

## ASSOCIATION OF VITAMIN D DEFICIENCY WITH TUBERCULOSIS IN ADULT PATIENTS REPORTING TO A TERTIARY CARE HOSPITAL OF RAWALPINDI

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### ABSTRACT

**Objectives:** To compare the mean vitamin D levels in pulmonary tuberculosis patients and healthy controls and to find out the frequency and association of vitamin D deficiency in patients with tuberculosis.

**Study Design:** Case control study.

**Place and Duration of Study:** Pulmonology department, Military Hospital Rawalpindi from Jan 2013 to Dec 2013.

**Patients and Methods:** Fifty two incident outdoor pulmonary tuberculosis patients were selected with 52 age and gender matched controls. Tuberculosis was diagnosed by the sputum examination through gene Xpert technique from National Institute of Health (NIH), Islamabad. Serum 25-hydroxyvitamin D level <50 nmol/l (electrochemiluminescence assay) was taken as vitamin D deficiency.

**Results:** Mean vitamin D was much lower ( $20.688 \pm 14.065$  nmol/l) in cases as compared to the controls ( $57.917 \pm 18.197$  nmol/l) which was statistically significant ( $p < 0.001$ ). Forty nine (94%) cases and 18 (34.6%) controls were found to be vitamin D deficient. (Odd's Ratio = 3.8, 95% CI = 2.423—5.999,  $p < 0.001$ ). Sensitive tuberculosis patients had mean vit D levels of  $18.55 \pm 9.99$  nmol/l while multi-drug resistant tuberculosis patients had the mean serum vitamin D levels of  $22.38 \pm 16.60$  nmol/l but the difference was statistically insignificant ( $p > 0.05$ ).

**Conclusion:** Significant vitamin D deficiency was seen in newly diagnosed TB patients. It was found that vitamin D deficiency is associated with tuberculosis, but its causal role has not been established.

**Key words:** Pulmonary tuberculosis, Multidrug resistant tuberculosis, Vitamin D deficiency.

### INTRODUCTION

Tuberculosis (TB) a momentous disease since ages has been a worldwide public health challenge and was declared a global medical emergency in 1993. One third of the world's population is infected with TB<sup>1</sup>. Around ten million cases of tuberculosis occur every year and out of these three million die around the world. Developing countries carry 95% of the total burden of this disease<sup>1</sup>. Pakistan ranks fifth in the list of 22 highest TB burden countries, and contributes 61% of total TB cases occurring in the Eastern Mediterranean Region of WHO<sup>2</sup>. Incidence and prevalence rates of TB remained very high in the year 2012 being 231/100,000 and 376/100,000 respectively<sup>1</sup>. Pakistan ranks 4<sup>th</sup> among the 27 high multidrug resistant (MDR) TB burden countries and 5<sup>th</sup> among the countries

with the highest number of incident TB cases in 2012 (0.3–0.5 million)<sup>2</sup>.

In past few years several lines of evidence have suggested a possible link between TB and vitamin D deficiency<sup>3</sup>. Surprisingly, studies have shown asymptomatic deficiency of vit D in the healthy general population (hidden hunger) of our country<sup>4-7</sup>. In spite of this high load of TB and vit D deficiency, very restricted literature highlighting vit D deficiency and TB linkage in indigenous Pakistani population is available. This situation definitely merits further research and in depth analysis to find out any probable link between the two conditions. Present study was carried out to find any association between vit D deficiency with TB.

### PATIENTS AND METHODS

This case control study was carried out in the Pulmonology Department of Military Hospital Rawalpindi from January 2013 to December 2013. TB case was taken as the one in which, "a health worker (clinician or other medical practitioner)

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has diagnosed TB and has decided to treat the patient with a full course of TB treatment<sup>8</sup>. Pregnant ladies, lactating mothers and individuals suffering from abnormal serum calcium levels, renal and hepatic impairments, previous bone disease, malabsorption syndrome and taking medications that effect vitamin D metabolism e.g., phenobarbital, phenytoin, carbamazepine, rifampicin, antiretrovirals were excluded from the study. Informed verbal consent was taken from the study subjects and permission from the hospital ethical committee was sought before commencement of study. Sample size (n) was calculated as 104 (case control ratio = 1:1) using G Power 3.1.6 software (effect size = 0.5, alpha error probability = 0.05, power = 90%). Fifty two newly diagnosed non smoking patients of pulmonary tuberculosis who were yet to start anti tuberculosis treatment were selected through non probability consecutive sampling from outpatient department and grouped as cases. Pulmonary TB was diagnosed through Gene Xpert technique (from National TB Reference Laboratory in NIH, Islamabad) utilizing nucleic acid amplification procedure that identifies mycobacterium DNA in the sputum specimens and confirms sensitive / MDR TB in less than two hours. Fifty two age and gender matched non smoking controls after screening for TB (negative for TB on clinical and radiological examination) were selected from the general population. All the study subjects were asked to fill a questionnaire related to socio demographic details, nature of occupation, vitamin D rich food intake, exposure to sunlight and time of the day spent in the sunlight. Body mass index (BMI) was also recorded as kg/m<sup>2</sup>. Five ml of venous blood was collected in plastic serum tubes. Blood samples were placed in ice boxes and sent to the laboratory for analysis. Samples were centrifuged for serum separation and 25—(OH)—D<sub>3</sub> levels were measured by chemiluminescence assay using Diasorin diagnosis Liasion in Armed Forces Institute of Pathology, Rawalpindi. All the results were duly verified by pathologist. For research purpose

serum vit D levels <50 nmol/l were considered as vit D deficiency while >50 nmol/l were considered as desirable. Data analysis was performed by Statistical Package for Social Sciences (SPSS) version 20. Results were expressed as mean and standard deviation (SD) for all quantitative variables. For qualitative variables, frequency and percentages were used. Independent samples t-test / Mann Whitney U test (where appropriate) and chi-square test were applied according to the type of data and distribution of the variables. A *p* value < 0.05 was considered as statistically significant.

## RESULTS

There were 52 cases and 52 age and gender matched controls (62% men and 38% women in each group). Mean age (SD) of the cases was 39.48 (14.21) [median (IQR)=34.00 (27.00)] years while in the controls mean age was 38.41(14.12) [median (IQR)=33.00 (28)] years. Both the groups were comparable with respect to age (*p*>0.05) and gender (*p*>0.05). Thirty two (61.5%) cases while 33 (62.4%) controls belonged to the urban areas while the rest belonged to the rural areas (*p*>0.05). Forty nine (94.2%) cases while 18 (34.6%) controls were found to be vit D deficient (*p*<0.001). According to the Odds ratio (OR = 3.8, 95% CI = 2.423—5.999, *p*<0.001) TB patients were 3.8 times likely to be deficient in vit D as compared to the controls. Mean serum vit D level was found to be lower in cases as compared to the controls (Table). Females of both the groups had much lower vit D levels as compared to the males. Nineteen out of 20 (95%) female TB patients while 30 (93.5%) out of 32 male TB patients had vitamin D deficiency (*p*>0.05). Although, female patients had lower vitamin D levels as compared to the male patients, the difference was not significant (*p* > 0.05). Twenty nine out of 52 (56%) TB patients were found to have sensitive TB while 23 (44%) cases were found to have MDR TB in incident TB cases through gene Xpert. Mean serum vit D level was found to be lower in MDR TB patients as compared to sensitive TB patients but the difference was not statistically significant

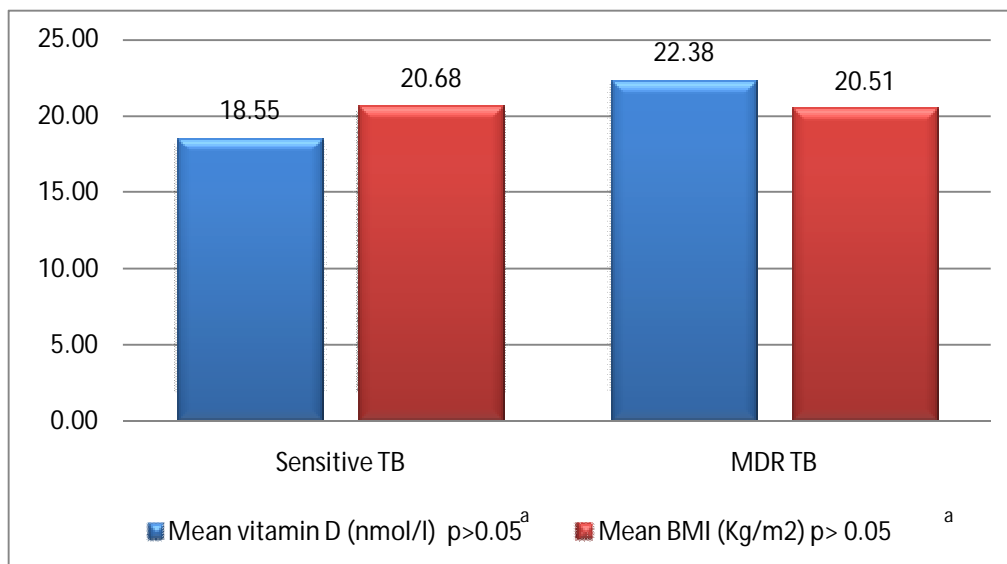
( $p > 0.05$ , figure). Mean (SD) BMI in cases was 20.313 (3.273) kg/m<sup>2</sup> while in controls it was 24.683 (2.414) kg/m<sup>2</sup> ( $p < 0.001$ ). Mean (SD) BMI in

and Prosser Thomas reported the treatment of this disease with oral vitamin D in 1946<sup>10</sup>. Vitamin D supplementation is being tried as

**Table-: Clinical characteristics of study subjects.**

Variable	Parameter	Cases	Controls	p value
Vitamin D (nmol/l)	Mean (SD)	20.68 (14.06)	57.91(18.19)	< 0.001 <sup>a</sup>
	Median (IQR)	16.25 (12.97)	65.50 (29.00)	
Vitamin D deficiency	n (%)	49 (94.2%)	18 (34.6 %)	< 0.001 <sup>b</sup>
Males	Mean Vit D (nmol/l) (SD)	21.29 (15.73)	62.96 (15.72)	< 0.001 <sup>a</sup>
	Median Vit D (nmol/l) (IQR)	16.80 (13.30)	68.95 (21.50)	
Females	Mean Vit D (nmol/l) (SD)	19.72 (11.21)	49.84 (19.34)	< 0.001 <sup>a</sup>
	Median Vit D (nmol/l) (IQR)	15.65 (11.58)	48.95 (31.87)	

a Mann Whitney U test, b Chi Square test



a Independent samples t test

**Figure-: Comparison of mean serum vitamin D levels and BMI among sensitive and MDR TB cases.**

cases with vit D deficiency (<50 nmol/l) was 20.314 (3.324) kg/m<sup>2</sup> while in cases with desirable serum vit D levels (>50 nmol/l) mean BMI was 20.289 (2.818) kg/m<sup>2</sup> ( $p > 0.05$ ). Mean (SD) BMI in sensitive TB cases was 20.629 (3.755) kg/m<sup>2</sup> while in MDR TB cases, it was 20.062 (2.879) ( $p > 0.05$ ).

**DISCUSSION**

There is a long history of using vitamin D to treat TB with some apparent success. Finsen discovered that UVB rays successfully treated the cutaneous form of TB, a discovery for which he was awarded the Nobel Prize in 1903<sup>9</sup>. Dowling

adjuvant to chemotherapy in pulmonary TB, enhancing antimicrobial responses<sup>11</sup> and accelerating clearance of bacilli in some patients. Significant role is played by vitamin D in bone homeostasis and calcium metabolism. It has also been proven to have some role in immunoregulatory mechanisms, especially in TB. Latest studies have demonstrated induction of antimicrobial peptide Cathelicidin by vitamin D indicating a possible anti mycobacterium tuberculosis action<sup>12</sup>.

Our study reveals that that serum vitamin D deficiency was markedly high in both male and

female TB patients. Incident cases of pulmonary tuberculosis were included who were yet to be treated with anti tuberculosis drugs as anti tuberculous chemotherapy lowers serum vitamin D levels<sup>13</sup>. Smoking is considered a risk factor for tuberculosis disease<sup>14</sup>. Vitamin D is imperative for calcium absorption (which is impaired by smoking), there is a suggestive evidence that vitamin D absorption is impaired indirectly by smoking<sup>15</sup>. It is pertinent to note that effect of smoking is not controlled in most of the studies conducted on vit D deficiency and TB linkage, making these studies biased. To control for the possible vit D lowering effect of smoking, only non smoker cases and controls were included in the our study. Present study also showed no significant relationship between BMI and change in vit D levels. Our patients were undernourished, with low BMI (lack of subcutaneous fat leading to depleted stores of vitamin D) which is further associated with vit D deficiency. Thus low BMI is important confounder for association of the low vit D tuberculosis which was controlled in the study through stratification. Our study has revealed that 44% of TB patients had MDR TB on gene Xpert and mean vit D level was found much lower than sensitive TB. This study needs to be verified by future studies upon MDR TB and vit D deficiency associations.

Different studies have been conducted in various parts of the world to find out the association of vit D deficiency with TB<sup>16</sup>. A number of studies in Gujarati Indian<sup>17</sup>, African residents in London<sup>18</sup>, indigenous Indian<sup>19,20</sup>, West African<sup>21</sup>, Malawi<sup>22</sup>, Chinese<sup>23</sup>, Vietnam<sup>3</sup>, Uganda<sup>24</sup> and Pakistani<sup>13</sup> populations have shown that TB patients had lower levels of 25 (OH) vitamin D and higher prevalence of vitamin D deficiency than non-TB individuals. In a case control study conducted by Iftikhar et al<sup>13</sup> on indigenous Pakistani population showed that there was significant vit D deficiency in TB patients as compared to the controls ( $p < 0.001$ ) with an Odd's ratio (CI) of 2.67 (1.67–4.25). Our study reports the Odds ratio (CI) between vit D

deficiency and TB disease as 3.8 (2.42-5.99). Iftikhar et al<sup>13</sup>, although included incident cases of TB but confounding effect of smoking was not controlled. Moreover no mention was made to the diagnosis of sensitive TB and MDR TB patients and the time taken by the diagnosis by culture and drug susceptibility testing by Iftikhar et al<sup>13</sup>. Our study confirmed TB diagnosis by gene Xpert giving the results in less than two hours.

Larger prospective studies to firmly establish the direction of the relationship between vitamin D and TB as well as evaluation of vitamin D supplementation in TB are needed. Asymptomatic vitamin D deficiency can be prevented by education of the population on change in dietary habits, educating parents to expose their children regularly to sunshine, prophylaxis through periodic dosing, vitamin D fortification of foods especially milk, butter and oil and attention of government towards food fortification policies is the need of the day to combat hidden hunger and lessen the double burden of disease in Pakistan.

There are a few limitations of this study. Other than being negative on screening, apparently healthy without any known co-morbidity were included, assessment of the dietary intake of vitamin D rich foods through dietary recall, seasonal variations and single centre sample selection through non probability sampling limit the generalize ability of this study.

## CONCLUSION

Significant vitamin D deficiency was seen in incident TB patients. It was found that vitamin D deficiency is associated with tuberculosis, but its causal role has not been established.

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