The Physiological Role of Nesfatien-1 And Its Relationship to Minerals in Patients with Osteoporosis

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ABSTRACT

Osteoporosis (OP) is a disease that affects the skeletal system, characterized by decreased bone density (BMD). This study aimed to estimate the level of nesfatin, minerals, and vitamin D3 in osteoporosis patients and to monitor and study the association of nesfatin with patient variability. We collected 150 samples from both sexes (30-65 years old) to achieve this goal. Ninety-three of them were osteoporosis patients. These patients were divided according to the severity of the disease, including osteopenia and osteoporosis. The remaining adults represented a control group, including 57 adults selected for comparison. Nesfatin, calcium (Ca), phosphorus, Ca/P ratio, and magnesium (Mg) were measured. The result indicates that a level of nystatin was highly significantly increased ($p \leq 0.0001$) in osteoporosis patients (35.5 ± 11.9 ng/ml) compared to the control group (9.5 ± 3.12 ng/ml). Nystatin was significantly increased ($p \leq 0.0001$) in osteoporosis (46.4 ± 4.2 ng/ml), more than in osteopenia (23.7 ± 2.59 ng/ml). There was a significant negative correlation between nesfatine with Mg in OP patients ($r = -0.855$, $p = 0.01$) and Vit. D3 ($r = -0.846$, $p = 0.01$) in OP. In contrast, there was no significant between nystatin with calcium and phosphorus. We can conclude nesfatin-1 can be considered one of the biochemical indicators of osteoporosis patients, indicating a significant difference between patients and healthy people.

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1. Introduction:

Osteoporosis is a disease of the skeletal system. It is a pathological condition that causes bones to be characterized by decreased bone density, leading to thin, weak bones. Even a minor accident can cause a broken bone[1, 2], It commonly affects more than fourteen million persons in the USA and more than two hundred million worldwide[3, 4]. OP is characterized by bone homeostasis, which results in decreased bone mass, poor bone quality, and increased susceptibility to fracture[5, 6]. The Bone is constantly turnover throughout life to maintain healthy bone mass[7, 8]. Bone homeostasis depends on a balance between osteoclasts (bone resorption) and osteoblasts (bone formation) [9, 10]. Osteoblasts are responsible for bone formation, while osteoclasts break down bone tissue[7, 8] [11, 12]. Hormone, cytokine, and growth factors directly and indirectly regulate bone homeostasis[13, 14]. Peak bone mass is achieved by the factors working together effectively[15, 16]. bone homeostasis is thought to be altered an imbalance in these molecular and cellular processes that lead to the pathophysiology of osteoporosis[9, 16]. Other factors such as gender, race, diet, and behavior can also affect susceptibility to osteoporosis and bone mass[17]. NESFATIN-1 Nesfatin-1 is an 82-amino acid peptide found in the brain derived from nucleobindin 2 (NUCB2), which regulates energy balance, appetite, and metabolism[18]. Nesfatin is widely expressed, with white adipose tissue being a major source of nesfatin in serum [19]. It was identified early as causing anorexia, and this neuroendocrine function [20, 21]. Recent studies have suggested that nesfatin has a role in bone metabolism. [22]. Recent studies have suggested that nesfatin may also affect bone metabolism[23]. It has been found that nesfatin can influence osteoblast and osteoclast activity, potentially impacting bone density. In osteoporosis patients, altered levels of nesfatin may be observed[23], which could correlate with the disease's progression and overall bone health[4, 17, 24]. During the bone-formation process, nutrients such as vitamin D, calcium, magnesium, and phosphorus must be properly and continuously supplied, of which magnesium affects bone strength and firmness[12]. The essential nutrient for the proper functioning of the human body is calcium[27]. It is a major element that affects many processes outside and inside cells. It is essential for the building, growth, and maintenance of bones and the stability of the cellular cytoskeleton[25]. Magnesium is an essential mineral ion for living organisms and constitutes the second most abundant cation inside cells, playing an important role in major physiological systems[26]. Magnesium is essential for converting vitamin D into an active

form for calcium absorption and stimulates the hormone calcitonin to transport calcium back from the blood and soft tissues to the bones, maintaining bone structure[27]. Therefore, maintaining an optimal level of magnesium is essential for calcium absorption and metabolism.[26]. Calcium and calcium phosphate complex give strength and rigidity to the skeletal system[28-30]. Calcium and phosphate are released to the blood during bone resorption and deposited into bone during bone formation[28, 30]. Therefore, bone remodeling is an integral part of calcium and phosphate homeostasis[32]. Adequate serum calcium concentration is maintained with the help of vitamin D and facilitates normal bone mineralization through intestinal calcium absorption[31],[32],[33], where vitamin D and calcium act synergistically on bone[27, 34]. Our study aimed to estimate serum levels of nesfatin, minerals, and vitamin D in patients and healthy subjects and to find a relationship between nesfatin and the studied variables. Minerals and vitamin D3 have a strong effect on bones, and therefore their levels have been studied to determine the effect of each on bones.

2. Materials and method:

A study current was started from 15/Oct/2023 to 15/Feb/2024.

2.1. Population study:

The study included 150 individuals; 93 of them were osteoporotic patients of both sexes (76 women, 17 men). The ages range from (30 – 65year). In addition, 57 healthy individuals of both sexes (28 females and 29 males) aged from 30 to 65 years were selected as a healthy people group. Samples of Patients were collected from the outpatient clinics of the joint unit of Al-Salam Teaching Hospital and Mosul General Hospital in Mosul, Iraq. Osteoporosis patients were divided according to the severity of the disease into two groups: osteopenia = 54, and osteoporosis = 48 after being diagnosed by a specialist physician through bone density examination using a DXA scan Patients with a medical history of other diseases, such as cancer, kidney disease, and thyroid disease, were excluded.

2.2. Sample collection:

Blood was withdrawn using a clean and sterile needle, and due to the nature of a sensitive calcium test, once blood was detected in the needle, the tube was opened, then Blood was drawn (about 5 ml) and placed in a clean gel tube, left for 10 minute at temperature room 25°C then separated

about centrifugation, at 3000g for 5 minutes, a serums were separate into a clean as well as sterile tube and then stored in a refrigerated place at -80 °C until measurements.The accuracy of calcium and magnesium measurements depends on proper handling of blood samples. When drawing blood, the tube must not be tied to prevent hemolysis.

2.3. Various Paramet ers Estimation:

2.3.1. Estimation of Nesfatin:

Nesfatin-1 was measured using an ELISA kit from BT LAB, and it contains Cat. No. E3063Hu of Chinese origin (bt-laboratory). It has sensitivity: 0.15ng/ml, Intra-Assay: CV<8% and Inter-Assay: CV<10%. The enzyme-linked immunosorbent assay (ELISA) principle is dependent on the sandwich ELISA and involves using a plate pre-coated with anti-human Nes-1. A sample containing Nes-1 is added, which binds to the antigens on the wells. Next, biotin-bound anti-human Nes-1 is added, which binds to the Nes-1 present in the sample. In the next step, streptavidin-HRP is added, which binds to the biotin-bound anti-Nes-1. After a period of incubation, the process is completed[35].

2.3.2. Estimation of calcium:

The serum's calcium level is measured using the " BIOLABO test kit of French origin" (Biolabs). The method allows for the determination of total Calcium concentration in serum. In alkaline solution, CPC reacts with calcium to form a dark-red colored complex, the absorbance of which is measured at 570 nm and is proportional to the amount of calcium in the specimen"[36].

2.3.3. Estimating phosphorus:

The serum's phosphorus level was measured using a BIOLABO test kit of French origin (Biolabs) .The principal methods without deproteinization are described by Daly et al. "Gamst O.K. modified it and Try K. In an acid medium, phosphate ions form a phosphomolybdic complex with the ammonium molybdate". "The absorbance measured at 340 nm is proportional to the concentration of phosphate ions in the specimen"REF 80015 (vial R2) for urines and manual method only[36]

2.3.4. Estimation of magnesium:

The serum's magnesium levels are measured by using a "MAGNESIUM LR" Italian production. "The principal endpoint analysis. Magnesium reacts with Xylidyl Blue, forming the Mg-Xylidyl

Blue complex. The increase in absorbance is proportional to the magnesium concentration in the sample and measured at 500 nm [36].

2.3.5. Estimation of vitamin D :

Vitamin D was determined in the Copas device using the kit Elecsys Vitamin D total ||| (REF: 09038078190), produced by Germany[37].

2.4. Statistical analysis:

Statistical analysis was done using the SPSS program version 25. The result is expressed as "mean ± standard deviation (SD); an independent T-test was used to compare the two groups". ANOVA test employed the differentiation between the three groups, and Pearson's correlation coefficient is used to explore the relationship between a nesfatin-1 level and the variables studied. P-values of 0.05 were considered statistically significant[36].

1. Results and discussion

3.1. Level of Nesfatin-1 in patients with osteoporosis:

The result in Table 1 and Fig. 1 showed a highly significant increase ($p \leq 0.0001$) on the levels of nesfatin-1 in OP patients (35.5±11.92 ng/ml) when compared to controls (9.5 ± 3.12 ng / ml). The study showed that serum nesfatin-1 levels were increased in osteoporosis patients compared to healthy people. This increase may be attributed to bone formation and mineralization, as nesfatin-1 inhibits osteoclast differentiation[40], affecting bone regeneration and osteoporosis onset. It is hypothesized that these elevated nesfatin levels represent a protective mechanism against bone structure and properties changes. This may help reduce the risk of osteoporosis and lead to decreased bone volume and resistance[24, 26].

Table 1: Level of nesfatin-1 hormone in osteoporosis patients and comparison with healthy individuals:

Variables	(Mean± SD)		P-value
	Control, n=57	Patients, n=93	
Nesfatin-1 (ng/ml)	9.53 ± 3.12	***35.47 ± 11.9	0.0001
*** a highly Significant at (p ≤0.0001); SD= standard deviation, ml=mliliter; ng= nanogram; n= number;			

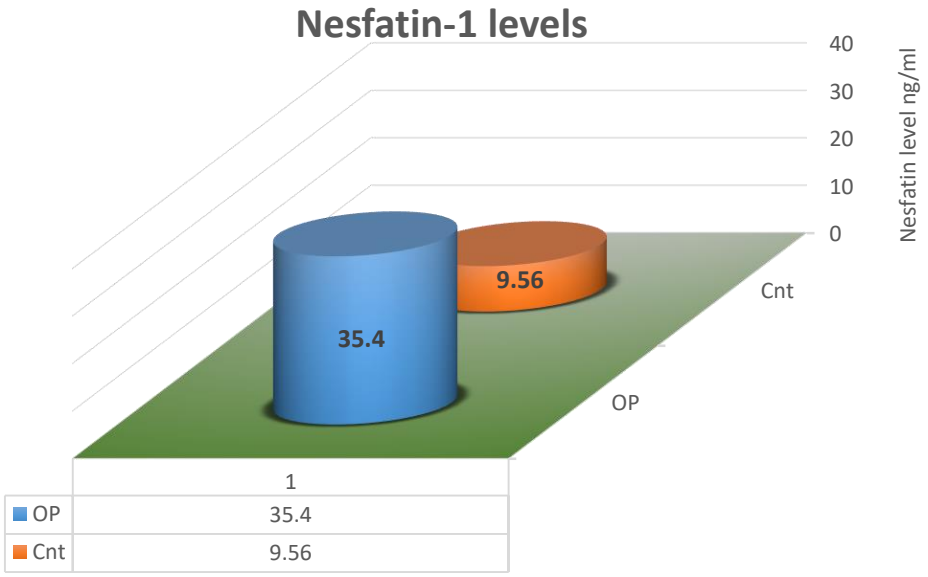


Figure 1: The level of Nesfatin-1 in OP patients compared with healthy individuals.

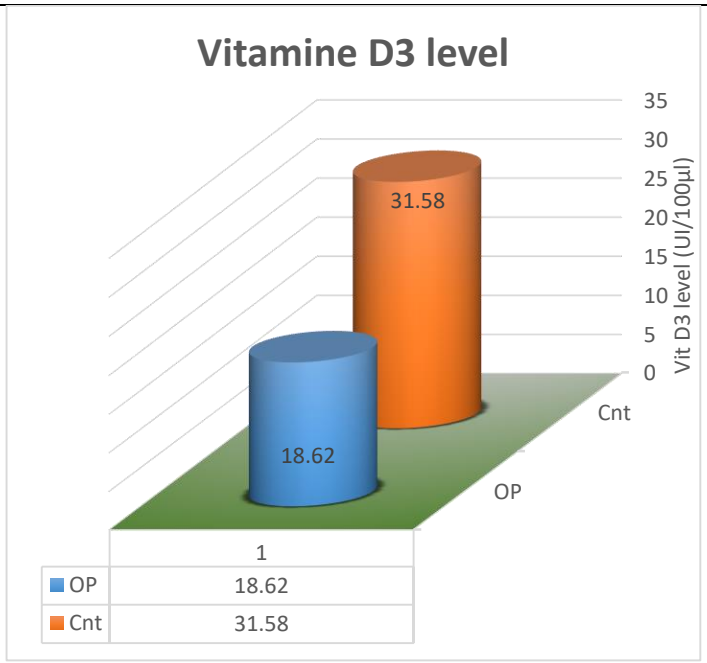
3.2. Study of the level of minerals in osteoporosis patients and comparison with healthy individuals:

Table 2 shows the study had a highly significant decrease (p≤0.0001) in magnesium levels in osteoporosis patients (1.07 ± 0.34 mg/ml) compared with the control group (1.94 ± 0.12 mg/ml), as reported in the research [26]. Studies have found a positive association between dietary magnesium intake and bone density and/or increased bone loss with lower dietary magnesium intake. This suggests that magnesium deficiency may be a risk factor for osteoporosis, which our study supports. In Table 2, the study shows a highly significant decrease (p ≤ 0.0001) in the levels

of vitamin D in patients (18.62 ± 4.8 UI/100µl) compared to the control group (31.58 ± 2.48 UI/100µl). These results were based on research [33] that found that low levels of vitamin D may lead to a decrease in calcium absorption in the intestine, which increases the risk of osteoporosis[27, 33, 39]. The study in Table 2 showed a significant increase ($p \leq 0.005$) in the level of phosphorus in osteoporosis patients (4.5 ± 0.25 mg/dl) compared with control individuals (3.3 ± 0.25 mg/dl). In contrast, a significant decrease ($p \leq 0.005$) in the levels of calcium in patients (8.01 ± 0.4 mg/dl) compared to the healthy individuals (9.5 ± 0.32 mg/dl). Table 2 shows that the Ca:P ratio has significantly decreased ($p \leq 0.0001$) in osteoporosis patients (1.7 ± 0.13) compared to the control group (2.8 ± 0.24). These results are consistent with the research of [27]. A study conducted in Korea on nearly 10,000 people showed that the lower the calcium-to-phosphorus ratio in the diet, the greater the risk of osteoporosis [39].

Table 2: Study of the level of minerals in osteoporosis patients and comparison with healthy individuals:

Variables	Control, n=57	Patients, n=93	P-value
	(Mean ± SD)		
Magnicium (mg/ml)	1.94 ± 0.12	***1.07 ± 0.34	0.0001
Vitamine D3, UI/100µl	31.58 ± 2.48	***18.62 ± 4.8	0.0001
Calcium, mg/dl	9.51 ± 0.32	***8.01 ± 0.4	0.0001
Phosphorus, mg/dl	3.3 ± 0.25	***4.5 ± 0.22	0.0001
Calcium/Phosphorus	2.8 ± 0.24	***1.7 ± 0.13	0.0001
Note: *** significant at ($p \leq 0.0001$); n= number; SD= standard deviation; ml=Milliliter ; mg= milligram; dl=disliter			



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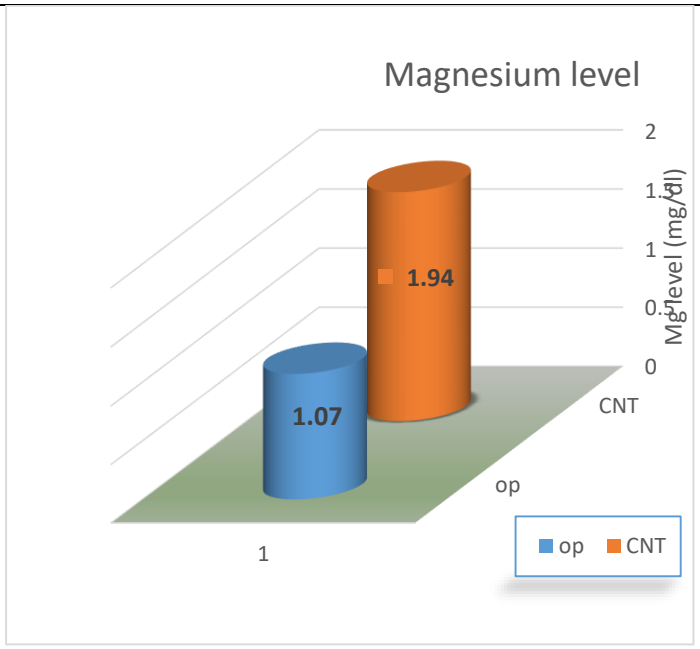

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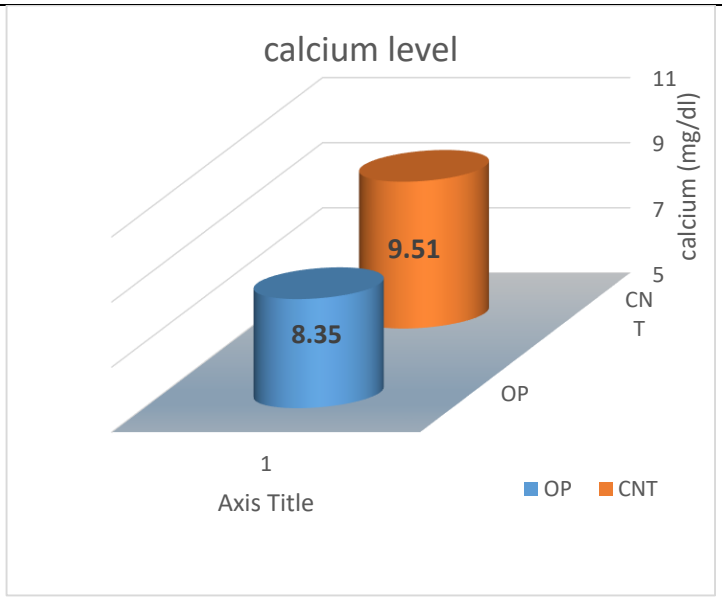

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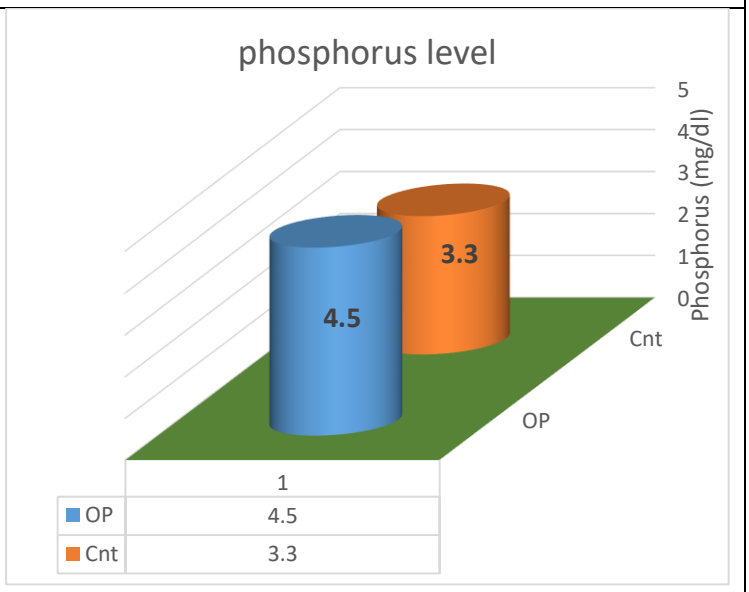


(D)

Figure 2: (A) shows that Vitamin D_3 level in OP patients compared to control; Figure 2 (B) shows the Mg level in Op patients compared to control; Figure 2 (C) shows the Calcium level in OP patients compared to control; Figure 2 (D) shows the Phosphorus level in OP patients compared to control. Mg = Magnicium

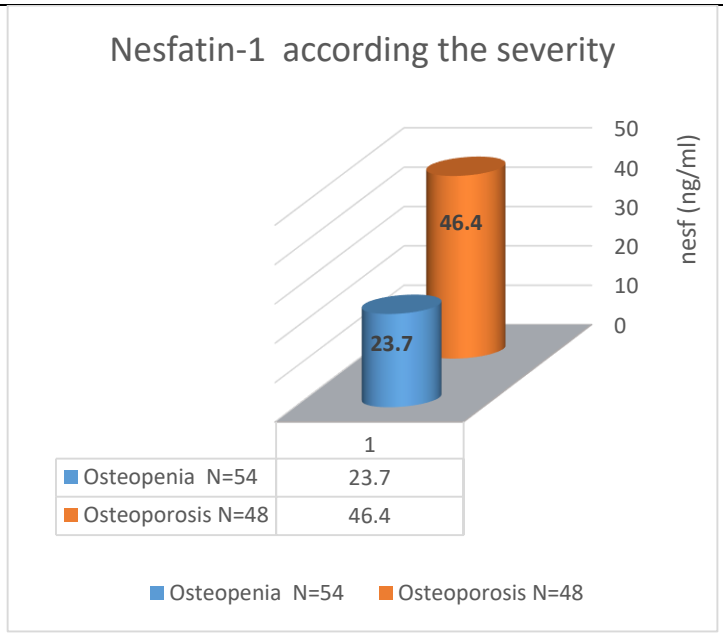
3.3. Effects of the disease severity and osteoporosis on the level of minerals, vitamins D3:

In Table (3) and Fig. (A), A study shows a highly significant increase ($p \leq 0.0001$) in the levels of nesfatin in cases of osteoporosis (46.4 ± 4.29 ng/ml) compared to osteopenia cases (23.77 ± 2.59 ng/ml)[40]. In contrast, the study showed in Table 3 and Fig. 3 (C) a highly significant decrease ($p \leq 0.0001$) of magnesium levels in an osteoporosis case (0.786 ± 0.15 mg/dl) compared to the case of osteopenia (1.39 ± 0.16 mg/dl). The study showed in Table 3 and Fig. 3 (F) a highly significant decrease ($p \leq 0.0001$) of vitamin D3 levels in the osteoporosis case (14.5 ± 2.41 IU/100µl) compared to the case of osteopenia (22.9 ± 2.1 IU/100µl). The study showed in Table (2) and Fig 3 (D), (B), (G), no significance of each of the calcium (($p \leq 0.356$), OP case (8.32 ± 0.26 mg/dl), osteopenia case (8.37 ± 0.2mg/dl)), phosphorus (($p \leq 0.493$), OP case (4.55 ± 0.21mg/dl), osteopenia case (4.55 ± 0.29mg/dl)) and calcium/phosphorus ratio ($p \leq 0.349$), OP case (1.7 ± 0.1), osteopenia case (1.84 ± 0.1).

Table 3: Effects of the severity of osteoporosis in the levels of nesfatin-1, minerals, vitamin D3:

Variables	Osteopenia , n = 45	Osteoporosis , n = 48	P- Valu
Nesfatien-1, ng/ml	23.7 ± 2.56	22.6 ± 4.3	0.0001
Calcium, mg/dl	8.1 ± 0.3	7.8 ± 0.5	0.356
Phosphate, mg/dl	4.55 ± 0.212	4.54 ± 0.298	0.493
Magnesium, mg/ml	1.389 ± 0.162	0.786 ± 0.15	0.0001
Vit. D3, IU/100µl	22.99 ± 2.06	14.5 ± 2.41	0.0001
Calcium/phosphate	1.84 ± 0.1	1.7 ± 0.1	0.349

Note: *** significant at ($p \leq 0.0001$); n= number; SD= standard deviation; ml=Milliliter ; mg= milligram; dl=disliter



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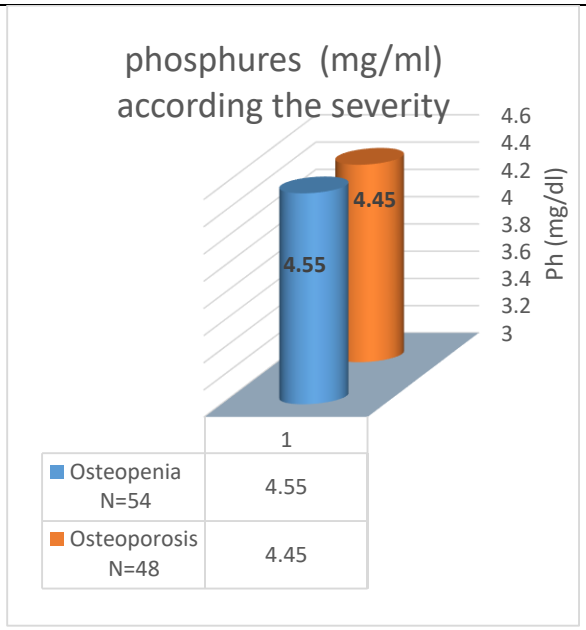

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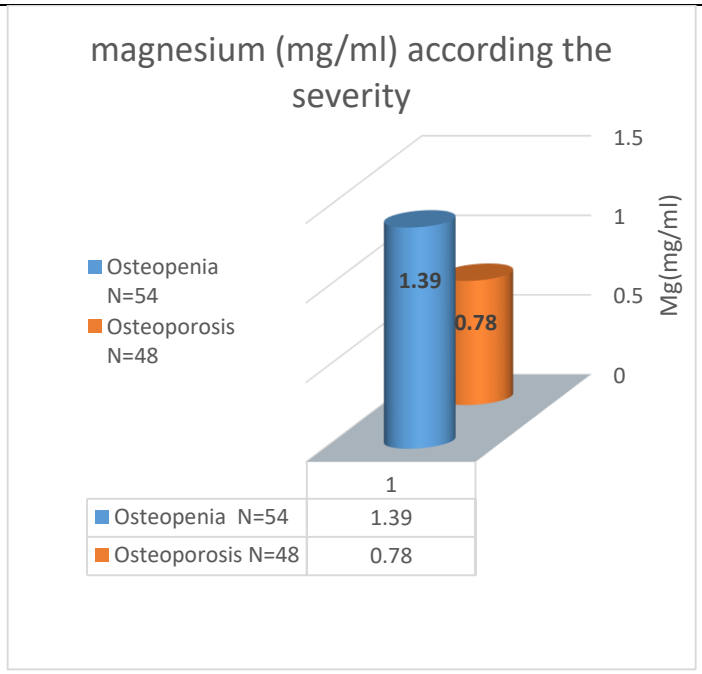

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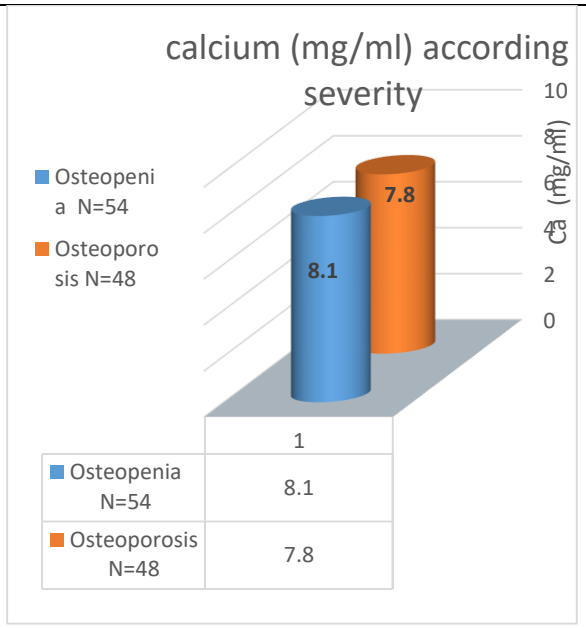

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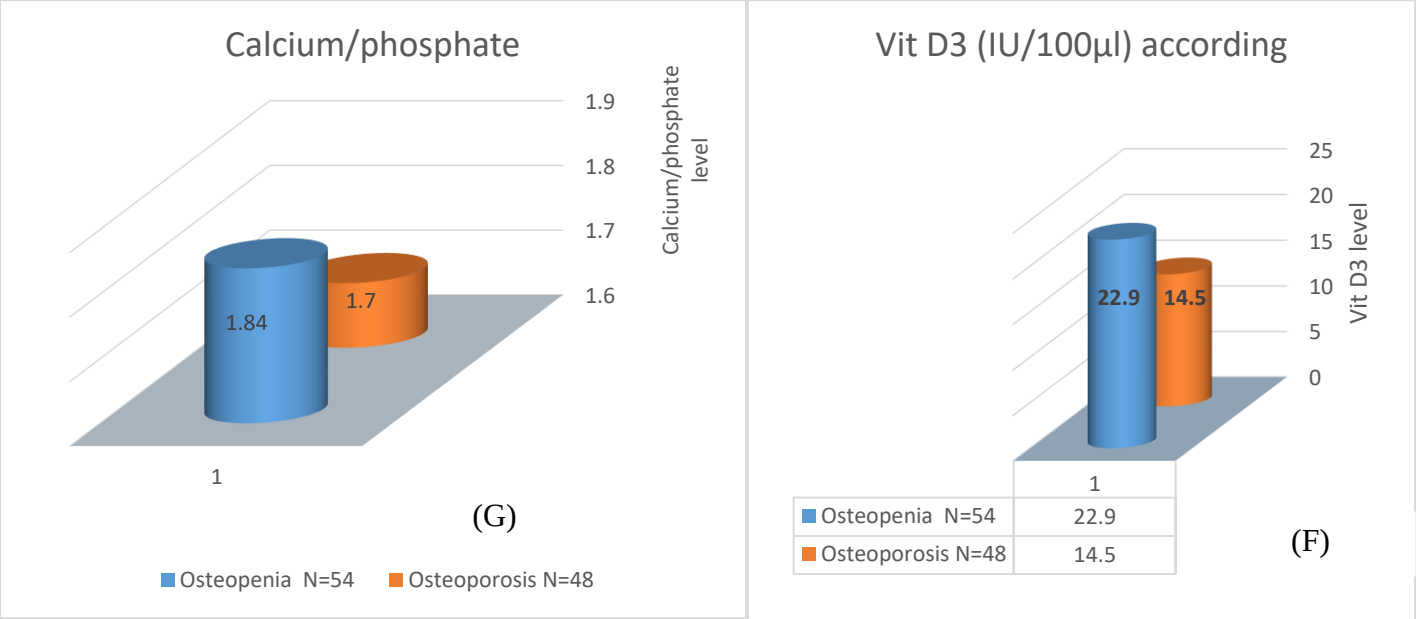

Figure 3: (A) Effects of a Severity Disease in osteoporosis in a nesfatin-1 ; Fig 3 (B)Effect of a Severity Disease in osteoporosis in a Phosphorase; Fig 3 (C) Effect of a Severity Disease in osteoporosis on the Mg; Fig 3 (D) Effect of a Severity Disease in osteporosis in a calcium, Fig3 (F) Effect of a Severity Disease in osteoporosis in a vitamin D3, Fig 3 (G) Effect of the Disease Severity of osteoporosis in a calcium/phosphorase.

3.4. Correlation of Nesfatin-1 biochemical parametars study:

The results, as shown in Table (4), showed that the relationship of nesfatin-1 with the studied variables, Ca, phosphorus, magnesium, vitamin D3 showed a highly significant difference relationship with each of the Magnicium ($r=-0.855$; $p\leq0.0001$), Vitamins D3 ($r=-0.846$; $p\leq0.0001$) in the OP patients and the relationship was negative, and showed no significant difference relationship with each of the calcium ($r=0.522$; $p\leq0.01$), phosphorus($r=-0.048$; $p\leq0.646$); calcium and phosphorus ratio ($r= -0.083$, $p\leq 0.428$) in the OP patients.

Table 4: Correlation of nesfatin-1 and biochemical variables study in OP patients:

Biochemical Variable	Nesfatin-1, Pearson correlation (r),p °	
	control	Osteoporosis
Magnesium, mg/dl	0.308 ; 0.02	**-0.855; 0.0001
Calcium, mg/dl	0.173 ; 0.198	-0.102; 0.331
Phosphorus, mg/dl	0.027 ; 0.842	0.048 ; 0.646
Calcium/Phosphorus	0.048 ; 0.723	-0.083; 0.428
Vit D3 IU/L	-0.186; 0.167	**-0.846; 0.0001
**correlation is significant at the 0.001 level; mg= milligram; dl= disiliter; L=liter U= unit.		

2. Conclusions

The results presented in this study indicate that nesfatin-1 is a physiological factor that mediates bone metabolism. Nesfatin-1 also exerts a protective effect on bone tissue properties. Based on the results obtained, nesfatin-1 can be considered one of the biochemical indicators of osteoporosis patients, indicating a significant difference between patients and healthy people, in addition to the relationship of nesfatin-1 with magnesium and vitamin D.

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الدور الفسيولوجي لنسفاتين-1 وعلاقته بالمعادن لدى مرضى هشاشة العظام

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المستلخص:

هشاشة العظام مرض يصيب الجهاز الهيكلي، ويتميز بانخفاض كثافة العظام (BMD. هدفت هذه الدراسة إلى تقدير مستوى النسفاتين والمعادن وفيتامين د3 لدى مرضى هشاشة العظام، ومراقبة ودراسة ارتباط النسفاتين بتغيرات المرضى. لتحقيق هذا الهدف، جمعنا 150 عينة من كلا الجنسين (30-65 سنة). كان ثلاثة وتسعون منهم مرضى هشاشة العظام. تم تقسيم هؤلاء المرضى وفقًا لشدة المرض، بما في ذلك هشاشة العظام وهشاشة العظام. يمثل البالغون المتبقون مجموعة ضابطة، بما في ذلك 57 بالغًا تم اختيارهم للمقارنة. تم قياس النسفاتين والكالسيوم (Ca) والفوسفور ونسبة الكالسيوم إلى الفوسفور والمغنيسيوم (Mg). تشير النتيجة إلى أن مستوى النسفاتين زاد بشكل كبير ($p \leq 0.0001$) لدى مرضى هشاشة العظام (35.5 ± 11.9 نانوغرام/مل) مقارنة بالمجموعة الضابطة (9.5 ± 3.12 نانوغرام/مل). زاد النسفاتين بشكل كبير ($p \leq 0.0001$) في هشاشة العظام (46.4 ± 4.2 نانوغرام/مل) أكثر من هشاشة العظام (23.7 ± كان هناك ارتباط سلبي ذو دلالة إحصائية بين النيسفاتين والمغنيسيوم لدى مرضى OP ($r = -0.855$، $p = 0.0001$) وفيتامين د3 ($r = -0.846$، $p = 0.0001$) لدى مرضى OP. في المقابل، لم يكن هناك ارتباط ذو دلالة إحصائية بين النيسفاتين والكالسيوم والفوسفور. ويمكننا أن نستنتج أن النسفاتين-1 يمكن اعتباره أحد المؤشرات الكيميائية الحيوية لمرضى هشاشة العظام، مما يشير إلى وجود فرق كبير بين المرضى والأشخاص الأصحاء.