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RESEARCH ARTICLE

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The relationship of cathepsin-k level with some biochemical and hematogical variables in women with osteoporosis, pregnant and menopausal

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Abstract

In this study, the biochemical and hematological variables in 180 patients (women) between 17 and 75 years old were hospitalized at Al-Sadr Teaching Hospital in Najaf Iraq between April and July 2023. The patients were devised in 4 different group studies with 180 patients in each group consisting of 45 women: female with osteoporosis in the range age 22-35, a pregnant female in range age 17-37, female menopause range age 45-75, and healthy females in range

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age 19-65 as the control group. The biochemical variables such as level of Cathepsin K (Cath-k) concentration, Estradiol (E2) concentration, vitamin D (Vit -D), and parathyroid hormone (PTH) concentration in women with osteoporosis, menopause, pregnant and healthy women were evaluated. In addition, the hematological variables: red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), number of platelets (PLT), and number of white blood cells (WBCs) were evaluated in different study groups. Also, the concentration of phosphate (P) and Body Mass Index (BMI) was estimated. In the end, the correlation test with each variable in different group studies was calculated by the STATISTICA program. The obtained results showed that there was a significant increase in the numbers of WBCs, the concentration of Cath-k, PTH, and BMI for the study groups compared to the control group, as well as a significant decrease in the concentration of P and Vit -D for the study groups compared to the control group. There is a significant increase in the E2 hormone in the group of pregnant women and a significant decrease in women with osteoporosis who are in menopause compared to the control group, as well as the presence of a significant decrease in RBCs, and Hb in the group of pregnant women compared to the control group. There is a significant increase in the numbers of PLT in the group of women with osteoporosis and women in menopause compared to the control group, as it is concluded from the current study that there is a close correlation between some different variables, including a good correlation between BMI and PLT was found to be 0.83. This may confirm that BMI is associated with increased cardiovascular morbidity and mortality through various molecular mechanisms possibly linking metabolic syndrome to hemostatic and vascular abnormalities in addition, a good correlation was observed between PTH and Cat-k (0.89), which indicated there is a relation between these two parameters.

Keywords: Cat-k, Vit-D, Osteoporosis, Correlation, STATISTICA program, Hematological variables, Biochemical variables.

1. Introduction

According to the WHO definition, osteoporosis is a generalized skeletal disease, characterized by low bone density and alterations in bone microarchitecture, responsible for excessive bone fragility and therefore a high risk of fracture [1]. This pathology particularly affects women aged 50 and over. In France, 3 million people are affected. It is a disease that is constantly increasing around the world but more particularly in Europe, linked to the increasing aging of the population. Osteoporosis is a serious disease, poorly understood by most patients, although established knowledge has recently emerged [1].

The severity of osteoporosis is directly linked to the consequences caused by osteoporotic fractures. Indeed, these fractures often compromise the quality of life of patients with an increase in morbidity. Patients often present with chronic pain, impaired mobility, increased disability, and dependence. To a greater degree, osteoporotic fractures are also responsible for an increase in mortality, especially in the first year following the fracture [2].

Early therapeutic management of osteoporosis could considerably reduce the cost generated by this disease (hospitalizations, long-term care at home, institutionalization in expensive adapted structures, etc.). Currently, this treatment is too insufficient, to the point that only around 20% of patients benefit from it after a fracture. The management of this disease thus becomes a public health objective set by the law of August 9, 2004 [3].

Cathepsin K (Cath-k) was recently proposed as a possible alternative bone marker [4]. Cath-k is a cysteine protease that breaks down type I collagen in bone through the action of osteoclasts [5]. It was discovered that mutations in the Cath-k gene cause pycnodysostosis, an autosomal recessive bone sclerosing condition [6]. On the other hand, Cath-k overexpression in osteoclasts sped up the trabecular bone turnover in mice [7]. In addition, in nonhuman primates, suppression of human Cath-k inhibits bone resorption in vivo [7].

Osteopetrosis is caused by decreased osteoclastic bone resorption in mice missing Cath-k, as shown by Saftig et al. [7]. Cath-k may be a helpful and precise biochemical marker of osteoclastic activity as it is produced and secreted by osteoclasts during active bone resorption.

According to Henriksen et al.[8], circulating levels of Cath-k can be utilized as a surrogate measure of osteoclast number since they are equal to the total amount of osteoclasts. The qualification of Cath-k serum levels in the diagnosis

of osteoporosis is examined in this study. Serum Cat-k may be helpful in detecting postmenopausal women with osteoporosis, according to several researchers who have reported increased levels of this protein in osteoporotic people [9][10].

This study aimed to evaluate the biochemical and hematological variables in patients women between 17-75 years old hospitalized in hospitalized at Al-Sadr Teaching Hospital in Najaf , Iraq between April and July 2023, Iraq between April, and Jully 2023. The patients were devised in 4 different group study with 180 patients in each group consist of 45 women: Female with osteoporosis in range age 22-year, Pregnant female in range age 17-37, Female menopause range age 45-75, and Healthy females in range age 19-65. The biochemical variables such as level of Cath-k concentration, estradiol (E2) concentration, vitamin D (Vit -D) and parathyroid hormone (PTH) concentration in women with osteoporosis, menopause, pregnant and healthy women was evaluated. In addition, the hematological variables: red blood cells (RBCs), hemoglobin (Hb), and hematocrit (HCT), number of platelets (PLT) and number of white blood cells (WBCs) were evaluated in different study groups. Also, the concentration of phosphate (P) and Body Mass Index (BMI) was estimated. In the end the correlation test with each variable in different group study was calculated by STATISTICA 10.0 program.

2. Materials and Methods

2.1. Study design

In this work the biochemical and hematological variables were evaluated in patients between 17-75 years old hospitalized Al-Sadr Teaching Hospital in Najaf, Iraq between April and Jully 2023.

The patients were devised in 4 different group studies with 180 patients in each group consisting of 45 women: females with osteoporosis in the range age 22-35, pregnant females in range age 17-37, females with menopause range age 45-75, and healthy females in range age 19-65 as the control group.

BMI was estimated. The correlation test with each variable in different group studies was calculated by the STATISTICA program.

2.2. Blood collection

Venous blood samples were obtained in the sitting position by using a disposable syringe (5 mL). Five ml of blood was obtained from each person by piercing the vein and slowly pushing it into two tubes (3 mL blood in the plan tube and the other 2 mL blood in the EDTA tube for CBC). The plane tube was centrifuged for 10-15 minutes at a speed of (10,000 × g) then the serum was divided into two parts and stored at -20˚C until analysis.

2.3. Evaluation of hematological variables

A blood test was performed on all patients in the group studied by collecting venous blood samples after a fast of more than 8 hours, as well as WBCs, RBCs, Hb, HCT, PLTs were tested. Blood tests were measured using the LABGEO PT 9 liver test (Samsung Electronics).

2.4. Evaluation of biochemical variables

2.4.1. Determination of E2-level

To prepare the reagent, the wash solution was diluted with distilled water the 1:20. Enzyme conjugate was prepared by adding the contents of the bottle (25 ml) to 475 ml of distilled water. Solutions were stored at room temperature. All reagents and samples were brought to room temperature before starting. Then volume of 25 µL of standard, specimens and controls was added to the appropriate wells, and a volume of 50 µL of working solution of Estradiol Biotin Reagent was added to each well and was mixed well by placed on a shaker for 20 seconds. Then plate was covered and incubated for one hour at 25 ºC. After that, a volume of 100 µL of Estradiol Enzyme Reagent was added

to all wells and mixed well by placing them on a shaker for 20 seconds and then incubated for 45 minutes at 25 ºC. The microplate was discarded from the solution and washed using 300 µl wash buffer three times, a volume of 100 µl of tetramethyl benzyl (TMB) reagent was added to each well and incubated for 20 minutes at 25 ºC. A volume of 50 µl of stop solution was added to each well for topping solution. Then the microplate contents were mixed gently for 30 seconds to make sure that all the blue color changed to yellow color completely. The absorbance of each well was determined at 450 nm with a microplate reader within 15 minutes after adding the stop solution.

2.4.2. Determination of Vit -D in Blood Serum

Vit -D was estimated in the blood serum using the ready-made kit from the Chinese company BIONT, by following the steps attached to the kit, and using the ELISA technique. The detailed experiment was mentioned in support information.

2.4.3. Determination of PTH levels

PTH levels were determined using ELISA technique according to the kit procedures.

2.4.4. Determination of Human Cat-k Levels

Cat-k levels were determined the spectrophotometric method. For the standard solution, a volume of 120 µL of the standard (16 ng/ml) was diluted with 120 µL of standard diluent to generate an 8 ng/mL standard stock solution. The standard solutions were allowed to sit for 15 minutes with gentle agitation prior to making dilutions. A volume of 50 µL standard was added to standard wells, then a 40 µL of serum was added to sample wells then 10 µL of anti- Cat-k antibody was added to sample wells. A volume of 50 µL of streptavidin-HRP was added to sample wells and standard wells and mixed well, then the plate was covered with a sealer and incubated for 60 minutes at 37 ºC. The sealer was removed, and the plate was washed 5 times with 0.35 mL of wash buffer, 50 µL of substrate solution A was added to each well, and then 50 µL substrate solution B was added to each well and the plate was incubated after covering with a new sealer for 10 minutes at 37 ºC in the dark. A 50 µL of stop solution was added to each well, and the tube color changed from blue to yellow immediately. The absorption was determined of each well using a microplate reader set at 450 nm within 10 minutes after adding the stop solution.

2.4.5. Determination of P concentration in blood serum

P concentration in blood serum was determined by spectrophotometric method. The principle of the method is based on the reaction of phosphate in serum with ammonium molybdate and changing color. Color intensity was measured at 340 nm and calculated in mg/dL by comparison with the standard curve.

2.5. Statistical analysis

The statistical study based on patient responses transcribed in the form of an Excel table. The ANOVA TEST was used to find the arithmetic mean and standard deviation, and the correlation test was determined. Qualitative variables were obtained and compared using the Fischer exact test. The chosen threshold of statistical significance was $p < 0.05$, i.e. a risk of concluding that there was an inaccurate difference between groups.

3. Results and Discussion

3.1. Standard curves

3.1.1. E2 level

The standard curve is generated by plotting the absorbance obtained for each reference standard on the vertical axis (Y) against the corresponding concentration (pg/ml) on the horizontal axis (X). Figure 1a. shows the absorption values for each sample versus the corresponding E2 concentration of the standard curve.

3.1.2. Human Cat-k Levels

A standard curve was created by plotting the absorption of standard solutions on the vertical (y) axis versus their concentration on the horizontal (x) axis. Test concentration was calculated by drawn an appropriate curve and by point to point on the graph.as shown in Figure 1b.

3.1.3. Vit -D in blood serum

The rates of the optical absorption values were calculated for each group, then a curve was drawn by means of the absorption rate that is obtained from each measurement against its concentration, as the absorbance is on the vertical Y axis and the focus is on the horizontal X axis, as shown in the Figure 1. Then the absorbance values decreased to the values obtained from the samples on the curve showing the hormone concentration (pg/mL).

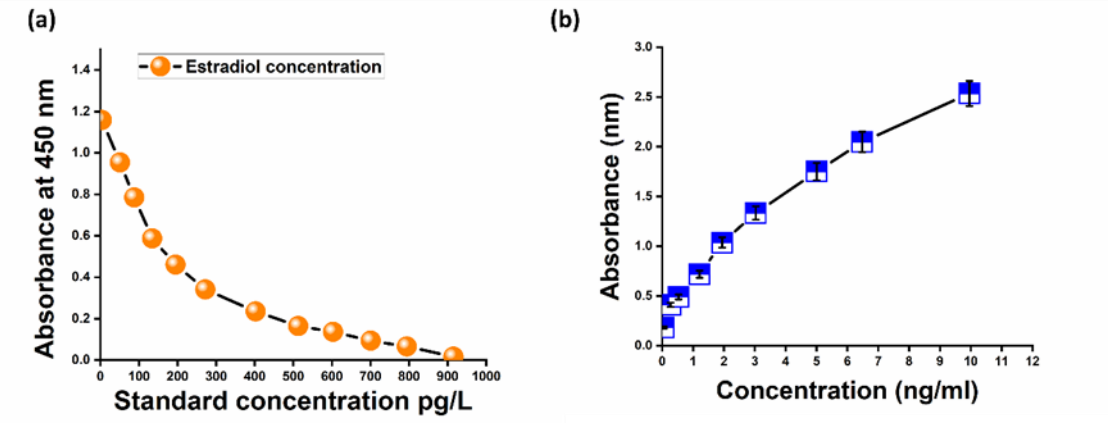


Fig 1. (a) The standard curve of E2 and (b) standard curve of the concentration of Cat-k.

3.2. Statistical analysis

3.2.1. Level of biochemical variables in blood serum

The results of the Cath-k level in different study groups were illustrated in Figure 2(a) and showed significant results ($p>0.05$). The increase in the activity of the Cath-k enzyme in the study groups of women was observed, which includes each of the pregnant women group, groups of menopausal women, and the group of women with osteoporosis, and the values were respectively (0.24±11.50, 12.73±0.17, 11.99±0.15) compare with a healthy woman (blank) (3.95±0.01) while decrease activity Cath-k level in pregnancy group compares in menopause group and osteoporosis group for woman this obtained results are compatible with reference study [11].

A study shows that the Cat-k enzyme is associated with estrogen in an inverse relationship with respect to the effect on bone metabolism, and the decrease in estrogen concentration in postmenopausal women and young women who suffer from a decrease in the hormone for several reasons, including those related to polycystic ovaries, have an increase in the activity of the Cat k enzyme due to the weak protective effect of estrogens on bones, as they increase bone resorption in reducing the number and activity of osteoblasts and accelerating programmed death [11]. An other study shows that the increased activity of the Cat-k enzyme during menopause in women is the result of their hyperparathyroidism, knowing that the high concentration of PTH and its catabolic effect on bones led to an increase in the effectiveness of the Cat-k enzyme in them and an increase in their incidence of fractures [12]. According to Adami and his colleagues, drugs might sometimes be the primary factor contributing to the detrimental effects on bones, making them more brittle and susceptible to breakage. Patients on glucocorticoids have higher levels of Cat-k enzyme because the drug's side effects have increased osteoporosis rates. These side effects include increased osteoblast cell programmed death and inhibition of osteoblast progenitor cell formation, while osteogenic cell secretion of Cat-k has increased [13].

The concentration of Vit-D in blood serum in different groups presented was shown in Figure 2(b). A significant decrease in Vit -D levels was observed in the groups of women, which include both the group of postmenopausal women, pregnant women, and the group of women with osteoporosis and the values were as follows (12, 75 ± 2.81), (11.94 ± 2.52), (7.88 ± 2.48) compared to the Vit -D level in healthy women groups (34.62 ± 4.64). The results obtained showed that the rate of Vit -D levels in osteoporosis groups is lower compared to women. at menopause, pregnant women. The obtained results are compatible with other references [14]. Vitamin D deficiency in pregnant women can be caused by decreased calcium, and this decreased calcium is due to the high need of the fetus to build its skeleton, especially during the last trimester. During pregnancy, if the mother does not obtain adequate calcium requirements, the mother`s body withdraws calcium from her bones and provides it to the fetus, but the persistence of the deficiency without treatment is a dangerous factor for the fetus during its growth and its long-term negative effects, even after birth, result in increased susceptibility to diseases, including rickets and diabetes [15]. In this work, Adit et al. demonstrate that adolescent pregnant moms had greater rates of osteoporosis and bone density loss than their peers, and that the age of the pregnant woman may be a significant factor in vitamin D shortage equivalents throughout puberty to expectant mothers [16]. The primary cause of osteoporosis in postmenopausal women is the decline in ovarian function and menopause, as well as the low level of vitamin D in them. The interruption causes a decrease in skeletal mass due to an imbalance in bone metabolism because the decline of ovarian functions is the most important factor in the development of osteoporosis after menopause. In postmenopausal women, the decrease in vitamin D is explained by the low consumption of milk and dairy products, the high percentage of body fat, the lack of sun exposure, and the significantly lower concentration of estrogens [17].

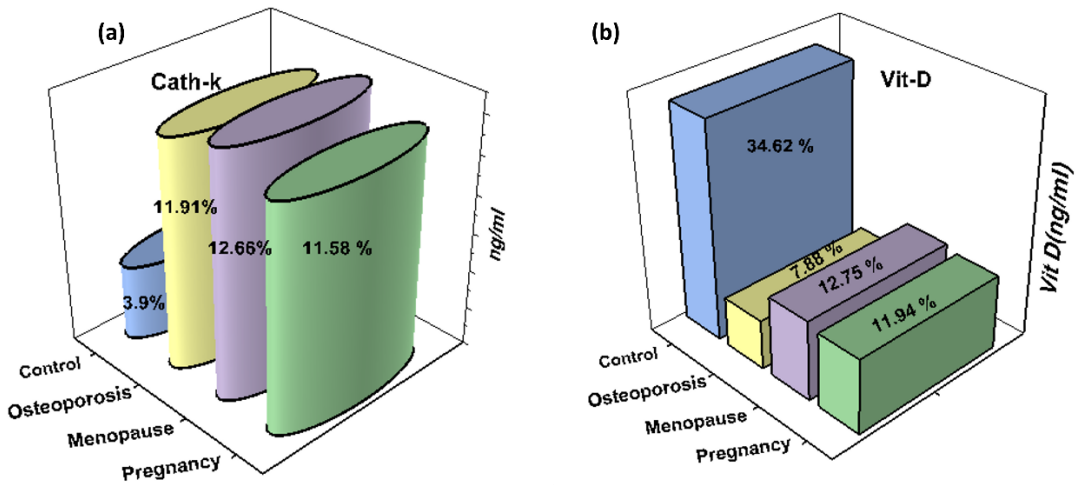


Fig 2. (a) The evaluation of Cath-k level and (b) level of Vit -D in different group study.

The Figure 3a, and b illustrates the concentration of E2 hormone and PTH hormone in different study groups respectively. The concentration of E2 hormone decreased significantly in the menopausal and osteoporotic women groups and the concentrations were found to be 28.39 ± 6.46 and 51.11 ± 10.55, respectively. The results are shown in Figure 3b. On the other hand, a significant increase in the E2 hormone was observed in the pregnancy group (416.85 ± 38.35) compared to the control group (277.75 ± 78.61), so the results obtained from the study approved a significant increase in the E2 hormone in the pregnancy group. in relation to menopause and women suffering from osteoporosis. The obtained results are compatible with other studies [18]. The studies indicated that osteoporosis occurs in both sexes and all age groups and races, but it is found higher in women After menopause, due to estrogen deficiency, the onset of cell aging, decreased immunity, increased inflammation within the body, medication intake, calcium and vitamin D deficiency, and an increase in thyroid hormones [19].

In addition, the estrogen deficiency increases the secretion of interleukin IL-6, interleukin IL-1, and tumor necrosis factor-α- TNF and thus the occurrence of bone resorption, as the lack of estrogen works to reduce the work of osteoblasts and increase the activity of osteopenia, which leads to the removal of minerals and the occurrence of osteoporosis after menopause. During pregnancy, the concentration of the estrogen hormone rises to levels much higher than the normal limit, and for several reasons [20].

Calc-spar et al. [19] indicated that during pregnancy the mammary glands undergo a series of structural and functional changes to prepare for milk production, as there are three hormones that have a major role in the development of tissue in the breast. The breast gland, which is estrogen, progesterone, and prolactin, as estrogen mainly works to promote and develop the lactiferous duct and prepare it until milk comes out after childbirth. It stimulates the secretion of the prolactin hormone and increases the receptors for this hormone in the breast glands [21]. A study by Winter et al. showed that the protective effect of estradiol on the bones decreases during pregnancy and lactation, which causes weakness in building new bone cells, and during the lactation period, the rise in prolactin causes a decrease in bone minerals[22].

The results of PTH levels in different groups of study are illustrated in Figure 2b. According to the study results, a significant increase in PTH concentration was observed in all female groups. The value of PTH hormone was found to be 111±1.23 in the pregnant women group, 95.46±1.36 in the group of menopausal women, and 88.14±2.32 in the group of women with osteoporosis compared with the concentration of PTH hormone in the control group (58.11±2.32). Where the results showed that there was a significant decrease in the concentration of PTH in groups of pregnant and menopausal women, compared with women with osteoporosis. This results are compatible with other references [23]. PTH regulates calcium balance by acting on many body organ systems in order to maintain a normal blood calcium concentration, while high levels of the hormone that occur due to low blood calcium concentration cause bone resorption and may be due to increased PTH in women with osteoporosis[24]. According to Bover et al.[25], Vitamin D deficiency is caused by either inadequate nutrition or lack of exposure to sunlight, which results in a lack of calcium in the blood. In response to this deficiency, more hormones are secreted. When vitamin supplements prescribed by the attending physician are taken, the concentration of vitamin D in the body and, accordingly, the hormone concentration return to normal values.

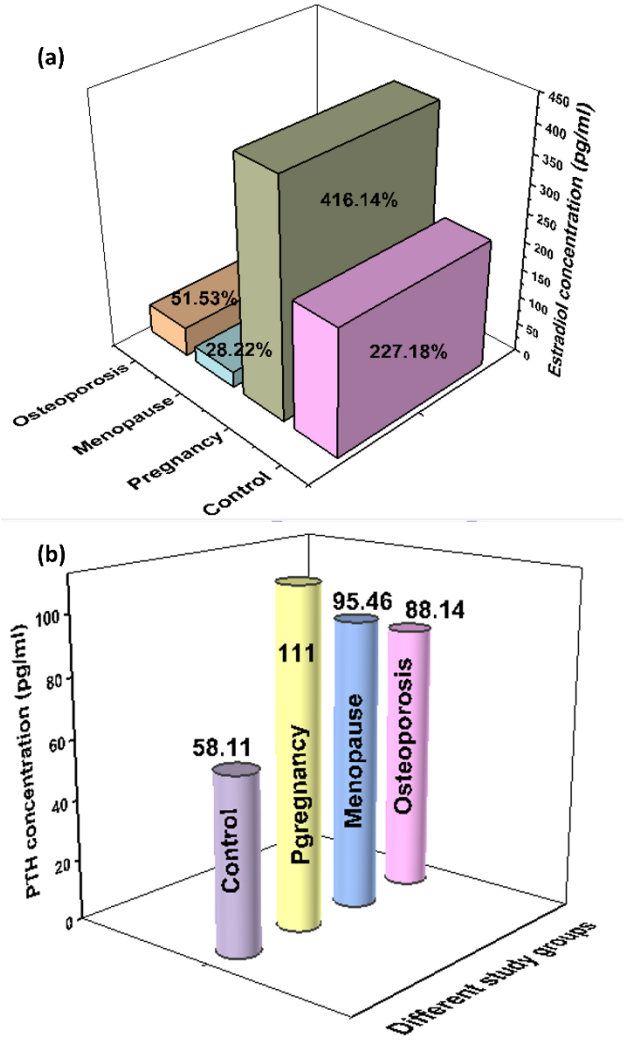


Fig 3. (a) Level of E2 and (b) level of PTH in different group study.

3.2.2. Determination of the Hematological profile in the study groups.

Concentration of RBCs, Hb, and HCT

The results obtained for the number of RBCs, Hb, and HCT in the different study groups are illustrated in Figure 4. A significant decrease in the number of RBCs, Hb, and HCT in the group of pregnant women, and the values were respectively: (4.29 ± 0.52, 34.60 ± 0.42, 11.20 ± 0.26) compared to the control groups (4.76 ± 0.95, 12.53 ± 0.4, 37.79 ± 0.83) Although no significant differences appeared in the two groups of postmenopausal women and women with osteoporosis compared to the healthy group of women (control group). A decrease in RBCs and Hb and HCT concentration was observed in the group of pregnant women, compared to both groups of postmenopausal women and those with osteoporosis.

The number of RBCs can decrease for several reasons, including genetic (hereditary) and others due to acquired (non-hereditary) diseases. This decrease affects the course of vital processes in the body as a whole because cells contain the protein hemoglobin, which carries oxygen to all cells of the body to produce energy when the number of RBCs

decreases. Hb and HCT (the percentage of RBCs in whole blood) will be affected, and therefore anemia.
Iron deficiency anemia is the most common nutritional deficiency, which the World Health Organization has highlighted as a serious health problem not only in developing but also in developed countries. Anemia is estimated to affect approximately one-third of the world's population, particularly women of childbearing age. One of the global nutrition goals set by the organization that must be achieved is to reduce the number of anemic women by 50% [26]. During pregnancy, the volume of blood increases for the growth of the fetus, but if the pregnant woman does not get enough iron or some other nutrients, the body's organs will not be able to produce enough RBCs that the mother and her fetus need, and thus she will suffer from anemia, which is one of the most common medical disorders during pregnancy Where the World Health Organization indicated that 60% of pregnant women will develop anemia, and the most vulnerable women are those with limited incomes and adolescent girls [27]. There is a correlation between vitamin D and iron, as some studies indicated that osteoporosis (vitamin D deficiency) causes anemia in some other studies, and other studies indicated the opposite, where anemia causes osteoporosis, as vitamin D plays an important role in the formation of blood cells, Erythropoiesis, which is the process of producing RBCs from within the bone marrow [28].

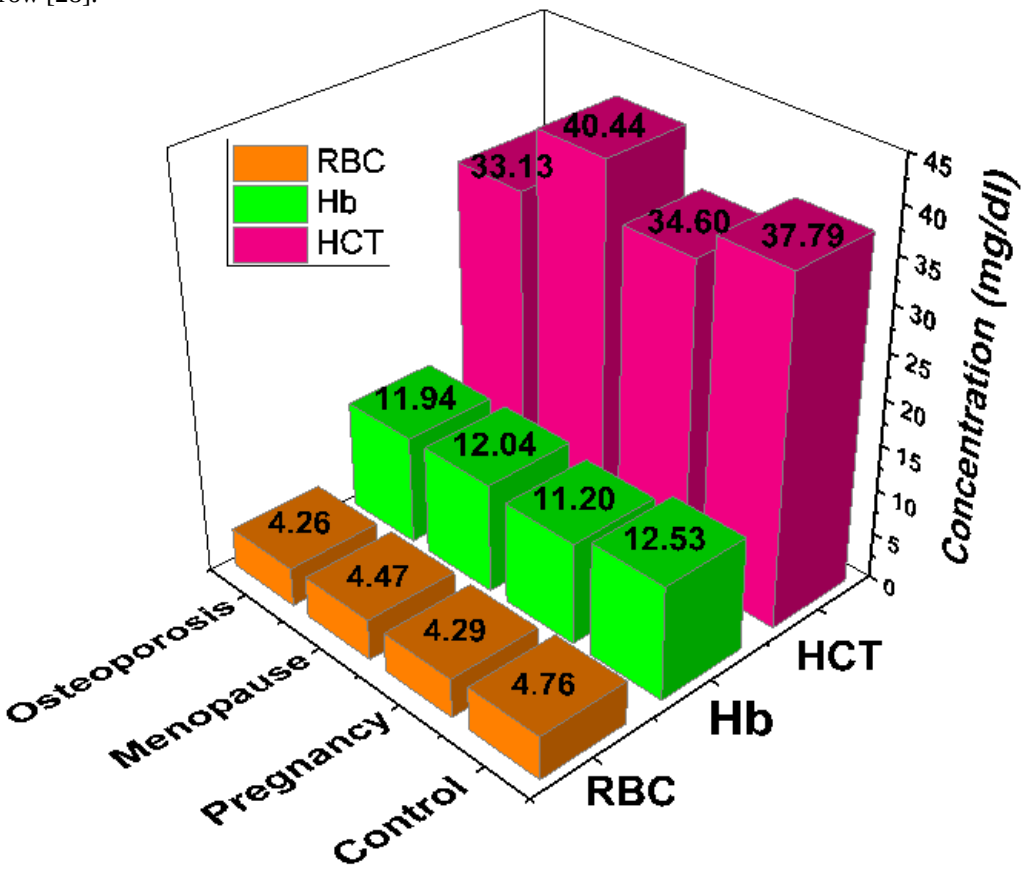


Fig 4. Concentration of RBCs, Hb, and HCT in different study groups.

Level of WBCs and the number of PLTs
The number of WBCs and the number of PLTs in the study groups of women were evaluated and the obtained results are shown in Figure 5. A significant increase in the number of WBCs in the study groups of women was observed, which includes each of the two groups of pregnant women, the group of menopausal women, and the group of women

with osteoporosis. The values were respectively (9.93±0.04, 8.64±0.21, 8.44±0.08) compared with the control group (7.95±0.02).

The results of PLTs showed a significant increase in the number of PLTs in the study groups of women, which includes each of the two groups of menopausal women, and the group of women with osteoporosis. The values were respectively (256.29±75.51,254.60±60.27) while the pregnant group of women did not show any significance in the number of PLTs (241.31±51.65) compared with the control group (242.38±70.92).

A study showed that blood cells are used as inflammatory markers to describe many diseases, including osteoporosis, and indicated that people with osteoporosis had high levels of granulocytes and non-granular WBCs with low counts. Elevated platelet at higher levels than their peers without osteoporosis. Because osteoporosis is characterized by a defect in bone metabolism which is more in bone resorption than in its structure, and during this process, inflammatory cytokines will be involved. It promotes, develops, and increases the number of osteoporosis, thus reducing bone mineral density and accelerating its catabolism[29].

In postmenopausal women, the decrease in estrogen plays a role in increasing the inflammatory process of their bones, as the researcher Kale et al. [29] indicated that with the decrease in the concentration of estrogen and the presence of indicators of a decrease in bone density, an increase in WBCs has been observed during menopause and loss of the protective effect of estrogen on bones.

It is normal for there to be an increase in the number of WBCs in pregnant women due to the physiological stress resulting from pregnancy, as well as to protect the body against microbes and fight certain diseases that some pregnant women may suffer from, such as urinary and genital tract infections and arthritis, as well as to prevent blood clots and help widen blood vessels when exposed to allergies [30].

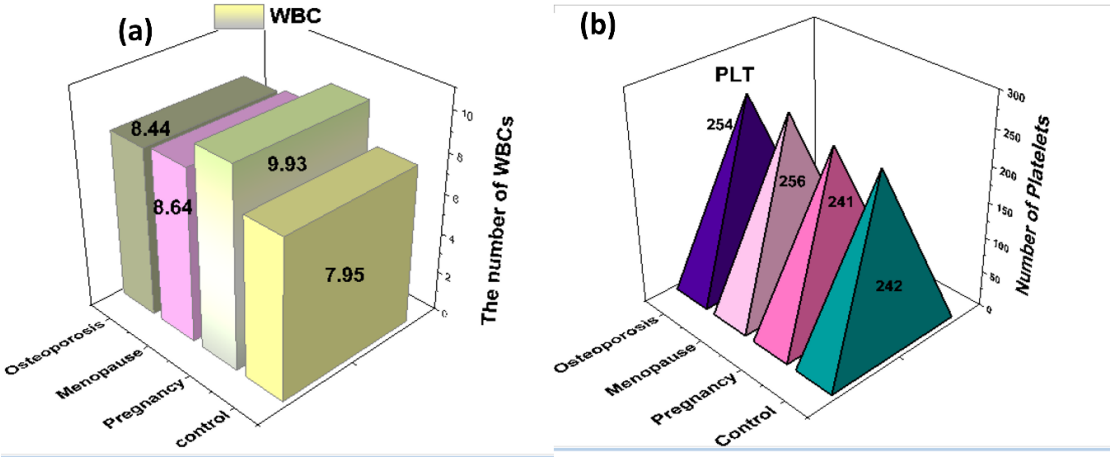


Fig 5. (a) Number of PLTs in different study groups and (b) number of WBCs in blood serum of different groups.

The concentration of P in the blood serum and BMI of the study groups

Figure 6 showed a significant decrease in the concentration of P in all groups of women, including each of the pregnant women group, the group of menopausal women, and the group of women with osteoporosis. The values were respectively (1.48 ±0.55, 1.42±0.91 and 2.05±0.49) compared with the control group (5.04±4.56). The results of the current study do not show any significance in all groups of women.

The reason for the decrease in phosphorus may be due to a deficiency of vitamin D, as all the researchers pointed out by Safari and Goltzman [31][32], Vitamin D has the optimal role in the absorption of both phosphorus and calcium through the intestines and re-absorption through the renal tubules. In case of deficiency, the concentration of both elements will be affected in a negative way.

Some studies have indicated the cause of low phosphorus and the occurrence of bone diseases as a result. It may be a defect in the activity of FGF-23H or PTH, as they work together to regulate the concentration of phosphorus in the

blood serum. The production of FGF-23H by osteoblasts and osteoblasts In response to an imbalance in the concentration of phosphorus in the blood to be regulated within normal levels [33] .

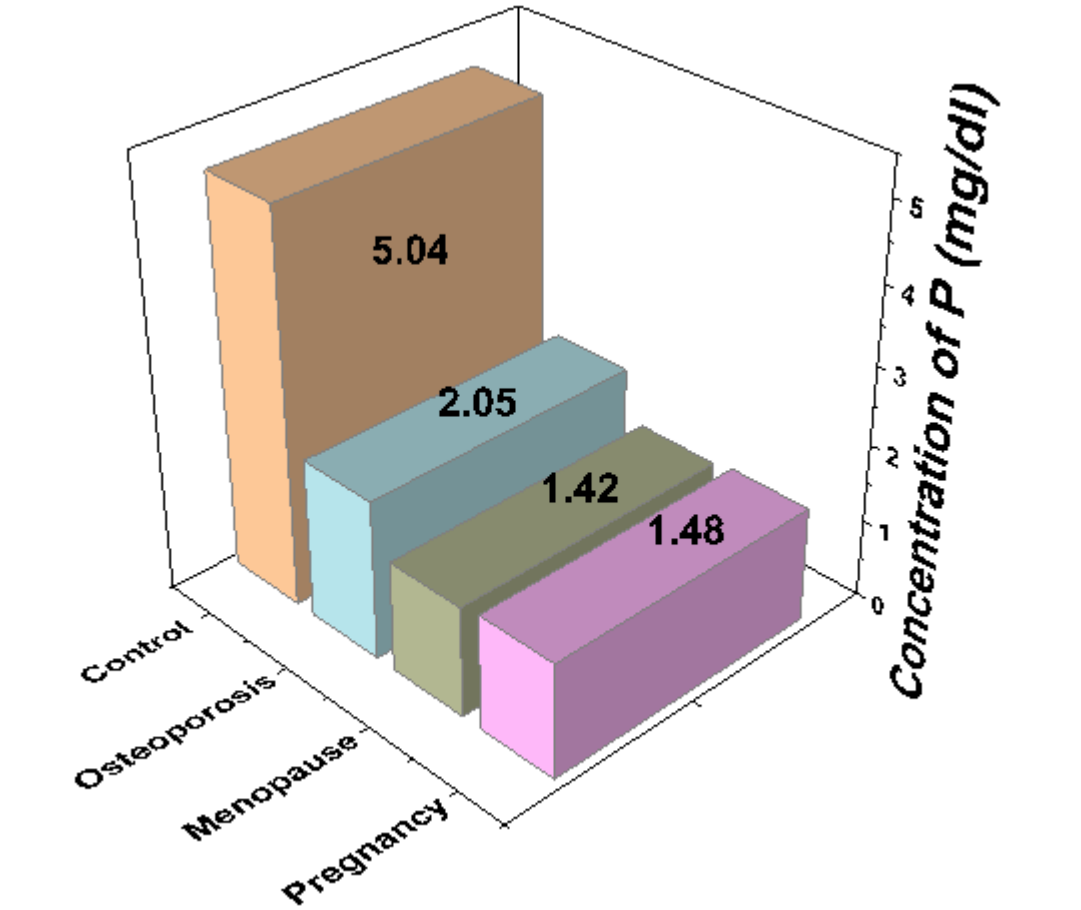


Fig 6. The P concentration in different group study.

The results of the BMI in the study groups of women (Figure 7). The obtained results showed a significant increase in the BMI in the pregnant group, the group of menopausal women, and the group of women with osteoporosis. The values were respectively (26.25±0.12,35.52±0.4, and 28.47±0.18) compared with a control group or healthy group (24.27±0.16) and the results of the current study showed a significant increase in the group of menopausal women compare with the pregnant group and the group of women with osteoporosis. This obtained results are compatible with other reference [34].

Many studies have investigated the relationship between obesity and osteoporosis. However, there is no consensus on this subject, as the skeleton is affected by many different factors such as age, sex, race, genetics, reproduction, calcium intake, BMI, and exercise. Among the most controversial of these factors is the BMI. Many women in menopause suffer from weight gain that may reach more than 2 kilograms per year, according to a study conducted by a team of American researchers, and what makes the matter more complicated is that the weight gained during this period usually accumulates in the form of fat in the abdomen, which represents a greater risk of cardiovascular disease [35]. Increasing BMI has multifactorial effects on bone metabolism, some studies indicated that it is generally accepted that increased body weight promotes bone production and has a protective effect on bone and that adipose tissue is a source

of estrogen in postmenopausal women, while other studies indicated that estrogen deficiency leads to excessive gain and that obese people have growth hormone less than their normal-weight peers. Fat cells produce leptin, which regulates appetite and body weight, and suggests that leptin may reduce bone formation in obese women as a result higher than normal levels and loss of insulin-regulating signals lead to down-regulation of glucose levels leading to an increase in BMI. It has recently been suggested that obesity is associated with low-grade chronic inflammation within adipose tissue and that inflammatory markers stimulate osteoporotic activity to accelerate osteoporosis [36][37].

In pregnant women, the BMI increases gradually with the progression of the months of pregnancy as a result of the physiological changes accompanying it. The natural weight gain is estimated between 10-16 kg. The increase appears in the last trimester of pregnancy. The increase is a result of the weight of the fetus, the weight of the amniotic fluid, the weight of the placenta, the increase in the muscle mass of the uterus, the increase in blood that nourishes the fetus, and organs, fluid retention in the pregnant mother's body, and the increase in stored fats that increase during pregnancy and have a role after birth to provide the body with energy for breastfeeding [38].

The researcher Gkastaris and his colleagues indicated in a study conducted on women that an increase in mineral density was positively associated with an increase in body mass and a decrease in exposure to osteoporotic fractures [39] .

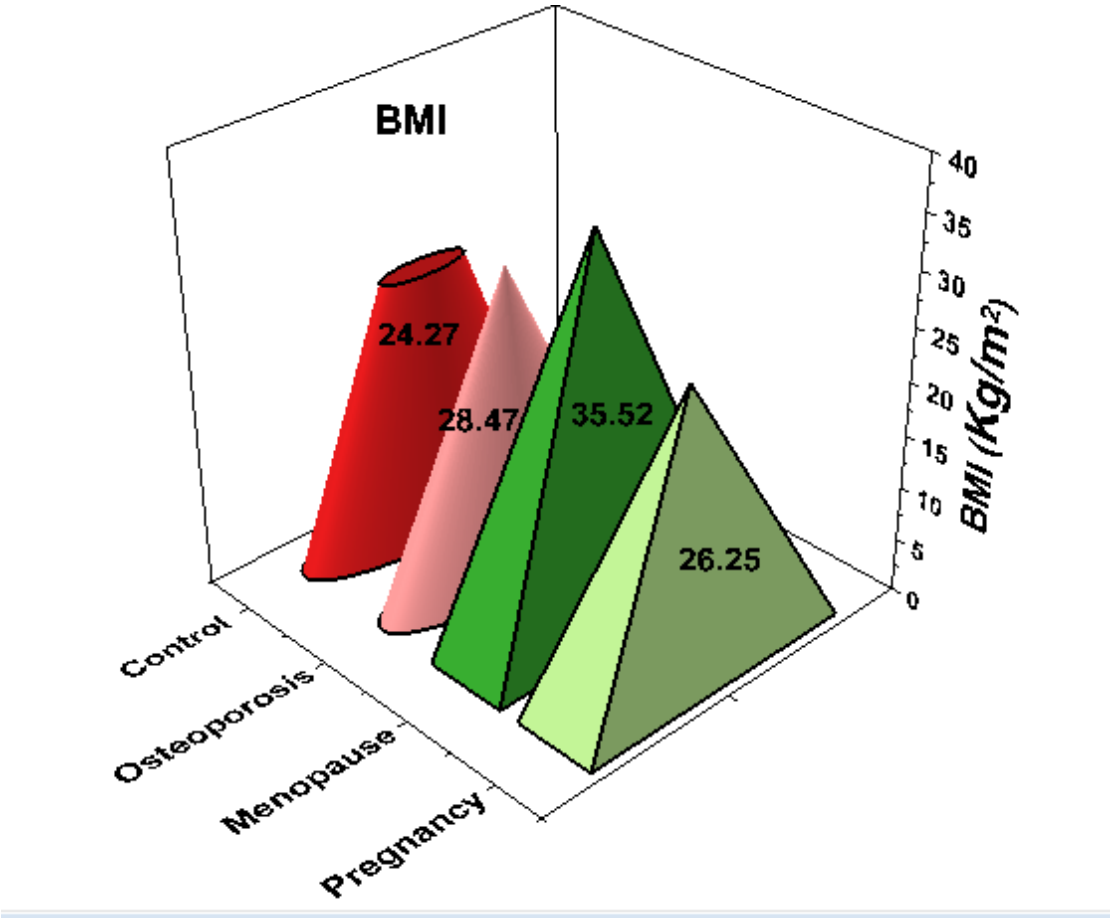


Fig 7. BMI level in different group study.

4. The Correlation Studies

The correlation between current variables was calculated using the STATISCA program (SPP) and the results are shown in Table (1). The high correlations were illustrated in Figure (8.9 and S2).
The direct correlations between some variables were observed, such as Vit -D with P, Hb, and RBCs, also, a good correlation was observed between PTH hormone, Cath-k, and BMI. In addition, between BMI, RBCs, and Hb, HCT. It also showed the existence of negative correlations between some variables such as between P with Cat-k and PTH, Vit -D with PTH, Cat-k, WBCs, BMI and between BMI and Vit-D, E2, RBCs, and P. There is no significant correlation observed between E2 between RBCs, Cat-k and Vit -D.

Table 1. Correlations between variables in different study groups.

	Cat-k	Vit-D	Estradiol	PTH	RBC	HB	HCT	WBC	PLT	P	BMI
Cat-k	1.000000										
Vit-D	**-0.971118**	1.000000									
Estradiol	-0.253255	0.222464	1.000000								
PTH	**0.899223**	**-0.955480**	-0.453183	1.000000							
RBC	**-0.863502**	**0.951414**	0.003641	**-0.892295**	1.000000						
HB	**-0.669912**	**0.691366**	**-0.542468**	-0.466629	**0.785855**	1.000000					
HCT	-0.170824	0.391621	-0.314745	-0.405465	**0.643174**	0.475104	1.000000				
WBC	**0.563705**	**-0.548614**	**0.652621**	0.286021	**-0.633739**	**-0.975224**	-0.328379	1.000000			
PLT	**0.600407**	**-0.560301**	**-0.925559**	**0.720994**	-0.334302	0.187731	0.206269	-0.317821	1.000000		
P	**-0.984656**	**0.938716**	0.104199	**-0.817474**	**0.843284**	**0.761489**	0.142397	**-0.685475**	-0.472472	1.000000	
BMI	**0.675760**	**-0.520006**	**-0.675964**	**0.536515**	-0.231831	-0.018251	**0.553083**	-0.006964	**0.830350**	**-0.633457**	1.000000

The correlation between Vit-D and Cat-K enzyme was illustrated in Figure 8 and showed a negative correlation found to be -0.97.

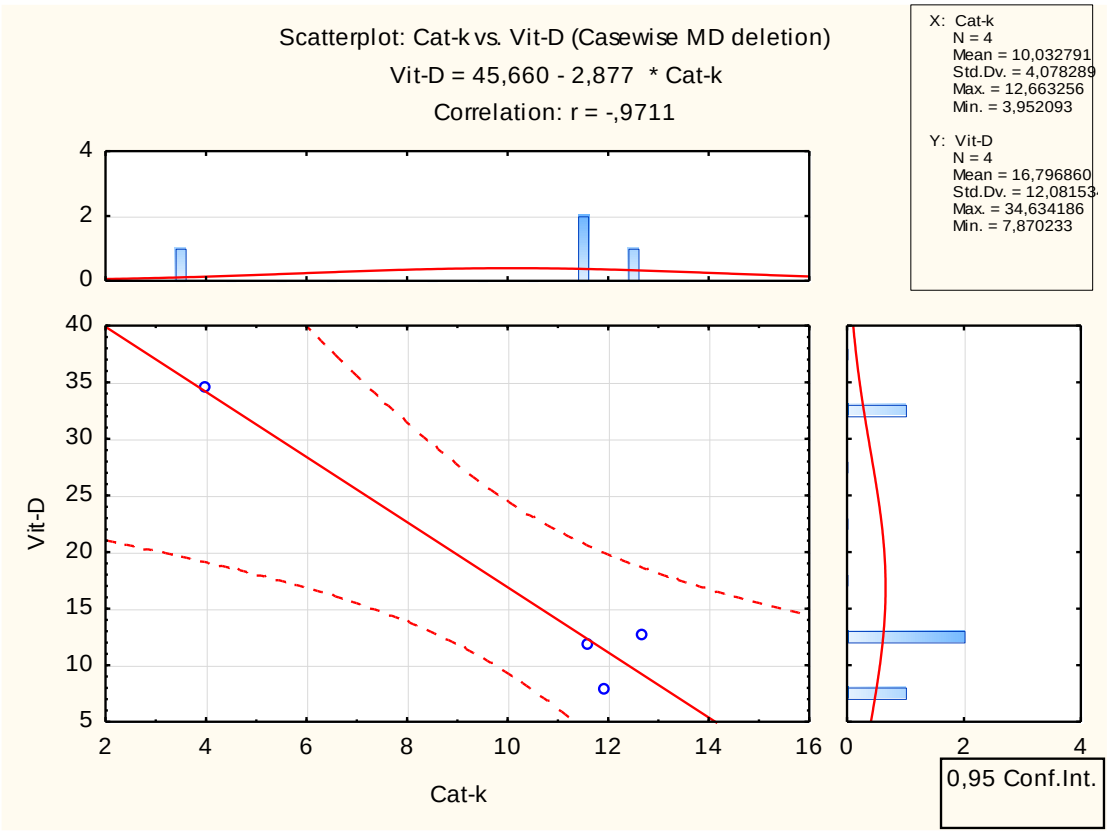


Fig 8. Correlation graphic between Vit-D and Cat-K.

A good correlation was observed between PTH and Cat-k (0.89), which indicated there is a relation between these two parameters. A study by Lotinun et al. showed that deletion of Cat-k in osteocytes increased bone parathyroid hormone-related peptide (PTHrP) and prevented the lactation-induced decrease in serum PTH but amplified the Increased serum 1,25-dyhydroxyvitamin D [1, 25(OH)2D] [40]. Another study confirms that various evidence exists demonstrating that PTH strongly induces Cathepsin-K, a cysteine protease present mainly in the lysosomes of osteoclasts and macrophages that promotes bone and extracellular matrix remodelling. Cathepsin-K levels are altered in various bone disorders, systemic inflammations [41].

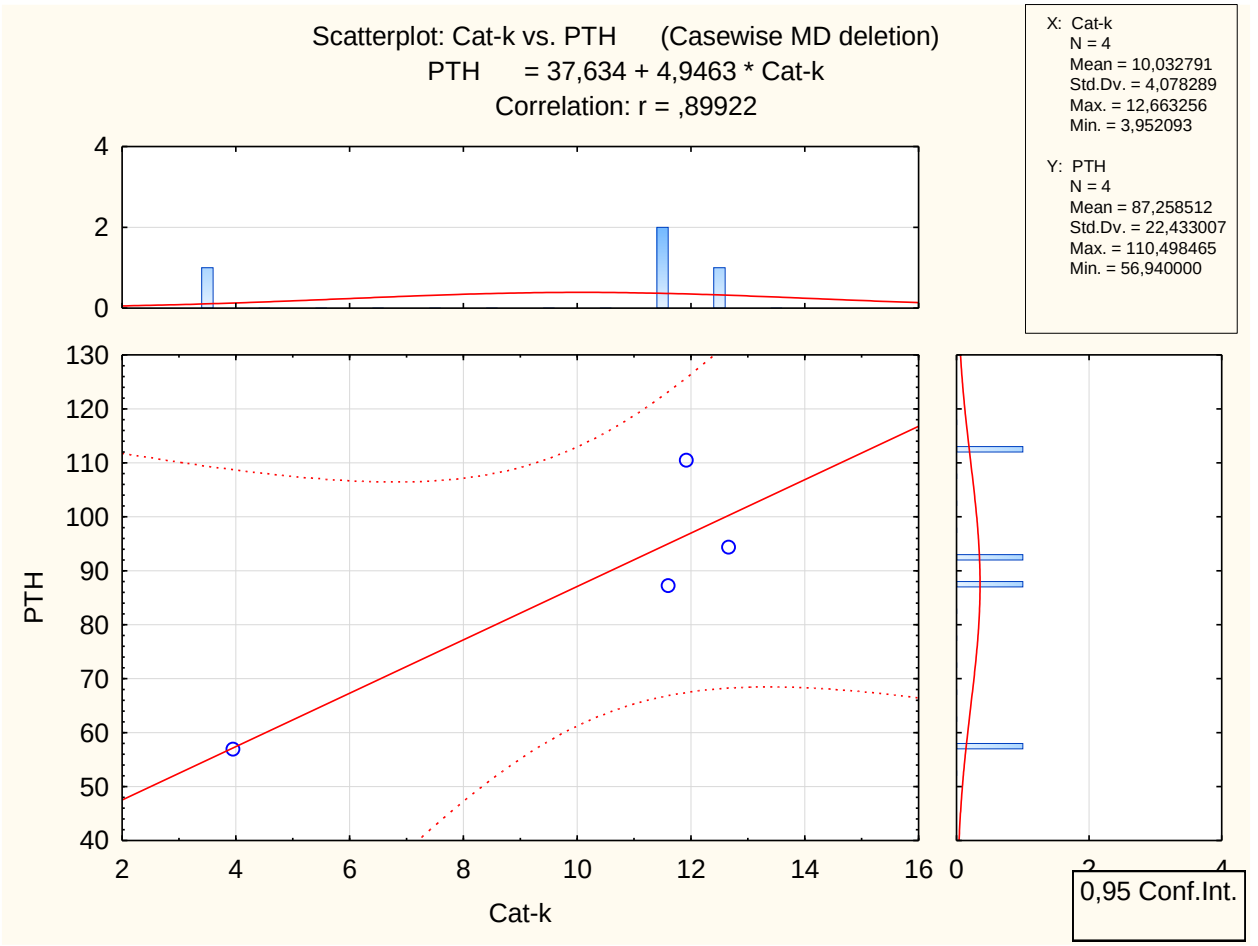


Fig 9. correlation graphic between PTH and Cat-K.

5. Conclusion

According to the obtained results, Cat-k was elevated in the study groups except for the control group, and this indicates the importance of this vital indicator in diagnosing osteoporosis. In addition, a decrease in the concentration of Vit -D in the study groups except for the control, and this indicates the onset of bone diseases, including osteoporosis if treatment is not done. Also, decrease in phosphorus in the study groups except for the control group, indicating a deficiency of mineral elements in the blood serum and the occurrence of bone weakness. High PTH in the study groups except for the control group, indicating a decrease in the calcium component in the blood serum, which causes bone weakness and fragility. Finally, decreased estrogen hormone in postmenopausal women and women with osteoporosis indicates the importance of this hormone in protecting bones from losing the basic mineral elements that makeup bones.

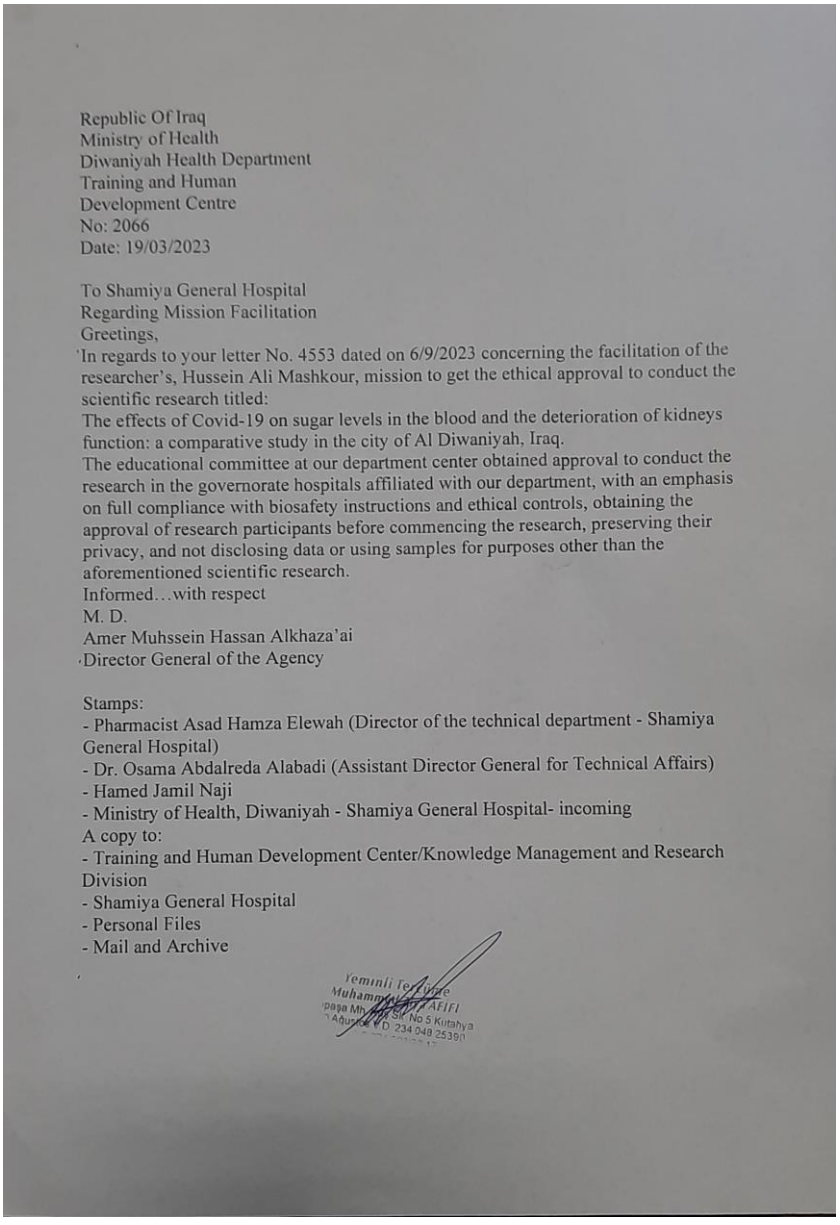
Based on this study, it is recommended to carry out a study on vitamin K and clotting factors released by blood platelets and their relationship with bone health at different ages of pregnant and postmenopausal women.

Thus, to carry out a study on the extent of exposure to the sun and its relationship with the level of vitamin D in women of different ages and its effect on bones and do a study of the relationship between cortisol and melatonin and osteoporosis in different age groups of women and also conduct a study on the relationship between heart disease and

vitamin D and calcium levels and osteoporosis.

Acknowledgement
This article is derived from the master's thesis titled "The Correlation of Cathepsin-k Level with Some Biochemical and Hematological Variables in Women with Osteoporosis, Pregnancy, and Menopausal at Al-Sadr Teaching Hospital in Najaf".

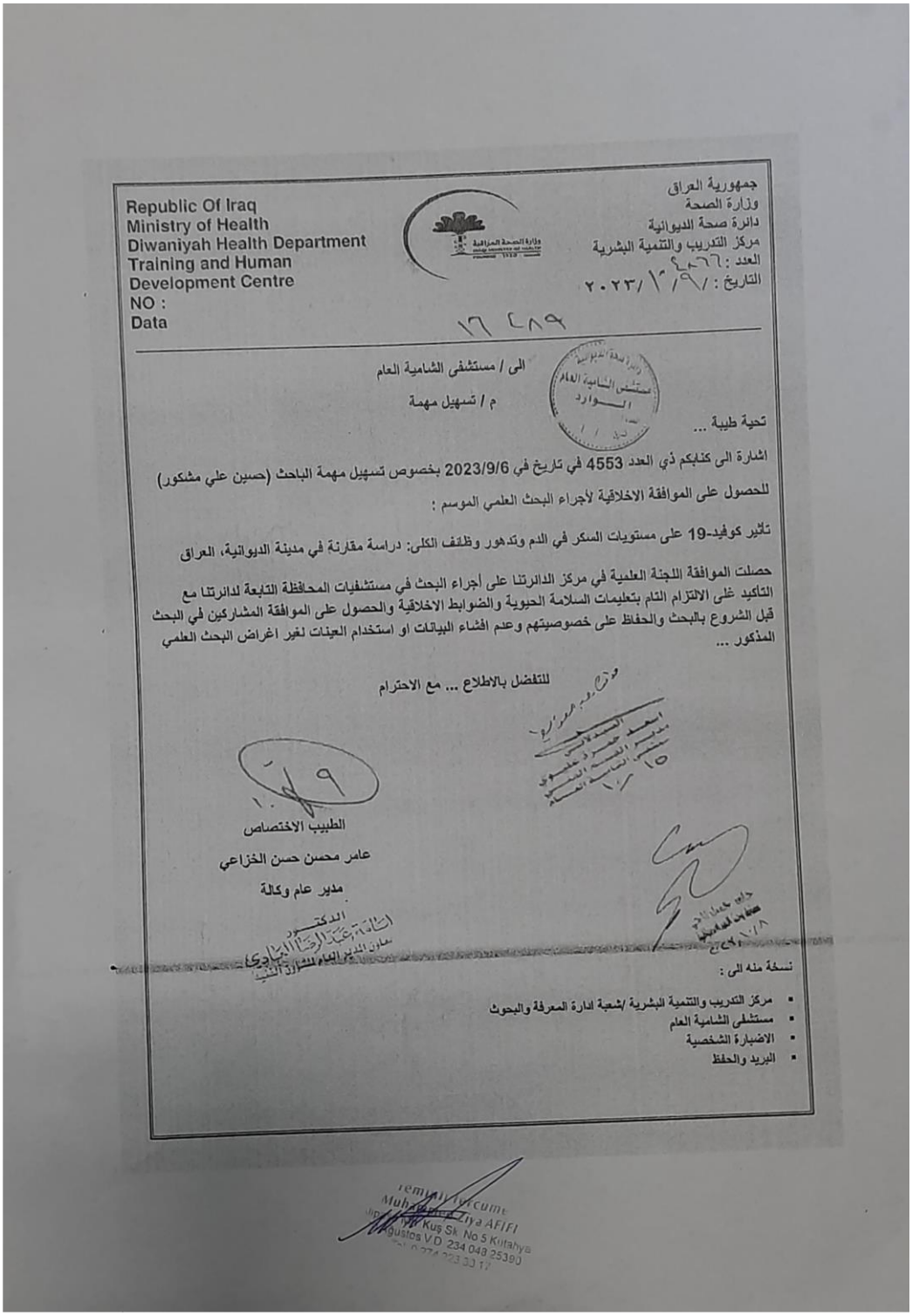
Appendix. Ethics Aproval



Republic Of Iraq
Ministry of Health
Diwaniyah Health Department
Training and Human
Development Centre
No: 2066
Date: 19/03/2023

To Shamiya General Hospital
Regarding Mission Facilitation
Greetings,
'In regards to your letter No. 4553 dated on 6/9/2023 concerning the facilitation of the researcher's, Hussein Ali Mashkour, mission to get the ethical approval to conduct the scientific research titled:
The effects of Covid-19 on sugar levels in the blood and the deterioration of kidneys function: a comparative study in the city of Al Diwaniyah, Iraq.
The educational committee at our department center obtained approval to conduct the research in the governorate hospitals affiliated with our department, with an emphasis on full compliance with biosafety instructions and ethical controls, obtaining the approval of research participants before commencing the research, preserving their privacy, and not disclosing data or using samples for purposes other than the aforementioned scientific research.
Informed…with respect
M. D.
Amer Muhssein Hassan Alkhaza'ai
Director General of the Agency

Stamps:
- Pharmacist Asad Hamza Elewah (Director of the technical department - Shamiya General Hospital)
- Dr. Osama Abdalreda Alabadi (Assistant Director General for Technical Affairs)
- Hamed Jamil Naji
- Ministry of Health, Diwaniyah - Shamiya General Hospital- incoming
A copy to:
- Training and Human Development Center/Knowledge Management and Research Division
- Shamiya General Hospital
- Personal Files
- Mail and Archive



جمهورية العراق
وزارة الصحة
دائرة صحة الديوانية
مركز التدريب والتنمية البشرية
العدد : ٤٣٦٦
التاريخ : ٢٠٢٣/١٢/٩

Republic Of Iraq
Ministry of Health
Diwaniyah Health Department
Training and Human
Development Centre
NO :
Data

١٢٤٨٩

الى / مستشفى الشامية العام

م / تسهيل مهمة

تحية طيبة ...

اشارة الى كتابكم ذي العدد 4553 في تاريخ في 2023/9/6 بخصوص تسهيل مهمة الباحث (حسين علي مشكور) للحصول على الموافقة الاخلاقية لأجراء البحث العلمي الموسم :

تأثير كوفيد-19 على مستويات السكر في الدم وتدهور وظائف الكلى: دراسة مقارنة في مدينة الديوانية، العراق

حصلت الموافقة اللجنة العلمية في مركز الدائرتنا على أجراء البحث في مستشفيات المحافظة التابعة لدائرتنا مع التأكيد غلى الالتزام التام بتعليمات السلامة الحيوية والضوابط الاخلاقية والحصول على الموافقة المشاركين في البحث قبل الشروع بالبحث والحفاظ على خصوصيتهم وعدم افشاء البيانات او استخدام العينات لغير اغراض البحث العلمي المذكور ...

للتفضل بالاطلاع ... مع الاحترام

الطبيب الاختصاص

عامر محسن حسن الخزاعي

مدير عام وكالة

الدكتور
انابة عبد الرضا الساعدي
مدير الشؤون الفنية

نسخة منه الى :

- مركز التدريب والتنمية البشرية /شعبة ادارة المعرفة والبحوث
- مستشفى الشامية العام
- الاضبارة الشخصية
- البريد والحفظ

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