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Cut off Value for Parathormone Level in Children with Vitamin D Deficiency

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Abstract

Background: When serum level of 25-hydroxy vitamin D [25(OH) D] decreases, intact Parathormone (iPTH) level increases compensatory. This study aimed to determine the cut off value for iPTH level in 2-14-year-old children with vitamin D (VD) deficiency.

Methods: This cross-sectional study was performed on 153 children aged 2-14 years old who referred to the endocrinology clinic of Amirkola Children's Hospital for growth assessment. Census sampling was conducted from January 2016 to June 2017 according to the eligibility criteria including height and weight above the percentile of 3% of growth charts and normal serum of calcium level (>8.5 mg/dl). Laboratory parameters such as serum calcium, 25(OH) D and iPTH levels were assessed. The children were divided into three groups based on serum levels of 25(OH) D as mild, moderate and severe VD deficiency. The Receiver Operating Characteristic (ROC) curve was used to analyze the cut-off point of iPTH and 25(OH) D. P-Value< 0.05 was considered significant.

Results: The mean VD and iPTH levels in children were 11.8 ± 4.59 ng/ml and 28.3 ± 13.3 pg/ml, respectively. At the iPTH serum level of 23.5 pg/ml, with a sensitivity of 66.1%, there was a possibility of moderate to severe VD deficiency. In the severe deficiency group, at the iPTH serum level of 23.5 pg/ml and above, with a sensitivity of 78.9%, there was a possibility of severe VD deficiency. The cut-off point of 25(OH) D and iPTH, at the serum VD level ≤10 ng/ml were determined.

Conclusions: The results of the present study showed that the cut-off value for iPTH in children with VD deficiency is serum level of 25OHD≤10ng/ml.

Key Words: Children, iPTH level, Serum level of 25(OH) D, Parathormone, Vitamin D deficiency.

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1- INTRODUCTION

Vitamin D (VD) is one of the fat-soluble vitamins, supplied by the synthesis in skin epithelial cells under the influence of ultraviolet radiation or absorption through the digestive system (1). VD has several functions in the body. One of the most important functions of VD is to participate in the homeostasis of calcium and phosphorus, consequently bone metabolism (2). Naturally, skin synthesis is the most important source of VD in the body due to the conversion of 7-dehydrocholesterol to vitamin D3 (3-cholecalciferol) by effect of ultraviolet B (UVB) of sunlight. The active metabolite of vitamin D, namely 1, 25 hydroxy cholecalciferol [calcitriol= 1, 25 (OH) D], by its action on the intestinal area, increases the active transport of calcium and also stimulates phosphorus absorption. Because 1α-hydroxylase is a mitochondrial enzyme, it is strongly affected by negative feedback mechanisms; so after calcium and phosphorus return to normal levels, the calcitriol level decreases (1).

The World Health Organization (WHO) has defined VD deficiency as a serum level of 25(OH) D less than 20 ng/ml. According to this definition, about 1 billion people in the world are deficient in VD (3). The prevalence of VD deficiency in children is high in all parts of the world, including Iran (4). VD deficiency and subnormal VD level have been reported in Iranian children between 3-42% (4) and 69%, respectively (2). VD deficiency has several causes, including limited vitamin D-rich food sources, exclusive breastfeeding, reduced exposure to sunlight, and the use of certain medications, such as anticonvulsants (especially phenobarbital and phenytoin) - rifampin and antacids contain aluminum, some diseases such as intestinal fat malabsorption, endocrine diseases, liver failure and kidney diseases such as nephrotic syndrome or fanconi syndrome (2, 5).

When serum level of 25(OH) D decreases, iPTH level increases compensatory to keep calcium level in the normal range; which can lead to secondary hyperparathyroidism. The level of 25(OH)D at which iPTH begins to rise is not exactly known, probably due to the differences in geographical and cultural areas related to how much people are exposed to sunlight. Sai et al., in a systematic review and meta-analysis of 70 studies, assessed the association between serum 25(OH) D and iPTH levels. The results of their study showed that there is a significant variability from less than 10 ng/ml to 40-50 ng/ml of VD level in which the iPTH reaches the plateau level or is maximally suppressed (6).

PTH is secreted by the chief cells from parathyroid glands located at the upper and lower poles of the thyroid gland. The main target of PTH is the bones and kidneys. PTH reacts to calcium drop, so a positive feedback mechanism stimulates the production of 1, 25(OH) D (7).

The main signal that stimulates PTH secretion is a low level of circulating ionized calcium. Although calcium sensor receptor (CaSR) binds to extracellular ionized calcium with a low affinity, CaSR is very sensitive to changes in extracellular calcium. 0.2 meq/l decrease in calcium level increases the amount of PTH from baseline (maximum 5%) to maximum level, so regulates PTH level moment by moment in response to subtle fluctuations in calcium levels. On the other hand, PTH directly induces bone resorption and stimulates the movement of calcium from bone to blood; thus, secondary hyperparathyroidism can lead to osteomalacia and irreversible bone loss. PTH affects bone and kidney through binding to specific receptors on the target cell membrane, by the activation of the

transduction pathway (including G-protein related to adenylate cyclase system) (5).

Due to the high prevalence of VD deficiency in Iran and the existence of heterogeneity in previous studies regarding the point of VD level that leads to an increase in iPTH level, we decided to determine the cut off value of parathormone level in 2-14-year-old children with VD deficiency.

2- MATERIALS AND METHODS

2-1. Design and participant

This cross-sectional study was performed on 2-14-year-old children who referred to the endocrine clinic of Amirkola Children's Hospital for growth assessment. They were divided into three groups based on serum levels of 25(OH) D (8):

a) 10-20 ng/ml: mild VD deficiency

b) 5-10 ng/ml: moderate VD deficiency

c) < 5 ng/ml: severe VD deficiency

2-2. Sampling and sample size

Census sampling was conducted from January 2016 to June 2017 according to the eligibility criteria. Eligibility criteria included children with normal medical history and physical examination, height and weight above the percentile of 3% of growth charts, normal level of serum calcium (>8.5 mg /dl), no history of thyroid and parathyroid disorders or chronic kidney- liver and gastrointestinal diseases, and no history of consumption of VD in the last three months. The exclusion criteria included the voluntary withdrawal of parents or their children from this study.

The present study examined the cut off value of iPTH level in children to determine the correlation between iPTH and VD. According to the results of similar studies (3), the inverse correlation between serum levels of 25(OH) D and iPTH was considered 0.256. With 95% confidence and 80% test power based on the comparison formula, the sample size was estimated to be 120. Yet, with an attrition rate of about 20%, 153 samples were selected.

2-3. Data collection

In this study, at first, the height and weight, clinical examination and laboratory parameters of children were assessed.

a) Height and weight assessment:

Children's height and weight were measured with no shoes and with minimum possible clothing at the upright position using a side wall-mounted plastic band and analogue weight scale (MW84, EmsiG Co., Gm-bH), respectively. All children with height and weight above the 3% of percentile of growth charts, entered the next stage of the study. This measurement was done by a pediatric resident (first author).

b) Evaluation of clinical specialized examinations:

A pediatric resident (first author) under the supervision of a pediatric endocrinologist examined all children in terms of heart, lung, abdomen and genitalia. Healthy children entered the next stage of the study.

c) Evaluation of laboratory parameters:

In this study, serum calcium and 25(OH) D levels were examined. For measurement of these parameters, 5 cc of blood was taken from all children. Children with a normal serum calcium level (> 8.5 mg/dl) and a serum level of 25(OH) D less than 20 ng/ml (8) were included in the study. The serum levels of 25(OH) D and calcium were measured using the kit IDS-iSYS 25 (OH) (made in Boldon of England) and photometric method, respectively. For measurement of iPTH level, the samples were immediately centrifuged at 4 °C and then the serum was separated and frozen at minus 20°C and iPTH level was measured

using the chemiluminescent immunoassay (CLIA) and the Liaison N-Tact iPTH Gen II kit (made in Boldon of England).

d) Data analysis:

Data were statistically analyzed using SPSS V. 25.0 software. Pearson correlation test was used to determine the relationship between VD and iPTH levels. ANOVA test was used to analyze the differences in VD and iPTH levels in different age groups. The ROC curve was also used to obtain the cut-off point of iPTH and 25 (OH) D.

3- RESULTS

Out of 480 children referred to the endocrine clinic of Amirkola Children's Hospital, eventually 153 children were included in the study (**Fig. 1**).

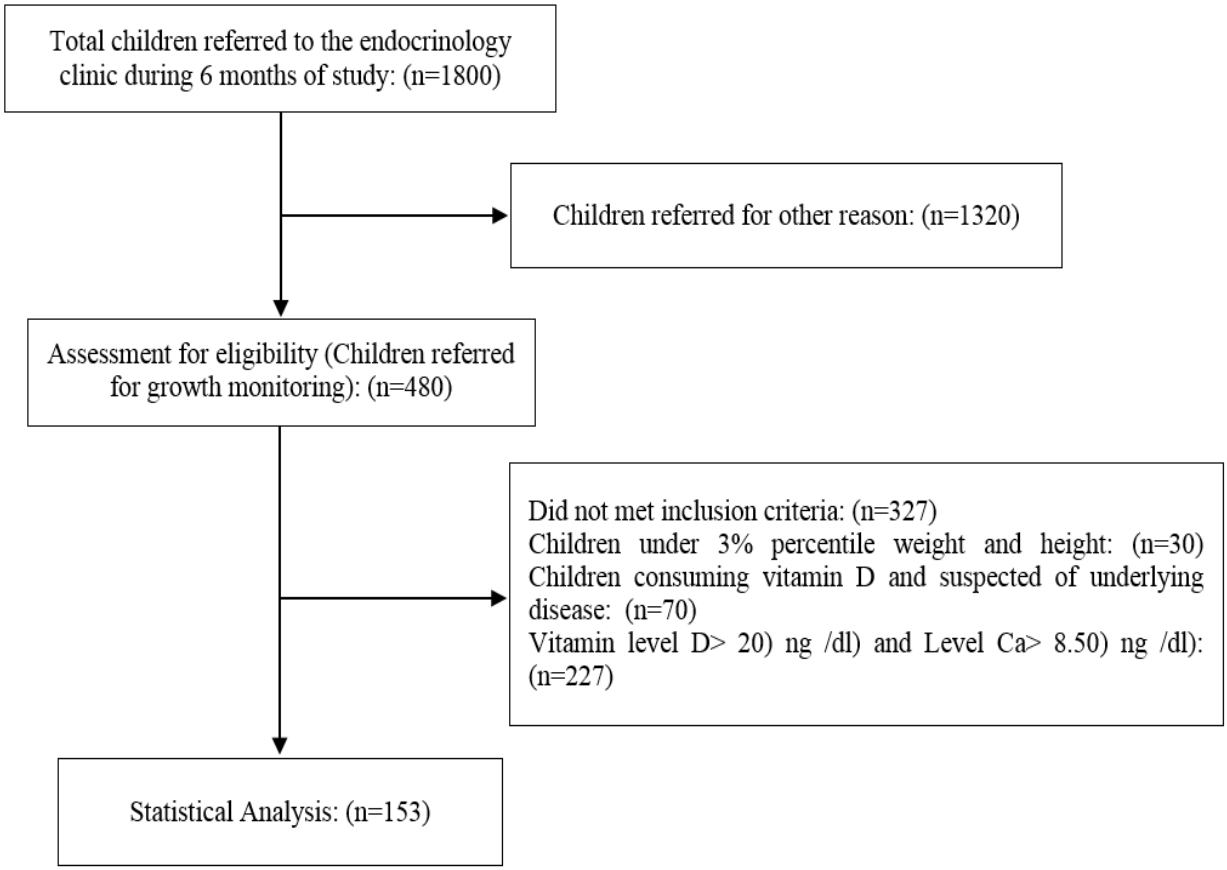

Fig. 1: Flow diagram of the study participants

3-1. Demographic Characteristics

Out of 153 participants, 69 children (45%) were boys and 84 (55%) were girls. The mean age of children was 98.85±38.91 months. Based on the severity of VD deficiency, 91 children were in the mild group (10–20 ng/ml), 43 children in the moderate group (5-10 ng/ml) and 19 children were in the severe deficiency group (less than 5 ng/ml).

3-2. Laboratory parameters

The mean level of VD in children was 11.8 ± 4.59 ng/ml. The mean level of VD in the mild deficiency group was 15 ng /ml, 8.3 ng/ml in the moderate deficiency group and 4.2 ng/ml in the severe deficiency group. The mean iPTH in all children in the study was 28.3 ± 13.3 pg/ml. The mean

iPTH in the mild deficiency group was 27.7 pg/ml, in the moderate deficiency group was 28.5 pg/ml, and in the severe deficiency group was 34.5 pg/ml (**Table 1**).

Table-1: Mean and Standard deviation of Vitamin D, PTH and age by groups

Variable		N	Mean ± S D	Lower Bound	Upper Bound
VD(ng/ml)	Mild	91	15.00 ± 2.64	.27	14.44
	Moderate	43	8.39 ± 1.32	.20	7.98
	Severe	19	4.26 ±.45	.10	4.04
PTH (ng/ml)	Mild	91	27.77 ±1 3.21	1.38	25.02
	Moderate	43	28.52 ± 12.51	1.90	24.67
	Severe	19	34.59 ± 17.72	4.065	26.05
Age (Months)	Mild	91	99.23 ± 41.74	4.376	90.53
	Moderate	43	94.93 ± 35.91	5.47	83.87
	Severe	19	105.89 ± 31.19	7.15	90.85

Abbreviations: Mean± SD, Mean ± Standard deviation; Parathormone: PTH; Vitamin D: VD

Pearson analysis showed that there was no significant correlation between iPTH and VD (close to significant = R = -0.157 and P = 0.053). There was no statistically significant relationship between VD and age. But a significant positive correlation was found between iPTH and children's age (R= -0.236 and P=0.003), which means iPTH increases by age (**Table 2**).

Table-2: Correlation between vitamins D, PTH, and age groups

Variable	VD	PTH	Age (Months)
VD (ng/ml)	1	R = -0.157 P = 0.053	R = 0.059 P = 0.469
PTH ng/ml	R = -0.157 P = 0.053	1	R = 0.236 P = 0.003

3-3. Cut-off point of iPTH and 25(OH)D

To obtain the cut-off point of iPTH and 25(OH) D using the ROC curve, the two groups of severe and moderate deficiency were first combined (which included 62 cases with VD ≤10ng/ml), at the iPTH serum level of 23.5 pg/ml, with a sensitivity of 66.1%, there was a possibility of moderate to severe VD deficiency. This result indicated that a significant increase occurs in iPTH level when the level of VD is lower than 10ng/ml. Additionally, the severe deficiency group was analyzed separately. In this group, at the iPTH serum level of 23.5 pg/ml and above, with a sensitivity of 78.9%, there was a possibility of severe VD deficiency. Therefore, according to the sum of the above results, the cut-off point of 25 (OH) D and iPTH at the VD serum level ≤10ng/ml was determined (**Fig 2** & **3**)

4- DISCUSSION

In the present study, the relationship between serum level of 25(OH) D and iPTH in 153 healthy children with 25(OH)

D deficiency was investigated and the cut-off point of 25(OH) D and iPTH was obtained at the serum level of VD ≤10ng/ml.

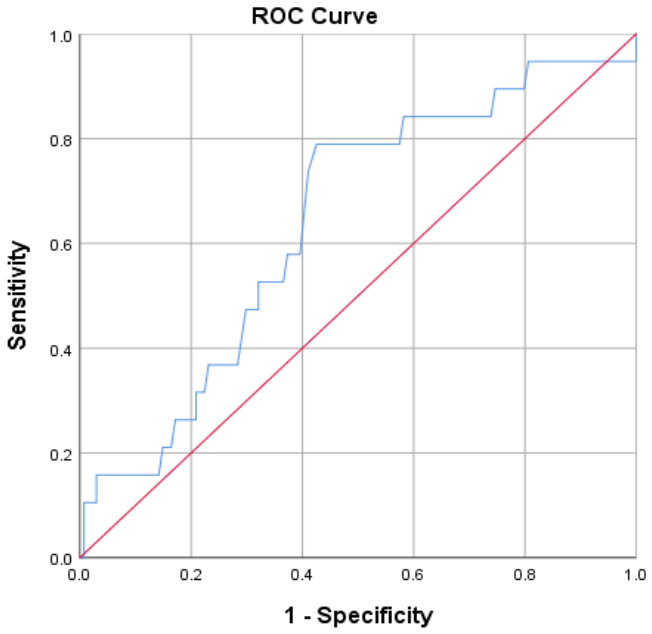

Fig. 2: ROC curve to determine the level of PTH cut off in the group of severe and moderate vitamin D deficiency

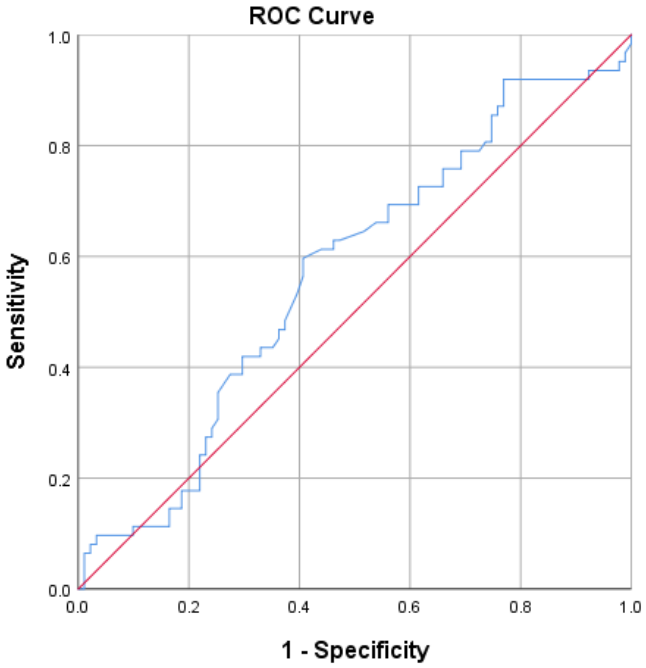

Fig. 3: ROC curve to determine the level of PTH cut off in the group of severe vitamin D deficiency

Memon et al. conducted a study on 500 children under 15 years old in 2012-2014 and based on serum level of 25 (OH)D, divided children into three groups of mild (10-20 ng/ml), moderate (5-10 ng/ml) and severe deficiency (<5 ng/ml); and iPTH> 65 pg/ml was considered as secondary hyperparathyroidism. They concluded that 30-40% of children with moderate to severe VD deficiency showed a secondary increase in iPTH. (9). According to the results of our study, the mean iPTH in the severe deficiency group was significantly higher than the mild and moderate deficiency group and the correlation between iPTH and 25(OH)D was significant and inverse.

In a study on 240 hospitalized children in 2012, Atapato et al. found that when the serum level of 25(OH) D falls below 13.6 ng/ml, the iPTH level rises above 50 pg/ml and an increase in its level occurs. The main difference between the study by Atapato et al. and the present study was the use of hospitalized children vs. healthy children (10).

Kong et al., in a retrospective study on 193 children aged 2.5 months to 18 years between 2010 and 2014, found that the mean iPTH level in children with a serum level of 25(OH)D <18 ng/ml is higher than that in children with a serum level of 25 (OH)D >18ng/ml (inflection point). The rate of secondary hyperparathyroidism was also higher in children with serum levels of 25(OH) D < 18 ng/ml. Therefore, they suggested that the serum level of 25(OH) D < 18 ng/ml could be defined as the inflection point for maximum iPTH suppression (11). The reason for the difference in cut-off point in the two studies can be attributed to the study method, the duration of the two studies, and the age difference between the participants.

In general, the cut-off point between serum levels of iPTH and VD has varied significantly in various studies around the world. For example, Karas et al (2016) conducted a cohort study in Greece and suggested a cut-off level of 20 ng/ml of VD as the ideal limit for preventing secondary hyperparathyroidism (12).

Sahin et al. in a study on 3525 children aged 2 months to 18 years in Turkey, considering the age and seasonal variations, determined that the starting point for increasing iPTH is at the serum level of 30 ng/ml for 25 (OH)D (inflection point). They concluded that the serum level of 25(OH) D decreases with age (13), which is contrary to the results of the present study. Because in the present study, iPTH was positively correlated with children's age, but there was no relationship between 25(OH) D and age. The possible reasons for this difference could be the higher sample size and differences in age ranges.

Hill.TR et al., in an analysis on 1015 Irish adolescents aged 12-15 years, concluded that the 25(OH) D level above 24 ng/ml in adolescent girls prevents an increase in serum iPTH level, and 25(OH) D level above 24 ng/ml improves bone health in both genders (14).

Asghari et al., in 2019, in a study on 198 boys and 180 girls with overweight or obesity status aged 6-13 years concluded that in girls, the inflection point of iPTH occurs in VD level less than 10 ng/ml, but in boys, a clear inflection point was not obtained (14). One of the important differences between this study and the current one and most other studies is the effect of gender on the results. Because in the present study, gender did not make a difference in iPTH and 25(OH) D levels in any of the groups.

To determine the threshold for VD deficiency to maintain bone health, Al-Quaiz et al., in Saudi Arabia, examined the association between iPTH and 25(OH) D in a cross-sectional study on 846 men and 1285 women (16). As in the current study,

they used the ROC curve to determine a threshold of VD at which iPTH begins to increase. They concluded that the iPTH cut-off point occurs when the serum level of 25(OH) D reaches 12 ng/ml in men and 9.6 ng/ml in women. In fact, by examining the level below the chart (which was 62% in men and 65% in women), with a sensitivity of 72% in men and 58% in women, an increase occurred in the above two thresholds. The main differences between this study and the current study were the age difference of the statistical population and the geographical area (angle of sunlight and amount of coverage).

4-1. Limitations

In the present study, the number of samples was limited in moderate (5-10 ng/ml) and severe deficiency (<5 ng/ml) groups. Using a larger sample size in these two groups could be useful to determine exactly at which level of VD, the iPTH increases.

5- CONCLUSION

The results of the present study showed that the cut-off point of iPTH in children with VD deficiency is at a serum level of 25 (OH) D ≤10ng/ml. If larger studies are performed with larger sample sizes (as a multicenter study), and if there is a consensus on the cut-off point between VD and iPTH, it may be possible to create a new definition for decreased level of VD; Then, the definition of VD insufficiency and VD deficiency would be adjusted accordingly. With the results of the present study, more appropriate and accurate treatment in terms of VD intake can be performed for children with VD deficiency and reduce the side effects of VD overdose.

6- ETHICAL CONSIDERATIONS

Written consent was obtained from all participants. This study was approved by the code ethics committee of Babol University of Medical Sciences, IR mubabol.HRI.REC.1398.265.

7- CONFLICTS OF INTEREST

None.

8- ACKNOWLEDGMENT

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