Prevalence of Vitamin-D and calcium deficiencies in female university students: An exploratory study in Taif, Kingdom of Saudi Arabia.

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ABSTRACT
Background: Osteoporosis is a widely prevalent health problem with substantial morbidity and mortality. Poor nutrition, inadequate exposure to sunlight and low vitamin-D status contribute to severe osteoporosis. A plethora of scientific evidence globally suggests that large proportions of people have vitamin-D deficiency and are not meeting recommended intakes.
Objectives: We conducted an exploratory study for estimation of vitamin-D and calcium levels in sera of volunteers from the young female students in Taif University, and effect of life style.
Subjects and Methods: We analyzed the level of vitamin-D and calcium in sera of young female volunteers using ELISA. In addition, FBG and BMI were measured. We conducted a questionnaire to study the effect of life style on the investigated parameters. Statistical analysis was done for entire study cohort, using GraphPad Instat.
Results: Young female students showed vitamin-D deficiency, with normal calcium and FBG (M±SE: 14.23±1.11 ng/ml, 9.2±0.1 mg%, 94.8±1.37 mg/dl respectively). There was a statistically significant correlation between FBG and vitamin-D in the studied volunteers of young Saudi female (r= 0.31 at p< 0.05).
Conclusion: The presented data confirms that despite young females in Taif showed normal calcium, vitamin-D was deficient, which renders them at higher risk of developing osteoporosis later on.

Key Words: Vitamin-D, Calcium, Young Females.

INTRODUCTION
Vitamin D “vit-D” is a fat-soluble vitamin that is synthesized in human skin exposed to ultraviolet radiation. Besides its classical effects on bone and calcium homeostasis, it has progressively become recognized as a pluripotent regulator of many other biological functions, has a crucial role in enhancing physiological functions both in skeletal and extra-skeletal tissues; its deficiency and insufficiency are associated with many acute and chronic illneses including calcium metabolic disorders, type 1 and 2 diabetes mellitus, some cancers, autoimmune diseases, cardiovascular disease, and infectious diseases.
Vit-D is an important steroid hormone with endocrine, paracrine, and autocrine effects. Its action is supported by vit-D receptor, which is widely distributed throughout different tissues of the body. Primarily, it is endogenously synthesized produced in the skin by exposure to UV rays and less than 10% obtained exogenously dietary sources. 
which naturally contain vit-D in significant amounts are very limited\(^1\).

Vit-D is said to be deficient when 25 hydroxy vit-D “25(OH)D” is < 30 ng/ml, sufficient when 30-50 ng/ml. Optimal values are 50-70 ng/ml \(^{[1]}\). Recent studies have reported common Vit-D deficiency in the Middle East and in Saudi Arabia. Osteoporosis: is a serious irreversible\(^1\), silent disease that develops slowly over so many years, often diagnosed after the fragility fracture happens. The disease development depends on many factors like genetic predisposition, aging, dietary habits, physical activity, endocrine changes, lifestyles, general health condition and as a result of using some medications\(^3\).

The WHO defined osteoporosis as an epidemic of the 21\(^{th}\) century\(^1\). It is a progressive systemic skeletal disease characterized by low mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture.

Osteoporosis has significant impact on patient's life; it could limit mobility and restrict daily activities. Many studies showed osteoporosis complications, most recent was that done by Awwad et al. (2017), to reassess osteoporosis related femoral fractures and economic burden in Saudi Arabia, who reported that it has become one of the most causes of morbidity and mortality in the world generally and in Saudi Arabia specifically\(^1\).

Osteoporosis includes several controllable and uncontrollable risk factors; the controllable risk factors (environmental) include low activity level, sedentary lifestyle over many years, smoking, alcohol abuse and inadequate diet including eating disorder, low calcium intake, low vit-D intake and excessive consumption of soft drinks\(^1\). It affects both sexes; its prevalence in the Kingdom of Saudi Arabia is about 34\% in healthy Saudi women aged between 50-79 years, and 30.7\% in men\(^3\).

Criteria for optimal vit-D status have been based primarily in the following: maximal suppression of PTH, adequate intestinal calcium absorption, and fracture prevention, calcium is advised only for those with known calcium insufficiency those who are already undergoing treatment for osteoporosis\(^2\).

We conducted an exploratory study for estimation of vitamin-D and calcium levels in sera of volunteers from the young female students in Taif University, and effect of life style.

**MATERIALS AND METHODS**

In an attempt to study the prevalence of vit-D and calcium deficiencies in young Saudi females, seventy two female students from the college of applied medical sciences volunteered to participate in our study. All participants gave an informed written consent for approval (PDF attached). The study adhered to the research and ethical regulations of Taif University which complies with the Helsinki declaration.

All participants filled out a questionnaire prepared by our team for recording the personal and demographic data, medical history, risk factors, and life style.

Early in the morning, blood samples were collected from volunteers after overnight fasting. Samples were processed and sera were separated for the analysis of 25(OH)D and total calcium by ELIZA and photometric techniques respectively, at Pioneers Laboratory, Taif. Serum total calcium was analyzed on a COBAS INTEGRA 800 analyzer using Calcium Gen2, Cat. No. 05061482190, Roche diagnostics, USA\(^5\). Serum 25(OH)D was analyzed on Architect system using 25.OH vitamin D, Catalog Number: 05061482190, Abbott diagnostics, Ireland) \(^6\).

Fasting blood glucose was estimated using strip test glucometer (Fine test). Anthropometric measures: weight, height and waist circumference were measured and BMI was calculated for each volunteer (using BMI calculator\%, © Timothy Oswald). We gave the volunteers informative brochures for awareness, in addition to drug education when ever needed. According to the endocrine society: 25(OH)D is said to be deficient when 25-hydroxy vitamin-D “25(OH)D” is < 20ng/ml, insufficient when 21-29 ng/ml. Optimal values when >30 ng/ml\(^1\).

Laboratory analysis, data sorting was done followed by statistical analysis and interpretation.

**Statistical analysis:**

Data were expressed as M±SD “whenever possible”, and calculated for entire study cohort, using GraphPad Instat tm (© 1992-2000 Graph software Inc., V 3.05, Ralf Stahlman, Purdue Univ. 931897 S). Graphs were plotted using GraphPad Prism 5 (For Windows, © 1992-2007 Graphpad software Inc,. V 5.01, USA). Correlation studies were done using multi-regression analysis. P< 0.05 was considered significant.

**RESULTS**

Table-1 demonstrates demographic, anthropometric & clinical data of 72 Saudi female students at Taif University: female students showed 25(OH)D deficiency with normal serum calcium (M±SE: 14.23±1.11 ng/ml, 9.2±0.1 mg% respectively), and FBG was 94.81±1.37 mg/dl. There was a statistically significant correlation between FBG and vit-D in the studied volunteers of young Saudi female (r= 0.31 at p< 0.05), as shown in figure-1. On studying effect of life style and health state: 95.4 were singles, 53%
walks daily for an hour, 80% have proper dairy nutrition, 59.4% had normal BMI (figure-2, figure-3, figure-4, and figure-5 respectively). 90% of them weren’t taking any medication, 20% were used to drinking carbonated beverages once weekly and 73% were residents of Taif city (figure-6, figure-7 and figure-8 respectively).

**DISCUSSION**

Osteoporosis is a widely prevalent health problem with substantial morbidity and mortality. Poor nutrition, inadequate exposure to sunlight and low vitamin D status contribute to severe osteoporosis. A plethora of scientific evidence globally suggests that large proportions of people have vitamin D deficiency and are not meeting recommended intakes\(^7\)\(^-\)\(^11\). Vitamin D is absolutely essential for the maintenance of a healthy skeleton throughout our lives. There is mounting evidence that vitamin D insufficiency and vitamin D deficiency in elderly people is a silent epidemic that results in bone loss and fractures\(^11\).

Alwahabbi reported that “No disease has attracted attention in the last 2 decades all over the world, such as osteoporosis. Situation is not different in the Kingdom of Saudi Arabia. An epidemiological analysis showed that 34% of healthy Saudi women, and 30.7% of men, 50-79 years of age are osteoporotic. With a reported increased life expectancy in KSA increasing from 45-67 years in 1960 to 75.7 years in 2013, the prevalence of osteoporosis is expected to increase even further\(^12\). After a deep literature review of the available data about KSA, we conducted an exploratory study and to our knowledge, we are the first to study vitamin D & calcium deficiency in young female university students, in a Taif population based study.

The present study, revealed that the volunteers had 25(OH)D deficiency, their normal (total) calcium and FBG. Our data came in accordance with Al-khenizanl et al.; who collected the laboratory and radiology, vit-D levels, calcium levels, and bone mineral density scan results\(^13\), and in line with Tayel et al.; who studied the role of calcium homeostasis and vit-D deficiency to determine its relation with disease activity\(^14\).

Although all our volunteers confirmed daily exposure to sun, skin wasn’t directly exposed due to social/clothing habits, and hot weathered climate which drives people to stay in closed conditioned areas. This is in agreement with Al-Daghri et al.\(^2\). Our opinion is in the contrary, Muslims respect their religious habits and believes, which is hard to be changed a long time and generations. This was the case since Islam started so why vit-D deficiency is increasing now???? We believe that modernization and hot atmosphere in such countries led to long stay in closed areas and air conditioner.

In line, Holick stated that there is mounting evidence that in the absence of exposure to sunlight the daily requirement of vitamin D should be at least 600 IU\(^15\).

**CONCLUSION**

The present data confirms that university female students in Taif city, are at higher risk of developing osteoporosis.

**RECOMMENDATIONS**

We recommend further investigation of vit-D prevalence and risk factors profile both in male/ female students in Taif city, compared to other cities at sea-level, with larger sample size.

**DECLARATION**

Some data of this study was poster presented in the 22\(^{nd}\) DUPHAT conference 7-9\(^{th}\) March 2017, and won the 7\(^{th}\) best pharmacy student poster award.

**ACKNOWLEDGMENT**

We appreciate the help of Dr. Nahed Hawasai, vice dean of college of applied medical sciences, staff members and students.
Table-1
Demographic, Anthropometric & Clinical Data of 72 Saudi Female Students at Taif University

<table>
<thead>
<tr>
<th>Parameter</th>
<th>№</th>
<th>M±SE</th>
<th>Range or %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>72</td>
<td>21.65 ± 0.14</td>
<td>20 – 26</td>
</tr>
<tr>
<td>Social status (Single)</td>
<td>72</td>
<td>-</td>
<td>95.4 %</td>
</tr>
<tr>
<td>Daily exposure to sun</td>
<td>72</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Anthropometrics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ht. (cm)</td>
<td>72</td>
<td>156.53 ± 0.63</td>
<td>145 – 173</td>
</tr>
<tr>
<td>Wt. (Kg)</td>
<td>72</td>
<td>58.68 ± 1.55</td>
<td>37 – 98</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>72</td>
<td>89.1 ± 1.1</td>
<td>73 – 114</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>72</td>
<td>23.86 ± 0.57</td>
<td>14.27 – 37.3</td>
</tr>
<tr>
<td><strong>Clinical Data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>68</td>
<td>9.2 ± 0.1</td>
<td>6.59 – 10.24</td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>68</td>
<td>14.23 ± 1.11</td>
<td>5.7 – 51.4</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>72</td>
<td>94.81 ± 1.37</td>
<td>62 – 123</td>
</tr>
<tr>
<td><strong>Diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Osteoporosis</td>
<td></td>
<td>-</td>
<td>0 %</td>
</tr>
<tr>
<td>– Prediabetic</td>
<td></td>
<td>-</td>
<td>1.6 %</td>
</tr>
<tr>
<td>– Diabetes /</td>
<td></td>
<td>-</td>
<td>4.7 %</td>
</tr>
<tr>
<td>– Others (hypothyroidism, asthma, dyslipidemia, migraine, polycystic ovary)</td>
<td></td>
<td>-</td>
<td>16.9 %</td>
</tr>
<tr>
<td><strong>Familial History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Osteoporosis</td>
<td></td>
<td>-</td>
<td>26.6 %</td>
</tr>
<tr>
<td>– Diabetes</td>
<td></td>
<td>-</td>
<td>76.9 %</td>
</tr>
<tr>
<td>– Other hereditary disease</td>
<td></td>
<td>-</td>
<td>30.8 %</td>
</tr>
</tbody>
</table>

– Studied population comprised volunteers from the College of Applied medical sciences- girls section.
– №: total number of patients, FBG: fasting blood glucose,
– Normal values: FBG= optimal < 108, impaired 125- 140 & diabetic >140 mg/dl. Calcium (S)= optimal 8.5 - 10.5 mg/dl, 25(OH)D= optimal 50-70, insufficient 30 - 50 & deficient < 30 ng/ml.
– Data were approx. to the second decimal.
Figure 1
Correlation of FBG with Vitamin D in the Studied Sample (Volunteers) of Saudi Female Students at Taif University

Figure 2
Marital Status of the Studied Sample (Volunteers) of Saudi Female Students at Taif University

r = 0.31
p < 0.05
N = 68
Figure 3
Physical Activity (Daily Walking) of the Studied Sample (Volunteers) of Saudi Female Students at Taif University.

Figure 4
Proper Diary Nutrition of the Studied Sample (Volunteers) of Saudi Female Students at Taif University.
Figure-5
Obesity Risk (BMI Score) of the Studied Sample (Volunteers) of Saudi Female Students at Taif University

Figure-6
Medication Taken by the Studied Sample (Volunteers) of Saudi Female Students at Taif University
Figure 7
Frequency of drinking carbonated beverages in the Studied Sample (Volunteers) of Saudi Female Students at Taif University

Figure 8
Site of birth of the Studied Sample (Volunteers) of Saudi Female Students at Taif University
REFERENCES
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