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# Association of Serum 25-Hydroxyvitamin D Levels With Markers of Metabolic Syndrome in Adult Women in Ramsar, Iran

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**Background:** Epidemiological studies showed that vitamin D deficiency is associated with components of metabolic syndrome.
**Objectives:** The aim of the present study was to determine the association between serum 25 hydroxyvitamin D concentration and components of metabolic syndrome in Iranian adult women.
**Patients and Methods:** This study was comprised of 156 women aged ≥ 30 years with at least three of the five criteria of metabolic syndromes. Serum 25-hydroxyvitamin D (25 (OH)D) levels and components of the metabolic syndrome were determined. Metabolic syndrome was defined according to NCEP/ATP III criteria. The subjects were classified into three groups according to their serum concentration of 25 (OH)D.
**Results:** Mean of serum concentration of 25-hydroxyvitamin D was 20.5 ± 10.8 ng/mL with %54.5, 23.1% and 22.4% of subjects were deficient, insufficient and sufficient in vitamin D, respectively. After adjustment for age, BMI, physical activity, and ANCOVA, fasting blood sugar concentration was shown to be inversely associated with serum 25 (OH)D (P = 0.004). On the other hand HDL-C showed significant correlation across different groups of vitamin D status (P = 0.014). Waist Circumference had favorable changes, without any statistically significant correlation. Also no significant association was observed between other components of metabolic syndrome and 25 (OH)D in different groups.
**Conclusions:** The components of metabolic syndrome are influenced by serum 25 (OH)D concentrations. The finding of this investigation revealed that FBS and HDL-C concentrations related to serum 25 (OH)D. Therefore, further longitudinal studies and randomized clinical trials are necessary to determine the possible role of vitamin D in prevention of diabetes and cardiovascular disease.

*Keywords:* Blood Fasting Sugar; Women; Vitamin D; Metabolic Syndrome

## 1. Background

Vitamin D deficiency is a common health problem (1). The prevalence of hypovitaminosis D is increasing worldwide (2, 3), and is more dramatic in Asian countries (4). According to different studies the prevalence of vitamin D deficiency in Iran varied from 44.8% to 79.6% (5). Studies suggest that nearly 30–50% of adult populations are at risk of vitamin D deficiency (6). However, it is increasing with aging as, the prevalence of vitamin D deficiency in elderly has been reported about 50% worldwide (7). The natural food sources of vitamin D are limited. Much of vitamin D is produced through direct exposure to sunlight. On the other hand, the latitude and season affect the cutaneous synthesis of vitamin D, the extent of which is less known (8). Moreover, vitamin D deficiency can be caused by inadequate vitamin D intake and absorption, obesity, dark skin, aging and decline in physical activity (9, 10). Although, the main role of vitamin D is regulating calcium homeostasis and bone health, it is now known that vitamin D deficiency is associated with insulin resistance, metabolic syndrome, hypertension, diabetes, cancer and autoimmune diseases (11). Several recent reviews have reported associations between low levels of 25 (OH)D and components of metabolic syndrome (MetS) (12-14). The guidelines of the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATP III) MetS indicate the presence of at least three of the following five criteria. These include fasting blood sugar ≥ 110mg/dL, TG ≥ 150 mg/dL, low serum HDL-C (< 40mg/dL (males) or < 50 mg/dL (females), waist circumference > 102 cm in males and >88 cm in females, blood pressure ≥ 130/85mmHg or using antihypertensive drugs (15, 16).

Because of the differences in definitions and populations studied, precise prevalence of MetS is unknown (17). However, data indicate that the prevalence of this syndrome among adult Iranian population aged ≥ 20 years is higher than those in most developed countries

**Implication for health policy/practice/research/medical education**
This paper discusses about Vitamin D role in the development of metabolic syndrome

(5). Individuals with MetS are at greater risk of coronary vascular disease (CVD), coronary heart disease (CHD) and diabetes (18). Physical inactivity, aging, obesity especially the central type and unhealthy diet influence the development of MetS and related diseases. Vitamin D status may be regarded as a novel possible risk factor for MetS and relevant diseases (19, 20). Acceptable biochemical criteria for vitamin D are still a matter of debate. However, most experts have defined vitamin D deficiency as 25 (OH)D level lower than 20 ng/mL, and insufficiency as it is 21-29.99 ng/mL, the optimal concentration of 25 (OH) D is at least 30 ng/mL (21, 22). Epidemiological studies have shown different results. A cross-sectional study on 45 year old subjects, reported that serum 25 (OH)D was associated with all features of MetS (23). But, other studies showed a relationship between 25 (OH)D and fasting glucose or blood pressure and triglyceride (7, 12) Third National Health and Nutrition Examination Survey have reported an inverse association between 25 (OH)D and hyperglycemia, and abdominal obesity (24). Despite the increasing prevalence of metabolic syndrome and vitamin D deficiency among Iranian population, especially women, limited studies have been conducted to explore this condition. Thus, it is seemed necessary to carry out an investigation to establish the association between metabolic syndrome and vitamin D deficiency in the foregoing populations and to delineate this relationship. Our hypothesis is that serum 25 (OH)D levels are associated with components of MetS.

## 2. Objectives

Therefore, in this study we evaluated the relationship between serum 25 (OH)D and component of the metabolic syndrome among Iranian women with MetS.

## 3. Patients and Methods

### 3.1. Subjects

Based on the underlying sample size Equation 1:

**Equation 1.**

$$\frac{\left(z_{1-\frac{\alpha}{2}}+z_{1-\beta}\right)^2 \times 4}{\left(\ln\frac{1+r}{1-r}\right)^2}+3=156$$

$Z_{1-\alpha/2}$, Z for Type I error = 1.96; $Z_{1-\beta}$, Z for Type II error = 0.84; r: correlation coefficient = 0.22. 156 women with MetS were needed for adequate power. Thus, in this cross- sectional study 156 women aged ≥ 30 years were selected by consecutive random sampling. Obese women with high blood pressure referred to us by physicians in health centers. Women consuming vitamin D and/or calcium supplements, as well as drugs affecting the metabolism of vitamin D during past 3 to 4 months, or having a history of renal or hepatic disease, overt diabetes and malignancies and those with at least three criteria for metabolic syndromes, as defined by NCEP/ATP III, were excluded from the study. Subjects were categorized into three groups based on serum concentrations of 25 (OH)D. The first category included: women with a serum concentration of 25 (OH)D < 20 ng/mL which were considered as vitamin D deficient. The second group had 25 (OH)D equal to 20- 29.99 ng/mL regarded as insufficient, and third group with 25 (OH)D concentration of ≥ 30 ng/mL as vitamin D sufficient one (18). Sampling was conducted in the summer after filling demographic questionnaire.

### 3.2. Laboratory Measurement

Blood samples were collected after an overnight fasting of 12-h, and serum was promptly separated by centrifugation and kept at -20°C until used. After all the blood samples were taken, fasting glucose and lipid profile were measured by routine enzymatic methods (Pars Azmoon kits; Tehran, Iran) using an autoanalyzer (Tokyo Boeki Prestige 24, Japan). Serum 25 (OH)D concentration was determined by radioimmunoassay ELISA (Euroimmune, Germany).

### 3.3. Assessment of Dietary Intake

Usual dietary intake was assessed using a validated 168-item semi quantitative food-frequency questionnaire (FFQ) (25). Which evaluated dietary intake during the past year, based on their consumption frequency, weekly and monthly? That questionnaire included a list of foods with serving (26). Each food and beverage was then coded according to the prescribed protocol and analyzed for content of energy and the other nutrients using N4 nutritional software designed for Iranian foods. The mean dietary vitamin D was obtained. All FFQs were processed by a trained dietitian.

### 3.4. Assessment of Other Variables

Waist circumference (WC) was measured at the narrowest level between the lowest rib and the iliac crest by a nonstretch tape at the end of a normal expiration. Body weight was measured by Seca digital scale with light clothes and without shoes to the nearest 100 gr. Height was measured by non-stretch tape to the nearest 0.1 cm with bare feet while the shoulders were in a normal position body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Data were obtained by an interview based on International Physical Activity Questionnaire and expressed as MET hours per day (MET-h/day) (27). Body weight was measured by Seca digital scale with light clothes with bare feet to the nearest 100 g. Height was measured by non nonstretch tape to the nearest 0.1 cm while not wearing shoes and the shoulders were in a normal position body mass index (BMI) was calculated as weight (in kilograms) divided on the square of height (in meters). Blood pressure was measured two times waiting 15-minutes between measurements using a mercury sphygmomanometer on the right hand in a sitting position. MAP was calculated as [(2 × diastolic) + systolic]/3 (28).

### 3.5. Statistical Analysis

We analyzed all data by SPSS software 16. We used ANOVA for comparing variables between groups. However, because of significant correlation between HDL-C and triglyceride as two components of metabolic syndrome, we introduced ANCOVA for comparing their relationship to Vitamin D status by adjusting confounding factors. Kolmogorov–smirnov was used to evaluate normal distribution of data. All variables reported as Means ± SD and values less than 0.05 were considered statistically significant.

## 4. Results

Of 156 women participating in this study, those with at least three of the five specific criteria for metabolic syndrome, which was prepared according to NCEP/ATP III, were included in this investigation. The general characteristics of subjects are shown in Table 1.

Subjects were categorized into three groups based on serum concentration of 25 (OH)D. Eighty five subjects (54.5%) with 25 (OH)D < 20 ng/mL were vitamin D deficient. Thirty-six (23.1%) had 25 (OH)D = 20-29.99 ng/mL that were insufficient and and thirty-five (22.4%) women had sufficient 25 (OH)D ( ≥ 30 ng/mL). The Kolmogorov–Smirnov test was used for testing the normality of all variables. We compared metabolic syndrome components between different groups of is vitamin D status by using ANCOVA (Table 2).

ANCOVA showed an inverse association between fasting blood sugar with serum 25 (OH)D (P = 0.004). On the other hand, MANCOVA revealed significant correlation between HDL-C across different groups of vitamin D status (P = 0.014). Waist circumference had favorable changes, but with no statistically significant correlation. Regarding components of metabolic syndrome, no significant differences were observed across 25 (OH) D groups.

## 5. Discussion

In this study, more than fifty percent of the participants had serum 25 (OH)D levels below 20 ng/mL. Our findings indicated that some components of MetS are significantly associated with serum 25 (OH)D concentrations. FBS concentrations was inversely associated with increasing serum 25 (OH)D concentration (P = 0.004). Also HDL-C was positively correlated with 25 (OH)D concentrations (P = 0.014, a relationship independent from confounders. Also, WC had favorable changes across different vitamin D. status groups, but with no significant correlation. Furthermore, no significant relationship was observed between the mean blood pressure and triglyceride across 25 (OH)D concentrations. The results of several studies,

**Table 1.** Anthropometric, Biochemical, and Dietary Characteristics of Subjects (n = 156) [a,b]

| Variables | Results |
|---|---|
| **Age, y** | 46.1 ± 9.5 |
| **BMI, kg/m$^2$** | 34 ± 5.3 |
| **WC, cm** | 106.9 ± 9.9 |
| **MAP, mmHg** | 10.2 ± 0.9 |
| **Physical activity index, MET-h/day** | 33 ± 2.7 |
| **Diet** | - |
| Total energy, kcal/d | 2444 ± 830 |
| Protein, g/d | 84 ± 31.4 |
| Carbohydrate, g/d | 369 ± 122 |
| Fat, g/d | 74.1 ± 47.4 |
| Dietary Vitamin D, μg/d | 1.1 ± 1.3 |
| **Biochemical** | - |
| Triglyceride, mg/dL | 176 ± 66.1 |
| Fasting plasma glucose, mg/dL | 115.7 ± 30.2 |
| HDL-C, mg/dL | 50.4 ± 7.7 |
| 25 (OH)D, ng/mL | 20.5± 10.8 |

[a] Abbreviations: BMI, body mass index; HDL, high density lipoprotein; MAP, mean arterial pressure; MET, metabolic equivalent; WC, waist circumference.
[b] All variables reported as Means ± SD.

**Table 2.** Comparison of the Metabolic Syndrome Components Across Different Groups of Vitamin D Status by Analysis of Variance[a,b,c]

| | Deficient (12.25 ± 3.79) (n = 85 | Insufficient (24.55 ± 3.39) (n = 36) | Sufficient (36.71 ± 5.6) (n = 35) | P Value |
|---|---|---|---|---|
| **Triglyceride, mg/dL** | 188.27 ± 87.63 | 182.25 ± 67.10 | 162.57 ± 48.063 | 0.121[d] |
| **WC, cm** | 107.65 ± 10.266 | 107.63 ± 10.62 | 104.31 ± 7.64 | 0.142[e] |
| **FBS, mg/dL** | 127.28 ± 33.72 | 113.22 ± 47.08 | 97.22 ± 14.79 | 0.004[e] |
| **HDL-C, mg/dL** | 49.35 ± 8.67 | 50.44 ± 7.11 | 54.228 ± 7.48 | 0.014[d] |
| **MAP, mmHg** | 10.231 ± 1.144 | 10.184 ± 0.89 | 10.247 ± 0.79 | 0.66[e] |

[a] Abbreviations: FBS, fastin blood sugar; HDL-C, high density lipoprotein.
[b] Deficient, 25 (OH)D < 20 ng/mL; Insufficient, 20-29.99 ng/mL; Sufficient, ≥ 30 ng/ml.
[c] All variables reported as Mean ± SD. Controlled by age, BMI, PAL.
[d] P is obtained by analysis of covariance (ANCOVA).
[e] P is obtained by multiple analysis of covariance (MANCOVA).

although inconsistent, have reported increasing evidence about relationships between serum 25 (OH)D and components of MetS (4, 21, 23). Our findings of the relationship between 25 (OH)D and glucose homeostasis are in agreement with those of Brock et al. (12). The relationship between 25 (OH)D and MetS may be approached mechanisms including:1) increasing the secretion and insulin sensitivity; 2) stimulated expression of insulin receptor; 3) enhancement of glucose transport into the cells of target tissues 4) and regulation of intracellular calcium (20, 23). Respecting the relationship of blood glucose to 25 (OH)D indicated in most epidemiological studies, it is necessary to design randomized clinical trials in order to determine the therapeutic dose of vitamin D for glycemic control, particularly in patients with type 2 diabetes. The association between waist circumference and vitamin D might be due to: 1) solubility of vitamin D in adipose tissue, 2) slow bioavailability of circulating vitamin D and 3) inadequate intake of vitamin D due to low physical activity in obese subjects and lesser exposure to sunlight (12).

In contrast with most studies, our study showed an inverse and non-significant relationship between WC and 25 (OH)D concentrations. This may be due to our small sample size in insufficient and sufficient categories of 25 (OH)D vitamin groups. However, HDL-C concentrations showed a positive and significant positive relationship with 25 (OH)D levels. Our findings were in agreement with those of Brock et al. study, in that vitamin D deficiency was not associated with central obesity (12), but this relationship was significant in studies reported by Maki et al. who used a larger sample size (21). We found no relationship between dietary vitamin D and markers of the Mets, and also observed no association between 25 (OH)D and dietary vitamin D. Such negative relationships may be explained by the absence of correlation, the limited food sources of vitamin D and measuring error in dietary assessment of vitamin D by FFQ. However, fewer studies have been conducted on the effect of sunlight on the synthesis of vitamin D in skin. Thus, the questionnaire used was designed depending on the latitude of different areas.

The present study showed that sun exposure was not associated with levels of 25 (OH)D, a finding in agreement with Hashemipour et al. study on Tehranian adults (4). Possible reasons might be either due to imprecise oral answers to questions regarding exposure period to sunlight or the type of clothes worn by the subjects. The cutaneous synthesis of vitamin D levels are influenced by lattitude, aging, season, the different intensity of sunlight at different times of the day. It is therefore necessary to consider such entities in preparing a valid questionnaire. Studies have shown that, vitamin D deficiency may be associated with negligible physical activity (10). Approximately, the strategies for activity should be considered for at least 55% of subjects. We found no significant relationship between blood pressure and vitamin D status. Similarly, Forouhi et al. (27) did not find any such association after 10 years follow up and pointed out that association of blood markers of vitamin D with blood pressure was complex. Several studies have shown that vitamin D may regulate blood pressure by regulating the renin-angiotensin system and inhibits the renin mRNA expression (29, 30). But, most of the results in this area are conflicting. Our results are in contrast with those of some studies (7, 13) that found statistically significant inverse association between 25 (OH) D with blood pressure. According to these studies, significant relationship could be attributed to using predicted vitamin D levels, self-reported hypertension and using repeated measure analyses. There is little evidence to suggest that vitamin D status can affect the development of dyslipidemia, an area deserving further studies. Our study have some limitations including small sample size ,cross sectional design, single sex, using questionnaire lacking accurate exposure time to sunlight and precise assessment of vitamin D intake. In conclusion, vitamin D deficiency may have an important role in MetS components. Therefore, further longitudinal studies and randomized clinical trials are necessary to examine the association between vitamin D deficiency and features of MetS.

## Authors' Contribution

Zamzam Paknahad has designed the study, written and edited manuscript; Azam Ahmadi Vasmehjani has done data collection, references search; Mohammad Reza Maracy has contributed in statistical analysis. All authors read and approved the final manuscript. Thanks to all authors for their support and help in this study

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