

## Estimation of vitamin D Serum Level Among Adult Population In Misurata

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### Abstract:

Vitamin D is an important prohormone with several biological roles. The high prevalence of vitamin D deficiency around the world highlighted the need for studying vitamin D serum status in the community. This prospective study aimed to evaluate the serum vitamin D concentrations among adult population in Misurata and to explore how its concentration is affected by factors like gender, age and menopausal status. To achieve the aim of the study, a total of 125 adult samples were collected and divided based on gender and age into four groups (premenopausal women, postmenopausal women, men below and above 45 years). Samples were analyzed by ELISA.

According to the results, about 20% of the younger males had sufficient vitamin D levels, 45% with insufficient levels and deficiency was reported in about 35% of the cases. The results in the other three groups were similar and consistent with the hypothesis where no individual in the three groups found with sufficient vitamin D. As hypothesized, younger males had higher serum vitamin D concentrations in comparison to younger females which is probably due the limited exposure to sun light as a result of factors such as hijab, sun screen usage and reduced outdoor activities. Furthermore, age impact was critical in the male groups but not in the female groups where pre and postmenopausal women exhibited comparable results.

In few words, vitamin D deficiency is predominant among the population of Misurata which emphasizes the need for empirical treatment with vitamin D supplementation as an appropriate solution.

**Keywords:** Vitamin D deficiency, supplementation.

**المخلص:**

فيتامين (د) مركب مهم لعدة وظائف بيولوجية. إن الانتشار الواسع لنقصه حول العالم سلط الضوء على أهمية دراسة مستوياته في الدم. الدراسة الحالية صممت لتقييم مستوى فيتامين (د) في الدم لدى القاطنين بمدينة مصراتة، ولاستكشاف تأثير عوامل كالعمر، الجنس وحالة انقطاع الطمث على تركيز فيتامين (د) في الدم. للوصول لهدف الدراسة جمعت عينات دم من 125 شخص بالغ بمصراتة وقسمت إلى أربع مجموعات: (نساء قبل مرحلة انقطاع الطمث، نساء بعد مرحلة انقطاع الطمث، رجال أكبر 45 عام، ورجال أصغر من 45 عام)، كل العينات تم تحليلها بواسطة تقنية ELISA.

حسب الدراسة، 20% من مجموعة الرجال الأصغر سناً وجدوا بمستويات كافية من فيتامين (د)، بينما 45% من نفس المجموعة وجدوا بمستويات غير كافية و35% شخصوا بفقير فيتامين (د). النتائج في المجموعات الثلاثة الأخرى كانت متشابهة ومتوافقة مع فرضية الدراسة حيث لم توجد أي عينة في الثلاث مجموعات بمستوى كافي من فيتامين (د). وكما تم افتراضه، تركيز فيتامين (د) عند الرجال الأصغر سناً أعلى منه عند النساء من نفس الفئة العمرية، قد يفسر ذلك بقلة تعرض النساء لأشعة الشمس مقارنة بالرجال بسبب عوامل كالحجاب، استخدام واقبات الشمس وقلة الأنشطة خارج المنزل. كما لوحظ أن تأثير عامل السن كان أكثر وضوحاً في البالغين من الرجال عنه في مجموعتي النساء، حيث لم يوجد فرق واضح بين نتائج مجموعتي النساء. للتخيص، نقص فيتامين (د) بالدم كان شائعاً جداً لدى العينة المأخوذة من مصراتة، ما قد يضع العلاج التجريبي بمكملات فيتامين (د) كحل مناسب. الكلمات المفتاحية: فيتامين (د)، المكملات الغذائية.

**Introduction:**

Vitamin D deficiency is a global health issue. It has been an active area of research for the last decades, the number of PubMed published articles about vitamin D role and deficiency problems has increased from 2844 in 2000-2001 to 4635 in 2008-2009<sup>[1]</sup>. The established definition of vitamin D deficiency is a serum 25-hydroxyvitamin D level of less than 20 ng/ml, while vitamin D insufficiency is defined as lower than 30 ng/ml; on the other hand, a level of 30 ng/ml or greater can be considered as sufficient vitamin D. Vitamin D

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intoxication is observed when serum levels of 25-hydroxyvitamin D are greater than 150 ng/ml<sup>[2-5]</sup>. Vitamin D was discovered in 1922 while searching rickets etiology.

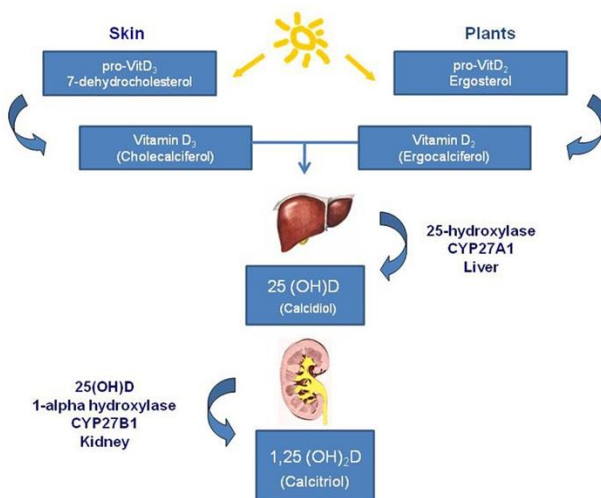


Figure 1: Vitamin D Biosynthesis<sup>[6]</sup>

Vitamin D<sub>2</sub> (ergocalciferol) is photochemically synthesized in plants, and vitamin D<sub>3</sub> (cholecalciferol) is synthesized from 7-dehydrocholesterol in the skin in response to sunlight exposure (Figure 1)<sup>[6]</sup>.

The medical community has in recent years increased its attention to vitamin D, unmasking its importance not only in bone abnormalities, but also in many pathological conditions including type 1 diabetes, multiple sclerosis (MS), irritable bowel syndrome (IBS), heart failure and tumors. The role of vitamin D in bone hemostasis is highly understood as it was intensively studied while searching causes of rickets and osteomalacia<sup>[7]</sup>. Furthermore, several studies have explained the close relationship between vitamin D insufficiency and a number of misdirected immunity syndromes such as IBS, MS and type 1 diabetes<sup>[1]</sup>.

In fact, vitamin D deficiency has attracted tremendous attention between health care providers as well as scientific researchers. The global understanding of the biological importance of vitamin D has been translated in to big advisory programs directed toward increasing social awareness of the issue of vitamin D deficiency. Particularly, gynecologists and midwives directed their efforts to ensure that pregnant and menopausal stage women are receiving amounts of vitamin D that meet their daily needs to avoid unfavorable deficiency effects on mothers and fetus. Also, another aspect that is widely considered is lactation

periods since lactating women require higher doses of vitamin D to meet both mother and baby needs<sup>[8,9]</sup>.

Moreover, several studies discussed factors influencing vitamin D status, these factors include age, gender, pregnancy, physical abilities and chronic disease status<sup>[10,11]</sup>. The biosynthesis of vitamin D in the skin drops with ageing, thus older individuals are expected to have low vitamin D levels. Moreover elderly are not able to produce adequate vitamin D from solar UV irradiation as they spend less time outdoors than younger people do; one reason for the limited outdoor activities is chronic diseases such as cancers and osteoporotic fractures which keep seniors in bed most of the day<sup>[10,12]</sup>. Also, in female population in particular, the effect of age and menopausal status have been discussed by several researchers who found out that postmenopausal women have decreased vitamin D and calcium storage due to the lack of estrogen produced by the ovary<sup>[13]</sup>.

In addition, several studies have proved that gender has a considerable impact on vitamin D levels. A number of studies in USA<sup>[14]</sup>, the United Arab Emirates<sup>[15]</sup> and the Middle East<sup>[16]</sup> have revealed that 30 to 50% of adult females had 25hydroxyvitamin D levels under 20 ng/ml due to the use of sun screen. Although sunscreen blocks the harmful UV rays that cause skin cancer, but it also blocks most of the skin's production of vitamin D. Furthermore, studies conducted in Islamic countries such as Iran and Saudi Arabia<sup>[17,18]</sup>, have revealed that females who cover most of their bodies with hijab, have lower levels of vitamin D due to the limited sunlight exposure.

Moreover, geographical location could have great influence on serum vitamin D levels. Several data obtained from African countries showed higher base line of vitamin D in comparison to other parts of the world, this difference is strongly related to the sun light exposure throughout the year. On the other hand, cultural and gender differences might have an impact on serum vitamin D levels. Type of clothing, particularly the hijab, could decrease the amount of vitamin D obtained from sun light exposure<sup>[19-21]</sup>. Basically, older people from both genders and younger females are expected to have low levels of vitamin D due to aging, use of sunscreen and type of clothing.

Indeed, the previously discussed factors can be applied in the city of Misurata; therefore, a hypothesis was set that vitamin D deficiency might be prevalence among the population of Misurata. In addition, number of questions needed to be answered such as whether age, gender and woman menopausal status have the same significant impact on vitamin D status or not. The presented hypothesis was rigorously examined and discussed in the following sections.

### **Methodology:**

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In this prospective study, a total of 125 adult samples were divided into four groups based on gender and age; premenopausal women, postmenopausal women, males older than 45 years and males younger than 45 years. Pregnant women, patients with diagnosed bone problem and individuals who receives vitamin D supplementation were excluded to avoid the deficiency due to extra need, already diagnosed inadequacy and treated patients respectively. In addition, All samples were taken in narrow time range, July and August 2017, to minimize the effect of different seasons on vitamin D level. Patient information forms were submitted to two medicine clinics to collect old age samples (men older than 45 and post-menopausal women) and two gynecology clinics for collecting younger age samples from both gender. Suitable subjects were directed to the chosen lab, Lames lab, accompanied by a completely filled and signed information form where free tests were performed.

Samples were analyzed in Lames laboratory and serum vitamin D was determined based on a competitive enzyme linked immunosorbent assay (ELISA), utilizing ELISA KIT LOT NO. 1709732. Vitamin D concentration in all samples was photometrically measured at 450 nm, and the intensity of the yellow color was inversely correlated to the concentration of vitamin D in the sample. All samples were analyzed by the same operator and using the same, only one, analysis kit to minimize the analytical variation.

Microsoft Excel 2016 was used for the summarization and graphical illustration of all data; Excel was also used to calculate central tendency and dispersion parameters. Student T-test was utilized to compare the means of groups. Furthermore, all descriptive analysis and t-test were performed via Excel functions. SPSS was used to draw boxplots.

### Results and discussion:

Table 1 and Figure 2 summarize the obtained results of vitamin D serum concentration in the studied groups. In the boxplots (Figure 2); the X-axis represents serum concentration in ng/ml. The lines inside the boxes represent the median, the borders of the boxes are the third and first quartiles while any value outside the whiskers regarded as an outlier.

Table 1: Results of vitamin D serum concentration of participants

	<b>Premenopausal women (ng/ml)</b>	<b>Postmenopausal women (ng/ml)</b>	<b>Males above 45 years (ng/ml)</b>	<b>Males below 45 years (ng/ml)</b>
Mean conc. ± SD	10.96 ±4.59	12.47 ±5.96	14.12 ±4.74	21.90 ±9.56
Max	24	24	25.51	45
Min	3	4	8.6	9
Median	11.7	11	12	22

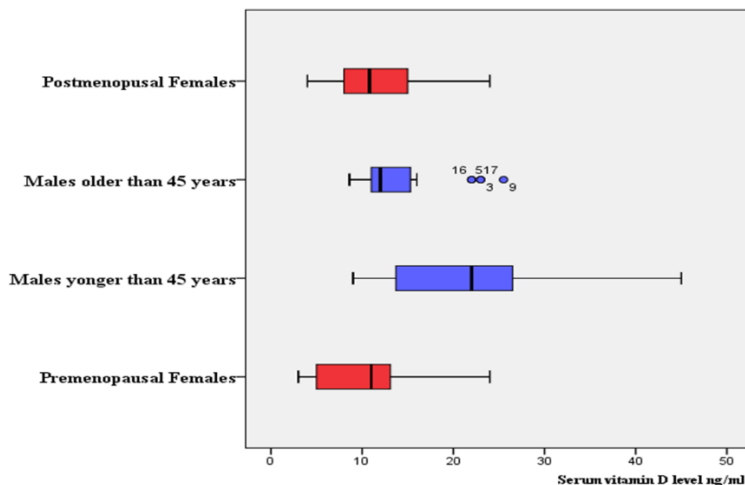


Figure 2: Boxplot graph summarizes serum vitamin D results

In order to obtain better illustration, the results were classified into sufficient (above 30-50 ng/ml), insufficient (20-30 ng/ml) and deficient (below 20 ng/ml) as shown in Figure 3. The presented data clearly demonstrate that the mean and the median of serum vitamin D concentration in males below 45 years can be considered as insufficient level; However, this level was significantly higher than the other three groups which are regarded as deficient in vitamin D. This difference could be due the fact that younger males spend more time outdoors in comparison to females and older males.

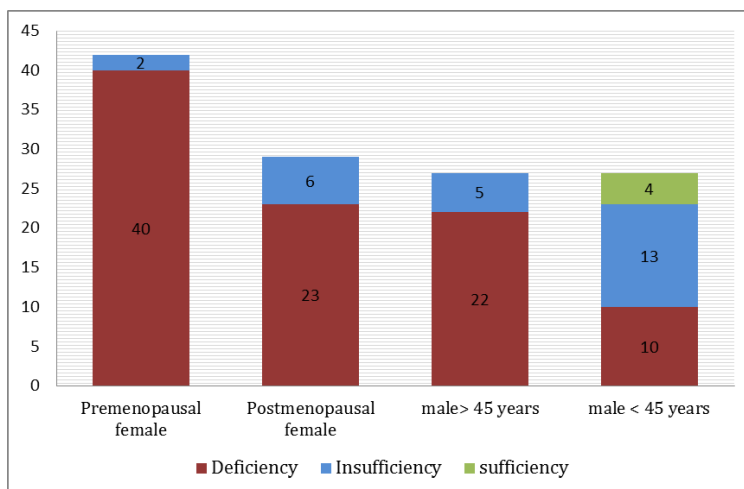


Figure 3: Bar graph representation of the prevalence of vitamin D status

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Figure 3 shows that neither females nor males above 45 years were found with sufficient vitamin D; in fact, deficiency was predominant in about 90% of premenopausal women and in about 80% of both older males and postmenopausal females. Hijab and application of sunscreen could be the reason behind high percentage of deficient vitamin D results among younger females, whereas lower skin performance in seniors could explain the inadequate vitamin D levels in this group. Moreover, in postmenopausal females' hormonal changes and the type of clothing are the most relevant factor resulted in the vitamin D deficiency. On the other hand only 35% of males below 45 years were found deficient in vitamin D; however, less than 20% of the cases had sufficient vitamin D level in this group. The results of all groups support the research hypothesis where serum vitamin D inadequacy was predicted. Actually, the obtained results were not unique hence the deficiency was reported frequently around the world; for example, similar results were reported in Saudi Arabia, Tunisia, Morocco, the United Arab Emirates, Australia, Turkey, and Lebanon, where more than 50% of adult populations had 25-hydroxyvitamin D levels under 20 ng/ml<sup>[18, 22-27]</sup>.

To further evaluate the effect of gender on vitamin D status excluding the age and postmenopausal stage factors, a comparison was made between males younger than 45 years and premenopausal females (Table 2). As previously stated, the mean serum concentrations of vitamin D is higher in males younger than 45 years than in premenopausal females, CV% and SD values are large in both groups denoting wide variation, also median and mean are close in both groups indicating that data are normally distributed.

Table 2: Summary of vitamin D serum concentration (ng/ml) in males < 45 years and premenopausal women

	<b>males &lt; 45 years (ng/ml)</b>	<b>premenopausal women (ng/ml)</b>
No. of subjects	27	42
Mean ± SD	21.90 ±9.56	10.96 ±4.59
CV%	43.66%	41.92%
Median	22	11.7
Research hypothesis	Mean of younger males □ Mean of younger females	
Two tailed t-test result	0.00000335972	
Interpretation of t-test results	As the result is smaller than 0.05, the difference between the two groups is significant	

Although vitamin D deficiency is predominant in both groups, the values of the two means and t-test results support the research hypothesis where lower

vitamin D level was expected in female serum than in male due to limited outdoor activity and hijab factors affecting the female population in Misurata. Comparing the study outcome to the reviewed literature, in one hand, there are some studies in which no significant difference was found between the two genders<sup>[28,29]</sup>. On the other hand, the majority of the reviewed articles results were consistent with research hypothesis and with the obtained results<sup>[30]</sup>.

In order to evaluate the effect of age and menopausal status on vitamin D levels while excluding the impact of gender, the group of males below 45 years was compared to males above 45 years. In addition, premenopausal women were compared to postmenopausal women as presented in table 3 and table 4.

Table 3 : Summary of vitamin D serum concentration (ng/ml) in men below and above than 45 years

	<b>Male below 45 years (ng/ml)</b>	<b>Male above 45 years (ng/ml)</b>
No. of subjects	27	27
Mean $\pm$ SD	21.90 $\pm$ 9.56	14.12 $\pm$ 4.74
CV%	43.68%	33.56%
Median	22	12
Research hypothesis	Mean of male below 45y $\square$ Mean of male above 45y	
Two tailed t-test result	0.000528	
Interpretation of t-test results	As the results is smaller than 0.05, the difference between the two groups is significant	

As shown in table 3 and figure 3, mean serum concentrations of vitamin D is significantly higher in younger than in older males. However, participants in both groups demonstrate predominant deficiency, Concentrations of both groups are widely distributed as it can be noted from high CV values. The significant difference in mean values between the two groups is supported by the result of t-test which shows statistical significance of the difference between the two means. The obtained results are consistent with the research hypothesis and can be explained by differences life style as older people have less outdoor activities. Another reason for low serum vitamin D level in males above 45 years could be the reduced liver function that would affect the absorption of vitamin D if the liver is not producing normal amounts of bile<sup>[31]</sup>. In addition, the ability of skin to produce vitamin D decrease in seniors<sup>[32]</sup>.



Table 4: Summary of vitamin D serum concentration (ng/ml) in premenopausal and postmenopausal women

	<b>Premenopausal women</b>	<b>Postmenopausal women</b>
No. of subjects	42	29
Mean $\pm$ SD	10.96 $\pm$ 4.59	12.47 $\pm$ 5.96
CV%	41.92%	47.8%
Median	11.7	11
Research hypothesis	Mean premenopausal $\square$ Mean postmenopausal	
Two tailed t-test result	0.255985	
Interpretation of t-test results	As the results is larger than 0.05, the difference between the two groups is insignificant	

Oppositely to male groups, no significant difference was observed among female groups according to age/menopausal state, which contradict the research hypothesis of lower vitamin D level in postmenopausal women. In males, factors like outdoor activities and skin ability to produce vitamin D were the proposed explanation, same factors can explain the insignificant difference among female groups. Firstly, according to the social and cultural theme of Misurata, similar outdoor activities can be proposed in female of all ages. Secondly, skin ability to produce vitamin D will not be important effector if hijab is taken in consideration. In other words, low skin exposure to sunshine will minimize the produced vitamin D regardless the skin ability hence most of the adult females in Misurata cover their bodies. In contrast, young females may have less sunshine exposure as they use sunscreens on uncovered skin more frequently than older women do, especially for samples collected in summer which represent substantial portion of all collected samples. The obtained findings of no significant difference among female based on their menopausal state is not unique as similar results were reported in two studies conducted in Iran where Hijab is predominant<sup>[30]</sup>. Supportably, the significant difference that can be caused by the Hijab can be noticed in many studies conducted in Tunis, Jordan and Turkey where higher vitamin D level found in female with western dressing style<sup>[22-27]</sup>. In summary, low sunlight exposure has more impact on vitamin D levels among women population in Misurata in comparison to age and menopausal status.

**Conclusion:**

In conclusion, vitamin D is an essential element which could affect general health status. Similarly, to most parts of the world, vitamin D deficiency was

found predominant among the population of Misurata. Although higher serum concentration of vitamin D was observed in younger males, all studied groups exhibit inadequate levels of vitamin D in their blood. Higher serum vitamin D in males in comparison to females is probably due to limited exposure to sun light among females; for the same reason the effect of age differences was intense among the males as older males are less engaged in outdoor activities. Empirical treatment with vitamin D supplements could be a beneficial solution for the deficiency problem; as vitamin D has a wide margin of safety. Also, health care providers are encouraged to develop advisory programs to increase the public awareness of the harmful effects of vitamin D deficiency as well as emphasizing the importance of adequate sun light exposure as a natural source of vitamin D. In addition, more studies should be conducted in order to obtain better understanding of different factors affecting levels of vitamin D among other populations in community such as pregnant women, people with chronic diseases and children.

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