VITAMIN D DEFICIENCY PANDEMIC, A REALITY OR AN OVER DIAGNOSIS? NEED TO RETHINK VITAMIN D DEFICIENCY CUT OFF LEVELS

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ABSTRACT

Objective: To determine clinical decision limits (cut off points) for vitamin D deficiency based on secondary hyperparathyroidism.

Study Design: Cross sectional study.

Place and Duration of Study: This study was conducted in the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), from Dec 2016 to May 2017.

Methodology: One hundred and sixteen subjects, aged 18 to 60 years, of either gender, with vitamin D levels less than 50nmol/L, were consecutively included in the study.

***Results*:** Serum 25(OH) D had inverse relation with plasma iPTH level (r=-0.597, p<0.0001). Thirty five percent of the subjects with vitamin D level below 50nmol/L had secondary hyperparathyroidism. However, the frequency of secondary hyperparathyroidism was 52% in subjects with 25 (OH) D level less than 25nmol/L compared with 28% having serum vitamin D levels of 25-40nmol/L. None of the patient with Vitamin D level above 40nmol/L had secondary hyperparathyroidism. Receiver operating characteristic (ROC) curve showed that 25(OH) D level of 25.5nmol/L as an optimal cut off level for vitamin D deficiency based on Secondary Hyperparathyroidism with area under the curve (AUC) was 0.761.

Conclusion: High frequency of secondary hyperparathyroidism in adults with vitamin D levels under 25nmol/L necessitates reconsideration of vitamin D cutoff limit for bone health.

Keywords: Cut off levels, iPTH, Secondary hyperparathyroidism, Vitamin D deficiency, 25(OH) D.

INTRODUCTION

Vitamin D deficiency has reportedly become a pandemic over the last few decades[1]. Deficiency of vitamin D is on the rise even in sunny countries like Australia, Saudi Arabia, UAE and India[2,3,4]. Various studies reported that vitamin D insufficiency is much more prevalent in pregnant women with different ethnic and geographical backgrounds[5]. Prevalence of vitamin D deficiency in Pakistan is documented to be 70 to 97% in a study conducted in Karachi, Pakistan, and high parathyroid hormone (PTH) levels were seen in 30% of these individuals[6]. In most of the centers standard test for vitamin D status is serum 25(OH) D levels[7], measured by a standardized assay, however this does not give any insight to the parathyroid status, i.e.; whether it is increased or within the normal range[8]. According to the literature whenever vitamin D decreases beyond a certain limit in the body there is decrease in serum calcium levels, which leads to increased secretion of PTH by parathyroid gland[9]. So without knowing parathyroid status we cannot comment on vitamin D levels for body's metabolic needs and also whether they are effecting bone health.

Definition of sufficient vitamin D level is controversial across the globe[10]. There is no agreement on the criteria for vitamin D deficiency. A number of studies conducted in the last couple of decades illustrated an association between decreased 25 hydroxyvitamin D levels in blood and the risks for cardiovascular diseases, fractures, cancers and mortality[11]. Approximately a decade ago, on the basis of these studies various professional societies including endocrine society recommended that definition of vitamin D deficiency

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should be changed from less than 50nmol/L to less than 75nmol/L which was recommended by Institute of Medicine (IOM) in its report of 2011. Use of this cut off, i.e.; less than 75nmol/L to define vitamin D deficiency resulted in approximately half of the tested population in different parts of the world being vitamin D deficient[12].

Based on inverse correlation between 25(OH) D and serum iPTH, it is quite reasonable to accept the level of serum 25(OH) D that would be sufficient to maintain PTH at normal levels for the bone health point of view, as a cutoff point for vitamin D deficiency[8].

Multiple studies have been conducted throughout the world. As far as Pakistan is concerned data about vitamin D deficiency leading to secondary hyperparathyroidism is lacking. Therefore, there is dire need for further studies to bring the true picture into account. On the basis of our study we can highlight the actual status of vitamin D and secondary hyperparathyroidism in our population, and set cut off points for vitamin D deficiency. This would help in formulating guidelines for testing and supplementation of vitamin D to prevent multidimensional ill effects of vitamin D deficiency on health.

METHODOLOGY

Current study was conducted at Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from December 2016 to May 2017. After taking approval from Institutional Review Board (FC-CHP14-1/READ-IRB/17/325). It was a cross-sectional study based on 116 adult subjects of both genders with ages, between 18 to 60 years with vitamin D levels under 50 nmol/L. Sample size was calculated by WHO sample size calculator by taking 95% confidence interval, anticipated population (26%)[8] and precision 8%.

Patients with history of choronic kidney disease (CKD), choronic liver disease (CLD), metabolic bone diseases, hypo or hypermagnesemia, malignancy, malabsorption disorders, using drugs (anticonvulsants or glucocorticoids) or taking any kind of calcium or vitamin D supplements in the previous three months before the start of study and pregnant and nursing women were excluded.

Samples were collected by nonprobability consecutive technique, from patients visiting the AFIP Endocrine Clinic after taking informed consent. All the participants were allotted the hospital ID numbers and relevant demographic data was recorded.

Samples were collected in gel tube for 25(OH) D level and for iPTH in precooled EDTA tube. Prechilled syringes were used for the collection of samples for iPTH. Specimens for iPTH were transported with ice to the processing room within fifteen minutes. Plasma for iPTH was separated immediately in processing room using refrigerated centrifuge[13].

Serum vitamin D was measured by anti body competitive (Chemiluminiscence) immunoassay on ADVIA Centaur (SIEMENS Germany). Plasma iPTH was measured by a solid phase, two site chemiluminescent enzyme labeled immunometric assay on Immulite 2000 (Siemens healthcare diagnostics, USA). Serum total calcium, phosphorous, magnesium and alkaline phosphatase were measured using Siemens assays on ADVIA 1800 chemistry analyzer, (SIEMENS Germany) by spectrophotometry, and ionized calcium was measured on E-Lite automated analyzer by potentiometry.

Data Analysis

Data was analyzed using SPSS version 21.0. Descriptive statistics for qualitative variables like gender and secondary hyperparathyroidism were calculated in percentages. Mean and SD were also calculated for all quantitative variables like age, serum vitamin D, iPTH, total calcium, ionized calcium, phosphorous, magnesium and alkaline phosphatase. Effect modifiers like age and gender were controlled by stratification, and post stratification independent sample t-test was applied and p-value ≤ 0.05 was considered as significant. Correlation between serum vitamin D and iPTH was determined by using Pearson correlation. ROC curve was used to determine cutoff value

for vitamin D deficiency based on secondary hyperparathyroidism.

RESULTS

Out of 116 subjects, 55 (47%) were males and 61 (53%) were females. Mean ± SD of age was 36.8 ± 12.2 years. Baseline characteristics like serum PTH, vitamin D, calcium, phosphorous, magnesium and alkaline phosphatase (table-I).

Subjects were labeled to have secondary hyperparathyroidism on the basis of serum iPTH levels more than 6pmol/L. 35% of the subjects with vitamin D level below 50nmol/L had secondary hyperparathyroidism, while 65% had normal parathyroid status. Nineteen females out of 55 had secondary hyperparathyroidism and 22 males out of 61 had secondary hyperparathyroidism.

Table-I: Baseline descriptive characteristics.

Parameters	Mean ± SD
Serum Vit D (nmol/L)	26.84 ± 10.2
Serum PTH (pmol/L)	5.2 ± 2.3
Serum Calcium (mmol/L)	2.22 ± 0.27
Serum Phosphorous (mmol/L)	1.04 ± 0.18
Serum Magnesium (mmol/L)	0.89 ± 0.08

Table-II: Comparison of mean vitamin D levels in males and female and different age groups with and without secondary hyparparathyroidism.

Intact PTH (pmol/L)	No. of individuals (n)	Mean ± SD Vitamin D (nmol/L)	*p*-value
Male			
>6	19	19.17 ± 7.92	0.0001
<6	36	31.88 ± 9.23	
Female			
>6	22	21.8 ± 8.36	0.001
<6	39	30.32 ± 9.62	
18-25 Years			
>6	10	19.17 ± 9.5	0.06
<6	14	26.92 ± 9.4	
26-35 Years			
>6	12	21.07 ± 8.18	0.0001
<6	26	32.71 ± 9.33	
36-45 Years			
>6	6	23.43 ± 9.39	0.271
<6	17	27.44 ± 7.09	
46-55 Years			
>6	10	20.60 ± 7.10	0.002
<6	6	36.50 ± 9.26	
56-60 Years			
>6	3	16.33 ± 0.577	0.016
<6	12	33.67 ± 10.49	

Correlation between serum 25(OH) D and iPTH was assessed by pearson correlation and significant inverse relation was found between serum 25(OH) D and iPTH levels (r=-0.596, *p*=0.0001).

Patients were divided into two groups on the basis of serum intact PTH. In one group there were patients with serum iPTH <6pmol/L, while in other group patients were included with iPTH >6 pmol/L.

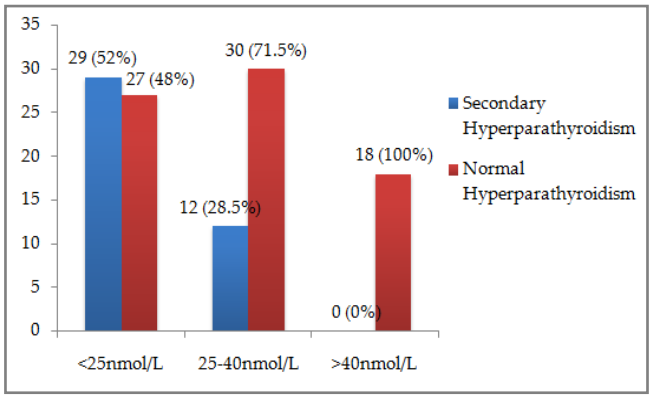

Figure-1: Frequency of secondary hyperparathyroidism in subjects after stratification of vitamin D status.

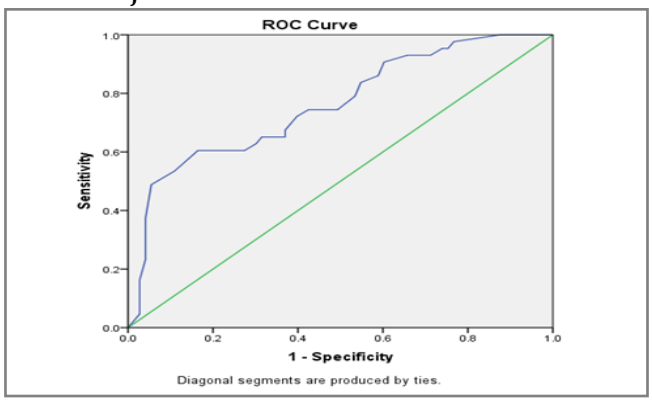

Figure-2: Receiver operating characteristic curve references.

Patients were divided into five groups on the basis of age and mean vitamin D levels. They were compared in each group (with iPTH >6 pmol/L and less than 6pmol/L) and significant difference was noted in all groups (table-II).

Fifty two percent of the subjects with vitamin D level below 25nmol/L had secondary hyperparathyroidism while 28% had secondary hyperparathyroidism with vitamin D level from 25-40nmol/L. None of the subject had secondary hyperparathyroidism with vitamin D level above 40nmol/L.

Thirty five percent of the males with vitamin D level below 50nmol/L had secondary hyperparathyroidism and 36% of the females with vitamin D level below 50nmol/L had secondary hyperparathyroidism.

The optimal cutoff value of serum 25(OH) D, to distinguish subjects with serum iPTH concentration of >6pmol/L as indicator of secondary hyperparathyroidism, was 25.5nmol/L showing the highest sensitivity and specificity (67.4% and 63% respectively). Area under the curve was 0.761 (confidence interval 0.67-0.85, p=0.0001) (table-III). Applying this cutoff point, 25(OH) D level was more than 25.5nmol/L in 60 participants, while it was less than 25.5nmol/L in 56 participants. At this cut off vitamin D deficiency is present in 56 (48.2%) individuals of the study population.

Table-III: Area under the curve.

Cut off Vitamin D (nmol/L)	AUC	Sensitivity	Specificity
24.5	0.761	67.4%	63%

DISCUSSION

Current study provided estimates of vitamin D status and prevalence of secondary hyperparathyroidism in representative sample of our adult population and reports association between 25(OH) D levels and iPTH level. It has revealed following significant findings; first, lower serum 25(OH) D levels were positively associated with secondary hyperparathyroidism in adults. Second, there is more risk of developing secondary Hyperparathyroidism in adults when vitamin D level is below 25.5nmol/L.

Vitamin D deficiency is reported to be common in various countries of the world[14]. Although a number of studies have reported its prevalence, different cut offs were used in these studies and there are no universal criteria for defining vitamin D deficiency. Optimal concentration for vitamin D is not known and majority of the studies have only assessed elderly and house bound individuals[15]. Limited literature is available on its prevalence in normal healthy individuals.

A significant inverse relation between serum vitamin D and intact Parathyroid hormone levels have been reported in a number of studies[11]. Similar correlation has been observed in our study (r=-0.596). We used pearson correlation to assess the correlation between vitamin D and intact parathyroid hormone. In a study conducted on medical students in Iran there was a significant inverse relation between plasma iPTH and vitamin D (r=-0.36)[8].

Both the IOM and the endocrine society's practice guidelines recommend that vitamin D deficiency associated with bone health is described as a 25(OH) D levels below 50nmol/L[12]. Priemel *et al*[16], also reported that osteomalacia due to vitamin D deficiency was not noted in any of the adult ileac crest biopsies with vitamin D levels of 75nmol/L.

In our study we found much less prevalence of vitamin D deficiency (48.2%) as compared to 50-90 and 100% reported by different studies conducted in different parts of the world[2,3]. Reason is that we used lower cut off point for vitamin D deficiency as we have used secondary hyperparathyroidism as an indicator of vitamin D deficiency.

In our study elevated parathyroid hormone levels were detected in 35% of the subjects with vitamin D levels below 50nmol/L, 34.5% of men and 36% of women. Hassan *et al*[17] has reported secondary hyperparathyroidism in 30.4% of their study population, 17.9% of men and 42.6% of women. A study conducted in Iran[8], reported elevated parathyroid hormone levels in 26% and

another study conducted in USA[18] reported elevated parathyroid hormone levels in 29.6% of the study population. A study conducted in Pakistan earlier in 2010 demonstrated that higher parathyroid hormone levels were present in 30.8% of the Pakistani adults[6]. Results of all of these studies are almost comparable to our study except for few differences. We observed that 48.2% of the study population had 25 (OH) D levels below 25nmol/L, whereas 61% of the healthy Syrian adults has vitamin D levels below 25nmol/L[19]. By decreasing the cut off point for vitamin D deficiency the prevalence of hypovitaminosis D reduces significantly. Currently very high cut off points are being used for vitamin D deficiency that has resulted in this epidemic or over-diagnosis of Vitamin D deficiency worldwide.

LIMITATION OF STUDY

There are few limitations of our study, cross-sectional design of our study limited our ability to examine causal relationship between levels of 25(OH) D and secondary hyperparathyroidism. Secondly, our sample is not true representative of all the ethnic groups of Pakistan. That is why difference in prevalence of Vitamin D deficiency among various ethnic groups cannot be assessed.

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RECOMMENDATION

A multi center prospective study should be undertaken to determine the cut off points for Vitamin D deficiency for our population.

CONCLUSION

High frequency of secondary hyperparathyroidism in adults with vitamin D levels under 25 nmol/L necessitates reconsideration of vitamin D cutoff limit for bone health.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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