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The effects of light on vitamin D level in human serum during laboratory processing



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ABSTRACT

This study aimed to analyze the impact of light on the amount of 25- hydroxyvitamin D [25-(OH) D] in the processed serums of the participants that have been referred to the diagnostic lab (King Diagnostic Laboratory) which process tests for around 150 collection sites around Erbil, Iraq. Scientists and researchers have declared a strong association between vitamin D status and various health issues. Many studies have now shifted to the interaction among/between genes and environment that impact different illnesses correlated with vitamin D. During the periods between 01/April/2020 till 01/ April/2021, the total number of cases (N = 5800) of vitamin D content detection in serum have been performed. However, this investigation found that there was a high deficiency (%) in vitamin D levels (nearly 80 %) among the participants contributed to our study and data analysis revealed that they had 25-(OH) D levels very less at 20 µg/L concentration. The amount of vitamin D levels did not vary about with respect to gender and no statistical significance between female and male participants were observed ($P > 0.05$), moreover, the variation in the group that was subjected to light differed significantly ($P > 0.05$). The present research work reveals that there is a significant variation in the use of dark and light collection and testing tube in the process of detecting serum vitamin D levels.

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1. Introduction

Biochemical and histopathological alterations were induced by subchronic exposure of various metals (Ramadan et al., 2022; El-Beeh et al., 2022). Bioactive vitamin D which is also called calcitriol plays a major role in regulating body phosphorus and calcium levels, and in bone mineralization (Demer et al., 2018). Moreover, the biological effect of this hormone has been further proved by the presence of several cell receptors for vitamin D which extend far beyond the role of the metabolism of minerals. Vitamin D is one of the unique vitamins and is biologically synthesized by the human body in the skin by using the sunlight. This vitamin exists in two different forms, namely, D2 and D3. The main dietary source of Vitamin D2 is yeast, mushrooms and the result of

UV irradiation. Humans synthesize vitamin D3 using the sun's UVB light and this is considered the natural form of vitamin D. Generally, human beings do not prepare vitamin D2 and the major source of vitamin D2 for human is fish such as mackerel, salmon and herring. In the human body, vitamin D is ingested into chylomicrons, and is absorbed, by the lymphatic system of the body and finally transported to venous blood. Vitamin D required to undergo a biological processes and human body accumulates after various hydroxylation process. In the human body, the available 1,25(OH)₂D induced the absorption of intestinal calcium. The lack of Vit-D in the human body leads to the loss of absorption of dietary calcium and phosphorus from the diet. The high available vitamin D levels showed improved phosphorus and calcium absorption. Vitamin D can be a steroid hormone and is fat-soluble. Vitamin D has several functions, including, immune regulation and functions of the brain, muscle, pancreas, and the control of cell cycles (Pilz et al., 2019; Abdul-Hassan Masser et al., 2021).

In recent years the prevalence of vitamin deficiency, especially vitamin D deficiency and its consequences appears to be increasing globally, and the impact on human health is crucial because of the relationship between vitamin D deficiency with increased risk of many diseases including cardiovascular disease, osteoporosis and

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a certain type of cancer (Jolliffe et al., 2019). A comprehensive understanding of the genetic factors involved in determining vitamin D status is, therefore, necessary to assess potential interactions between the gene and the environment of vitamin D (Shea et al., 2009). Previous studies of twins and families have shown that variations in genetic levels significantly contribute to differences in 25-OH-D levels, with an estimated heritability of up to 53%. Although many rare Mendelian disorders cause functional vitamin D deficiency. The gene of interest was studied to investigate the effects of a gene of interest in the vitamin D pathway, moreover, these investigations were limited by a small number of variables and the sample size was also less (Ramos-Lopez et al., 2007). Several studies in humans have announced genetic variants related to vitamin D levels. Studies are classified according to their purpose, such as contributing genes in the vitamin D pathway linkage studies and genome-wide association studies (GWAS).

In recent years, there is tremendous development and changes in human life which are changed food habits and the environment. The variations in lifestyle, food intake, use of highly processed food, and avoidance of sunlight led to the deficiency of vitamin D among populations (Roth et al., 2018). The decreased level of vitamin D is correlated with a decreased level of calcium in the human body. The decreased level of calcium in the bones leads to poor mineralization of bones and further development of diseases like osteoporosis in adults and rickets in children (Peterlik et al., 2009). The deficiency of calcium in the bones induces bone deformation, and increase in susceptibility to bone fractures in adult and old age groups. Hence, adequate vitamin D supplement is required to maintain body health and to contribute to metabolism (Abdul-Hassan Masser et al., 2021; Abdulrahman and Rahman, 2018). In recent years tremendous efforts were made to analyze the impact of vitamin D deficiency among the human population. However, the molecular mechanism and vitamin D and the role of pleiotropic hormone remains unclear. The clinical significance of 25(OH)D3 and the molecular mechanism in vitamin D biosynthesis is not evaluated. Hence, various randomized experimental trials are required to determine the significance of vitamin D and the level of serum 25(OH)D3 (Pilz et al., 2019). The main objective of the study is to analyze the effect of sunlight on vitamin D synthesis in male and female cases.

2. Materials and method

2.1. Subject

This study has been performed for one year starting 01/April/2021 in King Diagnostic Laboratory based in Erbil, Iraq. These samples from 5800 participants have included based on dividing samples taken from the patient into a dark tubes and light tubes. Both SST tubes were from the same company only the dark tubes has been covered with black tape to prevent lights from entering the serum. SST tube is used for a wide variety of tests including hormones, electrolytes, biochemical and vitamins.

2.2. Sample

The blood sample was collected from the participants (2 mL) using a sterile syringe rinsed previously with an anticoagulant. Then blood sample was collected using a trained professional. The collected blood sample was centrifuged and the serum sample was separated. The centrifuged pale yellow serum sample was used for the determination of vitamin D using the Roche E411 Analyzer. A vitamin D diagnostic kit was used for the quantification of serum vitamin D.

2.3. Analysis of vitamin D

The vitamin D level in the serum sample was evaluated using a clinical diagnostic vitamin D kit using an Analyzer (Cobas E411 model, Roche). The two tubes one with tape-covered (Black Tube) and another without (Transparent Tube) contained serum from the same patient has been gently applied to the analyzer. The amount of vitamin D was tested using this automated machine. The results were registered and the patients were categorized based on the amount of vitamin D in the serum sample. It was classified as, adequate group (30 – 70 µg/l), intoxication (≥ 70 µg/l), optimal group (20–30 µg/l), and deficient group (10–20 µg/l) and those with insufficient levels of < 0.05 has been considered as statistically significant.

2.4. Statistical test

The data were analyzed between the groups and compared with a statistical package (SPSS, version 14.0) using Microsoft windows. The standard deviation was tested using a *t*-test and the *p*-value < 0.05 was considered statistically significant.

3. Results

In the present study the samples of patients referred to King Diagnostic Laboratory during the periods of 01/April/2021 till 01/April/2022 were included. About 5800 tests of vitamin D have been performed during this time. In this study, the patients were categorized based on gender type. It was divided into the adequate group 30–70 µg/l, Intoxication ≥ 70 µg/l, Optimal group 20–30 µg/l, Deficient group 10–20 µg/l and those with insufficient levels of < 10 µg/l. The ratio of participants has been calculated based on gender and the study found that the female rate was higher than a male with 63.2% and 36.8% respectively (Table 1).

Paired sample *t*-test were used to analyze the vitamin D content within serums in the dark tube to the light tubes. The analyses revealed significant variation between the mean of the dark tube groups (M = 16.45, SD \pm 11.62) and Light tube groups (M = 16.01, SD \pm 11.28) conditions, *t* (5800), *P* = 0.856. The Dark tubes is 11.62 while the Std. Error Mean is 1.31, the standard deviation of light tubes is 11.28, the Std. Error Mean is 1.34, and the standard deviation (Table 2).

4. Discussion

DHCR7/NADSYN1 is an important locus and is associated with vitamin D levels in the human body. DHCR7 gene encodes 7 dehydrocholesterol reductase and this enzyme converts 7-dehydrocholesterol (7-DHC) to cholesterol by eliminating the required substrate in the vitamin D3 synthesis pathway. A precursor to 25-OH D3 is involved in the vitamin D3 synthesis pathway. The gene-level mutation in DHCR7 results in the development of Smith-Lemli-Opitz syndrome. This disease is characterized by low cholesterol levels, accumulation of 7-DHC, low level of 7-dehydrocholesterol reductase enzyme activity and multiple birth defects (Tint et al., 1994). DHCR7 mutations have also been suggested to have several advantages to heterozygous carriers and

Table 1
The average of the participant based on gender.

Gender	Frequency	Percent	Valid Percent
Female	3700	63.8	63.8
Male	2100	36.2	36.2
Total	5800	100	100

Table 2
Pared sample *t*-test showing the differences between Means of both tubes.

Group	Mean	N	Std. Deviation	Std. Error Mean
Dark	16.45	5800	11.62	1.31
Light	16.01	5800	11.28	1.34

an increased amount of 7-DHC may protect from the development of osteomalacia and rickets due to vitamin D deficiency. Moreover, there are data on vitamin D content in individuals with known mutation carriers or the syndrome of Smith Lemli-Opitz. The differences in the level of DHCR7 that was reported previously and were associated with circulating 25-OH D revealing that this enzyme may play a more important role than previously thought in the regulation of vitamin D status (Rossi et al., 2005).

The main objectives of the present investigations were to improve the laboratory quality in processing vitamin D level detection for the patient and to evaluate the influence of light on the serum vitamin D level. For such purpose, the patient sample has been divided into two tubes before centrifugation. One was covered with dark tape which is used for dark detection, and another had been left as it is which represented the light treated samples. The two tubes have been loaded onto the analyzer at the same time to eliminate any differences related to time and other cofactors. There are various studies revealed the protective property of vitamin D on heart diseases. Patients with a reduced levels of vitamin D pose a serious risks of cardiovascular diseases. The human population diagnosed with a low levels of vitamin D is associated with hypertension. In the human body, vitamin D receptor is reported. The reported 1,25(OH)₂D has several biological functions, such as inducing terminal differentiation, inhibition of cellular proliferation, stimulating insulin production inhibiting angiogenesis, stimulating macrophage, and inhibiting rennin production. The available, 25-dihydroxyvitamin D participates in various metabolic pathways which is useful to determine the significance of vitamin D. Vitamin D deficiency increased the risk for various chronic diseases including, infectious disease, type 2 diabetes, cardiovascular diseases and certain types of cancers. In the human population adolescents and infants are high at risk of vitamin D deficiency because of the increased requirement of vitamin D. Breastfed children are highly susceptible to the development of rickets. Generally, breast milk contains vitamin D, however, vitamin-deficient mothers are at high risk for the development of rickets. In elderly cases, vitamin D deficiency was reported. Infants required less exposure to sunlight than adults for the biosynthesis of vitamin D.

Erbil city of Iraq is evidenced to be semi-arid climatic conditions and during summer very dry and hot, however, in winter season it is very cold and wet climatic conditions. Moreover, the study area has four different seasons and most of the time there are sunny day that mean there is a lot of sun light. Hence, usually the amount of vitamin D biosynthesis and the level of vitamin D is extremely high and have been reported previously (Fields, Linnville and Hoyt, 2016). In this study, we have tested vitamin D content in patients admitted to the King Diagnostic Laboratory during the periods starting from 01/April/2021 till 01/April/2022. Thus, the total sample processed during this year and included in this study were 5800 blood samples from different age and gender as well as within 150 collection points around Erbil city.

The amount of analyzed vitamin D level in the serum sample varied widely and classified based on the availability of vitamin D. The vitamin D level, which is less than 20 µg/l is considered a deficient group, and the amount less than 10 µg/l was considered as an insufficient group. This has been used to determine the mean difference within the grouped genders. In this study, the number of female participants was higher than in male cases. A total of 63.8 %

of participants were females and the remaining 36.2 % of cases were males (Table 1). The selected study area is almost anytime hot with the sunlight that cannot stand under it and all these make the females stay indoors and have very little chance to expose to sunlight. The decreased exposure to sunlight leads to vitamin D deficiency.

Then we grouped our samples based on the light source that we used to collect the samples into light tube and dark tube to analyze the influence of light source on vitamin D level in the serum sample. The mean for each of them has been calculated and found that for the light is 16.01 and for the dark is 16.45 (Table 2). The mean level of vitamin D in Light tube was decreased than the mean level of serum vitamin D within the dark tube this is because of the lack of light in a dark tube. This is might be due to the UV light destroying the crystalline 25(OH) vitamin D or 1,25OHD within a few minutes. This finding is opposition to (Alnagar, 2018). The variation of vitamin D levels in relation to light and dark tubes is not statistically significant ($p > 0.05$).

5. Conclusions

In recent years, a large population of males and females are diagnosed with vitamin D deficiency. The analysis of vitamin D levels to the light and dark cycles is very important to conclude the use of vitamin D supplements and the application of sunscreen creams. Naturally, our skin is a major source of vitamin D and various dermatological factors influence the production of vitamin D. Hence, skin health is very important in the production of vitamin D. There are various factors involved in maintaining an optimum level of vitamin D in the human body. So, sunny climate is not only a single factor in maintaining adequate vitamin D levels in the human body. Adequate research is required to elucidate the impact of dermatological factors along with light factors on vitamin D levels in the serum.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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