Vitamin D status in healthy female individuals

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Abstract
Background: Vitamin D3 deficiency is highly prevalent in the population throughout the world and it's underestimated due to lack of screening tests. It's also associated with numerous diseases, including those characterized by inflammatory processes.

Aim of study: The study aims to examine the prevalence of vitamin D3 deficiency among young healthy females in Kerbala province. If we prove the high prevalence of vit D deficiency, we would consider writing a policy to the governmental bodies to start screening for Vitamin D3 deficiency and increase public awareness about this public health problem.

Materials and methods: This study was conducted at Al Zahra Medical Center, Kerbala, Iraq. The eligible candidates for this study were randomly selected; they were 305, 129 out of them were excluded due to having various medical issues and a further 96 excluded due to having anaemia; therefore, 80 final healthy subjects included in this study. Subjects selected from the patients and relatives attending the outpatient clinic during the period from Nov, 2015 to June, 2016, age ranged between (18- 50) years. Subjects interviewed and reviewed clinically, body mass index (BMI) calculated, Vitamin D3, creatinine, parathyroid hormone PTH, calcium, Alkaline phosphatase ALP, phosphorus and complete blood count were tested.

Results: The results showed that serum vitamin D3 level were significantly decreased in the selected healthy females (18.86 ±9.91 ng /ml). There is a significant differences were detected when the levels of serum Vitamin D3 were compared according to age, body mass index, skin pigmentation and place of residence.

Conclusions: There is a significantly low level of serum vitamin D3 in healthy female subjects in holy City of Kerbala province; High prevalence rate of hypovitaminosis is observed in spite of good sun light in Iraq which might attribute to the cultural habit and life style. Well-structured steps need to be taken by the governing bodies to tackle this health problem through primary and secondary care. Increase public awareness regarding adequate exposure to the sun, increase intake of food rich with vitamin D3 or use supplementations and screening for its deficiency especially in females in childbearing age. Key words: deficiency D3, vitamin D, prevalence

1. Introduction
Vitamin D is a fat-soluble steroid hormone. It obtained from foods or by synthesis in the skin after sunlight exposure (Ultraviolet rays) and converted to the active form in the liver and kidneys. 1 Vitamin D3 is the only form produced cutaneously while exposure of plants steroids and ergosterol to UV lights forms Vit D2. 2

Vit D has skeletal effects, including mineralization of bones and maintaining its turnover; It may also provide protection against health problems like cancer, hypertension, type 1 diabetes and multiple sclerosis. 3 It plays a vital role in the proliferation and differentiation of different cell type. 4 Vit D cause suppression of specific inflammatory pathways and reduces inflammatory cytokine production by human periodontal ligament cells. 5

Modulation of immune response has been linked to Vit D through number of studies that showed the relation between low serum 25(OH)2D3 level and many autoimmune diseases such as, atopic dermatitis, inflammatory bowel disease, asthma , systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. 6 , 7 Vitamin D also showed an effect in bone marrow function. 8
Evidences are increasingly showing that vitamin D deficiency is an unrecognized and common health problem in various age groups;9–12 and in different settings like adult medical inpatients, postmenopausal women presenting with hip fracture, and homebound elderly people.10, 12, 13 However, there are no data available concerning the prevalence of Vit D deficiency among healthy females in Kerbala province.

2. Aim: The main objective of this study was to investigate Vit D status and measure the prevalence of hypovitaminosis among healthy females in Holy city of Kerbala. Secondly, to determine if seasonal variation had an impact on Vit D and PTH levels. Lastly, to identify some factors like female occupation, place of residence and some biological factors such as age and skin type that represent predictors of hypovitaminosis D.

2. Methods

2.1. Subjects of the study
I studied 80 individuals (aged 18–50 years), they were randomly selected from the patients and relatives who presented to the medical outpatient clinic at Alzahra Medical Center, Kerbala, Iraq. Participants were classified according to season, winter from December 2015 – March 2016 and Summer from April- July 2016. Approximately 305 patients were selected potentially for the present study. One-hundred and twenty-nine candidates were excluded due to various reasons like obesity more than (30kg/m2), history of various diseases such as endocrine, renal, gestational, liver disorder or some elements deficiencies like iron, folate, and vitamin B12 or different types of anaemia such as sickle cell, aplastic, hemolytic anemias and thalassemia. Also individuals taking medications that influence bone metabolism and current vitamin D and calcium intake. A final sample of (176) women was studied. I further excluded 96 patients who found to be anaemic

Table (1): Participants in this study

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (year)</th>
<th>Age (year) (Mean ± SD)</th>
<th>BMI (Kg/m2) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>15-50</td>
<td>29.86 ±10.51</td>
<td>23.35 ±2.58</td>
</tr>
</tbody>
</table>

2.2 Data Collections
Questionnaire was designed and individuals interviewed. At the out-patient clinic data were collected from the patients this including age, sex, ethnicity, past medical history, drug history, exercise, cigarette smoking and place of residence (urban or rural areas). Physical examination performed including the general examination which involved the observational signs like blood pressure, pulse rate, temperature and respiratory rate and systematic physical examinations of cardiovascular, respiratory, gastrointestinal system. Height and weight measured to calculate BMI.

2.3 Laboratory investigations
After complete the examination an 8 mL of blood was obtained for each participant. All tests were done at Al-Hussiany Medical City laboratory using kits to confirm participant's healthy status and measure Vit D3 and PTH levels.

The Human 25(OH)2D3 ELISA is a solid phase Enzyme Linked Immunosorbent Assay performed on microtiter plates.14-17 The PTH Immunoassay is an adapted two-site sandwich ELISA. Using end point analyzer serum magnesium, calcium and phosphorus levels were measured. Complete Blood Count (Sysmex XP-300 Atomic Hematology Analyzer).

According to Vit D level the patients were divided into 3 subgroups; Deficiency< 10 ng/ml, Insufficiency 10- 29 ng/ml; Sufficiency from 29 -100 ng/ml. This definition of vitamin D deficiency was according to previous studies.18

The classification of patients BMI was according to WHO;19, 20 Underweight (BMI < 18.5),
normal weight (BMI 18.50-24.99), over weight 25-30, obese >30. Waist to hip ratio of > 0.85 in women indicates presence of central obesity.

2.4 Statistical analysis
Data were presented in simple statistical measures of number, percentage, mean, standard deviation and quartiles. The analysis was done by using SPSS software version 22 (statistical package for social sciences). Statistical analysis for the significance of differences of the quantitative data was done by using Student's t-test for two independent means and ANOVA test for more than two independent means while the Pearson's correlation coefficient was used for the determination of the correlation between two quantitative data in varied groups. Statistically significant probability value was p <0.05

Results
Participants who involved in this study were 80 Females (Table 1). Blood results of serum ALP, creatinine, GFR, magnesium, phosphorus, calcium, hemoglobin and complete blood counts were all normal as shown in table 2

Table 2: clinical characteristics and laboratory findings of the participants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Deficiency: &lt;10 (ng/mmol) Insufficiency: 10-30(ng/mmol) Normal&gt;30(ng/mmol)</td>
</tr>
<tr>
<td>25-(OH) D3 (ng/ml)</td>
<td>18.86 ±9.91</td>
<td>16–75 pg/mL</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>61.38 ±33.91</td>
<td>0.68–1.36 mg/dL</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.73 ± 0.18</td>
<td>8.5–10.5 mg/dL</td>
</tr>
<tr>
<td>Serum Ca (mg/dl)</td>
<td>9.07 ± 1.04</td>
<td>2.5 to 4.5 mg/dL</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.58 ±1.21</td>
<td>45 - 150 U/L</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.04 ± 0.66</td>
<td>80-95 u 3</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>83.66 ± 3.96</td>
<td>27-31 pg</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.22 ± 1.69</td>
<td>32-36 g/dl</td>
</tr>
<tr>
<td>MCHC (mg/dl)</td>
<td>33.80 ± 1.55</td>
<td>90 to 120 mL/min/1.73 m²</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>130.01 ± 25.18</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.35 ± 2.54</td>
<td>Underweight (BMI &lt; 18.5) Normal weight (BMI 18.50-24.99), over weight 25-30, obese &gt;30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.26 ±11.61</td>
<td>--</td>
</tr>
</tbody>
</table>

3.1 Vitamin 25 (OH)_2 D₃
Study results showed significant reduction in the level of vitamin D among the participants (18.86 ±9.91 ng/mmol) as shown in table (3). A 32.4 % is below the normal level (6.8% deficient, 25.6% insufficient). There was an inverse correlation with PTH as shown in table 2.

Table (3): Distribution of the values of serum 25 (OH)_2D₃ level among the participants

<table>
<thead>
<tr>
<th>Participants</th>
<th>D3</th>
<th>Insufficiency 10-30 (ng/mmol)</th>
<th>Normal&gt;30ng/mml</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>12</td>
<td>45</td>
<td>23</td>
<td>80</td>
</tr>
<tr>
<td>% of Total</td>
<td>15%</td>
<td>56.25%</td>
<td>28.75%</td>
<td>100%</td>
</tr>
</tbody>
</table>

3.2 Deficiency of 25(OH)D3 and Skin Pigmentation.
The results were shown a significant increase in vitamin D3 level in white skin research population compared with lower concentration of vitamin D3 in dark colour skin and other skin types(brown) (p <0.001), as shown in Table (4) and figure 1

Table (4): Relationship between deficiency 25(OH)D3 and skin pigmentation

<table>
<thead>
<tr>
<th>Skin pigmentation</th>
<th>Mean</th>
<th>SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark colour skin</td>
<td>12.20</td>
<td>4.88</td>
<td>0.42</td>
</tr>
<tr>
<td>White (reference)</td>
<td>21.90</td>
<td>10.31</td>
<td>0.355</td>
</tr>
<tr>
<td>Others</td>
<td>18.32</td>
<td>9.81</td>
<td>0.000</td>
</tr>
</tbody>
</table>

3.3 Seasons correlation with Deficiency of Vitamin D3.
There was significant association between vitamin D level and seasons (p <0.05), high concentration of vitamin D3 in summer compared with low concentration of vitamin D3 in winter (p<0.05), in contrast to the non-statistically significant association between serum vitamin D in winter compared to spring (p >0.05) as shown in Figure (2).
3.4. Relevance of 25-Hydroxyvitamin D3 and City Life.

In evaluating the relationship between the city life and 25(OH)D3 levels; A significant increment (p<0.005) in 25(OH)D3 level in participants who lived in rural areas compared to those who lived in the city as shown in table (5).

**Table 5: Relationship between 25-Hydroxyvitamin D3 level in rural and urban areas**

<table>
<thead>
<tr>
<th>Place of residence</th>
<th>(D3 ng / mmol) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>18.23 ± 9.35</td>
</tr>
<tr>
<td>Rural</td>
<td>25.34 ± 13.74</td>
</tr>
</tbody>
</table>

3.5. Serum Vitamin D3 Levels and Age

Non-significant correlation was shown when compared serum vitamin D3 levels and different age group in healthy individuals, as shown in table 5

**Table 5: Association between 25 (OH) D3 deficiency and age groups**

<table>
<thead>
<tr>
<th>Age</th>
<th>(D3 ng / mmol) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-22 years</td>
<td>18.55 ± 10.56</td>
</tr>
<tr>
<td>23-30 years</td>
<td>17.52 ± 8.78</td>
</tr>
<tr>
<td>31-40 years</td>
<td>20.78 ± 9.37</td>
</tr>
<tr>
<td>41-50 years</td>
<td>19.04 ± 11.88</td>
</tr>
</tbody>
</table>

3.6. Serum Vitamin D3 Levels and Occupation

A significant correlation (p<0.05) demonstrated when compared serum 25(OH)D3 levels in students and part time group in healthy individuals, as shown in table 6

**Table 6: Relationship between serum vitamin D3 levels and occupation**

<table>
<thead>
<tr>
<th>Occupation</th>
<th>(D3 ng / mmol) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housewives (reference)</td>
<td>18.72 ± 9.71</td>
</tr>
<tr>
<td>Student</td>
<td>16.50 ± 10.75</td>
</tr>
<tr>
<td>Part time</td>
<td>22.19 ± 10.78</td>
</tr>
</tbody>
</table>

Discussion

This is the first population-based study proved that vitamin D3 deficiency or insufficiency status was prevalent in healthy females in holy city of Kerbala. It account to 71.25% of the sample under the study. There are growing evidences of the significance of vitamin D in
human health; also there are researches across the globe to determine its level and various health impacts due to its deficiency. It was few Iraqi studies dealt with identifying this major health issues, none in Kerbala province; Therefore, the present study could add other values in this field.

A Meta-analysis showed that Vitamin D can be used for the treatment of a various disease.\textsuperscript{21,22} It has a vital role in bone mineralization and increment in calcium and phosphorus serum levels.\textsuperscript{23}

This study results similar to study found the levels of circulating 25(OH)\textsubscript{2} D\textsubscript{3} are also commonly well below the normal range, contributing to secondary hyperparathyroidism and bone loss.\textsuperscript{24} Also this study showed a significant association between vitamin D3 deficiency and season; this findings were matching several studies results which revealed that skin exposure to solar ultraviolet radiation is a significant source of vitamin D, and vitamin D deficiency in most cases occurs due to either not get enough exposure to or lack of the vitamin in the diet.\textsuperscript{25} It also compatible with several studies outcomes in finding the prevalence rate of Vitamin D deficiency is high in the sunniest part of the world like in middle east; in Iran, India, Turkey, Qatar, United Arab Emirates and Saudi Arabia due to cultural factors that reflect the conservative approach in life such as types of clothing and lack of outdoor activities and be indoors most of the times.\textsuperscript{25-28}

In addition, this study results in agreement with a reported study showed low Vit D level was 49\% in winter and 29\% in summer.\textsuperscript{29,30}

The other interesting finding is the significant correlation between Vit D level and skin types. It showed significantly that vitamin D level was higher in white skin pigmentation in comparison to dark or black and other pigmentation. May be the mechanism of vitamin D inadequacy is also caused by certain factors, one of the biological factors is skin pigmentation generally known as melanin. Individuals with dark skin need more time exposure to sunlight to produce the same amount of Vitamin D compared to light skin ones. As I highlighted earlier the significance of UVB rays in the synthesis of vitamin D in the skin; However, the presence of melanin decrease the UVB penetration and result in inadequate vitamin D level in subjects with darker skin. This results in harmony with a UK study that showed the elevation in serum 25(OH)\textsubscript{2}D\textsubscript{3} concentrations in white British and South Asian ethnicity adults after exposure to a dose of simulated summer sunlight.\textsuperscript{31}

Similar results from Saudi Arabia where a study conducted on residents found a prevalent hypovitaminosis D during both winter and summer seasons, dark skin considered as one of the factors in this study. A reduction in the synthesis of cholecalciferol likely due to melanin as majority of the people in this region are brown to dark skin.\textsuperscript{32}

This study clarified significantly the lower level of vitamin D3 in females living in urban areas of the holy city of Karbala urbans compared to those live in rural once. This may be attributed to the difference in the number of sun exposure hours in urban and rural areas.\textsuperscript{33}

As regards Vitamin D3 and BMI the study showed that lower level of Vit D significantly correlated with the healthy underweight females

5.1. Conclusions

According to the findings of the current study, it can be concluded that:-

URL: http://www.uokufa.edu.iq/journals/index.php/ajb/index
http://iasj.net/iasj?func=issues&jId=129&uLanguage=en
Email: biomgzn.sci@uokufa.edu.iq
Hypovitaminosis D is prevalent in healthy females in Holy City of Kerbala, Iraq. This major health issue is completely overlooked.

Parathyroid hormone level was significantly inversely correlated to Vitamin D3 level.

A significant correlation between seasons and low vitamin D3 concentration.

Skin types were significantly associated with low vitamin D3 levels

Vitamin D3 level was significantly correlated to females live in urban areas as compared to those who live in rural ones.

5.2. Recommendations

Based on this study results my recommendation would be:-

1. Conduct another study with a larger sample size to investigate the same aim and show the most common clinical implications on general health.
2. I recommend that ministry of health issue a policy to check vit D level in all child bearing age females specially the pregnant ones as deficiency of Vit D level is associated with plenty of health problems.
3. A work studies to find the relation of the gender and vitamin D3 deficiency in Iraqi population.

REFERENCES


19. WHO 2000

20. WHO 2006


