Research Article

Main Factors Contributing To Vitamin D Deficiency In A Sample Of Iraqi Population

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Abstract

Vitamin D is known to play a vital role in each of; calcium metabolism, bone health, and immune function. The present study aimed to evaluate serum vitamin D levels in a cohort of individuals recruited randomly from Iraqi population from Diyala Province for the period from January to April 2025 and validated the performance characteristics of the Mindray CL-series VD-T test. A total of 163 apparently healthy individuals were enrolled from outpatient clinics. Serum samples were collected and analyzed using chemiluminescent immunoassay (CLIA) methods.

Results revealed a relatively high prevalence of vitamin D deficiency among the participants, with more than 84% showing vitamin D serum levels less than 20 ng/mL, indicating clearly a widespread serum vitamin D deficiency in the studied sample group. Test performance evaluation demonstrated an excellent precision, linearity, and specificity, with a detectable range of 3.0–130 ng/mL and a correlation coefficient (r) of 0.990. Cross-reactivity studies proved the strong specificity of the test for 25-hydroxyvitamin D3 and D2, with least interference from other vitamin D metabolites. In conclusion, the results revealed the urgency for public health awareness in aspects of; lifestyle, dietary habits, and sun exposure, particularly among young people. The VD-T test has proven to be a reliable tool for accurately tracing of serum vitamin D levels in various clinical settings.

Keywords: Factors, Vitamin D Deficiency, Iraqi Population.

1. Introduction

Vitamins are occasionally described as the micronutrients, and in spite of their small size, they perform giant functions. Getting enough, but not too much vitamin quantity, is crucial, due to both deficiency and toxicity can have a harmful effect (Asensi-Fabado & Munne´-Bosch, 2010).

The generic term vitamin D specifies a group of chemically related compounds that retain antirachitic activity. Formerly, Vitamin D was first described as a vitamin in the twentieth century, but nowadays it classified as a prohormone. Variety of vitamin D forms are available; Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are the two most common forms of vitamin D (Benedik, E. 2026).

Vitamin D_3 is built in human skin and also can be taken from animal-based meals, primarily fish oils, but vitamin D_2 is acquired from plant sources, it is not completely human-made, and can be added to diets. Only difference is in the side chain structure of vitamin D_2 and D_3. These differences have no obvious effects on their metabolism (i.e. activation), and the two versions carry out the same functions as a prohormone (Wang et al., 2006), (Azizova, U. H., et al. 2022).

Vitamin D plays a well-known role in maintaining calcium homeostasis, which is associated with sustaining bone health. It enhances the efficiency of intestinal absorption of the dietary calcium, reduces calcium loss in the urine, and act to mobilizes stored calcium in the skeleton. However, vitamin D receptors are prevalent in the human body, and additionally vitamin D has an abundance of non-calcium functions (Psaty et al., 2010), (Šimůnek J, et al. 2022).

In contrast to majority of other vitamins, plenty of vitamin D can be generated in human skin. However, its synthesis can be effectively reduced due to multiple factors such as clothing, sunscreen, intentionally avoiding direct sunlight, or the elevated latitude of place of residence (Psaty et al., 2010).

Actually, it estimated that more than one billion people around the world can suffer from the vitamin D deficiency, and this state often goes without optimum diagnosis (Janoušek, J., et al; 2022). Vitamin D long standing defect is not only accompanied by rickets / osteomalacia / osteoporosis, but can further associated with an elevated risk of inititing high blood pressure, type 1 diabetes mellitus, multiple sclerosis, or even cancer. In fact, administering the vitamin D supplements can be beneficial, but receiving vitamin D dosage forms should be under supervision of the health professionals since taking an exaggerated doses of the vitamin D may lead to toxicity associated with variety of serious health consequences (Psaty et al., 2010)

Recently, multiple studies tracing the prevalence of vitamin D deficiency have markedly increased in each country across the world. Despite, there is remain no study measuring each of global and regional frequency of the vitamin D deficiency. Furthermore, the

process of estimation is time-consuming, toilsome, and costy to perform a large-scale trial on the global population (Cui A, et al; 2023).

Socioeconomic status of individual may be accompanied by vitamin D deficiency. So, the individuals living in Lower-middle-income and Low-income countries receive better care. Laboratories with different detection assays are may be also factors affecting the prevalence, therefore, studies manipulating similar detection assays will enhance the comparability of the results. Another significant risk factors include the genetic traits, dietary habits, clothing style, time intervals spent outdoors, skin pigmentation, and others (Black L. J., et al; 2021).

The present study aimed to examine the prevalence of vitamin D deficiency in our population and focusing on the major lifestyle concerns behind the deficiency, as well as, we examined the reliability and accuracy of currently depended approach for serum vitamin D estimation (the VD-T test).

2. Methodology

Study Design and Setting:

A cross-sectional assessment of the reference range was performed to determine the normal values of total 25-OH vitamin D using the VD-T test. Samples from 163 patients were collected from outpatient clinics, and other demographic data were recorded in a pre-designed questionnaire after obtaining patient consent.

Primary objective:

The aim of this experiment is to determine the concentration of 25-hydroxyvitamin D (VD-T) in human serum and plasma samples using the Mindray CL-series Chemiluminescence Immunoassay (CLIA) reagent kit (Catalog NO. VD-T112).

Operational Definitions:

25-OH Vitamin D Total : Refers to the total concentration of 25-hydroxyvitamin D (both D2 and D3 forms) in human serum or plasma. It is considered the most reliable indicator for assessing vitamin D status due to its longer half-life compared to the active form, $1,25(OH)_2D$. (Holick, 2007)

Vitamin D Deficiency : Defined as a serum 25-OH Vitamin D concentration below 20 ng/mL, based on current clinical guidelines **(Holick, 2011).**

Vitamin D Sufficiency :Defined as a serum 25-OH Vitamin D concentration between 30–100 ng/mL, representing adequate levels for bone and overall health. (Holick, 2011)

Chemiluminescent Immunoassay (CLIA) : An analytical technique used for quantitative measurement of 25-OH Vitamin D. It involves competitive binding reactions and chemiluminescence signal detection, with results expressed in relative light units (RLU).

Reference Range :The interval between the 2.5th and 97.5th percentiles of serum 25-OH Vitamin D concentrations in a healthy population. (Lips, 2001)

Ethical issue:

This study was anonymous, voluntary, beneficial, and harmless.

Human subject protection:

Taken information will be secured and without names and they will be well informed about the purposes of the study.

Assay Principle:

The CL-series VD-T assay is a competitive binding immunoenzymatic assay designed to determine the level of 25-OH Vitamin D Total in human serum or plasma.

Step 1: Sample Preparation: The sample, along with pretreatment reagent 1 and pretreatment reagent 2, are added into a reaction cuvette. After incubation, the bound 25-OH Vitamin D is released from the Vitamin D binding protein (DBP).

Step 2: Antibody Binding: The pretreated sample is then mixed with the anti-25-OH Vitamin D antibody - alkaline phosphatase conjugate in a separate reaction cuvette. After incubation, a complex is formed between 25-OH Vitamin D and the antibody.

Step 3: Microparticle Binding : Paramagnetic microparticles coated with streptavidin and biotinylated 25-OH Vitamin D are added into the reaction cuvette. After a third incubation, the biotinylated 25-OH Vitamin D competes to bind to the antibody-alkaline phosphatase conjugate. The resulting biotinylated 25-OH Vitamin D: antibody complexes are bound to the streptavidin on the microparticles. These complexes are magnetically captured, and any unbound substances are removed by washing.

Step 4: Substrate Reaction: The substrate solution is added to the reaction cuvette. The substrate is catalyzed by the anti-25-OH Vitamin D antibody - alkaline phosphatase conjugate in the immunocomplex retained on the microparticles. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by a photomultiplier built into the system.

The amount of VD-T in the sample is inversely proportional to the RLUs generated during the reaction. The VD-T concentration is determined through a calibration curve.
The samples were handled carefully to prevent cross-contamination. Grossly hemolyzed specimens and heat-inactivated specimens were not used. The specimens were tested as soon as possible after collection. If testing was not completed within 8 hours, specimens were stored at 2-8°C or colder. Specimens were stable for 4 days at 2-8°C and for 180 days at -20°C.

Assay Procedure:
For optimal performance of this assay, the related system operation manual was read carefully to obtain sufficient information such as operation instructions, sample preservation, safety management, precautions, and maintenance. All required materials for the assay were prepared. Before loading the VD-T (CLIA) reagent kit onto the machine for the first time, the unopened reagent bottle was inverted gently at least 30 times to resuspend the microparticles that had settled during shipment or storage. The bottle was visually inspected to ensure the microparticles had been resuspended. If the microparticles remained adhered to the bottle, inverting continued until they were completely resuspended. If the microparticles did not resuspend, the reagent bottle was not used, and Mindray Customer Service was contacted for assistance.

Table.1: Cross-Reactivity of VD-T Assay with Various Vitamin D Metabolites

Substance	Concentration (ng/mL)	Cross-reactivity (%).
25-Hydroxy-vitamin D3	100	100
25-Hydroxy-vitamin D2	100	100
Vitamin D3	0.60	0.60%
1α,25-Dihydroxy-vitamin D2	Not detectable	97.60%
1α,25-Dihydroxy-vitamin D2	Not detectable	detectable

This table illustrates the cross-reactivity of the VD-T assay with different forms of vitamin D. The assay shows full cross-reactivity with 25-hydroxy-vitamin D2 and D3, while the response to other metabolites such as 1α,25-dihydroxy-vitamin D2 and D3 is minimal or not detectable, indicating high specificity for 25(OH)D.

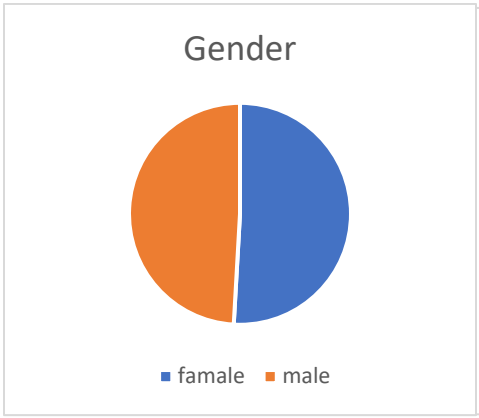
3. Results

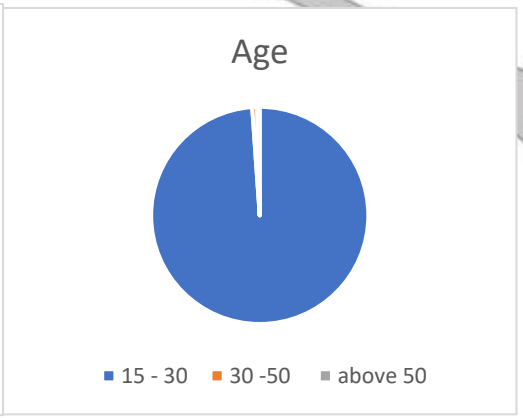
Section 1 - Demographic data:
Table. 6: Demographic data of participants

Variables		Number of participants	Frequency
Gender	Female	83	50.9%
	Male	80	49.1%
	Total	163	100%
Age	15-30	148	90.8%
	30-50	10	6.1%
	Above 50	5	3.1%
	Total	163	100%

The survey results showed a relative balance between males and females within the sample of 163 participants, with females accounting for 50.9% (83 participants) and males for 49.1% (80 participants). This balance provides the study with good gender comparability without apparent bias towards either party. It also provides a suitable opportunity to analyze potential differences in behaviors or health symptoms between males and females in a more statistically fair manner.
In terms of age distribution, the overwhelming majority of participants were within the 15-30 age group, representing 90.8% (148 participants). Only 6.1% (10 participants) were between the ages of 30 and 50, while the percentage of those over 50 did not exceed 3.1% (5 participants). This distribution demonstrates that the data extracted from this survey largely represents the youth population. Therefore, any health or behavioral findings or conclusions apply primarily to this specific group and cannot be accurately generalized to older age groups without an additional, expanded sample that includes those groups.

Fig.3: Demographic data of participants





Section 2 - Lifestyle and Health Behavior:

Q1: How much sun exposure do you get daily

Table 7: Distribution of Sun Exposure Duration Among Participants

Answer	Number of participants	Percentage
Less than 15m	40	24.5%
15m -1h	80	49.1%
More than 1h	43	26.4%
Total	163	100%

*p = 0.0001

Most participants were exposed to sunlight for 15 minutes to an hour per day, which is an ideal amount of time to stimulate vitamin D production in the body.

Q2: How often do you eat fish

Table. 8: Frequency of Fish Consumption Per Week Among Participants

Answer	Number of participants	Percentage
Don't eat	42	25.8%
Ones in week	70	43%
More than 3 in week	51	31.3%
Total	163	100%

*p = 0.0233

About 44% of participants eat fish regularly, which is an important source of vitamin D. However, a large percentage (25.8%) never eat fish.

Q3: How often do you drink milk

Table. 9: Frequency of Milk Consumption Among Participants

Answer	Number of participants	Percentage
Yes	92	56.4%
No	45	27.6%
Often	26	16%
Total	163	100%

*p ≈ 0

More than half of the participants regularly consumed milk, a major source of vitamin D. However, a significant proportion of participants (27.6%) did not consume milk at all.

Q4: How often do you eat egg

Table. 10: Frequency of eating eggs weekly

Answer	Number of participants	Percentage
Don't eat	92	56.4%
Ones in week	45	27.6%

More than 3 in week	26	16%
Total	163	100%

*p ≈ 0

About 82.7% of participants ate eggs regularly (daily or weekly), but a small percentage (3.1%) did not eat eggs at all.

Q5: Do you regularly eat white bread

Table. 11: Eating white bread weekly

Answer	Number of participants	Percentage
Yes	148	85.9%
No	18	11%
Without Answer	5	3.1%
Total	163	100%

p = 0

A large percentage (85.9%) of participants ate white bread, a type of food that is often low in important nutrients such as vitamin D.

Q6: Have you experienced hair loss

Table. 12: Percentage of Participants Experiencing Hair Loss

Answer	Number of participants	Percentage
Yes	130	79.8%
No	33	20.2%
Total	163	100%

*p ≈ 0

This finding suggests that hair loss is a common health problem in this sample, with 79.8% of participants experiencing it.

Q7: Do you experience muscle pain

Table. 13: Percentage of Participants Experiencing Muscle Pain

Answer	Number of participants	Percentage
Yes	112	68.7%
No	51	31.3%
Total	163	100%

*p = 0.00000177

Muscle pain is a common complaint among participants, with 68.7% of the sample experiencing it.

Q8: Do you feel frequent dizziness

Table. 14: Percentage of Participants Experiencing Frequent Dizziness

Answer	Number of participants	Percentage
Yes	94	57.7%
No	69	42.3%
Total	163	100%

*p = 0.0502

Approximately 57.7% of participants experienced frequent dizziness.

Q9: Do you have dental problems

Table. 15: Percentage of Participants Experiencing Dental Issues

Answer	Number of participants	Percentage
Yes	109	66.9%
No	54	33.1%
Total	163	100%

p = 0.000016

The results of the current study were exhibited a high percentage of participants undergoing dental problems (66.9%).

Q10: Do you suffer from dry mouth

Table. 16: Percentage of Participants Experiencing Dry Mouth

Answer	Number of participants	Percentage
Yes	87	53.4%
No	76	46.6%
Total	163	100%

*p = 0.3889

Nearly half of the participants suffered from dry mouth, a health problem that may be linked to vitamin D deficiency.

Q11: Have you previously had COVID-19

Table. 17: Percentage of Participants Who Have Had COVID-19

Answer	Number of participants	Percentage
Yes	43	26.4%
No	120	73.6%
Total	163	100%

*p = 0.0000000016

Less than 30% of participants developed COVID-19, a rate consistent with community infection rates at the time the survey was conducted.

Section 3 -Vitamin D Levels:

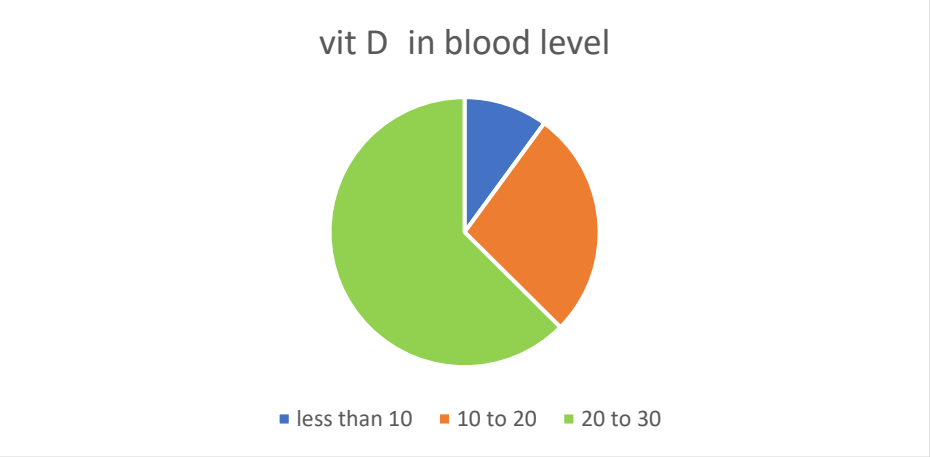
Table. 18: Vitamin D blood level

Vitamin D	Number of participants	Percentage
Less than 10	36	22.5%
10-20	101	61.3%
20-30	26	16.2%
Total	163	100%

*P= 0.0000000000000555

The study results show that the majority of participants suffer from varying degrees of vitamin D deficiency.

Fig.4: Vitamin D blood level



The category representing very severe vitamin D deficiency (less than 10 ng/mL) accounts for 22.1% of the total sample.

When combining the participants in the "less than 15 ng/mL" group with those in the "10–20 ng/mL" baseline group, this combined category accounts for 62% of the total sample, making it the largest proportion. This level indicates a moderate deficiency in vitamin D.

Participants with vitamin D levels in the range of 20–30 ng/mL make up 16% of the sample. Overall, the findings indicate that more than 84% of the participants have insufficient vitamin D levels (less than 30 ng/mL),

4. Discussion

The findings of the current study highlight a widespread nutritional deficiency of vitamin D among Iraqi population particularly youths. Over 84% of participants were found to suffer from vitamin D deficiency or at least insufficiency, a noticeably high figure

within an age group is typically proposed to be at minimal risk for nutritional imbalances. The unexpectedly high prevalence appoints a marked shift in lifestyle and increases serious concerns about the long-term implications of this deficiency, particularly in view of mounting scientific evidence linking hypovitaminosis D levels to skeletal, muscular, immune, and psychological consequences. (Bener et al., 2016; Matsuoka et al., 2020).

Approximately, a half of the participants reported sun exposure ranging between about 15 minutes and one hour per day. Nevertheless, the amount is proved to be insufficient for achieving an optimal vitamin D levels. This can be attributed to to several factors like; wearing of clothing that covers majority of skin, spending a prolonged time interval indoors, and lack of sun exposure at peak UVB hours. Added to the darker skin pigmentation that is common among the Iraqi population reducing the extent of skin's efficiency in synthesis of vitamin D and give rise to prolonged exposure requirement to produce an adequate vitamin levels. Previous research was suggested that individuals with a darker skin may require three times the sun exposure as compared to fair-skinned persons for the same magnitude of vitamin D (Palella et al., 2017).

Regarding the dietary perspective, the studied population revealed a low usual intake of vitamin D-rich foods such as milk, fish, and eggs, with a mass reliance on white bread (reported by 86% of participants), a food category that low in micronutrients. This common dietary pattern fails to achieve the daily recommended intake for vitamins especially D, particularly in lack of national food fortification programs applied. Those trends mirror a broader dilemma of nutritional monotony and poorer dietary habits, aggravating the risk of deficiency and the accompanied health sequela (Al-Saadi et al., 2019).

In terms of health manifestations, the participants revealed a high rates of hair loss (79.8%), muscle pain (68.7%), dizziness (57.7%), and dental issues (66.9%). These symptoms are clinically consistent with vitamin D deficiency consequences (Brito et al., 2017; Lee, W. C. et al., 2018; De Martinis M, et al. 2021). The presence of such symptoms in individuals without chronic illnesses strengthens the hypothesis that modifiable lifestyle behaviors—such as poor diet, physical inactivity, and insufficient sun exposure—are key contributing factors (Leite Â, 2025). Furthermore, these symptoms can adversely impact the mental well-being and quality of life of young subjects, emphasizing the significance of the ways of addressing this public health concern (AlGhamdi SA. 2024).

The current study further reveals that vitamin D deficiency is not merely a biological aspect, but a multifactorial state figured by various behavioral, environmental, and socioeconomic contributors. Vashi and colleagues for example, were noticed that many participants cited the scarcity of access to fortified foods species or the costly supplements as a means to enhance their health status. Furthermore, a general absence of public awareness and the lack of centered health education campaigns give rise to the underestimated significance of this vitamin's deficiency, even among educated ones (Vashi et al., 2018).

The literature also suggests that vitamin D deficiency may result in compromised immune function and enhancing susceptibility to infections as COVID-19, but the current study did not reveal a direct causation between vitamin D serum levels and COVID-19 infections, as with preceding studies, the link still merits more investigations, particularly in presence of a growing body of evidence unveiling the contribution of vitamin D in mental and immune health status (Grant et al., 2020).

Symptoms like hair falling and the muscle pain were mainly reported among participants with diminished vitamin serum D estimates, emphasizing a potential clinical relevance. Despite the numerical correlation tests in this study did not conducted such as the Pearson's or Spearman's coefficients, the current observations in accordance with preceding studies, ascertain the urgency for expanded, more detailed statistical analyses in future studies (Liu et al., 2017). Added to the need of inclusion of other continuous variables (e.g., sun exposure time versus vitamin D levels), chi-square tests or logistic regression for categorical variables (e.g., gender, dietary frequency), and Spearman's rho for ranked or non-parametric data analyses (Zhang et al., 2022).

Regarding gender aspect, while there is no cross-tabulated data were analyzed, previous research proposed that women are at an increased risk of deficiency owing to sociocultural barriers against sun exposure (Goyal et al., 2013), (Faghih S, et al. 2014). This state was also shown qualitatively in the current study's population.

This study presented a detailed overview of vitamin D status in a sample population predominantly comprised of young adults aged between 15 and 30 years. Despite the expectation that this age category should present an optimal health and nutritional status, the findings were alarming: more than 84% of the participants exhibited serum vitamin D levels under 20 ng/mL, signifying the widespread deficiency. This indicates a serious public health issue, especially when taking into account the long-lasting sequela of vitamin D deficiency on immune function, mental health, and chronic disease acquisition, the results were consistent with the findings of previous studies (Arfaj LK. et al, 2017), (Sanjay K. et al, 2025).

The study also demonstrated that vitamin D deficiency is not restricted to a specific body weight or gender group, boosting the assumption that lifestyle habits are the preliminary contributing factors. Most enrolled subjects reported a diminished intake of natural vitamin D sources (such as fatty fish meals, eggs, and fortified milk and dairy products), along with behaviors that minimizes sun exposure—such as spending a prolonged time indoors or the coverage of majority of the skin at time they outdoors, in accordance with other studies (Al-Ameer, M. 2024).

Despite vitamin D deficiency look like a minor issue in young populations, its long-term consequences are substantial. More efficient strategies involving education, nutrition, and justifying the individual lifestyle are essential to reverse the current trends and to promote better health outcomes for the future generations.

Furthermore, the findings of the current study underscore the urgency for national health programs represented by incorporation of awareness campaigns targeting communities of schools, universities, and workplaces. Furthermore, encouraging minor changes—such as motivating brief daily exposure to sunlight, improving of diet quality, and a routine vitamin D testing —can clearly reduce deficiency proportions in the population.

In conclusion, state of vitamin D deficiency in Iraqi youth should be handled as a public health priority. The asymptomatic nature of deficiency in its early-stage underestimates the significance of screening, public education, and encouraging of safe sun exposure and well-balanced diet. Introducing vitamin D testing within primary healthcare services along with manipulating targeted nutritional interventions could markedly alleviate the extended burden of deficiency-related consequences and ameliorating the quality of life for Iraqi population.

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