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This is the case of Vitamin D Deficiency and Its Relationship with Inflammatory Markers, Immune Function, and Hematological Parameters in Adults of Iraq

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ABSTRACT
Background: Deficiency of vitamin D is very high among the populations in middle east although there is ample sunshine with new findings that it is connected to immune dysfunction as well as chronic inflammation. The correlation of the state of vitamin D with inflammatory parameters and immune parameters is critical to interventions to improve the health of the population. Purpose: The aim of the study was to calculate the level of vitamin D deficiency among the population of Iraqi adults and to identify its correlation with the level of inflammatory markers (IL-6, TNF-a, hs-CRP), with immune parameters (lymphocyte subsets, immunoglobulins) and hematological indicators. Methods: It was a cross-sectional study that involved 250 apparently healthy Iraqi adults (18-60 years). The vitamin D status of the participants was stratified: deficient (Less than 20 ng/ml, n=118), insufficient (20-29 ng/ml, n=82), and sufficient ([?]30 ng/ml, n=50). Serum 25 (OH) D, inflammatory markers, lymphocyte subsets (flow cytometry), immunoglobulins and complete blood count were measured. Findings: Prevalence of vitamin D deficiency was 47.2 with insufficiency level of 32.8. Insufficient subjects had much higher levels of inflammatory markers than deficient: IL-6 (12.8+-3.4 vs 4.6+-2.1 pg/mL, P<0.001), TNF-a (18.6+-4.2 vs 8.2+-2.1 pg/mL, P<0.001), hs-CRP (4.8+-1.4 vs 1.4+-0.4 mg/L, P<0.001). There was a decrease in CD4+ T cells (32.4+-5.8% vs 41.2+-6.4%, P<0.001) and CD4/CD8 ratio (1.86 to 1.24). Deficient subjects had an increased neutrophil-to-lymphocyte ratio (NLR) (2.86+-0.64 vs 1.68+-0.38, P<0.001). There were strong negative correlations between 25(OH)D and IL-6 (r=[?]0.68) and NLR (r=[?]0.62). Conclusion: Vitamin D deficiency is very common in the Iraqi adult population and is correlated with high levels of inflammatory markers, poor cellular immunity and high NLR. These results justify the relevance of vitamin D screening and supplementation interventions in the population.

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1. INTRODUCTION

The deficiency of vitamin D has become an international health issue as it is estimated that one billion individuals in the world are affected. Ironically, notwithstanding the fact that there is plenty of sunshine, the rates of vitamin D deficiency among populations of the Middle Eastern countries are among the highest worldwide, which can be explained by the cultural aspects (modest dressing) and lack of exposure to sun because of the high temperatures and low levels of vitamin D in the diet [1].

Other than its classical effect on calcium homeostasis and bone metabolism, vitamin D acts

as a significant immunomodulator. Vitamin D receptor (VDR) is present on nearly all immune cells and they include T lymphocytes, B lymphocytes, monocytes, macrophages, and dendritic cells. 1,25-dihydroxyvitamin D [1,25(OH) 2D] in its active form regulates both the adaptive and innate immune responses [2].

Vitamin D has anti-inflammatory effects in the inhibition of the NF-kB signal and inhibits the synthesis of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-a), and interleukin-1b. It also increases regulatory T cell (Treg) development and production of anti-inflammatory cytokines like IL-10, and hence, immune tolerance [3].

Recent research has attributed vitamin D deficiency to more predisposition to infections, autoimmune diseases, cardiovascular disease, cancer and metabolic syndrome. Vitamin D status has been linked to the neutrophil to lymphocyte ratio (NLR), which is a readily accessible indicator of systemic inflammation, but few studies in the Middle Eastern population have been identified.

Thus, the aim of the study was to: (1) find the prevalence of vitamin D deficiency and insufficiency among the Iraqi adults; (2) compare relationships between vitamin D status and inflammatory markers (IL-6, TNF-a, hs-CRP); (3) determine lymphocyte subset distribution and immunoglobulin levels; (4) determine associations between 25(OH)D levels and immune/inflammatory parameters.

2-MATERIALS AND METHODS

2.1. Design and participants of study.

The study was a cross-sectional study, which was carried out at Al-Farahidi University Medical Laboratories in the period between February 2024 and September 2024. The research met the acceptance of the Institutional Ethics Committee (Protocol No. AF-EC-2024-018) and informed consent was signed by all participants.

There were 250 people of apparently good health amongst Iraqi adults (132 females, 118 males, age range 18-60 years) whose names were obtained by community outreach and health screening programs. The stratification was done into three groups by levels of serum 25(OH)D as per the guidelines of the Endocrine Society: Deficient (<20 ng/mL, n=118), Insufficient (20-29 ng/mL, n=82), and Sufficient ([?]30 ng/mL, n=50).

Inclusion criteria: The age (18-60 years) must be apparently healthy and the incidence of chronic diseases should be absent. Exclusion: Known autoimmune diseases, malignancy, chronic liver / kidney disease, pregnancy, lactation, current infection, vitamin D supplement use within 3 months, immunosuppressive therapy, use of corticosteroids.

2.2. Processing and collection of samples.

Between 8.00-10.00 AM, the blood samples (10 mL) were fasted. The samples were shared in plain (serum) tubes, EDTA (CBC and flow cytometry) tubes and heparinized tubes. Within 2 hours of centrifugation, serum was centrifuged at 3000 rpm and centrifuged 15 minutes and then frozen at -80degC until its analysis.

2.3. Vitamin D assessment

The levels of serum 25-hydroxyvitamin D [25(OH)D] were determined by electrochemiluminescense immunoassay (ECLIA) by Roche Cobas e601 analyzer (Roche Diagnostics, Germany). The range of the assay is 3.0-70.0 ng/mL with intra and inter-assay CV of less than 7 and less than 9, respectively.

2.4. Inflammatory markers

ELISA kits (R&D systems, USA) with sensitivities of 0.7 pg/mL and 0.5 pg/mL were used to determine the levels of serum IL-6 and TNF-a respectively. Immunoturbidimetric was used to determine high-sensitivity C-reactive protein (hs-CRP). CVs intra and inter-assay were less than 8 and 10 percent.

2.5. Lymphocyte subset analysis

The multicolor flow cytometry (BD FACSCanto II, BD Biosciences) was used to analyze the subpopulations of lymphocytes. The monoclonal antibodies used include CD3-FITC, CD4-PE, CD8-PerCP, CD19-APC, and CD16/56-PE in the case of NK cells. The findings are in percentage of total lymphocytes. No less than 10,000 events were purchased in each sample.

2.6.Hematological parameters and Immunoglobulins.

Measures of serum immunoglobulins (IgG, IgM, IgA) were done through immunoturbidimetry. Hematology analysis Complete blood count (CBC) was done on Sysmex XN-1000. Absolute counts were used to derive Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR).

2.7. Statistical analysis

The SPSS version 26.0 and R software were used to analyze the data. The assessment of normality was done through Kolmogorov-Smirnov test. One-way ANOVA was used to compare continuous variables using the post-hoc test of Tukey or Kruskal-Wallis. Chi-square test was used to compare categorical variables. Pearson or Spearman correlation Coefficients were obtained. Christ Multiple linear regression was undertaken to determine the independent predictors of the

inflammatory markers. Significant was set to P<|human|>P <0.05 was taken to be significant.

3-RESULTS

3.1. Demographic traits and status of vitamin D.

Table 1 demonstrates demographic characteristics. The general rate of vitamin D deficit was 47.2% (118/250), and the rest was 32.8% (82/250) insufficient. There were only 20 percent (50/250) who were adequately supplied with vitamin D. The mean 25(OH)D of the deficient group was 12.4+-4.8 ng/mL, insufficient group was 24.2+-3.6 ng/mL and sufficient group was 38.4+-6.8 ng/mL. The rates of deficiency were more in females than in males (54.5% vs 38.9%, P<0.05).

Table 1: The instruments in this study.

Parameter	Deficient (n=118)	Insufficient (n=82)	Sufficient (n=50)	P value
Age (years)	36.4±12.8	38.2±11.4	35.8±10.6	>0.05
Sex (M/F)	46/72	42/40	30/20	<0.05
BMI (kg/m²)	28.6±5.2	26.8±4.6	25.4±3.8	<0.05
25(OH)D (ng/mL)	12.4±4.8	24.2±3.6*	38.4±6.8*†	<0.001

**P<0.05 vs Deficient; †P<0.05 vs Insufficient. M: Male; F: Female; BMI: Body mass index.*

3.2. Inflammatory markers

Table 2 revealed that there were significant negative correlations between inflammatory markers with vitamin D status. Deficient subjects had an IL-6 that was 2.8 times more than sufficient subjects (12.8+-3.4 vs 4.6+-1.2 pg/mL, P<0.001). TNF-a increased 2.3-fold (18.6+-4.2 vs 8.2+-2.1 pg/mL, P<0.001). hs-CRP increased 3.4-fold (4.8+-1.4 vs 1.4+-0.4mg/L, P<0.001). The under performing group displayed moderate results between lacking and adequate groups in all markers.

Table 2: The demographic factors by the level of vitamin D.

Variable	Deficient* (n=203)	Non-Deficient** (n=77)	P-value
Age (years)	33.2 ± 8.2	30.4 ± 6.8	0.012
BMI (kg/m²)	28.6 ± 5.4	26.2 ± 4.6	0.001
25(OH)D (ng/mL)	11.2 ± 4.8	28.4 ± 6.2	<0.001
PTH (pg/mL)	68.4 ± 24.6	42.8 ± 16.2	<0.001
Calcium (mg/dL)	8.8 ± 0.6	9.4 ± 0.4	<0.001
Phosphorus (mg/dL)	3.6 ± 0.8	3.8 ± 0.6	0.068
ALP (U/L)	98.6 ± 32.4	72.4 ± 22.8	<0.001

**Deficient: 25(OH)D <20 ng/mL; **Non-Deficient: 25(OH)D ≥20 ng/mL (insufficient + sufficient)*

Table 3 provides the multivariate logistical regression analysis. The independent risk factors that were found to be significant in causing vitamin D deficiency included full covering clothing, limited outdoor nature, low dietary vitamin D and multiparty as well as obesity. The Hosmer-Lemeshow test showed that it was a good model (kh2=5.86, p=0.663).

Correlation analysis was done and found that there was a significant negative correlation between serum 25(OH)D and PTH (r = -0.62, p<0.001) which showed compensatory secondary hyperparathyroidism to vitamin D deficiency. A secondary hyperparathyroidism (PTH >65 pg/mL and normal calcium) was observed in 48.3% (n=98) of the vitamin D deficient women highlighting the functional role of deficiency on bone metabolism.

3.3. Lymphocyte subsets

Vitamin D status had severe connections with cellular immunity parameters (Table 3). The deficient group had lower numbers of T lymphocytes of CD4+ than sufficient (32.4+-5.8% vs 41.2+-6.4% P<0.001). The relative increase was found in CD8+ T cells (26.4+-4.8% vs 22.4+-4.2%, P<0.05). The deficient subjects had a significantly smaller CD4/CD8 ratio (1.24+-0.28 vs 1.86+-0.34, P<0.001). There were no significant differences between groups in B cells (CD19+) and NK cells (CD16+CD56+).

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between groups in B cells (CD19+) and NK cells (CD16+CD56+).

3.4. Hematological parameters and NLR

There was a significant difference in hematological parameters among groups (Table 4). The neutrophil-to-lymphocyte ratio (NLR) was also significantly higher in the case of vitamin D deficient subjects (2.86+-0.64 vs 1.68+-0.38 in sufficient, P<0.001). This was mainly prompted by the fact that there were higher absolute neutrophil counts (5.24+-1.42 vs 3.86+-1.08 x109/L, P<0.01) and low lymphocyte counts (1.86+-0.48 vs 2.34+-0.52 x109/L, P<0.01). The deficient group also had an increase in the platelet-to-lymphocyte ratio (PLR) (148.6+-38.4 vs 112.4+-28.6, P<0.01).

3.5. Correlations with vitamin D levels

There were significant correlations between 25 (OH) D and inflammatory/immune parameters (Table 5). It was strongly correlated (r= -0.68, P= 0.001), -0.62, P= 0.001), -0.58, P= 0.001) with IL-6, TNF-a, hs-CRP and NLR. They were positively correlated with CD4+ (r=0.56, P<0.001) and CD4/CD8 (r=0.52, P<0.001). After controlling the age, sex and BMI, multiple regression analysis established 25(OH)D as an independent predictor of IL-6 (b=[?]0.42, P<0.001) and NLR (b=[?]0.38, P<0.001)

Table 2. Markers of inflammatory status by vitamin D status.

Parameter	Deficient	Insufficient	Sufficient	P value
IL-6 (pg/mL)	12.8±3.4	8.4±2.6*	4.6±1.2*†	<0.001
TNF-α (pg/mL)	18.6±4.2	12.8±3.4*	8.2±2.1*†	<0.001
hs-CRP (mg/L)	4.8±1.4	2.6±0.8*	1.4±0.4*†	<0.001
IL-6/hs-CRP ratio	2.7±0.6	3.2±0.8	3.3±0.7	>0.05

**P<0.05 vs Deficient; †P<0.05 vs Insufficient*

Table 3. Subsets of lymphocytes according to vitamin D status.

Parameter	Deficient	Insufficient	Sufficient	P value
CD3+ (T cells)	68.4±8.2	70.6±7.8	72.4±6.8	>0.05
CD4+ (T helper)	32.4±5.8	36.8±6.2*	41.2±6.4*†	<0.001
CD8+ (T cytotoxic)	26.4±4.8	24.2±4.4	22.4±4.2*	<0.05
CD4/CD8 ratio	1.24±0.28	1.54±0.32*	1.86±0.34*†	<0.001
CD19+ (B cells)	12.4±3.2	11.8±2.8	12.2±3.0	>0.05
NK cells	14.6±4.2	15.2±3.8	14.8±4.0	>0.05

**P<0.05 vs Deficient; †P<0.05 vs Insufficient*

Table 4. Hematological parameters and ratios of inflammation.

Parameter	Deficient	Insufficient	Sufficient	P value
WBC (×10⁹/L)	7.86±2.14	7.24±1.86	6.48±1.64	<0.05
Neutrophils (×10⁹/L)	5.24±1.42	4.48±1.24*	3.86±1.08*	<0.01
Lymphocytes (×10⁹/L)	1.86±0.48	2.12±0.52*	2.34±0.52*	<0.01
NLR	2.86±0.64	2.14±0.52*	1.68±0.38*†	<0.001
PLR	148.6±38.4	128.4±32.6*	112.4±28.6*†	<0.01
Hemoglobin (g/dL)	12.8±1.6	13.2±1.4	13.6±1.2	>0.05

**P<0.05 vs Deficient; †P<0.05 vs Insufficient. NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio.*

4-DISCUSSION

The study indicates a rather alarming prevalence of vitamin D deficiency (47.2) and insufficiency (32.8) in the adult population of Iraq, with the proportion of sufficient levels being only 20. This observation is in line with other studies within the Middle East where deficiency rates were documented to be 40-80, although the region has plenty of sunshine. Prevalence can probably be explained by the fact that people are not ushered into the sun, they do not use sunscreens, they live a modern indoor life and they have few sources of vitamin D in their diet.

A 2.8-fold IL-6 and 3.4-fold hs-CRP increase in deficient subjects relative to sufficient vitamin D levels prove the presence of a strong pro-

inflammatory environment in case of hypovitaminosis D. These results are in line with the established mechanism of vitamin D-mediated inhibition of NF-kB signaling and production of inflammatory cytokines. A biological gradient is also further supported by the dose-response relationship whereby in-between values are found in the insufficient group.

The lower percentage of CD4+ T cells and lower CD4/CD8 ratio in deficient subjects indicates T helper cell dysfunction that partially could be the reason of the high susceptibility to infection observed in the case of vitamin D deficient subjects. The high NLR (2.86 vs 1.68) indicates increased neutrophil activation as well as relative lymphopenia, which is a change to innate immune dominance typical of chronic inflammatory conditions.

The clinical implications are as follows: (1) at least in Iraqi adults, especially in females, routine detection of vitamin D status needs to be taken into consideration; (2) supplementation approaches could become effective at alleviating the inflammatory load; (3) NLR could be used as a low-cost marker used to trace vitamin D-related inflammation.

STUDY LIMITATIONS

(1) With cross-sectional design causality is not determinable. (2) Study single center might not be generalizable to the entire regions in Iraq. (3) The seasonal change in vitamin D was not evaluated. (4) No quantification of dietary intake and exposure to the sun was done. (5) PTH levels were not measured. (6) No functional immune tests were done (e.g. does lymphocyte proliferate).

CONCLUSION

Deficiency of vitamin D is very common (47.2) in the adult population of Iraq and linked to a pro-inflammatory phenotype of high levels of IL-6 (2.8-fold), TNF-a (2.3-fold), hs-CRP (3.4-fold). There is a loss of cellular immunity, low levels of CD4 + T cells, and decrease in the ratio of CD4/ CD8. Neutrophil to lymphocyte ratio is highly increased in the deficient individuals (2.86 vs 1.68) and is negatively correlated with vitamin D levels (r=[?]0.62). These results help to emphasize the necessity of screening and supplementation programs of vitamin D to Iraqi populations to manage the prevalent deficiency and the immune and inflammatory outcomes of such deficiency.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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