ORIGINAL ARTICLE

Estimation of Vitamin D Level in Severe Variant of TB against Less Severe Variant of TB in Paediatric Patients: A Comparative Study

Sunil Piruwa[1], Ishrat Ali Ansari[2], Pankaj K Chaudhary[3,*]

[1]Associate Professor, Department of TB and Chest, GMC, Azamgarh, Uttar Pradesh, INDIA.
[2]Associate Professor, Department of Surgery, GMC, Azamgarh, Uttar Pradesh, INDIA.
[3]Assistant Professor, Department of Pharmacology, GMC, Azamgarh, Uttar Pradesh, INDIA.

ABSTRACT

Background: Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis killing nearly 2 million people every year. In India, over 100,000 children die from tuberculosis every year. The best-understood function of Vitamin D is in the absorption of calcium from the small intestine, which helps to prevent diseases such as osteoporosis and osteomalacia in adults and rickets in children. The present study was done with an aim to estimate the level of Vitamin D in severe against less severe paediatric tuberculosis patients. **Materials and Methods:** This present prospective observational study was conducted in Department of TB and Chest of GMC, Azamgarh from February 2022 to March 2023 after approval from Ethical Committee GMC, Azamgarh. The study was done till the sample size of 96 patients was achieved in children up-to 18 years of age presenting with tuberculosis at our hospital. **Results:** Out of total 96 patients, 63 (53.23%) were male and 33 (46.77%) were female. Majority of patients were age group of >10 years. 33 (46.77%) of them had positive history of contact, 63 (53.23%) had no history of contact. Majority 53 (85.48%) of TB were diagnosed through CBNAAT. Most common presentation was pulmonary TB (43.75%). 53 (55.20%) patients were having deficient Vit D. 26 (27.08%) had severe variant of TB and 70 (72.91%) had less-severe TB. Mean + SD level of serum Vitamin D3 in severe variant of TB was 13.4 + 7.8 and 22.6 + 8.9 in less-severe variant TB. Hypovitaminosis D was seen in 53 (55.20%) TB patients in which majority of case were of pulmonary TB 31(60.78%). 13 (13.54%) were in the age group 0-5 years, 24 (25%) were in the age group 5-10 years and 59 (61.45%) were of age >10 years. **Conclusion:** The study findings support the assumption that hypovitaminosis D potentiates susceptibility to TB. This relationship should be investigated further in a large-cohort longitudinal study. We advocate for longitudinal studies across various geographical regions to accurately define the roles of Vitamin D on TB treatment and prevention.

Keywords: Vitamin D deficiency, Tuberculosis, Diagnosis, Pulmonary.

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Correspondence
Dr. Pankaj K Chaudhary
Assistant Professor, Pharmacology, GMC, Azamgarh, Uttar Pradesh, INDIA.
Email: chaudharypankaj847@gmail.com

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. Tuberculosis is one of the deadliest diseases in the world killing nearly 2 million people every year. In India, over 100,000 children die from tuberculosis every year.[1] Global TB report 2018 reports that in India, an estimated 2.2 lakh children become ill with Tuberculosis (TB) each year (22% of global TB burden), with a slightly higher burden among males. Vitamin D is a prohormone with numerous functions in the body. The best-understood function of Vitamin D is in the absorption of calcium from the small intestine, which helps to prevent diseases such as osteoporosis and osteomalacia in adults and rickets in children.

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In the pre-antibiotic era, the role of Vitamin D in prevention of infectious diseases has been very important. The cod liver oil, one of the most important nutritional sources of Vitamin D, was used both for treatment of rickets and Tuberculosis (TB). Vitamin D is readily metabolized in the liver, to form 25 hydroxy-vitamin D [25(OH)D], the accepted measure of Vitamin D status.[2] Calcitriol, the active metabolite of Vitamin D induces anti-mycobacterial activity *in vitro* along with modulating the host response to Mycobacterial infection by induction of reactive nitrogen and oxygen intermediate and induction of antimicrobial peptide catheliciden.[3,4] This peptide has potent bactericidal capacity against a number of important bacteria and viruses, including *M. tuberculosis* and influenza-virus.[5] Since, there is scare of data regarding Vitamin D level in pediatric tuberculosis patients. The present study was done with an aim to estimate the level of Vitamin D in severe against less severe paediatric tuberculosis patients.

AIM AND OBJECTIVES

To estimate the levels of Vitamin D in Tuberculosis and to compare Vitamin D level in severe case of tuberculosis against less severe case of tuberculosis in pediatric patients.

MATERIALS AND METHODS

This present prospective observational study was conducted in Department of TB and Chest of GMC, Azamgarh from February 2022 to March 2023. The present study was initiated after the approval from Ethical Committee GMC, Azamgarh. The study was done till the sample size of 96 patients was achieved. The study includes children up-to 18 years of age presenting with tuberculosis at our hospital.

Patient selection

It was based on following criteria:

Inclusion Criteria

1. All patients aged below 18 years who presented with symptoms and signs of Tuberculosis and in whom the diagnosis was documented by laboratory findings both indoor and outdoor patient.
2. All newly diagnosed cases of Tuberculosis.

Exclusion Criteria

1. All patients above 18 years of age presented with Tuberculosis.
2. All patients presented with tuberculosis with HIV.
3. Patients who were not willing to sign the inform consent.

Method of diagnosis

All sputum or alternative specimens (Gastric lavage, Induced sputum, bronchoalveolar lavage) microscopy acid fast bacilli positive tuberculosis, All CBNAAT positive tuberculosis and Patient diagnosed as TB clinically by radiological findings with blood investigations and histopathology finding etc. were included in our study.

Methodology

Informed consent or assent in case of a minor was taken from the parents or the guardians of the children was taken before collection of blood sample. Details such as age, sex, demographic data, personal and family history, immunization history, nutritional supplementation history and detailed history of clinical features of Tuberculosis were recorded on pre- designed and pre-tested proforma. Anthropometric measurement like weight, height was recorded in all cases. Patient was not pricked separately for Vitamin D levels. Patients who were deficient in Vitamin D were given standard regimen for treatment of Vitamin D deficiency.

Vitamin D level estimation

Before starting treatment, venous blood samples of were collected for 25 (OH) D_3 levels in plastic serum tubes. Samples were placed in ice boxes and sent immediately to laboratory where Serum was separated by centrifugation and 25 (OH) D levels measured using beckman coulter access 2 auto analysers by Total ELISA assay which is a solid phase Enzyme Linked Immunosorbent Assay. All the results were duly verified by pathologist.

Statistical Analysis

Data was entered in Microsoft Excel Sheet and analysis was done using SSPS statistical package. Parameters such as rate, ratio and percentage were calculated. To test the significance suitable statistical test were applied. A probability of less than 0.05 was considered statistically significant.

RESULTS

Our study results are as follows.

Table 1: Distribution of the patients according to gender profile.

Gender	No. of Patient	Percentage
Male	63	65.62%
Female	33	34.37%
Total	96	100.00%

Out of total 96 patients, 63 (53.23%) were male and 33 (46.77%) were female.

Table 2: Distribution of the patients according to age group.

Age Group	No. of Patient	Percentage
0-5 Years	13	13.54%
6-10 Years	24	25%
>10 Years	59	61.45%
Total	96	100.00%

Out of 96 patients, 13 (13.54%) patients were in the age group of 0-5 years, 24 (25%) were in the age group of 6-10 years and 59 (61.45%) were of age >10 years.

Table 3: Distribution of the patients according to H/O contact.

H/O Contact	No. of Patient	Percentage
Positive	33	65.62%
Negative	63	34.37%
Total	96	100.00%

H/O- History of Out of 96 patients, 33 (46.77%) of them had positive history of contact, 63 (53.23%) had no history of contact.

Table 4: Distribution of the patients according to laboratory findings.

Laboratory findings	No. of Patient	Percentage
Mantoux Test	24	38.71%
CBNAAT	53	85.48%
XRAY (TB findings)	39	62.90%
OTHERS	33	53.23%

(Note-One patient may have more than one test positive), (Others =biopsy positive, MRI and CT findings, raised ESR).
Out of total patients, 24 (38.71%) were Mantoux test positive, 53 (85.48%) were CBNAAT positive, 39 (62.90%) had positive X-ray findings and 33 (53.23%) were Others (biopsy positive, MRI brain and CT findings of TB, raised ESR).

Table 5: Distribution of the patients according to Diagnosis.

Diagnosis	No. of Patient	Percentage
Pulmonary TB	42	43.75%
TB lymphadenopathy	16	16.66%
Abdominal TB	03	3.125%
Pleural TB	09	9.375%
Miliary TB	03	3.125%
TB Meningitis	17	17.70%
Disseminated TB	06	6.25%
Total	96	100.00%

In our study 96 patients diagnosed with TB were enrolled out of which most common presentation was pulmonary TB (43.75%), Extrapulmonary TB was in order of frequency TB lymphadenopathy (16.66%) followed by TB Meningitis (17.70%), pleural TB (9.375%), Disseminated TB (6.25%), Abdominal TB (3.125%) Miliary TB (3.125%) respectively.

Table 6: Distribution of the patients according to levels of Vitamin D and gender profile.

Level of Vitamin D	Male	Female	Total Patients
Deficient	34 (53.96%)	19 (57.57%)	53 (55.20%)
Insufficient	17 (26.98%)	10 (30.30%)	27 (28.12%)
Sufficient	12 (19.04%)	4 (12.12%)	16 (16.66%)
TOTAL	63	33	96

Serum Vit D3 (Mean +SD) among all 96 patients =21.4 + 9.0.
In our study we observed that out of 96 patients, 53 (55.20%) patients including 34 (53.96%) male and 19 (57.57%) female had deficient (<20 ng/mL) Vitamin D level, 27 (28.12%) patients including 17 (26.98%) male and 10 (30.30%) female had insufficient (20-30 ng/mL) Vitamin D level and only 16 (16.66%) patients including 12 (19.04%) male and 4 (12.12%) female had sufficient (>30 ng/mL) Vitamin D levels. Mean+SD serum Vitamin D in our study group of TB patients was 21.4 + 9.0 which is Vitamin D levels in paediatric population.

Table 7: Distribution of the patients according to Severity of TB.

Severity of TB	No. of Patient	Percentage
Severe	26	27.08%
Less severe	70	72.91%
Total	96	100.00%

Out of 96 total patients, 26 (27.08%) had severe variant of TB (Tubercular meningitis, miliary tuberculosis and disseminated tuberculosis) and 70 (72.91%) had less-severe TB (pulmonary TB, tubercular lymphadenopathy etc.).

Table 8: Distribution of the patients according to Serum Vitamin D and Severity of TB.

	Less severe disease (*n*=70)	Severe disease (*n*=26)	*p* value
Serum Vit D3 (Mean + SD)	22.6 + 8.9	13.4 + 7.8	0.04*

*Statistically significant.
We observed that out of 96 total patients mean + SD level of serum Vitamin D3 in severe variant of TB was 13.4 + 7.8 and 22.6 + 8.9 in less-severe variant TB, *p* value is 0.04. So, we can infer that Vitamin D level in severe variant of TB is significantly low than mean Vitamin D level in less severe variant of TB.

DISCUSSION

We conducted a study on paediatric tuberculosis patients of less severe and severe category patients. In our study we found that out of total 96 patients, 63 (53.23%) were males and 33 (46.77%) were females while in a study conducted by Dipti Sarma *et al.* it was observed that females 254 (50.8%) were more in comparison to males 246 (49.2%) which is contradictory to our study findings.[6]

In our study we observed that 24 (25%) were in the age group of 6-10 years while majority of patients 59 (61.45%) were in the age group of >10 years. A study done by Bindusha *et al.* revealed that 42 (45.7%) patients were in the age group of >10 years which is less than our study findings. Our study findings revealed

Table 9: Distribution of the patients according to Vitamin D level and Diagnosis.

Diagnosis		Vitamin D Level			Total
		Deficient (<20)	Insufficient (20-30)	Sufficient (>30)	
Pulmonary TB	Count	31	14	6	51
	% within Diagnosis	60.78%	27.45%	11.76%	100.0%
TB lymphadenitis	Count	4	2	2	8
	% within Diagnosis	50.0%	25.0%	25.0%	100.0%
Abdominal TB	Count	2	1	1	4
	% within Diagnosis	50.0%	25.0%	25.0%	100.0%
Pleural TB	Count	3	3	1	7
	% within Diagnosis	42.85%	42.85%	14.28%	100.0%
Miliary TB	Count	3	1	2	6
	% within Diagnosis	50.0%	16.66%	33.33%	100.0%
TB Meningitis	Count	7	5	4	16
	% within Diagnosis	43.75%	31.25%	25%	100.0%
Disseminated TB	Count	3	1	0	4
	% within Diagnosis	75%	25.0%	0.0%	100.0%
Total	Count	53	27	16	96
	% within Diagnosis	55.20%	28.12%	16.66%	100.0%

In our study out of 96 patients hypovitaminosis D was seen in 53 (55.20%) TB patients in which majority of case were of pulmonary TB 31(60.78%). Even in less severe variant of TB (n=70) minimum no of patients had sufficient vid D levels.

Table 12: Distribution of the patients according to level of Vitamin D and age groups.

Age Group		Vitamin D Level			Total
		Deficient (<20 ng/mL)	Insufficient (20-30 ng/mL)	Sufficient (>30 ng/mL)	
0-5yr	Count	4	06	03	13
	% within Age group	30.76%	46.15%	23.07%	100.0%
5-10yr	Count	10	08	06	24
	% within Age group	41.66%	33.33%	25.0%	100.0%
>10yr	Count	39	13	07	59
	% within Age group	66.10%	22.03%	11.86%	100.0%
Total	Count	53	27	16	96
	% within Age group	55.20%	28.12%	16.66%	100.0%

Chi square-2.113, Degree of freedom-3, p=0.601 (Not significant).
In our study, out of 96 patients, 13 (13.54%) were in the age group 0-5 years, 24 (25%) were in the age group 5-10 years and 59 (61.45%) were of age >10 years. Majority of patients had deficient vid D levels in all age groups.

that 33 (46.77%) of them had positive history of contact and 63 (53.23%) had no history of contact which is contradictory to findings done by Kirenga BJ *et al.* in Kampala and Uganda where 42 (11.5%) patients had positive history of contact.[8]

In our study we observed that 24 (38.71%) were Mantoux test positive, 53 (85.48%) were CBNAAT positive, 39 (62.90%) had positive X-ray findings and 33 (53.23%) were Others (biopsy positive, MRI brain and CT findings of TB, raised ESR). Not much of the studies were found in the literature comparing diagnosis of Tb patients according to laboratory findings.

In our study we found that most common presentation was pulmonary TB (43.75%) i.e., severe variant of TB while least were Abdominal TB (3.125%), Miliary TB (3.125%) i.e., less-severe TB respectively which was contradictory with the study done by Vijay Jaimni *et al.* who reported 50% patients diagnosed with pulmonary TB. Our study results revealed that mean + SD level of serum Vitamin D_3 in severe variant of TB was 13.4 + 7.8 and 22.6 + 8.9 in less-severe variant TB, p value is 0.04. So, we can infer that Vitamin D level in severe variant of TB is significantly low than mean Vitamin D level in less severe variant of TB. A study done by Rizvi I *et al.*

showed that Vitamin D deficiency was significantly more common in TBM compared to controls and less severe pulmonary tuberculosis (TBM versus controls $p<0.001$; TBM versus pulmonary tuberculosis $p<0.001$).[10]

In our study out of 96 patients hypovitaminosis D was seen in 53 (55.20%) TB patients in which majority of case were of pulmonary TB 31 (60.78%). Even in less severe variant of TB ($n=70$) minimum no of patients had sufficient vid D levels which was in accordance with the study done by Venturini, E *et al.* in 2014 in which 996 children screened for TB suggested that hypovitaminosis D was found respectively in 80 (58%) latent TB and 33 (75%) active TB patients.[11] Another study by Sasidharan PK *et al.* also observed that Vitamin D deficiency was present in all patients with active Tuberculosis.[12] In our study, out of 96 patients, 13 (13.54%) were in the age group 0-5 years, 24 (25%) were in the age group 5-10 years and 59 (61.45%) were of age >10 years. Majority of patients had deficient vid D levels in all age groups which was in accordance with study was done by Francis L Weng showed that in age group of 6-21 year were associated with low Vitamin Dconcentrations.[13]

CONCLUSION

In our study 96 patients diagnosed with TB were enrolled in our study. It was observed in our study that there is no statistically significant difference in Vitamin D levels among TB patients of different pediatric age group and gender. 27.08% of patients presented with severe TB and 72.91% presented with less severe TB. Commonest presentation of TB in our study was Pulmonary TB (43.75%). Most common presentation in severe variant of TB was TB meningitis. 55.20% of patients had deficient and 28.12% had insufficient Vitamin D level.

In our study mean + SD level of serum Vitamin D_3 in severe variant of TB was 13.4 + 7.8 and 22.6 + 8.9 in less-severe variant TB. Hypovitaminosis was observed in 100% of severe variant of TB. The current study shows significantly lower levels of Vitamin D in adults with newly diagnosed active TB. The study findings support the assumption that hypovitaminosis D potentiates susceptibility to TB. This relationship should be investigated further in a large-cohort longitudinal study. We advocate for longitudinal studies across various geographical regions to accurately define the roles of Vitamin D on TB treatment and prevention.

Limitation of study

Further more extensive study with apparently healthy children of the same ethnic background as a control group is required to establish a reference range of Vitamin D levels, but in our study apparently healthy children as control group were not included. Other biochemical parameter like calcium, phosphorus, alkaline phosphate, parathyroid hormone, could not be included which would have given a better picture of Vitamin D status in study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

The study was approved by ethical committee.

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