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SERUM 25-HYDROXY VITAMIN D;
PARATHORMONE AND BONE MINERAL DENSITY: CO-RELATION IN CHILDREN

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ABSTRACT: Deficiency of vitamin D is an emerging issue in children worldwide. It has been observed that all patients with vitamin D deficiency does not manifest clinical features or hyper-parathyroid response. In this study we have evaluated the interaction of serum vitamin D level, parathyroid hormone (PTH) level and bone mineral density (BMD) in children. **Objectives:** Our objectives were to determine the frequency of Vitamin D deficiency in children and association of low serum D level with serum parathyroid level and bone mineral density (BMD). **Study Design:** Descriptive cross sectional study. **Period:** June 2012 to May 2014. **Setting:** Pediatric and Ortho-pediatric out-patient departments. **Material & Methods:** A total of 500 children up to 15 years with low serum vitamin D level were enrolled to analyze the interaction of Serum vitamin D, PTH and BMD. Patients were divided in groups on the basis of serum PTH. We have categorize the deficiency of Vitamin D on the basis of level of 25OHD. It was defined as severe (25OHD ≤ 5 ng/ml), moderate (25OHD≤ 10 ng/ml) and mild (25OHD ≤ 20 ng/ml) and hyperparathyroidism (SHPT) was valued if level >65 pg/ml. All children with 25OH ≤ 20 ng/ml were included and association with SHPT and BMD were measured. **Results:** It has been observed that 30–40% of patients with moderate and severe deficiency of vitamin D respectively had shown increased level of PTH. Bone mineral density has demonstrated decline pattern from PTH Quartile 1to Quartile 4 at all sites in children, with only minimal difference (decreasing trend) in serum 25OHD levels between these quartiles. The critical level of parathyroid hormone beyond which BMD going to decline is 35 pg/ml. No demonstrable difference has been observed in BMD within each PTH quartile according to categorization of Vitamin D Deficiency. **Conclusions:** Around 40% of the patients having low serum vitamin D level demonstrated SHPT. Regarding the BMD levels, it begins to decreases at PTH levels currently well thought-out to be normal. So there is a need to re-define SHPT among different age groups considering the relationship linking PTH and BMD. This may also affect guidelines regarding vitamin d supplementation in patients with vitamin D deficiency.

Key words: Vitamin D deficiency, secondary hyperparathyroidism, bone mineral density, Children.

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BACKGROUND

Rickets caused by deficiency of vitamin D known as nutritional rickets is the universal problem and common among all ages.[1] Vitamin D acts vital role in maintaining blood levels of calcium and phosphorus required for appropriate bone mineralization, muscle tone, nerve conduction and stabilization of cellular functions. Its sufficient level during the childhood and adolescent years, is very essential for bone health, and also play an important role for the prevention of many chronic diseases, including malignancies, cardiac problems and immune mediated disorders.[2]

Clinical manifestations of vitamin D deficiency are different from patient to patient and depends on the length of vitamin D deficiency period. The clinical findings are more specific to the bones undergoing rapid growth at the time of onset of rickets. In infant older than 2-3 months, initially there were mainly hypocalcaemic features, when VDD continues the bony abnormalities become evident which includes cranio-tabes, delayed closure of anterior fontanel, enlarged/widened wrist, rachitic rosary and typical X deformity of the legs.[3] As the activated form of vitamin D begins to fall, the first hormonal change is the rise of PTH

to increase the active vitamin D by mobilizing the calcium stores from bones, with further fall in vitamin D bone demineralization occurs to maintain calcium levels, while gastro-intestinal calcium absorption is affected in severe Vitamin D deficiency.[4]

Then growth rate decreases in direct proportion to the degree of malnutrition. Consequently in cases of severe malnutrition the clinical features of rickets cannot be manifested due to decreased or arrested growth of growing bones. Therefore the diagnosis of rickets solely on physical features may be misleading particularly during early infancy. Majority of studies on analyzing the influence of serum vitamin D (25OHD) levels on serum parathyroid hormone level and calcium absorption have concluded that highest calcium absorption occurs at 4–8 ng/ml,[3] and maximum PTH suppression occurs at15–50ng/ml.[4] Another observation that has been drawn from different studies by literature search that all subjects with VDD will not manifest physical features of rickets or osteomalacia, and also do not mount a PTH response.[5-6] Ideally, to assess the effects of vitamin D deficiency, one must measure serum PTH, bone mineral density (BMD) and calcium intake and absorption.[7] Studies to assess calcium absorption are practically difficult to perform and facilities are not uniformly available at all laboratories. Therefore, we had selected to measure serum PTH and BMD to observe the effects of Vitamin D deficiency. We have designed this study to measure the effect of low serum Vitamin D on serum PTH level and bone mass density.[8] Out hypothesis is that all subjects with deficiency of vitamin D will not develop SHPT, those who developed will have lower BMD.

MATERIAL AND METHODS

The objective of our study was to determine the association of vitamin D deficiency with SHPH and bone mineral density in children. Comparative cross sectional study approved by Medical research center LUMHS was conducted from June 2012 to May 2014. Our sample size was 800, which was calculated with the help of WHO manual for estimation of sample size for cross-sectional comparative descriptive studies. We had recruited 500 children (70% of estimated sample) due financial constrain. The subjects were recruited from out-patient department LUH Hyderabad. Every subject was assessed for Vitamin D deficiency and those having 25OHD ≤ 20 ng/ml were included in the study. At the time of enrollment their required data including age, sex anthropometry, and detailed physical examination were recorded. Patients with liver, kidney or other disorders, and those receiving drugs expected to affect vitamin D level were excluded. After taking their written consent from the parents they advised to brought the child back on next morning for fasting blood samples collection (with minimum fasting of 6hours). There were clear instruction to the lab technician that the fasting samples should be centrifuged at 4 °C and immediately frozen at -8 °C till the assays were performed (within 2weeks from date of sample collection). All the required biochemical assessment was performed by using automated analyzer (Hitachi 902)[9] and commercial kits (Roche, Manheim, Germany).[9] The investigations that were done in all cases were given in Table-I with their reference range and analytical sensitivity.

Regarding the vitamin D deficiency we had estimated the 25OHD level. Level of <20 ng/ml was considered as deficiency[10] and <30 ng/ml was classified as insufficiency.[11] Deficiency state was further categorize as severe (<5 ng/ml), moderate (<10 ng/ml) and mild (<20 ng/ml).[12] Regarding estimation PTH, Secondary hyperparathyroidism (SHPT) was considered with serum PTH levels >65 pg/ml.[13] Patients were grouped according to quartiles of serum PTH (≤35.1, >35.1– 48.1, >48.1–66.2 and >66.2 pg/ml) having inter-quartile range of 31.3. BMD was measured by Prodigy Oracle (GE Lunar Corp., Madison, WI) according to manufacturer's recommendation.[10] Data was analyzed by using SPSS version 16. Differences in BMD among the four quartiles of PTH was detected by applying ANCOVA test. Categorical variables were analyzed by Chi-squared test. P value of <0.05 was considered statistically significant.

RESULTS

During study period from June 2012 to May 2014 total 500 children with males 56% and females 44% were enrolled in the study. Demographic characteristics of study population is presented in Table-II. To evaluate the secondary hyperparathyroidism we had measured serum PTH level, percent increase in serum PTH (defined as the increase in PTH above that seen in individuals with normal 25OHD levels) and those having SHPT were categorized according to severity of VDD (Table-III). SHPT was seen in approximately 30% and 40% subjects with moderate and severe VDD respectively. Bone mineral density decreased significantly with progression from Quartile 1 to Quartile 4, at all sites including radius, femoral neck, femur total, lumbar spine and total body (Table-IV). This significance was maintained even after weighted adjustment done for BMD for age, sex and BMI.

S. No.	Test	Normal range	Analytical sensitivity
1	Serum total calcium	8.8–10.2 mg/dl	0.2 mg/dl
2	Ionic calcium,	1.12–1.32 mM	
3	Inorganic phosphorus	2.7–4.5 mg/dl	0.3 mg/dl
4	Alkaline phosphatase	females: <240 U/L males: <270 U/L	5 IU/L. [9,10]
5	25OHD	30.0–37.6 ng/ml	1.5 mg/dl
6	PTH	10–65 pg/ml	0.7 pg/ml

Table-I. Biochemical tests done in all cases with VDD.

Parameters	CHILDREN (N = 500)
Age (years	13.3± 2.5
BMI (kg/m2)	19.3± 3.8
Sex (M:F-%)	45:55
S. Calcium (mg/dl)	9.9± 0.5
Ionic calcium (mg/dl)	1.14± 0.3 1
Inorganic phosphorus (mg/dl) 5	4.2 ± 0.5
ALP (IU/L)	307± 185
S. 25 OHD (ng/ml)	8.3 ± 5.2
S. PTH (pg/ml)	56.5 ± 33.7

Table-II. Basic anthropometric and biochemical parameters of study:

Vitamin D deficiency	PTH levels	PTH % Increase	SHPT*
Severe (<5 ng/ml) (125)	69.10± 41.10	55.8%	40 %
Moderate (5 to <10 ng/ml) (250)	54.28± 30.15	22.4%	30 %
Mild (10 to <20 ng/ml) (N=125)	47.99± 27.35	8.3%	10%

Table-III. PTH levels according to Vitamin D status *Cut-off of PTH 65 ng/ml. Number and percentage with secondary hyperparathyroidism.

DISCUSSION

Co-relational study conducted at out-patient department LUH had discovered that 40% of children has shown rise of PTH level response to severe vitamin D deficiency. According to referenced article <50% of patients had demonstrated increased level of PTH with moderate VDD.[16]

BMD	PTH Quartile 1 (≤35_1) N = 100	PTH Quartile 2 (>35_1–48_1) N = 120	PTH Quartile 3 (>48_1–66_2) N = 120	PTH Quartile 4 (>66_2) N = 160	P value
Radius 33%	0.581± 0. 091 (0.573–0.590)	0.573 _ 0.090 (0.565–0.581)	0.563 _ 0.090 (0.554–0.571)	0.525 _ 0.095 (0.516–0.534)	<0.0001
Femur neck)	0.934± 0.147 (0.920–947	0.911± 0.147 (0.897–924)	0.868± 0.157 (0.853–883)	0.900± 0.150 (0.886–913)	<0.0001
Femur	0.952 ± 0.146 (0.938–965)	0.935± 0 .141 (0.922–949)	0.925± 0.139 (0_911–939)	0.894 ± 0.162 (0.879–911)	<0.0001
Spine L1-L4	0.975± 0.190 (0.957–992)	0.945± 0.186 (0.931–965)	0.919± 0.177 (0.902–935)	0.862± 0.191 (0.843–880)	<0.0001
Total body	1.029± 0.105 (1.019–1.039)	1.013 ± 0.107 (1.003–1.023)	0.999± 0.107 (0.989–1.009)	0.58 ± 0.114 (0.947–969)	<0.0001
S. 25 OHD (ng/ml)	9.3± 5.2 (8.8–9.8)	9.3 ± 5.4 (8.8–9.8)	8.1± 5.3 (7.6–8.6)	6.3 ± 4,2 (5.9–6.7)	<0.0001

Table-IV. Association of PTH level and BMD.

It has not been cleared why the rise in parathyroid hormone will not be manifested in all patients having vitamin D deficiency. One possible explanation given in literature is decreased calcium intake as suggested in a study among children with rickets.[14] According to referenced study the levels of vitamin D were equivalent among patients and controls; but the dietary calcium intake was considerably lower in children with rickets, who also had mounted PTH response, whereas PTH level was normal in controls. Another likely justification can be impaired calcium absorption, which is not totally dependent on the vitamin D level, as other factors like diarrhea, nutritional status and volume and quality of milk consumption interfere with calcium absorption. In this study it has been observed that serum 25OHD levels were significantly lower in Quartile 3 and Quartile 4 compared with other groups, but the numerical difference in PTH level was seen to be very narrow. Literature has shown that the significant level of serum 25OHD required for maximal calcium absorption is 4–8ng/ml, levels above that will have little impact on calcium absorption.[15] Our study also supports that idea as in our study Quartile 3 and Quartile 4 of PTH had serum 25OHD levels <8 ng/ml.

Another study though conducted on elderly population to assess calcium absorption in relation to 25(OH)D levels ranging from 4 to 20 ng/ml, has shown similar observation that calcium absorption decreased only when serum 25OHD levels were below 8 ng/ml.[16] Regarding the effectiveness of Vitamin D supplementation in subjects with baseline 25OHD > 8 ng/ml, vitamin D supplementation had shown only 2–3% increase in calcium absorption.[11,16] while studies performed on subjects with 25OHD level of 4 ng/ml showed 21% increase in calcium absorption with vitamin D supplementation.[16]

In our study, BMD was appreciably lower in Quartile 3 and Quartile 4 as seen with Quartile 1 and Quartile 2. This observation favors the supposition that patients who manifest rise in PTH level will show lower BMD compared with those who do not. BMD at all sites was considerably lower in Quartile 3 compared with Quartile 1 while both groups had equivalent serum 25OHD levels. The possible mechanism may be that the subjects who do not mount PTH response, are spared from de-mineralizing consequence of VDD on bone.[17] To define the specific level for Secondary hyperparathyroidism there were different levels mentioned in literature.[18] Operational definition to define SPHT in our study was >35pg/ml, it was the level beyond which there was significant decline in BMD. It was notably lower in Quartiles 2, 3 and 4 compared with Quartile 1 (PTH approximately 35 pg/ml). The studies that were conducted on adults, have shown significant decline in BMD on Quartile 3 (PTH > 53 pg/ml).[19] This raises an interesting question about currently used normal range of PTH. The upper limits of PTH provided by manufacturers are probably too high because subjects with VDD may have been included while generating the norms.[20]

We conclude that our study verified that around 50% of subjects with Vitamin D deficiency had not manifested SHPT and had higher BMD compared with children with similar 25OHD levels. Children with Vitamin D deficiency with normal PTH have not manifested adverse effect on bones. This observation is further supported while looking for association of BMD with PTH level which had showed that within each quartile of PTH, there was no difference in BMD even with different levels of serum 25(OH) D levels. Therefore, it is perhaps the rise in serum PTH levels which decide bone health at any given level of serum 25(OH) D.

We recommends that serum 25(OH) D levels should always be analyzed in perspective of raised serum PTH. Further studies are required to assess the skeletal effect of vitamin D supplementations in children with VDD and normal PTH. Finally, there is also a need to identified criterion for defining biochemical hyperparathyroidism, as various studies had shown that BMD significantly declines even at levels which were currently considered as normal range.[19]

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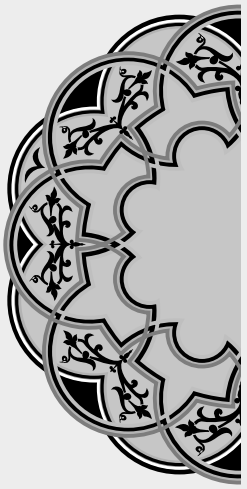

"Knowing other is wisdom,
knowing yourself is enlightenment."

Lao Tzu

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Shazia Memon	Contribution for conception and design, analysis and interpretation of data	
2	Dr. Farzana Shiakh	Collected data from pediatric department	
3	Dr. Asadullah Makhdoom	Collected and interpreted the data on bone mineral density by Dexa in outpatient department	
4	Dr S. M. Tahir	Final reversion of draft.	