

Original Research Article

Serum 25-Hydroxy Vitamin D Levels in Indian Infants and Mothers from Sub Himalayan Region

ABSTRACT

Intoduction : Vitamin D is a prohormone that is essential for normal absorption of calcium from the gut. Apart from rickets vitamin D deficiency has many other negative health associated conditions like infectious diseases, autoimmune diseases, diabetes, dilated cardiomyopathy and malignancies. Nowadays in children, there is a growing evidence of association of vitamin D deficiency with increase in acute lower respiratory tract infections (ALRTI), impaired neurological function and possibly mental health conditions, namely schizophrenia.

Objective: To find out serum 25-Hydroxy vitamin D (25 (OH) D) levels in healthy infants and mothers in a tertiary health care set up in a hilly terrain of Himachal Pradesh , India.

Methodology: An observational study planned to find the 25-Hydroxy vitamin D (25 (OH) D) levels among healthy infants and their mothers who came for routine health check up after taking written consent.

Results: Total 80 subjects studied and 40 infants who came for routine health check up the median 25 (OH) D levels was 12.70 ng/ml. The median 25 (OH) D levels of mothers of infants were 11.15ng/ml . The observations showed vitamin D deficiency in majority (78.75%) of our studied subjects.

Conclusion: This study showed vitamin D deficiency in healthy infants and their mothers. The observations of the study indicate that vitamin D deficiency is prevalent in high risk population group in Himachal Pradesh.

Keywords: 25 (OH) D; Vitamin D; Vitamin D deficiency

1. INTRODUCTION

Vitamin D is a prohormone that is essential for normal absorption of calcium from the gut. Solar UVB radiations transform thermally pre-vitamin D in the skin to vitamin D₃. Studies in recent years have demonstrated many non-classical roles for vitamin D in the immune, cardiovascular, muscular, reproductive and integumentary systems, as well as in cancer prevention¹⁻⁵. Its best-characterized target organs are the intestine, kidney and the bone, but nuclear receptors for the hormone have been identified for 36 tissues. Nowadays in children, there is a growing evidence of association of vitamin D deficiency with increase in acute

lower respiratory tract infections (ALRTI), impaired neurological function and possibly mental health conditions, namely schizophrenia. As per WHO report of 2010, 64.0% in children younger than 5 years died of infectious causes. Of all infectious disorders, pneumonia, diarrhoea and malaria were the leading causes of death worldwide. Due to high morbidity and mortality in infants due to these causes this study was undertaken to find out the baseline serum 25 (OH) D₃ levels in healthy infants and mothers⁶.

We conducted an observational study in Department of Pediatrics, Dr. Rajendra Prasad Government Medical College, Kangra at Tanda, Himachal Pradesh from Aug 2014 to July 2015 with the aim to find out the baseline serum 25 (OH) D₃ levels in healthy infants and mothers in our society.

2. MATERIALS AND METHODS

Participants

This was an observational study where we have studied infants and their mothers. The studied population was divided into 2 groups [Study Group (Group A₁) and Study Group (Group A₂)]. Group A₁ included all infants aged less than 1 year reporting for routine health check up. Group A₂ included all mothers of healthy infants enrolled in group A₁ (reporting for routine health check up).

Selection criteria

Inclusion criteria

- i. For Group A₁, infants aged less than 1 year reporting for routine health check up.
- ii. For Group A₂, mothers of infants enrolled in group A₁ (reporting for routine health check up)
- iii. Willingness to participate in the study
- iv. Informed written parental/ guardian consent

Exclusion criteria

- i. Infants who had received calcium and/or vitamin D supplementation in last 6 months
- ii. Infants with severe malnutrition [Grade III and IV as per IAP classification of Protein Energy Malnutrition (PEM)]
- iii. Infants with bronchial asthma and with other co-morbidities like heart disease, tuberculosis, epilepsy, liver disease, chronic lung disease and renal disease
- iv. Mothers of infants having any significant illness like epilepsy, liver diseases, renal diseases, chronic lung disease, tuberculosis, asthma, heart disease, diabetes and

received mega dose of vitamin D in last 6 months based on history and clinical examination

- v. Non- willingness to participate in the study
- vi. Failure to get informed written consent

After taking approval from the institutional ethics committee and protocol review committee, the study was commenced with enrolment of all the subjects fulfilling the inclusion criteria in 2 groups. Detailed history and clinical examination of all enrolled infants and mothers was done History of breastfeeding, initiation of complimentary feeding and exposure to sunlight for infants was taken. Clinical examination was done to find out any significant finding. This was followed by quantitative estimation of 25 (OH) D₃ of all the enrolled infants along with their mothers. 3 ml blood was collected by venipuncture in properly covered test tube to avoid sunlight exposure. Then it was allowed to clot and serum from it was separated after centrifugation at room temperature. This serum was subjected for quantitative analysis of serum 25(OH) D which was done by chemiluminiscence macroparticle enzyme immunoassay (CMIA).Classification of vitamin D status as sufficient/ insufficient/ deficient was done as suggested by Veith et al. (Table I)⁷.

Statistical Analysis

Results obtained were analyzed statistically. Continuous variables were presented as mean or median.

Ethics

There was no drug trial or human / animal experiment involved. All the subjects were counseled and results informed.

3.RESULTS

Characteristics of study participants

In Group A, total of 80 subjects were enrolled of which group A₁ had 40 healthy infants and group A₂ had 40 mothers. In Group A₁, out of 40 infants, there were 20 males and 20 females with mean age of 6.07 months. The mean age of mothers in Group A₂ was 26.93 yrs. As per modified Kuppuswamy scale, in Group A, 15% subjects belonged to class II, 70% subjects to class III and 15% subjects to class IV.

In group A₁, the mean duration of sunlight exposure with clothes during summers and winters in age group 0-2 months was 2.50 hours and 3.50 hours respectively, in age group 2-6 months, 4.40 hours and 4.50 hours respectively and in age group 6-12 months, 5.08 hours and 6.23 hours respectively.

In Group A₁ in infants aged 0-6 months , 19 (100%) were EBF(Exclusive Breast Feeding) and in infants aged 6-12 months, 13 (61.9%) were BF(Breast Feeding) with CF(Complimentary Feeding).

Vitamin D deficiency was observed in 32 (80%) infants and vitamin D insufficiency in 4 (10%) infants. Similarly vitamin D deficiency was observed in 35 (87.50%) mothers and vitamin D insufficiency in 3 (7.50%) mothers out of 40 mothers in Group A₂.

The median Vitamin D level in Group A₁ was 12.45ng/ml (deficient). The median Vitamin D level in Group A₂ was was 11.15 ng/ml (deficient).

4.DISCUSSION

This preliminary study revealed that vitamin D deficiency is present in healthy infants and their mothers . To our knowledge, this is the first study conducted in India to find out the status of vitamin D levels in healthy infants along with their mothers .

The baseline characteristics in infants of study group are compared with study conducted by Zuo et al in children from 6-36 months in which there was male predominance⁸.

In our study the mean age of mothers in Groups A₂ was very similar to the study conducted by Mirzaei et al where they compared the 25 (OH) D levels between mothers and their appropriate for gestational age (AGA) newborns and between mothers and their small for gestational age (SGA) newborns with mothers⁹.

The majority of our subjects belonged to class III followed by class II and class IV in our study which shows the strata of population coming to our hospital.

From our study, it is evident that duration of sun exposure was more during winters. The duration of sun exposure also increased with the increasing age of infants. This can be explained with cultural practice of less exposure to the young infant as compared to the older infants.

It has been observed that in our study population, the infants in age group 0-6 months were on EBF (100%) . The infants in age group 6-12 months were predominantly on breast feeding with complimentary feeds (61.9%). Wayse et al studied 150 Indian children aged 2-60 months as part of a case control study. They reported that subclinical vitamin D deficiency and non exclusive breast feeding on the first 4 months of life as significant risk factors for severe ALRTI in Indian children¹⁰.

The vitamin D deficiency was observed in 32 (80%) infants and vitamin D insufficiency in 4 (10%) infants. This suggests the magnitude of the problem of vitamin D deficiency in infants present in our society. In a prospective observational study, Prasad et al observed vitamin D

deficiency in 67 (83.8 %) out of 80 children aged 2mo to 12y admitted with medical conditions including pneumonia admitted at the pediatric intensive care unit of a tertiary care hospital¹¹. In a case control study conducted by Roth et al in Bangladesh found that in subgroup of 29 community control participants aged 1-6 months were all either vitamin D deficient or insufficient¹². Ritu et al reported that vitamin D deficiency prevails in epidemic proportions all over the Indian subcontinent, with a prevalence of 70%–100% in the general population. They also reported that subclinical vitamin D deficiency is highly prevalent in both urban and rural settings and across all socioeconomic and geographic strata¹³.

The vitamin D deficiency and insufficiency was observed in 35 (87.50%) mothers and 3 (7.50%) mothers respectively in Group A₂. This suggests that vitamin D deficiency is prevalent among mothers in our society. The lower levels of vitamin D in infants can be correlated with the lower levels of vitamin D in mothers as suggested by the study conducted by Sachan et al. Sachan et al studied 207 urban and rural pregnant women at term in India. 138 out of 207 (66.67%) of women were vitamin D deficient (<15 ng/ml) and maternal serum 25 (OH) D correlated positively with cord blood 25(OH)D. They observed a high prevalence of vitamin D deficiency among pregnant women and their newborns¹⁴.

Our finding that serum vitamin D level in mothers were either deficient or insufficient not consistent with the study done by Dinlen et al in which they observed that the median serum 25 (OH) D levels in the mothers of the study group were lower than those in the mothers of the control group¹⁵. Mirzaei et al compared the 25-hydroxy vitamin D levels between mothers and their small for gestational age (SGA) newborns with mothers and their appropriate for gestational age (AGA) newborns. Vitamin D deficiency was statistically higher in women with SGA newborns in comparison to women with AGA newborns. The relationship of vitamin D deficiency levels between mothers and infants in both the SGA group and the AGA group was significant⁹.

There was no statistically significant difference between vitamin D level distribution according to socioeconomic status. Vitamin D levels in socioeconomic class I was 41.70 ng/ml (sufficient). There was only 1 infant out of 40 infants among cases who belonged to socioeconomic class I and hence it does not reflect the exact nature of vitamin D distribution. Also there was no significant difference between vitamin D level distribution according to socioeconomic status in mothers. Ritu et al reported that subclinical vitamin D deficiency is highly prevalent in both urban and rural settings, and across all socioeconomic and geographic strata¹³.

Infants in Group A₁ and mothers in Group A₂ were having vitamin deficiency .Our observations showed deficient vitamin D levels in 78.75% of population and insufficient vitamin D levels in 12.50% of population in the whole studied population which suggests the high prevalence of vitamin D deficiency in our high risk population group (mothers and their infants).

5.CONCLUSION

In the present study, association of infant and maternal serum 25 (OH) D levels in relation to severity of pneumonia in infants was evaluated. This study showed vitamin D deficiency in both infants and mothers although there was no causal relation between the vitamin D levels and severity of pneumonia. The observations of the study indicate that vitamin D deficiency is prevalent in high risk population group in Himachal Pradesh and as a consequence this must be taken into consideration with future planning of strategies to prevent vitamin D deficiency during antenatal period in mothers and during infancy. However keeping in view the role of vitamin D in preventing many infectious, inflammatory and neoplastic diseases, a screening program of this high risk group can be undertaken.

Limitations

- Our observations showed low levels of serum vitamin D in the studied population which suggests the high prevalence of vitamin D deficiency in our high risk population (mothers and infants).
- Our sample size was small and hence a population based study is required to find out prevalence of vitamin D deficiency in high risk population to formulate the strategy to prevent it.

Recommendations

It appears from our study that vitamin D deficiency is not a risk factor for ARI. The low vitamin D levels in the studied population are reflecting vitamin D deficiency as a public health concern. Keeping this in view, it is recommended that a screening program is to be introduced to find out the vitamin D level status in the high risk population, to prevent illnesses caused due to vitamin D deficiency.

There were no baseline differences in the age characteristics in infants of study group and control group in our study when compared with study conducted by Zuo et al in children from 6-36 months in which case to control ratio was 2:1 and there was male predominance⁸.

In our study there was no difference between the mean age of mothers in Groups A₂ and B₂ as far as age was concerned. These findings were very similar to the study conducted by

Mirzaei et al where they compared the 25 (OH) D levels between mothers and their appropriate for gestational age (AGA) newborns and between mothers and their small for gestational age (SGA) newborns with mothers⁹.

The majority of our subjects belonged to class III followed by class II and class IV in our study which shows the strata of population coming to our hospital.

From our study, it is evident that duration of sun exposure in both groups was more during winters. The duration of sun exposure also increased with the increasing age of infants. This can be explained with cultural practice of less exposure to the young infant as compared to the older infants.

It has been observed that in our study population, the infants in age group 0-6 months were on EBF in cases (90%) and controls (100%). The infants in age group 6-12 months were predominantly on breast feeding with complimentary feeds in both cases (70%) and controls (61.90%). So more controls were given breastfeeding when compared with cases. This may suggest more ARI among infants in cases. Wayse et al studied 150 Indian children aged 2-60 months as part of a case control study. They reported that subclinical vitamin D deficiency and non exclusive breast feeding on the first 4 months of life as significant risk factors for severe ALRTI in Indian children¹⁰.

The vitamin D deficiency was observed in 26 (65%) infants and vitamin D insufficiency in 7 (17.50%) infants among cases whereas vitamin D deficiency was observed in 32 (80%) infants and vitamin D insufficiency in 4 (10%) infants among controls. This suggests the magnitude of the problem of vitamin D deficiency in infants present in our society. In a prospective observational study, Prasad et al observed vitamin D deficiency in 67 (83.8 %) out of 80 children aged 2mo to 12y admitted with medical conditions including pneumonia admitted at the pediatric intensive care unit of a tertiary care hospital¹¹. In a case control study conducted by Roth et in Bangladesh found that in subgroup of 29 community control participants aged 1-6 months were all either vitamin D deficient or insufficient¹². Ritu et al reported that vitamin D deficiency prevails in epidemic proportions all over the Indian subcontinent, with a prevalence of 70%–100% in the general population. They also reported that subclinical vitamin D deficiency is highly prevalent in both urban and rural settings and across all socioeconomic and geographic strata¹³.

The vitamin D deficiency and insufficiency was observed in 33 (82.50%) mothers and 6 (15%) infants respectively in Group A₂ whereas in Group B₂, vitamin D deficiency and insufficiency was observed in 35 (87.50%) mothers and (7.50%) mothers respectively. This suggests that vitamin D deficiency is prevalent among mothers in our society. . The lower

levels of vitamin D in infants can be correlated with the lower levels of vitamin D in mothers as suggested by the study conducted by Sachan et al. Sachan et al studied 207 urban and rural pregnant women at term in India. 138 out of 207 (66.67%) of women were vitamin D deficient (<15 ng/ml) and maternal serum 25 (OH) D correlated positively with cord blood 25(OH)D. They observed a high prevalence of vitamin D deficiency among pregnant women and their newborns¹⁴. Our finding that serum vitamin D level in mothers were not significantly different between cases and controls was not consistent with the studies done by Dinlen et al and Mirzaei et al. Dinlen et al conducted a case control study consisting of 30 term newborns with ALRTI who were admitted at neonatal intensive care unit as cases and 30 healthy newborns with the same age as controls along with their mothers in the same respective groups. They concluded that the median serum 25 (OH) D levels in the mothers of the study group were also lower than those in the mothers of the control group¹⁵. Mirzaei et al compared the 25-hydroxy vitamin D levels between mothers and their small for gestational age (SGA) newborns with mothers and their appropriate for gestational age (AGA) newborns. Vitamin D deficiency was statistically higher in women with SGA newborns in comparison to women with AGA newborns. The relationship of vitamin D deficiency levels between mothers and infants in both the SGA group and the AGA group was significant⁹. There was no statistically significant difference between vitamin D level distribution according to socioeconomic status in cases and controls in infants. Vitamin D levels in socioeconomic class I was 41.70 ng/ml (sufficient). There was only 1 infant out of 40 infants among cases who belonged to socioeconomic class I and hence it does not reflect the exact nature of vitamin D distribution. Also there was no significant difference between vitamin D level distribution according to socioeconomic status in cases and controls in mothers. Ritu et al reported that subclinical vitamin D deficiency is highly prevalent in both urban and rural settings, and across all socioeconomic and geographic strata¹³. Infants in both Group A₁ and Group B₁ were having vitamin deficiency but there was no statistically significant association was found between vitamin D levels and severity of pneumonia. Also mothers in Group A₂ and Group B₂ were having vitamin D deficiency but there was no statistically significant association between vitamin D levels and severity of pneumonia. Our observations showed deficient vitamin D levels in 78.75% of population and insufficient vitamin D levels in 12.50% of population in the whole studied population which suggests the high prevalence of vitamin D deficiency in our high risk population group (mothers and their infants).

CONCLUSION

The present study was done to find out the baseline serum 25 (OH) D₃ levels in healthy infants and mothers in a hilly terrain of Himachal Pradesh. This study showed vitamin D deficiency in healthy infants and their mothers. The observations of the study indicate that vitamin D deficiency is prevalent in high risk population group in Himachal Pradesh and as a consequence this must be taken into consideration with future planning of strategies to prevent vitamin D deficiency during antenatal period in mothers and during infancy. However keeping in view the role of vitamin D in preventing many infectious, inflammatory and neoplastic diseases, a screening program of this high risk group can be undertaken.

LIMITATIONS

- Our observations showed low levels of serum vitamin D in the studied population which suggests the high prevalence of vitamin D deficiency in our high risk population (mothers and infants).
- Our sample size was small and hence a population based study is required to find out prevalence of vitamin D deficiency in high risk population to formulate the strategy to prevent it.

RECOMMENDATIONS

The low vitamin D levels in the studied population are reflecting vitamin D deficiency as a public health concern. Keeping this in view, it is recommended that a screening program is to be introduced to find out the vitamin D level status in the high risk population, to prevent illnesses caused due to vitamin D deficiency.

ETHICAL APPROVAL

Ethical clearance was taken from the institution where the study was conducted.

REFERENCES

1. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am. J. Clin. Nutr. 2008; 88: 491S-99S.
2. Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB et al. The role of vitamin D in cancer prevention. Am. J. Public Health. 2006; 96: 252-261.
3. Holick MF. Vitamin D: molecular biology, physiology, and clinical applications. Totowa, NJ: Humana Press. 1999.
4. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease and osteoporosis. Am. J. Clin. Nutr. 2004; 79: 362-71.

5. Holick MF. Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease, type 1 diabetes, autoimmune diseases, and some cancers. *South. Med. J.* 2005; 98: 1024-1026.
6. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Adapted from global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012; 379: 2151-61.
7. Vieth R, Ferrari HB, Boucher BJ, Hughes BD, Garland CF, Heaney RP, et al. The urgent need to recommend an intake of vitamin D that is Effective. *Am J Clin Nutr* 2007; 85: 649-50.
8. Zuo XF, Li JX, Zhou WD. The nutrient status of Chinese infants with pneumonia. *Biomed Res- India* 2014; 25 (3): 317-20.
9. Mirzaei F, Moghadam TA, Arasteh P. Comparison of serum 25-hydroxy vitamin D levels between mothers with small for gestational age and appropriate for gestational age newborns in Kerman. *Iran J Reprod Med* 2015; Vol. 13. No. 4. pp: 203-208.
10. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical Vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5y. *Eur J ClinNutr* 2004; 58: 563-7.
11. Prasad S, Raj D, Warsi S, Chowdhary S. Vitamin D deficiency and critical illness. *Indian J Pediatr* 2015; 82: 991-95.
12. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status of infants in northeastern rural Bangladesh: Preliminary observations and a review of potential determinants. *J Health Popul Nutr* 2010; 28: 458-69.
13. Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. *Nutrients*. 2014; 6: 729-75.
14. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *Am J Clin Nutr* 2005; 81: 1060-4.
15. Dinlen N1, Zenciroglu A, Beken S, Dursun A, Dilli D, Okumus N. Newborn acute lower respiratory tract infection associated with low maternal vitamin D. *J Matern Fetal Neonatal Med.* 2015; 19: 1-5.

336 **Table 1: Classification of quantitative estimation of vitamin D⁷**

Vitamin D status	Serum 25(OH) D level	
	(ng/ml)	(nmol/l)
Deficiency	<20	<50
Insufficiency	20-<30	50-<75
Sufficiency	30-<100	75-<250
Toxic	≥100	≥250

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339 **Table 2: Characteristics of the subjects**

Characteristics	Controls
Age (months) of infants	
0-2 months	10
2-6 months	11
6-12 months	19
0-12 months (total)	40
Mean age (months) of infants	6.07
Sex	
Males	20
Females	20
Mean age (years) of mothers	26.93
Socioeconomic status	
Type I	0*
Type II	6
Type III	28
Type IV	6
Feeding	
Exclusive breast feeding (EBF)	19
Top feeding (TF)**	0*
Breast feeding with complimentary feeding	13

(BF with CF)	
Mixed feeding (MF)***	8

340 *There were no subjects in this group.

341 **Infants who were top fed (animal milk or formula milk) alone or top fed along with breast
342 feeding till 6 months of age.

343 ***Infants who were top fed alone or top fed along with breast feeding till 6 months of age
344 and continued with it along with complimentary feeds.