

Role Of Vitamin D Supplementation In The Clinical Course Of Pediatric Tuberculosis

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Abstract:

Aims and Objective: To investigate role of vitamin D in determining severity of tuberculosis and its supplementation in the clinical course of tuberculosis

Materials And Methods: Pilot intervention study done at tertiary care hospital in Hyderabad during 2014 - 2015. TB cases up to 14 years of age reported niloufer hospital. Sputum positive TB cases, or symptomatic cases with montoux positive + chest x ray finding consistent with TB + GENE EXPERT positive in pleural and pericardial fluid. primary or secondary immunodeficiencies, patients already on vitamin d supplementation and multi drug resistance tuberculosis are excluded. Every case is ascertained to one of three groups based on type of presentation as primary pulmonary complex (n=29), progressive pulmonary disease (n=56) extra pulmonary disease (n=2). base line height, weight, BMI, vaccination status, montoux, hemoglobin levels, vitamin D levels, chest X-ray, TB score, socio economic status were recorded. 60,000 IU vit D supplemented fortnightly for every alternate subject. Chest X-ray, sputum conversion, height, weight, TB score, BMI, is recorded at the end of intensive phase.

Results: out of 101 subjects 5 cases were dead, 7 subjects were dropped out, and 44 were subjected to vit D supplementation 45 not supplemented. Out of these 89, 49 were vit D deficient, 23 were vit D insufficient, and 15 were vit D sufficient. Progressive pulmonary disease were significantly higher in vit D deficient (73.47%) and in vit D insufficient (65.22%) when compared to vit D sufficient group (33.33%), chest X-RAY was 88% in supplemented groups and 68% in non supplemented groups. Difference in BMI was not significant between both groups however there was significant difference between both the groups in difference of weight ($p < 0.05$) and TB score ($p < 0.05$) after intensive phase

Conclusion: Low levels of vit D are associated with increased severity of tuberculosis but vit D supplementation has not significantly altered the clinical course of tuberculosis.

I. Introduction

In the pre antibiotic era, cod liver oil and sunlight exposure in sanatorium were used to treat tuberculosis (TB) [1], [2]. More recently, increasing evidences from *in vitro* studies suggest that vitamin D enhances anti mycobacterium immunity [3]. Several authors reported hypo vitaminosis D in TB patients [4]-[6], and serum level of vitamin D [25hydroxycholecalciferol] was found to be lower in TB patients than in healthy controls [7]-[14]. A recent study conducted among adult TB contacts found that 94% of recruits were vitamin D insufficient and that a single, oral 2.5 mg dose of vitamin D significantly enhanced their anti mycobacterial immunity *in vitro* [7]. Factors such as low socioeconomic status, poor nutrition, traditional/cultural traits, and little exposure to sunlight may contribute to vitamin D deficiency [15].

Mechanisms through which vitamin D modulates the immune system in the response to *Mycobacterium tuberculosis* infection are not completely understood, two possible mechanisms have emerged as the most likely. Stimulation of monocyte Toll like receptors (TLR1/2) by *Mycobacterium tuberculosis* (MTB) results in transcriptional induction of the vitamin D receptor (VDR) and 1 α -hydroxylase (CYP27B1). Circulating 25-hydroxyvitamin D (25[OH] D) enters the cell and is converted to 1, 25-dihydroxyvitamin D (1, 25[OH] 2D) by the CYP27B1 enzyme. VDR-bound 1, 25(OH) 2D then induces expression of LL37, an antimicrobial peptide of the cathelicidin family [16]-[19] and β -defensin 2 (DEFB4). In addition 1, 25(OH) 2D induces autophagy and downregulating metalloproteinases (MMPs), all of which help in the formation of phagolysosomes and killing of Mtb.

II. Aims And Objectives

- To study the correlation between vitamin D levels and severity of tuberculosis in children.
- To study the role of vitamin D supplementation in clinical course of pediatric tuberculosis

III. Materials And Methods

Study design: pilot intervention study

Setting: A tertiary care hospital in Hyderabad during 2014 -2015.

Participants: TB cases up to 14 years of age reported at Niloufer hospital

Inclusion criteria:

- (1). Sputum or gastric aspirate GENE EXPERT positive TB cases
- (2). Symptomatic cases with montoux positive + chest x ray finding consistent with TB + GENE EXPERT positive in pleural and pericardial fluid .

Exclusion criteria:

- (1). primary or secondary immunodeficiencies.
- (2). Cases taking vit D supplementation already
- (3). MDR TB case

IV. Intervention

Every case is ascertained to one of three groups based on type of presentation as primary pulmonary complex (n=29) , progressive pulmonary disease (n=56) extra pulmonary disease(n=2).Base line height, weight, BMI, vaccination status , montoux, haemoglobin levels , vitamin D levels ,chest X-ray, TB score , socio economic status were recorded. Levels of 25-hydroxy vitamin D were measured together for all samples after the study had ended. About 3 ml blood was collected prior to starting anti tubercular therapy in plain zinc free tubes for micronutrient estimation. Serum was separated within one hour and one aliquot of 300 µl was stored at -20°C till further analysis .Levels of 25-hydroxy vitamin D levels were measured from the stored serum samples by chemi luminescent immunoassay (CLIA)

60,000 IU vit D supplemented fortnightly for every alternate subject. Chest X-ray, sputum conversion, height, weight, TB score, BMI, is recorded at the end of intensive phase.TB score is calculated based on the presence of clinical signs and symptoms. The following are the 10 clinical signs and symptoms: cough, dyspnoea, chest pain, night sweats, pallor, tachycardia, fever, crepitations, reduced breath sounds, rhonchi. This tb score is used mostly in adults than in paediatric age group Mean increase in BMI, weight and TB score are noted at the end of 3 months and compared between the two groups

V. Observations And Results

A total of 101 children were identified and were included in study. 5 children expired and 7 children were lost follow up.

4.1 Age Distribution Of Subjects: The studied subjects belonged to age groups between 0 to14 yrs. Mean age of the studied subjects was 6.48 yr.

4.2 Distribution Of Study Population As Per Gender:

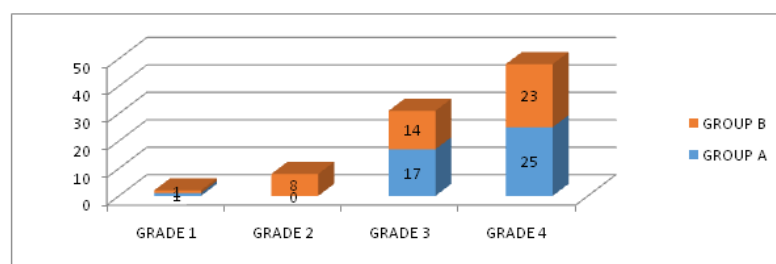
Among the studied subjects 62% (n=55) were female and 38% (n=34) were male.

4.3 Distributions Of Subjects As Per Locality Out of 89 subjects 35 were from rural area (39.32%), 25 were from urban area (28.08%), 29 were semi urban population (32.58%).

4.4 Status Of Immunization Among Subjects:

Out of 43 in group a 30 were vaccinated 13 were unvaccinated, Group B has 32 vaccinated and 14 unvaccinated subjects. 69.7 % and 69.5 % were vaccinated in group A and group B respectively

4.5 Severity Of Mal Nutrition In Subjects:



In group A 25 subjects were with grade 4 malnutrition 17 were grade 3, 1 child has grade 1 malnutrition. In group B 23 subjects were with grade 4 and 14 were having grade 3 malnutrition.8 subjects were with grade 2 and 1 subject has grade 1 malnutrition

4.6 Distributions Of Study Subjects Based On Type Of Tuberculosis:

Primary pulmonary complex 32.58% n=29

Progressive pulmonary disease	62.92%	n=56
Extra pulmonary	4.49%	n=4

4.7 Distributions Of Study Subjects Based On Vitamin D Levels

Out of 89 children in both groups 50 were vitamin D deficient, 24 were insufficient and 15 were sufficient. 56.17 % were vitamin D deficient,26.96 were vit D insufficient and 16.85 were vitamin D sufficient.

4.8 Comparison Of Severity Of Tuberculosis With Vit D Levels

TYPE OF PRESENTATION	VIT D DEFECIENT	VIT D INSUFFICIENT	VIT D SUFFICIENT
PRIMARY PULMONARY COMPLEX	22.46%	35%	67%
PROGRESSIVE PULMONARY DISEASE	73.54%	65%	33%
EXTRA PULMONARY DISEASE	04%	0%	0%

In children with vitamin D deficiency progressive pulmonary disease was in high incidence than primary pulmonary complex where as it is vice versa in vitamin D sufficient group where primary pulmonary complex .

4.9 Mean Base Line Vitamin D Levels Among 2 Groups :

Mean vitamin d levels were almost equal 12 and 12.3 in both groups.

4.10 Mean Parameters Among 2 Groups

Mean weight gain is 1.687 and 2.406 in group A and B respectively ANOVAs test is used with p value0.0005 which is highly significant(**P value <0.005**).Mean improvement in tb score is 4.206 and 4.655 in group A and B respectively .p value is 0.017 , ANOVAs test is used as test of significant.(**P value <0.05**.)Mean increase in BMI is 1.871 and 2.839 in group A and B respectively. Pvalue is 0.069, which is not significant (**Pvalue >0.05**)

PARAMETER	GROUP A	GROUP B	P VALUE
WEIGHT GAIN	1.687 KG	2.406 KG	0.0005
TB SCORE	1.871	2.839	0.017
BMI	4.206	4.655	0.069

4.11 Cxr Clearance In Subjects:

At the end of intensive phase chest x-ray was taken to check clearance. Clearance is present in 68% and 88% in group A and B respectively. P value is 0.036 which is significant (**P VALUE <0.05**)

4.12 Sputum Clearance:

Sputum / gastric aspirate was done in both groups at end of intensive phase mean time for sputum conversion was same for both groups and all subjects are sputum negative in both groups by the end of the intensive phase . Sputum for gene expert can be done at more frequent intervals such as fortnightly and response for early sputum conversion can be seen in between two populations

VI. Discussion

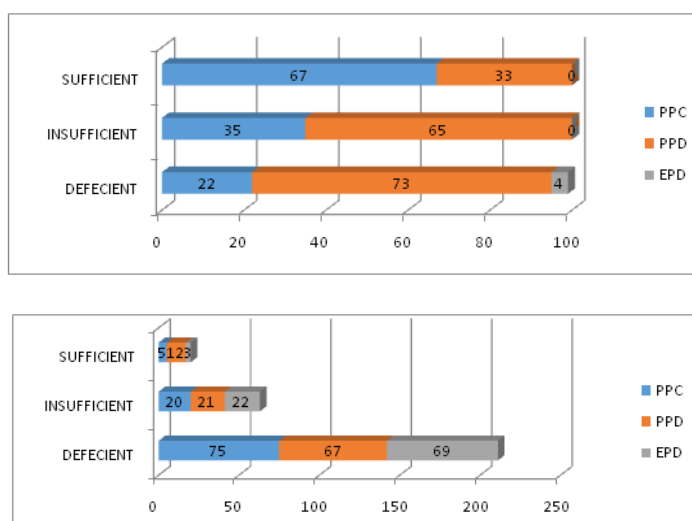
Tuberculosis is one of the major infections affecting children worldwide. Young children carry the greatest burden of disease. They are not only most likely to develop disease after infection but also more prone to develop extra pulmonary and severe disseminated disease. According to estimates, in industrialized countries childhood TB constitutes about 7 percent of all TB cases and the disease is much more prevalent in resource poor developing countries (15-40% of all TB).

25-hydroxyvitamin D is recognized as an important immune-modulator in TB. 1, 25 (OH) 2 D3 binding with VDRs activates cathelicidin-mediated mycobacterial killing whilst 25-hydroxyvitamin D deficiency increases the susceptibility and vulnerability to TB G interferon (IFN- γ) is a proinflammatory cytokine, which plays a critical role in resistance to MTB infection

5.1 Base Line Vitamin D Levels And Severity Of Tuberculosis

In this study, an attempt has been done to know the effects of vitamin D supplementation in tuberculous patients and also to study the relation between low levels of vitamin D and severity of tuberculosis. It is found that low levels of vitamin D have been associated with increased severity of pulmonary tuberculosis.73% and 65% of the deficient and insufficient groups have progressive pulmonary disease in contrast to sufficient group where only 33% are known to have progressive pulmonary disease More over sufficient groups have also shown to have more of primary pulmonary disease 67 % than that of deficient 22% and insufficient groups 35%

Similar study carried out by **khandelwal et al.**,^[20] in pediatric population has shown a different result. This study has shown that severity of vitamin d deficiency is not related to severity of tuberculosis. But concluded that tuberculosis patients have uniformly low levels of vitamin D.



5.2 Effects Of Vitamin D Supplementation In Tuberculosis

In the present study an attempt was also made to find the clinical outcome if vitamin D is supplemented to tuberculous patients. 60000 iu oral 25 hydroxy cholecalciferol was supplemented and differences in weight, BMI and TB score were noted after 3 months after completing supplementation. Following are the results tabulated.

parameter	group	mean	95% CI	Significance P value
ΔWEIGHT	Group A	1.687	1.436-1.937	=0.0001
	Group B	2.406	2.164-2.649	
ΔBMI	Group A	1.871	1.121-2.621	=0.069
	Group B	2.839	2.114-3.565	
ΔTBSCORE	Group A	4.206	3.943-4.470	=0.017
	Group B	4.655	4.400-4.910	

ANOVA test is used as test of significance. Hence from the above results it is evident that vitamin D supplemented group has significant improvement in weight and TB score where as BMI is not significant in between these two groups. Weight and BMI has been standardized in both groups for Age and sex p value 0.176 which is not significant (chi square test in used). Linear regression analysis is done to eliminate confounding factors like malnutrition and locality. (P<0.005). Chest x ray clearance was more in supplemented group than non supplemented group. All patients in both groups were sputum negative at the end of the study.

Many studies are carried out to know the role of vitamin D supplementation in tuberculosis. Most of these studies are of adults. Very few studies are available in pediatric age group. Most of pediatric studies have shown that low levels of vitamin D are associated with tuberculosis.

Morcos Et Al.,^[21] in Egypt has conducted a study where it was concluded that vitamin D supplementation has great value and dramatically improved the clinical outcome in children. 24 children were included in study and supplemented with 1000 iu of vitamin D daily for 6 wks. Specific statistical data is not available, however mean wt again was more in vitamin D group than placebo group. Draw backs of study are very low study population, no base line vitamin D levels.

Salahuddin et al.,^[22] in Pakistan conducted study in adults where 6 lakhs of vitamin D was supplemented one month apart. This study has taken weight, BMI, TB score and MTB induced gamma interferon levels in both groups. After 12 weeks, the vitamin D supplemented arm demonstrated significantly greater mean weight gain (kg) + 3.75, (3.16 – 4.34) versus + 2.61 (95% CI 1.99 – 3.23) p 0.009 and lesser residual disease by chest radiograph; number of zones involved 1.35 v/s 1.82 p 0.004 (95% CI 0.15, 0.79) and 50% or greater reduction in cavity size 106(89.8%) v/s 111 (94.8%), p 0.035. Vitamin D supplementation led to significant increase in MTBs-induced IFN-gamma secretion in patients with baseline 'Deficient' 25-hydroxyvitamin D serum levels (p 0.021). The results of this can be compared to present study.

parameter	group	mean	Std deviation	95% CI	Significance (P) value
ΔWEIGHT	Group A	1.687		1.436-1.937	=0.0005
	Group B	2.406		2.164-2.649	
ΔBMI	Group A	1.871		1.121-2.621	=0.069
	Group B	2.839		2.114-3.565	
ΔTBSCORE	Group A	4.206		3.943-4.470	=0.017
	Group B	4.655		4.400-4.910	

Salahuddin Et AL, Study

parameter	group	mean	Std deviation	95% CI	Significance P value
ΔWEIGHT	Group A	+ 4.02		3.18 – 4.86	0.007
	Group B	+2.61		1.99 – 3.23	
ΔBMI	Group A	+1.48,		1.17 – 1.78	0.008
	Group B	+0.96,		0.72 – 1.20	
ΔTBSCORE	Group A	- 2.79	±2.44	-3.23, -2.34	0.198
	Group B	- 3.19	±2.37	-3.61, -2.75	

In **Salahuddin study** TB score was not significant but there was significant change in weight, BMI, cxr clearance in between 2 groups. In addition salahuddin study has also carried out MTB induced γ interferon response which is more in supplemented group. However sputum clearance rate was same in both group's .Large study population and use of MTB induced γ interferon response have helped to more successfully establish the role of vitamin D in salahuddin study when compared to our study. However as this is an adult study this cannot be completely compared with the present pediatric study

Martineau et al., [7] had conducted a study in adults where 2.5 mg vit d supplementation was given at 7 ,14 ,28 ,42 days. It was concluded that vitd supplementation did not hasten the sputum smear conversion in whole study population but has hastened sputum smear conversion in taq 1 vitamin D receptor type(tt genotype).In the present study these genetic polymorphisms of vitamin D receptors are not taken in to consideration

Wejse et al., [23] has conducted a RCT where 1 lakh I u vitamin D was supplemented on 0, 5, 8 months .outcomes were mortality at end of 12 months and tb score, weight gain and sputum smear conversion .however none of the parameters are effected in both groups .This may be due to long intervals between dosages, inadequate dosage In intensive phase, and lack of effective parameters for assessment

Nursyam et al., [24] has .25mg/day vitamin D, or placebo supplementation during 6 weeks .primary out comes were sputum conversion and radiological improvements. There was significant differences in between both groups in both parameters .however limitations of this study are low study population and lack of clinical parameters like TB score, weight gain and BMI which are not taken in to consideration.

VII. Conclusions

The limitations of study are Low study population .Genetic polymorphisms in vitamin D receptors are not taken in to consideration. Lack of effective parameters to compare subjects in two groups .Lack of studies on the effectiveness of us of TB score for paediatric tuberculosis. This study recommends to do Sputum for Gene Expert at more frequent intervals such as fortnightly and response for early sputum conversion can be seen in between two population .Follow up can be done for longer period to assess mortality and other morbidities in between the two groups

This study concludes that Low levels of vitamin D are associated with increased severity of tuberculosis Vitamin D supplementation has significant improvement in TB score ,weight gain but not on BMI .Chest x ray clearance is more in vitamin D supplemented group than non supplemented group .No significant difference in sputum clearance in between 2 groups. Sputum was checked only once in both groups at the end of intensive phase .Studies in pediatrics with larger groups and sputum or gastric aspirate if done at frequent intervals with in intensive phase of treatment will yield more data on rate of sputum clearance

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