Study effects of vitamin D deficiency on hematological and some physiological parameters among population in Derna City, Libya

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Abstract

This work was carried out to investigate the changes in hematological and some physiological parameters among patients diagnosed with symptoms related to deficiency of vitamin D (VD) at Derna City, Libya in nine consecutive months (March to November 2020). A total of 110 adult patients were divided into two groups based on the deficiency and non-deficiency of VD. The percentages of positive group were 21 (19.09%) males, 69 (62.72%) females and that of the negative groups were 4 (3.63%) males and 16 (14.54%) females. Highest prevalence with positive VD deficiency was noted in age group 21-50 years. From all patients, blood samples were collected for multiple tests including complete blood counts (CBC), liver function and kidney functions. Red blood corpuscles (RBC) count and Hematocrit (HCT) were decreased with statistically different compared to control. Also Hemoglobin (HGB) concentrations and mean corpuscular hemoglobin (MCH) were decreased when compared to control subjects in both genders. Mean corpuscular hemoglobin concentration (MCHC) was significantly decreased in patients (males and females) compared to control subjects. The numbers of white blood cells (WBC), percentage number of lymphocyte and parameters of platelets were not changed in both genders. While number of granulocyte was increased with significantly difference in positive VD deficiency. Mean levels of liver function parameters Aspartate aminotransferase, Alanine aminotransferase (AST and ALT), Total bilirubin (T.bilirubin) and kidney function parameters (creatinine and urea) were in normal range in all patients. The mean Calcium and ferritin values at patients with VD deficiency were significantly lower in both female and male subjects. In conclusion, VD deficiency is now recognized as a pandemic diseases common and found in both genders a cross different age groups and has effect in hematological and physiological parameters that studied.

Keywords: VD deficiency, hematological and physiological parameters
دراسة تأثير نقص فيتامين د على المعايير الدموية وبعض المعايير الفسيولوجية لدى عينة سكانية في مدينة درنة - ليبيا

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الملخص:

الهدف من هذه الدراسة هو دراسة التغيرات الدموية وبعض المعايير الفسيولوجية بين المرضى الذين تم تشخيصهم بأعراض تتعلق بنقص فيتامين د في مدينة درنة، ليبيا في نسعة أشهر متتالية (مارس إلى نوفمبر 2022). تم تقسيم إجمالي 110 مريض بالعمر إلى مجموعتين بناءً على نقص وعدم نقص فيتامين د، وكانت نسبة المجموعة الموجبة 21 (19.09٪) ذكور و 69 (62.72٪) إناث، ونسبة المجموعات السلبية 4 (3.63٪) ذكور و 16 (14.54٪) إناث. لوحظ أعلى معدل انتشار مع نقص الـفيتامين في الفئة العمرية 21-42 سنة. تم جمع عينات الدم من جميع المرضى لإجراء فحوصات متنوعة بما في ذلك تعداد الدم الكامل والنبض والكبد والكلى. انخفض تعداد كريات الدم الحمراء (RBC) والهيماتوكريت (HCT) عند مقارنتها بأفراد المجموعة السلبية بشكل ملحوظ في المرضى الذكور والإناث. كما انخفضت تركيز الهيموجلوبين (HGB) ومتوسط المحمولة أو الهيموجلوبين العضلي (MCH) بشكل ملحوظ في المرضى الذكور والإناث. مقارنة مع الأشخاص مصابين. تم تغيير عدد الخلايا البيضاء (WBC) والكتلة النموذجية للخلايا البيضوية ومراقبة الخلايا المبهرة في كل الجنسين. بينما زاد عدد الخلايا المبهرة مع اختلاف معنوي. كان متوسط مستويات المعايير وظائف الكبد (T成就 and ALT)، Aspartate aminotransferase وظائف الكبد (T.bilirubin) وظائف الكبد (T.bilirubin) وظائف الكبد (T.bilirubin) (كالكلاسيوم والكربونات)) في النظام الطبيعي في جميع المرضى. كان متوسط قيم الكلافن في المرضى موجودًا عند مرضى نقص فيتامين D أقل بشكل ملحوظ في كل من الإناث والذكور. في الحقيقة ، يعتبر نقص فيتامين D الآن من الأمراض الشائعة والموجودة في كلا الجنسين عبر مختلف الفئات العمرية، ولها تأثير كبير في المعايير الدموية والفسيولوجية التي تمت دراستها.

الكلمات المفتاحية: فيتامين د، المعايير الدموية، معايير فسيولوجية و درنة - ليبيا.
Introduction

Vitamins are defined as a group of complex organic compounds present in minute amounts in natural foodstuffs that are essential to normal metabolism and lack of which in the diet causes deficiency diseases. It consists of a mixed group of chemical compounds and are not related to each other as are proteins, carbohydrates, and fats [1]. They are required in trace amounts (micrograms to milligrams per day) in the diet for health, growth and reproduction. Omission of a single vitamin from the diet of a species that requires it will produce deficiency sign and symptoms [1]. Vitamin D (VD) is a steroid hormone with a broad range of biological effects ranging from the classical role as a mediator of calcium and phosphate balance to cellular differentiation and immune modulation. These effects impact normal and dysfunctional hematopoietic and immune function, which may allow an avenue for improved treatment and support of patients suffering from hematologic disorders [2]. VD deficiency is a global problem and it is known as an essential factor involved in different immune functions besides skeletal and muscle development [3, 4]. Many study reported that most of the nonspecific etiologies of common symptoms can result from VD deficiency [5]; although some researchers emphasized that more studies need to be done to prove that VD deficiency can lead to common symptoms of unknown etiologies such as headache and fatigue [5]. Another study found that the prevalence of non-specific muscle pains among the Middle Eastern population might result from VD deficiency [6].

VD obtained from sun exposure, food, and supplements is biologically inert and must undergo two hydroxylations in the body for activation, first occurs in the liver and converts VD to 25-hydroxyVD [25(OH)D], also known as calcidiol. The second occurs primarily in the kidney and forms the physiologically active 1,25-dihydroxyVD [1,25(OH)2D], also known as calcitriol [7]. Several epidemiological studies have linked inadequate VD levels to a higher susceptibility of immune-mediated disorders, including chronic infections and autoimmune diseases [8]. VD deficiency is a worldwide epidemic and yet, it is a problem that is largely unknown by majority of
population, they at high-risks for VD deficiency [9]. During childhood, this deficiency can cause growth retardation and skeletal deformities, while in adults, muscle weakness and fractures may ensue [10, 11]. In addition to its importance for bone health, recent evidence suggests that VD is also useful in promoting cardiovascular health and preventing chronic diseases (diabetes mellitus, autoimmune disorders, and various cancers) [12, 13].

One of the major reasons for the worldwide spread of this nutritional disorder has been lack of awareness about the importance of VD, its health benefits, and prevention of deficient states across populations [14, 15]. Regardless, approximately 1 billion people worldwide are believed to have deficient 25D levels, with particularly stark statistics noted amongst females of Middle Eastern origin [16]. Cultural factors particularly related to dress wear and skin coverage are important. Equatorial countries that classically experience high levels of ultraviolet radiation year-round are now showing increasing rates of deficiency [16]. In these cases, factors such as obesity and sedentary lifestyle, darker skin pigmentation, use of sunscreen and UV avoidance may explain this trend. It is important to note here that sun-avoidance strategies have led to significant decreases in skin cancer rates, so there is an important ‘trade-off’ between UV exposure and skin cancer versus adequate VD [16].

According to the degree of its deficiency, lack of VD may lead to the development of rickets or osteomalacia. Besides its effects on bone metabolism or calcium homeostasis, VD modulates growth, inflammation, neuromuscular immune function and possibly hematopoiesis [17, 18]. Hematopoiesis occurs in the bone marrow and is strictly regulated with the help of various cytokines, hormones, growth factors, and even vitamins to supply for a steady state of the circulating red blood cells (RBC), white blood cells (WBC), and platelets (PLT). Lack or deficiency of any of these regulatory factors can potentially slow down the hematopoietic process and lead to a certain decrease in the production of any one or more of these three cell lines [19]. VD deficiency is generally associated with a slowed hematopoiesis and an outcome of varying degrees of peripheral blood cytopenia [18], iron deficiency anemia or anemia...
of chronic disorder [20, 21]. Besides anemia, VD has also some ambivalent effect on the production and life span of white blood cells and platelets [22]. VD deficiency is extremely common in chronic liver disease patients. Up to 93% of these patients have some degree of vitamin insufficiency [23, 24]. A systematic reviews has cast doubt on any causal link between VD deficiency and non-skeletal health outcomes, suggesting that VD deficiency is a marker of ill-health, rather than an important factor implicated in the pathogenesis of disease [25]. However, there is growing evidence that VD is involved in the decrease of inflammation, fibrosis [26-28] and kidney disease [29].

A predisposing factor for rickets is a low calcium intake. This suggests that there is an interaction between VD and calcium. The active interplay between calcium and VD is needed to prevent skeletal disease [30]. Ferritin level was correlated with the presence of anemia [31] which can be explained by its function as the indicator for total iron reserve for hematopoiesis [32]. In addition, ferritin is also known as an important angiogenic factor to enhance tissue growth including the bone [32]. The association between VD and ferritin is still being debated because the findings vary across studies. One study reported that VD is positively associated with ferritin [33]. However, other studies have reported that VD is not associated with ferritin [34, 35]. In addition, a study reported that the association of VD and ferritin differs in men and women [36]. There is limited evidence regarding the prevalence of VD deficiency determinants and the representative population sample method. No detailed study on such VD deficiency is being recorded so far in Derna City in Northeast of Libya. This study was aimed to find out effects of VD deficiency on hematological and some physiological parameters among population in Derna City, Libya.

**Material and Method**

The study protocol was reviewed and approved by Bioethics Committee at Biotechnology Research Center (BEC-BTRC) with Ref No: BEC-BTRC 47-2018. Inclusion criteria involve: patients agreement participation in the study. The study was performed between March to November 2020 at different private labs (Al-Raze Lab,
Al-Rasheed Lab and Al-Bara Lab) with Zoology department at Omar Al-Mukhtar University. A hundred and ten subjects were enrolled in this study. All adults’ subjects were diagnosed with symptoms of VD deficiency. Blood samples were collected from each patient. At the same time questionnaire was answered from each patient including: age, sex, and presence/absence of other diseases. Blood samples were obtained from patients (under doctor supervision). Serology tests (including levels of VD, calcium, liver function, T. bilirubin, kidney function and ferritin) were performed on the basis of specific laboratory criteria.

**Determination of VD levels**

Blood samples were taken from each patient and kept at room temperature for 30 minutes till they coagulated. Then, these samples were centrifuged at 3,000 rpm for 10 minutes to obtain serum. Measurement of VD (Independent variable) was done by an enzyme immunoassay method. At the laboratory, serum was prepared by centrifugation (1600 revolutions per minute for 10 min at room temperature). Serum 25-hydroxyVD (25(OH) D) level was measured using an enzyme immunoassay method (Roche Diagnostics, Mannheim, Germany) [37].

**Hematological parameters**

Venous blood was taken from each patient. Five ml was taken in EDTA for complete blood counts (CBC). All CBC tests were performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc [38]. CBC was performed on EDTA as anti-coagulated samples.

**Liver function parameters**

Liver function parameters were estimated in all subjects by a kit method on automatic analyzer. Commercially available test kits, from Analytic on Biotechnologies, Germany were used with the manufacturer's instructions strictly adhered to using spectrophotometers (Humalyzer Junior). Alanine aminotransferase (ALT) was measured using a ready to use kit from Archem Diagnostic (ALT; EC 2.6.1.2) was assayed by
Aspartate aminotransferase (AST) (AST; EC 2.6.1.1) was assayed by the method of [39].

Determination level of T. Bilirubin: Serum total bilirubin was measured using the method of [40].

Level of ferritin levels was measured using Immunoassay for the in vitro quantitative determination of ferritin in human serum. The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers. This kit was supplied by Roche Diagnostics GmbHMannheim, Germany).

Level of calcium was measured using the method of [41] which used a ready to use kit from Analyticon Fluitest® CA CPC (Lichtenfels, Germany). The calcium method was standardized by atomic absorption Spectrometry.

Kidney functions parameters
Creatinine: This was determined using ready to use kit from Archem Diagnostics industry. And the amount of urea is measured using a ready to use kit (Biomaghreb).

Statistical analysis
Statistical analysis was carried out in Minitab software 17 and Graph prism Pad; statistical significance was assessed using two samples T- test analysis after detection normal distribution to the data and appropriate P < 0.05 consider significant [42]. The numerical data were shown as number and percentage. To find the significant difference between the observed variable studied, Pearson Chi-Square Test for Association was used, P value was taken as level of significance at <0.05.

Results
110 was the total number of patients. Most people don't realize they have symptoms of VD deficiency, because the symptoms do not appear as evidence of VD deficiency; A total number of 110 patients (25 males (22.72%) 85 females (77.27%)) were enrolled in this study. Upon this study 110 patients who positive VD deficiency was found 90 (81.81%) and negative with VD deficiency was 20 (18.18%). In term of each gender, over all 110 patients who positive with VD deficiency was found 21 (19.09%) and 69
(62.72%) for male and female respectively, as compared to negative subjects with VD deficiency that found 4 (3.63%) and 16 (14.54%) for male and female respectively with not significant deference between gender and percentage number of cases P (0.57) as shown in Figure 1.

A total of 90 cases (who found with VD deficiency) were tested for comparison between 25 (OH)D concentration (Figure 2). The highest percentage (62%) was found an insufficiency with level of concentration between (10-30 ng/ml). Other status (Deficiency and Sufficiency) were found similar percentage around 20%. Results were shown significant deference between 25 (OH)D concentration and percentage number of cases P (0.000).

Results from distribution of cases upon age groups in each gender separately, (as each group consist of 10 years intervals) were shown in Table 1. Highest prevalence with VD deficiency was noted in age group 21-30 years (18.18%) followed by age groups 31-40 and 15-20 years with (16.36%) and (14.54%) for female subjects. Highest prevalence with VD deficiency was also noted at same age group 21-30 years (8.18%) followed with age groups 41-50 and 31-40 years with (4.54%) and (2.72%) for male subjects. Lowest prevalence was observed in the age group of >60 years for both genders.

Figure 1: Percentage number of patients with positive and negative cases with VD in each gender (Not significant deference between gender and percentage number of cases P (0.57)).
Figure 2: Percentage number of patients upon different 25 (OH)D concentration

Table 1: Age distribution of male and female subjects

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Female. Number of cases (%)</th>
<th>Male. Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>VD deficiency</td>
</tr>
<tr>
<td>15-20</td>
<td>1</td>
<td>(0.90)</td>
</tr>
<tr>
<td>21-30</td>
<td>3</td>
<td>(2.72)</td>
</tr>
<tr>
<td>31-40</td>
<td>5</td>
<td>(4.54)</td>
</tr>
<tr>
<td>41-50</td>
<td>4</td>
<td>(3.63)</td>
</tr>
<tr>
<td>51-60</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>2</td>
<td>(1.81)</td>
</tr>
<tr>
<td>71-80</td>
<td>1</td>
<td>(0.90)</td>
</tr>
<tr>
<td>81-90</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>91-100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>(14.54)</td>
</tr>
<tr>
<td>Ave. Age (year) Mean ± SEM</td>
<td>42.0±3.7</td>
<td>33.3±1.9</td>
</tr>
<tr>
<td>T (P-value )</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>(77.27)</td>
</tr>
</tbody>
</table>
Values derived from CBC, including differential cell counts were recorded for each patient who deficiency VD compared to the values of patient who not deficiency VD. Generally, the results were shown a lot of similarity between both genders in terms of effects. RBC counts were studied, and the results were shown in Table 2. The deficiency VD<10ng/ml and VD (10-30 ng/ ml ) insufficiency in female subjects had a mean count of (4.51 ± 0.10, 4.39 ± 0.06)×10^6/μl RBC, respectively. This result was not statistically different compared to not deficiency VD infection subjects. While in male subjects had a mean count of (5.38 ± 0.18, 4.65 ± 0.13) ×10^6/μl RBC, respectively. This result was statistically different compared to not deficiency VD subjects.

The level of HGB concentration in the female who deficiency VD <10 ng/ml and VD (10-30 ng/ml) insufficiency were 13.08 ± 0.34, 11.65 ± 0.23 g/dl, respectively. This result was statistically different compared to not deficiency VD subjects. However, in the male (who deficiency VD and insufficiency VD) cases were found non-significant with 14.75± 0.49, 13.02 ± 0.35g/dl, respectively.

The presence of deficiency VD had not significant effect on the percent HCT for female 37.58 ± 0.88, but significant effect for male 44.55 ± 1.65%. Meanwhile, levels of MCV for female were 83.37± 1.17 and male was 83.20 ± 5.57μm^3 in comparison with values of not deficiency VD subjects. MCH were significantly for female patients while in male were no significantly. Values of MCHC were also significantly (p>0.05) decrees in patients (female 26.67±0.46 and male 27.85 ± 1.70 pg) when compared with not deficiency VD subjects. Results of RDW-SD and RDW-CV (%) were not significantly change in both genders who positive deficiency VD status.

The count of WBC (× 10^3/μl) was found not significantly difference in female and male subjects with deficiency VD and insufficiency VD (Table 3).

However, percentage levels of Lymphocytes (%) were found none significantly deference both gender with positive deficiency VD (Lymphocytes in female 33.31±1.66 and male 36.05±2.25%).
The granulocytes (%) was found also not significantly difference in female with deficiency VD and insufficiency VD (54.74±1.70 and 55.75±1.11) but in male was significantly difference between VD status.

Results of platelets parameters were shown in Table 4. The mean PLT ($\times 10^3/\mu l$) number in patients with deficiency VD was found as not significant deference in both female and male subjects. and the mean platelets volume MPV (fl) number in female with deficiency vitamin d were not significant deference ($9.84 \pm 0.23$ ), but in male with deficiency vitamin d were significant deference ($9.30 \pm 0.36$ ). Mean while PDW (%) and PCT (%) were also not significant deference in both female and male subjects with deficiency VD infection to (13.05& 15.25) and (0.25& 0.21) respectively.

The mean calcium (mg/dl) values at number in patients with VD status were miner significant decreased with decreases level of VD in both female and male subjects. The mean level of ferritin (ng / ml) was decreased significantly with decreasing level of VD in female subjects. Similar effects were noticed in male subject without significant deference. Level of T, bilirubin was also measured in all subjects and was in normal range in both gender (Table 5).

The effect of the VD status with serum AST, ALT, and on kidney function parameters (creatinine and urea) were shown in Table 6. All mean level values were in normal range in both gender.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VD Deficiency</td>
<td>VD Deficiency</td>
</tr>
<tr>
<td></td>
<td>&lt;10 ng/ml</td>
<td>(10-30) ng/ml</td>
</tr>
<tr>
<td>RBC (×10⁶/μl)</td>
<td>4.51 ± 0.10 *</td>
<td>4.39 ± 0.06 *</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>13.08 ± 0.34 *</td>
<td>11.65 ± 0.23 *</td>
</tr>
<tr>
<td>HCT %</td>
<td>37.58 ± 0.88 *</td>
<td>35.35 ± 0.55 a</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>83.37 ± 1.17 *</td>
<td>81.39 ± 1.27 *</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>29.00 ± 0.53 a</td>
<td>26.67 ± 0.46 b</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>34.76 ± 0.47 a</td>
<td>31.91 ± 0.37 a</td>
</tr>
<tr>
<td>RDW-SD (%)</td>
<td>13.55 ± 0.51 a</td>
<td>13.42 ± 0.21 a</td>
</tr>
<tr>
<td>RDW-CV (%)</td>
<td>41.25 ± 0.86 a</td>
<td>40.83 ± 0.39 a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (a, b or ab) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05)
### Table 3: Values of WBC, Lymphocytes and Granulocytes of VD status in female and male subjects (Mean ± SEM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WBC (×10³/µl)</th>
<th>Lymphocytes (%)</th>
<th>Granulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD Deficiency &lt;10 ng/ml</td>
<td>9.10 ±0.87</td>
<td>33.31±1.66</td>
<td>54.74±1.70</td>
</tr>
<tr>
<td>VD Deficiency (10-30) ng/ml</td>
<td>8.13 ±0.30</td>
<td>34.95±1.36</td>
<td>55.75±1.11</td>
</tr>
<tr>
<td>Sufficiency (30-100) ng/ml</td>
<td>7.32 ±0.49</td>
<td>33.53± 2.02</td>
<td>58.82± 1.88</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD Deficiency &lt;10 ng/ml</td>
<td>7.35± 0.38</td>
<td>36.05±2.25</td>
<td>54.20±3.49</td>
</tr>
<tr>
<td>VD Deficiency (10-30) ng/ml</td>
<td>7.35± 0.45</td>
<td>32.16± 1.10</td>
<td>52.72±1.31</td>
</tr>
<tr>
<td>Sufficiency (30-100) ng/ml</td>
<td>6.12± 0.19</td>
<td>32.30 ±0.76</td>
<td>61.25±1.80</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM of each gender. Within each column for male or female separately, means with different superscript (a, b or ab) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

### Table 4: Values of PLT, MPV, PDW and PCT of VD status in female and male subjects (Mean ± SEM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PLT (10³/µl)</th>
<th>MPV(FL)</th>
<th>PDW (%)</th>
<th>PCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD Deficiency &lt;10 ng/ml</td>
<td>278.1±20.5</td>
<td>9.84 ± 0.23</td>
<td>13.05 ± 0.49</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>VD Deficiency (10-30) ng/ml</td>
<td>284.0 ± 12.4</td>
<td>9.70 ± 0.13</td>
<td>14.45 ± 0.21</td>
<td>0.27 ±0.008</td>
</tr>
<tr>
<td>Sufficiency (30-100)</td>
<td>243.4 ± 15.8</td>
<td>9.85 ±</td>
<td>14.63 ±</td>
<td>0.25 ±</td>
</tr>
</tbody>
</table>
Data are expressed as mean ± SEM of each gender. Within each column for male or female separately, means with different superscript (a or b) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

Table 5: Values of Calcium, ferritin and T. bilirubin of VD status in female and male subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Calcium (mg/dl)</th>
<th>Ferritin (ng/ml)</th>
<th>T. bilirubin (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>VD Deficiency &lt;10 ng/ml</td>
<td>7.15 ± 0.09 c</td>
<td>8.36 ± 0.11 a</td>
<td>0.505 ± 0.103 a</td>
</tr>
<tr>
<td>VD Deficiency (10-30) ng/ml</td>
<td>7.78 ± 0.05 b</td>
<td>8.71 ± 0.06 b</td>
<td>26.36 ± 1.73 b</td>
</tr>
<tr>
<td>Sufficiency (30-100) ng/ml</td>
<td>7.98 ± 0.11 b</td>
<td>8.36 ± 0.11 a</td>
<td>0.521 ± 0.039 a</td>
</tr>
<tr>
<td>Sufficiency (10-30) ng/ml</td>
<td>7.15 ± 0.09 c</td>
<td>8.90 ± 0.17 a</td>
<td>84.75 ± 5.41 a</td>
</tr>
</tbody>
</table>
Data are expressed as mean ± SEM of each gender. Within each column for male or female separately, means with different superscript (a, b or c) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

Table 6: Values of some liver functions enzyme (ALT, and AST), kidney function (Creatinine and urea) with three of VD status in female and male subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALT (U/L)</th>
<th>AST(U/L)</th>
<th>Creatinine (µg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD Deficiency &lt;10 ng/ml</td>
<td>11.82±0.56 a</td>
<td>16.52±0.800 a</td>
<td>0.729±0.031 a</td>
<td>28.94±0.424 a</td>
</tr>
<tr>
<td>VD Deficiency (10-30) ng/ml</td>
<td>13.78±0.82 a</td>
<td>17.96±0.65 a</td>
<td>0.728±0.017 a</td>
<td>31.09±0.52 a</td>
</tr>
<tr>
<td>Sufficiency (30-100) ng /ml</td>
<td>11.50±0.66 a</td>
<td>17.37±0.63 a</td>
<td>0.712±0.027 a</td>
<td>29.18±0.64 a</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VD Deficiency &lt;10 ng/ml</td>
<td>15.50±1.71 a</td>
<td>18.00±0.707 a</td>
<td>0.725±0.075 a</td>
<td>29.00±0.408 a</td>
</tr>
<tr>
<td>VD Deficiency (10-30) ng/ml</td>
<td>19.00±0.88 a</td>
<td>17.47±1.19 a</td>
<td>0.676±0.046 a</td>
<td>29.53±1.23 a</td>
</tr>
<tr>
<td>Sufficiency (30-100) ng /ml</td>
<td>15.75±2.75 a</td>
<td>15.75±1.93 a</td>
<td>0.675±0.047 a</td>
<td>32.75±2.06 a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM of each gender. Within each column for male or female separately, means with different superscript (a, b or c) were significantly different at p<0.05. Where means without superscripts mean that there is no significant difference (p>0.05).

**Discussion**

The prevalence of VD deficiency varies widely across several populations. From the effects of the current survey, the hypothesis was accepted that VD deficiency was common among the population, especially among 15 to 50 years groups for couple
genders. Among Libyan population, the prevalence of VD deficiency is not well known. There is limited evidence concerning the prevalence, determinants and mode of representative population sample as well as a lack recorder about this deficiency in main Hospital or in private clinical. In addition, no detailed study on such deficiency is being recorded so far in Derna City in Northeast of Libya. Therefore, the aim of this study was designed to study effect of VD deficiency on hematological and some physiological parameters among population in Derna City, Libya.

The result showed that majority of samples collected was female group. Over all 110 patients who positive with VD deficiency was found 19.09% and 62.72% for male and female respectively with significant deference between gender. The variation in VD deficiency among population might refer to different elements such as food habit, customs and clothing and the way of sun exposure [43]. Besides most of women may uneducated about the appropriate ways to get adequate amounts of VD during exposure of daily sunlight, regarding to the time of exposure and thickness cloths and type of skin influence on body responding to VD [44]. Current study agreed with a study conducted in the western region of the Kingdom of Saudi Arabia, where he was there is a high prevalence of VD deficiency among women. This result may be either due to the absence of public awareness or due to lack of exposure to sunlight due to our social habits from the way women dress [45]. According to studies in Canada on some students from the Middle East, it was observed that they have VD deficiency in [46], and this study was consistent with our study. The difference between sexes probably reflects the ethnic and religious practices leading to less skin exposure in adult females than in adult males.

Since female represented the most affected group at age 21-40 may indicate that women may display to the frequent pregnancy that could be the case of consuming all minerals and vitamins from the ivory of the mother to the fetus [47].

Despite ample sunshine, Africa recorded the highest rates of rickets worldwide [15], Some surveys have confirmed that dark skinned person as in North Africa are more susceptible to increased risk of hypo-vitaminosis D due to high melanin in their skin, which prevent ultra violet absorption [15, 43]. Another study also indicated that most
of the cases, collected and analyzed for VD status had an insufficient concentration of VD level about 53.8% and 13% had deficient level and just 12 were severe deficient [47]. These results are similar to our result since the percentages of severe deficiency and deficiency were high compared to normal values. The main cause may due to food habit in Libyan people they concentrate on food with low nutritional benefits.

Results from hematological parameters in this study were consistent with the study conducted by researchers in India [48], but there was a significant effect in males and this was consistent with a study conducted in Turkey [49]. VD deficiency significantly affects HGB in this study, and this is consistent with the study conducted by the researchers in the country of Brazil [50], also agreed with the study conducted by [51] and a study conducted in Turkey [49]. There is a significant association between VD deficiency and anemia among patients in Majmaah City, Saudi Arabia [52]. VD deficiency were found a risk factor for anemia in pregnant women [53]. The current study was found no significant effect on WBCs and lymphocytes in both sexes. These results were consistent with the study conducted by researchers in India [48], but present study was not in agreement with the study conducted by researchers in Brazil [54]. The current study also found that percentage of granulocytes did not have a significant effect in females while there was a significant effect in males.

VD deficiency had no significant effect on parameters of platelets in both genders. These results were consistent with the study conducted by researchers in India [48] and our study did not agree with the study conducted in Korea where they found an inverse relationship between VD deficiency, PLT and MPV [55]. Also, the present study did not agree with the study conducted in Turkey [56]. VD deficiency is generally associated with a slowed hematopoiesis and an outcome of varying degrees of peripheral blood cytopenia [18]. Although, there are conflicting reports about the role of VD on hematopoiesis, there are numerous reports indicating a correlation between VD deficiency and anemia [19]. Commonly proposed mechanisms, which relates VD deficiency to anemia, were the increased level of inflammatory cytokines
and hepcidin, which directly suppresses hematopoiesis and interrupts iron recycling, and reduction in the production of erythropoietin [19].

The observed association between VD and liver disease is insufficient to establish a causal effect between VD deficiency and the severity of chronic liver disease [25]. Results from current study did not match the study conducted by researchers in Iran [57]. VD deficiency also did not have a significant on kidney function (urea, creatinine) in both gender. These results were consistent with the study conducted by researcher [30]. Systematic and umbrella reviews has cast doubt on any causal link between VD deficiency and non-skeletal health outcomes, suggesting that VD deficiency is a marker of ill-health, rather than an important factor implicated in the pathogenesis of disease [25]. The presence study showed that VD deficiency significantly effects on levels of calcium and ferritin, in females these results were consistent with the study conducted by the researchers [58], but in males it did not have a significant effect, and these results were inconsistent with the study presented by researchers in Canada. [59]. Calcium and VD have long been recognized as important and required nutrients for bone health and maintenance. The continuation of calcium and VD in a patient with bone loss is critical for optimal care. Unfortunately, 90% of women may not be getting enough calcium and over 50% of women treated for bone loss have inadequate VD levels [44, 60].

The limited number of studies conducted on this issue have shown a positive correlation between the levels of VD and ferritin. Constantini, Arieli [61] and Andiran, Celik [62] have reported a positive correlation between the levels of VD and ferritin in athletes and dancers. Similarly, a positive correlation was reported between these two parameters in patients with Crohn’s disease [63]. In our study also, ferritin level was likely to be lower in individuals with low VD levels. This relationship may be explained by suboptimal dietary habits [61]. This link is further supported by studies showing that 1α, 25-dihydroxycholecalciferol, an active form of VD, leads to an increase in intestinal Fe absorption by elevating the erythropoietin level [64]. The previous studies indicate that periodic screening should be performed for both VD deficiency and low ferritin levels, two insidious conditions of high prevalence.
Conclusion

VD deficiency represents one of the most diseases common in worldwide. VD deficiency is now recognized as a pandemic diseases Health educational program should be developed and implemented for population to educate them about the importance of VD and the consequences of its deficiency during seminars to assure healthy population. Further studies need to be focusing on the associations between VD and health. More could be practiced for both sex to foster a warmness and to combat the problem of low degrees of VD for example, diet and dietary.

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Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.
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