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Evaluation of Preptin Hormone Levels as a Biomarker for Osteoporosis and its Association With Risk Factors in Mosul City

Elaf Ghazwn Nafi ^{1,a)} and Saba Zeki Al-Abachi ^{1,b)}

¹ Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.

^{a)} Corresponding author: Elaf.g.nafi@gmail.com

^{b)} saba-alabachi@uomosul.edu.iq

Abstract. Osteoporosis is a progressive bone disease that leads to reduced bone mass and increased fracture risk, especially in postmenopausal women. Often developing silently, it is usually diagnosed only after fractures occur. Preptin, a peptide co-secreted with insulin, has recently been identified as a potential biomarker for bone health. This study aimed to measure preptin levels in the serum of osteoporosis patients in Mosul and explore risk factors such as age, sex, marital status, vitamin D3 and calcium deficiencies, and oxidative stress markers. The study included 100 newly diagnosed patients (75 women, 25 men) and matched healthy controls. Preptin levels were measured using ELISA, and additional biochemical markers like calcium, vitamin D3, glutathione, malondialdehyde, and oxidative stress indicators were assessed. The results showed significantly lower preptin levels in osteoporosis patients, particularly in women, with associations to vitamin D3 and calcium deficiencies. This suggests preptin could be an important early biomarker for osteoporosis.

Keywords: Osteoporosis, Preptin, Vitamin D3 Deficiency, Oxidative Stress.

INTRODUCTION

Osteoporosis is a skeletal disease that leads to decreased bone density and deterioration of bone tissue, making bones more fragile and susceptible to fractures. [1] It is often considered a silent disease, [2] as it develops gradually without a person being aware of it until fractures occur. [3] Osteoporotic fractures are a leading cause of disability and death. [4] The most common method for measuring bone density is dual-energy radial absorptiometry (DEXA), which measures the sensitivity of calcium uptake by x-rays to determine the amount of bone and other tissue. However, absolute measurements can vary between devices, necessitating the use of standardized reference ranges. [5] These reference ranges are compared with a patient-specific database generated by the device. [6] The risk of developing osteoporosis increases significantly with age. [7] It is estimated that more than 200 million people worldwide suffer from the disease. According to statistics from the International Osteoporosis Foundation, one in three women over the age of 50 and one in five men will experience an osteoporotic fracture in their lifetime. [8] Gender is a largely non-modifiable factor in the risk of developing the disease, with women being more susceptible, especially after menopause, when there is a rapid loss of bone mass, which stabilizes after about 10 years[9].

Osteoporosis has multiple causes, both natural and unnatural. These include aging, poor nutrition, deficiencies of vitamin D, calcium, phosphate, and other elements vital for bone mineralization, lack of exercise, and hormonal changes in postmenopausal women.[10] The World Health Organization defines osteoporosis as a progressive, systemic skeletal disease characterized by decreased bone mass and microstructural deterioration, leading to fragility and fractures.[11] Prepeptin, a member of the insulin family, is a 34-amino acid peptide derived from the pro-peptide E of insulin-like growth factor 2 (pro-IGF2). Prepeptin is secreted with insulin and promotes glucose-mediated insulin secretion.[12] Discovered by Buchanan and his team in 2001, it is secreted with insulin and plays a role in glucose regulation.[13] Although the systemic effects of prepeptin on glucose metabolism remain largely undefined, it has been found to be secreted by various tissues, including pancreatic beta cells, kidney, liver, salivary glands, and mammary tissue[14]. Female mice that lost the prepeptin gene showed reduced glucose-enhanced insulin secretion, but this effect was not observed in males[15].

Calcium is essential for a wide range of biological functions, and any changes in its levels in the bloodstream trigger mechanisms to maintain calcium balance. When calcium levels drop, calcium-sensing receptors (CaSR) in

the parathyroid glands prompt the release of parathyroid hormone (PTH). This hormone helps the body retain calcium by increasing its reabsorption in the kidneys and promoting the production of 1,25(OH)₂D, which then binds to osteoblasts to help restore calcium levels[16]. To balance this, negative feedback mechanisms kick in, such as the release of calcitonin from the parathyroid glands. Calcitonin reduces the absorption of calcium in the kidneys and intestines and inhibits bone resorption, helping maintain calcium homeostasis[17]. The biomarkers you mentioned—glutathione (GSH), malondialdehyde (MDA), peroxynitrite (ONOO⁻), and aryl esterase—are all associated with oxidative stress. This stress is an important factor in the development of osteoporosis[18].

EXPERIMENTAL PART

In this study, high-quality analytical reagents and diagnostic tools were carefully selected to ensure accurate and reliable results. Blood samples were collected from patients referred by Ibn Sina Teaching Hospital, Al-Salam Hospital, and Al-Jumhuri Hospital in Mosul between October 2024 and March 2025. Participants fasted for at least eight hours before the blood draw. Osteoporosis diagnosis was confirmed using dual-energy X-ray absorptiometry (DEXA), a widely used, safe, and rapid imaging technique to assess bone density and monitor disease progression. A total of 100 newly diagnosed osteoporosis patients, including 75 women and 25 men aged 15 to 45 years, were enrolled after giving informed consent and receiving ethical approval from the Ninawa Health Department. The participants were grouped into three age categories: 15–30, 31–45, and over 45 years, with corresponding age-matched healthy controls included for comparison. Clinical examinations and family histories were documented to assess risk factors, with special focus on pain-related areas. Around 10 mL of venous blood was collected from each participant using sterile syringes, and 3 mL of this sample was transferred into polystyrene gel tubes for serum separation. The serum was incubated and centrifuged before being stored at −4 to −8°C for future analysis. Biochemical and hormonal assessments were conducted immediately, including glucose and lipid profiles. Preptin hormone levels were measured using an enzyme-linked immunosorbent assay (ELISA), while calcium concentrations were determined spectrophotometrically. Vitamin D3 was measured using electrochemiluminescence, and oxidative stress biomarkers, such as aryl esterase, peroxynitrite radicals, reduced glutathione, and malondialdehyde, were analyzed using standard methods. These analyses provided a comprehensive evaluation of bone metabolism, endocrine regulation, and oxidative stress in osteoporosis patients compared to healthy controls.

RESULTS AND DISCUSSION

The results shown in figure (1) indicated tht the highest percentage of patients participating in study is women while the lower percentage is men Researcher has demonstrated that a patients women is more than men due to the sexual hormonal differences Males and females ,menopause and aging[29] , Women, especially postmenopausal, are more susceptible to osteoporosis due to a sharp decline in estrogen levels, which directly impacts bone metabolism. Some evidence indicates that preptin shows greater osteogenic effects in females compared to males [19].

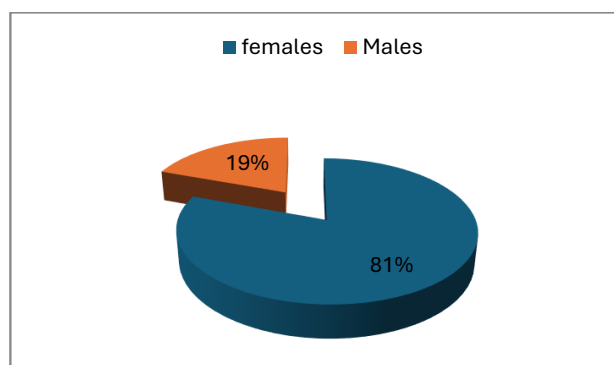
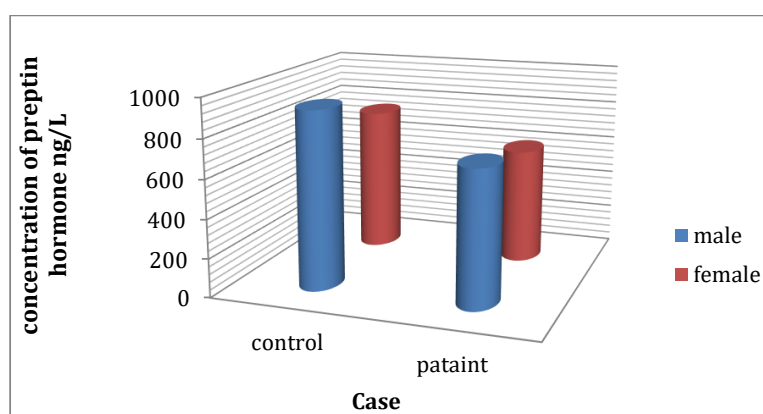


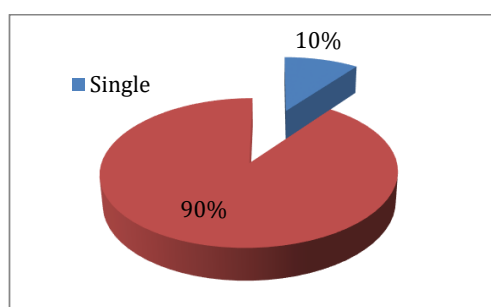
FIGURE 1. Gender Distribution of Osteoporosis Patients.

TABLE 1. The level of the preptin hormone in serum of the according to sex control and osteoporosis group

Preptin ng/L		
Sex	Control (mean \pm SE)	Patients (mean \pm SE)
Females	757.96 \pm 165.3	680.4667 \pm 100.22
Males	920.87 \pm 69.49	706.4283 \pm 65.71

**FIGURE 2.** The Level of The Preptin Hormone in Serum of the According to Sex Control and Osteoporosis Group

The table (1) shown indicate that there Is a significant difference in the concentration of the hormonal priptin and at the probability level of (0.024) in patients compare with control group probability level(0.039) based on sex, It was noted that the concentration of the hormone Male priptin Is higher than female priptin concentration. This difference in the concentration of the hormonal hormonal priptin between males and females may be due to the sexual hormonal differences Males and females, as researcher ,noted with a study conducted on females before and after despair, and between males and females in a stage of despair, there is a decrease in the concentration of the priptin hormone in females after Despair compared to females before menopause and a decrease in the hormones and preptin in females compared to males In menopause. The results shown in figure (4) indicated patients women that married is have low concentration of preptin compare with single women Multiple pregnancies may increase the risk of osteoporosis due to repeated calcium depletion in women, particularly in the absence of adequate nutritional compensation. This may affect preptin levels as well [19],The women during pregnancy need calcium and vitamin d3 to build the fetus is bones .if this not enough for women and their fetus lead to development osteoporosis ,breastfeeding also effects mothers bone heath , parity effect also [20].

**FIGURE 3.** Effect the marital status to concentration of preptin hormone in osteoporosis patients

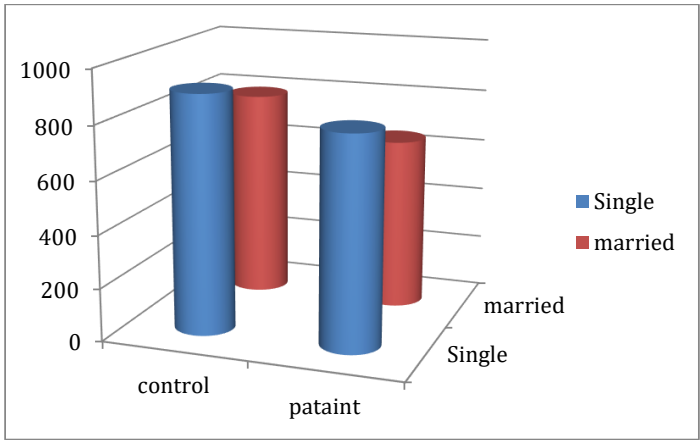


FIGURE 4. The level of the preptin hormone in serum of the according to marital status control and osteoporosis group

TABLE 2. The level of the preptin hormone in serum of the according to marital status control and osteoporosis group

Preptin ng/L		
marial status	Control (mean ±SE)	Patients (mean ±SE)
Single	849.3667± 102	740.1350± 83.69
Married	764.61± 97.36	596.0167±80.24

The results shown in figure (5) indicated patients women who >45 years have low concentration of preptin hormone compare with young women Has been demonstrated to have osteogenic effects both in vitro and in vivo. In the present study, serum preptin levels were measured in pre- and postmenopausal women with similar body mass indexes (BMIs) to elucidate its link with bone mineral density (BMD). Sixty women (30 premenopausal and 30 postmenopausal) with low bone mineral density were studied. The BMD scores, serum preptin levels and serum estradiol levels were measured. The correlation between serum preptin and estradiol levels with BMD was assessed. Serum preptin and estradiol levels were significantly lower in the postmenopausal women than the premenopausal subjects [21]

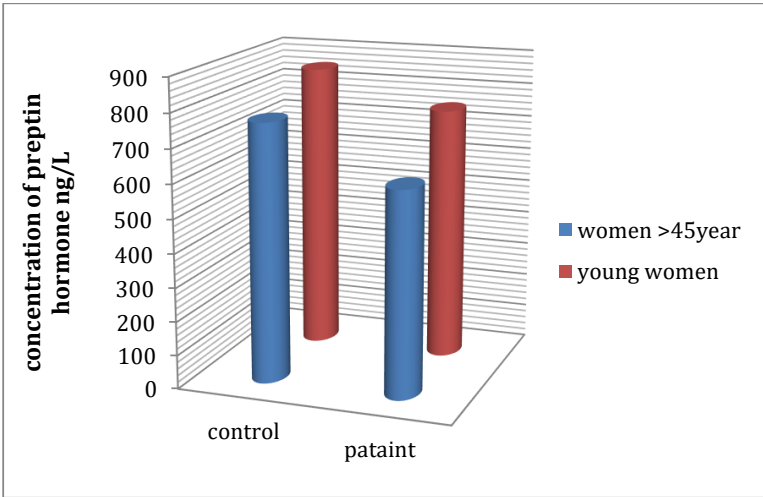


FIGURE 5. The level of the preptin hormone in serum of the according to >45 Women control and osteoporosis group.

TABLE 3. The level of the preptin hormone in serum of the according to >45 Women control and osteoporosis group

Preptin ng/L		
Menopausal	Control (mean \pm SE)	Patients (mean \pm SE)
Women >45 years	849.3667 \pm 258.73	596.0167 \pm 80.24
Young women	763.9804 \pm 359	763.9804 \pm 73.74

TABLE 4. Shown concentration of Vitamin D3 in control and patients

Vitamin D3	
Sample	Vitamin D3 Mean \pm SE
Control	34.14 \pm 0.54
Patients	23.51 \pm 0.47

The results shown in Table 4. indicated that there was a decrease in the average concentration of vitamin D3 in the blood serum of patients with osteoporosis at a probability level of ($p < 0.001$), reaching (23.51 \pm 0.47) compared to its average concentration in the control group, which reached (34.14 \pm 0.54). This may be due to the low concentration of 7-dehydrocholesterol in the elderly, which causes a deficiency of this vitamin in the body and thus affects the absorption of calcium in the body, leading to osteoporosis. These results were consistent with the results of previous studies that indicated vitamin D3 deficiency in older people in many countries [22]. When vitamin D3 is low, there is a clear risk to muscle and bone health, osteomalacia, hyperthyroidism with muscle weakness and osteoporosis. The amount of exposure to sunlight is a possible factor that contributed to this result [23]. Absolute vitamin D3 deficiency also leads to an increase in the amount of the skeleton that is replaced by unmineralized bone. As a result, the weight-bearing bones begin to bend. Although this is imperceptible, it is painful because the periosteum expands and the patient suffers from vague pain in the extremities [24]. From studying the relationship between the hormone preptin and vitamin D3, the results shown showed a directly relationship, as the value of the correlation coefficient reached (0.096).

TABLE 5. The Concentration of Oxediative Stress in Control and Patients

Oxediative Stress				
Sample	GSH	MDA	ONOO	Aryl esterase
Control (Mean \pm SE)	6.2650 \pm 3.37	4.2050 \pm 0.14	297.4950 \pm 117,12	712.9700 \pm 59
PatientS (Mean \pm SE)	1.2263 \pm 0.22	7.1201 \pm 0.13	463.9557 \pm 53.42	617.7438 \pm 89.03

Glutathione (GSH) is the most abundant antioxidant in the cell, and it is responsible for neutralizing reactive oxygen species (ROS). ROS can promote osteoclast differentiation and stimulate bone resorption and are some of the primary drivers of bone loss with aging and loss of sex steroids. Despite this, the role of GSH biosynthesis during osteoclastogenesis remains controversial. Here, we show that the requirements for GSH biosynthesis during osteoclastogenesis in vitro and in vivo are unique. Using a metabolomics approach, we discovered that both oxidative stress and GSH biosynthesis increase during osteoclastogenesis. Inhibiting GSH biosynthesis in vitro via the pharmacological or genetic inhibition of glutamate cysteine ligase (GCLC) prevented osteoclast differentiation. Conversely, the genetic ablation of GCLC in myeloid cells using LysMCre resulted in a decrease in bone mass in both male and female mice. The decreased bone mass of the LysMCre;Gclcf^{fl}/fl mice was attributed to increased osteoclast numbers and elevated bone resorption. Collectively, our data provide strong genetic evidence that GSH biosynthesis is essential for the regulation of osteoclast differentiation and bone resorption in mice. Moreover, these findings highlight the necessity of complementing in vitro studies with in vivo genetic studies [25].

Glutathione (GSH) is a major intracellular antioxidant crucial for protecting osteoblasts from oxidative damage. Research shows that decreased glutathione levels lead to impaired osteoblast differentiation and enhanced osteoclast activity, contributing to bone loss [26]. Malondialdehyde (MDA) is a final product of the oxidative decomposition of polyunsaturated fatty acids, initiated by free radicals. Therefore, it is commonly used as a biomarker of oxidative stress. Some justifications for the effect of oxidative stress on bone density have been provided by relevant cellular-molecular studies. For instance, oxidative stress not only increases osteoclastogenesis but also inhibits osteoblast differentiation and thus bone formation. The major causes of low BMD in women are menopause and low steroid levels. Postmenopausal women are more vulnerable to oxidative stress than reproductive-age women because their oxidative balance is disrupted not only by aging but also by a decrease in 17 β -estradiol, an antioxidant [27].

MDA is a byproduct of lipid peroxidation and serves as a marker of oxidative stress. Studies have found elevated MDA levels in patients with osteoporosis, indicating increased oxidative damage in bone tissues. The most common protein in the body, albumin, serves as an abundant and important circulating antioxidant and is an example of an intrinsic non-enzymatic antioxidant. Albumin acts as a powerful scavenger neutralizing both ROS and RNS in the forms of hydroxyl radicals and peroxynitrite (ONOO⁻). The disulfide bonds in the albumin are readily available to neutralize these dangerous free radicals. There are other valuable intrinsic nonenzymatic antioxidant scavengers. Estrogen, mostly lacking in postmenopausal women, is also a powerful antioxidant through both direct scavenging and stimulating increased expression of antioxidant enzymes. Furthermore, other endogenous nonenzymatic antioxidants include glutathione (GSH) and alpha-lipoic acid. GSH is the most abundant nonenzymatic antioxidant produced in cells. Changes in GSH homeostasis may be an important contributor to uncontrolled oxidative damage. Each of these endogenous antioxidants helps to maintain a healthy balance of oxidative stress [28]. Aryl esterase activity is associated with the enzyme paraoxonase 1 (PON1), which plays an important role in the body's antioxidant defense system. In individuals with osteoporosis—especially postmenopausal women—studies have reported reduced aryl esterase activity, suggesting a diminished antioxidant capacity. This reduction may contribute to the increased oxidative stress seen in osteoporosis, further exacerbating bone resorption and weakening bone structure [29].

TABLE 6. The concentration of Calcium test in control and patients

Electrolyte	
Sample	Ca
Control (mean \pm SE)	9.4571 \pm 0.1
Patients (Mean \pm SE)	8.7486 \pm 0.07

The results shown in Table 6 indicated a decrease in the average calcium concentration in women with osteoporosis, reaching (8.7486 \pm 0.07) compared to the average concentration in the control group (9.4571 \pm 0.1). The results indicated a significant decrease in the calcium level at the probability level ($p < 0.05$) in the serum of patients with osteoporosis compared to the serum of women in the control group. This may be due to the fact that low levels of vitamin D in the body negatively affect the intestinal absorption of calcium, leading to low levels of calcium in the plasma and an increase in the level of parathyroid hormone, which works to compensate for this absorption by withdrawing calcium from the bones, leading to their osteoporosis. These results are consistent with the results of previous studies indicating that calcium deficiency leads to a decrease in bone mineralization [30]. A decrease in both testosterone and estrogen, which affect the intestinal absorption of calcium from the bloodstream, leads to the recovery of calcium stores from spongy and compact bone to maintain the necessary levels of calcium in the blood, thus contributing to bone resorption and osteoporosis [7]. From studying the relationship between preptin and calcium, the results a direct relationship, with the correlation coefficient reaching (0.041). This may be due to a decrease in the level of calcium in the blood, which leads to an increase in the level of PTH and a decrease in the level of preptin, which works to meet the body's need for calcium by activating osteoclasts [31].

CONCLUSIONS

Based on the results of this study, it can be concluded that Preptin hormone is a promising biomarker for bone health and its association with osteoporosis. The study found significantly lower levels of Preptin in osteoporosis

patients compared to healthy controls, especially in postmenopausal women and married individuals. Additionally, deficiencies in Vitamin D3 and calcium were strongly linked to reduced Preptin levels, which in turn contribute to bone fragility. The findings also highlighted the role of oxidative stress, with elevated markers such as MDA and ONOO⁻, and decreased levels of antioxidants like GSH, further exacerbating bone deterioration. These results emphasize the potential of Preptin as an early biochemical marker for assessing osteoporosis risk, especially in vulnerable groups.

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