Original Article

CLINICAL AND BIOCHEMICAL PROFILE OF VITAMIN D DEFICIENCY

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Abstract

Aim: To study the clinical symptoms and signs of vitamin D deficiency and their correlation with 25-Hydroxy vitamin D [25(OH)D] levels.

Materials and methods: It is a prospective Observational study. All children age group of 6months to 13 years who visited the department of pediatrics with Hypovitaminosis D from September 2020 to October 2022. Ethics clearance was obtained before the start of study. Written and informed consent was obtained from all patients.

Results: The mean Vitamin D levels in males and females were 21.13 ± 6.15 ng/ml and 21.36± 5.64ng/ml. There was no significant difference between the Vitamin D levels in males and females (P=0.99). Patients with Vitamin-D level less than12ng/ml show 83% skeletal manifestation. The data shows X-ray changes in 100% of the patients with vitamin D levels below 8ng/ml and in 75% of patients with Vitamin D levels between 8-12ng/ml. The mean age of the patients was 6.27 ± 3.53 years. 12 % participants had vitamin D levels less than 12 ng/ml. Of these, the majority (33.3%) belonged to 2-5 years age group, followed by 25 % each in the 6months to 2 years and 10 to 13years age group. The least deficiency was seen in the 5-10 years age group. 25 % participants had Vitamin D levels in the range of 12-20 ng/ml. Of these, 36 % belonged to the age group 2-5 years and 5-10 years each. 24 % were in the age group 10-13 years and 8% were in the age group 6months to 2 years. 63 % participants had Vitamin D levels more than 20 ng/ml. The majority (47.6 %) belonged to the age group 5-10 years, followed by 23.8 % participants in the 10-13 years age group, 15.9 % participants in the age group 2-5 years and 12.6 % participants in the 6 months to 2 years age group. The correlation of Vitamin D deficiency with a particular age was not statistically significant (P= 0.2664).

Conclusion: Vitamin D is a modifiable risk factor for stunted growth etc. Hence supplementation at an appropriate age can go a long way in promoting good health among children in a resource limited country like ours. With so many studies all over the world showing a near universal presence of low vitamin D levels, it is a matter of concern whether low levels really cause so many problems. Routine supplementation as recommended by several agencies needs to be periodically reviewed and revised, based on the impact on functional outcomes and changing prevalence patterns of deficiency in target population.

Introduction

Vitamin D- cost free and freely available to anyone whose skin is adequately exposed to sunrays. Surprisingly, in spite of ample sunlight vitamin D deficiency is common in India[1].

The clinical syndrome of vitamin D insufficiency includes rickets in children and osteomalacia in adults. Joint pains, non-specific backaches, and broad body aches are further signs of vitamin D deficiency. With the development of a technique to precisely test 25-hydroxyvitamin D [25(OH) D], it is now possible to diagnose hypovitaminosis D without the presence of overt signs and symptoms of vitamin D deficiency. [2,3].

It is challenging to determine the amount of vitamin D provided by sun exposure and nutrition because the length and intensity of sunlight exposure vary on the location's latitude, the recipient's age, clothing, and use of sunscreens[4]. One of the factors contributing to the development of bone disease is the inadequate intake of vitamin D[4,5,6].

The reason for this inadequate vitamin D consumption is a combination of variables. These include genetic factors[8,9], Skin pigmentation[7], which ostensibly interferes with ultraviolet ray transmission via epidermis, social customs such as avoiding sun exposure, consumption of chapattis that are phytate rich and it binds calcium in the alimentary canal and thereby interferes with the absorption of calcium[10], lack of exposure to sun[11,12], and malnutrition[13,14,15] to name a few significant contributing factors.

Without treatment, some patients are likely to go on to develop frank nutritional rickets[1]. Low serum 25-hydroxyvitamin D levels, which may result in other illness outcomes, are now referred to as vitamin D insufficiency. Due to the large individual variations in the functional effects of vitamin D and its interactions with calcium intakes, it is not possible to set a single threshold number to establish vitamin D deficit or insufficiency[16].

Materials and methods

Type of study: Prospective Observational study

Place of study: Department of pediatrics, DR. D.Y. Patil Medical College, hospital and research Centre, Pimpri, Pune.

Period of study: September 2020 to October 2022, after approval from institutional ethical committee.

Ethical clearance: Ethics clearance was obtained before the start of study.

Written and informed consent was obtained from all patients. The patients or informants were informed regarding the purpose, procedures, risks and benefits of the study in their own vernacular language.

Source of data: All children age group of 6months to 13 years who visited the department of pediatrics with Hypovitaminosis D from September 2020 to October 2022.

Statistical methods: The data was extracted and recorded in MS EXCEL2016. The statistical analysis was performed using Graph PAD PRISM 8.0.2. Pearson correlation test was used where ever required. Statistical significance $P<0.05$. Student T-test was used where ever required.

Inclusion criteria:

- Children of age between 6 month and 13 years with signs and symptoms of rickets/hypocalcemia.
- Children of both sexes.
- Children with low vitamin D levels.
- Children with hypocalcemia.

Exclusion criteria:

- Age group of more than 13 years.
- Non nutritional rickets

- Renal rickets

Sample size: 100

Confidence level = 95%

Acceptable diff = 9 per 100

Assumed rate = 69per 100

Required sample size =100 if the rate is 69 per 100.

Software used to calculate sample size was winpepi.

Data analysis:

Data will be collected using preformed data collection as well as case record form for new cases. Data entry will be done in Microsoft Excel and analysis using GraphPad Prism 8.0.2 software.

Method:

- Patients were explained the purpose of study and informed consent was taken.
- Children aged 1 to 13 years with Hypovitaminosis D visiting the pediatric OPD and admitted patients were screened by history and clinical examined. 2-3ml blood was drawn by venipuncture for 25 (OH) D.
- In those children 25 OH VITAMIN D levels are done by FULLY AUTOMATED CHEMILUMINESCENT IMMUNOASSAY (CLIA)
- Further investigations like X-ray of left wrist till fingers in florid rickets.
- Laboratory parameters like alkaline phosphatase, serum phosphorus, serum calcium.

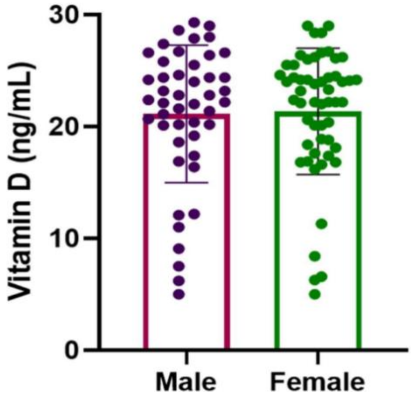
Results and observations

Table 1: General statistics of patients in the study.

Age (yr.)	6.27 ± 3.53
Percentage of Males	46
Percentage of females	54
Height (cm)	110.14±25.1
Weight (kg)	24.61 ± 4.48
25(OH)D (ng/ml)	21.25 ± 5.86
20-30 ng/ml, *n* (%)	63 (63%)
12-20 ng/ml, *n* (%)	25 (25%)

< 12 ng/ml, *n* (%)	12 (12%)
Study Population (N) = 100	

Figure 1: Vitamin D levels in males and females in the study.



Observation: There was no significant difference in the mean Vitamin D levels. (P = 0.99)

The mean Vitamin D levels in males and females were 21.13 ± 6.15 ng/ml and 21.36± 5.64ng/ml. There was no significant difference between the Vitamin D levels in males and females (P=0.99).

Table 2: vitamin D levels and relation with age

Vitamin D	**0-2yrs.**	**2-5yrs.**	**5-10yrs.**	**10-13yrs.**	**Total**
<12	3	4	2	3	**12**
12-20ng/ml	2	9	9	5	**25**
>20	8	10	30	15	**63**
Total	**13**	**23**	**41**	**23**	**100**

Figure 3: Distribution of study population based on the Vitamin D levels in different age groups

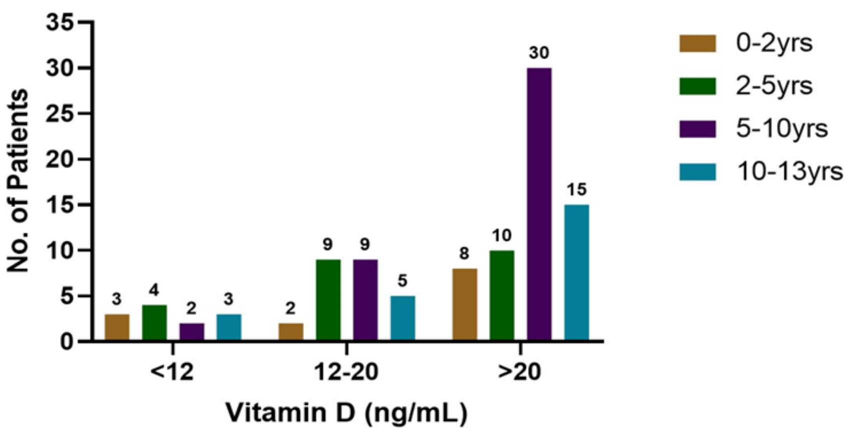
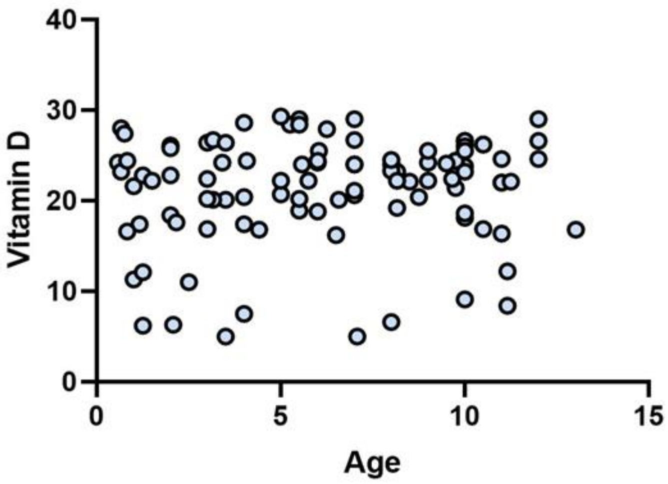
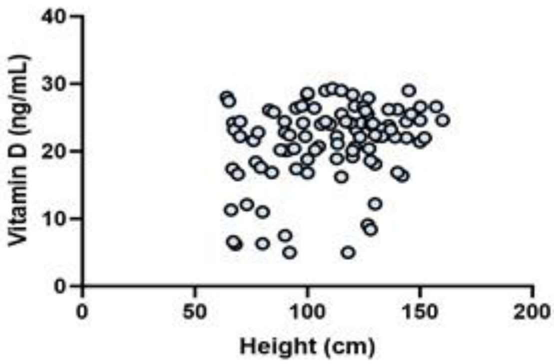


Figure 4: Scatter plot of Correlation between Vitamin D levels and age in children.



The data was analysed using Pearson correlation and it suggests that there is no correlation between the Vitamin D levels and age (N=100, P= 0.2664)

Figure 5: Scatter plot of Correlation between Vitamin D levels and height in children.

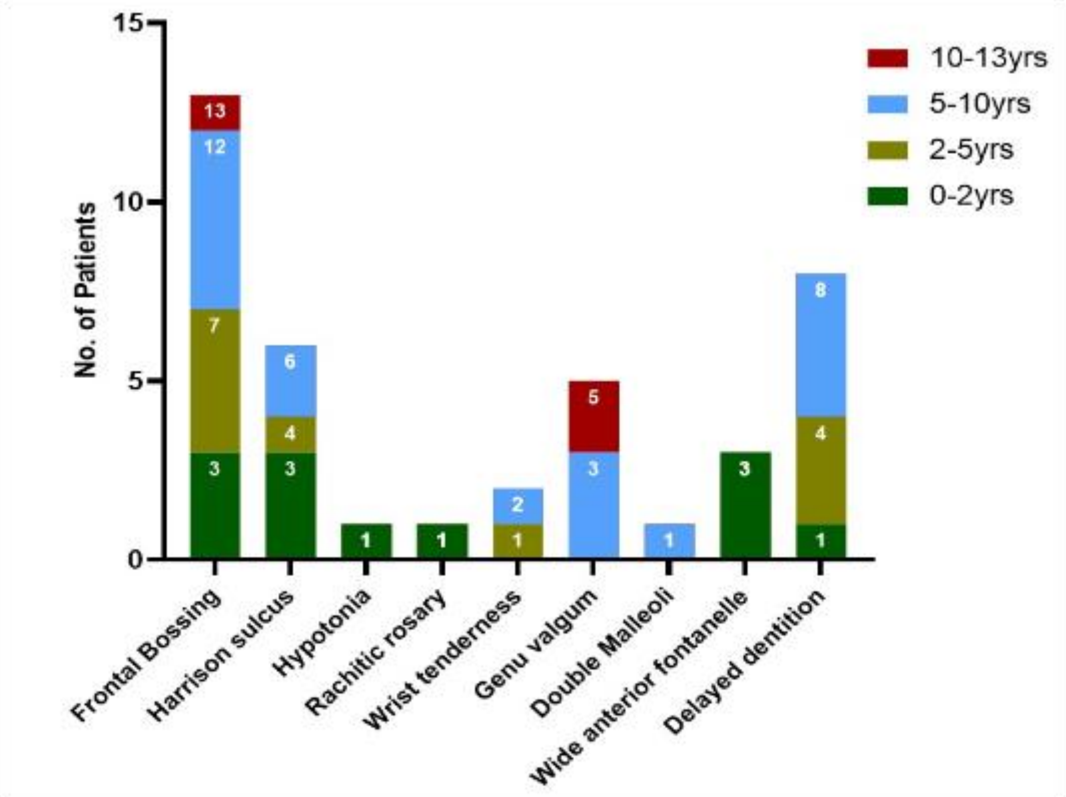


The data was analysed using Pearson correlation and it suggests that there is significantly fair correlation between the Vitamin D levels and height (P= 0.0090 ,N =100)

	0-2yrs.	2-5yrs.	5-10yrs.	10-13yrs.	Total
Frontal Bossing	3	4	5	1	**13**
Harrison sulcus	3	1	2	0	**6**
Hypotonia	1	0	0	0	**1**
Rachitic rosary	1	0	0	0	**1**
Wrist tenderness	0	1	1	0	**2**
Genu Valgum	0	0	3	2	**5**
Double Malleoli	0	0	1	0	**1**

Wide anterior fontanelle	3	0	0	0	**3**
Delayed dentition	1	3	4	0	**8**
Total	**12**	**9**	**16**	**3**	**40**

Table 3, Figure 6: Distribution of study population based on Skeletal manifestation in different age groups.



Skeleton Manifestation	**Vitamin D levels (ng/mL)**			
	<12	**12-20**	**>20**	**Total**
Frontal Bossing	3	6	4	**13**
Harrison sulcus	4	1	1	**6**
Hypotonia	1	0	0	**1**
Rachitic rosary	1	0	0	**1**
Wrist tenderness	2	0	0	**2**
Genu valgum	3	1	1	**5**
Double Malleoli	1	0	0	**1**
Wide anterior fontanelle	3	0	0	**3**
Delayed Dentition	2	3	3	**8**
Total	**20**	**11**	**9**	**40**

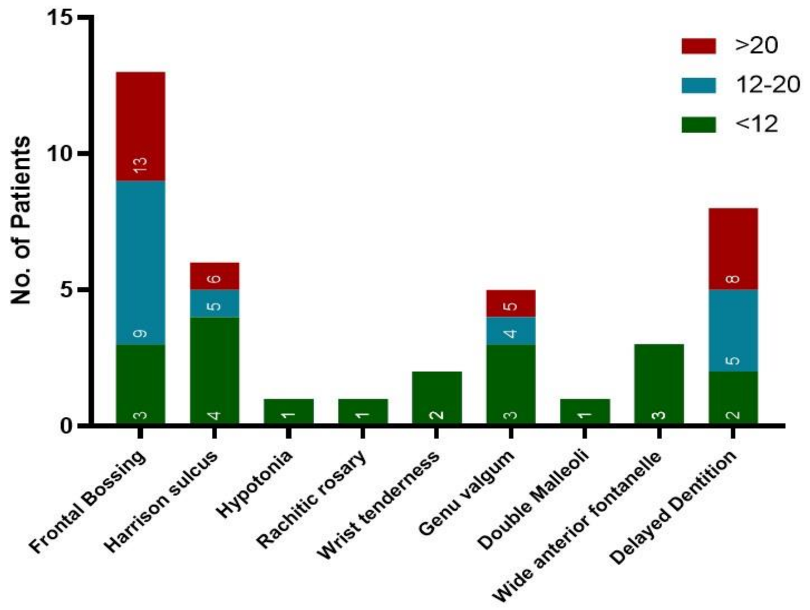


Table 4, Figure 7: Distribution of study population based on skeletal manifestation and vitamin D levels

Table 5: Skeletal Manifestation against Vitamin-D profile

Vitamin-D (ng/ml)	**No. of Patients**	**Skelton Manifestation n (%)**
5-12	12	10(83)
13-20	25	8 (32)
> 20	63	11 (17)
Total	100	29 (29%)

Observation: Patients with Vitamin-D level less than12ng/ml show 83% skeletal manifestation.

Table 6: Percentage of Patients showing X ray changes (Cupping and fraying of metaphysis) under different Vitamin D levels.

Vitamin-D Levels ng/ml	**Cupping and fraying of metaphysis n (%)**	**Total number of patients**
5-6	2 (100)	2
6-7	3 (100)	3
7-8	1 (100)	1
8 -12	3(75%)	4
Total	**9**	**10**

The data shows X-ray changes in 100% of the patients with vitamin D levels below 8ng/ml and in 75% of patients with Vitamin D levels between 8-12ng/ml.

Figure 8: Scatter plot showing correlation between Vitamin D level and Calcium level. (N=100, P value = 0.001)

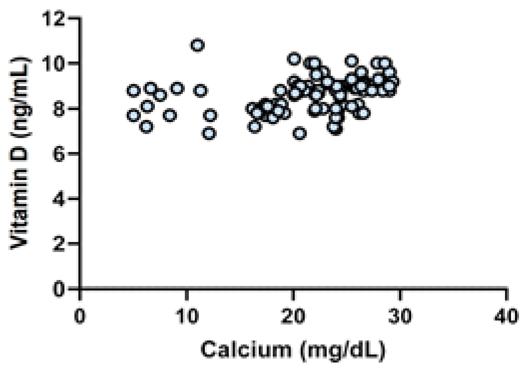


Figure 9: Scatter plot showing correlation between Vitamin-D level and phosphorus level. (N=100, P value = <0.0001)

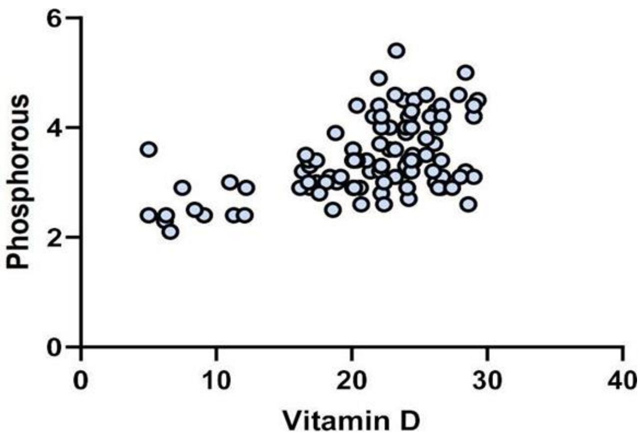


Figure 10: Scatter plot showing correlation between Vitamin D level and Alkaline phosphatase level. (N=100, P value = 0.03).

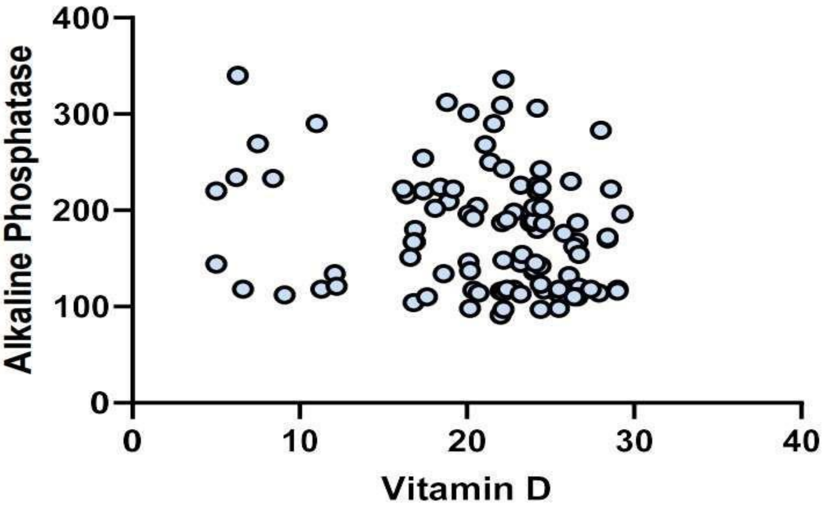


Figure 8-10 show graphical representation of correlation between variables. There is a significant positive correlation between Vitamin D levels and calcium levels (P value = 0.001), statistically significant between vitamin D levels and serum alkaline phosphatase levels, P value = 0.03), and statistically strongly significant between vitamin D levels and Phosphorous levels (P value = <0.0001)

Table 7: Distribution of study sample based on a) Vitamin D and Calcium levels b) Vitamin D and serum alkaline phosphatase level c) Vitamin D and serum phosphorous levels.

Vitamin D (ng/mL)	**Calcium (mg/dl)**	**Alkaline Phosphatase (U/L)**	**Phosphorous (mg/dL)**
<12	**8.34 ± 1.04**	**194.42 ± 79.58**	**2.61 ± 0.41**
12-20	**8.24 ± 0.70**	**183.96 ± 57.68**	**3.18 ± 0.41**
>20	**8.79 ± 0.67**	**169.97 ± 60.49**	**3.70 ± 0.65**

2-year-old female child showing frontal bossing with vitamin D level of 18.4ng/ml

10-year-old male child with genu Valgum with vitamin D level of 9.1ng/ml

3-year-old showing generalized osteopenia with cupping of metaphysis with vitamin D level of 5ng/ml

8-month-old female child with vitamin D level 6.6 ng/ml showing Cupping and fraying of metaphysis with generalized osteopenia.

Discussion

Rickets and osteomalacia have been widely researched, but there is little information on vitamin D deficiency without obvious bone disease.

This study was conducted on 100 children with Hypovitaminosis D of age group of 6 months to 13 years who visited the Department of pediatrics of Dr. D.Y. Patil Vidyapeeth (DPU), Hospital and Research Centre, Pune from September 2020 to October 2022.

Age

In present study, the mean age of the patients was 6.27 ± 3.53 years (Table 2). 12 % participants had vitamin D levels less than 12 ng/ml. Of these, the majority (33.3%) belonged to 2-5 years age group, followed by 25 % each in the 6months to 2 years and 10 to 13years age group. The least deficiency was seen in the 5- 10 years age group. 25 % participants had Vitamin D levels in the range of 12-20 ng/ml. Of these, 36 % belonged to the age group 2-5 years and 5-10 years each. 24 % were in the age group 10-13 years and 8% were in the age group 6months to 2 years. 63 % participants had Vitamin D levels more than 20 ng/ml. The majority (47.6 %) belonged to the age group 5-10 years, followed by 23.8 % participants in the 10-13 years age group, 15.9 % participants in the age group 2-5 years and 12.6 % participants in the 6 months to 2 years age group. (Table 2 and Figure 4). The correlation of Vitamin D deficiency with a particular age was not statistically significant (P= 0.2664) (Figure 8), while a study by Gonzalez-Gross et al.[17] reported that Vitamin D concentrations increased with age (P < 0·01). In contrast, a study by Kelly et al.[(18)] reported that in a multivariable model, older age was negatively associated with 25-OH D. Andiran et al. [(19)] also reported that 25 (OH)D levels correlated negatively with age p<0.001). In present study, 12 % children had deficient Vitamin D levels (< 12 ng/ml), while 25 % children and insufficient levels (12-20 ng/ml). 63 % children had sufficient levels of Vitamin D (> 20 ng/ml).

Gender

In present study, 46% patients were males and 54 % were females (Table 1). The male: female ratio was 1: 1.17. There was no significant difference in the Vitamin D levels between males and females in the study (P=0.99) (Figure 1). In a study by Dhillon et al.[50] in Amritsar, 79.4% of boys and 75.7% of girls had vitamin D deficiency. As in present study, Vierucci et al.[46] observed that gender was not associated with vitamin D status. However, a few studies have reported that the female sex is associated with hypovitaminosis D.[47,48,49] It is observed that studies reporting an association of female gender with hypovitaminosis are from countries where girls wear full clothing to cover their body due to religious reasons, thereby minimising the exposure of the skin to the sun, leading to hypovitaminosis D.

Height

In present study, the mean height of the patients was 110.14 ± 25.51 cm. (Table 1) There was a significantly fair positive correlation between the Vitamin D levels and height (P= 0.0090) (Figure 5). Similar to present study, a Japanese study[51] found that a definitive vitamin D deficiency (<10 ng/mL) restricted height growth by 0.6 cm per year even in young children, irrespective of their short stature. While the evidence base is much weaker at present, less severe vitamin D deficiency, which although not causing rickets, may prevent children and adolescents from reaching their genetically programmed height and peak bone mass [(26)]. Considering that vitamin D aids calcium absorption in the gut and is necessary for maintaining the blood calcium concentration and normal bone metabolism, the present result was reasonable.

Skeletal manifestations

In present study, frontal bossing, Harrison sulcus and wide anterior fontanelle were the major skeletal manifestations seen in 25 % of participants in the age group 6 month to 2 years. 8.3 % participants showed hypotonia, rachitic rosary and delayed dentition each. In the age group 2-5 years, frontal bossing was the major manifestation (44.4 %), followed by delayed dentition (33.3 %) and Harrison sulcus and wrist tenderness in 11.1 % participants. In the age group 5- 10 years, frontal bossing was the major skeletal manifestation (31.3 %) followed by delayed dentition (25 %), Genu Valgum (18.75 %), Harrison sulcus (12.5 %) and wrist tenderness and double malleoli (6.25 % each). In the older age group of 10 to 13 years, Genu Valgum (66.6 %) was the major manifestation followed by frontal bossing in 33.3 % participants (Table 7). The same is pictorially depicted in Figure 10. A study in Amritsar by Dhillon et al.[50] reported that only clinical features of vitamin D deficiency found in the study were frontal bossing (67 %), parietal bone prominences (62 %), pot belly (4 %) and widening of wrist epiphyses (4 %). A study by Gordon et al.[42] reported that a third of vitamin D-deficient subjects had radiological signs of demineralization, emphasizing the detrimental skeletal effects of this condition. In a study by Torun et al.[36], Group 1 (1-3 years age) presented with chief complaints on admission of low weight gain (failure to thrive) (89 %), muscle weakness (91 %), bone deformity (enlargement of wrist and ankles) (29.7 %) and head deformity (frontal bossing) (35.6 %). Group 2 (4- 6 years) exhibited muscle weakness (76 %) and low weight gain (failure to thrive) (68 %). The major symptoms in Group 3 (7 -11 years) were leg and chest pain (57 % and 28 %, respectively) and in Group 4 (12-17 years) (26 % and 55 %, respectively). High rates of obesity were observed in Group 3 and 4 (31 % and 63 %, respectively). The researchers observed that the biochemical findings of vitamin D deficiency were apparent in the Group 1 who acquired vitamin D deficiency due to the lack of vitamin D supplementation. However, in older groups, the majority of the patients had low 25 hydroxyvitamin D values without signs of biochemical findings of rickets. The study concluded that based on the degree of deficiency and insufficiency, and the age of the patients, the biochemical and clinical findings differed extensively. Children below 3 years of age who had either never been given vitamin D supplementation or who had been receiving supplementation that was stopped too early were at a higher risk for developing clinically and biochemically proven vitamin D deficiency. In older children, low vitamin D levels often led to mild complaints without abnormal biochemical findings.

Cupping and fraying of metaphysis

In present study, X ray changes, i.e., cupping and fraying of metaphysis was seen in 100% of the patients with Vitamin D levels below 8 ng/ml and in 75% of patients with Vitamin D levels between 8-12ng/ml. A study by Gordon et al.[42] reported that 7.5 % vitamin D-deficient participants showed rachitic changes on radiographs, whereas 32.5% had signs of demineralization. However, a study by Torun et al.[36] reported that in the age group 4 to 17 years, the major proportion of patients had low 25 hydroxyvitamin D values without proof of biochemical findings of osteomalacia. Osteoblasts, bone forming cells, lay down osteoid, whereas Osteoclasts ae involved in bone remodeling. Calcium salts are required for further mineralization of osteoids. In Vitamin D deficient conditions, the mineralization defect causes the osteoid to accumulate in the bone tissue, below the growth plate (metaphysis)[53]. Thus, the distal ulna in the upper limbs is where the changes are most obvious, whereas the metaphysis above and below the knees are where the changes are most apparent in the lower limbs. Unmineralized osteoid causes the epiphyseal plate to widen, and the zone of provisional calcification at the junction of the epiphyseal and metaphyseal bones loses its definition. The epiphyseal end of the metaphysis shows cupping and splaying, as well as cortical spurs and stippling is formed. Epiphyseal bone centers, which are small and osteopenic, may take longer to develop. Long bones in both children and adults have osteopenic shafts and thin cortices. The trabecular design is rough, fuzzy, and appears to be made of ground glass.[53].

Vitamin D and Calcium levels

Table 7 gives the distribution of study sample based on Vitamin D levels and calcium. The mean calcium level of the 12 patients having vitamin D levels < 12 ng/ml was 8.34 ± 1.04 mg/dl. For the patients with Vitamin D levels between 12-20 ng/ml, the mean serum calcium level was 8.24 ± 0.70 mg/dl. For patients with Vitamin D levels >20 ng/ml, the mean serum calcium levels were 8.79 ± 0.67 mg/dl. There is a fairly significant positive correlation between Vitamin D levels and calcium levels (P value = 0.001) Figure 12 gives the scatter plot showing correlation between Vitamin-D level and Calcium level. Similar to present study, Roh et al. [(25)] reported that average serum calcium levels were 9.67 ± 0.36 mg/dL for the vitamin D deficient patients, while the Vitamin D sufficient group had 9.76 ± 0.32 mg/dL, and it was significantly lower in the deficient patients (P=0.018). A similar result was observed by Andiran et al. [(19)]. However, a study by Dhillon et al.50 reported that 98% of vitamin D deficient subjects had normal serum calcium levels while only 2% cases had low serum calcium levels (p =0.73). Similarly, a study by Heaney et al. [(27)] in 2003 reported that there is no relation between serum calcium levels and of 25 (OH) vitamin D.

Vitamin D and Phosphorus levels

Table 7 gives the distribution of study sample based on Vitamin D levels and serum phosphorous levels. The mean serum phosphorous level of the 12 patients having vitamin D levels < 12 ng/ml was 2.61 ± 0.41 mg/dl. For the patients with Vitamin D levels between 12-20 ng/ml, the mean serum phosphorus level was 3.18 ± 0.41 mg/dl. For patients with Vitamin D levels >20 ng/ml, the mean serum phosphorus levels were 3.70 ± 0.65 mg/dl. We found a strongly significant, moderately positive correlation between vitamin D levels and serum Phosphorous levels (P value = <0.0001). Figure 13 shows a scatter plot of the same. A similar result was reported by Andiran et al. [19] . However, Roh et al. [25] reported that the mean serum phosphorus was not statistically different, between the deficient and sufficient Vitamin D levels groups in their study. For healthy bones, vitamin D is essential. It promotes enterocyte development and intestinal calcium and phosphorus absorption, which results in efficient bone mineralization (Ca2+ HPO42). Vitamin D promotes bone resorption in conditions of hypocalcemia or hypophosphatemia, keeping serum calcium and phosphorus levels stable. Thus, hypophosphatemia and hypocalcaemia are brought on by vitamin D insufficiency.

Vitamin D and Alkaline phosphatase levels (ALP)

Table 7 gives the distribution of study sample based on serum alkaline phosphatase levels and Vitamin D levels. Serum alkaline phosphatase level of the 12 patients having vitamin D levels < 12 ng/ml was 194.42 ± 79.58 U/L. For the patients with Vitamin D levels between 12-20 ng/ml, the mean serum alkaline phosphatase level was 183.96 ± 57.68 U/L. For patients with Vitamin D levels >20 ng/ml, the mean serum alkaline phosphatase levels were 169.97 ± 60.49 U/L. We found a significant correlation between the levels of vitamin D and serum alkaline phosphatase (P value = 0.03). Similar to present study, a study by Dhillon et al.[50] reported that, 2.5% of vitamin D deficient cases showed increased levels of alkaline phosphatase (ALP) but statistically insignificant (p=0.23). Mansour et al. [28] reported a positive correlation between vitamin D levels and ALP levels. Roh et al. [25] observed that ALP levels were higher in the cases with vitamin D deficiency group and also had a lower calcium level.

Since it is involved in the mineralization of bone and growth plate cartilage, alkaline phosphatase (ALP) is considered to be a reliable indicator of disease activity. ALP levels typically range from 500 IU/L in newborns to 1000 IU/L in kids up to age 9 before declining after puberty. Both hypophosphatemic and hypocalcemic rickets have elevated serum ALP levels. With the caution that occasionally rickets has been reported with normal ALP levels, ALP can be used to diagnose rickets. [29]. In order to achieve and maintain bone health in adults, as well as for optimal skeletal growth in utero and during children, vitamin D sufficiency is crucial. Vitamin D deficiency with 25(OH)D levels 15 ng/mL results in chondrocyte disorganisation and hypertrophy at the site of mineralization in children whose epiphyseal plates haven't closed, as well as skeletal mineralization defects, which induce bone malformations and short stature. [30]

Conclusion

Thus, Vitamin D is a modifiable risk factor for stunted growth etc. Hence supplementation at an appropriate age can go a long way in promoting good health among children in a resource limited country like ours. With growing evidence of extra skeletal role of vitamin D, generation of more evidence is required on whether everyone needs to maintain a vitamin D or whether its a bubble waiting to burst. With so many studies all over the world showing a near universal presence of low vitamin D levels, it is a matter of concern whether low levels really cause so many problems. Routine supplementation as recommended by several agencies needs to be periodically reviewed and revised, based on the impact on functional outcomes and changing prevalence patterns of deficiency in target population.

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