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A study of some Immunological and Physiological indicators for patients with Osteoporosis in Anbar Province

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ABSTRACT

Background: Osteoporosis is a degenerative, progressive bone condition that causes widespread fragility and an increased risk of fractures. This study was designed to determine some levels of Immunological parameters, such as Interleukin (IL) IL-17, IL-1, IL-15, IL-6, Hematological parameters (Hb and WBC), and some biochemical parameters (Vit D3) in patients with osteoporosis and compare their parameter levels with the control group. **Methodology:** This cross-sectional study was conducted among the general population who attended Ramadi Teaching Hospital in Ramadi city from the 1st of November 2023 to the 1st of January 2024. The total number of blood samples was 80 samples which are included 50 samples for patients with osteoporosis with ages ranging from 16 to 50 years and 30 samples for healthy individuals as a control group. **Results:** There was significant deference in all parameters with p-value (<0.05), the means levels of IL-1, IL-17, IL-15, IL-6 and Vit D3 respectively were: (8.467pg\mL), (89.32pg\mL), (83.84pg\mL), (60.34pg\mL), (4.667pg\mL); As well as the concentration of Hb, and mean number of WBC were: Hb(9.16g/dL) and\ WBC(14.19*10^3 c/mm3) respectively. **Conclusion:** We conclude that patients suffering from osteoporosis showed a significant increase in the levels of IL-1, IL-17, IL-15, IL-6, and WBC compared with controls. However, there was a significant decrease in vitamin D3 and Hb in patients compared to controls.

Keywords: Osteoporosis, Cytokines, VIT D3.
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INTRODUCTION

Bone is a dynamic, complex tissue. Numerous variables, including nutrition, age, hormones, and the body's inflammatory status, impact bone health. For example, in the elderly population, osteoporosis is also associated with age-related issues, indicating that aging has a significant impact on the immune system (1,2). Osteoporosis is a degenerative, progressive bone condition that causes widespread fragility and an increased risk of fractures (3). Men and women alike experience an increased risk of fractures and brittle bones as a result of osteoporosis. According to estimates, over 50% of women and 30% of men over 50 are vulnerable to these problems (4). The term "lymphokine" was initially used to describe a product of hematological cells, other than immunoglobulins, that acts as part of the host defense against infection and injury. However, the term cytokine was later suggested, as it was found to be produced by several cell types throughout the body. The role of cytokines is not limited to immunology and infection, but has also expanded into hematology, oncology, endocrinology, and other systems. New cytokines have been discovered in the past two decades, including interleukins, interferons, colony-stimulating factors, tumor necrosis factors, and leukemia inhibitory factors; however, the list will undoubtedly continue to grow as new cytokines are

discovered, identified, and purified in the bone and bone marrow (5,6). People with osteoporosis may have higher levels of inflammatory markers than those with normal bone mass, if proinflammatory cytokines are indeed linked to decreased bone mass; however, the evidence supporting this theory is conflicting. Peripheral blood mononuclear cells (PBMCs) have been observed to secrete IL-1 at significantly higher levels in osteoporosis patients (7). Many metabolic activities and hormone development, such as sex steroid production, are influenced by gut flora and play a critical role in skeletal turnover. Serotonin and vitamin D metabolism are also regulated by the microbiome, which affects bone health. Calcium channel activity, calcium absorption, and bone calcification are all controlled by Vit. D3, which plays a vital role in bone metabolism (8).

METHODOLOGY

This study included 80 individuals, comprising 30 healthy individuals as the control group and 50 patients with osteoporosis who attended the general teaching hospital in Ramadi city from the 1st of November 2023 to the 1st of January 2024, and ranging in age from 16 to 50 years old.

Ten milliliters of venous blood were drawn from an appropriate vein. About 2.5 mL of the blood sample was quickly transferred to a clean, dry EDTA tube. It was then gently shaken and used straight for the blood count test. The remaining blood sample was placed in a glass tube without an anticoagulant and allowed to coagulate. Then, it was centrifuged at 4000 rpm for 5 minutes in a centrifuge to separate the serum. To be employed for serological research, the separated serum was collected and stored at -20 °C in sterile, clean white tubes (9).

The diagnostic kits and chemical reagents used in the present study were employed for the quantitative determination of serum levels of IL-1, IL-17, IL-15, and IL-6, utilizing commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biosource Inc., USA). Serum levels of 25-OH Vit. D, as measured by commercially available ELISA kits (Biosource Inc., USA), was used for the quantitative determination of 25-OH Vitamin D concentration in human serum. The determination of Hb and WBC parameters was performed by a complete blood count (CBC) test in duplicate using a Coulter HMX Inc. instrument.

Statistical Analysis

The Statistical Analysis System (SAS) (2018) program was used to detect the effect of the different factors on the study coefficients. The T-test, the least significant difference (LSD) test, and the analysis of variance (ANOVA) were used for significant comparison between means. The Chi-square test was used for a significant comparison between the counters. The probability levels in this study were 0.05

RESULTS

Immunological parameters

IL- 1

The results of this study demonstrated a significant increase ($P < 0.05$) in IL-1 levels in osteoporosis patients compared to the control group. The means of IL-1 in the cases and control groups were, respectively, 8.467 pg/mL and 1.217 pg/mL, as indicated in Figure (1).

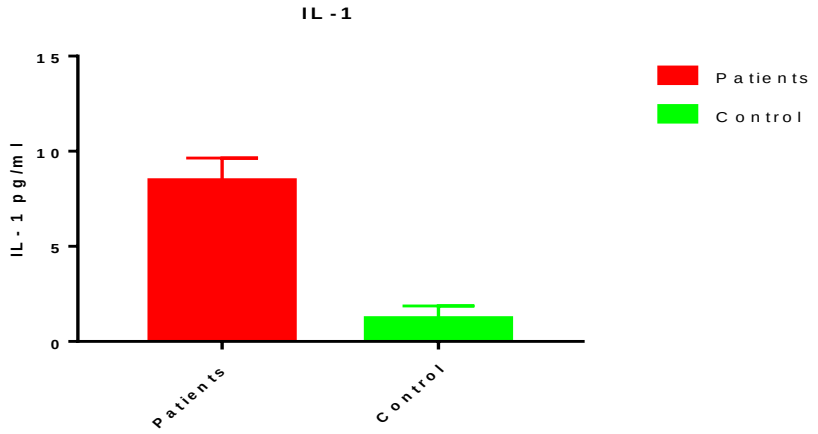

Figure (1): Mean levels of IL-1 (pg/mL) between patient and control groups of the population study

IL- 17

The current study demonstrated a significant increase ($P < 0.05$) in IL-17 levels in osteoporosis patients compared to the control group. The means of IL-17 in the patients and control groups were, respectively, 89.32 pg/mL and 10.82 pg/mL, as indicated in Figure (2).

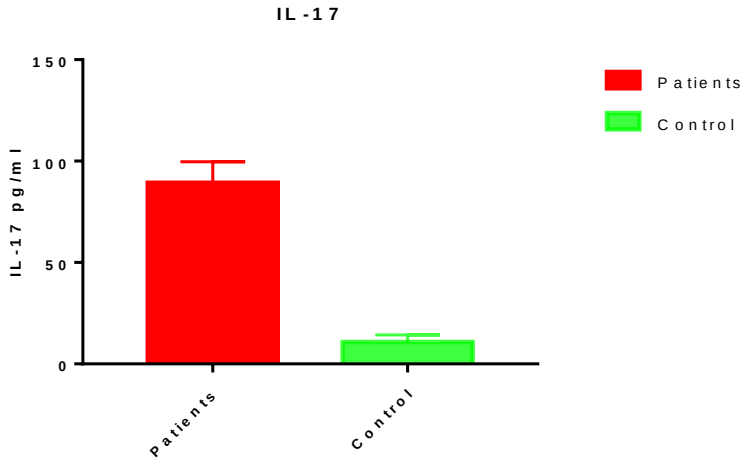

Figure (2): Mean levels of IL-17 (pg/mL) between patient and control groups of the population study

IL- 15

The current study demonstrated a significant increase ($P < 0.05$) in IL-15 levels in osteoporosis patients compared to the control group. The means of IL-15 in the patients and control groups were, respectively, (83.84 pg/mL) and (15.49 pg/mL), as indicated in Figure (3).

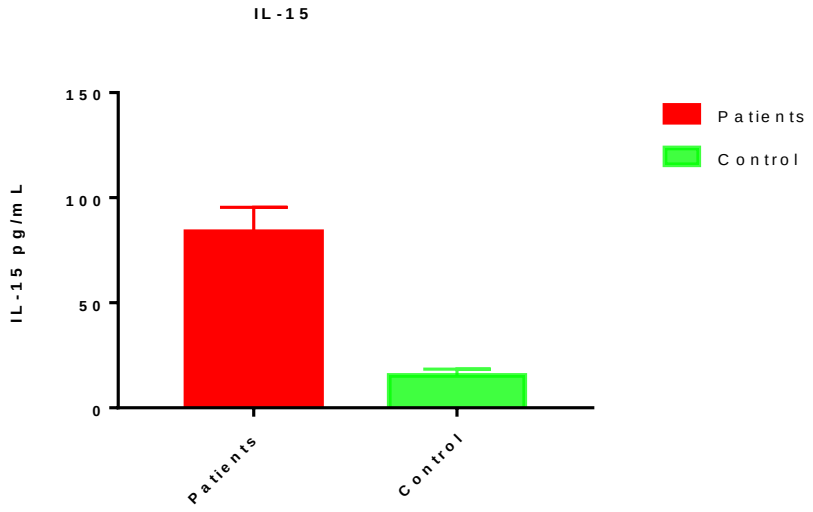

Figure (3): Mean levels of IL-15 (pg/mL) between patient and control groups of the population study

IL- 6

The results of this study demonstrated a significant increase ($P < 0.05$) in IL-6 levels in patients with osteoporosis compared to the control group. The means of IL-6 in the patients and control groups were, respectively, (60.34 pg/mL) and (9.05 pg/mL), as indicated in Figure (4).

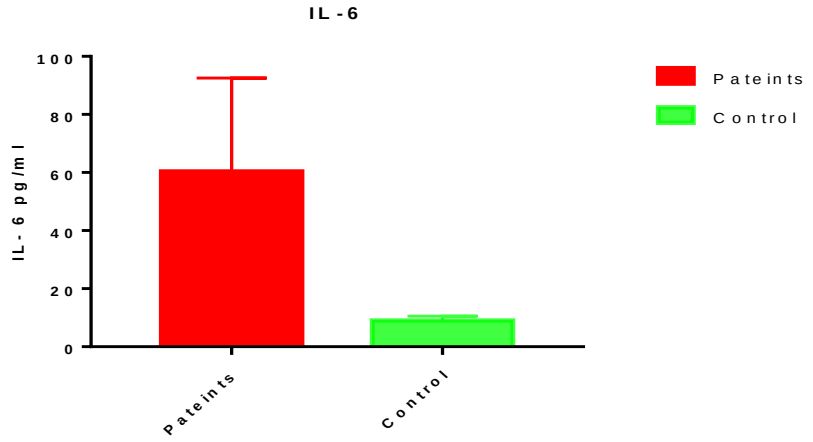

Figure (4): Mean levels of IL-6 (pg/mL) between patient and control groups of the population study

Biochemical alterations

Levels of Vit.D3

The current study demonstrated a significant reduction (P<0.05) in vitamin D3 levels in osteoporosis patients when compared to the control group. The level of vitamin D3 in healthy controls was higher than in patients by a mean of 19.9 ng/mL and 4.667 ng/mL, respectively, as shown in Figure (5).

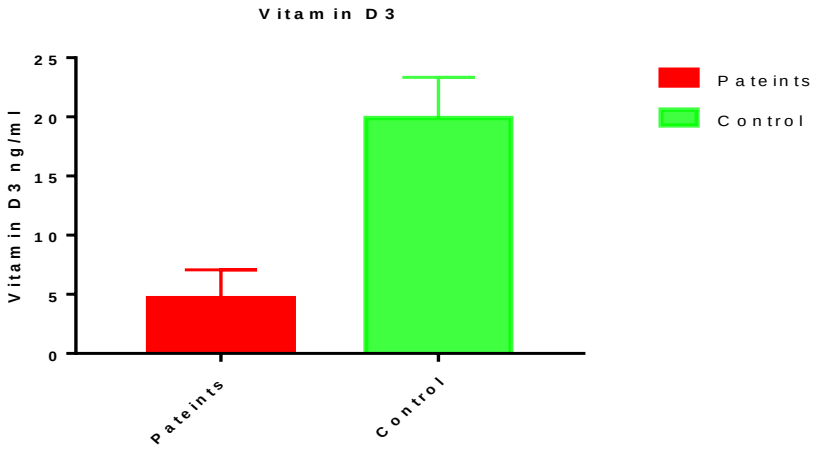

Figure (5): Mean levels of Vitamin D3 (ng/mL) between patient and control groups of the population study

Hematological parameters

Concentration of Hemoglobin (Hb)

The results of this study indicate that there was a significant difference (P < 0.05) in Hb concentration between the patient and control groups. The mean Hb levels for the two groups were 9.16 g/dL and 14.96 g/dL, respectively, as shown in Figure (6).

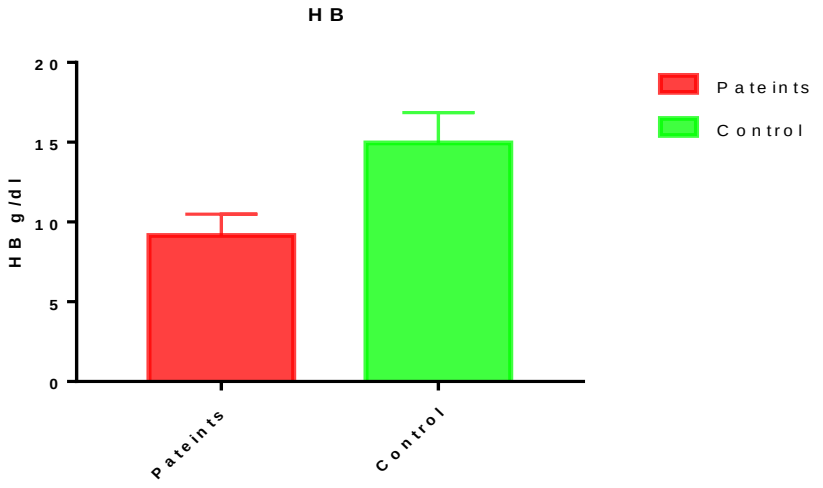

Figure (6): Concentration of Hb (g/dL) between the patient and control groups of the population study

Total number of WBC

In the current study, a significant difference ($P < 0.05$) was observed in the number of WBCs between the patient and control groups. The means of WBCs for the two groups respectively were 14.19* 10^3 c/ mm^3 and 7.01* 10^3 c/ mm^3, as shown in Figure (7).

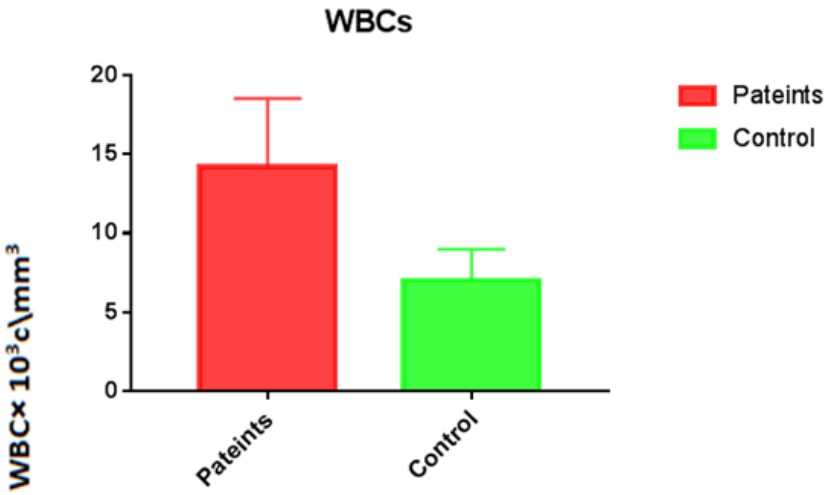

Figure (7): Mean number of WBC between the patient and control groups of the population study

DISSCUSION

Cytokines are low-molecular-weight proteins that have intrinsic or adjuvant effects on many tissues, ranging from the immune system to the nervous system, by interacting with their various receptors. (6, 10) Many studies showed that the expression of these cytokines increased with disease. IL-10 increases the expression of inflammatory mediators by producing anti-inflammatory cytokines, soluble TNF-α and IL-1 receptors (IL-1Ra), and inhibitory cytokines, such as TGF-β receptor 1 competitors (11). Among the most important roles of IL-1 in bone inflammation is its increased stimulation of collagenase and proinflammatory-stimulating PGE in the case of bone inflammation; it also stimulates the secretion of IL-2, rGM-CSF, and IL-6 cytokinin-expressing microalbuminuria cells from chondrocytes III and II, plus it reduces collagen production(12).IL-1, through the activation of transcription factors NF-κB and AP-1, another extremely pro-inflammatory cytokine, promotes osteoclast differentiation that is dependent on RANKL(13). Promotes osteoclast differentiation that is dependent on RANKL (13). The activation of osteoclasts produced by C5a (complement protein) requires IL-1. Additionally, it has been observed that IL-1β increases the activity of proteolytic enzymes, such as collagenases, plasminogen, and cathepsin B, which degrade bone matrix proteins and cause bone loss (14).

The synovial fluid contains proinflammatory cytokines (IL-6, IL-1β, TNF-alpha, and IL-17A), which are essential in cartilage degradation. Numerous cell types, including neutrophils, mast cells, Th17 cells, gamma-delta T cells, NKT cells, and ILC3, secrete IL-17A, which stimulates chondrocytes and synovial fibroblasts to produce and release additional proinflammatory cytokines (12). The majority of research on IL-17's function in the articular environment has been done on patients with osteoarthritis or rheumatoid arthritis. In all of these scenarios, the proportion of cells secreting IL-17A in synovial tissue and the frequency of chondrocytes expressing the IL-17 receptor appear to be rather similar (15). Furthermore, IL-17A promotes NO production, inhibits the synthesis of proteoglycans, and acts in tandem with TNF-alpha to break down the cartilage matrix. Other studies have shown that IL-17F induces cartilage degradation by upregulating the expression of ECM components (type II collagen and aggrecan), their inhibitors (TIMP-2 and TIMP-4), and stromelysin-1 (MMP-3) and collagenases (MMP-1 and MMP-13) (16).

IL-6 is a significant contributor to the development of osteoporosis. An increase in IL-6 levels in the body leads to an increase in osteoclastogenesis by inducing osteoblasts to produce more RANKL (17). The leading cause of systemic bone loss is IL-6, which promotes the transmigration of osteoclast precursors from the bone marrow into the blood by upregulating the S1PR2 Sphingosine-1-phosphate (S1P) receptor on its surface. Moreover, two inflammatory chemokines, CXCL8 and CCL20, enhance osteoblast-induced osteoclastic activity through the production of IL-6 (18) (19).

In individuals with osteoporosis and other chronic inflammatory diseases associated with bone loss, the IL-15 receptor alpha (IL-15RA) is more prevalent in the synovial fluid.NK cells have been shown to turn over and activate osteoclasts through the use of IL-15. The absence of IL-15 signaling prevents the loss of trabecular bone by inhibiting osteoclast activity (20).

Mineralization occurs naturally as long as the food contains enough calcium and vitamin D. Reduced 1,25 (OH)2D levels result from insufficient vitamin D consumption. Because calcium is less available for this usage, bone mineralization decreases as a result. In the kidney, parathyroid hormone will quicken the conversion of 25(OH) D to 1,25(OH)2D (PTH). PTH elevation in the blood causes bone turnover, and it's probable that although bone resorption increases, the new steady state will permit serum 1,25 (OH)2D levels to revert to normal. Deficits in vitamin D for extended periods can lead to osteoporosis (21). Osteoid tissue in high-turnover bone is greater than in normal bone because of the increased surface remodeling (not yet calcified bone). Bone with a higher concentration of minerals, such as bone that has been mineralized, tends to be less dense. Because osteons have a shorter lifespan than other bone tissue, mineral deposits can persist for up to two years after formation. When vitamin D deficiency is long-term ,and chronic, the amount of osteoid tissue builds up to more than 5% resulting in osteomalacia. Lips *et a*l. (22) identified significant bone turnover in 20% of individuals with hip fractures but no overt osteomalacia in 119 bone biopsy samples.

In adults, low hemoglobin levels were linked to a higher risk of osteoporosis. The model that was adjusted for several possible confounders, such as obesity, smoking, alcohol use, laboratory results, and comorbidities, confirmed the link between low Hb levels and osteoporosis. Low Hb levels were also consistently linked to osteoporosis in patients who also had comorbid diseases such as obesity, smoking, hypertension, diabetes, and dyslipidemia. Previous research suggested a link between osteoporosis and anemia, and an additional cross-sectional investigation found that in individuals with osteoporosis, low BMD was associated with low Hb levels (23). Hematopoietic cells, including osteoclasts, proliferate more readily when blood volume is lowered. Increased osteoclast numbers could encourage bone resorption. Blood loss can potentially increase osteoblast development, but it can also stimulate bone resorption, which can disrupt cycles of bone remodeling and lead to osteoblast fatigue (24).

Osteoporosis and anemia may be associated; however, inflammation may also act as a mediating factor. Hematopoiesis is influenced by proinflammatory cytokines, and osteoclast activation and differentiation have been reported to be promoted by IL-6 (25). Erythropoietin (EPO) may play a role in the physiology of skeletal remodeling, which would be another reason for the results. EPO may have both anabolic and catabolic effects on bone. EPO promotes osteoclastogenesis, which quickens the loss of bone mass. Anemia is more likely in individuals who do not respond well to EPO, which can be caused by a decrease in EPO concentration, an EPO functional defect, or EPO resistance (26, 27).

Chronic diseases are caused by inflammation, which is linked to abnormal WBC counts. As of now, associations exist between aberrant WBC counts and long-term illnesses. Therefore, there is unquestionably a connection between WBC numbers and chronic illnesses, which is mediated by inflammation. Still unclear, though, is how WBC levels have changed (28). The current study agrees with the research (29) conducted by which demonstrated that compared to control patients, those with inflammation had greater rates of anemia and WBCs.

CONCLUSION

According to the study's results, we conclude that when compared to normal individuals, patients with osteoporosis showed a considerable increase in IL-1, IL-17, IL-15, and IL-6, as well as a notable decrease in Vit-D3 and Hb in osteoporosis sufferers compared to healthy individuals. Additionally, compared to normal individuals, patients with osteoporosis had significantly higher WBC.

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دراسة بعض المؤشرات المناعية والفسيولوجية لمرضى هشاشة العظام في محافظة الانبار

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الخلاصة

خلفية البحث: هشاشة العظام هي حالة صحية تقدمية في العظام تسبب هشاشة واسعة النطاق وزيادة خطر الإصابة بالكسور. **الهدف من هذه الدراسة:** هو دراسة المتغيرات المناعية التي تشمل (IL-17، IL-1، IL-15، IL-6، والمعلمات الدموية (Hb و WBC) وبعض المعلمات الكيميائية فيتامين د3 في المرضى الذين يعانون من هشاشة العظام ومقارنة هذه المستويات مع مستويات مجموعة السيطرة. **المواد وطرق العمل:** أجريت هذه الدراسة على الاشخاص المرضى الذين حضروا إلى مستشفى الرمادي التعليمي في مدينة الرمادي في الفترة من 1 تشرين الثاني (نوفمبر) 2023 إلى 1 كانون الثاني (يناير) 2024. وقد بلغ العدد الإجمالي لعينات الدم 80 عينة شملت 50 عينة لمرضى هشاشة العظام الذين تتراوح أعمارهم بين 16 إلى 50 سنة و 30 عينة للأفراد الأصحاء كمجموعة سيطرة. **النتائج:** أظهرت نتائج الدراسة الحالية وجود اختلاف كبير في جميع المعلمات و بمستوى معنوية($p < 0.05$) حيث كانت مستويات المتوسطات للمعلمات IL-1 وIL-17 وIL-15 وIL-6 وVit D3 على التوالي: (8.467 بيكوغرام/مل)، (89.32 بيكوغرام/مل)، (83.84 بيكوغرام/مل)، (60.34 بيكوغرام/مل)، (4.667 بيكوغرام/مل)، بالإضافة إلى تركيز الهيموغلوبين ومتوسط عدد خلايا الدم البيضاء كانت: WBC(14.19X 103 c/mm3 \Hb(9.16g/dL)) على التوالي، ويمكن **الاستنتاج**: أن المرضى الذين يعانون من هشاشة العظام لديهم زيادة معنوية في مستويات الإنترلوكين-1، والإنترلوكين-17، والإنترلوكين15، والإنترلوكين-6 ونسبة خلايا الدم البيضاء مقارنة مع السيطرة. ومع ذلك، كان هناك انخفاض ملحوظ في فيتامين د3 والهيموغلوبين في المرضى مقارنة بمجموعة السيطرة.

الكلمات المفتاحية: Osteoporosis, Cytokines ,VIT D3.