

Association of Zinc and Vitamin A with Pulmonary TB Infection and PTB-HIV CO-Infection

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ABSTRACT

Objectives of Study: To assess serum zinc and vitamin A levels among patients of PTB and PTB-HIV co-infection.

Study Setting: Physiology department, Chest Medicine department, HIV center and Medical Research Centre, LUMHS Jamshoro and the Institute of Chest Diseases in Kotri TB Sanatorium Hospital.

Material and Methods: In this cross-sectional comparative study, patients having PTB and PTB-HIV co-infection were recruited from Chest Medicine Department and the Institute of Chest Diseases in Kotri after approval from Ethical Review Committee, LUMHS, Jamshoro. Study participants divided in two groups; one group (n=114) included diagnosed cases of PTB having no co-infection while in other group (n=9) there were patients having coinfection of PTB and HIV. The sampling method was non probability purposive. Blood samples were obtained intravenously after informed consent. Serum zinc levels were determined by zinc ELIA kit and vitamin A by vitamin A ELISA kit.

Results: Mean age of study participants was 28.88 years and among them 69.11 percent were AFB positive. To find out drug resistance, gene Xpert was done that was positive in 95.93 percent of patients. Vitamin A and zinc levels were decreased in the main but furthermore they were compared between PTB and PTB-HIV coinfection. Mean of vitamin A in PTB and PTB/HIV co-infection groups were 0.07 and 0.02 respectively, (p-value=0.03 and t value=2.09) that show statistically significant difference in vitamin A levels in two groups. Mean of serum zinc in PTB and PTB/HIV co-infection groups were 2.84 and 1.14 respectively, (p-value<0.01 and t value=2.89) that show highly significant difference statistically.

Conclusion: Vitamin A and zinc are significantly less in coinfecting people.

Keywords: Co-infection, HIV, PTB, vitamin A, Zinc

INTRODUCTION

Pakistan is among those twenty countries that had highest burden of pulmonary tuberculosis (PTB) all over world.¹ Association of PTB with HIV imposes a huge challenge on health care system globally. The diagnostic ways has been improved globally but still there hassles have been observed in the developing countries because of their behavior of seeking health care in later stages of disease.² Healthy and optimal immune system is very important to combat the infections specifically the co-infections and best working immune system depends on the better nutrition to combat the invading agents. It has been notified that vitamin A and zinc are the micronutrients that play the pivotal part in making optimal immune system and their deficiencies might lead to augmented risk of infections, oxidative stress with worse clinical outcomes.³ Deficiency of zinc affects the T-lymphocytes, especially their maturation and disturbs steadiness in various T-cell sub populations. Vitamin A in the form of retinol might had control over humoral Th-2 cellular reactions by means of antigen-presenting cells and is considered to be concerned with tolerance via its nuclear receptor 'RAR' plus kinase signaling cascades.⁴ PTB bears negative brunt on the immune system to infection by human immunodeficiency virus (HIV), and accelerates the sequence from HIV infection towards developing AIDS (autoimmune deficiency syndrome). Managing PTB-HIV co-infections needs incorporation of effectual anti-TB treatment (ATT), usage of parallel antiretroviral (ARV) therapy, anticipation of HIV infection associated co-morbidities, managing drug cyto-toxicities, encompassing the preventive measures or managing immune reconstitution inflammatory syndrome.⁵ In one of the studies carried in Karachi, Pakistan, it has been accomplished that PTB/HIV co-infected patients has lower hemoglobin concentrations with severe decline in CD4 levels as compared to those having mono-infection of HIV.⁶ Alveolar macrophages are the cells that are most abundantly present in the pulmonary alveoli and these are the prime line of defense to combat the micro-pathogens and airborne invading agents. Alveolar macrophage in the untreated patients of HIV became transformed to compromised functions state, which is supposed to be contributing to the amplified

vulnerability to get PTB infection into active phase from latent PTB. The lungs are considered as primary site for replication of HIV, and it has been revealed that alveolar macrophages in early stages of infection contain HIV DNA in addition to RNA, yet though HIV at initial stage does not enter macrophages competently in vitro.⁷ HIV is thought to be affecting helper T lymphocytes and alveolar macrophages, which play pivotal part in proper performance and regulation of cell mediated immunity. Cell mediated immune responses are worthy in defensive for individuals suffering from diseases encompassing PTB. HIV is the for the most part, essentially recognized risk factor to facilitate progression to active PTB in patients carrying mycobacterium tuberculosis. CD4 T-cell count is considered as the paramount pointer of the immediate immune capability of HIV infected patients.⁸ HIV infection can possibly influence antigen presentation by antigen presenting cells in PTB co infected dendritic cells and consequently restrain the acquired immune responses. The option to increase or improve the function of innate immunity may possibly show to be significant at some point in an HIV co-infection, at what time the functioning of acquired immunity is lacking.⁹ Meanwhile, unfavorable outcomes and adherence in treatment has been reported in the PTB-HIV co-infections as compared to people with PTB without HIV infection especially, MDR-TB (multiple drug resistance tuberculosis).¹⁰

There is limited data existing regarding the levels of zinc and vitamin A in the PTB patients with or without HIV co-infection. Many studies have been carried worldwide in different ways but not yet such study has been carried in Pakistan in young age group patients irrespective of gender and race. Several of the epidemiological studies has been carried on zinc and vitamin A but no specific emphasis has been given in past studies regarding the roles in PTB and PTB-HIV co-infections in adults. Past work has been carried on pregnant women and in pediatric set-ups; however there is lack of evidence regarding the deficiencies of just aforementioned micronutrients in patients of PTB and PTB co-infections. Deficits in the levels of vitamin A and zinc are the issues to address in PTB patients; moreover, under-nutrition may lead to the development of PTB & other co infections like HIV. This study

may help the physicians that micronutrients supplements, i.e., vitamin A and zinc in PTB patients play a major function in fighting against PTB & HIV infections.

It was hypothesized that vitamin A and zinc levels are altered in patients of PTB and PTB-HIV co-infected patients. Present study has been thought to evaluate serum zinc and vitamin A levels among patients of PTB and PTB-HIV co-infections.

METHODOLOGY

This Cross sectional study was carried out in Department of Physiology in collaboration with Department of Chest Medicine, LUMHS Jamshoro/Hyderabad, HIV Centre, LUMHS Jamshoro, Medical Research Centre, LUMHS Jamshoro and the Institute of Chest Diseases in Kotri TB Sanatorium Hospital. The way of sampling was non probability purposive sampling. Duration of study was six months after approval from Ethical Review Committee, LUMHS, Jamshoro.

Prevalence of active TB among adults of Sindh was 8.1 percent,¹¹ sample size for group A calculated by Rosoft with 95% confidential interval and 5% margin of error is 114. (n=114)

Prevalence of TB/HIV co-infection in Sindh was 0.58%,¹² so sample size for Group B, calculated by Rosoft with 95 confidential intervals and 5% margin of error is 9. (n=9)

Total sample size for this study was 123(n=123) Study participants divided into two groups, one encompassing diagnosed cases of PTB(n=114) and other group included diagnosed cases of PTB/HIV co-infection (n=09). **Inclusion Criteria** were diagnosed cases of TB and TB/HIV co-infection with age group of 18-40 years and of either gender. Patients who were HIV sero-negative, not suffering from TB, COPD, Children, Pregnant and other than above co-morbidities were eluded from this study.

The patients with diagnosis of TB and TB/HIV co-infection were recruited from department of Chest Medicine, LUMHS, Jamshoro /Hyderabad and the Institute of Chest Diseases in Kotri. At recruitment, each participant was assessed, using questionnaires including age, gender and medical history. Blood samples were obtained from all study participants meeting the selection criteria. Serum Zinc was measured on 'Human Zinc finger CCCH domain- containing protein 15, ZC3H15 ELISA Kit' CAT# 'E1985Hu' by Bioassay China and vitamin A was measured on 'Human vitamin A (VA) ELISA KIT' CAT# 'E1985Hu' by Bioassay China. Vitamin A levels less than 196 ng/ml or less than 0.70 miromol/litre is considered as vitamin A deficiency.^{13, 14}

PTB patients were recruited for study from TB sanitorium and chest ard , LUMHS. After informed consent demographic data was taken and then blood samples taken after aseptic measures. Blood samples used for determining zinc and vitamin A levels in medical research, LUMHS, Jamshoro. Data was entered in predesigned proforma. Data was analyzed on IBM, SPSS version 22.0. Mean and standard deviation were determined for quantitative variables like age, weight, height, body mass index, hemoglobin, serum zinc and vitamin A levels. Different quantitative variables compared among two groups by applying independent t test. P-value <0.05 was taken as significant with 95% confidential interval. Frequency (%) was determined for qualitative variables like, education status, living area, TB group and TB/HIV co-infection groups etc.

RESULTS

Mean age of study population that included both PTB patients and PTB/HIV co-infection was 28.88 years±5.9. Mean±SD of hemoglobin concentration, height (in cm), weight (in kg) and body mass index (in kg/m²), in study population was 7.50±0.85, 169.0±5.76, 62.81±6.85 and 21.96±1.95 respectively. Among the study population 85(69.11%) were AFB positive and 38(30.89%) were AFB negative. To find out the drug resistance, gene Xpert was carried and that revealed positive in 118(95.93%) patients.

Table 1

Among PTB patients, 48(30.0%) were residing in urban area and 66(53.7%) were residing in rural setups, while in patients with PTB/HIV coinfection, 6(4.9%) were living in urban area and 3(2.4%) were of rural areas. Generally, more people were found living in rural areas but when living areas of two groups of PTB and PTB/Hiv co-infection compared, and there were no statistically significant difference found. (p value=0.14 and x²=2.04). **Similarly**, when education status of study participants compared then , it was revealed that mostly the patients were never went to school, i.e., (75.4%) and among the 7.4% of PTB/HIV co-infection patients, 4.9 % found never went to school. (p value < 0.01 and x²=11.77) **Table No.2**

In PTB patients, Radiologic findings in chest X ray showed cavitations in 1.6%, cavitation with hilar lymphadenopathy in 9.8%, cavitation with infiltrate in 51.2%, cavitation, infiltrate with pleural effusion in 29.3% and cavitation with fibrosis in 0.8%. In PTB/HIV coinfection patients, chest X ray showed cavitations only in 0.6% patients, cavitation with hilar lymphadenopathy in 0.8%, cavitation with infiltrate in 1.6%, cavitation, infiltrate with pleural effusion in 4.1% and cavitation with fibrosis in 0.8%. **Figure 1**

Table 1: Descriptive statistics in study population (n=123)

	Mean±SD	Frequency (%)
Age (In Years)	28.88±5.9	--
Hemoglobin(g/dl)	7.50±0.85	--
Height(cm)	169.0±5.76	--
Weight(kg)	62.81±6.85	--
Body mass index	21.96±1.95	--
PTB	--	114(92.7)
PTB-HIV Co-infection	--	9(7.3)
Urban		54(43.9)
Rural		69(56.1)
AFB Positive		85(69.11)
AFB Negative		38(30.89)
Gene Xpert positive		118(95.93)
Gene Xpert negative		5(4.07)

Table 2: Living area and Education-based distribution of PTB patients and PTB/HIV co-infection

		PTB n(%)	PTB/HIV Co-infection n(%)	Total	p-value
Living area	Urban	48(39.0)	6(4.9)	54(43.9)	0.14
	Rural	66(53.7)	3(2.4)	69(56.1)	
Education Status	Never went school	86(70.5)	6(4.9)	92(75.4)	0.008**
	Primary	12(9.8)	1(0.8)	13(10.7)	
	Secondary	13(10.7)	0(0.0)	13(10.7)	
	Graduate	2(1.6)	2(1.6)	4(3.3)	

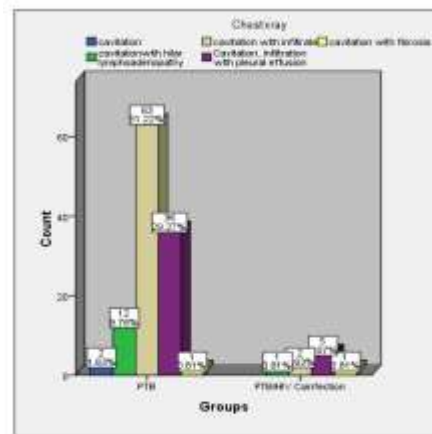


Figure 1: Radiological findings on chest X-ray in PTB and PTB/HIV Co-infection

Serum vitamin A and serum Zinc levels measured in unit ng/ml and then were converted in SI units of measurement for vitamin A and Zinc, i.e., µmol/L. Mean of serum vitamin A and Zinc in study population were 0.07µmol/L and 2.72 µmol/L respectively. Vitamin A levels and Zinc levels were decreased generally in study population (n=123) but further they compared in two groups of (PTB) and (PTB/HIV coinfection) by applying independent t test. Mean of vitamin A in PTB and PTB/HIV co-infection groups were 0.07 and 0.02 respectively, (p-value=0.03 and t value=2.09) that show statistically significant difference in vitamin A levels in two groups. Mean of serum zinc in PTB and PTB/HIV co-infection groups were 2.84 and 1.14 respectively, (p-value<0.01 and t value=2.89) that show highly significant difference statistically.

Table 3

Table 3: Comparison of vitamin A and zinc levels in PTB and PTB-HIV co-infection

	PTB		PTB-HIV Co-infection		p value
	Mean	Standard error of Mean	Mean	Standard error of Mean	
Vitamin A levels(µmol/L)	0.07	0.01	0.02	0.01	0.03*
Serum Zinc(µmol/L)	3.73	0.21	2.17	0.43	0.04**

*shows statistically significant p-value <0.05

** shows highly significant p-value <0.01

DISCUSSION

Pakistan is one of the country with concentrated epidemic of PTB-HIV coinfection. ¹⁵ Similarly has been found by Qadeer AS et al¹⁶ in Pakistan, they advise researchers and the health professionals for continuous close watch to control the growing epidemic. Patti G et al.¹⁷ revealed that the role of micronutrients is also undeniable in controlling this newly budding epidemic. Prescribing zinc and vitamin A may prove much more beneficial in better prognosis. In present study, mean age of PTB and PTB/HIV coinfection was 28.88 years±5.9 that is the young age group and among PTB patients, 80.5% were males and 12.2% were female, while in patients with PTB/HIV coinfection, 5.7% were male and only 1.6% were female. Generally, more people were found living in rural areas. These findings are similar to the study by Marskole P et al,¹⁸ who also revealed more cases in young age and more prevalent in males as compared to females. According to them co-infected individuals were residing in peripheral areas and this finding is also in constancy with present study. In present study, levels of vitamin A and zinc have been found significantly decreased in PTB-HIV coinfection.

When comparing the educational strata of patients then , it was discovered that among the affected proportion, 75.4 percent never went to school, and in 7.4 percent of PTB/HIV co-infection patients, 4.9percent found never went to school. This is similar to the study by Shereen MA,et al.¹⁹ who accomplished that adolescent, single and illiterate males were more affected by HIV and PTB, due to sexual contacts and injecting intravenous drugs by contaminated syringes.

Levels of vitamin A and zinc have been found significantly decreased in present study population generally, in both groups with PTB mono-infection as well as in PTB-HIV coinfection. Parallel to these findings, Gebremicael G, et al.²⁰ found decreased vitamin A levels in PTB patients but no significant difference between two groups with PTB-HIV co-infection and without HIV co-infection. Jain P et al., ²¹ revealed significantly decreased levels of zinc in PTB patients and it was suggested that normal levels of zinc are worthy to help in cell mediated immunity. The individuals without disease are required to take zinc in diet to enhance cell mediated immunity. Vitamin A plays immune regulatory role, cause growth and differentiation of B cells with production of antibodies. Zinc prevents from cytokine storm by limiting the excessive release of pro-inflammatory cytokines, IL-2, 6 and TNF alpha. ^{22,23} Scarce

vitamin A and zinc levels are the grave concerns to be tackled in patients of PTB mono-infection for resisting the progression towards PTB-HIV coinfection and other comorbidities.

CONCLUSION

Vitamin A and zinc are found decreased in both groups, PTB mono-infection and PTB-HIV co-infection but instead vitamin A and zinc has been found significantly decreased in PTB patients with co-infection of HIV.

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