Divalent Metal Ions; Key factor towards Pulmonary Tuberculosis

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Abstract

Objective: The purpose of this study was to compare demographic profile in Tuberculosis and Non-Tuberculosis individuals. We also aimed to compare divalent metal ions in tuberculosis and Non-Tuberculosis Individuals

Methods: This case control study was carried out on 345 subjects. Sample size was calculated through open EPI. Patients were grouped into two categories i.e., Pulmonary Tuberculosis (cases group) with 115 patients and without Pulmonary Tuberculosis (control group) with 230 subjects. Demographic Profile was recorded through already designed proforma. 5 ml sterile gel tube was used to collect blood samples. The samples, which were then centrifuged, and the serum were examined to check the levels of iron and copper. Data was evaluated using SPSS version 25.00.

Results: Male patients in productive age groups are more likely to have TB in comparison of female patient. In comparison of patient with normal serum copper, patient with serum copper below normal are less likely for TB and patient with serum copper above normal are more likely to develop TB. Patient with serum iron below normal are also more likely to develop TB in comparison of patients with normal serum iron.

Conclusion: Male patients in productive age groups were commonly affected with tuberculosis. In tuberculosis patients, a low serum iron concentrations along with significantly high concentration of serum of copper were found. Subsequently, it may increase the human susceptibility to the infection of mycobacterium tuberculosis.

Keywords: Mycobacterium tuberculosis, Iron, Copper, BMI, Nutritional status.

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Introduction

Tuberculosis (TB) is a communicable infectious disease which spreads with the close contact of people through small droplets when disease-ridden people cough or sneeze. According to World Health Organization (WHO) statistics mycobacterium tuberculosis has infected nearly one third population of the world, out of which above 80% of individuals belong to the developing countries[1,2]. Geographically, in 2019, most TB cases were in the World Health Organization (WHO) regions of South East Asia (44%), Africa (25%) and the Western Pacific (18%), with smaller shares in the Eastern Mediterranean (8.2%), the Americas (2.9%) and Europe (2.5%). Eight countries accounted for two thirds of the global total: India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%)[3]. In terms of absolute numbers of TB cases Pakistan is ranked 5th among 22 high burden countries. Above this poverty, malnutrition and other illness are directly related to high preva-

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lence of TB[4]. Tuberculosis is more linked with congested areas with low-income group, low literacy rate and inadequate health care facilities[5]. Trace elements (micronutrients) such as iron, copper, lead, arsenic, selenium, calcium and zinc are necessary nutrients that effects in various biological systems[6]. The role of these divalent metals is decisive to the growth of tissues and organs of a human[7]. Likewise, these elements now have been recognized as a prospective key factor in numerous contagious diseases. It has been witnessed that some modifiable metals support immune system and abolish microorganisms like mycobacterium tuberculosis[8]. A number of studies have reported that the extra iron supports mycobacterium tuberculosis and HIV development tin human body which can cause co-infection of HIV-TB in several patients[9]. Iron works significantly in the growth, pathogenicity, and metabolism of mycobacterium tuberculosis which depends on the attainment of iron from resources of host, as a result high frequency of iron deficiency (anemia) is experienced frequently among the patients infected by TB[10]. The function of copper in biological systems is activation of several enzymes that work in oxidative and reductive reactions[11]. Even though, copper has a lot of biological benefits, but its excess is toxic for infection and might obstruct the growth of mycobacterium tuberculosis[12]. Keeping in view the above consideration, this study was designed to compare the demographic features and divalent metal ions such as iron and copper between pulmonary tuberculosis and non-tuberculosis individuals. To the best of our knowledge, previously these parameters were not compared among TB and Non-TB individuals in Pakistan. Thus, our study would highlight the most prevalent risk parameter of TB among target population.

Patients and Methods

This case control study was carried out between January 2019 to December 2019 at the department of chest medicine, the sample was collected by using simple random sampling technique. Sample size was calculated through open EPI requiring 98 cases and same number of controls, however we included 115 cases and 230 controls in the study. Patients were grouped into two categories i.e., Pulmonary Tuberculosis (cases group) with 115 patients and without Ethical approval was taken from Institutional Review Board (IRB#8255/). The study subjects were grouped in two categories i.e. Tuberculosis Patients (Cases group) with 115 patients and Non-TB Individuals (Control group) with 230 subjects. The patients of both genders with age between 18 years to 60 years were included in the study. Those patients were suffering from extra pulmonary, MDR & XDR tuberculosis, and failing to give consent were excluded from the study. Demographic profile was recorded through a pre-designed proforma. BMI was computed as weight (Kg) / height $(m)^2$. BMI less than 18.5 Kg/m^2 was considered underweight. Five ml sterile gel tube was utilized to collect the blood samples. The collected samples were then centrifuged and the obtained serum was investigated for Iron (cat#ACN 8661,COBAS, Roche Germany) and Copper (cat#6K93-30, Architect c Sytems, Abbot, U.S.A) levels through Chemilumenisence method.

The collected data was evaluated by using SPSS version 25.00 Descriptive statistics were calculated for quantitative and qualitative variables. Qualitative variables were presented in terms of frequency and percentages while quantitative variables were presented in terms of mean ± standard deviation. To evaluate association of demographic parameters among study groups, Chi-square test and Fisher exact test (as appropriate) were applied. Apart from this, independent t-test was applied to determine significant mean difference of quantitative variables amongst study groups. Odd ratios were calculated through Uni-variate Binary Logistic Regression. Adjusted odd ratios were determined through Multi-variate Binary Logistic regression. P-value$\leq$0.05 was considered significant in all analysis.

Results

Total 345 individuals were enrolled in study out of which 230 subjects without Tuberculosis were included in control group and 115 Tuberculosis patients in case group. The results showed that mean age was 30.96 ± 10.10 years (15 years to 60 years) in control group and 28.17 ± 12.72 years (15 years to 60 years) in case group. In control group

most of the patients (55.2%) were belonged to age group between 26-50 years while in case group, most of the patients (58.3%) were from age group 25 years. In both control group and case group, 57.8% and 66.1% were male respectively. Detailed comparison of control and case group are presented in Table 1.

Uni-variate binary logistic regression shows that male patient is more likely to have Tuberculosis in comparison of female patient (OR=1.421, p=0.140). Patient with age >50 years (OR=0.344, p=0.018) are more likely to have Tuberculosis in comparison of patient with age <50 years. In comparison of patient with normal serum copper, patient with serum copper below normal are less likely for Tuberculosis (OR=0.114, p=0.000) and patient with serum copper above normal are more likely to develop Tuberculosis (OR=3.566, p=0.001). Patient with serum iron below normal are also more likely to develop Tuberculosis in comparison of patients with normal serum iron (OR=4.305, p=0.000). Detailed odds are presented in Table 2.

Mean iron serum was observed 80.00 ± 39.04 mcg/dL and 71.58 ± 46.15 mcg/dL in control group and case group, respectively with the insignificant difference (p=0.077), while mean serum copper was 93.53 ± 25.62 μg/dL and 121.43 ± 31.05 μg/dL in control group and case group, respectively with a significant difference (p<0.001), as presented in Table 3 and Table 4.

Discussion

This study was an attempt to compare the association of demographic features and divalent metal ions between pulmonary tuberculosis and non-tuberculosis individuals. In a study, the occurrence of underweight in TB patients was 57.17% as compared to non-TB controls i.e., 23.37%[13]. Gupta et al in their study concluded that the odds were around 2.5 times greater in female than male TB patients[14]. However, in contrast to their finding our current study result reported that in odds of gender, males were 1.4 times higher than the females TB patients. Our findings are supported by the work of Watkins et al, who in their study also reported a similar ratio and higher frequency of male individuals affected with tuberculosis. The Cause of this high proportion of TB cases in men in comparison to women has been cited as social behavior since females remained in houses, while the male spends most of times outside and have more chances of contracting the disease. The biological differences such as immunity, exposure to tuberculosis associated with social behaviors including smoking also have significant impact[15].

The current study revealed that (36.5%) cases of TB patients were underweight with low BMI. Shukla et al in their study reported that the frequency of under nutrition was 79 % i.e., BMI <18.5 among the patients suffering from TB. Further, 41 (20.5%) TB patients had BMI <14, that was considered as highly underweight. The study discovered the relationship of nutritional status among TB patients with socio-economic status, living style, and the factors related to health. The outcomes of multivariate analysis presented that those patients had difficulty in breathing, has history of smoking, and consuming other substances were expected to have lower BMI[16].

Dye et al in their study revealed that diabetes was found to be significantly related to high BMI in patients of TB with a multifaceted relation between contagion, non-infectious diseases, and nutrition[17]. However, our study demonstrated (12.2%) cases of diabetes in TB affected individuals whereas (11.7%) cases of diabetes were found in the control group with a non-significant difference (p=0.906).

Iron deficiency and excess, both can compromise immune and cellular functions. Low iron status may impair motor activity and enhance disease susceptibility whereas excess iron contributes to toxicity by catalyzing reactive oxygen species. A study by Boelaert et al. demonstrated the lower iron concentration in serum of TB individuals as compared to the healthy control group. They further specified that mycobacterium tuberculosis ability of reproduction in the host macrophages rely upon the existing iron. The deficiency of iron in TB patients might be owing to the mycobacterium iron intake[18]. Our study findings are consistent with the above-mentioned studies and reveal that iron serum level was low in TB patients with respect to gender, age group, nutritional status and diabetes mellitus with an insignificant difference while significant difference

Table 1. Comparisons of frequency of Basic Demographic Characteristics for TB and Non-TB Individuals

		Non-TB Individuals n (%) (n=230)	TB Patients n (%) (n=115)	Total Individuals n (%) (n=345)	P-Value
Gender	Male	133(57.8)	76(66.1)	209(60.6)	0.139
	Female	97(42.2)	39(33.9)	136(39.4)	
Age Group	≤ 25 years	90(39.1)	67(58.3)	157(45.5)	<0.001**
	26-50 years	127(55.2)	37(32.2)	164(47.5)	
	>50 years	13(5.7)	11(9.6)	24(7)	
BMI Group	Underweight	19(8.3)	42(36.5)	61(17.7)	<0.001**
	Normal	65(28.3)	48(41.7)	113(32.8)	
	Overweight	45(19.6)	7(6.1)	52(15.1)	
	Obese	101(43.9)	18(15.7)	119(34.5)	
Diabetes Mellitus	Yes	27(11.7)	14(12.2)	41(11.9)	0.906
	No	203(88.3)	101(87.8)	304(88.1)	
Marital Status	Single	103(44.8)	52(45.2)	155(44.9)	0.939
	Married	127(55.2)	63(54.8)	190(55.1)	
Serum Copper	Below Normal	49(21.3)	3(2.6)	52(15.1)	<0.001**
	Above Normal	11(4.8)	21(18.3)	21(18.3)	
	Normal	170(73.9)	91(79.1)	91(79.1)	
Serum Iron	Below Normal	16(7)	28(24.3)	44(12.8)	<0.001**
	Normal	214(93)	87(75.7)	301(87.2)	

Chi square test was applied. *Fisher exact test was applied. ** Significant at 0.01 levels

Table 2. Odds Ratio of Cases Compared for TB and Non-TB Individuals

		Unadjusted Odds Ratio (95% CI)	P-Value
Gender	Male	1.421(0.891-2.266)	0.140
	Female®	1	
Age Group	≤ 25 years	0.880(0.371-2.085)	0.771
	26-50 years	0.344(0.142-0.832)	0.018
	>50 years®	1	
BMI Group	Underweight	12.404(5.928-25.953)	<0.001
	Normal	4.144(2.218-7.741)	<0.001
	Overweight	0.873(0.341-2.237)	0.777
	Obese®	1	
Diabetes Mellitus	Yes	1.042(0.524-2.074)	0.906
	No®	1	
Marital Status	Single	1.018(0.649-1.596)	0.939
	Married®	1	
Serum Copper	Below Normal	0.114(0.035-0.377)	0.000
	Above Normal	3.566(1.647-7.723)	0.001
	Normal®	1	
Serum Iron	Below Normal	4.305(2.218-8.352)	0.000
	Normal®	1	

®Reference Group Univariate and multivariate binary logistic regression was applied.

Table 3. Mean comparison of iron serum (mcg/dL) for TB and Non-TB Individuals according to demographic characteristics.

		STUDY GROUP Mean±SD Non-TB Individuals (n=230)	TB Patients (n=115)	P-Value
Overall		80.00±39.04	71.58±46.15	0.077
Gender	Male	82.43±41.08	72.18±45.80	0.098
	Female	76.67±36.00	70.41±47.41	0.406
Age Group	≤ 25 years	76.77±37.96	72.95±46.53	0.572
	26-50 years	82.38±40.62	70.81±47.36	0.144
	>50 years	79.07±30.70	65.81±43.18	0.390
BMI Group	Underweight	83.42±43.56	63.28±43.89	0.102
	Normal	84.83±37.46	75.91±46.13	0.260
	Overweight	78.68±41.76	86.57±59.00	0.663
	Obese	76.84±38.15	73.55±46.97	0.746
Diabetes Mellitus	Yes	65.66±27.36	65.00±53.91	0.958
	No	81.91±40.01	72.49±45.20	0.065
Marital Status	Single	77.69±38.32	78.86±46.52	0.868
	Married	81.87±39.68	65.57±45.33	0.012**

Independent t-test was applied. ** Significant at 0.05 levels

Table 4. Mean comparison of serum copper (μg/dL) for TB and Non-TB Individuals according to demographic characteristics.

		STUDY GROUP Mean±SD Non-TB Individuals (n=230)	TB Patients (n=115)	P-Value
Overall		93.53±25.62	121.43±31.05	<0.001**
Gender	Male	94.74±27.64	117.86±27.71	<0.001**
	Female	91.87±22.60	128.38±36.08	<0.001**
Age Group	≤ 25 years	96.44±26.12	118.32±31.99	<0.001**
	26-50 years	91.44±26.19	125.81±29.56	<0.001**
	>50 years	93.76±12.47	125.63±30.62	0.007
BMI Group	Underweight	89.89±16.58	115.38±32.04	<0.001**
	Normal	91.40±23.36	124.00±23.36	<0.001**
	Overweight	92.06±27.02	138.42±25.29	<0.001**
	Obese	96.62±27.64	122.05±26.01	<0.001**
Diabetes Mellitus	Yes	94.55±12.49	126.71±38.19	<0.001**
	No	93.39±26.91	120.70±30.09	<0.001**
Marital Status	Single	95.41±28.50	115.28±31.68	<0.001**
	Married	92.00±23.03	126.50±29.82	<0.001**

Independent t-test was applied. ** Significant at 0.01 levels

was explored with low iron serum in TB patients with respect to marital status and blood group.

Copper is an essential nutrient that is widely distributed in food and water. Elevated Cu levels are characteristic of most acute and chronic infections as well as tuberculosis. Serum Cu levels have been reported to be higher in tuberculosis patients[19]. In a study carried out in Korea, it was reported that the patients suffering from pulmonary TB possessed significantly higher serum copper as well as cobalt than healthy control group, whereas zinc and selenium concentrations were significantly low[20]. There is a reasonable clarification for the relationship of high concentration of copper in TB patient. The reduction in the levels of zinc, which takes place in TB patient, avoids the entry of copper to the tissues, and this contributes to increase of serum copper level[21]. The findings of current study are in agreement with the above cited studies as we also found significantly raised copper levels in serum of TB affected individuals when compared with the controls.

Conclusion

Male patients in productive age groups were commonly affected with tuberculosis. In tuberculosis patients, a low serum iron concentrations along with significantly high concentration of serum of copper were found. Subsequently, it may increase the human susceptibility to the infection of mycobacterium tuberculosis.

Conflict of Interest

Authors have no conflict of interest and no grant/funding from any organization

References

1. Issoufou I, Sani R, Belliraj L, et al. Pneumonectomy for tuberculosis destroyed lung: a series of 26 operated cases. Revue Pneumol Clin.2016;72:288-292.
2. Organization WHO. Tuberculosis. Saudi Med J. 34:1205-7.
3. World Health Organization. Global tuberculosis report 2020.
4. Ambrus JL. (2004). Nutrition and infection diseases in developing countries and problems of acquired immuno deficiency syndrome. Exp Biol Med. 229: 464-472.
5. Mohan G, Kulshreshtha S, Sharma P. Zinc and copper in Indian patients of tuberculosis. Biol Trace Elem Res.2006;111:63-9.
6. Hegazy AA, Zaher MM, Abdel-hafez MA, et al. Relation between anemia and blood levels of lead, copper, zinc and iron among children. BMC Res Notes.2010;12;3:133.
7. Pfaender S, Fohr K, Lutz AK, et al. Cellular zinc homeostasis contributes to neuronal differentiation in human induced pluripotent stem cells. Neural Plasticity. 2016;3760702.
8. Neyrolles O, Mintz E, Catty P. Zinc and copper toxicity in host defense against pathogens: Mycobacterium tuberculosis as a model example of an emerging paradigm. Front Cell Infect Microbiol. 2013;3:89.
9. Kerkhoff AD, Meintjes G, Opie J, et al. Anaemia in patients with HIV-associated TB: relative contributions of anaemia of chronic disease and iron deficiency. Int J Tuberc Lung Dis. 2016;20:193-201.
10. Lun S, Tasneen R, Chaira T, et al. Advancing the Therapeutic Potential of Indoleamides for Tuberculosis. Antimicrob Agents Chemother. 2019;63:e00343-19.
11. Grammer TB, Kleber ME, Silbernagel G, et al. Copper, ceruloplasmin, and long-term cardiovascular and total mortality (the Ludwigshafen Risk and Cardiovascular Health Study). Free Radic Res.2014;48:706-15.
12. Liu T, Ramesh A, Ma Z, et al. CsoR is a novel Mycobacterium tuberculosis copper-sensing transcriptional regulator. Nat Chem Biol. 2007:3:60-8.
13. Feleke BE, Feleke TE, Biadglegne F. Nutritional status of tuberculosis patients, a comparative cross-sectional study. BMC Pulmonary Medicine.2019:19:182-191.
14. Gupta KB, Gupta R, Atreja A, et al. (2009). Tuberculosis and nutrition. Lung India. 2009: 26:9-16.
15. Watkins RE, Plant AJ. Does smoking explain sex differences in the global tuberculosis epidemic? Epidemiol Infect.2006:134, 333-39.
16. Shukla A, Pandey S, Singh SP, et al. Nutritional status of pulmonary tuberculosis patients: A hospital?based cross?sectional study. Indian J Community Fam Med. 2019;5:134-40.
17. Dye C, BourdinTB, Lönnroth K, et al. Nutrition, Diabetes and Tuberculosis in the Epidemiological Transition. PLoS One. 1;6: e21161.
18. Boelaert JR, Vandecasteele SJ, Appelberg R, et al. (2007). The effect of the host's iron status on tuberculosis. J Infect Dis. 2007;195:1745-53.
19. Ciftci TU, Ciftci B, Yis O, et al. (2003). Changes in serum selenium, copper, zinc levels and cu/zn ratio in patients with pulmonary tuberculosis during therapy. Biol Trace Elem Res. 2003;95:65-71.
20. Choi R, Kim HT, Lim Y, et al. (2015). Serum Concentrations of Trace Elements in Patients with Tuberculosis and Its Association with Treatment Outcome. Nutrients. 2015;7:5969-81.
21. Wolschendorf F, Ackart D, Shrestha TB, et al. (2011). Copper resistance is essential for virulence of Mycobacterium tuberculosis. Proc Natl Acad Sci. 2011;108:1621-6.