Original Article

Assessment of risk factors of multidrug resistant tuberculosis with emphasis on serum zinc

Barman N[1], Ghosh D[2], Rahman Q[3], Uddin MN[4], Ahmed S[5], Paul D[6], Sultana T[7], Ahmed ANN[8], Haque MA[9]

Abstract

Multi-drug resistant tuberculosis (MDR-TB) is a form of TB resistant to isoniazid and rifampicin and considered as a major threat to TB control worldwide. Limited data are available regarding risk factors and serum zinc status of MDR-TB in Bangladesh. Tuberculosis is a disease which blocks a key part of the body's immune defense. As zinc deficiency causes immune suppression, we assume that it may play a role in the development of MDR-TB. So, this study was carried out to evaluate and assess the serum zinc level as well as different risk factors in patients of MDR-TB. This case control study was carried out in the Department of Clinical Pathology, in collaboration with Department of Public Health and Informatics, Department of Biochemistry, BSMMU and National tuberculosis referral laboratory (NTRL) in NIDCH, Mohakhali, Dhaka from March' 2012 to February 2013. We enrolled 50 newly diagnosed cases of MDR-TB patients from NTRL and 50 age matched healthycontrols from a selective community Sirajdikhan Upazilla, Munshiganj District. The two groups were compared based on various demographic factors, behavioral factors, factors related with personal illness history and also laboratory related factors. Serum zinc deficiency, changing trend of number of family members, presence of smoking history, absence of BCG scar and past history of contact TB were statistically significant risk factors for development of MDR-TB in logistic regression model. In our study, we found the mean (SD) serum zinc level in case group 60.40 (8.91) µg/dL and 84.00 (13.62) µg/dL in healthy controls which was significant statistically. The risk factors that we found, specially lower level of serum zinc, are the major concern for the development of MDR-TB and attempt to minimize them might contribute to control TB.

Key words: multidrug-resistant tuberculosis, risk factor, zinc, immune status

1. *Dr Nilima Barman, Assistant Professor of Pathology, Dr Sirajul Islam Medical College, Dhaka

2. Dr Debabrata Ghosh, Project Director, Santi Sir Memorial Diabetes Centre, Extension of Diabetes Care (EDC) Center, Bangladesh Diabetic Association (BADAS), Sirajdikhan, Munshigonj

3. Dr Md Quddusur Rahman, Professor, Department of Clinical Pathology, BSMMU, Dhaka

4. Dr Md Nasir Uddin, Assistant Professor, Department of Biochemistry, National Institute of Cardiovascular Diseases, Dhaka

5. Dr Sarmeen Ahmed, Lecturer, Department of Microbiology, Anowar Khan Modern Medical College, Dhanmondi, Dhaka

6. Dr Debatosh Paul, Assistant professor, Department of Clinical Pathology, BSMMU, Dhaka

7. Dr Tuhin Sultana, Associate professor, Department of Clinical Pathology, BSMMU, Dhaka

8. Dr A N Nashimuddin Ahmed, Professor and Chairman, Department of Clinical Pathology, BSMMU, Dhaka

9. Dr Md Atiqul Haque, Assistant professor, Department of Public Health and Informatics, BSMMU, Dhaka

*For correspondence

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis which remains a major health problem worldwide. Among the 22 highest TB burden countries in the world, Bangladesh ranked 6th.[1] Multidrug-resistant tuberculosis (MDR-TB) is defined as TB resistant to at least isoniazid and rifampicin, the two most potent anti-TB drugs. It results from either primary infection with resistant bacteria or may develop in the course of a patient's treatment. According to WHO report 2012, MDR-TB was found in 1.4% in new TB cases and in 29% in previously treated TB cases in Bangladesh.[2]

Cell mediated immunity (CMI) plays a major part in the pathogenesis of tuberculosis. But the vital elements of CMI i.e. T lymphocyte, absolute numbers and percentages of CD3 (+) and CD4 (+) cells, cytokines level specially TNFα and IL-1 levels were significantly found lower in MDR-TB patients than in non resistant-TB cases. The total burden of bacteria also increases in MDR-TB patients. As a result the immune system is suppressed in MDR-TB patients.[3,4]

Zinc is a vital micronutrient which has an important role in immunity. In zinc-deficient persons, increased susceptibility to a variety of pathogens including M. tuberculosis occur.[5] Zinc is essential for normal development and function of cell-mediated immunity, maturation of T lymphocyte, neutrophils and natural

killer cells. Macrophages are also affected by zinc deficiency. The growth and function of T and B cells are also affected adversely due to zinc deficiency. Zinc deficiency adversely affects the secretion and functions of cytokines, the basic messengers of the immune system.[6]

As zinc deficiency causes immune suppression, we assume that it may play a role in the development of MDR-TB. Now a days, the MDR-TB is a frightening condition for TB control in our country.[7] The risk factors for multidrug resistant TB in Bangladesh are also largely unknown. Therefore, the present study was carried out to assess the risk factors of MDR-TB with emphasis on serum Zinc.

Methods
This case control study was conducted in the department of Clinical Pathology in collaboration with department of Public Health and Informatics & department of Biochemistry, BSMMU and NTRL (National Tuberculosis Reference Laboratory) in National Institute of Diseases of the Chest Hospital (NIDCH), Mohakhali, Dhaka from March 2012 to February 2013. We considered multidrug-resistant tuberculosis patients as case and similar age matched healthy adults from community as control. Fifty newly diagnosed adult MDR TB patients (≥ 18 years age) who were confirmed by Line Probe Assay (LPA)[8] and having both isoniazid and rifampicin resistance were included from NTRL in NIDCH, Mohakhali, Dhaka. Patients who were already on second line anti-tubercular therapy, non-tuberculous pulmonary infections and extra pulmonary tuberculosis were excluded from the study. As serum zinc levels are affected by many physiological and pathological states and drugs, we excluded pregnant women, patients with malabsorption syndrome, nephrotic syndrome, chronic liver disease, diabetes mellitus, malignancy, chronic renal failure, patients who were on zinc medication & women taking oral contraceptive pill. Fifty age-matched healthy adults who were free from prolonged cough (90 days) and fever as well as having no previous history of tuberculosis including normal chest radiograph and negative Mountax test, were enrolled as control. Those controls were taken from health camp in Sirajdikhan thana, Munshiganj district. Informed consent was obtained from each subject before interview.

Blood samples (6 ml) were collected from the respondents after overnight fasting via venipuncture to estimate the level of serum albumin, serum creatinine, blood glucose, serum zinc and Hb%. For serum zinc 2 ml blood was transferred immediately in a clean deionized screw capped plastic tube. Serum samples were obtained from the spontaneous coagulation of blood. The blood was then centrifuged at 2500 rpm for 10 min to obtain serum. The serum was stored at -20^0c until analysis. The test serum was stored at -20^0c until analysis. The tests were done on several occasions in the department Biochemistry, BSMMU, Dhaka using atomic absorption spectrophotometry method. The accepted reference range for serum zinc level is 70-120 µg/dl (10.7-18.4 mmol/L), considering cut off point below the level of 70 µg/dl (10.7 mmol/L) as an indicator of zinc deficiency.[9] For other tests 4 ml blood was used to rule out the exclusion criteria that previously mentioned and those were done in the department of Clinical Pathology of the same university.

The data were analyzed through SPSS version 21.00. We analyzed categorical variables by using chi-square test and for continuous variable by independent sample t-test. Odds ratios and their corresponding 95% confidence limits for categorical variables were determined by logistic regression. A p-value <0.05 was considered statistically significant. We calculated crude odds ratio (ORS) between case and control. All significant variables were entered into a multivariate conditional logistic regression model and a final fitted model was determined by enter method.

Study protocol was reviewed and approved by the Institutional review board (IRB) of Bangabandhu Sheikh Mujib Medical University, Dhaka.

Results
In our study, 50 (28 male and 22 female) newly diagnosed MDR-TB and 50 (30 male and 20 female) healthy controls with a mean age of 31.45 ±11.71 yrs and age ranging from 18 to 65 years were included. The higher percentage (11%) of the patients was in 21-25 year age group. The mean (SD) years of schooling of MDR-TB was statistically significant [5.84 (4.05) vs. 9.9 (4.38), p< <0.0001]. Twenty-three cases (46.0%) had known past history of contact TB. All cases had previous tuberculosis. A total of 38% of MDR-TB cases had history of drug default and 10% had history of irregular treatment of antitubercular therapy. (Table - I) The mean (±SD) serum zinc level in cases and control groups were found to be 60.40 (8.91) µg/dL and 84.00 (13.62) µg/dL respectively. (Table - II)

In univariate model predictors of tuberculosis were respondent living in joint family [Unadj OR= 1.78, 95% CI (0.75-4.24)], living in family where numbers of members change [Unadj OR= 5.69, 95% CI (2.23-14.53)], having no BCG scar [Unadj OR= 2.43, 95% CI (1.05-5.63)], smoker [Unadj OR= 2.70, 95% CI (1.15-6.34)], having past history of contact TB [Unadj

Table I: Demographic characteristics of the study population

	Case (n=50)	Control (n=50)	p value
Gender			
Male	28 (56.0)	30 (60.0)	0.685
Female	22 (44.0)	20 (40.0)	
Type of family			
Extended family	38 (76.0)	32 (64.0)	0.19
Nuclear family	12 (24.0)	18 (36.0)	
Number of family members			
Change	26 (52.0)	8 (16.0)	0.0001
Not change	24 (48.0)	42 (84.0)	
BCG Scar			
Present	13(26.0)	23 (46.0)	0.037
Absent	37 (74.0)	27 (54.0)	
History of smoking			
Smoker	23 (46.0)	12 (24.0)	0.021
Non smoker	27 (54.0)	38 (76.0)	
Past history of TB contact			
Yes	23 (46.0)	5 (10.0)	<0.0001
No	27 (54.0)	(4590.0)	
Serum zinc level (µg/dL)			
Lower than normal (<70)	38 (76.0)	4 (8.0)	<0.001
Normal (70-120)	12 (24.0)	46 (92.0)	
Years of schooling	5.84 (4.05)	9.9 (4.38)	<0.0001
Monthly family income (BDT)	15100 (6525)	26700 (9605)	<0.0001

Table II: Distribution of the respondents by gender and serum Zinc (µg/dL) level (n=100)

Gender	Case (n=50)	Type of Respondents	Control (n=50)	p value#
Male	62.32 (10.40)*		90.50 (12.88)*	<0.001*(s)
Female	57.95 (5.9)		74.25 (7.65)	<0.001*(s)
Total	60.40 (8.91)		84.00 (13.62)	<0.001*(s)

*mean (±SD), # Independent samples t-test was done to determine the level of significance. s=significant

OR= 7.67, 95% CI (2.61-22.54)] and in patients with low level of serum zinc level [Unadj OR= 36.42, 95% CI(10.86-122.17)] When all significant variables from univariate model entered into the multivariate logistic regression model significant risks were frequent changes in amily members (adj. OR 14.49, 95% CI 2.39, 88.03), history of smoking (adj. OR 14.86, 95% CI 2.53, 87.46), past history of contact TB (adj. OR 6.251, 95% CI 1.08, 36.04) and serum zinc level lower than normal (<70µg/dL) (adj. OR 54.43, 95% CI 10.15, 291.88). (Table – III)

Table - III: Association of variables on MDR-TB: Logistic regression model (Enter Method)

Variables	UnAdj OR (95% CI)	p value	Adj OR	p value
Sex				
Male*	1			
Female	1.18 (.532, 2.61)	0.685		
Family type				
Nuclear *	1			
Extended	1.78 (0.75, 4.25)	0.193		
Number of family members				
Not change	1			
Change	5.69 (2.23, 14.53)	0.001	14.49 (2.39, 88.03)	0.04
BCG Scar				
Present*	1			
Absent	2.43 (1.05, 5.63)	0.39	5.85 (1.21,28.36)	0.28
History of smoking				
Non smoker*	1			
smoker	2.7 (1.15, 6.34)	0.23	14.86 (2.53, 87.46)	0.03
Past history of contact TB				
No*	1			
Yes	7.67 (2.61, 22.54)	<.0001	6.25 (1.08, 36.04)	0.040
Serum zinc level (µg/dL)				
Normal (70-120) *				
Lower than normal (<70)	36.42 (10.86, 122.17)	<.0001	54.43 (10.15, 291.88)	<.0001

Discussion

We assessed risk factors of MDR-TB patients in comparison with healthy adults. We also investigated serum zinc level in MDR-TB patients as we assumed that zinc deficiency may play an important role for developing MDR-TB. The risk factors for MDR TB are widely evaluated throughout the world but considering zinc deficiency as a risk for the development of MDR-TB are still unknown. So, it is dire need to detect risk factors for MDR-TB cases in Bangladesh to prevent disease transmission and subsequent mortality giving emphasis to serum zinc level.

The person living in extended family was more vulnerable to develop active pulmonary TB. In our study, we found extended family was a risk factor for development of MDR-TB (unadj. OR 1.78, 95% CI 0.75-4.25). There were few studies done in pulmonary nonresistant tuberculosis case but not in MDR-TB regarding this factor. Pakasi et al[10] found that living in an extended family was associated with the development of TB (OR 2.7, 95%CI 1.5–4.8). This result is consistent with our study.

In our study we considered the frequency of changes of the family members as a risk factor of multidrug-resistant tuberculosis. We found that the number of family members of MDR-TB change frequently than that of control. In unadjusted odd ratio model, it is about 5.69 times with 95% confidence interval ranging from 2.3 to 14.52 and in adjusted odd ratio model, it is about 14.49 times with 95% confidence interval ranging from 2.39 to 88.03.

We found cases in this study who had five or less years of schooling which was statistically significant (p value <0.001) in contrast to control group. Some other studies conducted on risk factor analysis of MDR-TB were consistent to this result. Patients with no or little education were more likely to develop of MDR-TB.[11] In another study by Ahmed et al[12] found low educational status as a risk factor for MDR-TB. In a different study by Barroso et al[13] found an association between MDR-TB and lack of school education by univariate analysis.

Our study revealed that the significant association between smoking and MDR-TB having unadjusted odd ratio 2.7 and 95% confidence interval ranging from 1.15 to 6.34 and in adjusted odd ratio model, it is 14.86 with 95 % confidence interval ranging from 2.53 to 87.46 (p=0.003). Marahatta et al[14] also found smoking as a risk factor of MDR-TB with odd ratio 2.35 and 95% confidence interval ranging from 1.071 to 5.159 (p=0.032). In a study done in western Nepal[15] 74% of the MDR-TB patients had a history of smoking (past as well as present). Barroso et al[13] revealed smoking as one of the risk factors for MDR TB. All these studies are consistent with our study result.

Our study also revealed that past history of contact TB was strongly associated with development of MDR-TB. In our result, adjusted odd ratio was found 6.25 with 95% confidence interval ranging from 1.08 to 36.04 (P < .05).

Our findings is similar with some other similar studies. Vashakidze et al[16] found history of TB contact associated with risk for having MDR-TB in Georgia. This study result is comparable to our study. In a study conducted in four European Union countries Casal et al[17] revealed tuberculosis contacts (odd ratio 2.01) as the most statistically significant risk factors of MDR-TB.

In our study estimation of serum zinc level was done by atomic absorption spectrophotometry (AAS) method. In our study mean (SD) value of serum zinc level was 60.40 (8.91) µg/dl and 84.00 (13.62) µg/dl in case and control group respectively and this difference was statistically significant (P<0.05). There are few studies regarding serum zinc level in pulmonary TB but no study was found in case of MDR-TB. Lower level of serum zinc was reported in these studies.[12,18] Serum zinc level in patients having pulmonary tuberculosis was 65.53 (9.8) µg/dl and 52.5 (19.5) µg/dl in Ramakrishnan et al[18] and Ahmad et al[12] study respectively and these values were lower than normal cut off value.

Infection induces a reduction in serum zinc level. The present study demonstrated diminished serum zinc level in MDR-TB patients compared to healthy controls which was statistically significant (p value < 0.001). In case group, normal serum zinc level (70-120 µg/dl) was found in 24% subjects and low level (< 70 µg/dl) in 76% subjects [adjusted odd ratio=54.42, 95% CI (10.150, 291.882)].

Now a days, MDR-TB is a big challenge to control TB in the world. We recommended further study to compare serum zinc level of MDR-TB with that of non resistant pulmonary tuberculosis in a large sample size.

References

1. WHO 2011. National Guidelines and Operational Manual for Tuberculosis Control, Fourth Edition. Available from:http://www.scribd.com/doc/573715/Bangladesh-National-Guidelines-and-Operational-Manual-for-Tuberculosis-Control

2.   WHO 2012. Global Tuberculosis Report. Available from: http://www.who.int/tb/publications/ global _report/gtbr12_main.pdf

3.   Cağatay T, Kiran B, Yurt S, Gülbaran Z, Kosar F, Cağatay P. Levels of tumour necrosis factor-alpha and IL-1 alpha in newly diagnosed and multidrug resistant tuberculosis. Respirology. 2005; 10(3):290-4.

4.   Yildiz P, Kadakal F, Tutuncu Y, Gunnur D, Gurel N, Adin S, Yilmez V. Natural killer cell activity in multidrug-resistant pulmonary tuberculosis. Respiration 2001;68: 590-4.

5.   Riccardi G, Milano A, Pasca MR, Nies DH. Genomic analysis of zinc homeostasis in mycobacterium tuberculosis. Federation of European Microbiological Society 2008;287: 1-7.

6.   Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. American Society of Clinical Nutrition. 1998; 68(suppl): 447S-63S.

7.   Zaman K, Rahim Z, Yunus M, Arifeen S, Baqui A, Sack D et al. Drug resistance of Mycobacterium tuberculosis in selected urban and rural areas in Bangladesh. Scand J Infect Dis. 2005; 37(1):21-6.

8.   Policy Statement, WHO 2010. Molecular Line Probe Assays for Rapid Screening of Patients at Risk of Multidrug-Resistant Tuberculosis (MDR-TB). Available from: http://www.who.int/ tb/features_ archive / policy_statement.pdf

9.   Milne DB. Essential trace elements. In: Burtis CA, Ashwood editors. Tietz text book of clinical chemistry. 3rd ed. Philadelphia (USA): WB Saunder; 1999. p. 1029-1051.

10. Pakasi TA, Karyadi E, Dolmans WM, Vander Meer JW, Velden K. Malnutrition and socio-demographic factors associated with pulmonary tuberculosis in Timor and Rote Islands, Indonesia. Int J Tuberc Lung Dis. 2009; 13(6); 755–59.

11. Lee JH, Chang JH. Drug-resistant tuberculosis in a tertiary referral teaching hospital of Korea. Korean J Intern Med. 2001; 16 (3): 173–179.

12. Ahmad I, Srivastava VK, Prasad R, Yusuf M, Saleem M, Ali W. Deficiency of Micronutrient Status in Pulmonary Tuberculosis Patients in North India. Biomedical Research 2011; 22 (4): 449-454.

13. Barroso EC, Salanimota RM, Oliveira RO, Oliveirasousa AL, Brasileirobarroso J, Nobrerodrigues JL. Risk factors for acquired multidrug-resistant tuberculosis. J Pneumol. 2003;29(2): 89-97

14. Marahatta SB, Kaewkungwal J, Ramasoota P, Singhasivanon P. Risk factors of Multidrug Resistant Tuberculosis in central Nepal: A pilot study. Kathmandu University Medical Journal. 2010; 8 (32): 392-95.

15. Pant R, Pandey KR, Joshi M, Sharma S, Pandey T, Pandey S. Risk factor assessment of Multi-drug Resistant TB. JNHRC. 2009; 7:89-92.

16. Vashakidze L, Salakaia A, Shubladze N, Cynamon M, Barbakadze K, Kikvidze M et al. Prevalence and Risk factors for Drug Resistance among Hospitalized TB Patients in Georgia. Int J Tuberc Lung Dis. 2009;13(9): 1148–1153.

17. Casal M, Vaquero M, Rinder H, Tortoli E, Grosset J, Rusch-Gerdes S et al. A Case-Control Study for Multidrug-Resistant Tuberculosis: Risk Factors in Four European Countries. Microbial Drug Resistance 2005; 11(1): 62-67.

18. Ramakrishnan K, Shenbagarathai R, Kavitha K, Uma A, Balasubramaniam R, Thirumalaikolundusubramanian P. Serum zinc and albumin levels in pulmonary tuberculosis patients with and without HIV. Journal Infectious Disease. 2008; 61: 202-4.