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The correlation between the of Vitamin D and the presence of anemia in Iraqi pregnant women

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Running title: Vitamin D level among pregnant women with anemia

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Abstract

Background: There is an association between iron status and vitamin D, suggesting an interplay between these nutrients. Vitamin D is linked to erythropoiesis and iron absorption regulation. However, evidence on its association with hemoglobin levels in pregnant women is limited and conflicting. **Objective:** Study correlation between Vitamin D and anemia in pregnant women. **Methods**: Health clinics at Fallujah General Hospital in Anbar, Iraq, conducted a case-control study involving 100 pregnant women in their first and third trimesters. The study assessed Vitamin D, serum ferritin, serum iron, and hemoglobin (HGB) using electrochemiluminescence immunoassay, cobas c311, and autoanalyzer hematology **Results**: In a study comparing pregnant women in the 1st trimester (n=50) and 3rd trimester (n=50), significant findings : serum vitamin D were lower in the 3rd trimester group (14.70±6.164 ng/mL) compared to the 1st trimester group (33.4±4.813 ng/mL, $p = 0.016$). Conversely, iron levels were higher in the 3rd trimester(58.4±13.71 µg/dL) compared to the 1st trimester (31.5±7.85 µg/dL, $p = 0.001$). No correlations were found between hemoglobin and vitamin D ($r = 0.039$, $p > 0.05$), vitamin D and ferritin ($r = 0.056$, $p > 0.05$), vitamin D and iron ($r = -0.187$, $p > 0.05$). **Conclusions**: The vitamin D is decreased in 3ed trimester pregnant women and There is no direct correlation between Vitamin D and anemia in pregnant women.

KEYWORDS

Pregnant, anemia , vitamin D, hepcidin, ferritin

INTRODUCTION

Vitamin D is well acknowledged as a crucial biological regulator of calcium balance and has a significant impact on bone and mineral metabolism in mammals. Nevertheless, numerous studies have demonstrated that the importance of vitamin D extends beyond bone health, as evidenced by the widespread expression of the vitamin D receptor in various cells and tissues, as well as the broad distribution of 1-alpha-hydroxylase, a crucial enzyme for the formation of 1,25-dihydroxyvitamin D [1].

Vitamin D insufficiency is a widespread public health issue that affects a large portion of the general population and is particularly common among pregnant women. It is defined by low levels of 25-hydroxyvitamin D (25[OH]D) in the blood, specifically below 20 ng/mL (50 nmol/L). This condition may be linked to chronic diseases, impaired immune function, and inadequate production of red blood cells [2]. A population's vitamin D status can be influenced by a multitude of personal and environmental factors. These include, but are not limited to, having dark skin, living in a high-latitude or low-sunlight climate, applying sunscreen, one's ethnic heritage, certain clothing choices, cultural customs, and many more. More recently, a comprehensive analysis of iron's impact on vitamin D metabolism found a favourable correlation between iron status and vitamin D concentration [3].

Some have hypothesized that vitamin D and haemoglobin are related. There is conflicting and scant evidence that vitamin D influences erythropoiesis in pregnant women, despite its ability to regulate the iron-hepcidin-ferroportin axis in monocytes and increase iron intake [4]. On the other hand other studies showed no significant association between vitamin D, anemia (HGB), and iron markers was found [5, 6].Important nutritional health issues include iron and vitamin D deficiencies. Iron deficiency and vitamin D insufficiency go hand in hand when one's diet is inadequate [7, 8].

MATERIALS AND METHODS

Study groups

Participants in this study were pregnant women who visit health clinics at Fallujah General Hospital in Anbar, Iraq, to assess their vitamin D levels. During the first and third trimesters of pregnancy because third-trimester vitamin D deficiency was widespread among pregnant women despite the fact that Iraq gets year-round sunshine according to the Idan (2028)(2), one hundred pregnant women participated in the case-control study. They are in the 18–40 age bracket. A pregnant woman in different stages of pregnancy were tested, and their results were recorded as 50 females during the first trimester. Fifty female volunteers served as patients throughout the third trimester, with data recorded at the chemical and hormonal center.

Criteria for inclusion

Pregnant women from Iraq who are in either their first or third trimesters and are 18 years of age or older.

Exclusion criteria

Chronic ailments, including Cardiovascular disorders, neurological issues, diabetes mellitus, kidney disorders, polycystic ovarian disorder, thyroid conditions, and abortion are all examples of long-term medical conditions.

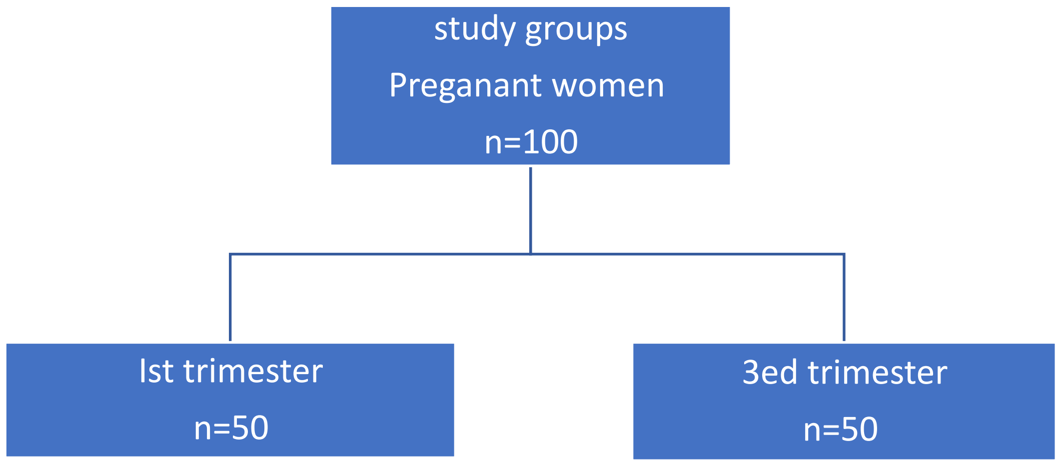

Figure 1: Study design illustrated the different pregnant stage included in this study

Sampling

Each woman who took part in the study had a sample of venous blood collected using a sterile venipuncture technique. Blood samples are analysed for biochemical indicators such as Vitamin D, Iron, Ferritin, and Haemoglobin (HGB). A volume of 5 cubic centimeters of blood was necessary. The blood samples underwent centrifugation to isolate the serums from the plasma. For a duration of 10 minutes, the engine was running at a speed of 3000rpm. The samples were removed from the refrigerator and left to reach room temperature once they had finished the required number of samples. Subsequently, the samples underwent a sequence of specialized examinations. The questionnaire and data collected by clinicians were used to identify potential variables, including age, week of pregnancy, and the usage of vitamin D-containing supplements.

Estimation of HGB g/dl using 3-part differential Autoanalyzer Hematology using (CBCswelab 110270/ Sweden)

White blood cell (WBC) counts and differentiation into three subpopulations—lymphocytes (LYM), Granulocytes (GRAN, primarily neutrophils, eosinophils, and basophils) and mid-sized white cells (MID, primarily monocytes) are within the scope of this analyzer's capabilities., in addition to determining haemoglobin (HGB) concentration and counting RBCs and platelets (PLT). The Swelab Alfa Plus uses spectrophotometry to quantify HGB and impedance to estimate cell counts.

Detection of Vitamin D and serum Ferritin level

The serum vitamin D levels were tested using an electrochemiluminescence immunoassay on automated analyzers, such as the immunoassay analyzer cobas e 411 (Roche/Hitachi, Germany). The assay requires three stages and 27 minutes of incubation. The VDBP releases bound 25-OH vitamin D after pretreatment with a reagent. Second, ruthenium-labelled VDBP is applied to the preprocessed sample, forming a compound with 25-OH vitamin D. In the third incubation step, streptavidin- and biotin-labeled 25-OH vitamin D microparticles are added.

The biotinylated 25-OH vitamin D and ruthenium-labeled vitamin D binding protein create a complex when the VDBP's empty sites are filled. Streptavidin and biotin immobilize the complex to solid. Serum level Electrochemiluminescence immunoassay detects serum ferritin levels in the Roche/Hitachi cobas e411 automated immunoassay analyzer in Germany. In the initial 9-minute incubation, a sandwich complex was created by combining 10µL of sample with biotin-labeled ferritin-specific monoclonal antibody and ruthenium-labeled antibody. After adding streptavidin-coated microparticles, the biotin-streptavidin combination was attached to a solid surface. Magnetic forces drew microparticles to the electrodes when the reaction mixture was drawn into the measurement cell. Photomultiplier was utilised to detect chemiluminescent light from electrode voltage.

Working method of serum iron

Manufactured by Roche/Hitachi in Germany, the cobas c311 is a diagnostic instrument. Human serum can be evaluated using IRON on Roche/Hitachi Cobasc311apparatus in an in vitro investigation. Iron is liberated from trans-ferrin in an acidic solution. Detergent is employed to clarify lipemic samples. Ascorbate facilitates the conversion of Fe3+ ions to Fe2+ and FerroZine, resulting in the formation of a visually striking complex. The iron concentration of 23 has a clear correlation with the intensity of color, as assessed through photometric analysis.

Ethical permission

Ethical approval was obtained for this investigation, ensuring adherence to the ethical principles derived from the Declaration of Helsinki. Prior to obtaining a sample, the patient's verbal and analytical consent was obtained.

Statistics Analysis

Application of SAS (Statistical Analysis System-2019) is utilised to ascertain the impact of different circumstances on parameter-related inquiries. One way to compare two elements' fundamental assumptions is via the t-test. Less than p 0.05 was used to define statistical significance in each study.

RESULTS

In this study, 100 pregnant women participate 50 patients in their 3ed trimester, whereas 50 in their 1^{st} trimester. From August 2021 to April 2022, this case-control study was conducted. The comparison between the pregnant women in different stages. According to Figure 2, the mean age of the study groups was (27.0 ± 6.285 y.) for pregnant in 3ed trimester and (27.1 ± 6.20 y.) for pregnant in 1^{st} trimester . (P0.90) indicates no difference.

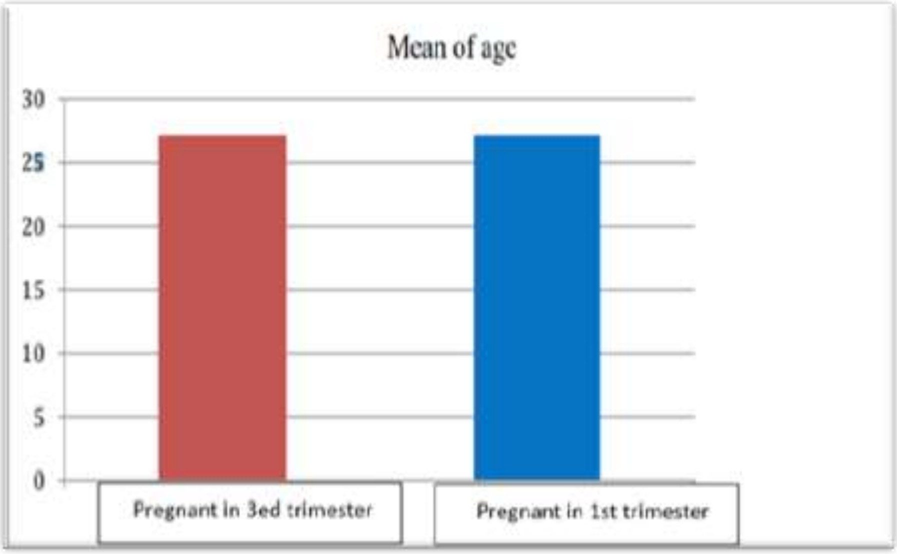

Figure 2: The chart of age in study

Hemoglobin concentration result

The pregnant ladies who participated in this research were all anemic. Mild anemia was indicated by the mean HGB level of 10.28 ± 0.73 g/dl. Eleven.61 ± 0.589 g/dl was recorded in the control group. Standard range is 11.5-16.5 g/dl. As in Table 1.

Vitamin D levels

In 1st trimester women the vitamin D were significantly higher (P 0.016) than the pregnant in 3ed trimester (33.4 ± 4.81 ng/mL) (mean = 14.7 ± 6.16 ng/mL, normal = 30-70 ng/mL), as shown in Table 1. The current study found that third-trimester patients had vitamin D deficiency more than first-trimester group. The pregnant in 1st trimester their vitamin D was normal due to early pregnancy**.**

Serum ferritin

The mean concentration of the hormone in the pregnant in 3ed trimester was 38.35 ± 17.28 ng/dL, while the concentration in the pregnant in 1st trimester was 40.46 ± 16.12ng/dL. As seen within Table 1. The standard range was between 13 and 150 nanograms per deciliter (ng/dL). The study did not observe a significant impact of pregnancy on ferritin levels (P0.71), except in some subgroups of pregnant women in first and third trimester. This difference may be attributed to the iron consumption of pregnant women.

Serum iron

The mean concentration of the substance in third trimester pregnant women was 31.59 ±7.85ug/dL while the usual range was 37-145ug/dL, there was statistically significant difference (P 0.001) when compared to the pregnant women in 1st trimester (58.43 ± 13.71 ug/dL) as indicated in Table 1.

Table 1: The parameters between different pregnancy stages

Parameters	Mean ±SD		p- value
	pregnant in 3ed trimester (n=50)	pregnant in 1st trimester (n=50)	
HGB (g/dl)	10.2±0.73	11.6±0.58	0.34
Vitamin D (ng/ml)	14.7±6.16	33.4±4.81	0.016
S. Ferritin (ng/dl)	38.3±17.28	40.4±16.21	0.71
S. Iron (µg/dl)	31.5±7.85	58.4±13.71	0.001

The correlation between study parameters

The correlation analysis between the parameters—Hemoglobin (HGB), Vitamin D, Ferritin, and Iron—revealed several significant associations:

There was a strong positive correlation between Hemoglobin (HGB) and Ferritin ($r = 0.874$, $p < 0.01$), indicating a robust relationship between these two variables. A moderate positive correlation was found between Iron and Hemoglobin ($r = 0.369$, $p < 0.05$), suggesting a moderate association between iron levels and hemoglobin synthesis. Additionally, there was a weak positive correlation between Ferritin and Iron ($r = 0.369$, $p < 0.05$), indicating a modest association between iron storage and iron levels.

However, no significant correlations were found between Hemoglobin and Vitamin D ($r = 0.039$, $p > 0.05$), Vitamin D and Ferritin ($r = 0.056$, $p > 0.05$), and Vitamin D and Iron ($r = -0.187$, $p > 0.05$), As shown in Table 2.

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Table 2 : The correlation between study parameters among patients group

Correlations					
Parameters		HGB	Vitamin D	Ferritin	Iron
HGB	r	1	0.039	0.874**	0.369*
	P- value		0.828	0.001	0.035
Vitamin D	r	0.039	1	0.056	-0.187
	P- value	0.828		0.756	0.298
Ferritin	r	0.874**	0.056	1	0.369*
	P- value	0.001	0.756		0.034
Iron	r	0.369*	-0.187	0.369*	1
	P- value	0.035	0.298	0.034	

**. *Correlation is significant at the 0.01 level (p value).*

*. *Correlation is significant at the 0.05 level (p value).*

DISCUSSION

Vitamin D is essential for sustaining optimal health. Vitamin D inadequacy during pregnancy is an acknowledged health risk. There is a lack of data about this group in Iraq.
In a study comparing vitamin D levels in pregnant women in first and third trimester , it was found that the mean vitamin D level For women in the third trimester of pregnancy 14.70 ± 6.164 ng/mL, significantly lower than the normal range of 30-70ng/mL. This difference was highly significant (P0.016) in comparison to the first trimester pregnant women which had a mean of 33.40 ± 4.8133ng/mL. Women in the third trimester of pregnancy were particularly prone to vitamin D deficiency compared to those in the first trimester.

Despite Iraq's ample sunshine, there was a widespread prevalence of vitamin D deficiency among pregnant women, with 82 percent of the patient group being insufficient. These findings align with previous studies by Christoph et al. (2020) [10], Woon et al. (2019) [11], Krieger et al. (2018) [12], Al-Rubaye et al. (2021) [13], and Idan (2018) [14], which reported high rates of vitamin D deficiency among pregnant women in various regions. Future nutrition education initiatives should focus on promoting the consumption of vitamin D-fortified foods among pregnant women to mitigate the long-term health implications of vitamin D deficiency.

Serum ferritin levels serve as a crucial biomarker for assessing iron reserves in the body, with low levels often indicating anemia due to their role in hematopoiesis. Furthermore, ferritin plays a significant role as an angiogenic agent, supporting tissue growth including bone formation (Yuniati et al., 2020) [15]. However, high ferritin levels are more commonly associated with poor iron status, as stated by Brown et al. (2021) [16], No significant differences were found between the research groups in these prior studies, which are congruent with the current investigation.

Because of their role in hematopoiesis, serum ferritin levels are an important indicator of iron stores; low levels are often indicative of anemia.. Additionally, ferritin serves as a crucial angiogenic factor, facilitating tissue growth such as bone formation (Yuniati et al., 2020) [15]. Conversely, elevated ferritin levels are typically indicative of poor iron status, as highlighted by Brown et al. (2021) [16]. These earlier findings resonate with the current study, which similarly did not identify any notable discrepancies between the study groups.

In this study, mean serum iron levels in third trimester pregnant women were (31.59 ± 7.85ug/dL), significantly lower (P 0.001) compared to the first trimester pregnant women (58.43 ± 13.71ug/dL), as depicted in these findings parallel those of (Yuan et al. 2019) [17], who reported a 51.82% prevalence of iron deficiency in pregnant women, expressed by low blood serum ferritin levels (< 12μg/L). Pregnant women are markedly exposed to iron lack due to increased iron requirements. In the U.S., about 35% of women experience reduced or depleted iron levels, categorizing them as iron deficient [18]. Physiological iron requirements fluctuate during pregnancy. Iron needs decrease in the first trimester due to menstruation cessation, with potential increases in iron reserves. However, the second trimester witnesses heightened iron needs due to expanded blood volume and red cell mass. Rising placental iron deposition supports fetal erythropoiesis, with iron needs steadily increasing into the third trimester [16]. Iron demands rise in the second and third trimesters due to expanded blood volume catering to fetal and placental demands, potentially masking first-trimester anemia [19].

The correlation analysis conducted on the (HGB), Vitamin D, Ferritin, and Iron—revealed several significant associations that provide important insights into their interrelationships within physiological contexts.

The strong positive association observed between Hemoglobin (HGB) and Ferritin ($r = 0.874$, $p < 0.01$) indicates a robust relationship between these variables. This finding suggests that higher levels of Ferritin, which is a marker of iron stores, are associated with increased levels of Hemoglobin, reflecting enhanced erythropoietic activity and potentially reduced risk of anemia [20]. Moreover, the moderate positive correlation between Iron and Hemoglobin ($r = 0.369$, $p < 0.05$) suggests a moderate association between iron levels and hemoglobin synthesis. This finding is consistent with the well-established role of iron in hemoglobin production, as iron is an essential component of hemoglobin molecules [20].

However, no significant correlations were found between Hemoglobin and Vitamin D, Vitamin D and Ferritin, and Vitamin D and Iron. These results suggest that there may be no direct associations between Vitamin D levels and erythropoiesis or iron metabolism in the studied population [1]. Vitamin D's potential to raise HGB via lowering hepcidin and enhancing iron absorption is one probable explanation. Taking iron supplements may increase the body's absorption of the mineral by providing a source of vitamin D [4].

Multiple cross-sectional studies provide evidence of a correlation between vitamin D levels and iron levels in the blood. A comprehensive study was conducted on 2526 Korean children and adolescents, revealing a notable disparity in the prevalence of vitamin D deficiency and anemia between females and males. The researchers concluded that the observed positive relationship between vitamin D and iron levels may be attributed to the inhibitory effect of vitamin D on hepcidin, an iron regulatory hormone [21].

CONCLUSIONS

Overall, the findings suggest that the third trimester pregnant women may be at a higher risk of anemia, vitamin D deficiency, and iron deficiency compared to the first trimester pregnant women. Also, there were no significant correlations were found between Hemoglobin and Vitamin D, Vitamin D and Ferritin, and Vitamin D and Iron. These results suggest that there may be no direct associations between Vitamin D levels and erythropoiesis or iron metabolism in the studied population. Vitamin D, Ferritin, and Iron levels in the context of erythropoiesis and iron metabolism, they underscore the importance of maintaining adequate iron stores and iron levels for optimal hemoglobin synthesis and erythropoietin function, particularly in preventing anemia during pregnancy.

RECOMMENDATIONS

Future studies should explore potential mechanisms underlying the observed associations and lack of correlations between vitamin D levels and erythropoiesis or iron metabolism in the studied population. Additionally, longitudinal studies assessing the impact of interventions such as nutritional supplementation on maternal and neonatal outcomes are warranted to inform evidence-based practice.

Vitamin D, Ferritin, and Iron levels in the context of erythropoiesis and iron metabolism, they underscore the importance of maintaining adequate iron stores and iron levels for optimal hemoglobin synthesis and erythropoietin function, particularly in preventing anemia during pregnancy.

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