The Hidden Link between Calcium Deficiency and Osteoporosis Risk During Pregnancy: An In-depth Study in Samarra

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Abstract
The aim of this study is to evaluate calcium deficiency and its relationship to some biochemical variables during the three stages of pregnancy for some pregnant women in the city of Samarra. Blood samples are collected from (66) pregnant women who were diagnosed by specialized doctors. Their ages ranged between (17-50) years, from the Samarra General Hospital and outpatient clinics, and (24) healthy women of similar ages. The level of calcium, vitamin D, parathyroid hormone, alkaline phosphatase, phosphorous and albumin were measured. The results show a high level of vitamin D and Parathyroid Hormone (PTH), alkaline phosphatase (ALP) and albumin in pregnant women compared to the control group, while calcium decreased compared to the control group. Where the thyroid hormone PTH and albumin are more low in the first period of pregnancy, while calcium and phosphorus are lower in the third period of pregnancy, while ALP was lower in the second period of pregnancy, while vitamin D is significantly lower in the third trimester of pregnancy, as the results showed that the correlation between calcium and PTH is a negative relationship, while the relationship between calcium, vitamin D, albumin, and phosphorus is poorly positive, and the relationship between calcium and ALP is weak positive relationship. From this, we can conclude that there is a relationship between the low level of calcium and pregnancy and therefore can be considered as indicators of an increased risk of osteoporosis in women.

Keyword: Calcium deficiency, Osteoporosis risk, Pregnancy stages, Vitamin D, Parathyroid Hormone.

Introduction:

During pregnancy, there is no doubt that a woman's nutritional needs are very different from the normal situation. All the elements that the fetus obtains for growth during the nine months of pregnancy come from the mother's body and its stores of vitamins and minerals, in addition to what she eats during this delicate period for her and her fetus.In the following, we review and you the changes that occur in the body of a pregnant woman with regard to her needs for calcium, in addition to the most important sources through which she can be provided with this important

element.Calcium is an important component of bone health throughout life. Although diet is the best way to get calcium, supplements containing calcium may be a possible option to replace a deficiency in the diet.Before considering the use of calcium supplements, make sure you know how much calcium you need and the pros and cons of any calcium supplement to choose.

Calcium is one of the necessary elements for living organisms, especially in cell physiology, as the movement of calcium ion Ca + 2 to and from the cytoplasm is important for many processes that occur in cells, and it is one of the important minerals for bones. Ca+2 is (2.2 105) of the body mass, 99% of which is stored in the bones and 1% is found in the blood and extracellular fluid.Calcium ion is an important form for most functions in the body, including the effect of calcium on the heart, nervous systems, and bone formation, and it is believed that parathyroid hormone affects calcium metabolism [(1-5)]. Calcium-regulating hormones are:

1- Parathyroid hormone: The effect of the hormone in increasing calcium ion levels in the blood is through the activity of osteoclasts, leading to bone destruction and inhibiting the activity of osteoblasts [(6-8)].

2- Calcitonin hormone: The hormone calcitonin is secreted from C- cell cells in the thyroid gland, which reduces the concentration of calcium in the plasma in two ways: it reduces the absorption activity of osteoclast cells, then it causes a state of balance in favor of calcium arrangement in the bone, and it reduces cells Osteoblasts and osteoclasts, and the overall result is a significant decrease in biological activity [(9-11)].

3- Vitamin D: The presence of vitamin D is essential in the process of calcium absorption, as the presence of vitamin groups to help the absorption process, and on the contrary, the absorption process is greatly reduced, which leads to a disturbance in the metabolism of vitamin D, which leads in patients with renal failure to a decrease in calcium absorption from the intestine, and an increase in the secretion of thyroid hormone [(12-15)].

Liver enzymes are complex protein substances that catalyze chemical reactions in vital systems and are the key factors that enable many of the biochemical reactions that make up life to occur naturally in the body's cells. Most enzymes work inside the cells that produce them, and they are in a concentration much greater than the

concentration of the enzyme in the serum, and this change in the concentration of the enzyme in the normal serum reflects both disease and health conditions by reversing the change in the balance between the rate of enzyme production within the cell and the rate of enzyme release rate And its leakage from the cell during normal cell turnover or catabolism and cell secretions [16-20]. The defect in liver function is diagnosed by estimating the activity of liver enzymes, including alkaline phosphatase (ALP) enzyme, alanine aminotransferase (ALT) enzyme Aspartate aminotransferase (AST), in serum [21-25].

Materials and Methods:

- **Samples of the study**

Sixty-six pregnant women, their ages ranged from 17-50 years, participated in the visits to Balad General Hospital and outpatient clinics who did not have clinical signs of calcium and vitamin D deficiency according to the special questionnaire. Patient group and 24 healthy women of similar age were drawn 5 ml of blood and from Then the serum was separated by centrifugation for 10 minutes at a speed of 3000 rpm, then the serum was withdrawn and placed in sterile tubes and kept in a state of freezing at a temperature of 20 until biochemical tests were performed.

- **Biochemical examinations**

Determination of calcium concentration in blood serum

Calcium concentration was estimated using the Colorimetric Methods, in which ready-made solutions were used, which depend on the reaction of the compound (O-Colorimetric) with the compound (8- HydroxyQuinoline) with calcium ions and the formation of a complex with a violet color with the highest absorbance at a wavelength (572) nanometer, as the intensity of the color is directly proportional to the concentration.

Determination of total protein concentration in blood serum

This method is based on the basis that the proteins in the basal environment are with copper ions ll a blue-violet complex (Colored Complex blue-Violet) and the color intensity of this complex is directly proportional to the concentration of total proteins

in the serum) and the color intensity of this complex is directly proportional to the concentration Total proteins in serum.

Determination of the total albumin concentration in the blood serum

The serum albumin concentration was estimated using the BromocresolGreenMethod (BCG) method in which ready-made solutions were used.This method depends on the amount of albumin bound with Tetrabromo 5-5-3-3 M-Cresol Sulphonphthalien reagent called bromocresol green to form an albumin-bromocresol green complex.Complex Albumin-BCG `has a green color, whose intensity is measured at a wavelength of 630 nm in the spectrophotometer.

Determination of the activity of the enzyme aspartate aminotransferase (AST) in the blood serum

The activity of the enzyme AST in the blood serum as in the following equation:Oxalacetate+ L-glutamate ALT L- Aspartate+ oXaglutarate.

A test tube was taken and (0.5) ml of the reagent solution (1 R) was placed in it, then the tube was placed in the incubator at (37) C for 5 minutes, after that it was extracted from the incubator and (0.1) ml of serum was added to it and the tube was mixed well. It was returned to the incubator for 60 minutes, then it was extracted again from the incubator and 0.5 ml of reagent solution (2 R) was added to it. The contents of the tube were mixed well, and left for 20 minutes at room temperature, then added. To it (5) ml of sodium hydroxide and left for (5) minutes, then the absorbance was read at a wavelength of NM (505) after measuring the intensity of the absorbance in a spectrophotometer that locked the absorbance of the ALT enzyme.

Determination of the activity of the alanine aminotransferase enzyme in the blood serum.

A test tube and put in it (0.5) ml of the reagent solution (1 R), then the tube was placed in the incubator at (37) C for 5 minutes. After that it was extracted from the incubator and (0.1) ml of serum was added to it and the tube was mixed well. It was returned to the incubator for (30) minutes, then it was extracted again from the incubator and (0.5) ml of reagent solution (2 R) was added to it, the contents of the

tube were mixed well, and left for (20) minutes at room temperature, then added To it (5) ml of sodium hydroxide and left for (5) minutes, then the absorbance was read at a wavelength of NM (505) after measuring the intensity of the absorbance in a spectrophotometer that locked the absorbance of the ALT enzyme.

Determination of the concentration of 25-Hydroxy vit. D in serum

The enzyme horseradish peroxidase (HRP) marked with Avidin is added, and selectively arranged with the biotin complex, followed by another washing step where the color is revealed using the dye substrate (TMB) Tetramethylbenzidine. With a concentration of vit. D.25-hydroxy vit antibody. D at Microplate micro-calibration drilling sites.Concentrated solution of 25-D biotin.A package contains (50) ml of 25-hydroxy vit. D dissociated from bound proteins.A bottle containing (22) ml of phosphate buffer salin bound to Horseradish peroxidase (HRP).Two packages, each containing (1) ml, one with a high concentration and the other with a low concentration.A bottle containing (28) ml of Tetramethylbenzidine (TMB) and hydrogen peroxide.A bottle containing (13) ml of hydrochloric acid at a molar concentration (0.5).A bottle containing (50) ml of phosphate buffer salin.

The calibration and control solutions and the sample were prepared by adding (25)µl to each of them in test tubes. 1 mL of 25-D biotin was added to the test tubes and the nose, then the solutions were shaken for 10 minutes.200µl of the previous mixture was added into the micro-calibration holes, covered with tape, and incubated for two hours at (25)°C.The pits were washed three times with diluted washing solution.200µl of bound enzyme was added to all titration pits, covered with tape and placed in the incubator for half an hour at (25)°C.The pits were washed three times with diluted washing solution.200nµl of TMB primer solution was added, the holes were covered with tape and incubated for (30) minutes at (25)°C.After the end of the incubation period, the enzymatic reaction was stopped by adding (100)µl of the solution to each pit.The optical density of the etch was read at a wavelength of (450) nm.

- **Statistical Analysis**

The statistical analysis of the results of the study was conducted according to the analysis of variance (ANOVA) test, using the statistical program Minitab, and the

average sensitivity of the groups was compared using the Duhcun Multiple Range test to show the difference between two groups at the level of probability ($P \leq 0.05$), [27-30].

Results:

The results shown in Table 1 compared between pregnant women and the healthy group showed a low level of calcium in pregnant women, as there was a significant difference between pregnant women and the control group, and it showed a significant increase in the level of vitamin D in pregnant women compared to healthy ones.

The study also showed a significant increase in the concentration of PTH and ALP in pregnant women compared with the control group and as shown in Table 1, and it was found that hyper-PTH poses a threat to the mother and fetus, and that up to 80% of pregnant women with primaryPhosphorus.Italsoshowed that there was an insignificant increase of phosphorous in pregnant women compared to the control group as shown in Table 1.

Table 1: shows the comparison between the biochemical levels studied for the pregnant and the control group.

Parameters	Control (No.24)	Pregnant (no.66)	P.Value
	$Mean \pm SD$		
PTH	23.301 ± 10.093	74.046 ± 13.685	0.001**
Vit D	5.211 ± 0.30	6.401 ± 0.33	0.01*
Calcium	8.552 ± 0.265	7.577 ± 0.630	0.001**
Phosphorus	2.7 ± 0.281	2.768 ± 0.382	0.822
Albumin	3.408 ± 0.573	3.692 ± 0.545	0.03*
ALT	7.920 ± 1.629	9.638 ± 2.627	0.004**

The biochemical indicators were studied during the three stages of pregnancy, is shown in Table 2.

Figure 1: Visual Comparison of Biochemical Levels Studied for the Pregnant and Control Groups

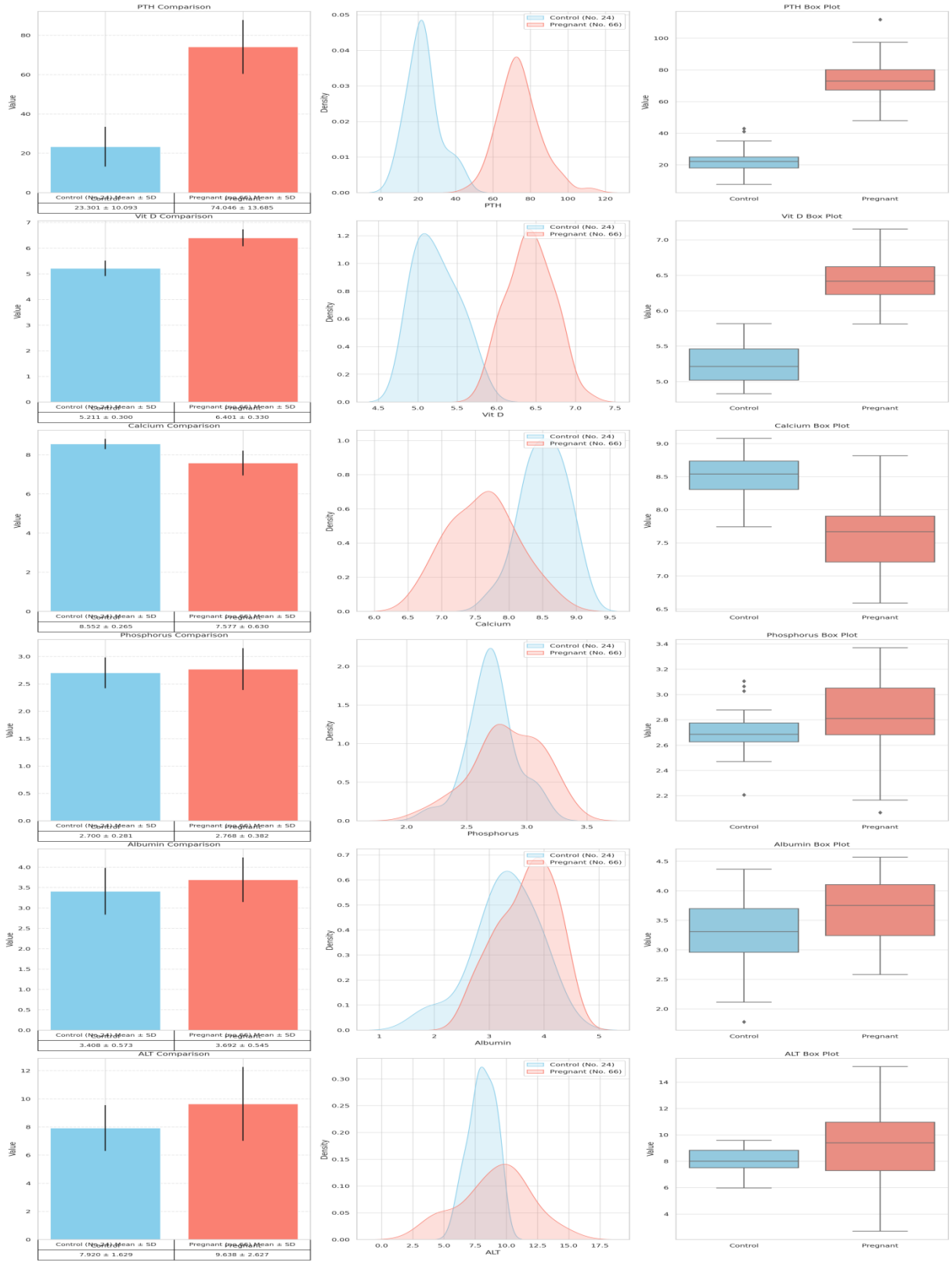
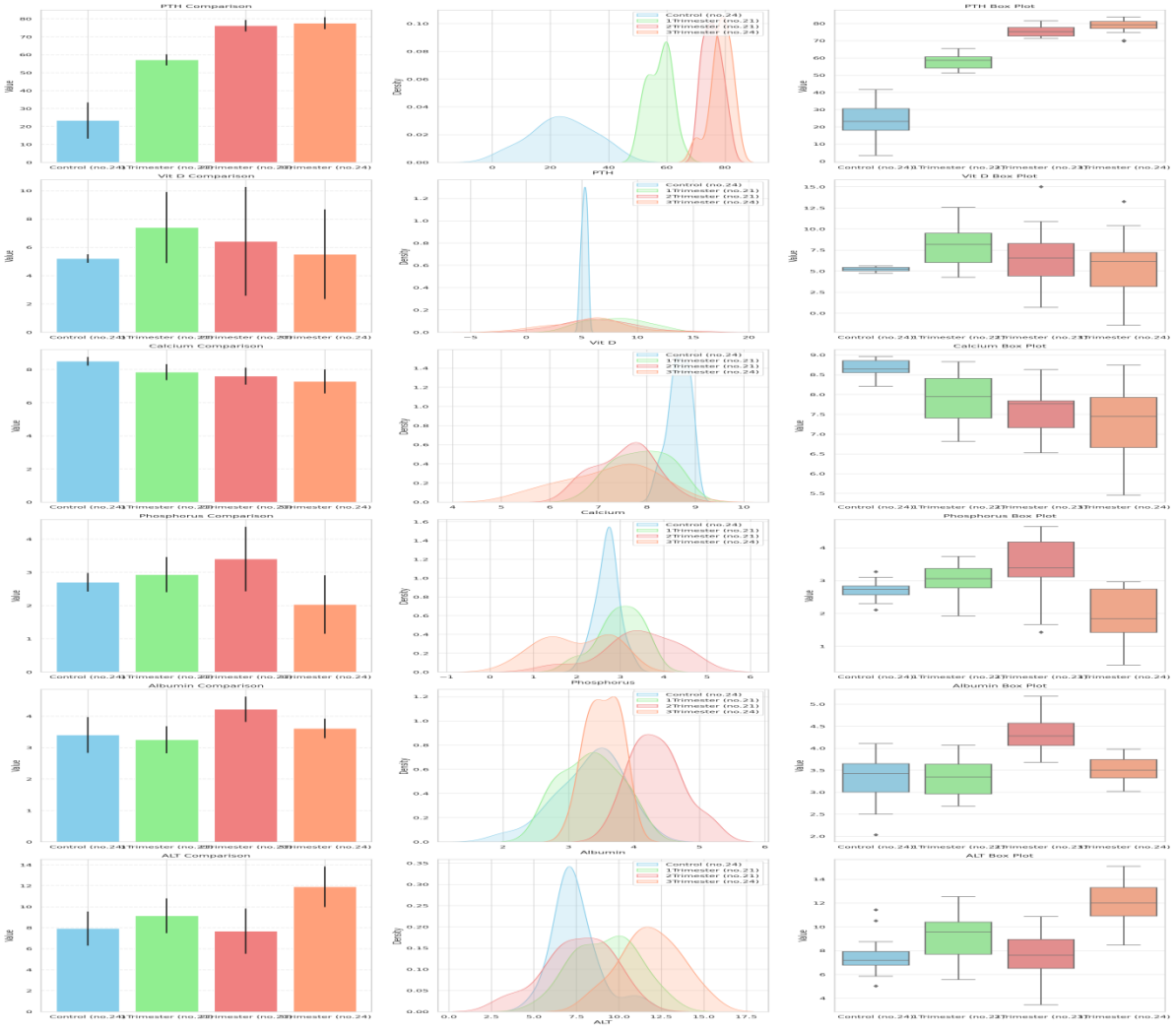


Table 2: Comparison between the studied biochemical indicators for the pregnant group and the control group according to the stage of pregnancy.

Parameters	***Control (no.24)***	***1Trimester (no.21)***	***2Trimester (no.21)***	***3Trimester (no.24)***	***P Value***
$Mean \pm SD$					
PTH	23.3 ± 10.09				0.001**
Vit D					0.03*
Calcium					0.001**
Phosphorus					0.001**
Albumin					0.001**
ALT					0.001**



Discussion:

The reason for the low level of calcium leads to an increase in the transfer of calcium to the fetus for use in building the skeleton of the fetus and as a result of the increase in blood volume and the occurrence of dilution and the increase in the transfer of calcium from the bones and the increase in its excretion in the urine, which stimulates the parathyroid hormone (PTH) to stimulate calcium absorption from the intestine and release calcium from the bones to compensate for the deficiency in Calcium The cellular calcium level increases [(31-40)], the decrease in the nutritional content of calcium exposes the pregnant woman to the risk of pregnancy complications such as spasticity of the limbs, preeclampsia, or osteoporosis. And taking calcium supplements during pregnancy reduces the incidence of pregnancy complications, so calcium has a role mainly in bone formation, muscle contraction, transmission of nerve impulses, maintaining the integrity of muscle and bone functions, and works with magnesium in regulating blood pressure and affects the permeability of cellular membranes and has a role in iron absorption [(42-50)].

The results in Table 1, and this result differs with a study conducted by [(11)]. whose results showed a decrease in vitamin D in pregnant women. Many studies have been conducted on the measurement of vitamin D because there are various consequences of its deficiency, and that there is a relationship between vitamin D deficiency and various negative pregnancy outcomes, and the reason for the difference in results with the previous study may be that the control group that was taken as a sample for the study had a deficiency.

Vitamin D. It has been hypothesized that vitamin D deficiency can be associated with an increased risk of pre-eclampsia and disease Diabetes during pregnancy, caesarean section and bacterial vaginosis during pregnancy, and vitamin D may play an indirect cycle through its effect on intracellular extracellular calcium regulation, which is necessary to mediate glucose transport in target tissues [(51-53)]. The results showed a high level of albumin in pregnant women compared to the control group, and this increase may explain hyperalbuminemia in pregnant women, including: liver disease, dehydration, and impaired kidney function [(54-59)].

Hyperparathyroidism do not suffer as a symptom, the diagnosis of this condition is more difficult. Complications associated with primary hyperparathyroidism in pregnancy have been reported in 67% of mothers and 80% of fetuses. As well as symptoms, complications include nephrolithiasis, bone disease, pancreatitis, hyperemia, muscle weakness, mental status changes and hypercalcemia crisis, and reported fetal complications include intrauterine growth retardation, low birth weight, preterm delivery, and intrauterine death [(60,61)]. And there is insufficient evidence for the effectiveness of ALP in the serum of pregnant women as a control marker for the risk of preterm birth, and this is related to the fact that the level of ALP grows during pregnancy (placental fracture) [(15)].

In a study by [(16)] that focused on renal treatment of phosphorous during pregnancy and its relationship to factors that regulate metabolism. A significant decrease of phosphorus was observed and the change was Most notably due to high cholesterol levels, high cholesterol enhances intestinal absorption of calcium and phosphorous to compensate for the increased needs. And its low in pregnant women is due to its use in the process of building the skeleton of the fetus.

The biochemical indicators were studied during the three stages of pregnancy, and as shown in our results indicated a continuous decrease in the calcium level in the second and third trimesters of pregnancy, which was associated with the study [(17,18)]. and this is due to a decrease in the calcium concentration in the blood. This decrease is attributed to this decrease. To the widening of the intravascular space during pregnancy, and the increased transfer of calcium to the fetusthrough the placenta to build the fetal skeleton, the fetus begins intensive calcium consumption early in pregnancy and increases further, as the fetus begins its nervous system, skeletal system, and internal organs to form. In the fifth month of fetal development, only 15-35% of the calcium is used by the fetus. For this reason, the calcium level in a pregnant woman decreases at 8 to 15 weeks of pregnancy and this progression in pregnancy. Increases calcium consumption by the fetus explains the high incidence of caries in the mother in late pregnancy [(19)].

Our study differed with the study conducted by [(20)], which indicated that vitamin D deficiency is in the early stages of pregnancy compared with the third age stage, where low vitamin D was observed in the second and third age stages, and low

vitaminD is due to several reasons, including the level of Education, community environment, insufficient intake of vitamin D. The results showed elevated PTH in all three pregnancy periods and it was more elevated in the third pregnancy, and in a study conducted by [21] on a group of pregnant women, an increase in PTH was observed in the third trimester compared to With the first and second trimester. Our results showed that ALP was more elevated in the thirdpregnancy term compared to the rest of pregnancy, and this result was in agreement with [22].

The albumin concentration was lower in the first trimester of pregnancy compared to the rest of the pregnant period, and this result agreed with [23] where the albumin concentration decreases by 8-10% in the first week of pregnancy and we find that the reason is that the album is pregnant or associated with many nutrients and when the concentration of nutrients leads to a decrease in the concentration of albumin, and the level of phosphorus in the blood was lower during the third trimester of pregnancy, which can be explained by the increased demand for the growing fetus and exposing the mother to the risk of complications associated with low phosphorus in the blood serum [24].

Conclusions:

There is a relationship between the low level of calcium and pregnancy and therefore can be considered as indicators of an increased risk of osteoporosis in women.

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