12963 (21745.7)	18117 (34080.8)	21693 (37632.6)
Gujarat	4765 (7197.6)	5067 (7656.5)	3696 (5470.5)	5687 (10616.2)	12246 (44944.2)	6787 (7963.3)*	7642 (7766.9)	6585 (8148.6)	11420 (18510.3)	21236 (62035.4)
Maharashtra	4080 (17441.2)*	2922 (5866.8)	7500 (19269.1)	9693 (27225)	6064 (11103.9)	9521 (19534.9)*	10521 (19416.9)	11344 (17106.9)	14289 (27908.5)	23074 (49007.2)
Andhra Pradesh	2362 (4931.6)*	6017 (13337.6)	5443 (18059.8)	3652 (8425.1)	9223 (17089.5)	6906 (7437)*	8635 (13516.5)	8819 (11274.6)	12703 (22134.1)	21113 (37920.7)
Karnataka	1582 (2005.8)*	3077 (6178.8)	3081 (4295)	3354 (3785.5)	9327 (21267.9)	7546 (8465.3)*	9599 (16290.6)	9864 (16079.9)	10081 (17082.8)	21109 (34531.8)
Kerala	4411 (7828.4)	5859 (15643.4)	5238 (9880.4)	6579 (15496.3)	8076 (23117.7)	8674 (10928.3)	9593 (18889.1)	10535 (27298.7)	10245 (18229.2)	13612 (22818.7)
Tamil Nadu	1604 (6167.6)*	1843 (4058.2)	2274 (6982.1)	5368 (24340)	5281 (11336.3)	5427 (8021.7)*	8810 (12158)	11609 (18573.8)	17268 (37661.4)	29750 (62971.1)
All India	4197 (8549.6)*	5134 (10160.8)	6502 (15342.4)	9106 (3811.9)	13016 (34608.6)	8225 (11851)*	10220 (17854.4)	11671 (19893.5)	14239 (25384.1)	22974 (50489.1)

* Indicates significant difference across the quintile groups at $p<0.05$ significance level

TABLE IV. Determinants of non-communicable disease hospitalization using binary logistic regression

Characteristic	B	Standard error	Wald	Significance	Adjusted OR	95% CI for Exp (B)	
						Lower	Upper
Q1* (reference value)	—	—	250.379	0.000	—	—	—
Q2	0.124	0.023	30.378	0.000	1.132	1.083	1.184
Q3	0.189	0.023	70.538	0.000	1.209	1.156	1.263
Q4	0.258	0.023	125.832	0.000	1.294	1.237	1.353
Q5	0.344	0.023	219.241	0.000	1.411	1.348	1.477
Elderly	0.098	0.011	85.512	0.000	1.103	1.081	1.127
Doctor–population ratio	–0.009	0.004	3.875	0.049	0.991	0.983	1.000
Literacy	–0.009	0.002	17.453	0.000	0.991	0.987	0.995
Age	0.022	0.000	4473	0.000	1.022	1.021	1.023
Sex (male)	–0.112	0.014	64.044	0.000	0.894	0.870	0.919

* Q wealth quintile

TABLE V. Factors affecting the total expenditure during hospitalization using linear regression

Characteristic	Unstandardized coefficients		T	Significance
	B	Standard error		
Wealth quintile	2239.770	91.544	24.466	0.000
Age	98.710	8.226	12.000	0.000
Sex	–244.195	317.391	–0.769	0.442
Elderly	–73.765	187.437	–0.394	0.694
Doctor–population ratio	348.864	77.659	4.492	0.000
Literacy	–55.667	36.439	–1.528	0.127
Proportion of public expenditure on health	–24.711	5.920	–4.174	0.000
Gross state domestic product per capita	0.014	0.013	1.082	0.279

private sector the utilization patterns favoured the rich, both in urban and rural areas. The overall mean OOP expenditure on inpatient services for NCDs was higher in the private sector compared with public facilities across all areas, with the richer quintile uniformly spending more than poorer quintiles. The range of expenditure in the private sector (₹14 749) was significantly higher than within the public sector (₹8819). This could be due to the largely heterogeneous private sector ranging from single practitioners to multispecialty corporate hospitals.[18] This highlights the need to regulate the private sector; the Clinical Establishment bill is an effort in that direction.

The pro-poor trend of utilization of inpatient care for NCD in public sector may be an outcome of the subsidies offered by the government in the public sector. In the private sector, however, there was inequity in the utilization of inpatient services for NCDs with the pattern of utilization being pro-rich, slightly more pronounced in the urban than rural areas. One reason for this pattern could be the profile and range of services available in the private sector, which are also quite different from that in the public sector.[19] Another reason could be the higher concentration of private sector hospitals in the urban sector.[20] The choice of services varies between the rich and the poor as a function of awareness, access and affordability. Hence, the pro-rich utilization of the private sector could also be due to the greater awareness among the rich and literate population about the latest treatment options available for NCDs compounded by their higher cost. Similar pattern was observed in our previous analysis for all diseases, where the poor utilized inpatient services at public hospitals at a slightly higher rate than the rich, suggesting potentially more equitable use than the private sector. In some states the rich were also utilizing the public sector more than the poor. This trend was earlier documented by Mahal *et al.*, e.g. in Punjab, 66% of the bed days utilized by people below the poverty line were in the private sector.[11]

The mean OOP expenditure was found to be more in the private than in the public sector both in rural and urban areas. Even within the public sector a wide variation in expenditure was noted across the states. This could be attributed to the capacity of health system in providing such care itself in the first place, and once it had the capacity in terms of human resources and logistics then it exposes the huge scope of impoverishment even at public facilities due to inadequate medicines and other supplies for which patients have to pay. The profile of the private sector, which is a highly heterogeneous group,[18] could be different in urban and rural areas, accounting for the difference in their clientele, type and cost of services. The rural private set-up might not offer a different set of services compared to the rural public sector whereas this differential is quite marked in urban areas.

In the public sector, the Q5/Q1 ratio was found to be higher in rural areas while in the private sector, the ratio was almost equal in urban and rural areas. This may be because of the utilization of public sector differently in rural and urban areas. In rural areas where there is limited access to advanced, complex, state-of-the-art care, the rich also, within certain limits, may utilize the curative services for NCD care from the public sector itself. Such reasons have been found to play a role in determining the differences between the rural rich and the urban poor/rich in utilization of maternity care.[21] On the other hand, in urban areas with better access to complex, expensive, tertiary level care, the rich utilized the private sector to avail these services. The utilization of the public sector in urban areas was more by the poor, which is more likely for primary or secondary level care that costs much less than tertiary level care in the private sector.[11] Hence, as described above in the utilization pattern, this could also be due to the higher concentration of private sector hospitals in the urban sector which are utilized more by the rich; hence the higher Q5/ Q1 ratio.

The higher OOP expenditure among the rich could be a function of the type and cost of services that the rich avail for NCDs. This could also be explained by our results which show the utilization of private services to be more by the rich and the cost of services under the private sector is higher than that in the public sector. Such a finding could be due to the fact that the poor cannot even afford smaller costs and thus the care for NCD remains an unmet need, or that a treatment which would be administered in a hospital in the ideal way is preferred in outpatient settings by the poor due to financial constraints. This was in contrast to what Chuma *et al.*[22] observed in Kenya where the poorest quintiles were observed to spend more than the richest quintiles on NCD care. Shobhana *et al.*[23] and Ramachandran *et al.*[24] have also reported similar findings with reference to diabetes care with the poorer quintiles spending a greater proportion of the household income as compared to their richer counterparts.

One major implication of our paper is that given that the utilization of the public sector for NCDs is more by the poor, this trend has to be encouraged by provision of appropriate technology, essential logistics, drugs and the introduction of an effective package of these interventions in the public sector. It has been documented that low cost and highly effective interventions are available for prevention and treatment of NCDs, which may be within the reach of the poor. Other factors affecting utilization of inpatient care for NCDs as identified by the results of our logistic regression were demand side factors such as the proportion of the elderly in the population, literacy rate and supply side factors such as the doctor–population ratio. These factors have to be addressed by interventions targeting NCD prevention and control. India has a national programme addressing chronic diseases named 'National

programme for prevention and control of cancer, diabetes, CVD and stroke'(NPCDCS), which has been expanded to cover cancer as well in 100 districts and upscaled to cover the entire country under the 12th Five-Year Plan. The programme targets both prevention and treatment of NCDs with more stress on the former. While control of risk factors, early diagnosis and treatment of NCDs is the prime focus there are also provisions under this programme for upgradation of medical college hospitals and district hospitals for inpatient service delivery for NCDs. Facilities for provision of NCD preventive and curative services are generally poor at all levels of the healthcare system because of poor health system response to meet the challenge of NCDs. Little progress has been made in this direction to strengthen inpatient care for NCDs with no medical college and only 100 district hospitals upgraded as of March 2012.[25] Given the high hospitalization rates for NCDs and the high catastrophic expenditure that the household incurs, there is a need to focus more on health system strengthening especially provision of trained human resources, essential drugs and technology under this programme alongside efforts to strengthen preventive and outpatient services.

However, just availability of inpatient services does not improve utilization unless these are also made financially accessible. Among the major challenges in the NCD scenario is the need to address the impact of NCDs on domestic economies.[26] One such attempt to reduce the adverse financial impact of ill-health on households in India has been the *Rashtriya Swasthya Bima Yojana* (RSBY), a health insurance scheme launched by the Ministry of Labour and Employment for families below the poverty line. The benefit package, though designed to include pre-existing ailments in the beneficiaries, does not cover outpatient department (OPD) consultations and medications unless they involve hospitalization. Given that most NCDs require regular follow-up in OPDs and medication prescribed in an OPD setting and that the major share of OOP expenditure on NCDs is on medication,[16] this is an issue that has to be addressed to make NCD care more accessible to the poorer sections of society. Further, RSBY has empanelled more private than public facilities as of date,[27] which also needs to change given that it is the public facilities that are utilized more by the poor for NCD care.

A limitation of our study was that we have not classified and analysed the exact nature of the treatment that was utilized. This could have been important as Xavier *et al*. have reported that the use of key treatments differed by socioeconomic status accounting for differences in mortality.[12] This could have helped us substantiate our study findings better. We have reported interstate variations in the utilization of and expenditure on inpatient services for NCDs but have refrained from exploring reasons for the difference given sample size limitations of the study. We have calculated the OOP as a proportion of the MPCE and not the annual household income, which would have enabled us to classify the expenditure as catastrophic and otherwise.

Conclusion

With the rapidly progressing epidemiological transition and the reversing social gradient, it is essential that we are prepared to protect the vulnerable poor from facing adverse outcomes of NCDs. Overall, the utilization of the public sector for inpatient care services for NCDs was found to be equitable. This is a good opportunity to make these services more affordable and accessible to the poor by introducing tailor-made provisions into existing programmes.

Conflicts of interest. None declared

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Original Article

Effect of a Brief Smoking Cessation Intervention on Adult Tobacco Smokers with Pulmonary Tuberculosis: A Cluster Randomized Controlled Trial from North India

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Abstract

Background: An association between smoking and poor tuberculosis (TB) treatment outcomes has been globally established. Various smoking cessation interventions (SCIs) have been proven worldwide to curb smoking behavior. There is a need for evidence to assess if SCI increases the chance of successful treatment outcome among TB patients. **Objectives:** To assess the effectiveness of a brief SCI; The Ask, Brief, Cessation support (ABC) package, on treatment outcomes and smoking cessation in smear-positive adult pulmonary TB patients. **Methods:** A cluster, randomized controlled trial was conducted wherein 17 designated microscopic centers of Chandigarh, India were randomly assigned using a computer-generated randomization sequence to receive SCI within directly observed treatment, short (DOTS) services, or existing standard of care. Eligible and consenting smokers (15 + years) registered as smear-positive pulmonary TB for DOTS (n = 156) between January and June 2013 were enrolled. Smoking cessation (self-reported) was assessed at intervals till the end of treatment. End TB treatment outcomes were extracted from patient records. **Results:** Treatment success was lower in intervention arm (83.6%) as compared control arm (88.2%), but the difference was statistically insignificant (P = 0.427). Smoking cessation was higher in intervention arm (80.2%) compared to comparison arm (57.5%) (adjusted incidence risk ratio = 1.56; 95% confidence interval = 1.24–1.93; P < 0.0001). **Conclusions:** SCI is effective in inducing smoking cessation among TB patients. No association of SCI with TB treatment outcomes could be detected.

Key words: Advice, India, smoking cessation, tobacco control, tuberculosis

Introduction

Tuberculosis (TB) is a leading cause of death worldwide, alongside HIV and was responsible for 1.4 million deaths in 2015.[1] India accounts for more than a quarter of the global TB load.[1] As an established risk factor of TB, tobacco smoking has increased substantially over the past three decades, especially in developing countries.[2] The WHO has strongly recommended coordination between national TB and tobacco control programs.[3] Observational studies have shown association between smoking and poor TB treatment outcomes such as increased loss to follow-up rate, increased severity of disease.[2,4-10] So logically drawing from it, smoking cessation efforts should lead to quitting and thus reduce the incidence of unfavorable TB treatment outcomes.

However, Jeyashree *et al*. in their systematic review had identified no randomized controlled trials to support the effect of smoking cessation on TB treatment outcomes and had stressed the need for the same.[4] The authors hypothesized that smoking cessation intervention (SCI) would be associated with favorable TB treatment outcomes. Hence, among smear-positive pulmonary TB patients in Chandigarh city, India, the present study aims to assess the effect of SCI on smoking cessation and TB treatment outcomes.

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Materials and Methods

Study design

This was a two-arm parallel cluster randomized controlled trial.

Study settings

The study was carried out in all the seventeen Designated Microscopy Centre (DMC) under three TB units (TUs) of Chandigarh. DMC is a health facility with TB diagnostic facility through sputum smear examination, catering to a population of 50,000–100,000 which is routinely staffed with a medical officer, TB health visitor (TBHV), and a laboratory technician. Cluster randomization technique was used in the study, and the clusters were defined at the DMC level so as to reduce contamination between clinics. DMCs were randomized based on computer-generated block randomization sequence (block size 4) ensuring equal allocation in both arms.

Study population

All sputum smear-positive pulmonary TB patients, males and females, aged 15 years and above, registered for treatment under Revised National TB Control Program (RNTCP) in two-quarters (January till June 2013) in various DMCs of Chandigarh were the target population in the study. Among those registered, consenting current and occasional smokers (n = 152) were enrolled into the study. They were followed up till completion of the study period (i.e., December 2013).

Data collection

TBHV of the intervention DMCs were trained by the investigators to deliver ABC package[11] while those in the control arm continued to provide the existing standards of care for the smokers in their area. Data were collected by two field investigators who were blinded to the allocation of DMCs.

Enrolling tuberculosis patients and classification into smokers and nonsmokers

The smoking history among TB patients was obtained from all smear-positive pulmonary TB patients using a structured questionnaire, and the smokers were thereafter enrolled in the study. Their basic sociodemographic characteristics, prevalence, and smoking history were also obtained.

Follow-up of smokers in two arms

The participants from both arms were followed till the end of 6 months follow-up period. The outcomes for the two arms were analyzed and compared for assessing the effectiveness of the SCI on TB treatment outcomes.

ABC intervention: Ask, brief advice, and cessation support

The intervention by the International Union against TB and Lung Disease (The Union) "Smoking Cessation and Smoke-free Environments for TB Patients 2010" was used in the study.[11] "ABC for TB" is an intervention that guides the health-care provider to Ask about smoking habit, give brief advice on smoking cessation and provide cessation support. It can be delivered by any health-care worker. The intervention is delivered systematically within the existing program activities and can be done within as little as 2–5 min. These services are delivered at the time of registration of the patients and during his/her sputum reexamination visits (2 months, 5 months, end) [Figure 1].

Outcome measures

Treatment outcome (as per WHO 2013 revised guidelines)

Treatment outcomes were recorded as treatment success or treatment failed.

Treatment success was a sum of cured (a pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion) and treatment completed (a TB patient who completed treatment without evidence of failure but with no record to show that sputum smear or culture results in the last month of treatment either because tests were not done or because results are unavailable and on at least one previous occasion were negative). Treatment failed was a TB patient whose sputum smear or culture is positive at month 5 or later during treatment.

Smoking behavior

Smoking cessation was recorded as positive and negative. Smoking cessation was recorded as positive if patient had not smoked at all in the last 2 weeks and negative if patient smoked in the last 2 weeks and did not attempt to quit smoking since their last sputum examination visit (quit attempt was defined patient who tried to quit and succeeded for at least 24 h).

Data analysis

Two key statistical approaches used for the analyses of trial results were "clusterlevel" parametric analysis of summary measures estimated for each cluster and "individual-level" parametric analysis adjusted for clustering at TU level. The intraclass correlation coefficient (ICC) of baseline smoking habit was calculated using Stata (loneway command). At individual-level univariate analysis, Chi-square test was used. Multivariate analyses of incidence risk ratios (IRRs) or simply relative risks for quit of smoking at follow-up in the 2 study arms adjusted for TU-level clustering as well as important covariates were carried out by generalized estimating equations (GEE; using log Poisson as well as binomial regression models with exchangeable correlation and robust variance estimates). The linear mixed model was also used to predict follow-up treatment outcome in the two-study arms adjusted for covariates. Analyses were both per protocol (PP) and intention-to-treat (ITT; latter included all randomized participants). Missing smoking status data on follow-up were imputed by the "last observation carried forward" method. Using two-tailed statistical tests, $P < 0.05$ was taken as statistically significant. Stata version 11.0 (Lakeway Drive, College Station, TX 77845, USA) was used for analyses.

Ethical considerations

Appropriate permissions were taken from the State TB cell Chandigarh to access the data of the TB patients. The data were

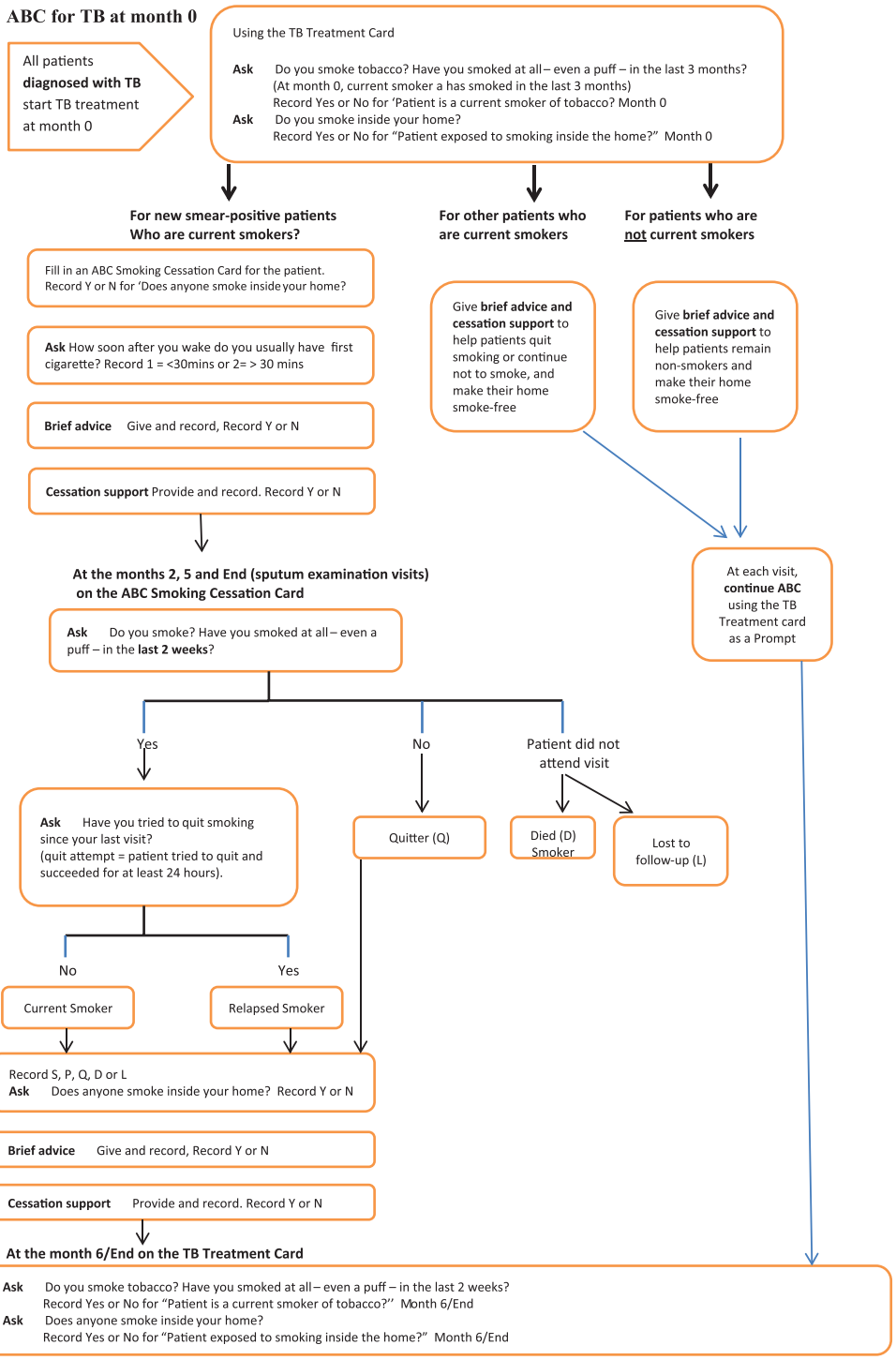

Figure 1: Flow diagram of ABC intervention.

kept confidential with access restricted only to the principal investigator. The trial was ethically approved by the Institute Ethics Committee, PGIMER (Histopath/NK/EC/46-102 DATED 8/1/2013).

Results

There were a total of 17 clusters (DMCs) with 685 smear-positive pulmonary TB patients enrolled from January to June 2013. Of 685, 152 (22.7%) smokers (current daily and occasional smokers) were enrolled to the study. The smokers were assigned in the intervention (n = 78) and control arm (n = 74). Postintervention status of smoking could not be done in 13.8% (21/152) subjects–16.6% (13/78) in intervention arm and 10.8% (8/74) in comparison arm [Figure 2]. Nonetheless, baseline characteristics did not differ statistically between subjects with and without missing follow-up data ($P > 0.05$) when analyzed by Chi-square

test of significance for each variable separately. The ICC was estimated from a one-way random effects model using the analysis of variance method. The mean sum of squares between TU clusters was 0.304, and mean sum of squares within the centers was 0.170, which resulted in an ICC of 0.019 (95% confidence interval (CI) = 0.00–0.050). The majority of participants were males (96.7%), in the age bracket of 30–44 years (37.9%), educated (70.5%), and having some occupation (97.3%). The baseline characteristics of smokers in intervention and control arm did not differ significantly except for the age [Table 1]. Factors determining nicotine dependence in both arms were found to be statistically insignificant, reflecting similar smoking habits in both arms.

Smoking cessation and treatment outcome

Treatment outcome was defined as cured (n = 113), treatment completed (n = 6), treatment failed (failure/on treatment/MDR) (n = 12), died (n = 6), loss to follow-up (n = 7), and not evaluated (n = 8). The intervention group reported 83.6% successful outcomes compared to the control group that reported 88.2% successful outcomes. More adverse outcomes were noted in the intervention (11, 16.4%) as compared to the control group (8, 11.8%), but the difference was not statistically significant (P = 0.427).

To measure the association between quitting and treatment outcomes, regression model was run between the last status of smoking (noted on month 7 or end sputum examination day) and treatment outcome between the two arms. The association between smoking and treatment outcome was done adjusting for age and nicotine dependence variables to exclude the possibility of confounding by these variables. The

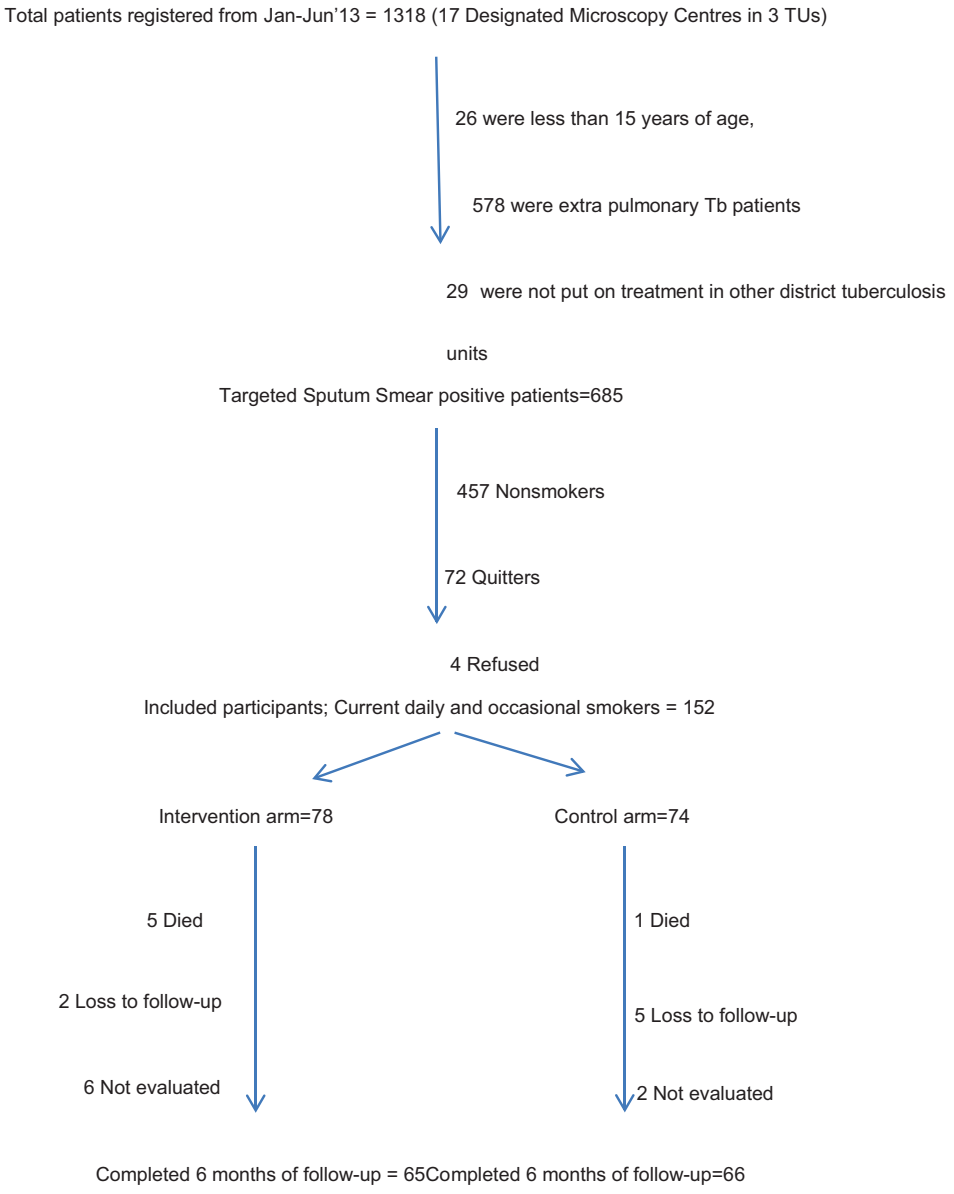

Figure 2: Patient flow diagram for cluster trial.

treatment success was found higher among quitters (74.7%) as compared to smokers (25.2%), the difference was found to be significant (IRR = 1.31; 95% CI = 1.30–1.32; $P \leq 0.0001$).

Smoking cessation and intervention

At the end of the treatment, 57 (80.2%) patients in the intervention arm had quit smoking as against 42 (57.5%) in the control arm. After adjusting for confounders such as age, socioeconomic, and nicotine dependence variables [Table 1], the relative risk of quitting on follow-up was significantly higher in the intervention arm as compared to the comparison arm for both PP analysis (adjusted IRR = 1.56; 95% CI = 1.24–1.93; $P < 0.0001$) and ITT analysis (adjusted IRR = 1.52; 95% CI = 1.19–1.87; $P < 0.0001$) by GEE analysis with log Poisson regression [Table 2].

It was also found that the percentage of quitters were significantly higher in intervention arm (as compared to comparison arm); after first counseling session ($P < 0.0001$), after second counseling session ($P < 0.0001$), and after third counseling session and ($P = 0.008$). Overall, 69.2% ($n = 54$) of smokers had quit and the quit rate increased progressively from the first follow-up to the end of anti-TB treatment.

Discussion

This is the first randomized controlled trial conducted in India that investigated the effect of a SCI on tobacco smoking behavior and on the treatment outcomes of new smear-positive pulmonary TB patients. The intervention did not affect the treatment outcome of the patient difference in treatment outcome was found to be insignificant between intervention and control arm. This finding could be due to the fact that for an intervention to bring positive treatment outcome, the total time spent for each session should be relatively more. This idea can be verified with the finding from a study done in Rio de Janeiro, Brazil, which stated that a dose–response relationship exists between the length of a session by person-to-person contact and successful treatment outcomes.[12] Repeated brief cessation advice has been shown to be a feasible and inexpensive addition to routine TB case management in this study and others.[13] Basu *et al*. had also concluded that in spite of evidence that tobacco control may be highly relevant to the future control of TB; such control has not been integrated into most TB control programs.[14]

We also found that the smokers who received SCI were more likely to quit smoking as compared to those who received

Table 1: Baseline characteristics of smokers among new smear positive tuberculosis patients registered in tuberculosis units of Chandigarh from January to June 2013

	Intervention ($n=78$), n (%)	Comparison ($n=74$), n (%)	Total ($n=152$), n (%)	Pearson χ^2	P^a
Age (years)					
15-29	14 (18)	20 (27)	34 (22.4)	7.57	0.109
30-44	37 (47.4)	21 (28.4)	58 (38.2)		
45-59	17 (21.8)	24 (32.4)	41 (27)		
60+	10 (12.8)	9 (12.2)	19 (12.5)		
Sex					
Males	77 (98.7)	70 (94.6)	147 (96.7)	2.03	0.154
Females	1 (1.3)	4 (5.4)	5 (3.3)		
Education					
Yes	53 (68)	55 (74.3)	108 (71.1)	0.75	0.386
No	25 (32.1)	19 (25.7)	44 (29)		
Occupation					
Yes	76 (97.4)	72 (97.3)	148 (97.4)	0.003	0.957
No	2 (2.6)	2 (2.7)	4 (2.6)		
Number of cigarettes and or bidis per day					
<15	55 (70.5)	58 (78.4)	113 (74.3)	1.6	0.206
≥15	23 (29.5)	15 (20.3)	38 (25)		
Missing	0 (0)	1 (1.4)	1 (0.7)		
Time after waking to the first cigarette					
Within 30 min	43 (55.1)	35 (47.3)	78 (51.3)	0.627	0.429
More than 30 min	34 (43.6)	37 (50)	71 (46.7)		
Missing	1 (1.3)	2 (2.7)	3 (2)		
Quit attempt in the last 12 months					
Yes	47 (60.3)	41 (55.4)	88 (57.9)	0.496	0.481
No	29 (37.2)	32 (43.2)	61 (40.1)		
Missing	2 (2.6)	1 (1.4)	3 (2)		

[a]*P* values are calculated from Pearson's Chi-square test of significance for categorical variables

Table 2: Smoking cessation status among intervention and comparison arm using regression model adjusted for clustering and covariates at individual level

Generalized estimating equation		Adjusted for age, sex, education, occupation, nicotine dependence variable [Table 1]		
Predictor	**Outcome**	**IRR**	**Robust SE**	***P* (95% CI)**
Intervention versus comparison arm	Smoking status at follow-up (per protocol)	1.56	0.18	<0.0001 (1.24-1.93)
Intervention versus comparison arm	Smoking status at follow-up (intention to treat)	1.52	0.17	<0.0001 (1.19-1.87)

IRR: Incident risk ratio, CI: Confidence interval, SE: Standard error

standard advice on smoking in TB case management under RNTCP. The findings observed in our study with respect to smoking cessation are consistent with other studies conducted in Bangladesh (82%), Indonesia (66.8%), Sudan (67%), and Malaysia (78%) which has also showed increase in quit percent in TB patients by simplified SCI.[15-18] A study conducted on TB patients in Kerala, India observed that almost one-third patients relapsed among the quitters during the first 4–8 weeks of treatment, unlike our study which had zero relapse during intervention.[19] The high quit rate and absence of relapse during the entire course of treatment in our study may have occurred due to many reasons. First, standardized cessation intervention supplemented with adequate training support to existing health force may have led to positive outcomes in our study. Second, person-to-person delivery of intervention for four or more sessions appeared to be especially effective in increasing abstinence rates. A study in Brazil has also documented that minimal interventions lasting <3 min increases overall tobacco abstinence rates.

A study by Kaur *et al*. conducted in India found that by offering "brief advice" for tobacco cessation based on five A's approach advocated by the WHO and the Union, resulted in a quit percentage of 67.3% among smokers at the end of 6 months treatment period from baseline.[20] A cohort of newly diagnosed TB cases was followed up from their discharge after completion of treatment, and it was found that smoking was associated with the relapse of TB with odds ratio = 2.53 (95% CI: 1.23–5.21), even after adjustment for the socioeconomic variables.[10] Although there is lack of evidence on the direct effects of smoking cessation on TB treatment outcomes, available data suggest that smokers are less adherent to TB treatment, and thus at higher risk for default and persistent infectivity.[16,21]

We found that there was not much difference observed in the percentage of quitters from month 5 till the end of treatment in the intervention arm. This could be because of the fact that repeated reinforcements on quitting are most effective during the initial period of treatment. None of the sociodemographic factors contributed as a factor to quit smoking which is unlike the findings of many other studies where high socioeconomic status, high family income, literacy, and sex are potential determinates for quit attempts of tobacco.[22-24]

The treatment success was found to be higher among quitters (74.7%) as compared to smokers (25.2%) in the present study; the difference was found to be significant. Merely, one study documented that advising patients with TB to stop smoking also help them to complete their TB treatment and to respond better to that treatment.[25]

Strengths

The study was conducted under routine program conditions, and the wide coverage with disaggregated data of patients registered in RNTCP of an entire Union Territory makes the study conditions representative of programmatic conditions in India. Second, the study used robust methodology, which included predefined operational definitions for the study population and variables, and also adhered to CONSORT guidelines for conducting and reporting on randomized controlled trials. Third, involvement of health workers throughout the treatment of patients helped in sustenance of smoking cessation care within RNTCP. The intervention is not time or resource intensive as it can be done by existing health-care staff in routine settings. All health centers were located in the community which made the informal visits of health workers to monitor progress easier.

Limitations

In intervention arm, we approached family members to ascertain whether patient quit smoking or not, however in control arm we just relied on the response of the patient. This may be the reason of over reporting of quit percent in control arm. Smokeless tobacco users were excluded from the study. While the sample size was adequate to detect an association between SCIs and smoking cessation, the power of the study to detect an existing association between SCI and TB treatment outcome was small.

Conclusions

Interventions such as "ABC intervention" that are based on the principal of stepped approach of health education have proved to be useful in reducing smoking practices among smokers and should be recommended as a part of national TB control programs.

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Conflicts of interest

There are no conflicts of interest.

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RESEARCH ARTICLE

Effect of glycemic control and type of diabetes treatment on unsuccessful TB treatment outcomes among people with TB-Diabetes: A systematic review

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Abstract

Background

Stringent glycemic control by using insulin as a replacement or in addition to oral hypoglycemic agents (OHAs) has been recommended for people with tuberculosis and diabetes mellitus (TB-DM). This systematic review (PROSPERO 2016:CRD42016039101) analyses whether this improves TB treatment outcomes.

Objectives

Among people with drug-susceptible TB and DM on anti-TB treatment, to determine the effect of i) glycemic control (stringent or less stringent) compared to poor glycemic control and ii) insulin (only or with OHAs) compared to 'OHAs only' on unsuccessful TB treatment outcome(s). We looked for unfavourable TB treatment outcomes at the end of intensive phase and/or end of TB treatment (minimum six months and maximum 12 months follow up). Secondary outcomes were development of MDR-TB during the course of treatment, recurrence after 6 months and/or after 1 year post successful treatment completion and development of adverse events related to glucose lowering treatment (including hypoglycemic episodes).

Methods

All interventional studies (with comparison arm) and cohort studies on people with TB-DM on anti-TB treatment reporting glycemic control, DM treatment details and TB treatment outcomes were eligible. We searched electronic databases (EMBASE, PubMed, Google Scholar) and grey literature between 1996 and April 2017. Screening, data extraction and

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

Competing interests: The authors have declared that no competing interests exist.

risk of bias assessment were done independently by two investigators and recourse to a third investigator, for resolution of differences.

Results

After removal of duplicates from 2326 identified articles, 2054 underwent title and abstract screening. Following full text screening of 56 articles, nine cohort studies were included. Considering high methodological and clinical heterogeneity, we decided to report the results qualitatively and not perform a meta-analysis. Eight studies dealt with glycemic control, of which only two were free of the risk of bias (with confounder-adjusted measures of effect). An Indian study reported 30% fewer unsuccessful treatment outcomes (aOR (0.95 CI): 0.72 (0.64−0.81)) and 2.8 times higher odds of 'no recurrence' (aOR (0.95 CI): 2.83 (2.60−2.92)) among patients with optimal glycemic control at baseline. A Peruvian study reported faster culture conversion among those with glycemic control (aHR (0.95 CI): 2.2 (1.1,4)). Two poor quality studies reported the effect of insulin on TB treatment outcomes.

Conclusion

We identified few studies that were free of the risk of bias. There were limited data and inconsistent findings among available studies. We recommend robustly designed and analyzed studies including randomized controlled trials on the effect of glucose lowering treatment options on TB treatment outcomes.

Introduction

Tuberculosis (TB) remains a major public health problem in low and middle income countries. [1] Meanwhile, the burden of diabetes mellitus (DM) is increasing to epidemic proportions in the same countries.[2,3] Worldwide, there are an estimated 10.4 million new people with active TB annually and of them, one million have both TB and DM (TB-DM). This double burden deserves attention. [2–4]

DM increases the risk of incidence of TB by 2–3 folds and bi-directionality of association between TB and DM has been studied. [5–7] Although there is no singular mechanism that has been identified as the cause of this bi-directional association, immune compromise is widely accepted as the reason for increased risk of TB among DM; and inflammation (mediated by IL6 and TNFα) whilst modulating a response to TB infection could cause an increase in insulin resistance. [8] Risk of TB is higher in DM patients with 'poor glycaemic control' as compared to those with 'optimal glycaemic control'. [9–11] Among people with TB, DM increases the risk of unfavourable treatment outcomes (delayed culture conversion, death, treatment failure, recurrence). [12,13] Hyperglycemia among people with TB is associated with more severe clinical manifestations during TB treatment like higher bacterial load in sputum, increased leucocyte count, increased acute phase response, more fever and atypical localization and cavity formation. [14–19] Glycemic control results in improvement in phagocytic activity [20], and avoidance of above-listed clinical complications. There is also evidence that enhanced management of DM reduces the risk of developing TB and improving TB treatment outcomes. [21]

To address the looming TB-DM epidemic, World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (The Union) developed a collaborative framework in 2010 for care and control of TB and DM. [22]

DM management among TB

Goals for glycemic control among people with DM are defined using various biochemical tests such as fasting/pre-prandial capillary blood glucose (FBG) or 2 hour post-prandial capillary blood glucose (PPBG) or glycosylated hemoglobin (HbA1c). Corresponding less stringent glycemic goals using higher cut offs of FBG, PPBG and HbA1c are available for those with extensive co-morbid conditions, less life expectancy and extensive micro/macro vascular complications. The same may be applied to TB people with DM. [23]

Rifampicin and Isoniazid have been documented to interact with OHAs and hamper glycemic control. [24] Insulin is more efficacious than OHAs in achieving glycemic control, though, the chances of hypoglycemic episodes increase with insulin use.[25,26] Using insulin treatment to replace or add to OHAs when TB is diagnosed among people with DM has been recommended by some for better glycemic control especially in severe tuberculosis. [24]

Why is it important to do this review?

There has been a call for integrating communicable and non-communicable disease care. [27] One of the four high priority research agendas recommended for reducing the joint burden of TB-DM has been to determine the impact of glucose lowering treatment on TB treatment outcomes with a detailed assessment on death. [28]

Though stringent glycemic control and using insulin as a replacement or in addition to OHAs have been recommended when DM is diagnosed in people with TB,[22,24,29,30] there is a need to systematically review whether this actually leads to improved TB treatment outcomes. Jorgensen et al found studies with mixed reports regarding effect of glycemic control on TB treatment outcomes. Some of the results quoted were not statistically significant, quality of studies included was not assessed and pooled estimates were not available. [31]

Specific objectives: Among people with drug susceptible TB and DM on anti-TB treatment (ATT), ***primary objective*** was to determine the effect of the following on unsuccessful TB treatment outcome(s) i) glycemic control (stringent or less stringent) when compared to poor glycemic control ii) insulin, alone or in combination with OHAs when compared to OHAs only. ***Secondary objectives*** were to determine the i) effect of glycemic control on emergence of multi-drug resistant TB (MDR-TB) at the end of TB treatment and recurrence of TB after successful treatment completion (after 6 months, after 1 year and beyond), ii) association of type of DM treatment and adverse outcomes (including hypoglycemic episodes) related to glucose lowering treatment.

The findings from this systematic review may provide evidence and guide the existing programmes, especially in low and middle income countries (with a high burden of TB-DM), regarding development of guidelines for management of DM during ATT and also guide future research priorities in TB-DM.

Material and methods

Protocol

The protocol was registered with PROSPERO (PROSPERO 2016:CRD42016039101) and is available online. There were no protocol deviations. The search is up to date as on 25 April 2017. [32]

Inclusion criteria for studies

Types of studies. We intended to include all interventional studies (with a control arm) on the topic (randomized or non-randomized; individual or cluster randomized) and all cohort studies (retrospective, prospective and ambispective). All studies between 1996 and 25 April 2017, published in any country and any language were included. We excluded single arm intervention studies, before-after design without control, cross-sectional studies, case control studies, case series, case reviews, subject reviews and ecological studies.

Types of participants. Participants were people of all ages and sexes with TB on ATT (not known to be drug-resistant at baseline) and diagnosed with diabetes (type I or type II or any other type) before or during TB diagnosis or during ATT. We included people on daily or intermittent anti-TB regimens (at least Isoniazid, Rifampicin, Pyrazinamide, Ethambutol); with or without HIV; managed either in programmatic or clinical (public or private facility) settings; managed in inpatient or outpatient settings; with pulmonary or extra pulmonary TB; with or without cavities at baseline chest radiograph (in case of pulmonary TB); with microbiologically or clinically diagnosed TB; and with new or retreatment TB.

Types of interventions / exposure. For primary and secondary objective one, 'stringent glycemic control' was pre-defined based on one time measurement of capillary FBG of <130 mg/dl (7.2 mmol/l) or capillary PPBG <180 mg/dl (10mmol/l) or HbA1c of <7%. Corresponding less stringent glycemic goals were capillary FBG of <178 mg/dl (9.9 mmol/l) or capillary PPBG <206 mg/dl (11.4 mmol/l) or HbA1c of <8%. [25,33] Glycemic control could be defined at various TB treatment phases: at baseline or any time during treatment (before / after intensive phase). People with 'poor glycemic control' were the comparison group.

For primary and secondary objective two, people on various glucose lowering treatment options (allopathic) were included. Individual level pharmacological interventions or exposures were included. The various glucose lowering treatment options could be 'insulin only', 'insulin with OHAs' or 'OHAs only'. People receiving 'OHAs only' were the comparison group.

Types of outcome measures. Primary outcomes were TB treatment outcomes, classified as favourable and unfavourable at the end of intensive phase and / or at end of TB treatment (minimum six months and maximum 12 months follow up). (Table 1) [34] Secondary outcomes were development of MDR-TB during the course of treatment, recurrence during follow up after 6 months and/or after 1 year post successful treatment completion and development of adverse event related to glucose lowering treatment (including hypoglycemic episodes).

Search methods for identification of studies

Electronic searches. We searched separately for interventional and cohort studies in PubMed, EMBASE, Google Scholar and the Cochrane Database of Systematic Reviews (search strategy and search results in S1 Appendix).

Searching other resources. We looked for cross references in the included studies, and reports; online and offline. We communicated with relevant experts in the fields for any article/research study (ongoing or recently completed) on this topic. A grey literature search included ISI Web of Science with conference proceedings, ClinicalTrials.gov, national and international trials registers, the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) search portal (apps.who.int/trialsearch/), metaRegister of Controlled Trials (mRCT) (controlledtrials.com/mrct/) and trial results registers, and guidelines and their reference lists. We contacted Jorgensen et al for the list observational studies they had listed for their review. [31]

Table 1. Operational definitions of TB treatment outcomes: end of intensive phase and end of treatment [34].

At the end of treatment	
Cured	A pulmonary TB patient with bacteriologically-confirmed TB at the beginning of treatment who was smear- or culture-negative in last month of treatment and on at least one previous occasion
Treatment completed	A TB patient who completed treatment without evidence of failure, but with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.
Lost to follow-up	A TB patient who did not start treatment or whose treatment was interrupted for two consecutive months or more.
Treatment failed	A TB patient whose sputum smear or culture is positive at month five or later during treatment.
Died	A TB patient who dies for any reason before starting or during the course of treatment
Not evaluated	A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.
Unfavourable outcome	Died, treatment failed, lost to follow-up, not evaluated
Favourable outcome	Treatment completed, cured
At the end of intensive phase (2 months for new patient, 3 months for a previously treated patient)	
Unfavourable outcome	Died, Lost to follow-up, extension of intensive phase and non-conversion at three/four months
Favourable	Microbiological conversion

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Data collection and analysis

Selection of studies. We removed duplicates and imported the bibliography to Rayyan for initial screening of title and abstracts. Rayyan is an open access web application (https://rayyan.qcri.org/). Once the bibliography was uploaded, it facilitated independent screening of bibliographic records (with blinding) followed by a providing a summary of screening (number of records included or excluded with consensus and number with no consensus). [35] During full text screen, the study was included for data extraction only if it fulfilled all the criteria (study type, participant, intervention/exposure with comparator, and outcome). If the study did not meet any one of the above criteria, it was excluded. Screening was done by two investigators (KJ and PM) independently. The investigators resolved any disagreements by mutual consent; with recourse to a third investigator (ANS for title/abstract screen; HDS for full text screen), if required.

Data extraction and management. Two investigators (KJ and PM) independently extracted study data from full text of the included studies into a data extraction form. (S2 Appendix) Any disagreement was resolved by discussion with a third investigator (HDS).

If primary data were unavailable in the supplementary file and/or no effect measures (adjusted/unadjusted) were reported, we requested the same form the authors. We sent three email reminders, fortnightly and awaited response for a maximum period of 1 month after writing the first mail to them.

Assessment of risk of bias in included studies. For interventional study, we planned to rate each included trial as being at high, low or unclear risk of bias under the following domains: sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting and other

sources of bias like carry-over, recruitment bias and contamination. [36] For cohort studies, we modified New Castle-Ottawa quality assessment scale and summarized it in a risk of bias table. [37]

Measures of effect. We planned to report the effect separately for interventional and cohort studies using adjusted or unadjusted Odds Ratio (OR/aOR) or Relative Risk (RR/aRR) or Hazard Ratio (HR/aHR) along with 0.95 confidence interval (CI). If the desired adjusted comparison was not available, the authors calculated the unadjusted effect based on the data available from narrative texts/tables. If primary data were available, we planned to perform analysis after appropriate adjustment followed by a pooled analysis, if possible.

Assessment of reporting biases. Considering possible reporting bias we contacted the study authors to get more information about the reported outcome. We also contacted the authors of registered trials/studies (but not yet published) in registries. Subject to inclusion of sufficient studies, we planned to plot the funnel plot to detect publication bias.

We followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA statement) and Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist to report this review. [38,39]

Results

We found 2326 articles after database search. After removal of duplicates, 2054 underwent a title and abstract screen and 56 were assessed for eligibility (full text screen). Nine studies were included for qualitative assessment (data extraction). Considering high methodological and clinical heterogeneity, we decided to report the results qualitatively. (Fig 1) Reasons for exclusion of studies during full text screen have been reported in Table 2. Characteristics of included studies are presented in S1 Annex.

Of the nine studies included in the review, all were cohort studies: eight dealt with glycemic control and its effect on TB treatment outcomes (Table 3) [40–47] and two dealt with type of DM treatment and TB treatment outcomes. (Table 4) [47,48] All studies included both sexes and adults, with children being included in one.[43] It was unclear whether children were included in the other two studies. [40,46] Four studies included patients with and without HIV, [40,42,44,47] three studies excluded patients with HIV [41,45,46] and in the remaining two information on HIV status was not available [43,48]. None of the authors responded with primary data or unreported outcomes. Hence, we did not perform any primary data analysis for adjusted effect measures. We derived unadjusted RRs from the data extracted from narrative texts/tables. As sufficient number of studies under each primary objective was less than 10, we did not plot the funnel plot to detect publication bias.

Glycemic control and TB treatment outcomes

Mahishale et al from India reported that compared to poor glycemic control (HbA1c $\geq$7%) at baseline, optimal glycemic control (HbA1c <7%) at baseline resulted in 88% reduction in sputum smear non-conversion at 2 months (unadjusted RR (0.95 CI):0.12 (0.06–0.23)); 30% reduction in unsuccessful treatment outcomes (aOR (0.95 CI):0.72 (0.64–0.81)) and 2.8 times higher odds of 'no recurrence' (aOR (0.95 CI):2.83 (2.60–2.92)). MDR-TB, confirmed by either culture or by Xpert MTB/RIF assay, was noted in 47 of 423 (11%) people with poor glycemic control as against none with optimal control group. [46] (Table 3, S1 Annex) Nandakumar et al, from India, found no association between glycemic control and end treatment outcomes. Glycemic control was considered as 'known' if FBG/RBG was available on three occasions, separated by one month and at least one of them was in continuous phase of ATT. If all three

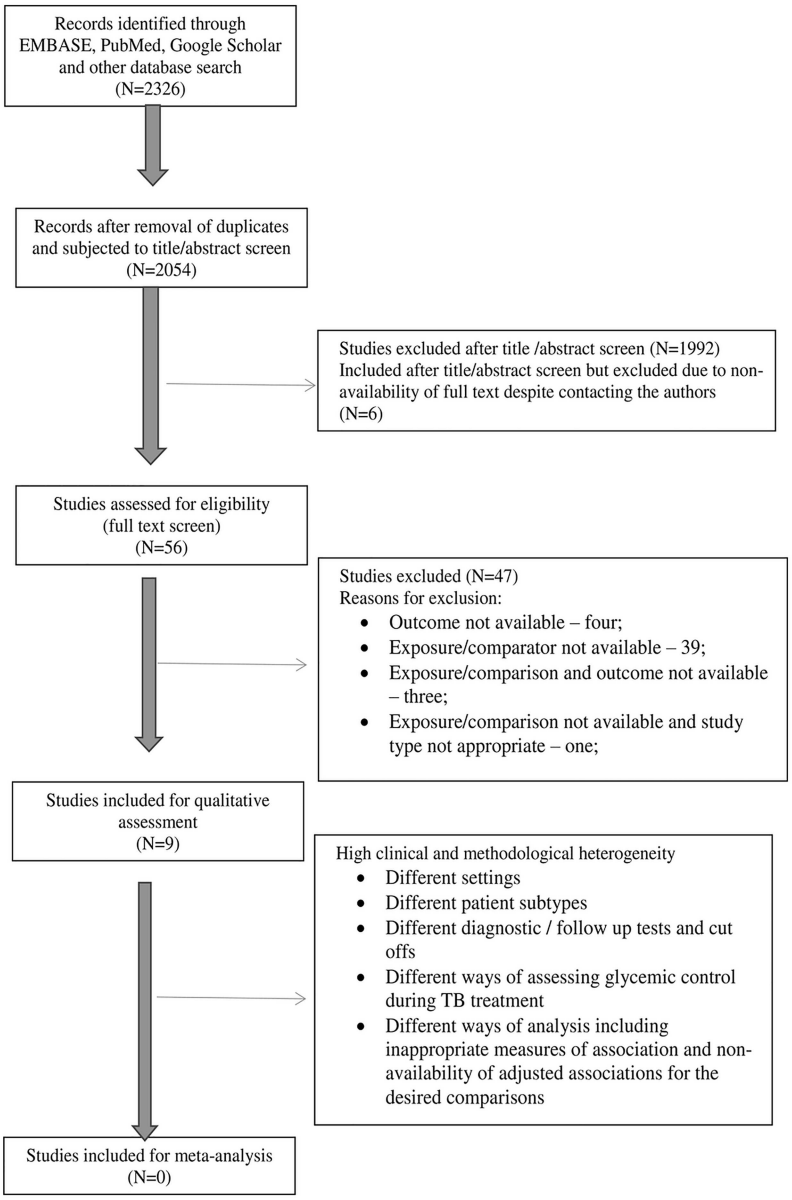

Fig 1. PRISMA flow diagram through different phases of the systematic review [39].

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values were below the cut off (FBG<100mg/dl; RBG<140mg/dl), then it was considered as 'optimal glycemic control' during ATT. [44]

Chiang CY et al from Taiwan reported that stringent glycemic control (HbA1c <7%) at baseline was associated with (unadjusted RR (0.95 CI): 1.89 (1.12–3.20)) higher risk of unfavourable treatment outcome when compared to poor glycemic control (HbA1c ≥9%); while less stringent glycemic control (HbA1c 7–9%) was not (unadjusted RR (0.95 CI): 1.53 (0.96 –2.46)). In the adjusted analysis, while DM was not associated with unfavourable TB treatment outcomes, DM related comorbidity was. [40](Table 3, S1 Annex)

Mi F et al from South China reported no significant association between stringent glycemic control (FPG<7 mmol/l) and less stringent glycemic control (FPG 7–10 mmol/l) with end IP

Table 2. Characteristics of the studies excluded from the systematic review*.

S.No	Study identifier	T	P	E/C	O
1.	Hongguang C_2015_Epidemiol Infect	Y	Y	N	Y
2.	Wang CS_2009_Epidemiol Infect	Y	Y	N	Y
3.	Banurekha VV_2007_IJMR	Y	Y	N	Y
4.	Chang JT_2011_J Formos Med Assoc	Y	Y	N	Y
5.	Kota SK_2011_Diabetes and Metabolic Syndrome Clinical Research and Reviews	Y	Y	N	Y
6.	Duangrithi D_2013_International J of Clinical Practice	Y	Y	N	Y
7.	Fielder JF_2002_Int J of Tub Lung Dis	Y	Y	N	Y
8.	Jabbar A_2006_Eastern mediterranean health Journal	Y	Y	N	Y
9.	Perez-Navarro LM_2015_J of Diabetes and its complications	Y	Y	N	Y
10.	Iseri AU_2010_Tüberküloz veToraksDergisi	Y	Y	N	Y
11.	B.E. Abdelbary_2016_Tuberculosis	Y	Y	N	Y
12.	Bachti Alisjahbana_2007_Clinical Infectious Diseases	Y	Y	N	Y
13.	Rani Balasubramanian_2007_Indian J of TB	Y	Y	N	Y
14.	Boillat_Blanco N_2016_The J of Infectious Diseases	Y	Y	N	Y
15.	Dobler CC_2012_BMJ Open	Y	Y	N	N
16.	Castellanos-Joya M_2014_Plos One	Y	Y	N	Y
17.	Chaudhry LA_2012_International J of Mycobacteriology	Y	Y	N	Y
18.	Faurholt-Jepsen D_2012_BMC Infectious Disease	Y	Y	N	Y
19.	Faurholt-Jepsen D_2013_TMIH	Y	Y	N	Y
20.	Suwampimolkul G_2014_Plos One	Y	Y	N	Y
21.	Gnanasan S_2012_University of Nottighamphd thesis	Y	Y	N	N
22.	Johnson HD_2016_American Journal of Infectious Disease and Microbiology	Y	Y	N	Y
23.	Dooley KE_2009_Am J Trop Med Hyg	Y	Y	N	Y
24.	Wang JY_2015_Chest	Y	Y	N	Y
25.	Lee PH_2016_Plos Medicine	Y	Y	Y	N
26.	Lo HY_2016_Int J of Tub Lung Dis	Y	Y	N	Y
27.	Wang JY_2013_Pharmacoepidemiology and drug safety	Y	Y	N	N
28.	Workneh MH_2016_Infectious Disease of Poverty	Y	Y	N	Y
29.	Oceguera DM_2016_Lung Disease and treatment	Y	Y	N	Y
30.	Orofino RDL_2012_J Bras Pneumol	Y	Y	N	Y
31.	Pajankar S_2008_Oman Medical Journal	Y	Y	N	Y
32.	Sahakyan S_2015_not a peer reviewed publication	Y	Y	N	Y
33.	Jimenex-Corona ME_2013_Thorax	Y	Y	N	Y
34.	Shariff NM_2015_Int J of mycobacteriology	N	Y	N	Y
35.	Sulaiman SAS_2013_American J of medical sciences	Y	Y	N	Y
36.	Vellalacheruvu BN_2015_International J of Scientific and Research Publications	Y	Y	N	Y
37.	Wang JY_2009_Respirology	Y	Y	N	Y
38.	Yusupova S_2016_Public Health Panorama	Y	Y	N	Y
39.	Siddiqui AM_2009_Journal of Taibah University Medical Sciences	Y	Y	N	Y
40.	Kornfield H_2016_Chest	Y	Y	N	Y
41.	Gil_Santana L_2016_Plos One	Y	Y	N	Y
42.	Mukhtar F_2016_BMJ Open	Y	Y	N	Y
43.	Salindri AD_2016_Open Forum Infectious Diseases	Y	Y	N	Y
44.	Barss L_2016_Chest	Y	Y	N	Y
45.	Wu Z_2016_J of Diabetes and its complication	Y	Y	N	Y
46.	Lee EH_2017_Lung	Y	Y	N	Y

(*Continued*)

Table 2. (*Continued*)

S.No	Study identifier	T	P	E/C	O
47.	Perez-Navarro LM_2017_Tuberculosis	Y	Y	N	Y

*T: Type of study = all interventional studies on the topic (randomized or non-randomized; individual or cluster randomized) with a control arm. Among observational studies, cohort studies (retrospective, prospective and / ambispective); P: participant criterion = people with TB and DM on anti-TB treatment; E/C: exposure/comparator = for research question on glycemic control, glycemic status was the exposure of interest. For research question on insulin, type of DM treatment was the exposure of interest. If any one of the two was present in the study we included it; O: outcome = the study was included if any one of the primary outcomes were present; Y—yes; N—no

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Table 3. Effect of glycemic control (stringent or less stringent) on unfavourable TB treatment outcomes, summarized as unadjusted relative risk (RR)* [40–47].

Study ID	Reference group	Exposed group	Unadjusted RR	95% CI
All unfavourable end (TB) treatment outcome				
Chiang CY_2015_Plos One	Poor glycemic control (HbA1c>9)	Less stringent glycemic control (HbA1c 7–9)	1.53	0.96, 2.46
		Stringent glycemic control (HbA1c<7)	1.89	1.12, 3.20
Mi F_2013_TMIH^	Poor glycemic control (FPG>10mmol/l)	Less stringent glycemic control (FPG7-10mmol/l)	0.91	0.18, 4.43
		Stringent glycemic control (FPG<7mmol/l)	1.03	0.21, 5.07
Magee MJ_2013_International J of Infectious diseases	Poor glycemic control (no specific criteria)	Glycemic control	1.08	0.54, 2.16
Nandakumar KV_2013_Plos One	Poor glycemic control (FBG>100 mg/dl and PPBS/RBG>140 mg/dl)	Glycemic control	0.52	0.25, 1.07
Tabarsi P_2014_Journal of Diabetes and Metabolic Disorder	Poor glycemic control (HbA1c≥6.5)	Glycemic control	1.13	0.2, 6.44
Yoon YS_2017_Thorax	Poor glycemic control (HbA1c≥7)	Glycemic control	0.55	0.22, 1.36
Mahishale_2017_Iran J MS	Poor glycemic control (HbA1c≥7)	Glycemic control	0.18	0.09, 0.36
Culture non-conversion at 2 months				
Park SW_2012_Eur J ClinMicrobiol Infect Dis	Poor glycemic control (HbA1c>7)	Glycemic control	0.62	0.14, 2.71
Yoon YS_2017_Thorax	Poor glycemic control (HbA1c≥7)	Glycemic control	0.23	0.05, 0.94
Sputum smear non-conversion at 2 months				
Mi F_2013_TMIH^	Poor glycemic control (FPG>10mmol/l)	Less stringent glycemic control (FPG7-10mmol/l)	1.50	0.57, 3.90
		Stringent glycemic control (FPG<7mmol/l)	0.65	0.19, 2.15
Nandakumar KV_2013_Plos One	Poor glycemic control (FBS>100 mg/dl and PPBS/RBS>140 mg/dl)	Glycemic control	0.99	0.65, 1.51
Mahishale_2017_Iran J MS	Poor glycemic control (HbA1c≥7)	Glycemic control	0.12	0.06, 0.23

*data extracted from the narrative text/tables;

^End IP and end treatment outcomes reported among those with glycemic status at 2 months and 6 months respectively

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Table 4. Effect of glucose lowering treatment on unfavourable TB treatment outcomes, summarized as unadjusted relative risk (RR)* [47,48].

Study ID	Reference group	Exposed group	Unadjusted RR	95% CI
All unfavourable end (TB) treatment outcome				
Magee MJ_2013_International J of Infectious diseases	OHA only	Insulin	2.63	1.07, 6.46
		OHA+Insulin	0.81	0.23, 2.80
Viswanathan V_2014_Journal of Diabetes and its complications ^	-	-	-	-

*data extracted from the narrative text/tables;

^Unsuccessful TB treatment outcomes among those on OHA only, insulin only and both were 0/53, 2/18 and 0/3 respectively. Sufficient outcomes were not there to calculate RR.

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and end treatment outcomes. Though FPG values were available at baseline, 2 months and 6 months, 2 months glycemic status was compared with end IP outcomes and 6 month glycemic status was compared with end treatment outcomes. [43] (S1 Annex)

Magee MJ et al from Lima, Peru found that among TB-DM patients (30% had drug resistance), culture conversion among those with glycemic control (assessed while on ATT) was faster than those without control. (aHR (0.95 CI) = 2.2 (1.1,4)). [42](S1 Annex)

Yoon YS et al from South Korea, used HbA1c <7% and ≥7% for glycemic control and poor glycemic control respectively in the adjusted analysis. However, people without DM were the reference. We extracted numbers for treatment outcomes among the subgroups of glycemic control in people with TB-DM. Culture non-conversion, but not end treatment outcomes, was associated with glycemic control when compared to poor control at baseline (unadjusted RR (0.95 CI):0.23 (0.05–0.94)). [41](Table 3, S1 Annex)

Data extracted from the study by Park SW et al from South Korea revealed no association between sputum conversion at 2 months and glycemic control (HbA1c<7) at baseline.[45] Though Tabarsi P et al did not exactly address our research question(s), we could extract the data that we required. HbA1c was measured at baseline and 3 months of ATT and was categorized as elevated if HbA1c was ≥6.5%. Those with 'Elevated-normal' and 'elevated-elevated' at baseline and 3 months respectively were included in our analysis to represent good control and poor control respectively. We found no significant difference in end treatment outcomes among these two groups.[47] (Table 3, S1 Annex)

Type of DM treatment and TB treatment outcomes

Data extracted from Magee MJ et al from Lima, Peru revealed that when compared to those receiving 'OHA only', those receiving 'Insulin only' had significantly higher risk of unfavourable end treatment outcomes (unadjusted RR (0.95 CI):2.63 (1.07–6.47)); while those receiving 'insulin and OHA' did not have significantly different unfavourable end treatment outcomes. [42] (Table 4, S1 Annex) Viswanathan V et al did not have sufficient number of unfavourable outcomes to enable calculation of RR.[48] (Table 4, S1 Annex)

Risk of bias assessment

Of the nine studies included, six had poor 'comparability', four of which did not report adjusted analysis. Four studies suffered inadequacies in follow up that was likely to induce bias. The study by Mahishale et al had no risk of bias. (Table 5)

Table 5. Summary of risk of bias in included studies assessed using New Castle Ottawa quality assessment scale for cohort studies [37].

Study ID	Selection (max 4 stars)	Comparability (max 3 stars)	Outcome (max 2 stars)	Overall comment
Chiang CY_2015_Plos One	****	-	**	Adjusted analysis done but for not of the comparison of our interest, glycemic control was not included in the adjusted analysis
Mi F_2013_TMIH	***	-	*	No description of derivation of TB DM glycemic control/uncontrolled cohort, cross sectional data used for analysis, no adjustment, incomplete follow up likely to introduce bias
Magee MJ_2013_International J of Infectious Diseases	***	**	*	Selected group of people with TB-DM (presumptive MDR), adjustment for two confounders only, incomplete follow up likely to introduce bias
Nandakumar KV_2013_Plos One	***	***	**	Of 667 TB-DM, exposure status was unknown for 427 (64%)
Park SW_2012_Eur J Clin Microbiol Infect Dis	***	-	*	Excluded extra pulmonary, pulmonary TB with HIV and age <15 years. Adjusted analysis not done, incomplete follow up likely to introduce bias
Tabarsi P_2014_Journal of Diabetes and Metabolic Disorder	****	-	**	adjusted analysis not done
Viswanathan V_2014_Journal of Diabetes and its complications	***	-	**	New smear positive TB cases, adjusted analysis not done
Yoon YS_2017_Thorax	****	-	*	Adjusted analysis done but for not of the comparison of our interest, incomplete follow up likely to introduce bias
Mahishale_2017_Iran J MS	****	***	**	No risk of bias

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Discussion

Summary of findings

The systematic review identified two studies which provided quality information on glycemic control during TB treatment among people with TB-DM and its effect on TB treatment outcomes. One study provided evidence on reduction in unfavourable end treatment outcomes including recurrence after initial treatment success. [46] The second study provided information on faster TB culture conversion among those with glycemic control. [42] Other studies, though provided some information, were not free of the risk of bias. We did not find a study where less stringent glycemic control's effect was exclusively assessed on TB treatment outcomes after adjusting for confounders. The two studies on effect of insulin (with or without OHA) on TB treatment outcomes were not free of the risk of bias.

Qualitative assessment of the studies

Except for Mahishale et al and Magee MJ et al, no study did adjusted analysis to measure the desired associations. Mahishale et al had the largest sample of people with TB-DM (n = 630). Participants were also followed up for two years after treatment initiation to measure recurrence post treatment success. [46] They could have used aRR instead of aOR as the former was most appropriate for the design.[49] One additional HbA1c measurement during treatment, besides baseline) could have helped estimate glycemic control during ATT better. [46] Magee et al used time to event analysis and summarized the association between glycemic control and time to culture conversion using aHR, however the adjustment was done only for drug-resistant status and previous ATT. Despite availability of data, there was no mention of effect (adjusted analysis) of glycemic control, DM care and type of DM treatment on end TB outcomes. The criterion for recording glycemic control was not specified. This study does provide estimates of TB outcome by degree of diabetic care as well as by type of anti-diabetic regimen used unlike Mahishale et al. Unadjusted RR derived from Magee et al revealed worse treatment

outcomes among those on insulin. However, we cannot infer much from this as the analysis was unadjusted. [42]

Chiang CY et al's primary research question did not match ours. But they had analyzed influence of glycemic control on TB treatment outcomes. The HbA1c cut offs used by them <7%, 7–9% and ≥9% did not meet our review's predefined cut offs, <7%, 7–8% and ≥8%. Of 705 people with TB-DM, 30% had no information on the glycemic control. Even though, HbA1c level was found to be associated (statistically significant) with unfavourable outcomes in unadjusted analysis, they did not include it in the adjusted analysis and the results were summarized as aOR instead of aRR. Non-DM was used as the reference group similar to the multi-centre study by Yoon S et al (n = 157). [40,41]

Nandakumar KV et al (n = 667) reported that control status was 'known' for 240 (36%) only. They used very strict operational definitions for 'known' diabetic status and control among known. They used RR/aRR which was appropriate for the study design. Multiple blood glucose values were available and the values were summarized into a single variable 'glycemic control during treatment'. Instead, they could have used the glycemic control data as it is at various points during TB treatment by performing longitudinal data analysis. [44] The same applies to Mi F et al. However, Mi F et al chose to have a cross-sectional comparison: two months glycemic control with end IP outcomes and 6 months glycemic control with end treatment outcomes. [43]

In the study by Park SW (n = 124), unadjusted comparison of culture conversion at 2 months among those with glycemic control (HbA1c <7%) and with poor glycemic control (HbA1c ≥7%) at baseline was made with non-DM as reference. The information was also not reliable as many did not have info on culture conversion. [45] Tabarsi P et al, instead of categorizing into 4 groups, could have used the individual HbA1c value as it is at two time points and looked at its association with end treatment outcome. [47]

The HbA1c test should be performed using a method that is certified by the NGSP (www.ngsp.org) and standardized or traceable to the Diabetes Control and Complications Trial reference assay. [25] With reference to the use of HbA1c in five studies (S1 Annex), there was no mention of NGSP certification or standardized to the DCCT assay.

Ongoing trials

We have summarized the ongoing trials (n = 5) whose results are awaited in S2 Annex. In all these trials, the primary objective does not meet our review's objectives. Four trials are looking at effect of Vitamin D supplementation on TB treatment outcomes among people with TB-DM. We hope to get the glycemic control related data from the authors as and when the results are available or published. Another trial is looking at the effect of intensive monitoring of diabetes on diabetes control up to six months during TB treatment when compared to standard diabetes monitoring. Here we hope to get the TB treatment outcome related data from the authors once available.

Implications for TB-DM management and future research

Among people with TB-DM, this review found a dearth of studies with minimal or no risk of bias for the effect of glycemic control and the effect of insulin (with or without OHA), when compared to OHA only, on TB treatment outcomes. There is a need for RCTs on effect of glucose lowering treatment options on TB treatment outcomes. As of now, the countries may continue to follow the existing guidelines of DM management among people with TB-DM. This also provides an opportunity for the national TB programmes to systematically record

glycemic control status at baseline, intensive phase and continuation phase of ATT. Routine reporting and setting up monitoring mechanisms for the same is the need of the hour.

Cohort studies are observational in nature which makes the analysis complex with multiple options. This also makes comparisons and pooling of results complex. In future, for cohort studies on this research question, we recommend a standard methodology which will ensure comparability among the studies. They are as follows: i) conducting a multicenter study which will ensure sufficient number of people with TB-DM; ii) using standard diagnostic criteria to diagnose DM [25]; iii) using TB-DM with uncontrolled glycemic status as the reference instead of TB people without DM; iv) assessment of glycemic status, preferably using HbA1c (standardized), at baseline, end of intensive phase and during continuation phase; v) use of standard HbA1c (7% and 8%) and FBG (130mg/dl and 178 mg/dl) cut offs for glycemic control, thus providing three arms of glycemia (stringent, less stringent, poor control); vi) if three or more FBG values are available during treatment, considering conversion of mean FBG during treatment to an estimated HbA1c during treatment; [25,33] vii) if time of follow up is consistent, then use of RR, and if time of follow up is not consistent and the dates are available, then use of HR as the measure of effect; ix) adjusted analysis for confounders (age, sex, TB site, TB microbiological status, new or old TB, baseline BMI, baseline anemia, HIV status, steroid use, tobacco and alcohol use,); x) ideally, a longitudinal data analysis making use of HbA1c or FBG value at each time point is recommended. This would also adjust for clustering for repeat measurements and not reduce the sample size, which usually happens when people with TB-DM are classified into groups based on baseline and during ATT HbA1c/FBG values. This way we can not only analyze the effect of glycemic control on TB treatment outcomes but also the effect of improving or worsening glycemic control with time.

Conclusion

This systematic review identified two studies that were free of the risk of bias and suggested glycemic control may have a favourable effect on TB treatment outcomes. However, in many studies, the variables to answer our research question were available, but the analysis was not meeting our review's objectives. The author group of this systematic review is in touch with the authors of the included studies and is working on a research consortium for either a re-analysis or a pooled analysis of data.

In the future, focused cohort studies are required on this topic, using a standard design and analysis plan. There is a need for RCTs looking at effect of glucose lowering treatment options on TB treatment outcomes.

Supporting information

S1 Annex. Characteristics of studies included in the review.
(DOCX)

S2 Annex. Characteristics of ongoing studies.
(DOCX)

S1 Appendix. Search strategy for electronic database search and search results.
(7Z)

S2 Appendix. Data extraction form containing information of studies included in the review.
(7Z)

S3 Appendix. PRISMA checklist.
(DOC)

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Brief Communication

Cross-cultural Adaptation of Jefferson Scale of Empathy-Health Professions Students Version: An Experience with Developing the Tamil Translation

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ABSTRACT

Background: Empathy is a key cognitive attribute among healthcare professionals that fosters better patient– healthcare provider relationships. The Jefferson scale of empathy (JSE) measures self-rated empathy among various groups of healthcare professionals–health professionals, medical students and health professions students (HPS). The authors present the experience in translating the JSE-HPS version into an Indian regional language with insights into the issues faced in every step. **Methods:** With official permission from the Thomas Jefferson University, the authors embarked on the translation proceeding through forward translation (three rounds of modification), back translation (two independent translates), and synthesis of a final translated version. While targeting literary accuracy, the simplicity and comprehensibility of the instrument by the study population were also ensured. Variations in regional dialects and accents across the population were considered. **Results:** The back-translated version was evaluated for semantic, content, cultural, and technical equivalence. It was then pretested on ten students followed by a group discussion with them to ensure the comprehensibility of the tool and the differences between written and spoken language were addressed through necessary modifications. **Discussion:** The Tamil translation of the HPS version of JSE is now approved by and officially available with the Thomas Jefferson University.

Keywords: Empathy, health professions students, Jefferson Scale, nursing, Tamil, translation

Background

Empathy is the physician's ability to understand the patients' concerns by placing himself/herself in the patient's position.[1] It is an essential skill to build good healthcare provider–patient relationships and reap better clinical outcomes.[2] Hojat *et al.* define empathy as as a cognitive attribute that involves an ability to understand the patient's inner experiences and perspective and a capability to communicate this understanding.[3]

The Jefferson scale of empathy (JSE) is the widely used instrument for objective measurement of self-rated empathy among physicians, medical students, and other healthcare professionals – health professions students.[4,5] Initially called the JSPE, it is now widely termed as JSE to include scales to measure empathy among various populations, namely, JSE physicians, medical JSE students, and other JSE healthcare professionals, and JSE health professions students (JSE-HPS). The HPS version has replaced the earlier JSE nursing students'

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version which was applicable only to nursing students.[6] The current JSE-HPS version has 20 items rated on a scale of 1–7 ranging from strongly disagree to strongly agree.

Tamil is an Indian regional language predominantly spoken by people in the state of Tamil Nadu in India and the Union territory of Puducherry. It is the official language of two countries, namely, Sri Lanka and Singapore. Besides these countries, it is also widely used in Malaysia, (where it is one of the languages of education), Tamil diaspora in the USA, UK, Canada, and few Asian countries.

Every year, in Tamil Nadu, India, over 10,000 students enter Bachelors/Diploma courses in nursing and allied health sciences after completing their higher secondary education. There are private and government colleges of nursing (around 170 in total) and other allied health sciences. Although the medium of instruction in these courses is English, the students are mostly from vernacular (Tamil) medium schools where English is taught as a second language. The widely spoken language by patients being Tamil, the healthcare providers' medium of interaction with the patients is also Tamil. Given that the English comprehension is limited among this population and the need for conceptual understanding of empathy to respond appropriately to the JSE, the authors embarked on a Tamil translation of the HPS version of JSE.

Methods

Forward translation

The official permission to translate the JSE-HPS to Tamil was obtained from the Thomas Jefferson University. The guidelines and references on translation and back translation provided by the Thomas Jefferson University were adhered to besides those proposed by Guillemin *et al*.[7] The three authors who were proficient in Tamil and English language performed the Tamil translation. Following three rounds of modification, the forward translated version was developed.

Back translation

The back translation was done by three other healthcare professionals who were proficient in both English and Tamil and unaware of the original English version of the instrument. One back translated version was synthesized by discussion among the three authors and compared with the original English version for semantic, content, cultural, and technical equivalence.

Pretesting

The instrument (Tamil and English version) was administered to ten HPS who were proficient in English and Tamil. Group discussion with the ten participants for pretesting was moderated by the author. Suggestions and experiences regarding the questionnaire's understandability, ambiguity of terms, and time taken were noted.

Discussion

Translation of instruments to other languages is vital to promote cross cultural applicability of tools and their widespread application for research.[8,9] The process of translation is neither straightforward nor easy.[10] Besides the challenges posed by technical terms, for example, health- or illness-related terms that abound in health measurement scales, the use of idioms, figures of speech, examples, and cultural context of the questions that cannot be literally and directly translated are also to be dealt with care.[11] While they have to be relevant in the new form, the construct that is being measured needs to remain unaltered. While translating JSE-HPS to Tamil, the authors have adhered to commonly recommended standards for translating health measurement scales.[10,12]

One of the foremost challenges faced by the authors was the difference between the spoken Tamil and the written form. The written form of Tamil has retained its original form since many centuries. Diglossia has led to the divergence between the spoken and written form over the years.[13] The literary Tamil or the written form is rarely used in informal oral communication between people in day to day life.[14] A compounding issue was the liberal mix of the English language with Tamil in its spoken form. The mix is so commonly used to make the Tamil words for certain terms sound alien to the common human, for example, history and patient.

While targeting literary accuracy, the simplicity and readability of the instrument by the study population should not be compromised. Hence, the authors were careful to strike a balance between retaining the meaning of the items and their understandability by the respondents. It is preferable that the translators translate into their mother tongue, which was the case with our authors. Besides the authors felt that the translation should be done by those who understood the construct of empathy and the purpose of the instrument.[7] The involvement of more than one person in the forward translation process led to critical evaluation of the translated version and it was revised thrice before the final version was arrived at.

Although two direct equivalents of the word "empathy" are available in Tamil, they are sparsely used in day to day life. There was also a risk of the term being misinterpreted as sympathy. Hence, the authors circumvented the problem by providing both the terms and the word "empathy" within brackets. The authors also included the translation of the definition of empathy used by Hojat *et al*. while developing JSE.[5]

Literal word-to-word translation was avoided while the overall content and meaning of the items were retained carefully, for example, phrases like "stand in their patients' shoes." Colloquial terms were used with caution keeping in mind the variations in regional dialects and accents of Tamil across different districts of the state and across various socioeconomic strata and literacy levels.

The patient–healthcare provider relationship and healthcare delivery systems and scenarios vary across the world. The JSE-HPS does not refer specific scenarios or examples of patient–healthcare provider interactions. Thus, retaining the cultural/local relevance of the tool was not a major challenge while translating it. The authors ensured that the meaning of each of the translated items was same in the cultural context as in the original English version.

Although JSE-HPS does not use too much of technical jargon, it was a challenge finding simple equivalents of few words and phrases into Tamil, for example, "history," "therapeutic in its own right."

The method of administration was to be retained as self-administration of printed questionnaires. The Tamil translation of the HPS version of JSE is now approved by and officially available with the Jefferson University. The translated instrument can be used after testing for psychometric properties.

Conclusion

The authors have translated the JSE-HPS version to Tamil language. A pragmatic approach to translation requires an understanding of the source and target language and cultures to effectively translate the instrument. One has to be cognizant of the differences between written and spoken language and aim to keep the instrument understandable by the target population. The cultural milieu has to be understood and the translation should be appropriate and relevant in the areas where the translated version will be applied. At every step of translation, the goal should be to retain equivalence between the original and translated versions which ultimately captures the construct.

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Conflicts of interest

There are no conflicts of interest.

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Letter to the Editor

National guidelines on screening for diabetes among patients with tuberculosis in India: Need for clarity and change in screening cut off?

Globally, diabetes mellitus (DM) contributes to 15% of tuberculosis (TB) burden and is a risk factor for unfavourable TB treatment outcomes [1]. A TB-DM collaborative framework was launched in 2011 by the World Health Organization and International Union Against Tuberculosis and Lung Disease with bidirectional screening of TB-DM as one component [2].

India is world's diabetes capital after China and has the highest burden of TB [1,3]. A policy decision to screen all TB patients within the programme for DM was taken in 2012.The detailed guidance has been provided in the 'National framework for joint TB-Diabetes collaborative activities' (2017) [4]. According to these, a patient with TB should be screened for random blood glucose (RBG) using a glucometer, which can be interpreted as random capillary blood glucose (RCBG). If RCBG is ≥7.8 mmol/l (140 mg/dl), then the patient should be offered fasting blood glucose (FBG). If FBG is ≥7.0 mmol/l (126 mg/dl) then s/he is referred to the nearest facility for definitive (confirmatory) diagnosis and management of DM.

For Asian Indians, RCBG cut off ≥6.1 mmol/l (110 mg/dl) has been recommended for further definitive testing., RCBG cut off ≥7.8 mmol/l (140 mg/dl) has 89% sensitivity for diagnosing DM (even lower sensitivity for diagnosing pre-diabetes) and the risk of false negative results are high [5]. This becomes even more important among patients with TB as they have a higher chance of DM. The public health implications of reducing the RCBG screening cut off are summarized in Table 1.

If we use the RCBG cut off ≥7.8 and ≥6.1 mmol/l as the screening criterion, approximately 10% and 60% of the patients with TB will screen positive respectively. [5] The latter would translate to 1,050,000 requiring confirmatory tests (fasting and 2 h post glucose load tests) annually among notified TB in the country: six times higher (875,000 additional confirmatory tests) than using the RCBG cut off ≥7.8 mmol/l [6] (Table 1).

Assuming a 13% prevalence of DM among TB in India, there are 227,500 TB-DM among the notified patients with TB [7]. With a RCBG cut off ≥6.1 mmol/l, we expect to detect most of them. While, with a RCBG cut off ≥7.8 mmol/l, 11% of those screened positive (11% of 175,000) will be negative on confirmatory tests: 155,750 will be detected with DM. We expect to miss 71,750 TB-DM among the notified patients with TB (Table 1).

The costs of missing TB-DM are very high: delayed sputum conversion, more MDR-TB, more relapse and more mortality. If we are of thinking of zero TB deaths and ending TB, then reducing RCBG cut off to ≥6.1 mmol/l is one of the ways. India should invest in 875,000 additional confirmatory tests to detect additional 71,750 patients with TB-DM (12 additional confirmatory tests for one additional patient with TB-DM by reducing RCBG cut off). This has to be coupled with investments in monitoring with indicators related to DM screening/confirmation among notified TB within the programme.

For confirmatory diagnosis of DM, the national guidelines mention the use of fasting and 2 h post glucose load tests, but do not mention whether venous or capillary blood has to be used [4]. If it is a venous sample, it is not clear whether blood or plasma has to be used. In fasting state, capillary blood glucose is equal to venous blood glucose. In non-fasting state, capillary blood glucose is higher than venous blood glucose. Also, plasma glucose levels are relatively higher than blood glucose [8–10]. Capillary blood glucose may therefore not be a replacement for venous plasma glucose which is mandatory for diagnosis. Hence, while the

Table 1
Public health implications of reducing the random capillary blood glucose (RCBG) screening cut off (from ≥7.8 mmol/l to ≥6.1 mmol/l) for screening of DM among patients with TB in India.

Sno	Indicator	Number	Comments
i.	Number of estimated TB cases [1]	2, 800, 000	–
ii.	Number of notified TB cases [6]	1, 750, 000	–
iii.	Number of estimated patients with TB-DM among notified TB cases [7]	227, 500	13% of (ii.)
iv.	Number of confirmatory tests required annually if RCBG cut off ≥7.8 mmol/l[a] [5]	175, 000	10% of (ii.)
v.	Number of confirmatory tests required annually if RCBG cut off ≥6.1 mmol/l [5]	1, 050, 000	60% of (ii.)
vi.	Additional number of confirmatory tests required annually if RCBG cut off decreases from ≥7.8 mmol/l to ≥6.1 mmol/l	875, 000	(v.) minus (iv.)
vii.	Number of patients with TB-DM detected if RCBG cut off ≥6.1 mmol/l is used followed by confirmatory tests	227, 500	Almost all estimated TB-DM among notified TB
viii.	Number of patients with TB-DM detected if RCBG cut off ≥7.8 mmol/l is used followed by confirmatory tests[b] [5]	155, 750	Of (iv.), 11% will be negative for DM on confirmatory tests
ix.	Number of patients with TB-DM missed due to RCBG cut off ≥7.8 mmol/l	71, 750	(vii.) minus (viii.)

[a] 10% interpreted from data presented in the study [5].
[b] Of those with RCBG ≥ 7.8 mmol/l, 89% are positive for DM based on confirmatory test [5].

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diagnostic cut offs provided do match with international guidelines, they are actually meant for plasma and not for blood samples [11].

Further, the diagnostic criteria for confirmation of DM have not been clearly spelt out. In the absence of a clear clinical diagnosis (classic symptoms of hyperglycaemia and random plasma glucose ≥11.1 mmol/l (200 mg/dl)), a second test is required for confirmation of DM i.e., i) both fasting plasma glucose (FPG) and 2-h plasma glucose (2-hPG) are above the cut off (FPG ≥7.0 mmol/l (126 mg/dl) and 2-hPG ≥11.1 mmol/l (200 mg/dl)) or ii) either FPG or 2-hPG is above the cut off and the repeat value is also above the cut off [11]. The public health implication of using a standard confirmatory diagnosis criterion is that we will get reliable and valid numbers of TB-DM among patients with TB in India.

For uniform implementation across the country, the guidelines have to be clear and specific with no scope for misinterpretation. For better clarity, we recommend the following: i) mention RCBG instead of RBG and FCBG instead of FBG; ii) the RCBG cut off for screening should be ≥6.1 mmol/l; criteria for confirmatory diagnosis of DM should be specified; and type of blood sample to be used for confirmatory tests for DM should be mentioned.

Authors' contribution

HDS and AMVK conceived the idea; HDS prepared the first draft; all authors provided critical comments and approved the final draft.

Conflict of interest statement

There is no conflict of interest

Ethics committee approval

Not applicable

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RESEARCH ARTICLE

Comparing yield and relative costs of WHO TB screening algorithms in selected risk groups among people aged 65 years and over in China, 2013

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Data Availability Statement: Data cannot be publicly shared due to restrictions imposed by The Institutional Review Board of Chinese Center for Disease Control and Prevention. In the signed Informed Consent Form of the primary study, there is a Confidentiality Clause saying for each participant: The investigation records and results of health examination will be kept secret. Without your agreement, no individual-level data can be publicly shared. Data requests may be sent to corresponding author Dr. Jun Cheng

Abstract

Objective

To calculate the yield and cost per diagnosed tuberculosis (TB) case for three World Health Organization screening algorithms and one using the Chinese National TB program (NTP) TB suspect definitions, using data from a TB prevalence survey of people aged 65 years and over in China, 2013.

Methods

This was an analytic study using data from the above survey. Risk groups were defined and the prevalence of new TB cases in each group calculated. Costs of each screening component were used to give indicative costs per case detected. Yield, number needed to screen (NNS) and cost per case were used to assess the algorithms.

Findings

The prevalence survey identified 172 new TB cases in 34,250 participants. Prevalence varied greatly in different groups, from 131/100,000 to 4651/ 100,000. Two groups were chosen to compare the algorithms. The medium-risk group (living in a rural area: men, or previous TB case, or close contact or a BMI <18.5, or tobacco user) had appreciably higher cost per case (USD 221, 298 and 963) in the three algorithms than the high-risk group (all previous TB cases, all close contacts). (USD 72, 108 and 309) but detected two to four times more TB cases in the population. Using a Chest x-ray as the initial screening tool in the medium risk group cost the most (USD 963), and detected 67% of all the new cases. Using the NTP definition of TB suspects made little difference.

(chengjun@chinatb.org). According to the management requirements of the IRB of China CDC, data requests should be sent to the corresponding authors, then the authors apply to the IRB for data sharing to interested readers.

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Competing interests: The authors have declared that no competing interests exist.

Conclusions

To "End TB", many more TB cases have to be identified. Screening only the highest risk groups identified under 14% of the undetected cases,. To "End TB", medium risk groups will need to be screened. Using a CXR for initial screening results in a much higher yield, at what should be an acceptable cost.

Introduction

Tuberculosis (TB) is still a major global health problem and has been identified in the Sustainable Development Goals as one of the major diseases to be eliminated by 2030. Recent estimates from World Health Organization (WHO) give the global prevalence of TB as 174/100,000 and the incidence as 133/100,000 [1].

Despite China having a much lower prevalence (89/100,000) and incidence (68/100,000), there were still 1,200,000 TB cases and 930,000 incident TB cases in 2015. This accounted for almost 10% of the estimated new cases worldwide [1]. China therefore has one of the highest burdens of TB globally.

Passive case finding (PCF) and treatment of diagnosed TB disease are currently the principal means globally and in China, of controlling transmission of *Mycobacterium tuberculosis* and reducing TB incidence [2,3]. The standard PCF approach has not been successful in detecting all cases and globally it has been estimated that nearly 37% of new TB cases are undiagnosed or not reported [1].

Active case finding (ACF) is believed to contribute to the earlier detection of persons with TB and an earlier initiation of treatment, and to result in better outcomes for individuals with reduced transmission in the community [4–6]. Almost all ACF interventions rely on sputum smear-microscopy as the basis for diagnosis; but there is also growing evidence that screening through the use of chest radiographs is both effective and cost-effective in high-burden settings [7,8].

The results of most ACF studies show a predictable rise in the number of TB cases identified [9,10]. However, the individual and community-level benefits from active screening for TB disease remain uncertain, and the benefits of earlier diagnosis on patient outcomes and ongoing TB transmission have not yet been established [11].

WHO recently published operational guidelines on systematic screening for active tuberculosis [12,13]. These have been developed for use in settings with different diagnostic resources, and they acknowledge that the yield (number of new cases of PTB found by each screening algorithm) will vary depending on the prevalence of undiagnosed TB and will be greater in subgroups at higher risk of TB. The prioritization of high risk groups for screening should be based on potential benefits and harms, the feasibility of the initiative, the acceptability of the approach, the number needed to screen, and the cost of screening[12,13].

In China, the population of persons aged 65 years and over is rapidly expanding. They are at increased risk of TB due to a longer exposure to infection with *Mycobacterium tuberculosis* and declining immunity with age which allows latent infection to reactivate and cause disease [14,15]. Those 60 years and over have a high prevalence of TB (349/100,000). This is 2.6 times higher than those aged 45 to 59 [16]. Symptoms of TB may be non-specific or absent, and attendance at health facilities may be erratic [17].

The aim of our study was to compare four screening algorithms for TB in persons aged 65 years and over. Using data from a TB prevalence study conducted in China, we sought to

identify the risk groups with the highest yield and determine the relative costs of the different algorithms.

Methods

Ethical considerations

The prevalence study was reviewed and approved by the Institutional Review Board of Chinese Center for Disease Control and Prevention before commencing data collection. This study using data collected in the prevalence study, was approved by the Ethics Advisory Group of the International Union against Tuberculosis and Lung Disease, Paris, France.

Study design

This was an analytic study based on data from a cross-sectional study.

Study setting

China has a population of 1.37 billion, of whom 10.1% are people aged 65 years or over, and a GDP per capita of $7590 [18]. The level of affluence and urbanization varies greatly across the country.

There is a National TB control program (NTP) which develops the national protocols for detection and treatment of TB. The diagnosis of TB, including microscopy and X-ray examination, first-line anti-TB drugs and DOTS are all offered free of cost.

Study population

All persons aged 65 years and over who were interviewed in the TB prevalence study were included in the study.

TB prevalence survey

A TB prevalence survey in adults was conducted in 2013, the results of which will be published in full elsewhere. Sample size was estimated using a method appropriate to estimate a single population proportion. The 369/100,000 prevalence of bacteria-positive PTB among elderly people ($\geq$65 years) from the latest national tuberculosis prevalence survey was used as a reference. 95% confidence level and 0.2 allowable error were assumed. The formula was $n = pq/(d/z_\alpha)^2$ (p = 369/100,000, q = 1-p, d = 0.2p,α = 0.05, Z_α = 1.96,). A total of 25,931 elderly participants were requested. In consideration of 10% non-response, sample size should be 28,812.

Multi-stage cluster sampling was used, and the procedure of sampling is shown in Fig 1. 27 study fields (10 townships and 17 communities) from 10 counties of 10 provinces were selected. The 10 counties are shown in Fig 2. The chosen number of townships or communities of each county depended on the population of aged people. Finally, 38,888 participants were included in the study.

Within this survey, each resident aged over 65 years was interviewed by trained staff face-to-face in their home using a standard questionnaire. Data on participants' sex, age, marital status, education, past medical history, occupation, tobacco and alcohol use were collected. Their height and weight were used to calculate their Body Mass Index (BMI).

All participants were invited to have a full-size chest X-ray (CXR). Participants who had any suspected TB symptoms (cough for over 2 weeks or haemopytsis) or abnormal lung field shadows on CXR, were requested to submit 3 sputum samples (morning, night and spot sputum). The sputum samples were submitted and examined by smear microscopy for acid-fast

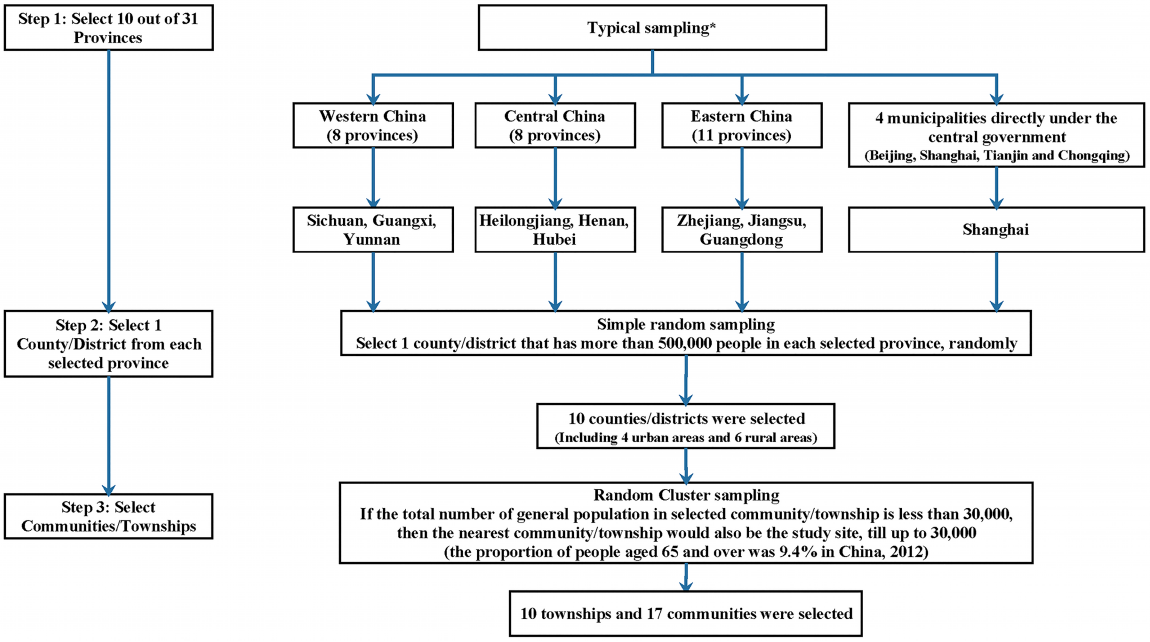

Fig 1. The sampling procedure of the TB prevalence survey in China in 2013. (*) 10 out of 31 provinces were selected, by considering the cooperative willingness, human resources and related abilities of each province.

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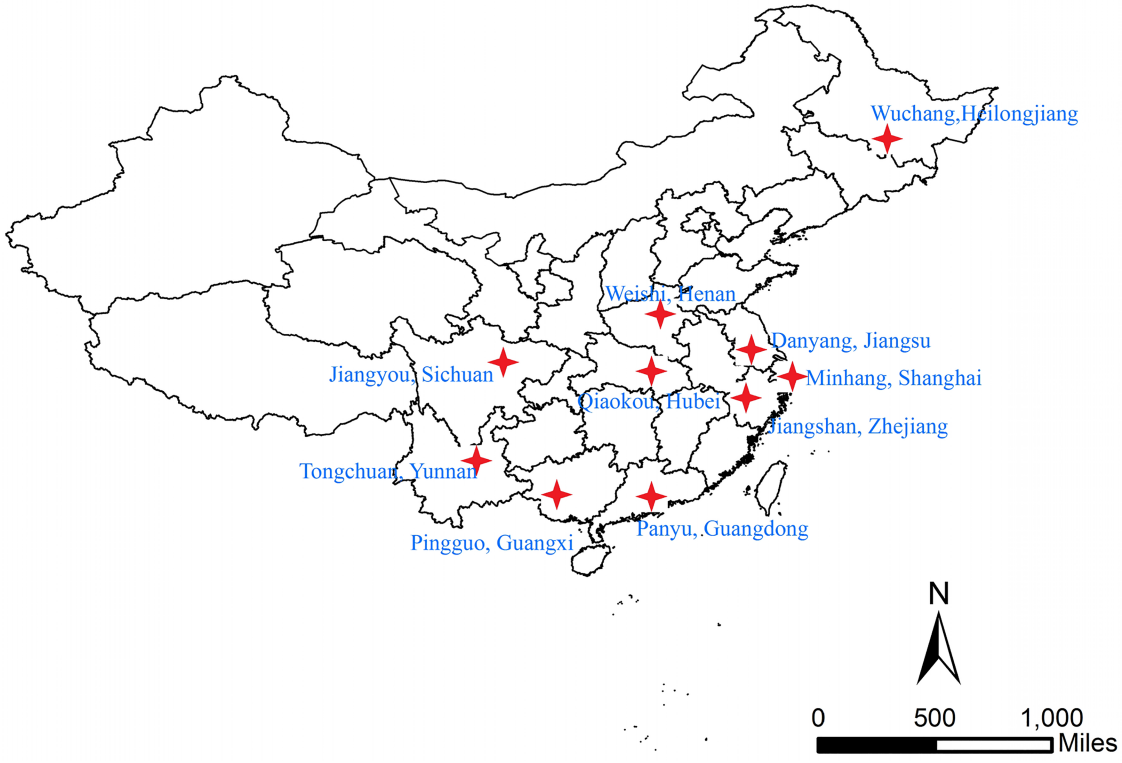

Fig 2. The location of 10 sample counties in the TB prevalence survey in China in 2013.

https://doi.org/10.1371/journal.pone.0176581.g002

Table 1. Cost of each component of active TB case-finding in this study in China in 2013.

Contents	Cost per unit (USD)
Household primary screening by village health workers	0.15
Chest X-ray	9.0
Sputum smear	3.9
Sputum culture	4.8

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bacilli and were assessed for mycobacterial culture using the solid Löwenstein—Jensen medium.

The cost of each component of the screening process is shown in Table 1. The cost of the household visits was based on the additional daily allowance, agreed nationally for work relating to infectious diseases, divided by the average number of households visited in a day. The price of a CXR, sputum smear and culture was the average market price paid.

Pulmonary TB was diagnosed as sputum smear-positive and/or culture positive, or it was diagnosed on CXR based on a decision by a group of clinical doctors and radiologists [16]. Quality checks were done according to the National Guidelines [19].

The data collected in the survey were double entered using an online input system developed by a local software company.

TB screening algorithms

WHO has published three different algorithms for use depending on the risk groups and the diagnostic resources available [12]. These WHO algorithms (A1, A1b, A2 and A3) are shown in Table 2. Algorithm A1b is similar to A1 but is altered to reflect national policy in China which is to screen those with cough for more than 2 weeks and/or haemoptysis, rather than cough alone.

The high risk groups [12] for TB identified from the literature are shown in Table 3.

Table 2. Algorithms to screen the population for TB aged 65 or over in the different high risk group in this study, based on the WHO recommendations.

Algorithms	Intervention 1	Intervention 2	Intervention 3	Intervention 4
WHO A1	Interview	CXR	Smear	Culture
	If cough lasting > 2 weeks, then	If positive, then	If positive = TB	If positive = TB
			If negative, then	If negative, then possible clinical diagnosis with CXR
WHO A1b	Interview	CXR	Smear	Culture
	If cough lasting > 2 weeks &/or haemoptysis, then	If positive, then	If positive = TB	If positive = TB
			If negative, then	If negative, then possible clinical diagnosis with CXR
WHO A2	Interview	CXR	Smear	Culture
	If any TB symptoms(cough of any duration, haemoptysis, weight loss, fever, night sweats), then	If positive, then	If positive = TB	If positive = TB
			If negative, then	If negative, then possible clinical diagnosis with CXR
WHO A3	CXR	Smear	Culture	NA
	If positive, then	If positive = TB	If positive = TB	
		If negative, then	If negative, then possible clinical diagnosis with CXR	

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Table 3. Definition of high risk factors for TB used in this study.

Previous TB cases: registered in TB Management Information System, and finished treatment or cured.
HIV/AIDS: registered in local CDC database.
Known Diabetes: recorded on the Citizen Health Management Files as diagnosed with Diabetes, plus those using medicine to control Blood glucose by self-report.
Close Contacts: living with new active PTB case for at least 7 days in the three months before diagnosis.
BMI<18.5: Weight (kg)/Height2 (m2) <18.5.
Tobacco use: ever smoked tobacco by self-report.
Drinking history: drinking more than one unit (21 grams pure alcohol) per week.

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Data analysis

We obtained the point prevalence (number of missing TB cases detected/ population screened) data from the TB prevalence study and transferred this into our electronic database. For each algorithm and for different high risk groups, we calculated the yield of TB screening.

Yield = number new TB cases (smear-positive PTB, culture-positive PTB and active TB)

Number needed to screen to detect one case (NNS) = total number screened / number of cases identified

The costs of each of the algorithms were applied to the number of TB cases diagnosed to give indicative costs per case of TB detected: this was done by dividing the relative total cost of the tests in the algorithm by the number of new cases of TB identified.

All tests were performed using SAS 9.3 (SAS Institute Inc., USA).

Results

From the TB prevalence survey, there were 38,888 eligible people aged 65 years and over in the ten sample areas. Demographic characteristics are shown in Table 4.

Nineteen people were excluded as they were known TB cases under treatment. 4,619 refused to participate. 34,250 (88.1%) agreed to participate in this study. Of those who agreed to participate 33,510 (97.8%) had a chest X-ray, and 1,534 submitted sputum for smear and culture.

The number of people diagnosed with TB by smear and/or culture is shown in Table 5. There were 172 new TB cases, of which 116 were diagnosed only by CXR and clinical diagnosis.

Table 4. Demographic characteristics of the population aged 65 or over in the sample population in China in 2013.

Characteristics	No.	%
Total	38,888	100.0
Sex		
Male	18,005	46.3
Female	20,883	53.7
Age group		
65–74	24,102	62.0
75–84	12,193	31.3
85-	2,593	6.7
Place of residence		
Urban	13,533	34.8
Rural	25,355	65.2

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Table 5. Total number of new TB cases found in the study population in China in 2013 and how diagnosed, smear positive TB and/or culture positive TB, or CXR and clinical alone.

		culture		Total
		+	-	
Smear	+	23	8	31
	-	25	116	141
Total		48	124	172

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The number, yield, and prevalence of new TB cases, in each risk group is shown in Table 6. The prevalence of new TB cases in males was 3 times higher than in females. The prevalence rates in "previous TB" and "close contacts" were very high, 3,698 and 3,192/100,000 respectively. Also, the groups "BMI<18.5" and "Tobacco use" had high prevalence of new TB cases. For all risk groups except "BMI<18.5", the new TB case prevalence in "rural areas" was 2 to 3 times higher than that in "urban areas"

Two specimen groups at increased risk of TB were identified to run the WHO algorithms. Group 1 "medium risk" was a group of 12,006, with a prevalence (between 936/100,000 and 4,651/100,000). This medium risk group comprised those living in a "rural area", who were "men" or a "previous TB case", or were a "close TB contact" or a "BMI <18.5" or "tobacco users".

Group 2 "high risk" totalled 668 people, comprised the groups with the highest prevalence (over 3,000/100,000), which were all "previous TB cases" and all "close TB contacts". (Table 7)

The numbers of each tests used in the screening algorithm, number of new TB diagnosed and relative cost per case for each group are shown in Table 8.

The yield for algorithms WHO 1, 1b and 2 increased slightly from 15% (25/172) to 17% (29/172) in the medium risk group with cost per case increasing from $221 to $298 and was unchanged in the high risk group, 7% (12/172) at a cost of between $72 and $108 per case. For all aged 65 and over, algorithms WHO 1, 1b and 2 found 18% (31/172) to 22% (37/172) at a cost of between $330 and $458 per case.

Table 6. Number in each risk group, number of new TB cases diagnosed and, prevalence of new TB cases, in the prevalence survey China, 2013.

Groups	Total			Urban areas			Rural areas		
	No. in risk group	No. of TB diagnosed	Prevalence of new TB cases (1/100,000)	No. in risk group	No. of TB diagnosed	Prevalence of new TB cases (1/100,000)	No. in risk group	No. of TB diagnosed	Prevalence of new TB cases (1/100,000)
All aged 65 and over	34250	172	502	12932	34	263	21318	138	647
Previous TB	595	22	3698	251	6	2390	344	16	4651
Close contacts	94	3	3192	20	0	0	74	3	4054
BMI<18.5	3632	39	1074	931	9	967	2701	30	1111
Tobacco use	6763	55	813	2168	12	554	4595	43	936
Male	16044	129	804	6044	25	414	10000	104	1040
Alcohol use	6543	40	611	1907	6	315	4636	34	733
Diabetes	2400	14	583	1306	3	230	1094	11	1006
Female	18206	43	236	6888	9	131	11318	34	300
HIV/AIDS	1	0	0	0	0	—	1	0	0

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Table 7. Risk group 1 and 2, and yield, and prevalence of new TB cases, for each group.

Groups	No. in risk group	No. of new TB cases diagnosed	Prevalence of new TB cases (per 100,000)
medium risk group 1*	12006	119	991
high risk group 2**	688	25	3,634

*Group 1 medium risk: Living in a rural area and male, or previous TB, or close contacts, or BMI<18.5, or tobacco use

**Group 2 high risk: previous TB or close contacts.

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WHO A3 diagnosed 67% (116/172), 14% (24/172) and 95% (164/172) of new TB cases respectively in two risk groups and all aged 65 and over, which were two and five times as many new TB cases as the other three algorithms, but the cost per case was three to five times higher at $963, $309 and $1,881 per case respectively.

Discussion

This is the first study that has applied the WHO TB algorithms to population data of a country. The study discussed the yield and relative cost per case when each algorithm was applied on a population aged 65 years and over.

The prevalence survey demonstrates the difficulty of screening 100% of a population. Only 88% agreed to participate and of those a small proportion did not attend to have a CXR.

Using the WHO algorithms, the NNS and the cost per case detected, varies depending on the prevalence in risk groups and which algorithm is used. But the lower cost per case detected may leave up to 93% of new cases undetected.

Using CXR as the first screening test as in algorithm WHO A3 in the medium risk group detects a much higher proportion of the new TB cases in the whole population (116/ 172) than the other algorithms (25 to 29/172) at a cost of $963 per case.

The strengths of this study were that it used a large dataset collected as part of a carefully designed and implemented survey, which used the current TB diagnostic protocols and tests in China for diagnosis. Using real data to model the WHO algorithms showed how they work in practice. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [20] and sound ethics principles for the conduct and reporting of this study [21].

The study had a few limitations. The costing data that was collected was basic and was only indicative of the relative costs of the different groups being screened. Only current occupation was recorded and most participants were retired, thus risk groups based on previous occupation could not be identified. The original prevalence survey identified 172 new cases of TB and this has been used in this study. This is likely be incorrect for several reasons 1) the reported sensitivity of CXR as a screening tool is 87% and the specificity 89% [12]. 2) There will also be false positives included particularly as 67% of the new diagnoses were not confirmed by smear or culture. 3) Only 88% of the target population was screened. In our study, no modeling of screening of people living with HIV was possible, as in the data there was only one person who was living with HIV.

Screening the high risk group only, of "previous TB cases" and "close contacts", gives a low cost per case. Studies from Africa and Cambodia also confirmed it was cost-effective to implement ACF among close contacts but the proportion of undiagnosed cases detected in the population is only 7% [22,23]. If the End TB target is to be achieved this is not effective.

It is being argued that the Stop TB proposal of ACF costing $350 per case [24] is too low to enable enough TB cases to be identified to reduce the prevalence in the community. It has

Table 8. Number of tests to be taken, yield and cost per case for each algorithm of the medium risk group 1, high risk group 2 and all aged 65 and over.

Algorithms	Group 1*						Group 2**						All aged 65 and over					
	No. of CXR	No. of Smear	No. of Culture	No. of new TB diagnosed	NNS	Cost per case (USD)	No. of CXR	No. of Smear	No. of Culture	No. of new TB diagnosed	NNS	Cost per case (USD)	No. of CXR	No. of Smear	No. of Culture	No. of new TB diagnosed	NNS	Cost per case (USD)
WHO A1	366	74	62	25	481	221	59	31	25	12	58	72	611	103	90	31	1,105	330
WHO A1b	386	79	67	26	462	221	61	32	26	12	58	74	643	110	97	32	1,071	331
WHO A2	683	107	94	29	414	298	97	41	35	12	58	108	1313	153	138	37	926	458
WHO A3	11953	551	531	116	104	963	677	160	154	24	29	309	33510	989	959	164	209	1,881

*Group 1 medium risk: Living in a rural area and male, or previous TB, or close contacts, or BMI<18.5, or tobacco use.

**Group 2 high risk: previous TB or close contacts.

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been suggested that $1000 per case is more realistic [24] and is more similar to the cost and benefit of ART.

Use of WHO algorithm 3 in China would detect 67% of the new cases in the elderly community, at a crude cost of under $1000. The actual cost will be higher, but if the screening was set up as a large scale program the cost per test may well markedly reduce, for example with increased use of digital CXR [25].

The National Project of Basic Public Health Service launched by Ministry of Health in 2011 has made it easier for China to implement ACF [26]. In this project, all elderly people have an annual interview and physical examination, and the information recorded in the citizen health management file. This means that high risk groups as in Table 2 can be identified from routine data, and ACF can be combined with the annual physical examination. A pilot study in China, which integrated TB screening into annual health examinations for the rural elderly, and targeted diabetes patients and close contacts, had a significant yield. But no TB case was identified from close contacts alone [27].

China NTP used a different definition of TB symptoms from those in the WHO algorithm in that it uses "cough for 2 weeks or more, and haemoptysis". This was used in the algorithm WHO A1b. The study results found there was little difference in the number of cases detected from using cough alone. Three more cases were identified when weight loss, fever and night sweats were added in algorithm 2. This shows that it is not necessary to change the nationally agreed TB symptoms used for screening.

Algorithm WHO A1 and WHO A2, using symptoms as the initial screening, will miss many undiagnosed TB cases when implemented in China, but it can still be used in some resource-limited areas, such as Western China.

This study has implications for other TB high burden countries which are also resource-limited, such as India and Indonesia. Choosing the optimal ACF strategy depends on the TB prevalence, economics, and human resources, etc. and it needs to fit with local health policies and available technology. This study has shown how the yield varies greatly and higher costs may need to be accepted in order to have an impact on the burden of TB.

To achieve the ambitious targets of ending the TB epidemic by 2035, ACF screening has to be implemented more widely.

Conclusions

WHO recommends that indiscriminate mass screening should be avoided, and the prioritization of risk groups for screening should be based on the prevalence of new cases [12]. Knowing the expected prevalence of TB in risk groups enables appropriate targeting of screening and in China risk groups can be identified from routine data. The cost per diagnosed case, and NNS increases as the prevalence reduces. However if just the highest risk groups are screened, only between 7 and 14% of the undetected cases will be found, depending on the algorithm used. To "End TB", appropriate medium risk groups will need to be screened. To obtain the highest yield, a CXR should be used for initial screening, as in WHO Algorithm 3.

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ORIGINAL RESEARCH

Competency-based tool for evaluation of community-based training in undergraduate medical education in India – a Delphi approach

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Introduction: A community-based training (CBT) program, where teaching and training are carried out in the community outside of the teaching hospital, is a vital part of undergraduate medical education. Worldwide, there is a shift to competency-based training, and CBT is no exception. We attempted to develop a tool that uses a competency-based approach for assessment of CBT.

Methods: Based on a review on competencies, we prepared a preliminary list of major domains with items under each domain. We used the Delphi technique to arrive at a consensus on this assessment tool. The Delphi panel consisted of eight purposively selected experts from the field of community medicine. The panel rated each item for its relevance, sensitivity, specificity, and understandability on a scale of 0–4. Median ratings were calculated at the end of each round and shared with the panel. Consensus was predefined as when 70% of the experts gave a rating of 3 or above for an item under relevance, sensitivity, and specificity. If an item failed to achieve consensus after being rated in 2 consecutive rounds, it was excluded. Anonymity of responses was maintained.

Results: The panel arrived at a consensus at the end of 3 rounds. The final version of the self-assessment tool consisted of 7 domains and 74 items. The domains (number of items) were Public health – epidemiology and research methodology (13), Public health – biostatistics (6), Public health administration at primary health center level (17), Family medicine (24), Cultural competencies (3), Community development and advocacy (2), and Generic competence (9). Each item was given a maximum score of 5 and minimum score of 1.

Conclusion: This is the first study worldwide to develop a tool for competency-based evaluation of CBT in undergraduate medical education. The competencies identified in the 74-item questionnaire may provide the base for development of authentic curricula for CBT.

Keywords: competency-based education, questionnaire design, Delphi technique, community medicine, community education, India

Introduction

Community-based training (CBT) program is a vital part of undergraduate medical education (UGME) where teaching and training are carried out in the community outside the teaching hospital.[1–3] In India, CBT is managed by the Department of Community Medicine or Preventive and Social Medicine. CBT is offered from the first year of Bachelor of Medicine and Bachelor of Surgery (MBBS) with the objective of orienting the students to community-based health care services. Through CBT, students are trained in all 4 core disciplines of community medicine: family medicine, epidemiology, health promotion, and health management.[4]

In a developing country like India where the predominant section of population is in the villages and suburban areas, teaching in tertiary care hospitals alone does not equip the students with skills essential to work in the community. They have to be trained to work at all levels of health care delivery system. The Reorientation of Medical Education scheme, though not as successful as it was conceived to be, is one of the notable attempts to deliver CBT effectively.[5,6]

In the UGME scenario worldwide, there is a shift toward competency-based training, and the same is also recommended by an expert group commissioned by the World Health Organization.[7,8] Medical Council of India (MCI), the apex body which regulates medical education in India, has in its Vision 2015 document recommended a shift toward competency-based approach.[9] The change in the approaches to teaching also necessitates a change in the assessment methods used.[10]

The National Health Mission in India emphasizes the need for competent health care providers in rural areas. Unlike secondary or tertiary care systems, medical officers working under primary care system do not have the opportunity/privilege to work under experienced health care team. They have to be equipped with skills in clinical judgment and administration of the health center. Apart from this, they have to train their team of paramedical workers and frontline workers in the community. In this case, it is the responsibility of the medical education system to ensure that the candidates have acquired these essential competencies before they graduate and venture into the community on their own.

Currently, there are no competency-based assessment tools available for CBT. We attempted to develop a tool that uses competency-based approach for assessment of CBT. In this paper, we describe the development of a 74-item competency-based questionnaire using Delphi technique. The psychometric properties of the 74-item questionnaire and the development of an abridged 58-item self-assessment questionnaire using exploratory factor analysis are described elsewhere.[11]

Methods

Study setting

In India, medical graduation or MBBS is covered over 4 and a half years (9 semesters) followed by a year of internship. The subject community medicine is taught since first year till 7th semester through theory sessions and CBT. There are 3 clinical postings under CBT where the undergraduate students are posted in rural/urban health training centers each for 4 weeks. In addition to clinical postings, students also undergo Family Health and Advisory Programme during which they follow up a family (in the community they serve) allotted to them through weekly home visits. The students appear for a final theory and clinical/practical examination in community medicine at the end of the 7th semester. Then, the students are posted in the community as part of Compulsory Rotatory Residential Internship (CRRI) for a period of 2 months where they are expected to practice all the competencies gained.

Questionnaire development – Delphi technique

Based on a review on competencies to be acquired under a CBT program, we developed a conceptual framework using 6 core competencies after adapting it to Indian context and prepared a preliminary list of major domains with items under each domain.[12] While deciding on the items, we considered the exposure of the student to the competency during his/her training and the practical requirement of the competency in his/her day-to-day practice of community medicine in future. We had designed it as a self-rated questionnaire where the student will be required to rate his/her competencies. The rating scale for each item was a Likert scale ranging from "much above average" (score=5) to "much below average" (score=1).

We used Delphi technique to develop the questionnaire and arrive at a consensus.[13–16] We purposively selected a panel of experts (n=8) in community medicine. The prerequisites were that the expert should have a minimum of 3 years' experience in health service provision and/or teaching and training and/or research, post his/her postgraduation (MD) in community medicine. The list of members who constituted the expert panel is presented in Table 1. The principal investigator (HDS) facilitated the process but was not part of the panel. Email was the medium of communication with the experts. All experts were aware of the list of experts constituting the panel, but anonymity of the responses was maintained.

Each Delphi round spanned over 2 weeks. During the 1st week, the Delphi panel experts gave their comments. During the 2nd week, the facilitator compiled the comments. In the light of the comments, the questionnaire was revised and recirculated among the experts.

In round 1, we shared the preliminary draft of the questionnaire prepared by us and a rating sheet. Experts rated each item (close-ended response) in part I of the rating sheet and gave suggestions (open-ended) in part II. The facilitator requested the experts to rate each item (rating scale was between 0 [poor] and 4 [good]) under the heads of relevance, sensitivity, specificity, and understandability (Box 1). We sought suggestions to improve understandabil-

Table 1 Panel of experts for consensus building (Delphi technique) to develop competency-based self-assessment questionnaire in community-based training program

SN	Affiliation	Age/sex	Place	Years of post-MD teaching experience
1	Professor, Department of Community Medicine, Post Graduate Institute of Medical Education and Research (PGIMER)	59/male	Chandigarh, India	33
2	Professor, Department of Community Medicine, Sri Manakula Vinayagar Medical College (SMVMC)	41/male	Puducherry, India	13
3	Public Health Specialist, John Hopkins School of Public Health	36/male	Baltimore, MD, USA	10
4	Professor, Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER)	56/male	Puducherry, India	27
5	Assistant Professor, Indian Institute of Public Heath	37/male	New Delhi, India	10
6	Assistant Professor, Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER)	35/male	Puducherry, India	6
7	Assistant Professor, Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER)	34/female	Puducherry, India	8
8	Associate Professor, Department of Community Medicine, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences (NEIGRIHMS)	40/male	Shillong, India	10

Abbreviation: SN, serial number.

ity, language of items, and wording. Comments on adequacy and any additional items for inclusion were also welcomed. We requested the experts to also suggest alternate options for the response scale used in the questionnaire. At the end of each round, the median ratings received by each of the items were shared with the experts. The comments were also anonymously shared.

The iterative process continued up to the consensus point. Consensus was predefined as when 70% of the experts gave a rating of 3 or above for each item under relevance, sensitivity, and specificity (content validity). An item that did not achieve consensus was allowed to be rated again by the experts in the light of the compiled ratings and open-ended comments at the end of the previous round. If an item failed to achieve consensus after being rated in 2 consecutive rounds, it was excluded. Any item that achieved consensus was not allowed for rating again in the consecutive rounds. Where they had given poor rating for items under the head "understandability", we requested the experts for suggestions to improve the same.

Box 1 Operational definition of the terms used by the Delphi panel to rate each item in the questionnaire: sensitivity, specificity, relevance, and understandability.

Relevance: Item considered relevant if the given item is found appropriate and important in assessing the student's competency under the given domain.
Sensitivity: Item considered sensitive if it shall be rated high by those who possess the competency under the given item and vice versa.
Specificity: Item considered having good specificity if it exclusively measures the student's competency under the given domain.
Understandability: Item considered having good understandability if the wording is simple and unambiguous to the student evaluating himself/herself.

Pretesting

The draft of the questionnaire at the end of Delphi process was shared with 3 students (2 female, 1 male). He spent 45 minutes with each discussing the relevance; adequacy of concepts, language, and responses; understandability; and any other difficulties he/she faced in interpreting the questionnaire.

Data entry and analysis

The scores assigned to each item under the 4 criteria were entered into Microsoft Excel. Median ratings were calculated at the end of each round. The extent of consensus arrived at the end of each round was calculated as the percentage of experts assigning a score of 3 or more under each characteristic of an item.

Ethics

The Institute Research Committee of Indira Gandhi Medical College and Research Institute (IGMCRI), Puducherry, approved the study. Written informed consent via email was obtained from each of the Delphi panel experts before including them in the panel.

Results

Questionnaire development

All eight experts participated in each round of Delphi. The preliminary draft of the questionnaire had 6 domains namely "Public health", "Family medicine", "Cultural competence", "Community development and advocacy", "Research and evidence-based practice", and "Generic competence" and 81 items.

The details of the modification of the tool in each of the Delphi rounds are presented in Table 2. At the end of round 1, 59 out of the 81 items had attained consensus. Nineteen new items were suggested. So, a total of 41 items were presented for rating by the expert group in the second round along with the compiled ratings and open-ended comments (blinded) from the previous round. There was rearrangement of items under some domains and rephrasing of certain items as suggested by the experts. Two domains namely "Public health" and "Research and evidence-based practice" were regrouped to form 3 domains namely "Public health – epidemiology and research methodology", "Public health – biostatistics", and "Public health administration at PHC level".

Table 2 Details of the modification of the tool in each of the Delphi rounds

Characteristics	Baseline	Round 1	Round 2	Round 3 (final)
Number of items that were presented for rating	81	81	22	0
New items added	NA	19	0	0
Number of items that were presented for re-rating	NA	NA	19	9
Total number of items for which consensus was achieved	NA	59	59+14 (4 out of 22 and 10 out of 19) =73	73+1
Number of items deleted	NA	0	18	8
Number of domains at the end of the round	6	7	7	7
Number of items at the end of the round	81	100	82	74
Any other	NA	Domains rearranged*	Changed to modified Miller's response scale	–

Note: *Two domains, namely "Public health" and "Research and evidence-based practice" were regrouped to form 3 domains namely "Public health – epidemiology and research methodology", "Public health – biostatistics", and "Public health administration at primary health center level".
Abbreviation: NA, not applicable.

At the end of round 2, 4 out of the 22 items rated for the second time achieved consensus leading to deletion of the remaining 18 items. Out of the 19 newly added items, 10 had achieved consensus. The remaining 9 were presented for re-rating in round 3. No new items were added in round 2. The response scale was changed to a modified form of Miller's response scale as suggested by the Delphi panel. The final response scale was an adaptation of the Miller's triangle to assess competency: "don't know", "know", "know how", "show how", and "do".[17] Miller's triangle, which may be applied as a part of Objective Structured Clinical Examination (OSCE) for rater assessment, was adapted and used in this self-assessment questionnaire. Each item in the questionnaire under all domains except "Generic competence" had this response scale. The domain "Generic competence" had the following response scale: "strongly agree", "agree", "neither agree nor disagree", "disagree", and "strongly disagree".

At the end of round 3, only 1 out of the 9 items had achieved consensus. The remaining 8 items were excluded. Like in round 2, no new items were added during round 3. Hence, the Delphi process came to a conclusion at the end of 3 rounds.

The final questionnaire prepared after completion of Delphi process is presented in Figure S1. All excluded items that did not achieve consensus are listed in Table S1. The questionnaire had 7 domains and 74 items. The domains (number of items) were "Public health – epidemiology and research methodology" (13), "Public health – biostatistics" (6), "Public health administration at PHC level" (17), "Family medicine" (24), "Cultural competencies" (3), "Community development and advocacy"(2), and "Generic competence" (9). Each item was given a maximum score of 5 and minimum score of 1. Higher score indicated better skill score for each item in the questionnaire. Hence, maximum and minimum possible scores for a student were 370 and 74, respectively.

There were no significant changes made to the questionnaire after pretesting with the students.

Discussion

This is the first study from India and worldwide to develop a tool for competency-based evaluation of CBT in UGME. Drawing from an existing conceptual framework, we designed the preliminary draft of the questionnaire. Though various inventories of competency classifications are available,[18–26] we have based our tool on a conceptual framework specific to CBT.[12] This draft evolved through 3 rounds of Delphi into the final version composed of 7 domains and 74 items.

Appropriateness of Delphi technique

With increasing use of Delphi to address different research questions, there have been variants of the classical Delphi, thus necessitating the term "Delphi techniques" or the "Delphi approach".[27,28] Fink et al have prescribed a clear "decision trail" as one of the key goodness criteria to judge the credibility of the evidence generated by Delphi.[29]

Delphi technique is considered as a structured way of assessing and synthesizing/combining human judgment. Rowe et al state that Delphi can be used when the researcher is convinced that the technique will generate more accurate assessments and judgments compared to that provided by individuals.[30] Delphi technique has also been used in UGME scenario worldwide.[31–33] We resorted to Delphi technique given that our objective was to develop a comprehensive tool for competency-based evaluation of CBT.

Delphi technique limits the inhibition that the participants may face in other informal group situations by promising anonymity. Thus, the technique encourages the expert to offer his/her frank and candid opinion(s) which is termed as "process gain".[30] We are quite sure that we would not have been able to achieve this using any other technique given the issues of seniority, interfering or inhibiting personality traits that are quite evident in other face-to-face meetings of experts.

We understand that Delphi does not offer a fool-proof solution to these issues. But it does circumvent these to a great extent. Hence, we had chosen Delphi as one of the steps in the development of the tool. Delphi was preceded by the use of a conceptual framework to develop a preliminary draft derived from review of literature in the field. After Delphi, the tool was informally discussed with students to obtain their feedback on the tool. This was followed by psychometric analysis to assess the validity and reliability of the tool, the results of which are reported elsewhere.

Recruitment of Delphi panel

We recruited a heterogeneous group of experts from across the country to contribute toward the development of the same. We aimed to draw from all their knowledge and experience while also achieving consensus. Given that there is no prescribed minimum size of the panel, we recruited 8 experts.[13]

Data collection procedures

We had adhered to all the 4 essential prerequisites of a Delphi technique namely anonymity of participants, iteration, controlled feedback, and statistical aggregation of the results of the rounds. Our panelists could modify their judgment based on feedback without being influenced by others in the group.[13,30] Although anonymity is reported to cause a lack of accountability of one's views, we believe that it is not a major drawback in our study as the outcome of this study has direct application and relevance to the practice of experts themselves who were chosen based on their experience and expertise.

We encouraged qualitative feedback also, so as to not restrict the experts to rating the existing items in the tool. This qualitative feedback enabled us to reclassify certain items under the domains, rephrase certain items and domain names, add relevant items, and delete redundant items or items beyond the scope of the tool.

Means of implementation

The competencies identified in the 74-item questionnaire may provide the base for development of authentic curricula for CBT. In India, community medicine is taught from 1st semester to 7th semester: the period is divided as preclinical (1st–2nd semester), para-clinical (3rd–5th semester), and clinical (6th–7th semester). Competencies pertaining to CBT may accordingly be divided over the preclinical, para-clinical and clinical training periods, with clinical competencies under CBT covered in the 6th and 7th semester. Practice of these competencies, under supervision, is expected during the CRRI period. The tool may find its best application at the end of the CRRI period, though it may also be administered at the end of the 7th semester.

Of the 74 competencies in 74-item questionnaire, 41 competencies (55%) were pertaining to "Family medicine" or "Public health administration at PHC level". This draws our attention to the primary domains of focus for faculty of community medicine–family medicine and community health administration.[34] It is their primary role to impart knowledge and skills in these domains to the students, and faculties need to be sensitized and reoriented in this regard.[35–37]

Competency-based CBT is likely to face challenges in terms of curricula design, faculty training, student assessment, and systematic institutional change, all of which require sustained, long-term commitment.[38]

Limitations

There were some limitations. This is a self-rated questionnaire which captures a student's perception about his/her competencies. Currently, self-assessment or rating does not figure in UGME scenario in India. We propose to develop an instructor-/teacher-rated version of this tool which can be routinely employed in tandem with the existing assessment methods.

Conclusion

This tool can be seen as the sign of entering into the realm of competency-based assessment in community-based UGME in India. It is a valuable addition to the existing assessment methods in India and can guide experts in a need-based design of curriculum and teaching/training methodology.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Dear Student,

For each of the items listed under the various domains please choose an option from those listed against each of the items. We request you to leave no missing columns and share specific reason if you choose not to rate any particular item. Each item has a maximum score of five and a minimum score of 1. Interpretation of the options for each item is as follows:

Don't know: Have no knowledge

Know: Have knowledge; but don't know how to apply the knowledge

Know how: Know how to apply the knowledge

Show how: Confident in performing in a simulated environment – say in classroom practical or simulated settings

Do: Do independently in the complex situation of an everyday context and real life scenario

Rate yourself under the following competencies mentioned below (Tick in the appropriate box)

I. Public health – Epidemiology and research methodology

SN	Item	Do not know	Know	Know how	Show how	Do
1.	Frame a research question for a cross-sectional study					
2.	Choose an appropriate study design to answer your research question					
3.	Design a proforma/ questionnaire to collect data for your research					
4.	Calculate sample size for a prevalence study					
5.	Perform probability sampling methods and recruit a sample					
6.	Interpret probability and odds					
7.	Calculate relative risk and odds ratio					
8.	List possible confounders in a given research article					
9.	Classify services delivered at your PHC under different levels of prevention					
10.	Classify services delivered at your PHC under different modes of Intervention					
11.	Make a community diagnosis of your catchment population					
12.	Carry out investigation of a suspected outbreak					
13.	Presentation of research findings					

II. Public Health - Biostatistics

SN	Item	Do not know	Know	Know how	Show how	Do
1.	Classify variables					
2.	Calculate measures of central tendency					
3.	Calculate measures of dispersion					
4.	Interpret Z scores in WHO growth charts					
5.	Interpret *p* value in a given research article					
6.	Clinical and statistical significance of research finding					

Figure S1 (*Continued*)

III. Public Health Administration at PHC level

SN	Item	Do not know	Know	Know how	Show how	Do
1.	Inventory control					
2.	Supervision of PHC staff					
3.	Maintenance of cold chain in PHC					
4.	Maintenance of registers at a PHC					
5.	Preparing monthly reports generated from PHC					
6.	Calculate vital rates of your PHC population- birth/ death rates etc					
7.	Conducting PHC monthly meetings					
8.	Implementation of national health programmes like RNTCP, NVBDCP etc at your PHC					
9.	Lead a healthcare team					
10.	Calculate vaccine requirement for a PHC for a year					
11.	Calculate IFA requirement for a PHC for a year					
12.	Disposal of hospital waste at PHC level					
13.	Identify various triggers for diseases according to Integrated Disease Surveillance Programme (IDSP).					
14.	Interpret an IDSP report of a PHC- S, P, and L forms					
15.	Perform o-Toluidine test to assess chlorination in drinking water in your PHC area.					
16.	Organizing health camps at a village level					
17.	Organize health education session for community members					

IV. Family Medicine

How would do you rate your ability to provide preventive and primary health care for the following diseases /conditions/special groups at PHC level.

SN.	Item	Do not know	Know	Know how	Show how	Do
1.	Management of common morbidities of children under five years of age					
2.	Management of antenatal case					
3.	Management of postnatal mother					
4.	Management of normal newborn					
5.	Management of tuberculosis					
6.	Management of diabetes Mellitus					
7.	Management of hypertension					
8.	Management of anaemia					
9.	Management of adult with fever					
10.	Management of adult with acute respiratory infection					
11.	Management of RTI/STI					
12.	Management of scabies					
13.	Management of adult with diarrhea/dysentry					
14.	Screening for NCDs					
15.	Contraception advice/prescription					
16.	Immunization advice/prescription					
17.	Breast feeding counseling					
18.	Preparation of thick and thin blood smears for malaria					
19.	Reporting of sputum AFB smears					
20.	Perform minor procedures like incision and drainage/ dressing/suturing					
21.	Conduct of spontaneous vaginal delivery of pregnant women who are not high risk					
22.	Primary health care in emergency including cardio-pulmonary resuscitation in adult					
23.	School health services					
24.	Integrated Child Development Services (Anganwadi)					

Figure S1 (*Continued*)

V. Cultural competencies

SN	Item	Do not know	Know	Know how	Show how	Do
1.	Interpret how social and cultural factors affect health in your area					
2.	Design different interventions for different populations with creativity and flexibility					
3.	Communicate with people of a different cultural background					

VI. Community development and advocacy

SN	Item	Do not know	Know	Know how	Show how	Do
1.	Use of appropriate tools for advocacy* for problems in your community say, sanitary latrines, high prevalence of NCDs, child rearing practices etc.					
2.	Mobilize community resources for change					

*advocacy: The act of pleading or arguing in favour of something, such as a cause, idea, or policy; active support

VII. Generic competence

Kindly indicate your level of agreement with the following statements regarding you generic competencies

SN	Item	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
1.	I can communicate effectively with patients					
2.	I am not approachable to community members					
3.	I can communicate with peers with ease					
4.	I feel uncomfortable/ apprehensive when communicating with program managers/ policy makers					
5.	I can individually handle operational problems in my PHC					
6.	I will have to always consult others for any decisions related to patient care					
7.	I am not creative and prefer to follow the usual ways					
8.	I can critically analyse my skills as a primary care physician					
9.	I can innovatively use available technology for better health care delivery					

Figure S1 The 74-item competency-based self-assessment questionnaire for assessing community-based training of undergraduate medical students

Abbreviations: AFB, acid fast bacillus; IFA, iron folic acid; L, laboratory; NCD, non-communicable disease; P, presumptive; PHC, primary health center; RNTCP, Revised National Tuberculosis Control Program; NVBDCP, National Vector Borne Diseases Control Program; RTI, reproductive tract infection; S, syndromic; SN, serial number; STI, sexually transmitted infection; WHO, World Health Organization.

Table S1 Item(s) that did not attain consensus after 2 rounds of Delphi, and hence, were excluded from the questionnaire (n=26)

SN	Item
1	Frame null and alternate hypothesis for your research question
2	Look for bias in a given research article
3	Interpret percentiles in WHO growth charts
4	Making a social map
5	Evaluating beat schedule of health workers
6	Knowledge of registers at PHC
7	Fund raising
8	Accessing and using information for health
9	Appraise available scientific evidence
10	Write a research proposal
11	Skills in scientific writing
12	Presentation of research results
13	Team work with staff
14	Life table
15	Use of multimedia/PowerPoint
16	Utilization and management of RKS fund
17	Disaster relief
18	Patient empowerment
19	Conduct training of staff on different aspects of national programs
20	Conduct rapid survey assessment method like for immunization coverage
21	Integrated medicine
22	Self-care
23	Counseling skills especially for adolescent health problems
24	Primary health care (general OPD)
25	Ability to carry out a cross-sectional study (descriptive/analytical)
26	Ability to prepare microplan for IPPI or any special activity

Abbreviations: IPPI, intermittent pulse polio immunization; OPD, outpatient department; PHC, primary health center; RKS, Rogi Kalyan Samiti; SN, serial number; WHO, World Health Organization.

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Case Report

Snakebite Induced Thrombotic Microangiopathy Leading to Renal Cortical Necrosis

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Renal complications from snakebite result in high mortality and morbidity. Acute kidney injury (AKI) occurs in 5–30% of cases. Renal manifestation could include acute tubular necrosis, cortical necrosis, interstitial nephritis, glomerulonephritis, and vasculitis. We present a case of thrombotic microangiopathy (TMA) resulting in renal cortical necrosis. Renal biopsy showed fibrin thrombi in glomeruli and arterioles with cortical necrosis. Our patient progressed to end-stage renal disease.

1. Introduction

A 60-year-old lady was admitted to our centre 48 hours after an unknown snakebite, having been treated in a peripheral hospital with antisnake venom. There was no relevant previous medical history.

She was intubated on admission with a background of ongoing mucocutaneous bleeding and epistaxis. Endotracheal aspirate was bloody and melena was noted. Her urine output was negligible with frank haematuria. There was an area of cellulitis at the site of snakebite on the left foot. She was hypertensive with BP of 180/70, accompanied with peripheral and pulmonary oedema.

Haematological findings include haemoglobin of 7.1 g/dl, platelet of 33000/cu mm, WCC of 10300/cu mm, bilirubin of 3.47 mg/dl, direct bilirubin of 1.57 mg/dl, SGOT 676 IU/L, SGPT of 165 IU/L, and ALB of 2.5 g/dl with normal clotting. Creatinine level was 4.9 mg/dl with metabolic acidosis. She was transfused with packed red blood cells, fresh frozen plasma, and platelets. Steroids were started under the impression of an acute interstitial nephritis and she was commenced on haemodialysis.

Ultrasound showed a right kidney of 10.3 cm, left kidney of 9 cm, bilateral pleural effusion, dilated IVC, and hepatic veins associated with ascites.

Renal biopsy revealed renal cortical necrosis with segmental necrosis and luminal thrombotic occlusion in the arteries and arterioles (Figures 1 and 2). Further blood tests revealed an unresolved thrombocytopenia and a blood film showing fragmented red blood cells, suggesting microangiopathic haemolytic anaemia (MAHA). The clinical presentation was therefore consistent with thrombotic microangiopathy (TMA).

LFT normalised on day 5 and haematological parameters including platelets normalised on day 8 with blood pressure control. Renal function did not improve, requiring long-term maintenance haemodialysis, and she was discharged with an arteriovenous fistula.

2. Discussion

TMA is characterised by the triad of acute renal failure, thrombocytopenia, and MAHA. At present, there is paucity of literature on TMA following snakebites in contrast to venom-induced consumptive coagulopathy (VICC), a commoner and well-known haematological complication of snakebites [1–3]. This is probably because TMA has been recognised as a complication of snakebites [4] and has been reported in this series of cases [5].

2.1. TMA in Snakebites. VICC is the commonest coagulopathy following snake envenomation. It arises from the activation of the coagulation cascade snake toxins including

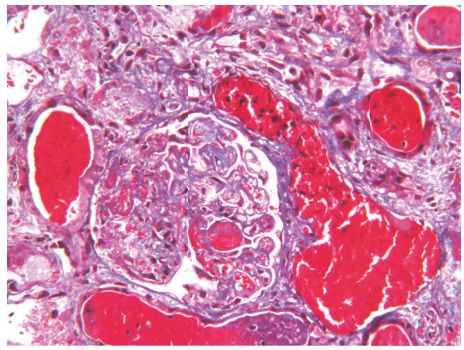

Figure 1: Arterial and arteriolar wall necrosis with luminal occlusion (thrombotic microangiopathy).

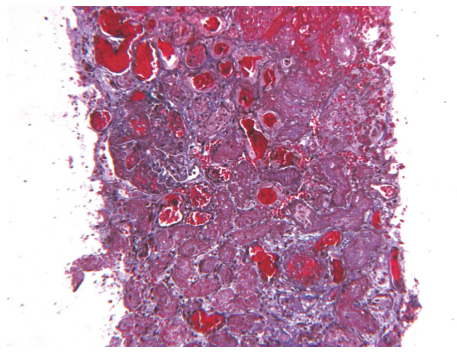

Figure 2: Necrotic process involving 60–70% of sampled cortex.

thrombin-like enzymes (most commonly), prothrombin, and factor X activators, resulting in a consumptive coagulopathy [2, 6, 7]. This results in an elevated D-dimer, prolonged prothrombin time, and low fibrinogen, all of which are features that overlap with DIC. However, the activation of the coagulation cascade via a snake procoagulant toxin rather than factor VIIa, the rapid onset and resolution of coagulopathy within 24–48 hours, and the absence of nonrenal end-organ damage seen in VICC make it a separate entity from DIC [1].

TMA tends to occur in conjunction with VICC. However, while the coagulopathy in VICC resolves rapidly, the triad of TMA persists for a longer period of time as seen in the case of our report. It is therefore suggested that the pathological process driving TMA may be distinct but related to that in VICC, although the exact mechanism of TMA in snakebites remains largely unknown. It has been suggested that, in all cases of VICC, there is a potential for TMA to develop but it only manifests in some patients [1]. However, cases of TMA have recently been reported to occur even in the absence of VICC, raising the possibility of an acquired HUS-like syndrome that is independent of VICC [3, 4]. This has led to the suggestion that a toxin in the venom may be precipitating endothelial damage that culminates to TMA [3, 7]. Future research is needed to ascertain the pathophysiology of TMA in snakebites and the relationship between the two conditions.

2.2. TMA and Renal Cortical Necrosis. Acute renal failure is a frequently reported complication of snakebites. The pathogenic mechanisms include circulatory collapse following massive haemorrhage, intravascular haemolysis, and VICC [7]. However, most of these cases are self-limiting and resolve completely within 1–8 weeks [1]. Acute tubular necrosis accounts for the large majority of acute renal failure following snakebites [7, 8]. Chronic renal impairment and mortality following TMA in snakebite coagulopathy are uncommon [1]. In this report, however, we observed a case of renal cortical necrosis (RCN) secondary to TMA following a snakebite, an uncommon and severe cause of acute renal failure, resulting in loss of kidney function and end-stage renal disease.

RCN is characterised histologically by ischaemic necrosis of large portions of the renal cortices which is irreversible [9, 10]. Declining urine output is the most common clinical feature described in the literature. The clinical suspicion of RCN should be raised in the context of ARF when oliguria or absolute anuria persists for more than 28 days [8, 10].

RCN is an uncommon cause of renal failure and is usually associated with obstetric complications [10, 11]. It has occasionally been implicated in snakebites [8, 9, 12–14]. However, the current literature available on RCN complicated by TMA is scant. At present, there are scattered reports of a HUS-like syndrome following snakebites, although they have not been recognised as TMA [1]. This could be due to TMA only being recognised recently as an entity on its own which is distinct from VICC. Moreover, since both conditions usually occur in conjunction, with VICC typically dominating the clinical presentation (i.e., haemorrhagic state in our case), a TMA-like syndrome can easily be missed. Furthermore, an isolated thrombocytopenic state in the absence of MAHA can also occur following snakebites [7], making it insufficient for a diagnosis of TMA.

Because of this wide variation in coagulation abnormalities following a snakebite, it would also be interesting to see if there is a correlation between the type and extent of haematological abnormalities and the histological findings in the kidneys.

2.3. Clinical Significance. Since the clinical features of TMA and VICC can be similar, prompt investigations should be carried out at the first instance. This includes coagulation studies and a blood film to identify the presence of MAHA. Abnormal results should indicate continuous close monitoring until normalisation. This would allow the early identification of a possible underlying TMA from VICC and guide the choice of treatment. The value of an early renal biopsy remains unknown. Plasmapheresis has been used in cases of TMA following snakebite [5] but there is no evidence to suggest its benefit. In our case, given the lack of clear mechanism for TMA, presence of extensive renal cortical necrosis, oliguria, and dialysis dependence, along with no evidence of benefit, plasmapheresis was not attempted.

Nevertheless, timely administration of antivenom is of paramount importance [15, 16]. Antivenoms are widely used in an attempt to reverse the coagulopathy associated with VICC. However, they cannot reverse any injury or organ

damage that has already been caused by the coagulopathy [16]. There is therefore an emphasis on the prompt delivery of antivenoms to limit the deleterious effects of the venom toxin. Interestingly, Isbister et al. also observed that patients who developed MAHA following snakebites tended to receive delayed administration of antivenom, although the study was underpowered to confirm this finding [3].

3. Conclusion

In summary, we report a case of TMA resulting in RCN following a snakebite. A better awareness of TMA and its early identification can contribute to a more tailored approach in the management and better prognosis for the patient. We therefore emphasise the importance of early and complete haematological investigations, including a blood film at presentation in cases following a snakebite.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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