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CATHEPSIN ASSESSMENT AND ITS CORRELATION WITH PARATHYROID HORMONE, ESTROGEN AND VITAMIN D LEVEL IN OSTEOPOROSIS PATIENTS

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Abstract: The study was conducted from September 20, 2022, to May 1, 2023. The study sought to assess the function of cathepsin and its correlation with various physiological and biochemical factors in women. A total of 90 samples were obtained from two hospitals, Medical City and Al-SADER Teaching Hospital, located in Hilla Governorate. The study was partitioned into four distinct groups, the first group consisted of 22 pregnant females, the second group consisted of 22 females experiencing menopause, the third group consisted of 22 females with osteoporosis (OP) and the fourth group consisted of 24 seemingly healthy females. This is accomplished by measuring the physiological, enzymatic, hormonal and biochemical factors present in the blood serum. These factors include the enzyme cathepsin, estrogen, parathyroid hormone (PTH), vitamin D. The study results indicated a notable rise in the cathepsin concentration, PTH among the study groups compared to the control group. Additionally, there was a significant decrease in vitamin D concentration in the study groups compared to the control group. Furthermore, the pregnant women exhibited a substantial increase in estrogen hormone levels. In contrast, women with osteoporosis in menopause showed a significant decrease in estrogen hormone levels compared to the control group. This finding suggests a strong association between fragility disease and various groups of women, including pregnant women, women in menopause and those affected by osteoporosis.

Keywords: Osteoporosis, Cathepsin, Estrogen, Parathyroid hormone, Vitamin D.

Introduction

Osteoporosis is a significant worldwide health issue and ranks in the top ten most widespread diseases globally, as reported by the World Health Organization. The global prevalence of osteoporosis has reached 400 million individuals. As defined by the International. Osteoporosis Foundation, osteoporosis is a skeletal illness characterized by a reduction in bone mineral density and degeneration of the bone tissue's fine geometry. This condition significantly increases the likelihood of fractures. Osteoporosis, often known as OP, is a significant public health issue and is currently a global concern[1]. Osteoporosis is a chronic skeletal disorder characterized by low bone mass and structural deterioration of bone tissue, which increases the likelihood of fractures in the wrist, spine, and hip[2]. Osteoporosis is commonly referred to as a silent ailment since it typically does not exhibit symptoms until a fracture occurs. This significantly impacts morbidity and death rates[3].

Furthermore, many individuals are unaware of it until issues arise. It is a prevalent occurrence in women following menopause. Osteoporosis is a challenging condition to manage and currently has no cure, making prevention of utmost importance. The operational description of osteoporosis relies on the T-score of bone mineral density (BMD). The T-score quantifies the deviation of an individual's BMD from the average value predicted in healthy young individuals, measured in standard deviations (SDs)[4].

The Z-score is used in dual-energy X-ray absorptiometry (DXA) surveys to determine the deviation of an individual's bone density from the reference group of the same age and gender. It is a valuable tool for investigating secondary causes of osteoporosis[5]. The objective of this study is to establish a connection between osteoporosis during different stages of pregnancy and menopause in women and various physiological and biochemical factors. This will be achieved by examining the activity of the cathepsin enzyme in the serum of selected women, assessing the impact of vitamin D, estrogen, and parathyroid hormones, as well as phosphate levels in the blood serum of these women. Additionally, the study will explore the relationship between osteoporosis and the complete blood picture.

Materials and Methods

Study Setting

This study was conducted on patients treated at the Bone Density unit in two hospitals, Medical City and Al-SADER Teaching Hospital, located in Hilla Governorate. Specimens in the investigation were gathered. The present investigation's analytical phase was conducted at the Department of Chemistry and Biochemistry laboratories. This study included a total of ninety female participants. The individuals were categorized into four groups. The first group consisted of 22 pregnant females, the second group consisted of 22 females experiencing menopause, the third group consisted of 22 females with OP and the fourth group consisted of 24 seemingly healthy females. The age of individuals in both groups was above 45 years and matched.

Socio-demographic and anthropometric measurement refers to the collection and analysis of data related to a population's social and demographic characteristics, as well as their physical measurements and proportions.

Questionnaire:

The socio-demographic parameters in the questionnaire appended include height, weight, age, equivalence, miscarriage, marital status and medical history.

Osteoporosis Diagnosis

Every woman in this study had bone mineral densitometry using the central DEXA method, specifically the DEXXUM 3 machine.

Methods

Measurement of Estrogen Concentration

Principle

The Enzyme Linked Immuno Sorbent Assay (ELISA) kit utilizes a delayed competitive binding technique to determine the concentration of (E2) quantitatively. This technique involves using a fixed amount of monoclonal anti-estradiol and coenzyme E2, which competes with E2 in the test sample. The assay sample and buffer were combined and treated with an anti-E2 antibody biotin reagent for 45 minutes at room temperature. Subsequently, E2 enzyme conjugate was added to the reaction mixture and incubated for over 45 minutes.

Measurement of Human Cat-k Levels

Principle

The Cat-k test, utilizing the Bioassay ® kit, employs a solid phase ELISA approach. The plate was previously treated with a human Cat-k antibody. The sample contained Cat-k, introduced and attached to the antibodies coated on the wells. Next, the biotinylated human Cat-k antibody was introduced and attached to Cat-k in the sample. Subsequently, streptavidin-HRP was introduced, and a bond was formed with the biotinylated Cat-k antibody. Following incubation, any unbound streptavidin-HRP was removed through a washing procedure. The substrate solution was introduced, and the color intensified directly correlated with the concentration of human Cat-k. The reaction was halted by introducing an acid-stop solution, and the absorbance was quantified at a wavelength of 450 nm.

Quantification of Vitamin D in Blood Serum

Vitamin D concentration in the blood serum was determined using a commercially available kit from the Chinese company BIONT. The procedure outlined in the kit instructions employed the ELISA technique.

Measurement of Parathyroid Hormone in Blood Serum

The blood serum's parathyroid hormone concentration was determined using a commercially available kit from the Chinese company BIONT. The measurement was conducted using the ELISA device technique, following the instructions provided with the kit.

Quantifying phosphorus in blood serum using phosphorus liquidated, a human-specific reagent manufactured in Germany

The concentration of phosphate in the serum was assessed using a commercially available kit designed to quantify phosphate, manufactured by the German company Human.

Quantitative analysis

The ANOVA program was utilized to determine the arithmetic mean and standard deviation. The Duncan multiple-range taste test was also employed to compare the four study groups. This comparison was conducted at a probability threshold of 0.05 to ascertain the significance of the variations between the means of the groups.

Results and discussion

Demographic characteristics of a biochemical study:

The study's findings, as presented in Table (1), indicate a statistically significant increase in the activity of the Cath-k enzyme among the groups of women studied. These groups include pregnant women, menopausal women, and women with osteoporosis. The respective values for Cath-k activity were (11.50±2.35, 12.73±0.91 and 11.99±1.11) compared to healthy women (3.96±1.20). Additionally, there was a decrease in Cath-k activity in the pregnancy group compared to the menopause and osteoporosis groups for women.

Table 1 displays the demographic information of the participants involved in the biochemical investigation.

Table (1) Demographic of biochemical study

Patient	Pregnancy	Menopause	Osteoporosis	Healthy	p-value
Cat-kng/mL	11.50 ± 2.35^{a}	12.73 ± 0.91^{a}	11.99 ± 1.11^{a}	3.96 ± 1.20^{b}	0.001
Vitamin D ng/mL	11.95 ± 3.81^{b}	12.45 ± 4.52^{b}	7.78 ± 3.48^{c}	34.37 ± 8.65^{a}	0.001
Estrogen pg/mL	416.85 ± 39.22^{a}	28.39 ± 5.59^{c}	51.11 ± 10.52^{c}	227.75 ± 78.52^{b}	0.001

PTH pg/mL	86.76±7.76[a]	93.30±10.53[a]	110.26±13.66[a]	55.48±10.31[b]	0.001

The different letters indicate the existence of significant differences.

The findings of the present investigation corroborated the results of the study conducted by Pepe[6] among postmenopausal women. Cat-k is predominantly found in osteoclasts and, to a lesser degree, in other organs. It plays a crucial function in breaking down bone, specifically type I collagen, and is implicated in cellular processes such as the immunological response, regulation of cell renewal, autophagy, and protein degradation[7].

The enzyme Cat-k is inversely associated with estrogen and its effect on bone metabolism. Macias and colleagues found that a decrease in estrogen concentration in postmenopausal women and young women with hormonal imbalances, such as polycystic ovaries, increases Cat-k enzyme activity. Estrogen cannot protect bones since it promotes bone breakdown by decreasing the quantity and function of osteoblasts and speeding up programmed cell death (apoptosis). Alternatively, the heightened activity of the Cat-k enzyme in women during menopause may be attributed to their hyperparathyroidism. Pepe and his colleagues[6], suggested that the elevated levels of parathyroid hormone and its breakdown of bones contribute to the enhanced effectiveness of the Cat-k enzyme in women, resulting in an increased risk of fractures

According to researchers Adami and Saag drugs[8] can be a primary factor in causing negative effects on bones, leading to increased vulnerability and fragility. For instance, patients taking Glucocorticoids experience heightened activity of the Cat-k enzyme, which further exacerbates their osteoporosis. One of the effects of these drugs is the inhibition of osteoblast progenitor cell formation and an increase in programmed cell death of osteoblast cells. Simultaneously, there is an increase in the secretion of Cat-k by osteogenic cells. Enhanced deterioration of bone.

During pregnancy and lactation, BMD diminishes due to vitamin D deficiency or inadequate calcium intake during breastfeeding. During pregnancy and lactation, the mother's bones experience a process where calcium and other minerals are taken out due to the growth of the fetus and the production of milk to nourish the newborn. This leads to bone metabolic breakdown and enhances these minerals' concentration. The Cat-k enzyme is secreted from osteoclasts and contributes to the release of enough calcium for the fetus and infant while also causing the breakdown of bone cells[9]. According to a study conducted by researcher Bolignano and his colleagues[10], it was found that the increased activity of the Cat-k enzyme in patients with chronic hemodialysis is often caused by kidney disease. These patients experienced an increase in the effectiveness of the Cat-k enzyme and higher levels of parathyroid hormone due to low calcium concentration. This puts them at a higher risk of developing osteoporosis. The study also revealed a positive relationship between Cat-k and parathyroid hormone in hemodialysis patients, known as mineral bone disease. This disease occurs due to the gradual deterioration in mineral balance caused by declining kidney function.

The study's findings, presented in Table 1, indicate a significant decrease in vitamin D levels among various groups of women. These groups include women in menopause, pregnant women, and women with osteoporosis. The specific values recorded were 11.95±3.81, 12.45±4.52, and 7.78±3.48, respectively. In comparison, healthy women had a vitamin D level of 34.37±8.65. Additionally, the study revealed that women with osteoporosis had lower vitamin D levels compared to women in menopause and pregnant women.

The study's findings were consistent with the findings of Md Isa [11] for both pregnant and postmenopausal women, as well as with Rozenberg[12] for women with osteoporosis. Vitamin D is endogenously synthesized within the body, and its levels are influenced by various factors such as nutrition, sunlight exposure, age, metabolism, genetic factors, and others[13].The World Health

Organization has reported a common vitamin D deficiency in pregnant women aged 18-40. Fetal development and ossification.

The pace of decline intensifies as the stages proceed and has been associated with an elevated likelihood of gestational diabetes, early deliveries or miscarriages, and various other illnesses. A drop in calcium levels causes vitamin D shortage in pregnant women due to the increased demand for calcium by the fetus to develop its skeleton, particularly during the final trimester. During pregnancy, if the mother does not receive sufficient calcium, her body will extract calcium from her bones to provide it to the fetus. However, if this deficiency is not addressed, it can pose a significant risk to the fetus's growth and have long-term negative effects even after birth. These effects include an increased vulnerability to diseases such as rickets and diabetes. Respiratory disorders and autoimmune diseases were identified by researcher Christoph and his colleagues [14], as well as other researchers, during their study. The pregnant woman's age may significantly influence vitamin D deficiency, as indicated by Várbíró and colleagues[15]. Their research suggests that pregnant mothers in their teens are more likely to experience bone density loss and osteoporosis compared to pregnant mothers in puberty.

Due to the ongoing expansion of their bone mass and the conflicting needs of the fetus, pregnant women may experience a large fall in calcium concentration, leading to a shortage in vitamin D. In their study, Kovacs and Balasubramanian [16], [17], suggested that the lack of vitamin D in pregnant women could be attributed to insufficient calcium intake during pregnancy and inadequate exposure to sunlight, possibly due to the use of sun protection products to prevent skin cancer. Foods enriched with vitamin D, together with skin pigmentation. In postmenopausal women, Liu and his colleagues[18], found that the decrease in vitamin D levels can be attributed to several factors, including low consumption of milk and dairy products, high body fat percentage, lack of sunlight exposure, and significant estrogen concentration. On the other hand, Gill and others[19], explained that the main cause of osteoporosis in menopausal women is the decline in ovarian function and menopause, which leads to low vitamin D levels. This interruption in ovarian function decreases skeletal mass due to an imbalance in bone metabolism. Therefore, the decline in ovarian function is the most crucial factor in the development of osteoporosis after menopause.

The study's findings, as presented in Table 1, indicate a noteworthy reduction in estrogen hormone levels among both menopausal women and women with osteoporosis. The concentrations were measured at 28.39±5.59 and 51.11±10.52, respectively. Conversely, there was a significant increase in estrogen levels among pregnant women, with a concentration of 416.85±39.22 compared to the healthy group's concentration of 277.75±78.52. Additionally, the study revealed a significant increase in serum estrogens among pregnant women compared to menopausal women and those with osteoporosis.

The study's findings were consistent with those of Cheng[20] and Calik-ksepka[21]. Foger-Oswald and his colleagues found that osteoporosis affects both sexes and all age groups and races but is more prevalent in women after menopause. This is due to estrogen deficiency, cellular aging, weakened immunity, increased inflammation, medication use, calcium and vitamin D deficiency, and elevated thyroid hormone levels. Sultana and his colleagues [22]also noted that estrogen deficiency leads to increased secretion of interleukin IL-6, interleukin IL-1, and tumor necrosis factor-α (TNF), resulting in bone resorption. The lack of estrogen reduces osteoblast activity and increases osteopenia, leading to mineral loss and the development of osteoporosis after menopause.

During pregnancy, the concentration of the estrogen hormone significantly exceeds the normal limit due to various factors. Calic-ksepka and his colleagues[23] stated that the mammary glands experience significant alterations in structure and function during pregnancy to facilitate milk production. This process is influenced by three key hormones that play a crucial role in breast tissue development. The breast gland, composed of estrogen, progesterone, and prolactin, primarily stimulates and develops the lactiferous duct, preparing it for milk secretion following childbirth. It enhances the production of the

prolactin hormone and amplifies the receptors for this hormone in the mammary glands. During pregnancy, elevated progesterone levels counteract the stimulating impact of estrogen on the hormone Prolactin. This avoids suppressing milk production until childbirth and the removal of the placenta.

In their study, Ni and his colleagues[24] found that the placenta produces estrogen during pregnancy, leading to elevated hormone levels. This increase prompts the formation of new blood vessels in the uterus and placenta, facilitating the transportation of nutrients to the fetus. Consequently, mothers experience discomfort during the first trimester of pregnancy while the hormone levels rise rapidly.

In a study conducted by Winter[25], it was shown that estrogen's ability to protect bones diminishes during pregnancy and lactation. This leads to a weakened ability to generate new bone cells. Additionally, the increase in prolactin levels during breastfeeding results in a decrease in bone minerals.

The present study's findings, as presented in Table 1, indicate a statistically significant rise in the levels of PTH across all groups of women, including pregnant women, menopausal women, and women with osteoporosis. The respective values for the groups were (110.26±13.66, 93.30±10.53 and 86.76±7.76), while the concentration of parathyroid hormone in the control group was (55.48±10.31). The results indicate a significant decrease in parathyroid hormone concentration in the groups of pregnant and menopausal women compared to women with osteoporosis.

The study findings corroborated the findings of Alyassin and Taher[26] and Hysaj[27] PTH plays a crucial role in regulating calcium balance by influencing various organ systems in the body to maintain a normal blood calcium level. Conversely, elevated levels of PTH resulting from low blood calcium levels lead to bone resorption. The potential cause of the observed increase in parathyroid hormone levels in women with osteoporosis is suggested by Bollerslev[28].

In their study, Rija and colleagues[29] highlighted that a reduction in calcium concentration leads to a decline in the production of Hydroxyapatite crystals within the bone. The increase in hormone levels may be attributable to the administration of some drugs. In a study conducted by Researcher Dittmer and his colleagues in 2021, it was found that patients who received Glucocorticoid treatment, commonly used for medical conditions like allergies, asthma, and autoimmune diseases, exhibited elevated levels of parathyroid hormone in their blood. This was accompanied by reduced calcium absorption and increased calcium excretion through urine, ultimately decreasing bone mass. Research conducted by Goltzman[30] revealed that elevated levels of the parathyroid hormone have detrimental effects on cortical bones. This increases the likelihood of gradual bone loss and the risk of fractures. Alternatively, an increase in the hormone may result from vitamin D deficiency in pregnant women. According to Hysaj[31], vitamin D deficiency can be attributed to malnutrition or insufficient sunlight exposure. This deficiency leads to a decrease in blood calcium levels, causing an increased hormone secretion in response to this deficiency. The hormone concentration returns to normal when vitamin D levels are restored by taking prescribed vitamin supplements.

Alternatively, it could be attributed to chronic kidney disease. According to a study conducted by researcher Tinawi[32], persons with chronic kidney disease experience a decline in blood calcium levels as the disease advances. This decline subsequently leads to a fall in renal cholesterol concentration. Simultaneously, the level of phosphate in the blood rises due to a decrease in glomerular filtration, resulting in a decrease in calcium and an increase in phosphate. This increase in phosphate concentration triggers an elevation in parathyroid hormone levels. Alternatively, an increase in this hormone can be caused by a benign parathyroid tumor, as mentioned by Gasparri and colleague [33]. They found that 80% of parathyroid gland hyperactivity is attributed to this tumor, which is three times more prevalent in women than in men. Secondary hyperparathyroidism typically arises from vitamin D deficiency, chronic kidney disease, or other factors leading to hypocalcemia.

Correlation

The findings of the present study, as presented in Table (2), indicate that there exist direct relationships among certain variables, specifically:

1- Vitamin D in combination with estrogen.

2- Estrogen with PTH.

3- PTH with Cat-k.

Additionally, it demonstrated the presence of inverse correlations among some variables.

1- Vitamin D levels along with PTH, Cat-k.

2- Estrogen with Cat-k.

3- PTH with estrogen.

Table (2) Correlations between variables

	Vitamin D	Estrogen	PTH	Cat-k
Vitamin D	1	0.20 0.05	-0.37 0.0002	-0.80 <.0001
Estrogen	0.20 0.05	1	0.73 <.0001	-0.25 0.01
PTH	-0.76 <.0001	-0.37 0.0002	1	0.73 <.0001
Cat-k	-0.80 <.0001	-0.25 0.01	0.12 0.22	1

Conclusions

The study groups showed increased Cat-k levels, except for the control group. This suggests that Cat-k is a crucial biomarker for detecting osteoporosis. The study groups, except the control group, had a decline in vitamin D concentration, which suggests the development of bone illnesses such as osteoporosis if left untreated. This suggests a deficit of mineral components in the blood serum and the presence of bone weakening. Additionally, postmenopausal women and women with osteoporosis exhibited decreased levels of estrogen hormone. This highlights the significance of estrogen in safeguarding bones against the loss of essential mineral elements that constitute bone structure. The study groups, excluding the control group, showed elevated parathyroid hormone levels, suggesting reduced calcium content in the blood serum. This decrease in calcium can lead to weakened and fragile bones.

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