

Distribution of TNF- α Among Antenatal Women with Varying Levels of Vitamin D

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ABSTRACT

Background and Objective: Vitamin D deficiency in antenatal mothers adversely affects not only the health of pregnant mother but also her child. Vitamin D is crucial for proper immune system functioning and management of cytokine environment. Relationship between vitamin D deficiency and TNF- α may prove useful in early detection of inflammatory conditions in pregnant mothers. Therefore, this study assessed serum vitamin D and TNF- α levels in antenatal mothers.

Materials & Methods: 78 serum samples of healthy pregnant mothers were included. Serum 25-OH Vitamin D and TNF- α levels were estimated using commercially available ELISA kits.

Results: 25-OH Vitamin D levels were inadequate (<32ng/ml) in 94.9% of antenatal cases. Majority (74.4%) had an insufficient (11-32 ng/ml) 25-OH Vitamin D level. Mean and median vitamin D levels were 16 ± 7.5 SD and 14.5 ng/ml (3.5-39.5) respectively in antenatal mothers. None or a very weak positive linear relationship was observed between serum 25-OH Vitamin D and TNF- α levels (r: 0.13). No correlation was seen between 25-OH Vitamin D & age and TNF- α & age (r: 0.04; r: 0.06 respectively).

Conclusions: In settings where subnormal vitamin D levels are prevalent in antenatal mothers, screening for vitamin D deficiency in early pregnancy with subsequent supplementation if needed is recommended. No correlation was observed between serum vitamin D and pro-inflammatory marker TNF- α levels. Further elaborate studies are required to investigate the effects of vitamin D on cytokine environment especially in pregnant mothers.

Keywords: Vitamin D, 25-OH Vitamin D, TNF- α , Antenatal, Cytokine

Introduction

Vitamin D plays a pivotal role in the maintenance of homeostasis of calcium and phosphate. It makes vitamin D crucial for proper bone mineralization and growth. Pregnancy is associated with enhanced demand of calcium as the maternal body adapts to meet the increasing foetal requirements. Several studies have reported an increased levels of vitamin D in antenatal and lactating mothers.¹⁻³ This emphasizes the enhanced demand of vitamin D during pregnancy.⁴ Vitamin D deficiency can result in adverse maternal outcomes, like recurrent abortions,⁵ preterm delivery,⁶ induced hypertension,⁷ gestational diabetes mellitus.⁸

Vitamin D deficiency in mother enhances the risk of vitamin D deficiency in neonate. The birth weight of neonates born to vitamin D deficient mothers are reportedly lower than the ones born to mothers having sufficient vitamin D levels.⁹ In foetus it can lead to delay in the ossification of cranial vertex, enlarged cranial fontanelles and impairment in foetal bone ossification.⁴ Vitamin D deficiency is also a significant cause of neonatal hypocalcaemic seizures.¹⁰ Deficiency of vitamin D in mother can influence the development of foetus in a more extensive manner. Vitamin D is now

also being acknowledged for its several other roles, like immuno-regulation, enhanced insulin secretion.¹¹ Vitamin D has a significant immune modulatory role. The deficiency of vitamin D enhances the risk of developing infectious diseases and malignancies.¹² Maternal vitamin D deficiency may also result in asthma in progeny.¹³ Thus timely corrective measures for vitamin D deficiency in antenatal mothers can potentially prevent vitamin D deficiency and hypocalcaemia in their neonates.¹¹

Tumor necrosis factor (TNF- α) is primarily produced by activated macrophages, T lymphocytes and dendritic cells.¹⁴⁻¹⁶ TNF- α also acts synergistically with interferon (IFN)- γ and enhances the production of reactive nitrogen intermediates (RNIs).^{17, 18} TNF- α plays an important role in the acute phase reaction. It acts by enhancing the inflammatory response to infection. Thus serum TNF- α levels may be used as a marker for an inflammatory infection. This multifaceted cytokine also plays a crucial role in the regulation of ovarian cycle in young women. TNF- α is necessary for the normal growth and development of ovarian follicle.¹⁹ It has also been reported that an increased serum levels of TNF- α is related with infertility and recurrent spontaneous abortions.²⁰

Although vitamin D deficiency is suspected to be a universal health issue, only fewer countries have the data available for the assessment of vitamin D deficiency in their antenatal population. There is need for the representative data for vitamin D deficiency in antenatal mothers in order to take the necessary steps to prevent the effects of vitamin D deficiency in the mothers and their neonates. Vitamin D deficiency is associated with enhanced risk of developing infectious diseases in particular tuberculosis (TB), which is endemic in our scenario and accounts for huge morbidity and mortality. The relationship between vitamin D deficiency and TNF- α may prove to be helpful in early detection of these inflammatory conditions in antenatal mothers. Therefore, in this study we have assessed the serum vitamin D and TNF- α levels in the antenatal mothers.

Materials and Methods

The present study was carried out in a tertiary care centre of east Delhi. The serum samples of the pregnant mothers availing antenatal care that were received in our serology lab for routine Venereal Disease Research Laboratory (VDRL) testing and were found to be non-reactive were included in the study. No additional sample was taken for this study. Only the samples with no pathological condition mentioned or suspected on requisition form were included. 78 such antenatal serum samples were collected. The information pertaining to demographic profile was collected from the requisition form.

The collected Serum samples were stored at -800C until further assayed. The commercially available kits were used for the quantitative determination of total 25-OH Vitamin D and TNF α in antenatal serum. Total 25-OH Vitamin D levels were estimated with Calbiotech: A life science company (Spring Valley, CA) and TNF α levels by Diaclone Human ELISA kit (France). The manufacturer's instructions were thoroughly followed throughout the procedure. The individual manufacturer for 25-OH Vitamin D claims the analytical sensitivity of 2.5ng/ml with standard range of 2.5-150ng/ml. The individual manufacturer for 25-OH Vitamin D claims the analytical sensitivity of 2.5ng/ml with standard range of 2.5-150ng/

ml. For TNF- α sensitivity was less than 8pg/ml. 25-OH Vitamin D assay was a solid phase ELISA, based on the principle of competitive binding whereas TNF α assay was based on the principle of solid phase sandwich ELISA. The levels of both the markers were determined by extrapolating OD values against the individual marker's standard concentrations using the standard curve. The antenatal serum 25-OH Vitamin D levels of <11, 11-32 and 32-100 ng/ml were classified as severe deficiency, insufficiency and adequacy respectively.²¹ The statistical analyses was performed using Microsoft Excel 2013.

Results

The age of cases ranged from 19-31 years with a mean age of 24.3 years. The majority (69.2%) of antenatal cases were in 19-25 years age-group. The 25-OH Vitamin D levels were inadequate (<32ng/ml) in 94.9% of the antenatal cases included in this study. Majority of cases had an insufficient (11-32 ng/ml) 25-OH Vitamin D levels (figure 1). Table 1 shows the age wise distribution of serum 25(OH) vitamin D levels in antenatal cases. The percentage of antenatal cases with severe 25-OH Vitamin D deficiency (<11 ng/ml) was higher in the younger (19-25 years) age group than the older (26-31 years) age group. None of the antenatal mothers in 26-31 years age group had an adequate (\geq 32ng/ml) serum level of 25-OH Vitamin D. The mean and median vitamin D levels were 16 ± 7.5 SD and 14.5 ng/ml (3.5-39.5) respectively in antenatal mothers. The mean serum levels of 25-OH Vitamin D were 15.4 ng/ml and 17.6 ng/ml in 19-25 and 26-31 years age groups respectively (figure 2).

Mean TNF- α level in antenatal mothers was 24.3 pg/ml. The mean TNF α serum levels were 21 pg/ml and 29.4 pg/ml in 19-25 and 26-31 years age groups respectively (figure 2). The mean serum TNF- α levels were 14.8, 25.8 and 26.4 in antenatal cases with serum 25-OH Vitamin D levels <11 ng/ml, 11-32 ng/ml and \geq 32ng/ml respectively. A very weak positive linear or no relationship was seen between the serum 25-OH Vitamin D and TNF- α levels (r : 0.13). The correlation coefficient implied none or a very weak positive linear relationship between 25-OH Vitamin D & age and TNF- α & age (r : 0.04; r : 0.06 respectively).

Table 1: Age wise distribution of serum 25-(OH) vitamin D levels in antenatal cases (n=78).

25(OH) vitamin D level (ng/ml)	Number	Age group	
		19-25 years N(%)	26-31 years N(%)
<11	16	14(25.9)	2(8.3)
11-32	58	36(66.7)	22(91.7)
32-100	4	4(7.4)	0
>100	0	0	0

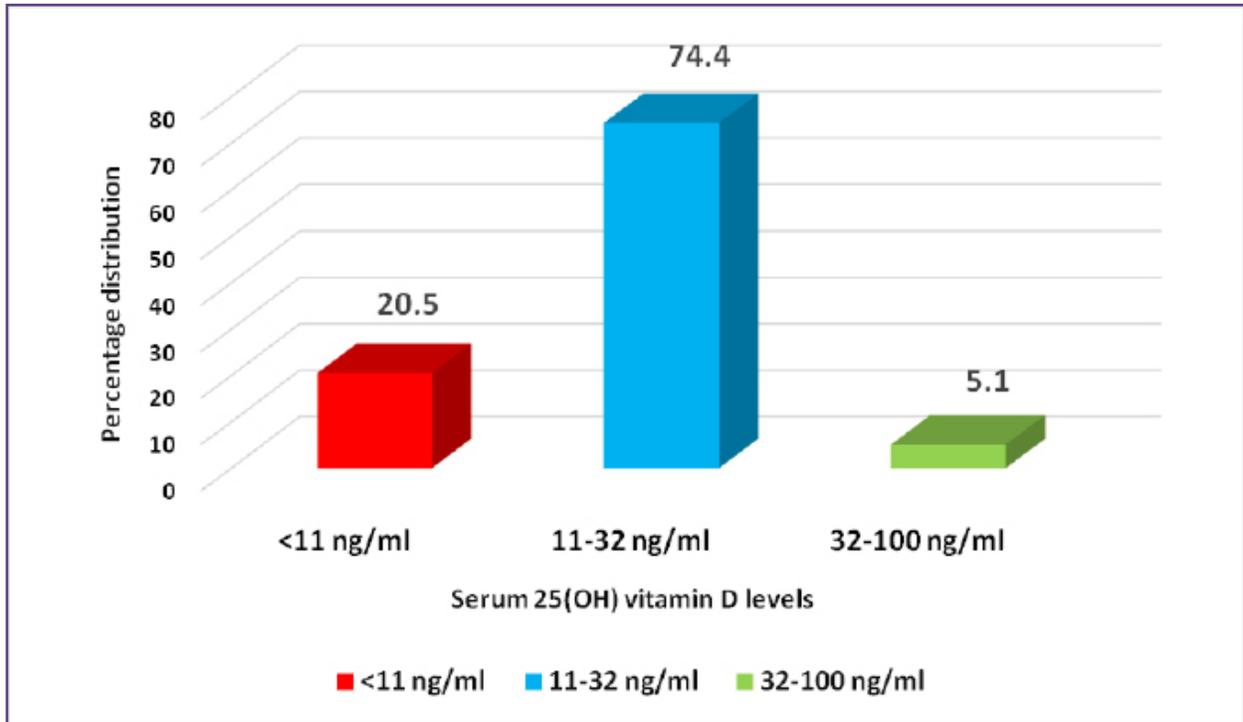


Fig. 1: Distribution of serum 25-(OH)vitamin D levels in antenatal cases (n=78).

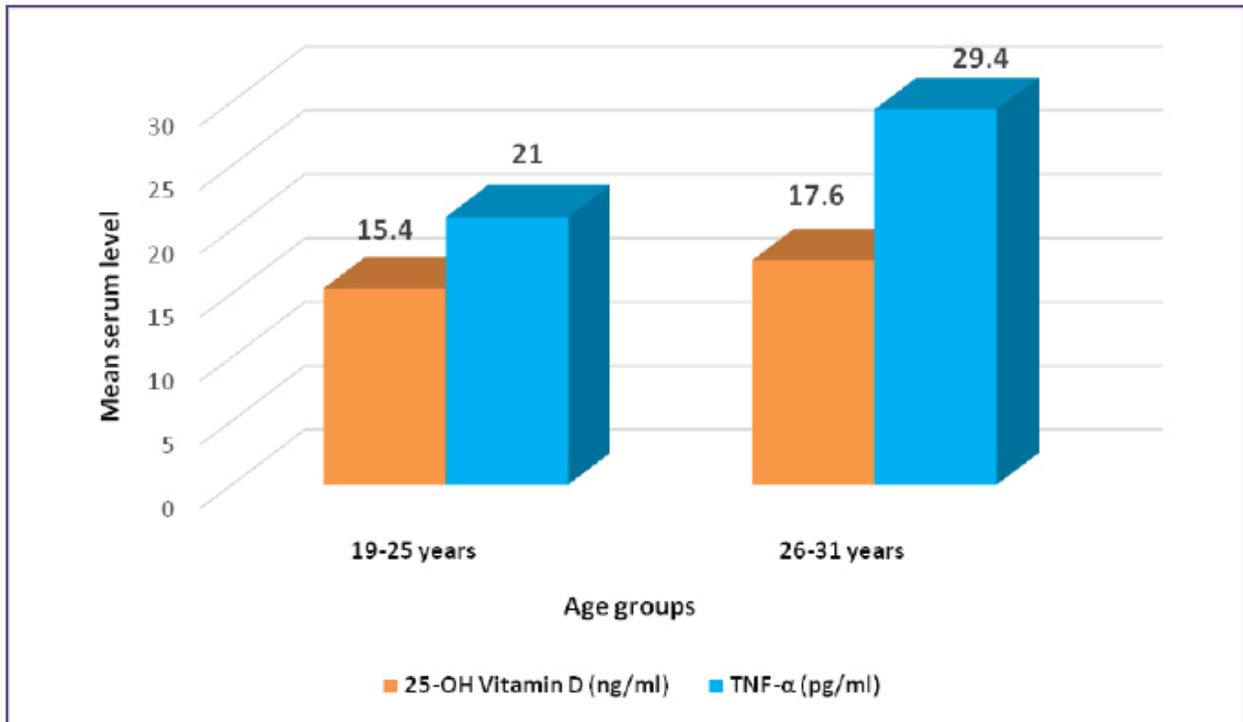


Fig. 2: Age wise distribution of mean serum 25-(OH)vitamin D and TNF-α levels in antenatal cases (n=78).

Discussion

Tuberculosis, a leading infectious disease, is responsible for 16% of maternal mortality worldwide.²² India alone accounts for nearly 21% of global load of TB among antenatal mothers.²³ Maternal TB has been associated with various adverse outcomes including enhanced risk of preterm labour and low birth weight babies.²⁴ In India we witness a dual threat of high incidence of TB along with widely prevalent vitamin D deficiency among antenatal mothers. The immunomodulatory and mycobactericidal role of vitamin D underline the importance of screening for infectious diseases, in particular TB, among antenatal mothers.²⁵ TNF- α plays a key role in the pathogenesis of mycobacterial infection. TNF- α , being pivotal in the formation of granulomas for walling off Mycobacterium tuberculosis, is an important biomarker for the detection of an early tubercular disease.²⁶

Institute of Medicine (IOM) 2010 Report defined the 25-OH Vitamin D cut off levels as below 12ng/L (30nmol/L) “persons are at risk for bone deficiency” and below 20ng/L (50nmol/L) “Some but not all are potentially at risk”.²⁷ The American Congress of Obstetricians & Gynecologists (ACOG) described the levels below 20ng/L (50nmol/L) as deficiency of 25(OH)D.²⁸ Majority agree that 25-OH Vitamin D levels below 20 ng/mL signify deficiency of vitamin D. There is no agreement among various studies about the optimal levels of 25-OH Vitamin D especially in antenatal mothers. A National Institute of Child Health and Human Development (NICHD) covered large trial has described vitamin D levels above 32 ng/ml safe in antenatal mothers.²⁹ Another large trial by Wagner C.L et al. has also supported the levels of 32 ng/ml as safe levels.³⁰ In line with these findings, we have considered serum 25-OH Vitamin D levels of <11, 11-32 and 32-100 ng/ml as severe deficiency, insufficiency and adequacy respectively in the present study.

Maternal vitamin D deficiency is associated with suboptimal bone development in foetus. Javaid MK et al. in the longitudinal study have demonstrated a positive association between maternal late pregnancy 25-OH Vitamin D levels and mineral content & bone density of their offspring at age 9 years as determined by dual energy X-ray absorptiometry (DEXA).³¹ Same study has reported circulating 25-OH Vitamin D concentrations of 27–50 nmol/L in 31% of the mothers.³¹ Farrant H.J. et al. in their study based on South Indian mothers have reported vitamin D deficiency in 66.5% of pregnant mothers.³² Agarwal N. et al. have documented vitamin D deficiency in 75%

of pregnant mothers in Delhi.³³ However, present study has demonstrated severe deficiency of 25-OH Vitamin D in 20.5% of antenatal mothers. This was probably due to the differences in the cut off values used in these studies to define the vitamin D deficiency. The studies by Farrant H.J. et al. and Agarwal N. et al. have defined Vitamin D deficiency as 25-OH Vitamin D < 20 ng/mL, whereas in our study we have considered serum 25-OH Vitamin D levels <11 ng/mL as severe deficiency.^{32, 33} The 25-OH Vitamin D levels of 32 ng/mL or more were seen in 5.1% antenatal mothers in our study, however another study has reported vitamin D levels of ≥ 30 ng/mL in 15% pregnant mothers.³³ Marwaha R.K. et al. have observed vitamin D deficiency in 96.3% of pregnant mothers.³⁴ Similarly, in present study we have reported low 25-OH Vitamin D levels in 94.9% antenatal mothers.

The mean 25-OH Vitamin D levels in antenatal mothers were 16 ± 7.5 SD ng/mL in our study. Our findings are in line with the study by Agarwal N. et al. that has reported 16.24 ± 4.4 SD ng/mL as mean vitamin D levels in pregnant mothers.³³ However, a study by Marwaha R.K. et al. has reported a lower (9.28 ± 4.9 SD ng/mL) mean vitamin D levels.³⁴ Another study has reported a median vitamin D level of 15.12 ng/mL (9.6 - 23.4) in pregnant mothers.³² Similarly in present study median vitamin D level was 14.5 ng/ml (3.5-39.5) in antenatal mothers. The present study reported a high proportion of antenatal mothers with low serum 25-OH Vitamin D levels. This emphasizes the urgent need of vitamin D deficiency screening in pregnancy and the follow up supplementation if needed. Despite the paucity of evidence, it is well accepted that vitamin D supplementation or treatment in pregnancy inflict no harm and may prove to be beneficial for maternal and foetal health.

TNF- α is a pro-inflammatory, multifunctional cytokine. TNF- α has been implicated as an inducer of embryopathic stresses and as a triggering agent for immunological pregnancy loss.^{35, 36} Several researchers have suggested a role of TNF- α in the pathogenesis of preeclampsia.^{37, 38} A relationship has been proposed between inflammatory processes and preeclampsia.^{39, 40} This is particularly relevant in developing countries where subclinical, latent, chronic infections are widely prevalent. In present study, mean TNF- α level in antenatal mothers was 24.3 pg/ml. However, another study has reported a higher serum TNF- α level of 51.9 pg/ml in normal pregnant women. Same study has reported no significant difference in serum TNF- α levels in preeclamptic and normal pregnant mothers ($p > 0.1$).⁴¹

Vitamin D is crucial for the proper immune system functioning. It also plays a role in the management of cytokines.⁴² Cantorna MT et al have demonstrated that vitamin D stimulates the production of anti-inflammatory cytokines.⁴³ Other studies have documented that vitamin D down-regulates the production of pro-inflammatory cytokines including TNF- α .^{44, 45} However in present study, no correlation was present between serum vitamin D and TNF- α levels in antenatal mothers. Our findings are in agreement with another study that has reported no significant correlations between serum vitamin D and TNF- α levels in healthy women.⁴⁶

We recognize that present study has many limitations. Our study focused on serum vitamin D and TNF- α levels in apparently healthy antenatal mothers. Similar studies involving larger number of subjects with inflammatory or chronic conditions would be very useful. Vitamin D deficiency is highly prevalent in developing countries, and recent researches support its importance in various diseases. Cytokines plays a key role in immune regulation, therefore further studies involving both healthy and diseased populations are required to elaborate on relationship between vitamin D and various cytokine levels.

Conclusion

Vitamin D deficiency adversely affects not only the health of pregnant mother but also her child. In our setting where subnormal vitamin D levels are prevalent in antenatal mothers, screening for vitamin D deficiency in early pregnancy with subsequent supplementation if needed is recommended. No correlation was observed between serum vitamin D and pro-inflammatory marker TNF- α levels in antenatal mothers. However, epidemiological studies on larger scale could be initiated for screening of levels of such cytokines along with few other key biomarkers with varying levels of vitamin D in antenatal mothers, in order to better manage and contain these infections. Further studies are required to investigate the effects of vitamin D on cytokine environment especially in pregnant mothers. Moreover, the outcomes of vitamin D supplementation in pregnancy on maternal and child health need to be thoroughly investigated.

Compliance with Ethical Standards

Conflict of interest Author Bineeta Kashyap declares that she has no conflict of interest. Author Nisha Goyal declares that she has no conflict of interest. Author Neha Gupta declares that he has no conflict of interest. Author SapnaJhanjhria declares that she has no conflict of interest.

Ethical Approval This study was conducted with sera that had already been received in routine for Venereal Disease

Research Laboratory (VDRL) testing in antenatal mothers. No additional sample was taken particularly for this study.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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