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EVALUATION OF VITAMIN D3 LEVELS AND SOME BLOOD PARAMETERS IN PREGNANT WOMEN DURING EARLY AND LATE PREGNANCY

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Abstract:

The study was conducted on pregnant women who visited Kirkuk General Hospital, during the period from March to May 2022. The total number of the study sample was 40 women, 20 women in the first trimester of pregnancy and 20 women in the third trimester of pregnancy, their ages ranged between (25-35). The results of the analysis of variance showed significant differences at ($P \leq 0.01$) between the two study groups (first and third period of pregnancy), and for all studied parameters (vitD3, HGB, HCT, MCV, PLT, WBC and Glucose). The first pregnancy period achieved the highest averages for the parameters vitD3, HGB, HCT, MCV and PLT, which were 18.38 ng/ml, 12.02 gm/dl, 37.56%, 88.04 FL and 260.85 x 10^3/L, respectively. While the third pregnancy period recorded the highest averages of the two parameters, WBC and Glucose, which amounted to 9.36 × 10^3/L and 92.36 mg/dl. The results of the analysis of Pearson's correlation coefficient between vitamin D levels and the rest of the parameters studied during the first and third pregnancies showed that there were positive and significant correlations at ($P \leq 0.05$) during the third pregnancy between vitamin D levels with both HGB and HCT, whose values were 0.349 and 0.332, respectively. It also found a negative and significant association at ($P \leq 0.05$) for the level of vitamin D with the level of WBC, and its value was -0.446.

Keywords: Vitamin D, pregnant women, early and late pregnancy.

INTRODUCTION

Vitamin D is a fat-soluble steroid hormone. It is obtained from foods or by synthesis in the skin after exposure to sunlight (UV rays) and converted to the active form in the liver and kidneys (McCarty *et al.*, 2013). Vitamin D3 is the only form that is produced on the skin when steroids and ergosterol are exposed to UV light (Pedroza-Tobías *et al.*, 2016). The skin is a chemical factory, fueled in part by sunlight, and converts cholesterol molecules into vitamin D in sufficient quantity, the vitamin D is released in the cold months from the electrodes of fat, because the vitamin cannot be modified from cholesterol in the skin. Sunbathing in the summer months needs a certain amount of time and duration to produce an adequate amount of vitamins and can be prevented or changed by many factors such as sunscreens even with an SPF of 30% and more, and also by wearing long clothing that covers the bottom and top. Skin tone also affects the formation of vitamin D, so formation is more in people with darker skin than in light skin (Golbahar *et al.*, 2014).

Significant changes in calcium homeostasis occur during pregnancy to meet the requirements of the fetus. The fetal skeleton contains about 30 g of calcium at birth (Kovacs *et al.*, 1997). These changes are particularly evident in the third trimester of pregnancy, when approximately 250 mg/day of calcium is transferred from the mother to the fetus (Specker, 2004). Increased intestinal absorption is the main mechanism for providing the increased demand for calcium (Kent *et al.*, 1991). Vitamin D and its active metabolite, 1,25(OH)2D, are essential for calcium absorption in the intestine. The concentration of 1,25(OH)2D increases by 50-100% during the second trimester of pregnancy and 100% in the third trimester (Kovas, 2008). At present, the general recommendation for giving vitamin D to pregnant women is 400 units/day. Some studies have shown, however, that this amount of vitamin D is insufficient during pregnancy, and higher doses are needed to prevent complications associated with vitamin D deficiency during pregnancy (Hollis *et al.*, 2011).

The current dietary recommendation for vitamin D during pregnancy is still outdated for a simple reason: the fear of vitamin D toxicity (Hollis *et al.*, 2011). To understand this phrase, it is important to look at the history surrounding vitamin D during pregnancy. In 1947, Dr. E. Obermer provided evidence that pregnant women need several thousand

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international units of vitamin D per day during pregnancy. This recommendation was barely 'seeing the light' before vitamin D was incorrectly associated with causing supravalvular aortic stenosis syndrome during pregnancy (Hollis and Wagner, 2013). Thus, vitamin D was seen as teratogenic to the developing fetus during pregnancy, and in response, the medical profession adhered to the largely insignificant recommendation of 200 IU/day for adults set by the Forbes Commission in 1963. Unfortunately, for the time being, it is still The lower recommendation is similarly valid to a large extent as shown in a recent IOM document (Food and Nutrition Board, 2010), although a recent recommendation from the Endocrine Society recommends higher doses taking into account emerging data surrounding the effect of the vitamin d on non-structural functions, and it should be noted that the IOM report is a guide for food manufacturers, while the Endocrine Society report is a guide to clinical care for patients (Holick *et al*., 2011).

Studies in pregnant women have shown a high prevalence of vitamin D deficiency worldwide, particularly in developing countries and women of Asian descent (Al Kalbani *et al*., 2011). Reports have shown vitamin D deficiency in 40-80% of pregnant women during different seasons of the year (Kazemi *et al*., 2009). Observational studies have reported an association between vitamin D deficiency and maternal complications (eg, preeclampsia (Bodnar *et al*., 2007), increased bone resorption markers (Haliloglu *et al*., 2011), and fetal complications (eg, increased risk of infection). (Dror, 2011), hypocalcemia in newborns and Craniotabes (Yorifuji *et al*., 2008)).

Vitamin D deficiency is prevalent in pregnancy, as recent studies have shown that vitamin D deficiency may be a modifiable contributor to the etiology of gestational diabetes mellitus (GDM), and that vitamin D plays a key role in glucose homeostasis and insulin resistance (Bener *et al*., 2013). Maternal vitamin D status affects the calcium balance of the mother and newborn during pregnancy. The supply of vitamin D to the fetus occurs through the transfer of the placenta from the mother. Newborn umbilical cord blood concentration of 25(OH)D3 correlates with maternal level (Halicioglu et al., 2012). Vitamin D deficiency is an important health problem during pregnancy that leads to bone abnormalities known as rickets, hypocalcemia with or without convulsions, muscle weakness, and heart failure in the newborn (Sachan *et al*., 2005). In several studies, the cause of vitamin D deficiency in pregnant women has been identified, the main factors being living location, dark skin color, pattern of covered clothing, winter pregnancies, low socioeconomic status, and lack of calcium and vitamin D supplementation during pregnancy (Andiran *et al*., 2002).

The current study aims to assess the levels of vitamin D in pregnant women during the first and third trimesters of pregnancy, in addition to some other parameters, and to study the relationship between them through the analysis of the correlation coefficient.

MATERIALS AND METHODS

The study was conducted on pregnant women who visited Kirkuk General Hospital, during the period from March to May 2022. The total number of the study sample was 40 women, 20 women in the first trimester of pregnancy and 20 women in the third trimester of pregnancy, their ages ranged between (25-35). Cases were selected after excluding those who had used vitamin D-containing supplements within the past two months.

Fasting venous blood samples were collected and preserved in the correct manner to ensure the most important components of the model study and through which reliable results in the assessment can be obtained. Therefore, many considerations were taken during sample collection, such as the use of disposable syringes, as well as the sterilization of the area with heptin, and samples were collected between 8-10 am, after which blood was drawn from the vein using (5) ml syringe, and (1) ml of drawn blood was put into fresh EDTA tubes containing anticoagulant for the purpose of measuring certain hematological parameters such as Hb. While 4 ml of the blood sample drawn in a normal tube remained at room temperature for 30 minutes and then placed in a centrifuge for ten minutes at (3000) rpm to separate the serum, the obtained serum was stored at -20 °C for measurement of vitamin D concentration by immune assay methods by Abbot equipment and by minividas equipment (Jumaa, 2019), as well as glucose measurement with a COBAS C 311 ROCH Analyzer (Burrin and Price, 1985).

Statistical analysis: After data collection and entry into the computer, it was classified using Microsoft Office Excel, and the averages for the first and third pregnancy periods were calculated for all studied parameters according to Duncan's mutable range test at a ($P \leq 0.05$), and the standard deviation was extracted using the SAS statistical analysis program (SAS, 2003). The graphs were also made and the Pearson correlation coefficient was estimated between the level of vitamin D and the rest of the other parameters using the Excel program.

RESULTS AND DISCUSSION

The results of the analysis of variance (Table 1) showed that there were highly significant differences at ($P \leq 0.01$) between the two study groups (the first trimester and the third trimester of

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pregnancy), and for all studied parameters (vitD3, HGB, HCT, MCV, PLT, WBC and Glucose) according to the results F-test, which necessitated a statistical depth to identify the group in which these parameters increased or decreased, as the averages of these parameters were tested by Duncan's multi-range method as shown in Table (2).

Table (1): Analysis of the variance between the two periods of pregnancy for the studied parameters.

Source of variance	**Df**	**Mean Square**						
		vitD3 (ng/ml)	**HGB (gm/dl)**	**HCT (%)**	**MCV (FL)**	**PLT ($\times10^3$/L)**	**WBC ($\times10^9$/L)**	**Glucose (mg/dl)**
Rep.	**19**	**9.524**	**1.506**	**10.64**	**46.678**	**2617.2**	**3.606**	**90.15**
Periods of pregnancy	**1**	**293.01****	**22.801****	**135.42****	**611.52****	**43560****	**65.792****	**960.4****
Exp. Error	**19**	**7.883**	**1.120**	**11.73**	**22.89**	**865.8**	**1.685**	**82.03**

** Significant at 1% probability level.

The results of testing the averages of the two study groups for all studied parameters by Duncan's multi-range method appear in Table (2) and Figure (1) at the 0.05 probability level.

Reports have shown vitamin D deficiency in 40-80% of pregnant women during different seasons of the year (Kazemi *et al*., 2009). Vitamin D is a steroid hormone that is synthesized in the epidermis by converting cholesterol to cholecalciferol by the sun, and has also been found in some food sources (Scwartz *et al*., 2011). With regard to the level of vitamin D (Table 2; Figure 1-a), it is clear that the first period of pregnancy was significantly superior in achieving the highest average, which amounted to 18.38 ng/ml, compared to the third pregnancy, which recorded 12.97 ng/ml. Maternal UV exposure is positively correlated with bone mineral content and infant bone volume, suggesting that maternal vitamin D has a direct effect on periosteal bone formation in infants (Sayers and Tobias, 2009). An association between low maternal vitamin D in late pregnancy and decreased intrauterine long bone growth has been reported (Viljakainen *et al*., 2010). However, the exact mechanism of action of maternal vitamin D deficiency on indices of fetal growth is unclear. The vitamin D level of the newborn correlates well with the mother's vitamin D level (Thomas *et al*., 2011). Osteoblasts have 1,25(OH)D receptors, and a decrease in 25(OH)D and/or 1,25(OH)D in the fetus results in decreased osteoblast activity, which reduces bone growth (Van Driel *et al*., 2004). Fetal vitamin D deficiency can also stimulate parathyroid hormone and parathyroid hormone-binding protein activity, resulting in decreased cortical bone growth (Tobias and Cooper, 2004). Specker (2004) reported that the amount of vitamin D drops significantly in the third trimester, when approximately 250 mg/day of calcium is transferred from the mother to the fetus. The cause of vitamin D deficiency in pregnant women has been identified, the main factors being living location, dark skin color, covered clothing style, winter pregnancies, low socioeconomic status, and lack of calcium and vitamin D supplementation during pregnancy (Andiran *et al*., 2002).

Table (2): The averages of the studied parameters during the first and third pregnancy periods.

Parameters / Periods	**vitD3 (ng/ml)**	**HGB (gm/dl)**	**HCT (%)**	**MCV (fl)**	**PLT ($\times10^3$/L)**	**WBC ($\times10^3$/L)**	**Glucose (mg/dl)**
1st Trimester	**18.38 a ±3.79**	**12.02 a ±0.94**	**37.56 a ±2.8**	**88.04 a ±4.45**	**260.85 a ±40.12**	**6.79 b ±1.32**	**83.15 b ±6.3**
3rd Trimester	**12.97 b ±2.03**	**10.51 b ±1.27**	**33.88 b ±3.66**	**80.22 b ±6.8**	**194.85 b ±42.02**	**9.36 a ±1.8**	**92.95 a ±10.33**

The values followed by the same letter are not significantly different from each other according to Duncan's multiple range test.

Vitamin D receptors are found in the bone marrow (Norman, 2006), and both 25(OH)D and 1,25-dihydroxyvitamin D (1-25(OH)D), calcitriol, the active form of vitamin D) cellular content of the marrow Bone (hematone) is much higher than in bone marrow plasma (Blazsek *et al*., 1996). Accumulating evidence indicates a role for vitamin D in erythropoiesis, as vitamin D deficiency has been associated with a higher risk of anemia, lower hemoglobin levels and the use of erythrocyte stimulating factors in individuals (Monlezun *et al*., 2015). Several potential mechanisms have been proposed for the association between vitamin D and anemia, such as the direct proliferative effect of vitamin D on bone marrow cells via vitamin D receptors, and the silencing of inflammatory reactions that lead to anemia or chronic disease (Lucisano *et al*., 2014). For blood status parameters between Table (2)

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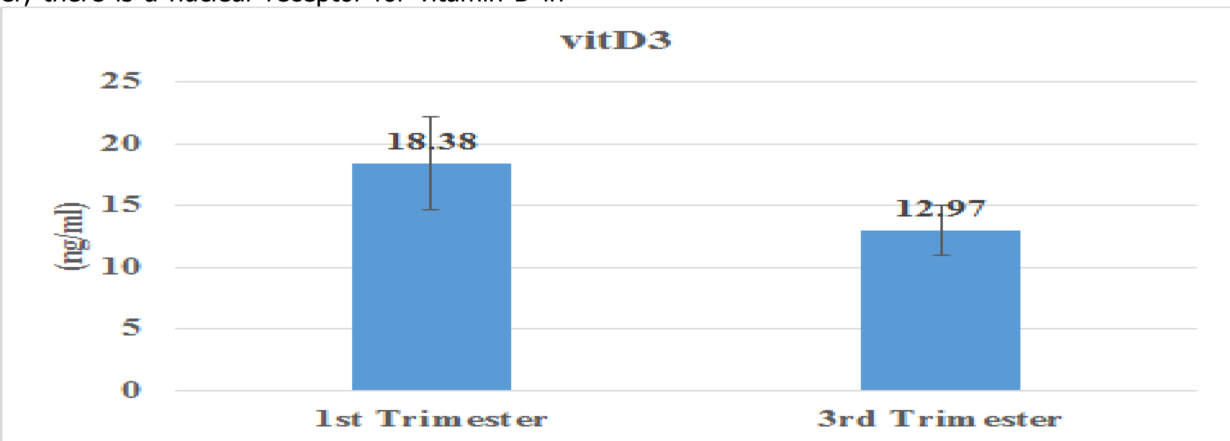
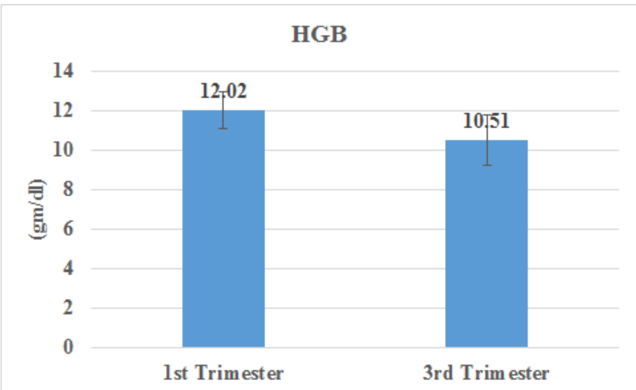
and Figures (1-b, c, d and e) that the first period of pregnancy gave the highest levels of HGB, HCT, MCV and PLT, which amounted to 12.02 gm/dl, 37.56% and 88.04 fl and 260.85 x 10^3/L, respectively, compared with the third trimester that gave 10.51 gm/dl, 33.88%, 80.22 fl and 194.85 x 10^3/L, respectively. Studies have shown the relationship between low serum 25(OH)2D3 levels and many autoimmune diseases such as impaired function of the bone marrow responsible for the production of red blood cells (Norman, 2006). In the study of Erkus et al. (2018) Results were Hb (13.5 gr/dl) in study group versus (13.6 gr/dl) in control group, Htc (39.8%) in study group versus (40.5%) in control group, MCV (88.4 FL) in study group versus (87.5 FL) in control group and PLT (259089 cells/mm^3) in study group versus (235825 cells/mm^3) in control group were not significantly different in vitamin D deficiency group compared to controls.

The anti-inflammatory effect of vitamin D has been reported in some studies (Talaei *et al.*, 2013). It is possible that the active form of vitamin D reduces the expression of pro-inflammatory cytokines such as IL-6, IL-1 and TNF-α involved in insulin resistance. Moreover, there is a nuclear receptor for vitamin D in some specific tissues during pregnancy such as the placenta. and decidua's (Hypponen and Boucher, 2010), vitamin D deficiency appears to have a role in inflammation (Neyesani *et al.*, 2012). The results for white blood cells (WBC) (Table 2) and (Figure 1-f) showed that the first pregnancy recorded the lowest mean, which was 6.79 x 10^3/L, compared to the third period of pregnancy, which recorded a significant increase in the WBC rate, which amounted to 9.36 x 10^3/L. Vitamin D has structural effects, including mineralizing bones and maintaining their turnover; It may also protect against health problems such as cancer, high blood pressure, type 1 diabetes, and multiple sclerosis (Holick, 2003). It plays a vital role in the proliferation and differentiation of different types of cells (Bikle, 2010). Vitamin D suppresses specific inflammatory pathways and reduces inflammatory cytokine production by human periodontal ligament cells. Studies have shown the association between low serum 25(OH)2D3 levels and several autoimmune diseases such as atopic dermatitis, inflammatory bowel disease, asthma, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and bone marrow function (Norman, 2006).



a



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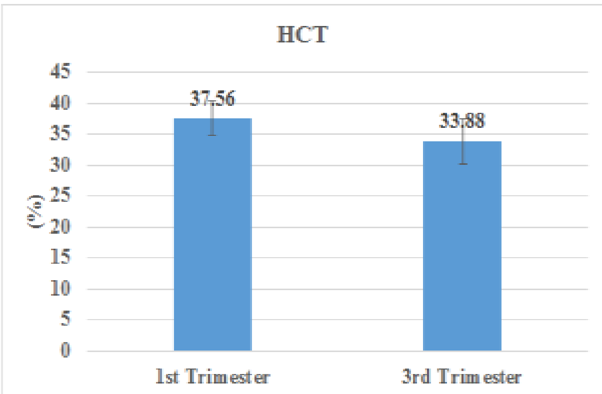

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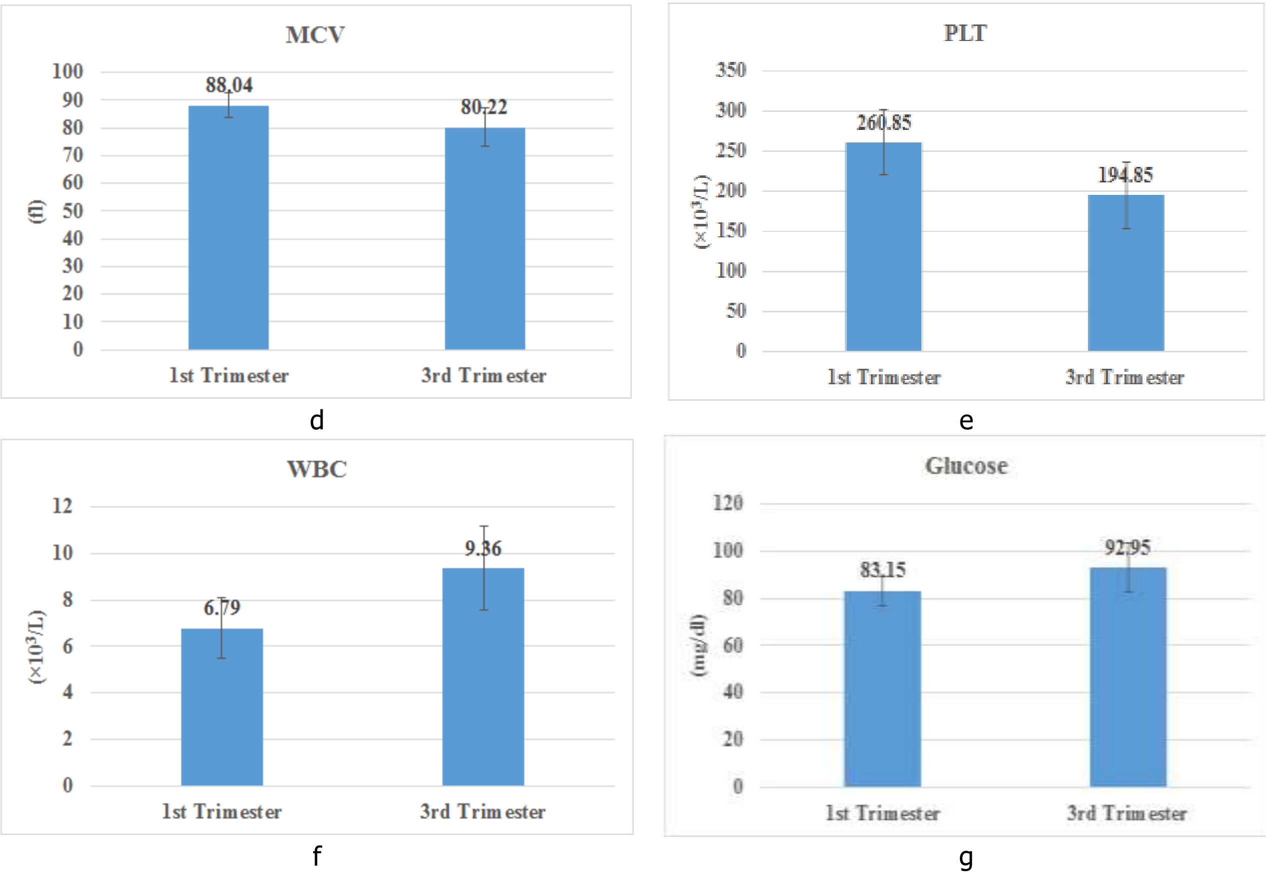

Figure 1: Graphic forms of the averages of the two study groups for all the studied parameters.

Hyperglycemia may occur during pregnancy and begin at 24 weeks of gestation, but reach its maximum in the last trimester. Pregnancy is prediabetes and is associated with glucose intolerance. In the second and third trimesters, there will be an increase in the levels of hormones primarily secreted by the placenta such as human placental lactogen, prolactin, progesterone and estrogen, which in addition to thyroid hormone secrete anti-insulin hormones, to increase glucose production in the liver, reduce tissue glycogen storage, and reduce the sensitivity of peripheral tissues to insulin. All of the above events will lead to what is called gestational diabetes (Triunfo *et al*., 2017). For blood glucose level, Table (2) and Figure (1-g) showed a significant increase in the progression of pregnancy, as the first and third pregnancies recorded rates of 83.15 and 92.95 mg/dl, respectively. Some studies suggest that vitamin D deficiency has a negative effect on insulin sensitivity and may lead to an increased incidence of type 2 DM in adults (Kayaniyil *et al*., 2010). There is a significant association between glucose and vitamin D metabolism, through increased sensitivity of insulin receptors as well as insulin release from the pancreas and overcoming or decreasing insulin resistance, at the level of peripheral tissues there will be an increase in response to glucose and enhanced is intracellular entry (Kostoglou – Athanassiou *et al*. 2013).

Table (3) shows the results of the analysis of Pearson's correlation coefficient between vitamin D levels and the rest of the studied parameters during the first and third pregnancy periods. Among them, it was shown that there were no significant correlations between the level of vitamin D and other parameters during the first pregnancy period, while during the third period of pregnancy, positive and significant correlations were found at ($P \leq 0.05$) for the level of vitamin D with both HGB and HCT, whose values were 0.349 and 0.332, respectively. It also found a negative and significant association at ($P \leq 0.05$) for the level of vitamin D with the level of white blood cells, and its value was -0.446. These results do not agree with Kostoglou – Athanassiou *et al*. (2013) who found a strong association between glucose metabolism and vitamin D, by increasing insulin receptor sensitivity as well as releasing insulin from the pancreas and overcoming or decreasing insulin resistance, at the level of peripheral tissues there will be an increase in

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response to glucose and enhanced is intracellular entry. Amiri *et al*. (2021) that HOMA, HC, MCV, MCHC had a negative correlation with vitamin D concentration, in assessing the association of blood components with vitamin D in the blood. Jastrzebska *et al*. (2017) link vitamin D deficiency with an increased risk of cardiovascular disease and anemia. Red and white cell counts, hemoglobin, hematocrit, MCHC, and ferritin, changed significantly.

Table (3): Analysis of Pearson's correlation coefficient between vitamin D levels and other parameters under study during the first and third pregnancies.

	HGB (gm/dl)	HCT (%)	MCV (fl)	PLT ($\times 10^3$/L)	WBC ($\times 10^9$/L)	Glucose (mg/dl)
vitD3 in 1st Trimester	**-0.155**	**-0.109**	**0.226**	**0.219**	**0.009**	**0.252**
vitD3 in 3rd Trimester	**0.349***	**0.332***	**0.251**	**0.202**	**-0.446***	**0.054**

*Sentimental at 5% probability level.

CONCLUSION: The results of the analysis of variance showed significant differences at ($P \leq 0.01$) between the two study groups (first and third pregnancy), and for all studied parameters (vitD3, HGB, HCT, MCV, PLT, WBC and Glucose). The first pregnancy period achieved the highest averages for the parameters vitD3, HGB, HCT, MCV and PLT, which were 18.38 ng/ml, 12.02 gm/dl, 37.56%, 88.04 FL and 260.85 x 10^3/L, respectively. While the third pregnancy period recorded the highest averages of the two parameters, WBC and Glucose, which amounted to 9.36 × 10^3/L and 92.36 mg/dl. The results of the analysis of Pearson's correlation coefficient between vitamin D levels and the rest of the parameters studied during the first and third pregnancies showed that there were positive and significant correlations at ($P \leq 0.05$) during the third pregnancy between vitamin D levels with both HGB and HCT, whose values were 0.349 and 0.332, respectively. It also found a negative and significant association at ($P \leq 0.05$) for the level of vitamin D with the level of WBC, and its value was -0.446. Thus, we find a clear effect for the period of pregnancy in the level of vitamin D, which decreased with the progression of pregnancy, and the decrease in the level of vitamin D was followed by a deterioration in the parameters of the blood condition. It was also found that the level of white blood cells and glucose increased as the level of vitamin D decreased, and thus the health status can be detected. For individuals by evaluating the level of vitamin D in the serum, care should be taken to take nutritional supplements containing vitamin D for women during pregnancy within safe concentrations.

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