EC ENDOCRINOLOGY AND METABOLIC RESEARCH
Research Article

Rethinking Vitamin D Deficiency Cut-Off Point: A Study Among Healthy Syrian Adults

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Abstract

Background: There is an ongoing debate on the cut-offs used to indicate vitamin D deficiency due to the discrepancies between the ones established by the Endocrine Society (ES) and the Institute of Medicine (IOM), as well as the populations used to determine these cut-offs, who were mainly Caucasians. This has led to confusion and lack of consensus among clinicians and researchers, and potentially the overestimation of Vitamin D deficiency. This study aimed to explore a vitamin D deficiency cut-off within a sample of Syrian adults, using parathyroid hormone (PTH) to determine the PTH-25OHD inflection point.

Methods: A convenient sample of 372 healthy subjects aged 18 - 62 years participated in this study, 51% of whom were females. A piecewise regression was used to determine the PTH-25OHD inflection point. The association between the newly identified 25OHD cut-off value and total alkaline phosphatase (TAP) was assessed using multivariate regression.

Results: The identified PTH-25OHD inflection point was 13 ng/ml; accordingly, 72% of the participants were vitamin D deficient in contrast to 90% using the established cut-off of 20 ng/ml. Participants with high TAP levels were only 8%, and no association was found between TAP and 25-OHD levels using both the established and identified cut-offs for 25-OHD.

Conclusion: The cut-off indicating vitamin D deficiency in Syrian adults may be different than the one established by the ES. Thus, further studies should be conducted on a larger representative sample of Syrians and using BMD as a biomarker for bone health instead of TAP for more accurate results.

Keywords: *Vitamin D Deficiency; 25 Hydroxy Vitamin D; Parathyroid Hormone; Cut-Offs; Inflection Point; Total Alkaline Phosphatase*

Introduction

Vitamin D deficiency is a major global concern because of its high prevalence worldwide in people of all ages, regardless of their socio-economic status and ethnicities [1-3] and its link with various ill-health outcomes, including injuries (i.e. risk for fracture, falls) [3,4], non-communicable diseases (i.e. CVDs, cancer, diabetes) [5-7], neuropsychiatric conditions (i.e. depressed mood, cognitive decline) [8-10] and autoimmune conditions [11,12].

Vitamin D deficiency has been commonly determined by measuring 25-hydroxyvitamin D (25-OHD) [13-16], using cut-off scores set based on the level at which parathyroid hormone (PTH) starts to rise [3,17-19]. Still, different cut-off values have been set by the Endocrine Society (ES) and the Institute of Medicine (IOM), whereby desirable 25-OHD levels were set at 30 ng/ml versus 20 ng/ml, respectively [13,20], resulting in confusion among clinicians as well as lack of consensus [13,20,21]. Though widely adopted, these cut-off points were set based on studies conducted in North America and Canada (NHANES and CaMOS) and on Caucasians [15,20,22]. Some researchers have thus argued that clinicians may thus be over-screening and over-diagnosing vitamin D deficiency, and are thus treating individuals unnecessarily [23-25]. Over-prescribing vitamin D can lead to vitamin D toxicity, associated with anorexia, polyuria, heart and kidney damage [26,27].

Several factors have been shown to affect vitamin D production and ultimately 25-OHD levels in healthy subjects, which vary widely both within and across populations due to environmental, behavioral and cultural differences. These factors include time of day and season (maximum UVB radiation at mid-day in Summer) [28], latitude (higher UVB radiation in lower latitudes) [29,30], skin pigmentation (those with higher melanin concentrations require longer UVB exposure) [31], pollution [32], clothing [33], smoking levels and patterns [27], obesity [34] and diet (fortified food and supplements) [35]. The Middle East and North African region is regarded as one of the sunniest regions globally, as well as the region with the highest proportions of vitamin D deficient populations [36,37]. Several explanations have been provided [38-40] including the possibility of the need for a more context-specific cut-off [41]. Alternative cut-offs have been explored in several populations (i.e. Blacks, Asians and have found PTH-25 OHD inflection points different (lower) than the established ones [23,42-45].

This study aimed to add to the published literature by exploring whether a different cut-off score for Vitamin D deficiency is warranted within the Syrian population, the first of its kind study from the MENA region.

Materials and Methods

Participants

Secondary de-identified data were used as part of a cross-sectional survey conducted in Damascus, Syria between April 2011 and March 2013. Participants were recruited from Al Assad University Hospital using a flyer advertising for the study at the Department of Internal Medicine. Initially, 412 volunteered to participate in the study. A series of laboratory measurements and tests were used to exclude participants who were suffering from any acute or chronic illness, impaired renal or liver function, and intestinal malabsorption; these tests included: serum calcium, phosphorus, albumin, creatinine, total alkaline phosphatase (TAP), alanine transaminase (ALT), aspartate transaminase (AST) and magnesium. Gamma-glutamyl transferase (GGT) was performed for those whose TAP levels were high to exclude any liver disease. Participants who had been taking vitamin D supplements in the preceding 6 months were also excluded, in addition to those taking medication that affect bone metabolism, having a family history of hypocalcemia or vitamin D disorders, pregnant or lactating women within the last 3 years and women who were taking oral contraceptives. The final sample comprised of 372 "healthy" participants, including184 males and 188 females aged 18 - 62 years.

A written informed consent was obtained from participants [41]. The original study was approved by the Damascus University Review Board (DURB), and the present analyses of secondary data by the American University of Beirut Institutional Review Board (IRB).

Data collection

Medical doctors in their final year of specializing in Endocrinology conducted the face-to-face interviews and drew the morning fasting, blood samples, divided into summertime (May to October) and wintertime (November to April).

Measures

iPTH was measured using automated electrochemiluminescence immunoassay (Elecsys 2010 analyzers, Roche Diagnostics GmbH, Mannheim, Germany). PTH values were recoded to reflect hyperparathyroidism (below and above 65 pg/ml).

Total 25OHD levels were measured using automated electrochemiluminescence immunoassay (Elecsys 2010 analyzers, Roche Diagnostics GmbH, Mannheim, Germany). Since there were only 3 participants with normal levels (above 30 ng/ml), all being males and below the age of 35 years, 25-OHD levels were grouped into those below 20 ng/ml and were categorized as deficient, and those with 25-OHD levels above 20 ng/ml and were categorized as non-deficient.

Total alkaline phosphatase (TAP) was measured by standard colorimetric methods using the Roche Hitachi 912 autoanalyzer (Roche Diagnostics, Mannheim, Germany). TAP was recoded into below 270 (normal) and above 270 (high) for males; and below 240 (normal) and above 240 (high) for females.

Several covariates were also considered, including age (< 35 years, ≥ 35 years), BMI (obese 30 kg/m^2 and above, not obese below 30 kg/m^2), time spent outdoors (≤ 3h/week, > 3h/week), season (winter, summer), phosphorus (below and above 2.5 mg/dl) and corrected calcium (below and above 8.6 mg/dl).

Statistical analysis

Descriptive statistics was reported as frequencies and valid percentages for all categorical variables. As for continuous variables, results were reported as mean± SD for normally distributed variables and as median and inter-quartile intervals for skewed ones.

To identify the 25OHD-PTH inflection point, first the association between 25-OHD and PTH was visualized using a LOWESS scatterplot (Figure 1) and a boxplot of PTH against 1 ng/ml 25-OHD strata (Figure 2). Since PTH is positively skewed, ln PTH was plotted against 25-OHD for the scatterplot. Following the visual depiction of the inflection point, a piecewise regression was performed between ln PTH and 25-OHD to estimate the inflection point reflecting a significant change in the slope between PTH and 25-OHD.

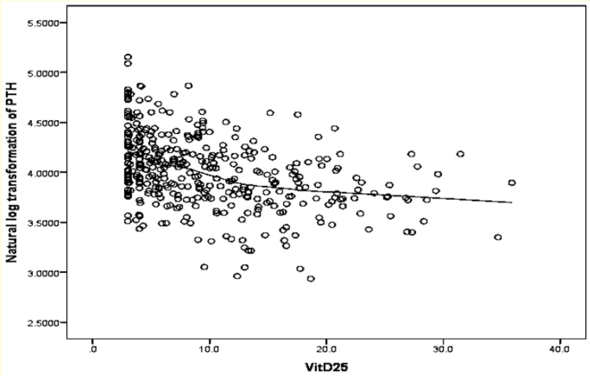

Figure 1: *LOWESS Scatterplot Showing the Relationship Between log iPTH and 25-OHD in 372 Participants Recruited from Al-Assad Hospital Between 2011 and 2013.*

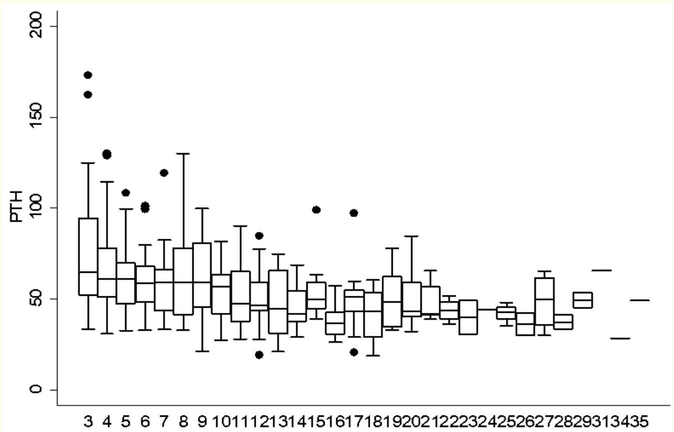

Figure 2: *Boxplot Showing the Relation of median iPTH to 1 ng/ml 25-OHD Strata.*

Following the identification of the inflection point, 25OHD levels were regrouped. Bivariate analyses were conducted between the ES established and identified 25-OHD levels and various co-variates and TAP. Covariates included were gender, age, BMI, season, time spent outdoors, corrected calcium, phosphorus, iPTH and the established/identified 25-OHD cut-offs. Then a multivariate regression analysis was performed to assess the magnitude of the association between Total Alkaline Phosphatase (TAP) and 25-OHD as per the identified cut-off, controlling for selected covariates statistically significantly related to both 25-OHD and TAP at the bivariate level. The same procedure was done with the cut-off established by the Endocrine Society (20 ng/ml) to compare the findings. Data was analyzed using Stata Software (version 13).

Results

Sample characteristics

Of the total sample, 51% (n = 188) were females, and 44% (n = 164) were aged 35 years and older. Approximately one in four (23.4%, n = 87) were obese. About half (n = 182) were recruited during the winter season (i.e. their blood was drawn in winter months), and 43% (n = 161) spent less than 3 hours per week outdoors (Table 1).

		Gender			Age		
Variables	All (372) N (%)	Males (184, 49.0)	Females (188, 51.0)	p-value	Below 35 (208, 55.9)	35 and above (164, 44.1)	p-value
Age group (35 and above)	164 (44.0)	64 (34.8)	100 (53.2)	< 0.001			
Gender (female)					88 (42.3)	100 (60.9)	< 0.001
Education (secondary or less)	99 (28.0)	37 (20.6)	62 (35.6)	0.002	30 (14.9)	69 (45.4)	< 0.001
Time spent outdoor (≤ 3h/week)	161 (43.3)	46 (25.0)	115 (61.2)	< 0.001	77 (37.1)	84 (51.5)	0.006
BMI (obese)	87 (23.4)	47 (25.5)	40 (21.3)	0.331	27 (13.0)	60 (36.6)	< 0.001
Season (winter)	182 (49.0)	87 (47.3)	95 (51.1)	0.466	94 (45.4)	88 (54.0)	0.101
Corrected Calcium (< 8.6 mg/dL))	23 (6.2)	12 (6.5)	11 (5.9)	0.788	13 (6.3)	10 (6.1)	0.952
Phosphorus (< 2.5 mg/dL)	7 (1.9)	4 (2.2)	3 (1.6)	0.682	2 (1.0)	5 (3.0)	0.139
iPTH (> 65 pg/mL)	113 (30.4)	33 (18.0)	80 (42.6)	< 0.001	44 (21.2)	69 (42.1)	< 0.001
TAP (high)	31 (8.4)	12 (6.5)	19 (10.2)	0.2	11 (5.3)	20 (12.3)	0.016
25-OHD (< 20 ng/mL)	335 (90.0)	153 (83.0)	182 (97.0)	< 0.001	183 (88.0)	152(92.7)	0.161
20 < 30 ng/ml	34 (9.0)	28 (15.0)	6 (3.0)		22 (10.6)	12 (7.3)	
≥ 30 ng/ml	3 (1%)	3 (2.0)	0		3 (1.4)	0	

Table 1: *Study population characteristics by gender and age.*

Clinical profile of the sample

As per the ES guidelines, 90% (n = 335) of the sample were vitamin D deficient (< 20 ng/ml), 9% (n = 34) had insufficient levels (20 - 30 ng/ml) and only 1% (n = 3) were considered to have normal levels (> 30 ng/ml).

Around 30% of the participants had hyperparathyroidism (iPTH levels above 65 pg/ml), which was more prevalent in females (42.6%) than in males (18%) (p-value < 0.001) and those 35 years old and above (42.1% versus 21.2% in < 35 years, p-value < 0.001).

As for TAP, the majority had normal levels (92%). No gender differences were observed for high TAP levels (10.2% females versus 6.5% of the males, p-value = 0.2), but differences by age groups were noted, whereby 5.3% of those below 35 had high levels of TAP compared to 12.3% of those above 35 (p-value = 0.016).

Hypocalcemia and hypophosphatemia were only seen in 6.2% and 1.9% of the sample, respectively, with no significant differences by gender or age (Table 1).

Laboratory results were also looked at as continuous variables as seen in table 2. All participants results' mean and median values were within the normal range except for 25-OHD (median = 8.02 ng/ml; IQR = 4.07, 13.49), which is much lower than the normal ranges set by both the ES and IOM (≥ 30 and ≥ 20 ng/ml respectively). As expected, median 25-OHD for samples drawn in winter time (median = 6.6 ng/ml; IQR = 4, 10.6) were much lower than those drawn in summer time (median = 10 ng/ml; IQR = 5.2, 16.32) since winter is an established risk factor for vitamin D deficiency.

	25-OHD (ng/ml)	25-OHD (ng/ml) in winter	25-OHD (ng/ml) in summer	iPTH (pg/ml)	TAP (U/L)	Corrected Calcium (mg/dl)	Phosphate (mg/dl)
Normal Reference Range used in Syria	≥ 30 (ES), ≥ 20 (IOM)	≥ 30 (ES), ≥ 20 (IOM)	≥ 30 (ES), ≥ 20 (IOM)	10 - 65	80 - 270 (M), < 240 (F)	8.6 - 10.1	2.5 - 4.5
Mean	9.89	8.2	11.48	58.94	180.30	9.14	3.61
SD	6.77	5.56	6.76	22.95	51.57	0.34	0.51
Median	8.02	6.6	10	55.02	170.00	9.15	3.60
IQ interval	4.07 - 13.49	4 - 10.6	5.2 - 16.32	43.06 - 68.84	144 - 209	8.93 - 9.36	3.30 - 3.97

Table 2: *Distribution of laboratory results in the total study sample.*

25-OHD and PTH: Determining the Inflection Point in the Syrian Population

As seen in figure 1, the association between ln iPTH and 25-OHDis not linear, and the slope changes at 25-OHD levels of around 12 ng/ml. This is also illustrated in figure 2, where there is a significant change in the median iPTH starting at 11ng/ml 25-OHD, whereby iPTH medians are high below 11 ng/ml 25-OHD levels and start decreasing at a steady state above it. The piecewise regression confirmed the above and estimated the slope to change at 13.26 ng/ml (Figure 3).

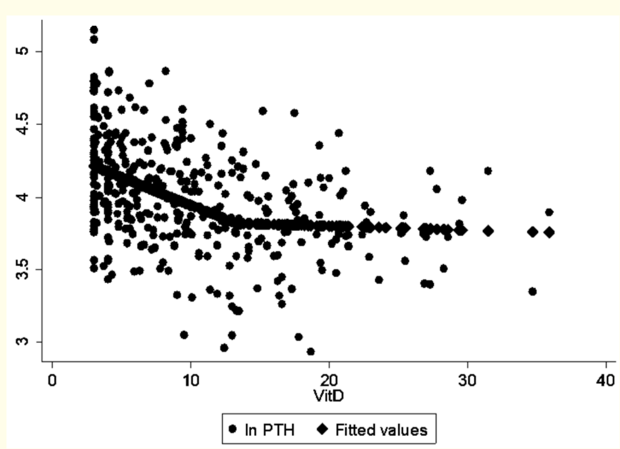

Figure 3: *Piecewise regression between log iPTH and 25-OHD.*

Association between sample characteristics and vitamin D deficiency using the established and identified cut-offs

According to the identified cut-off of 13 ng/ml, 72% of the participants were regarded as deficient compared to 90% as per the ES established cut-off of 20 ng/ml. In both cut-offs, factors that were significantly associated with 25-OHD were gender (females more likely to be deficient), time spent outdoors (less than 3h/week outdoors), winter season and iPTH levels (having hyperthyroidism) (Table 3).

	25-OHD concentration using a cut-off of 20ng/ml				25-OHD concentration using a cut-off of 13 ng/ml			
Variables	N (%) below 20 ng/ml	N (%) above 20 ng/ml	p-value	U-OR	N (%) below 13 ng/ml	N (%) above 13 ng/ml	p-value	U-OR
Gender (female)	182 (54.3)	6 (16.2)	< 0.0001	6.25 (2.50, 14.28)	158 (58.7)	30 (29.1)	< 0.0001	3.46 (2.12, 5.65)
Age 35 and above	152 (45.4)	12 (32.4)	0.132	1.73 (0.83, 3.57)	123 (45.7)	41 (39.8)	0.259	1.30 (0.82, 2.06)
Time spent outdoor (≤ 3h/week)	154 (46.2)	7 (18.9)	0.001	3.68 (1.57, 8.63)	126 (47.2)	35 (34.0)	0.022	1.74 (1.08, 2.78)
Secondary or less education	89 (28.0)	10 (27.8)	0.979	1.01 (0.47, 2.18)	75 (29.3)	24 (24.5)	0.367	1.28 (0.75, 2.18)
Winter season	172 (51.7)	10 (27.0)	0.004	2.88 (1.35, 6.15)	149 (55.8)	33 (32)	< 0.0001	2.68 (1.66, 4.32)
BMI 30 and above	80 (23.9)	7 (18.9)	0.499	1.34 (0.57, 3.17)	69 (25.7)	18 (17.5)	0.084	1.66 (0.93, 2.93)
Corrected Calcium (< 8.6 mg/dL)	23 (6.9)	0	0.1		19 (7.1)	4 (3.9)	0.243	1.90 (0.66, 5.48)
Phosphorus (< 2.5 mg/dL)	6 (1.8)	1 (2.7)	0.699	0.66 (0.07, 5.07)	4 (1.5)	3 (2.9)	0.375	0.51 (0.12, 2.07)
iPTH (> 65 pg/ml)	109 (32.5)	4 (10.8)	0.006	3.98 (1.43, 11.03)	101 (37.5)	12 (11.7)	< 0.0001	4.64 (2.44, 8.80)
TAP (high)	27 (8.0)	4 (10.8)	0.569	0.72 (0.25, 2.10)	21 (7.4)	10 (9.6)	0.584	0.80 (0.37, 1.75)

Table 3: *Association between 25-OHD and sample characteristics.*

When looking at the association between the sample characteristics and the established cut-off (25-OHD levels of 20 ng/ml), it was found that females were 6.25 times more likely to have vitamin D deficiency compared to males (p value < 0.0001; CI = 2.5, 14.28). Also, those who spent less than 3h/week outdoors were 3.68 more likely to be vitamin D deficient than those spending more than 3 h/week (p-value = 0.001; CI = 1.57, 8.63). In addition, participants who had their blood samples drawn in winter were 2.88 more likely to have vitamin D deficiency than those who had their samples drawn in summer season (p-value = 0.004; CI = 1.35, 6.15). As for iPTH, it was found that those with secondary hyperparathyroidism were 3.98 more likely to have 25-OHD levels below 20 ng/ml compared to those with normal iPTH levels (p-value = 0.006; CI = 1.43, 11.03).

As for the association between the sample characteristics and the identified 25-OHD cut-off, females were 3.46 times more likely to have vitamin D deficiency than males (p-value < 0.0001; CI = 2.12, 5.65) as opposed to 6.25 in the established cut-off. Spending less than 3 h/week outdoors remained significant (p-value = 0.022) but the association was relatively weak as the OR was 1.74 (CI = 1.08, 2.78). As for having the blood sample drawn in winter season, it also remained significantly positively associated with vitamin D deficiency (p-value < 0.0001; U-OR = 2.68; CI = 1.66, 4.32). Finally, the association between iPTH and vitamin D deficiency increased both in statistical significance and strength of association, whereby those with levels above 65 ng/ml were 4.64 times more likely to have vitamin D deficiency as opposed to those with normal iPTH levels (p-value < 0.0001; CI = 2.44, 8.8) (Table 3).

Exploring the association between TAP and 25-OHD levels using the established/suggested 25-OHD cut-off values

All correlates that had a p-value of < 0.2 in the bivariate analyses looking at associations with 25-OHD or TAP were included in the multivariate analyses. The association between TAP and 25-OHD was not statistically significant using both cut-offs of 20 ng/ml and 13 ng/ml (p-value = 0.247 and p-value = 0.163, respectively) (Table 4).

	25-OHD below 13 ng/ml	25-OHD below 20 ng/ml
Variables	**A-OR**	**A-OR**
25-OHD	0.52 (0.21, 1.30)	0.48 (0.14, 1.65)
Gender (female)	1.64 (0.66, 4.05)	1.56 (0.63, 3.87)
Age 35 and above	2.20 (0.95, 5.16)	2.27 (0.46, 3.09)
Time spent outdoor (≤ 3h/week)	0.94 (0.40, 2.20)	0.97 (0.41, 2.28)
Winter season	1.67 (0.72, 3.82)	1.52 (0.67, 3.46)
BMI 30 and above	1.61 (0.67, 3.81)	1.47 (0.62, 3.49)
Corrected Calcium (< 8.6 mg/dL)	1.48 (0.31, 7.17)	1.46 (0.30, 7.10)
Phosphorus (< 2.5 mg/dL)	2.54 (0.41, 15.71)	2.66 (0.45, 17.84)
iPTH (> 65 pg/ml)	1.05 (0.44, 2.52)	0.95 (0.41, 2.24)

Table 4: *Model predicting the association between 25-OHD and TAP in the total study sample of 372 participants, controlling for selected covariates.*

A-OR: Adjusted Odds Ratio.

Discussion and Conclusion

This study suggests that a 25-OHD level of 13 ng/ml may be warranted, which is lower than the established cut-off of 20 ng/ml by the ES [13], resulting in a lower percentage of Syrians being identified as vitamin D deficient (72% versus 90%, respectively).

Several other studies have also advocated for different cut-off points for vitamin D deficiency. In a study conducted to explore potentially different cut-offs for black and white females, it was found that the inflection point differed significantly for black women (14.8 ng/ml) compared to white women (23.6 ng/ml) [42]. In another study done on a Chinese population, it was found that the inflection point was 12.8 ng/ml [45], which is also lower than that proposed by the ES and very similar in value to the inflection point found in this study. In fact, the value found in this study is closer to the one established by the IOM [20]. Still, while the IOM suggests that at 25-OHD levels of 10 ng/ml bone health is affected, this study found no effect between the identified cut-off of 13 ng/ml and bone health. This might be due to the fact that TAP was chosen to reflect bone health instead of bone mineral density (BMD) or biochemical markers of bone turnover. While most studies use bone mineral density (BMD) as a measure of bone health due to its relationship with 25-OHD and PTH [8,20], still, ALP has been used as a biomarker for bone health [46]. Alternatively, it could be that even at low vitamin D levels, the population's bone health isn't affected. In fact, the effect of low vitamin D on bone health has been shown in Caucasians [23], but not African American immigrants in the US [47] and the authors concluded that race-specific guidelines need to be developed [48,49].

It is clear that the prevalence of vitamin D deficiency is affected by the cut-off chosen, where the percentage of people identified as vitamin D deficient in this study sample is 20% lower using the identified versus the established cut-offs. Nevertheless, there is still a high prevalence of vitamin D deficiency even when using the newly identified cut-off, potentially explained by other factors such as inadequate vitamin D intake, conservative clothing style, urban living, insufficient sun exposure and obesity, all of which are risk factors related to the high prevalence of vitamin D deficiency in the MENA region [36,39,40,50,51].

The findings need to be considered in light of some limitations. First, the study sample was conveniently sampled from a large university hospital and does not constitute a representative sample of the Syrian population. Still, the strict inclusion criteria and thorough examinations helped ensure that participants were healthy and that the results were internally valid. While not representative, the sample is assumed to be heterogeneous since Al-Assad hospital is a big public referral hospital that Syrians from different areas seek for health care. The sample size also impeded any sub-group analyses, particularly by gender. As for the measurement issues, the use of electrochemiluminescence immunoassay to measure 25-OHD instead of Liquid Chromatography tandem Mass Spectroscopy (LC-MS), the gold standard for measuring 25-OHD [52,53], may have also impacted the results due to the variability between the assays. Furthermore, financial constraints prevented the use of Bone Mineral Density (BMD), which is the "gold standard" for measuring bone health (osteoporosis) [54,55].

An off-setting strength is the contribution of this study to the debate surrounding vitamin D deficiency and the established cut-offs. To the best of our knowledge, no published studies from the MENA region have explored possible alternative cut-offs except one study in Iran, which aimed at finding a cut-off point for vitamin D deficiency based on insulin resistance in children, and found that the most appropriate vitamin D deficiency cut-off was 11.6 ng/ml [56].

It is recommended that further studies are conducted on representative samples of the population, using gold standard measures, and large enough samples to explore potential differences by gender and age [57].

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