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WORD COUNT 8841
TIME SUBMITTED 01-MAY-2024 11:27AM
PAPER ID 108662370

Word Count – Words: 8841

TYPE OF ARTICLE: Original Article

Vitamin D levels among Iraqi population: regional and seasonal variations

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ABSTRACT

Background and Objectives. fluctuated vitamin D (VITD) levels have impacted the healthy status of individuals across the globe. A proper understanding of the surrounding life events that affects the VITD status will enables correct assessment of the deficient cases and the associated clinical consequences. The objectives of the present work are to assess the levels of VITD across different geographical locations of Iraq and to highlight the magnitude of seasonal differences on VITD status among different age, and gender related population groups.

Materials and Methods. A total of 5,014 subjects all attending a single-consultation outpatient clinic for a routine blood test from three different regions of Iraq the northern (N=825), the middle (N=3,277), and the southern (N=912) region were recruited in the current study. Population from the middle region were enrolled through the seasons of the year (from January to December 2022), whereas population from the north and south were enrolled during the winter months.

Results. The study reveals a wide range level of VITD spanning from 3 - 110 ng/ml, with a mean of 26.5 ± 15.7 ng/ml. Deficiency (levels <20 ng/ml) was spotted in 39% of participants, with 11% of study population signified severe level of deficiency (<10 ng/ml). Additionally, 28% of participants exhibited insufficiency (20-30 ng/ml), while 33% had sufficient VITD levels (>30 ng/ml). Gender variations were observed, with significantly higher VITD levels in females (27.2 ± 15.9 ng/ml) compared to males (25.3 ± 15.2 ng/ml).

Seasonal differences revealed significantly reduced VITD levels during the winter season (24.8 ± 14.3 ng/ml) compared to spring and summer (28.3 ± 14.6 ng/ml and 28.5 ± 15.5 ng/ml, respectively), while significantly higher levels were observed in autumn (31.3 ± 16.4 ng/ml). Furthermore, VITD levels were significantly lower in the northern regions (21.7 ± 17 ng/ml) compared to the middle and southern regions (24.8 ± 14.3 ng/ml and 25.1 ± 14.2 ng/ml, respectively). VITD levels also varied by age, with adolescents and young adults showing lower concentrations. The youngest age groups consistently had the highest deficiency rates year-round, ranging from 36% in summer to 70% in winter. This trend was consistent across regions, the 16-30 age group were most affected, especially in the North region where 74% were deficient, compared to 60% and 49% in the Middle and South regions, respectively. The mean serum calcium was 9.33±0.7 mg/dl (range: 6.2-11.9), with a positive correlation with VITD. Participants classified as deficient in VITD had significantly lower mean calcium concentrations compared to insufficient and sufficient groups. Calcium levels were significantly lower in winter (9.8±0.5 mg/dl) compared to summer and autumn (9.8±0.5 mg/dl and 9.54±0.6 mg/dl), further indicating correlation with VITD.

Conclusions. deficient VITD status in Iraq scored high level which involves almost one third of population based on the current study. The level of deficiency was at the highest in the youngest age individuals in all regions and across seasonal comparisons. A clear regional and seasonal variations in the level of 25OHD were spotted among Iraqi population.

Keywords: VITD, supplementation, 25(OH)D, seasonal, regional

INTRODUCTION

The significance of vitamin D (VITD) as a key regulator in managing several metabolic processes, has emphasised its role as an essential element in supporting normal human health and function [1–3]. Diseases of bone and musculoskeletal system are linked, in many conditions, to a deficient VITD level especially in young personals [4,5]. Rickets in childhood and osteomalasia in adults are serious health problems related to disturbed VITD levels with a worldwide impact [6]. Recent studies indicated even more contribution of lower VITD levels to a reduced immunity and enhanced susceptibility to infections such as the human immunodeficiency virus diseases [7]. Keeping an adequate VITD level is fundamental to ensure healthy lifestyle [8]. The major form of VITD in the blood is the 25-hydroxyvitamin D (25(OH)D or calcidiol), which considered the best indicator of VITD status [9]. VITD levels can vary significantly owing to aspects such as exposure to sunlight, dietary habits, geographic location, and seasonal variations. Adequate sunlight exposure is essential for VITD synthesis, but populations at higher latitudes or in places with less sunlight intensity through certain seasons may be at greater risk of deficiency. Additionally, dietary sources of VITD including fish oil are crucial, but cultural food habits and availability can influence intake levels. Other influencers such as skin colour, age, and the fat percentage of body can also impact VITD status [10,11]. Measuring VITD levels requires consideration of factors affecting its concentration. Understanding these factors is essential for accurately assessing clinical conditions linked to VITD deficiency [10,12]. Accordingly, monitoring VITD in different population groups across a district, a country, or even a continent will enable the proper assessment of fluctuations in VITD in a worldwide prospective. This may constitute an ideal strategy to outline the deficiency status of VITD and the associated risks in addition to a balanced VITD supplementation protocol [8]. Just like other countries, Iraq represents a model that offers a variable climates and population diversity from the northern to the southern regions. Available studies on VITD are mostly focusing on a small population group in a single city or a rural region of Iraq which is sometimes limited to a specific season or timing of the year [13,14]. The current study intended to investigate and assess the levels of VITD (collected at the same timing) among population of Iraq based on different regional, and seasonal variations across the country. Also, to assess these variations based on age and gender differences and to highlight the effectiveness of these circumstances on the deficiency status of VITD.

MATERIALS AND METHODS

2.1- Study subjects

In this cross-sectional study, individuals aged four years and above who attended a single-consultation outpatient clinic for a routine blood test as part of their standard clinical care were invited to participate. Leftover blood samples, which are typically discarded, were utilized for this research. Participants were recruited from three main regions of Iraq: The North region included the cities of Nineveh (Lat: 36°30' N) and Erbil (Lat: 36°2' N), the Middle region included Baghdad City (Lat: 33°2' N), and the South region included cities of Basra (Lat: 30°3' N) and Najaf (Lat: 30°0' N). The participant from the Middle region were recruited over a period of one year from January 2022 to December 2022, while samples of the North and South regions were collected during the winter season only.

The study excluded individuals taking supplements or medications that affect bone health, as well as those with medical conditions that impact bone or VITD metabolism, such as liver or kidney disease, diabetes, immobility, cancer, pregnancy, and lactation. In total, 5,014 eligible subjects were enrolled: 3,277 from the middle region, 825 from the north, and 912 from the south.

2.2- Ethical issues

The study received ethical approval from the Collegiate Committee for Medical Research Ethics at the University of Mosul (code number: CCMRE-phA-23-13). All participants provided informed consent before taking part in the study. Those who agreed to participate were then asked to complete a simple questionnaire containing demographic and clinical information, including age, gender, medications, and any existing comorbidities.

2.3- Biochemical parameters

Collected serum samples were stored at -20°C until analysis. VITD (25(OH)D) levels were assessed using a chemiluminescent immunoassay (CLIA) on a Cobas E 411 analyzer (Roche Diagnostics GmbH). The Elecsys VITD (25(OH)D) total reagent kit from Roche Diagnostics was employed for this analysis. This highly sensitive and specific assay offers a measuring range of 4.0 to 160 ng/mL, with intra- and inter-assay coefficients of variation (CVs) below 5%. Concurrently, serum calcium levels were measured in samples with sufficient volume remaining after the VITD (25(OH)D) analysis on a Cobas c 501 analyzer (Roche Diagnostics) using the Roche Calcium Gen.2 reagent kit. This assay utilizes a colorimetric method with a measuring range of 2.00 to 12.00 mg/dL, and intra- and inter-assay CVs below 2%.

2.4- Subject grouping and statistical analysis

The study participants were initially categorized based on their VITD levels into three groups: deficient (<20 ng/mL), insufficient (20-30 ng/mL), and sufficient (>30 ng/mL). Simultaneously, participants were classified into distinct age groups, Adolescent (<15 years), Young Adult (16-30 years), Middle-Aged Adult (31-45 years), Older Adult (46-60 years), and Senior Adult (>60 years).

To investigate regional variations in VITD levels among the Iraqi population, the participants were divided into three groups based on their region of residence: South, North, and Middle. To get rid of the impact of seasonal variation, only samples collected during the winter from the Middle region were utilized to maintain consistency with the samples from the North and South regions, which were also recruited during the winter. Additionally, participants from the Middle region were analysed to investigate seasonal variations in VITD levels, with further subdivision into four subgroups based on the season of sample collection: Winter, Spring, Summer, and Autumn.

The statistical analysis was conducted using SPSS Statistics software version 22 (IBM, Armonk, NY), with a significance level set at $p < 0.05$. Descriptive statistics were employed to characterize the demographic features of the study participants. Normality of the data was assessed using the Shapiro-Wilk test and the Kolmogorov-Smirnov test. For normally distributed groups, comparisons were made using either Student's t-test or analysis of variance (ANOVA). Non-normally distributed data were analysed using the Mann-Whitney U test or Kruskal-Wallis test. Post hoc analyses were performed to further explore significant findings. Tukey test was applied for normally distributed data, while Dunn's test was used for non-parametric data.

RESULTS

3.1- Overall status of study population:

significantly different from each other ($p > 0.05$), both seasons exhibited higher VITD levels than winter ($p < 0.001$) and lower levels than autumn ($p < 0.001$), (Table 3, Figure 1).

The percentage of individuals with sufficient, insufficient and deficient VITD levels across the seasons was illustrated in Figure 2. During the winter, only 28% of the participants exhibited sufficient VITD levels (>30 ng/ml), while 43% had deficient levels (<20 ng/ml). As the seasons progressed, the proportion of participants with sufficient VITD levels gradually increased, reaching 38% in spring, 39% in summer, and attaining a peak of 43% in autumn. Notably, the percentage of individuals with VITD deficiency (<20 ng/ml) mirrored this trend, decreasing to 32% in spring and summer, and further diminishing to 27% in autumn, (Figure 2).

Further analysis revealed a clear age-related trend in VITD deficiency across the seasons. Individuals in the youngest age group (<15 years) exhibited the highest prevalence of VITD deficiency throughout the year, with deficiency rates of approximately 70% in winter, 55% in spring, 36% in summer, and 59% in autumn. The prevalence of deficiency decreased across older age groups, (Figure 3).

The observed seasonal fluctuations in VITD levels significantly influenced calcium levels. Participants recruited during the winter season exhibited the lowest mean calcium level (9.3 ± 0.6 mg/dl), which was significantly different from both summer ($p < 0.001$) and autumn ($p < 0.005$). Conversely, samples collected in summer showed the highest mean calcium level compared to all other seasons ($p < 0.001$), (Table 3, Figure 4).

3.3- Regional variation of VITD levels

The study samples were collected from three distinct geographical regions of Iraq (North, Middle, and South), resulting in the categorization of the study population into three groups based on the region of sample collection. Samples from the North and South regions were collected during the winter season. To ensure consistent comparison across regions, only winter season samples were utilized for the Middle region, despite year-round data availability. This winter-specific sampling strategy minimize the influence of seasonal variations on VITD concentrations, enabling a more accurate assessment of potential regional differences. The characteristics of participants from each region are presented in (Table 4).

Participants recruited from the north region of Iraq exhibited significantly lower VITD levels (21.7±17 ng/ml) compared to those from the middle (24.8±14.3 ng/ml) and south regions (25.1±14.2 ng/ml, $p < 0.001$). However, no significant difference was observed between the middle and south regions($p > 0.05$), (Table 4, Figure 5).

Consistent with these differences in VITD levels, the prevalence of VITD deficiency (<20 ng/ml) also varied significantly across the regions. Participants from the north region suffered from VITD deficiency at a significantly higher rate (58%) compared to the middle (43%) and south (42%) regions ($p < 0.001$), (Figure 6).

Further analysis revealed that the age groups most affected by VITD deficiency throughout the country were the youngest age group (<15 years). In the middle region, 70% of participants aged <15 years were deficient, with 55% and 57% experiencing deficiency in the south and north regions, respectively. Notably, the 16-30 age group also suffers significantly, especially in the north region where 74% of this age group were deficient, compared to 60% and 49% in the middle and south regions, respectively, (Figure 7).

DISCUSSION

Variations in VITD levels may significantly alter human health according to multiple prospective [1,15]. That's because VITD is known for its vibrant role in growth and development and is involved in several metabolic processes including immunological reactions [16]. Recently, Vit D deficiency has been reported as a global catastrophe which requires immediate intervention [17]. This is why it is tremendous to outline the circumstances and conditions that negatively or positively affect VITD status. Similarly, exploring VITD levels among population of multiple ethnic, regions, and ultimately, variable environments will aid in assessing the worldwide status of VITD and draw measures to limit the deficiency status [8]. Outcomes of the present study reveal a wide fluctuation in the levels of VITD based on gender, age groups, seasonal, and regional measures. Deficiency status has been recognized in less than half of the total study population (39% assigned at <20 ng/mL). This comes in association with numerous studies that highlighted the frequent incidence of VITD deficiency in a worldwide prospective [18,19]. Interestingly, the mean VITD level showed significantly higher value in females compared to males. This was opposite to the general concept, based on several studies, that VITD levels usually indicated lower value in female gender [20–22]. In a study conducted in the USA recruiting 15804 participants, VITD deficiency was elevated in men compared to w [23]. Another cross-sectional study by Vallejo et. al. to evaluate VITD in 1329 individuals, also reported higher values of VITD in females with respect to males [24]. Similar findings were also obtained by AlQuaiz et. al. in exploring VITD status among 2835 Saudi individuals [25]. The variations in VITD between the two genders, has been correlated with several factors including body fat percentage and hormonal differences [26,27]. Additionally, the common use of VITD supplementation in females more than in males pose another factor in shifting VITD [25,28]. The regional, ethnic, food (involving fish and fish oil consumption) and variable lifestyles among population are contributing factors affecting VITD status [29]. Samples of the present study outlined the population of Iraq in 3 distinct regions (north, middle and south) with variable latitudes. These may pose a noticeable variation in the level of VITD which possibly correlated to the different regional and also various food and lifestyle habits across the country. In certain studies conducted in northern Iraq (Dohuk, Erbil, and Sulaymania cities) and southern Iraq (Basra, and Najaf), the levels of VITD deficiency were reported higher in females compared to males which was contrary to our present findings [14,30–32]. This could be attributed to the larger sample size and the involvement of participant from several regions of Iraq in the current study. Also, in our study we included larger cities such as Baghdad and Mosul, both are highly populated cities of multiethnic communities. In one study to compare the level of VITD between Jordanian and Iraqi students, Iraqi females showed significantly higher ($p \leq 0.05$) level of VITD [33]. This may indicate the impact of some ethnic and regional variations between the populations.

The higher deficiency percentages were reported among two age groups (<15, and 16-30) with only 22-23% from each group has reached the sufficiency level (>30 ng/mL). More specifically, the majority of the severe deficiency status, defined by levels <10 ng/mL (11% of the total population), was observed in these two age groups. This indicates a major deficiency state existed within children, adolescents, and young adults reaching the age of 30. These results were in accordance with similar literatures reporting prevalence of VITD deficiency in children and adolescents [34–36]. In a cross-sectional survey by Arshad et. al. to monitor VITD deficiency in children and adolescents in Pakistan, a 56% of the total population included were reported as deficient (<20 ng/mL). the same study also reported a 22% insufficient versus only 24% as sufficient VITD level of the total children and adolescents (age 6-18) involved in the study [22]. This was in agreement with the current findings concerning the deficient levels of VITD reported in children and adolescents of below 15 years old. Conversely, in Germany Rabenberg et. al., in a study to track the VITD status following surveys from 1998-2011, reported lower deficiency of VITD among children and adolescents (1-17 years) which was 32-33% of the total population [37]. A 40% VITD deficiency was observed in children and adolescents below the age of 16 as stated by Andiran et. al., who conducted a study to assess VITD deficiency in 440 individuals in Ankara, Turkey [34]. Although the latter study showed lower deficiency level than the current findings it still signifies a deficiency state among this age group. A high percentage of VITD deficiency of up to 89% in young adults aged 19-25 years old has been reported according to Nadeem et. al. in assessing VITD deficiency and determinants among Pakistani medical students [38]. Although the latter study showed significantly higher level of deficiency, it may agree with the current findings in highlighting the prevalence of deficiency among young adults.

The condition reflects different prospective when moving above the age of 30 according to the present results. A progressively reduced deficiency profile was noted in adults of multiple ages (39%-33%) reaching to seniors' level (above the age of 60) with only 20% deficiency level. In the current observations, by monitoring different age groups, it was evident that the deficiency state scored higher level in children and adolescent while showcasing less deficiency towards elderly group of individuals. Studies assessing the deficiency state of VITD in different population were controversial in reporting high percentage of deficiency among old age groups of over 40 and 60 years [39,40]. In a study by Kmieć et. al. to assess the VITD status of 109 adults (>40 years old male and females) in northern Poland, over 50% of the studied population were deficient in VITD level (<20ng/mL) [41]. A randomized study conducted in Switzerland, to follow-up VITD level in 200 seniors of mean age 78, highlighted a higher deficiency rate of 58% which did not agree with current study [42]. Haitchi et. al. investigated the level of VITD in 478 (males and females) seniors of mean age 83 years old revealing around 22% of VIT Deficiency was existed among participants [40]. This was in accordance with the current findings showing a 20% deficiency in this age group. Similarly, Al Quaiz et. al. agreed with the current findings in revealing age related deficiency which was evident in younger age group compared to older age participants. The same study also highlighted the frequent use of VITD supplementation in older individuals which possibly contributed to the mean higher VITD level [25]. The deficiency status reported in children and adolescents may possibly attributed to poor control and monitoring in these age categories. The current changes in lifestyle with children and adolescents being at home most of their times may significantly reduce the exposure to sunlight which adversely impacted their VITD status. Added to that less motivation and poor nutrition at this age group could also impose an impact on VITD status. On the other hand, the reduced percentage of deficiency reported in adults and senior age individuals is mostly related to the routine VITD supplementations that has been increased in these age groups. The current results of calcium ion have come in favour to support the findings of VITD revealing significant difference between the deficient group and both the insufficient and sufficient groups. Moreover, the calcium findings were associated with age in a manner that meet the age-related VITD findings. Although calcium level may not be considered as an effective predictor of VITD level, its correlation with VITD in terms of metabolism and absorption makes it valuable evidence for the measured VITD. The positive correlation of calcium ions and VITD has been indicated according to certain studies, signifying the value of this marker as an assistive measure in reflecting VITD status [28,29,38].

In our attempt to investigate seasonal impact on VITD status while limiting regional differences, we focused on middle region of Iraq to reflect the multi-seasonal changes based on a single region. The middle region was represented by Baghdad which is a multi-ethnic group situated in fairly the centre of Iraq. Such a regional collection of population may constitute a good reflection of how multiple seasons could through their shadows upon VITD status. The current results demonstrated winter season as the one reflecting the lowest VITD level compared to other seasons ($p<0.01$). These findings may reflect the general concept of reduced VITD levels in winter season as a consequence of reduced daylight and exposure to sun. It also supported the findings of other studies across the globe in highlighting the effect of exposure to different intensities of sunlight through variable seasons upon the level of VITD [43,44]. This is reasonable considering the lower intensity of sunlight during the winter season (less UV rays), beside the heavy clothing in cold weather which could limit the exposure of skin to sunlight [4,45]. Even more, the reduced activity and sedentary lifestyle in winter could adversely lower the metabolic rate in general including VITD metabolism [46,47]. Moving to spring season along with summer, the level of VITD started to increase reaching to significant levels compared to winter. this increase is simultaneous with the enhanced intensity of sunlight and outdoor activities normally reported in these seasons (). The current results revealed significant elevation of VITD in spring in both genders compared to winter. The elevated VITD level was maintained in summer season with no significant elevation compared to spring. Also, the percentage of deficiency reduced from 43% in winter to 32% in spring which was maintained during summer. The elevated level of VITD in both spring and summer is a popular concept that has been referred to in certain literatures, and in many times the levels of both seasons are in accordance with each other [48–50]. In general, the weather in Iraq represents a typical fluctuation between seasons with predominance of hot seasons and sunny intervals across the whole year [30]. As such, spring time started early in March with temperatures up to 20°C. This encourages a surge of outdoor activities among populations and certainly more exposure to sun. By the end of spring, the temperatures exceed extreme levels of up to 50°C which limits outdoor activities to lowest level and hence less exposure to sun. This may contribute to the somewhat plateaued VITD level observed during the summer time. Although VITD in both spring and summer seasons where significantly higher than winter, autumn has come out with the highest mean value compared to all seasons. The deficiency state reduced to 27% with higher sufficiency level of up to 46% compared to all seasons. Some reports indicated a decline in the level of VITD in autumn compared to summer time (). Which is correlated with the fact that this season is associated with cold waves, reduced the intensity of sunlight, and thus less exposure rate to sun [10,20]. In fact, the latitude and geographical location may affect the weather characteristics, individuals' activities, intensity of UV radiation and thus VITD status [51]. The situation in Iraq may follow different regime as the extreme summer hotness is extended to almost the mid of September [52]. Once the temperatures begin to fall (reaching mid-twenties by October), the outside activities started to increase again in pattern that reassemble the spring time. This counts for enhanced exposure rate of skin to sun light and thus more VITD synthesis. Keeping in mind that the level of VITD was already high during summer, another increase may be spotted during autumn as the body stores of vitamin was maintained throughout the summer time. In a study conducted in Nepal to assess seasonal difference in VITD recruiting 3320 individuals, the autumn season highlighted increased level of VITD which was in accordance with the present findings. The study tested two groups of people in two geographical locations, the hills and plains. The findings revealed higher deficiency level in the people living in plain areas with limited sun exposure compared to high exposure spotted at the hills [53]. This is another evidence of how exposure to sun may variably affect VITD across the seasons of the year. In a study conducted in

Turkey to examine seasonal variations on food habits, activities, and vitamins level in 53 females, the level of VITD was significantly elevated ($p<0.05$) in summer compared to winter. Although this part was in agreement with the current findings, the same study reported no significant difference between spring and autumn. Also, the same study reported that the level of VITD in both spring and autumn was significantly lower than that of summer which did not agree with the present study [10]. A recent study in Baghdad to assess the optimal exposure time to sun for VITD synthesis, proved the prevalence of high level of UV rays in most times of the year including autumn season. The study also concluded that high temperature values in summer (and thus higher UV radiations) account for the less outdoor activities and thus less exposure rate among population [54]. This finding approved our previous assumption linking the fluctuations in VITD levels and outdoor activities across the different seasons in Iraq.

Interestingly, the age-related VITD deficiency observed earlier among all tested individuals, has shown a similar pattern when reflected across all seasons. The deficiency state was maintained higher in the youngest age group and reduced to the lower level in oldest age participants. All the age groups indicated higher level of deficiency in winter which was reduced in spring and summer then more reduction in autumn season. The tested age groups revealed fluctuation in VITD level across the seasons which was lower in winter and higher in summer. In all seasons, the younger age group showed higher rate of deficiency compared to other groups. The rate of deficiency in this age group was even higher in autumn compared to other age groups where the deficiency levelled down in the same season. Conversely, the senior age group was at the lower deficiency level across all seasons which may reflects the prevalence of VITD supplementations in the elderly. These findings may refer to an important prospective showing the dangerous situations of VITD deficiency spotted in adolescents and children in Iraq territory which was high across all seasons. These results may emphasize our previous declaration that current changes in lifestyle mostly affect children and adolescents as they spend more time in digital activities such as TV, electronic, and smart devices [55]. As such, there will be less exposure rate to sun in these younger age groups whom supposed to be engaged in plenty of outdoor activities which is an important factor to support healthy vitamin level [55,56].

Changes in VITD status has also affected the level of calcium ions across all seasons. It was obvious in summer and autumn time showing significantly higher levels compared to other seasons while summer indicated higher level even compared to autumn. This could be a proof of how VITD fluctuations may affect calcium ion status in individuals. The correlation between VITD and calcium has been indicated in several studies linking the fluctuation in VITD with the level of calcium which agreed with the current results [2,3]. The role of VITD in preserving body calcium level is crucial in maintaining normal functioning of human body. Eventually, adequately maintained calcium level supported a plethora of biological activities basically involving proper neural function and mineralization of bones [16,57,58].

The regional variations in VITD level spotted from north to south of Iraq according to the current findings unveil the impact of different latitudes and geographical locations on VITD status. The higher deficiency levels are mostly encountered in areas of higher latitudes which possibly linked to lower UV radiations' intensity in these locations [45,59]. This may explain the significant distinctions between north of Iraq (Lat. 36) and southern regions (lat. 30). Although samples from the current study were all collected from urban regions which represent a common city lifestyle, certain socioeconomic and food habits are existed. For instance, people of the southern region of Iraq (also some of the middle region) included higher percentage of sea food in their meals compared to northern places. This is a food habit which may contributes in elevating VITD in these regions as their food are richer source in VITD [3]. In a study conducted in Turkey by Yeşiltepe-Mutlu et. al. to inspect the VITD status in the population of Turkey, the greater percentage of deficiency was in the northern region scoring 42% which was reduced in the middle and southern regions to 24% and 26%. Although these finding may agree with our current results, the study highlighted other factors that may variably affect VITD level in some regions which involve economic factors and specific VITD supplementation protocol. This was obvious in the eastern Anatolia region which shows increased deficiency of up to 36% and 39% [60]. Another study by Ovesen et. al. to assess VITD level in Europe, identified certain factors to affect VITD status in addition to sunlight exposure and latitude. These involve fortified food, fish consumption, and VITD supplements. The study verified an increased deficiency status in countries such as France and Italy which are in the southern Europe which was a consequence of nutritional and sun exposure factors. This may disagree with the current study in showing higher deficiency of VITD in southern regions (sunshine regions), but also highlighted the value of proper VITD supplementations which was followed in Norway and Sweden [1,61].

It was clear, based on the current results, that the youngest age group (<15) carry the highest deficiency rate in all comparisons while the oldest (>60) age group maintained the least percentage. However, the regional differences indicated extreme deficiency in the young adults' age group (16-30 years) of up to 74% in the north region. Generally, the deficiency status in different age groups showcasing a supreme percentage in the northern regions of Iraq which was reduced in the middle and indicated lowest value in the south. Indeed, two age groups may deviate from this rule, that is the youngest age (<15 years), and the adults age (46-60). The youngest age group deficiency was outstanding in the middle part of the country (70%), while the adults age (40-60) scores the lowest percentage in the middle region. This may explain the impact of factors such as the nutritional, socioeconomic, and VITD supplementation rather than sun exposure in controlling VITD level [62]. A study in Korea to examine VITD deficiency in relation to age, sex, regional and seasonal measures, reveals enhanced deficiency in younger age across 13 cities involved in the study. The study also emphasised the regional variations showing higher percentage of deficiency in Seoul and Gyeonggi which both are based in the northern region [63]. An earlier study in the United Kingdom showed no significant variations in VITD level between 4 selected regions across the country. The study outlined the proper use of VITD supplementation among population and indicated evidence of reduced VITD level in population with less supplementation intake [64].

The current study may carry some limitations that we wanted to outline here. The study is missing data such as the body mass index (BMI), nutritional information, occupations, and supplementations of VITD are essential additives that may support the findings when addressing the deficiency status. However, in our study we focused on VITD concentration in different locations in Iraq and through variable seasons to highlight the deficiency status in nationwide level. We believe that our study was unique to involve high sample size from around the country and across several regions and seasons and provide a valuable data to be considered for future assessment of VITD status.

CONCLUSION

The deficiency state of VITD in Iraq indicated high level which reflected in almost one third of the population included in the present work. The level of deficiency was at the highest in the youngest age individuals which was observed in all comparisons. Seasonal differences clearly affected VITD status leading to fluctuations which was low in winter and higher in the rest of seasons. Regional differences impacted the level of VITD revealing increased deficiency in northern Iraq which was progressively reduced in the middle and southern regions of the country. Children and adolescents scored the highest level of deficiency in multiple latitudes of Iraq and also in all seasons. based on the current results, certain factors such as the season and geographical location are need to be considered when measuring VITD level. Nutritional, lifestyle and occupation are other variables that requires attention as it may interferes with the outcome VITD status. The findings also feature a serious situation in children and adolescents of Iraq as the deficiency status hits a critical level. Recommended monitoring and follow up for the VITD in these age groups are important to antagonize this condition. Proper supplementation of VITD tracked by medical professionals are necessary to obtain stable levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest concerning authoring or publishing of this article.

AUTHOR’S CONTRIBUTIONS

All authors contributed equally to the conceptualization and methodology; validation, Sameer M. Mahmood, Zaid M. Younus; formal analysis, Sameer M. Mahmood; investigation, Sameer M. Mahmood, Zaid M. Younus, Athir Kadhim Mohammed; resources, Athir Kadhim Mohammed; data curation, Sameer M. Mahmood; writing—original draft preparation, Zaid M. Younus; writing—review and editing, Sameer M. Mahmood, Zaid M. Younus. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

List/thank/acknowledge here individuals who contributed to the work but do not qualify for authorship

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TABLES

Table 1. Participants' demographics and measurements

	N (%)	Age (years) Mean ±SD	25(OH)D3 (ng/ml) Mean ±SD	Median (IQR)	Calcium (mg/dl) Mean ±SD (N)
Total	5,014 (100)	40.7 ± 17.9	26.5 ± 15.7	23.3 (19.5)	9.33 ± 0.7 (954)
Sex					
Female	3191 (63.6)	41.2±17.9	27.2 ± 15.9	24.7 (20.0)	9.35± 0.6 (623)
Male	1823 (36.4)	39.8±18	25.3 ± 15.2	21.6 (17.3)	9.36± 0.6 (331)

VITD Level					
Deficient (< 20)	1970 (39.3)	35.1±16.6	12.9±4.4	13.1 (7.6)	9.22±0.6 (347)
Insufficient (20-30)	1383 (27.6)	42.3±17.1	24.6±2.7	24.4 (5.0)	9.41±0.6 (218)
Sufficient (>30)	1661 (33.1)	46.1±18.2	44.3±13.2	40.8 (15.6)	9.45±0.6 (389)

Table 2. VITD and Calcium Levels (mean ± SD) and VITD Distribution Among Iraqi Population Stratified by Age

Age group	**25(OH)D3 (ng/ml)**		**VITD distribution**			**Calcium (mg/dl)**	
	N	**Mean ±SD**	**Deficient**	**Insufficient**	**Sufficient**	**N**	**Mean ±SD**
<15	422	22.8±15.6	54.3%	22.3%	23.4%	101	9.36±0.6
16-30	1084	22.3±13.8	53.2%	24.4%	22.4%	184	9.23±0.7
31-45	1527	26.1±15.6 $^{a, b}$	39.6%	30.3%	30.1%	231	9.25±0.6
46-60	1181	28±15.6 $^{a, b, c}$	33.3%	30.0%	36.7%	249	9.41±0.6 $^{c, b}$
>60	800	32.9±16 $^{a, b, c, d}$	20.8%	26.0%	53.2%	189	9.52±0.6 $^{c, b}$
P-value		<0.001					<0.001

Note: Significance levels denoted by letters a, b, c, and d represent post hoc comparisons following Kruskal-Wallis Test for VITD and ANOVA for Calcium comparisons: 'a' indicates significance with the <15 age group, 'b' indicates significance with 16-30, 'c' indicates significance with 31-45, 'd' indicates significance with 46-60 age group.

Table 3. Characteristics of the middle region population stratified by season

Season	**Participant N (%)**	**Gender F/M**	**Age (year) Mean±SD**	**25(OH)D3 (ng/ml) Mean±SD, Median (IQR)**	**Calcium (mg/dl) Mean±SD (N)**
Winter	807 (25%)	518/289	43.3±17.7	24.8±14.3, 21.9 (16.8)	9.3±0.6 (177)
Spring	740 (23%)	483/257	44.5±18.2	28.3±14.6, 25.4 (17.9)	9.4±0.4 (181)
Summer	938 (28%)	647/291	42±18.2	28.5±15.5, 26 (19.2)	9.8±0.5 (178)
Autumn	792 (24%)	531/261	43±18.7	31.3±16.4, 28.6 (22)	9.5±0.6 (176)
Total	3277 (100%)	2195/1082	43.1 ± 18.2	28.3±15.5, 25.5 (19.3)	9.5±0.6 (712)

Table 4. Characteristics of the study population stratified by region.

Region	**N (%)**	**Gender F/M**	**Age (year) Mean ±SD**	**25(OH)D3 (ng/ml) Mean ±SD**	**Median (IQR)**
Middle	807 (31.7)	518/289	43.3±17.7	24.8±14.3,	21.9 (16.8)
South	825 (32.4)	534/291	33.4±16.2	25.1±14.2,	22.0 (18)
North	912 (35.9)	462/450	38.8±16.6	21.7±17,	18.1 (16.7)
Total	2544 (100)	1514/1030	38.5±17.3	23.8±15.3,	20.5 (17.7)

FIGURES

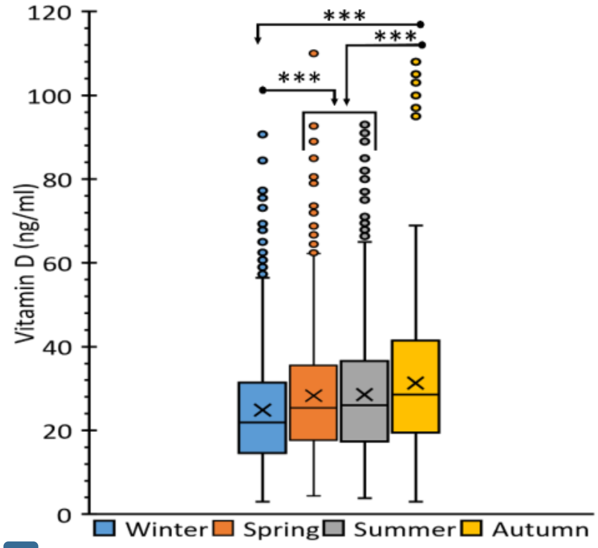

Figure 1. Box plots of 25(OH)D3 Levels (ng/ml) in the middle region population (N:3277), stratified by season of sample collection. The box represents the interquartile range, the line through the box indicates the median level, and × represents the mean. Whiskers extend up to 1.5 times the interquartile range, with (•) denoting outliers above this limit. The significance level ($p < 0.001$) is indicated by (***).

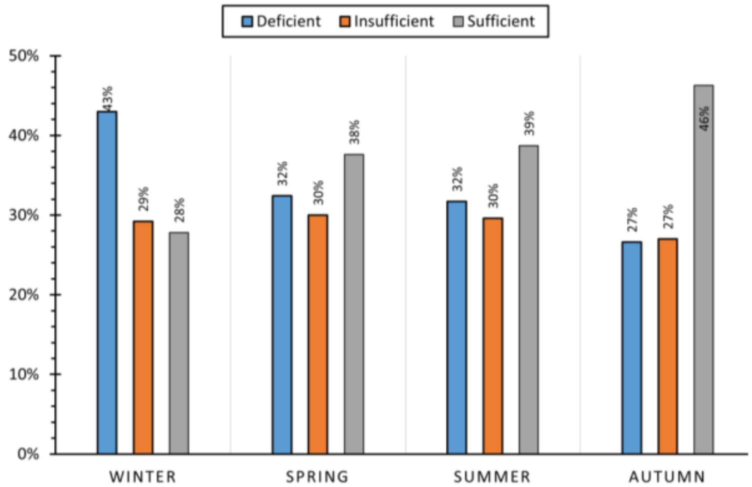

Figure 2. The percentage of individuals with sufficient, insufficient and deficient VITD levels across the seasons. The Y axis denotes the percentage of populations. Each set of 3 bars corresponds to a specific season reflecting deficient, insufficient, and sufficient population percentage.

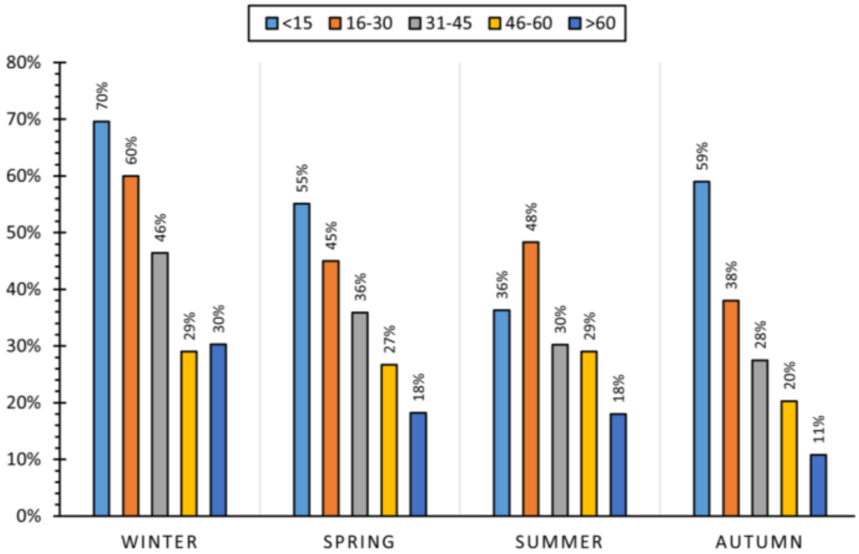

Figure 3. Seasonal distribution of VITD deficiency (<20 ng/ml) across different age groups. Y axis represents the percentage of population that reflects VITD deficiency. Each set of 5 bars correspond to different age groups in specific season.

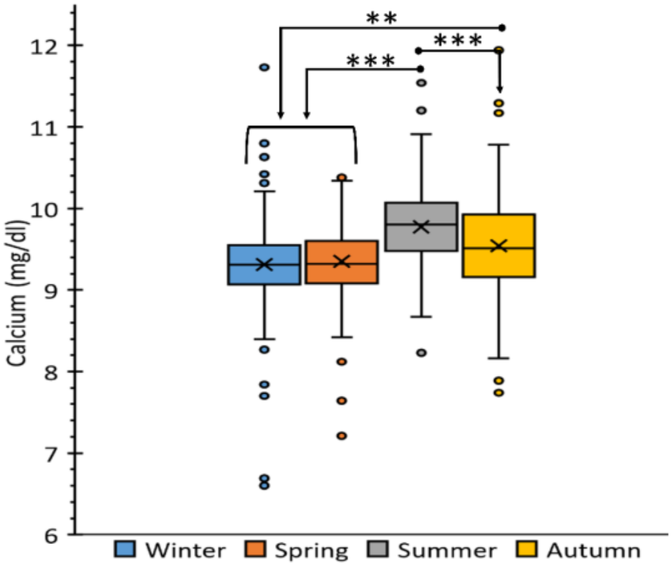

Figure 4. Box plots of Calcium Levels (mg/dl) in the middle region population (N:712), stratified by season of sample collection. The box represents the interquartile range, the line through the box indicates the median level, and × represents the mean. Whiskers extend up to 1.5 times the interquartile range, with (•) denoting outliers above this limit. The significance levels ($p < 0.001$) and ($p < 0.005$) are indicated by (***) and (**), respectively.

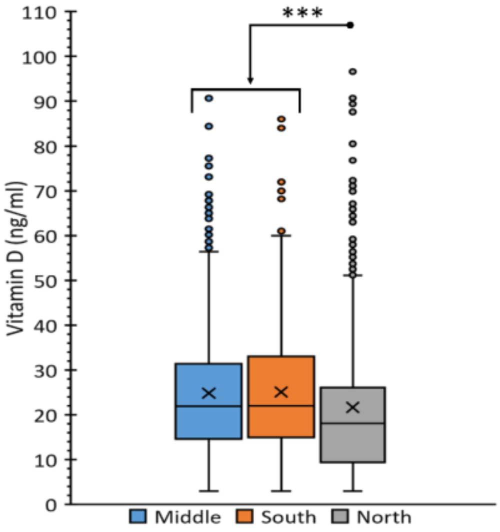

Figure 5. Box plots of 25(OH)D3 Levels (ng/ml) in the study population (N:2544), stratified by region of sample collection. The box represents the interquartile range, the line through the box indicates the median level, and × represents the mean. Whiskers extend up to 1.5 times the interquartile range, with (•) denoting outliers above this limit. The significance level ($p < 0.001$) is indicated by (***).

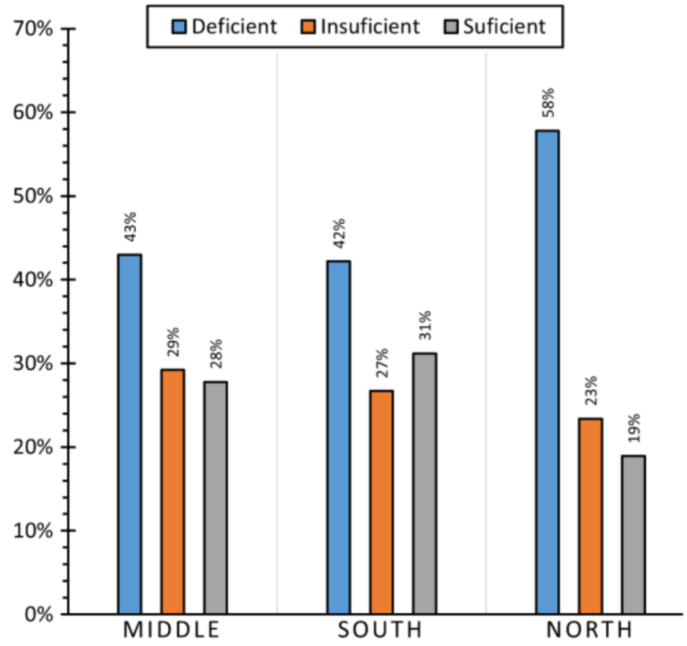

Figure 6. Percentage of individuals with sufficient, insufficient and deficient VITD levels across regions of Iraq.

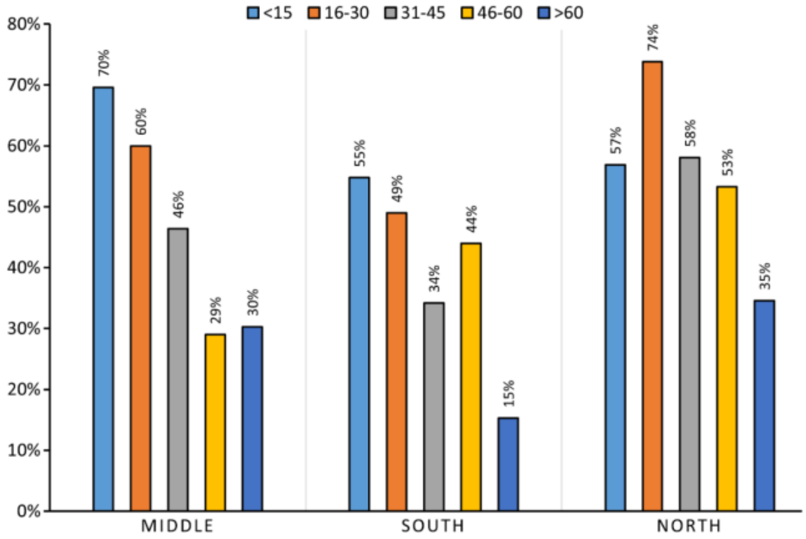

Figure 7. Regional distribution of VITD deficiency (<20 ng/ml) across different age groups