

**Republic of Iraq**

**Ministry of Higher Education & Scientific Research**

**University of Babylon**

**College of Science for women**

**Department of Biology**



**Study of some Hematological Parameters and Inflammatory biomarkers in Iraqi Osteoporosis patients**

A research

submitted to the Council of the College of Science for women\  
Department of Biology as part of the requirements for obtaining  
a bachelor's degree

By

**Furqan Aamer AbdalHassan**

**Fatima Makky Noor**

**Supervised by**

**Assis . Prof. Dr. Shaymaa Obiad Abdullah**

**College of Science for women**

**University of Babylon**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ نَرْفَعُ دَرَجَاتٍ مِّنْ نَّشَأٍ وَفَوْقَ كُلِّ ذِي عِلْمٍ

عَلِيمٌ ﴾

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

سورة يوسف- آية ( ٧٦ )



الإهداء :

الى سيدي ومعلمي وتاجي الذي توجني هلالا به ...أبي العزیز

الى من سعت لإسعادي إلى أعظم وألطف مخلوق بحياتي ... أمي العزیزة

الى من قدم لي العون والتشجيع والمساعدة في اتمام هذا البحث ..

الدكتورة شيما عبيد

الى اخوتي واسرتي وكل من لم يخنر جهدا في مساعدتي

الى جميع اساتذتي الكرام ممن لم يتوانوا في مد يد العون لي

اهدي اليكم بحثي





## شكر وتقدير

الى قسم علوم الحياة (رئاسة واساتذة )

الى الدكتورة شيما عميد ( مشرفة البحث )

الى كادر مستشفى مرجان في محافظة بابل ومدينة الطب في

بغداد السلام (اطباء وممرضين)

الى كل من ساعدني ولو بكلمة طيبة ..... لكم مني كل

الشكر والاحترام



## Summary

Osteoporosis is a skeletal disease that affects bone strength (bone quality and density). Osteoporosis is predisposing and risk factor of fractures which occur due to minor injuries, The osteoporosis showed the low density of the bone and loss of the maintenance of bone homeostasis. In this study, patients with osteoporosis were taken . The study included twenty patients whose ages ranged from (20-80) years old ( 5 male and 15 female ) , those visiting the rheumatoid Consultation Clinic in each of Babil governorate (Marjan city) - Iraq for diagnosis and / or treatment, also to supervise the disease , and ten healthy volunteers (5 male and 5 female ) as control groups..

The present study is divided into two main parts: the hematological parameters (RBCs count , WBCs count (total and differential) , platelets count , Hb level , and PCV) and inflammatory biomarkers C-reactive protein (CRP) and ESR . The results of the study showed an increase in the level of inflammatory biomarkers (CRP and ESR)

The results also showed a decrease in blood parameters, with the exception of the percentage of lymphocytes, which was high compared to its percentage in healthy people.



***Chapter***  
***One***  
***Introduction***



## **Definition of Osteoporosis:**

Osteoporosis is a metabolic illness of the bones in which the bones become so weak and unable to support the body due to the loss of the bone mass and the minerals over time. It begins to set in throughout the latter years, but it begins to develop much sooner and without warning. More than 25 million individuals worldwide have been impacted by this condition, which affects mostly women. It is the most prevalent disease of ageing. Every year in the United States, people are admitted to hospitals because of osteoporosis (Shakoor *et al.*, 2014).

## **Criteria for Diagnosis of Osteoporosis:**

An osteoporotic fracture and/or low bone mineral density (BMD) in postmenopausal females are required to diagnose osteoporosis in this population. Dual X-ray absorptiometry is the "gold standard" technique of measuring bone mineral density (DXA). Specifically, it is expressed as a number of standard deviations away from the population's mean in young adults (T-score) or away from the mean of a population of similar age (Z-score). Database of the National reference in Caucasian females aged 20–29 years is the reference range recommended by IOF, ISCD, WHO, and NOF for calculating the T-score in postmenopausal women (Shin *et al.*, 2015).

Osteoporosis is diagnosed in postmenopausal females and men over the age of 50 if the T-score of the lumbar spine, whole hip, or femoral neck is less than -2.5 on the International Skeletal Diagnostics (ISCD) scale. In certain cases, 33 % of the radius (also known as the 1/3radius) may be used to achieve the desired result (Shakoor *et al.*, 2014). The Z-score describes the number of standard deviations (SDs) by which a person's BMD differs from the average expected for his or

her age and gender. It is most often used in children, females, and adolescents who are premenopausal. The presence of a Z-score less than -2 is abnormal and should be "low for age." A low Z-score in a postmenopausal woman implies that she should be evaluated for secondary osteoporosis, which is more common after menopause (Varacallo and Fox, 2014).

<b>Table 1 T scores and WHO Diagnostic Criteria for Osteoporosis (Cosman <i>et al.</i>,2015).</b>	
<b>Interpretation</b>	<b>T-Score</b>
<b>Normal</b>	<b>-1.0 and higher</b>
<b>Osteopenia</b>	<b>-1.0 to -2.5</b>
<b>Osteoporosis</b>	<b>-2.5 and lower</b>
<b>Severe osteoporosis</b>	<b>-2.5 and lower with one or more fragility fractures</b>
<b>Reference values vary by geographical location. WHO = World Health Organization.</b>	

### **Bone Structure:**

The bones are the differentiated organ that originates from the mesenchyme tissues and are the most specialized in the body. It has a vibrant and lively feel about it. Function, the bone is primarily a structural organ that can withstand a great deal of pressure .

Three cell types in bone are found: osteocytes, osteoblasts, osteoclasts, and lining cells (Zang *et al.*,2017).

### **Osteocytes:**

Osteocytes are osteoblast-derived cells incorporated into the bone matrix. Between 5% and 20% of osteoblasts are converted into osteocytes (Carvalho, *et al.*,2018). The osteoblasts entrapped in the newly formed bone matrix developed into osteocytes. Osteocytes account for



approximately 95% of the bone cells; they are not divided and long-lived (Clarke 2008).

### **Osteoblast:**

It is the cells responsible for bone formation and located along the outer surface of the bone . The osteoblasts are cubic cells located along the surface of the bone, 4% of the total resident bone cells known for their role in bone formation . These cells demonstrate the morphological properties of protein-synthesizing, including prominent Golgi apparatus abundant, rough endoplasmic reticulum and various secretory vesicles (Lee et al., 2020).

### **Osteoclasts**

Osteoclasts are cells that line the inside of the bone that works as bone resorption. Osteoclasts dissolve bone mineral content (BMC) by producing an acidic environment, compromising the existing bone strength. Osteoclasts secrete enzymes that break down the residual collagen bone matrix, bringing the resorption process to a conclusion (Terndrup *et al.*,2016).

### **Risk Factor for Osteoporosis**

There are two types of OP risk factors:

#### **A- Non-modifiable risk factors:**

##### **1- Age:**

Premature age is the single most important risk factor for OP. Growing older causes bone mineral density (BMD) to decline in both sexes due to the influence of hormonal alteration on the bone remodelling process. The hormones estrogen and testosterone have an effect on the intestine absorption of Ca from the blood flow. When the gut absorption of calcium is reduced, calcium storage in

compact and cancellous bone is recovered in order to maintain serum calcium levels at a constant level. A loss in bone mass happens as a result of calcium absorption from the bones exceeding the quantity of calcium that can be replenished (Miller et al., 2020).

## **2- Gender:**

Women experience bone loss at a younger age and faster than men. Women aged 50 or more have four times the OP rate and two times the osteopenia rate compared to men (Miller et al., 2020).

## **3- Family History of Fragility Fracture:**

Fragility fracture causes extreme complications and even death for all menopausal women. About 61% of fragility fractures occur in women, with the female-male ratio reaching 1.6 (Akkawi and Zmerly , 2018).

## **4- Race:**

Conventionally, the Caucasian race has a higher risk factor for the occurrence of osteoporosis, while Asians showed the normal risk of osteoporosis (Miller et al., 2020).

## **B- Modifiable Risk Factors**

### **1-Medication:**

The use of prednisolone in a dose  $\geq 5.0$  mg/day for more than 3 months and other medications such as glucocorticoids, anticoagulants, anticonvulsants, aromatase inhibitors, cancer chemotherapeutic drugs, and gonadotropin-releasing hormone agonists are risk factors of OP (Al-Bashaireh and Alqudah , 2020).

### **2- Short Time of Sun Exposure:**

The short time of sun exposure has been supposed to afford low BMD, since enough sun exposure to ultraviolet light is essential for vitamin D synthesis, which is essential for calcium homeostasis in the human body (Al-Bashaireh and Alqudah , 2020).

### **3- Smoking:**

Smoking is harmful to the bone, reduce body weight, calcium absorption, reproductive hormones, and bone mineral density, induces early menopause, and elevates bone-turnover markers and fracture risk , Smokers tend to have spinal deformities more than non-smokers (Anastasilakis et al ., 2020).

### **4- Consumption of Alcohol:**

Consumption of alcohol increases the risk of OP by inhibiting the function of the osteoblast and reducing bone formation, therefore resulting in more hip fractures (Al-Bashaireh and Alqudah , 2020).

### **5- Nutrition:**

The abundant nutrients used in our daily diet can affect bone health. Bones can be affected by different mechanisms, including Adjusting bone structure, the Process of endocrine and Metabolism of bone (Miller et al., 2020).

## **Aim of study**

The aim of current study was to illustrate certain biological aspects of Iraqi Osteoporosis patients , and this achieved by using following objectives :

1- Study hematological parameters which included :

PCV , Hb level and CBC .

2- Study some inflammatory markers that included :

ESR and CRP



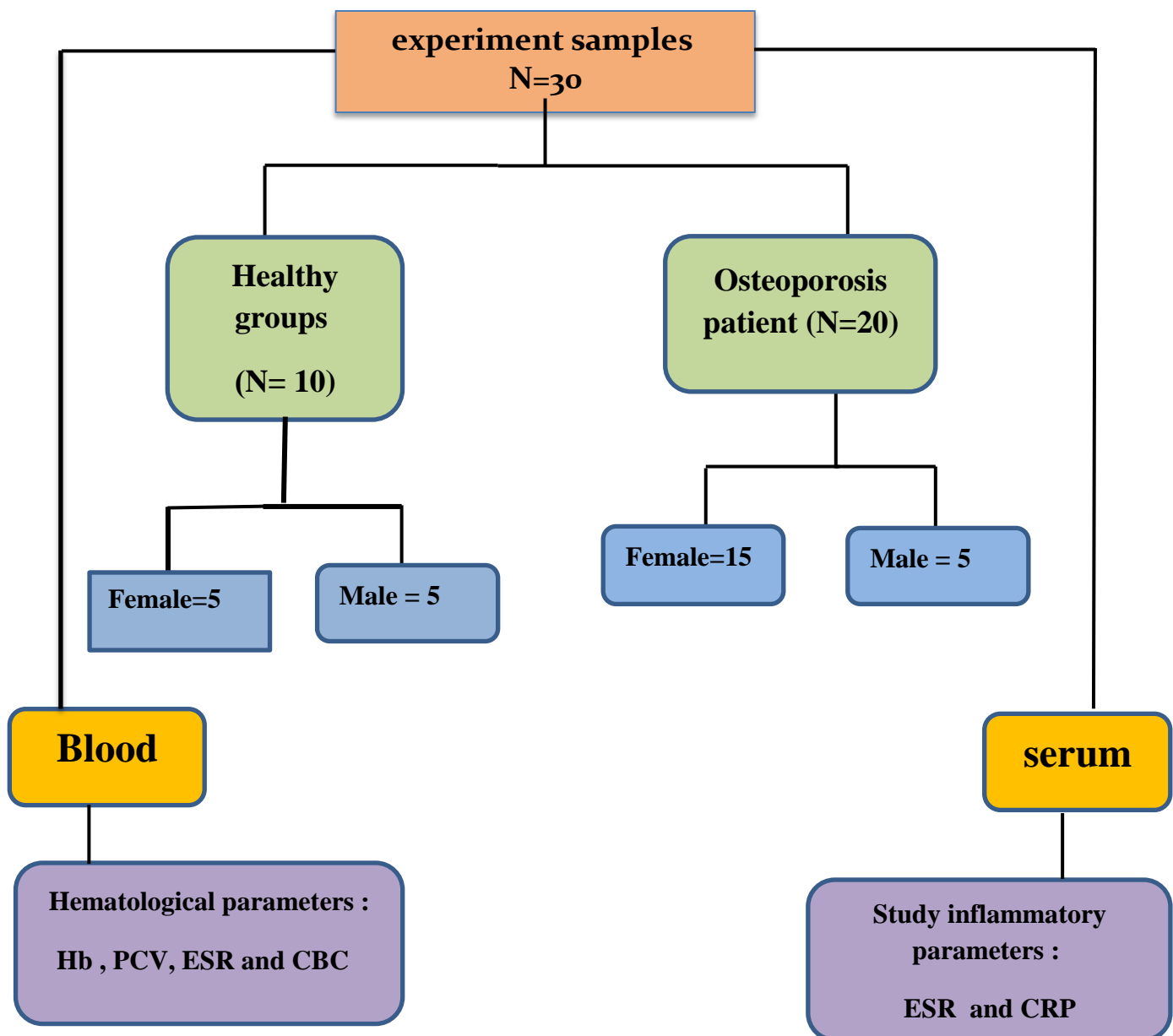
# *Chapter Two*

## *Materials and Methods*



## Materials and Methods :

**Experimental Design** :as in figure (1)



**Figure (1) : Experimental design of study**

### **Study subjects (Patients and Healthy) :**

The study subjects comprised of 20 osteoporosis patients (5 male and 15 female), age (mean  $\pm$  SD) = 61.88  $\pm$  0.86 (ranging from 20-80 years). These patients were suffered from rheumatoid arthritis and were referred to the rheumatoid Consultation Clinic. Those patients have been diagnosed by a specialized physician. The healthy group included

10 individual (5 male, 5 female) age (range 18 to 60) not suffer from any disease, served as a control group and this groups matched with patient group. All subjects in this study were taken consent before participation in this study.

### **Collection of blood samples :**

Venous blood samples were drawn from patients and control subjects by using disposable syringes . Five ml of blood was obtained from each subject , 3 ml was placed into EDTA tubes and the remaining (2 ml) pushed slowly into disposable gel containing tubes. Blood in the EDTA tubes was used to determine CBC and the ESR while blood in the gel containing tubes was allowed to clot at room temperature for 15 minutes and then centrifuged at 3000 rpm for approximately 10-15. minutes , after that sera was obtained and stored at -20°C until used.

### **Erythrocyte sedimentation rate (ESR):**

The Westergreen method as in figure (3) was used as described by (Cheebrough, 2004). When anticoagulated blood is left to stand undisturbed for 1 hour the red cells sediment gradually to the bottom of the tube leaving clear plasma on top and the distance occupied by the supernatant plasma is determined.

### **Procedure :**

- 1- Place 0.5 ml of Trisodium Citrate in test tube.
- 2- Add 2 ml of blood sample to the test tube.
- 3- Mix the contents of test tube gently.
- 4- Fill the westergreen tube to 0 mark.

- 5- Set up the westergreen tube right in a stand with a spring clip on top and rubber at bottom.
- 6- Read the highest of the clear plasma above the upper limit of the column of sediment red cells in mm\hr .



**Figure (2)**

### **Hematological Assessments :**

The counting of the cellular blood components, the Analyzer Ruby, uses the impedance technique only. A cyanide free spectrophotometry method was used to measure hemoglobin by formation of oxyhemoglobin at 555 nm. Packed cell volume (PCV) was measured by volume integration. The sample volume was 10  $\mu$ l. The instrument can determine the parameters in the research mode: white blood cells (WBC), the number of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit value (HCT), platelet count (PLT). For platelet counting a floating threshold was used, whereas for RBC and WBC counts the thresholds are predefined.

**Results** :are provided within 1 minute on the display, printed out on the printer and stored in the resident memory or on a USB key. Results were presented with flags; optionally reference ranges can be reported. the instrument uses three reagents: a diluent, a lysis reagent and a cleaning solution.

**Procedure :**

1. 10  $\mu\text{l}$  of the EDTA blood sample was placed in the aspirator on the instrument.
2. The start key on the instrument was pressed and the blood sample was aspirated.
3. Results were provided within 1 minute on the LCD display, printed out on the printer and stored in the resident memory.





# ***Chapter Three***

## ***Results***



**Table 1: General characteristics of the patients and healthy groups.**

Characteristics	Patients No. (%)	Control No. (%)
<b>Gender</b>		
Male	5(25%)	5(50%)
Female	15(75%)	5 (50%)
<b>Age (years)</b>		
20-40	4 (20%)	3 (30%)
41-60	13(65%)	5 (50%)
61-80	3 (15%)	2(20%)
<b>Smoking</b>		
Yes	4(20%)	
no	16(80%)	
<b>Diabetes</b>		
Yes	5 (25%)	
no	15 (75%)	
<b>Hypertension</b>		
Yes	7(35%)	
No	13(65%)	

**Table 2 :Inflammatory markers values in male arthritics as compared with healthy control males**

	patients (N=5)	control (N=5)
Parameters	Mean ± S.D	Mean ± S.D
CRP (mg\L)	65± 12.5	30±4.5
ESR \ hour	73.2 ±17.5	17.4 ±23 .6

**Table 3 : Inflammatory markers values in female patients as compared with healthy control females**

	patients (N=15)	control (N=5)
Parameters	Mean ± S.D	Mean ± S.D
CRP (mg\L)	58± 10.9	22±9.3
ESR \ hour	77.3 ±16.3	20.2 ±11.7

**Table 4 : Hematological values in male patients as compared with healthy control males**

	patients (N=5)	control(N=5)
Parameters	Mean ± S.D	Mean ± S.D
Haemoglobin	9.8±1.2	13.2 ± 0.9
PCV%	31.6±3.7	38.8±2.5
WBC x 10 <sup>9</sup> \ L	3.5 ±1.9	5.8 ±2.1
Platelet x 10 <sup>9</sup> /L	172.5 ±20.4	287 ±30.3
Neutrophil %	53.0 ±3.9	66 ±9.3
Lymphocytes %	41 ±7.6	35.5 ±10.5
Eosinophil %	1±1.6	1.1 ±1.2
Monocytes %	1.1 ±0.9	0.4 ±0.7
basophil	-	-

**Table 5 : Hematological values in female patients as compared with healthy control females**

	<b>patients (N=15)</b>	<b>control (N=5)</b>
<b>Parameters</b>	<b>Mean ± S.D</b>	<b>Mean ± S.D</b>
<b>Haemoglobin</b>	<b>9.6 + 1.3</b>	<b>12.2 ±0.6</b>
<b>PCV%</b>	<b>29.0 ±3.5</b>	<b>36.9 ±2.0</b>
<b>WBC x 10<sup>9</sup>/L</b>	<b>3.9 ±1.3</b>	<b>5.4 ±1.1</b>
<b>Platelet x 10<sup>9</sup>/L</b>	<b>179 ±31.9</b>	<b>267 ±46.0</b>
<b>Neutrophil %</b>	<b>55 ±8.0</b>	<b>65.5 ±6.1</b>
<b>Lymphocytes %</b>	<b>41 ±7.6</b>	<b>32.6 ±6.5</b>
<b>Eosinophil %</b>	<b>1±1.6</b>	<b>1.1 ±1.2</b>
<b>Monocytes %</b>	<b>1 ±1.0</b>	<b>0.7 ±0.9</b>
<b>basophil</b>	<b>-</b>	<b>-</b>

## **Discussion**

The results of the current study with regard to inflammatory indicators showed a clear increase in these indicators, and this indicates the presence of inflammation, which may extend to all bones. There could equally be premature destruction of red cells which may have caused secondary iron deficiency as was signaled by some microcytic cells observed. This was equally the observation made by Danesi and Taccam (2004). The highly elevated ESR observed could be attributed to response to inflammation caused by the disease. There may be elevation of gamma globulin and fibrinogen as seen in other diseases of chronic disorder. Similar observations were made in male arthritics where In the case of inflammatory diseases, CRP level represents the disease activity. Studies have suggested direct correlations of CRP with osteoporosis and inflammatory disease.

An erythrocyte sedimentation rate (ESR) test evaluates how much inflammation is present in your body. The test measures how quickly your red blood cells, called erythrocytes, separate from your other blood cells in a lab when they are treated with a substance that prevents clotting. Red blood cells clump together when there's inflammation in your body, making them separate from your other blood cells much faster. Low ESR levels indicate low levels of inflammation while high ESR results indicate high levels of inflammation. Doctors use this test to diagnose rheumatoid arthritis because this condition causes inflammation throughout your body. An ESR test on its own, however, is not enough to diagnose bone disease (Pincus , 2004) .

The results recorded changes in all hematological parameters, especially white blood cells. Similar unusual alterations in haematologic variables were documented by Bowman (2002), who worked on haematologic manifestations in osteoporosis . The values equally agreed with results obtained by Peters.(2020).Blood films analyses revealed normocytic cells with few microcytic cells. The causes for these alternations could be multifactorial due to disease activity, drug induced, bone marrow suppression or ineffective erythropoiesis as documented by Harrison(2001) who studied the clinical features of osteoporosis . There could equally be premature destruction of red cells which may have caused secondary iron deficiency as was signaled by some microcytic cells observed. This was equally the observation made by Danesi and Taccam (2004).The highly elevated ESR observed could be attributed to response to inflammation caused by the disease .

## References ...

- Akkawi I, Zmerly H.** Osteoporosis: Current concepts. *Joints*. 2018;6:122–127.
- Al-Bashaireh AM, Alqudah O.** Comparison of bone turnover markers between young adult male smokers and nonsmokers. *Cureus*. 2020;12(1):e6782.
- Anastasilakis AD, Polyzos SA, Yavropoulou MP, Makras P.** Combination and sequential treatment in women with postmenopausal osteoporosis. *Exp Opin Pharmacother*. 2020;21:477–490.
- Carvalho, M.S.; Poundarik, A.A.; Cabral, J.M.S.; da Silva, C.L.; Vashishth, D.** Biomimetic matrices for rapidly forming mineralized bone tissue based on stem cell-mediated osteogenesis. *Sci. Rep.* **2018**, *8*, 14388.
- Chan R, Chan D, Woo J. A .** cross sectional study to examine the association between dietary patterns and cognitive impairment in older Chinese people in Hong Kong. *J Nutr Health Aging*. 2013;17:757–765.
- Clarke, B.** Normal bone anatomy and physiology. *Clin. J. Am. Soc. Nephrol.* **2008**, *3* (Suppl. 3), S131–S139.
- Denesi ,T.H.,and , D.S** (2004).Haematology toxicity of Immunosuppressive Treatment. *Transplant* 36:703- 4.
- Haentjens P, Magaziner J, Colón-Emeric CS, et al.** Meta-analysis: excess mortality after hip fracture among older women and men. *Ann Intern Med*. 2010;152:380–390.
- Lee, P.; Chandel, N.S.; Simon, M.C.** Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 268–283.
- Miller PD, Pannacciulli N, Malouf-Sierra J, Singer A, Czerwiński E, Bone HG, et al.** Efficacy and safety of denosumab vs. bisphosphonates in postmenopausal women previously treated with oral bisphosphonates. *Osteoporos Int*. 2020;31:181–191.
- Peters HR.,** (2020). Effect of recombinant human erythropoietin on anaemia and disease activity in patients with rheumatoid arthritis and anaemia of chronic disease. *Annual Rheumatology* 55:739-45

**Pincus T.**, (2004). Clinical Trials in Rheumatic disease Designs and limitations. *Rheumatology* 30:7 \ 0-24.

**Shakoor S , Ilyas F , Abbas N , Aslam M and Arif1 S.** PREVALENCE OF OSTEOPOROSIS IN RELATION TO SERUM CALCIUM AND PHOSPHORUS IN AGING WOMEN . *J. Glob. Innov. Agric. Soc. Sci.*, 2014, 2(2): 70-75.

**Shin S, Sung J, Joung H.** A fruit, milk and whole grain dietary pattern is positively associated with bone mineral density in Korean healthy adults. *Eur J Clin Nutr.* 2015;69:442–448. 44.

**Varacallo MA, Fox EJ.** Osteoporosis and its complications. *Med Clin North Am.* 2014 Jul;98(4):817-31,

## الخلاصة....

هشاشة العظام هي مرض يصيب الهيكل العظمي ويؤثر على قوة العظام (جودة العظام وكثافتها).

هشاشة العظام هي عامل مهيب وخطر للكسور التي تحدث بسبب الإصابات الطفيفة ، وأظهر هشاشة العظام

انخفاض كثافة العظام وفقدان التوازن في الحفاظ على توازن العظام. في هذه الدراسة ، تم أخذ مرضى هشاشة

العظام. اشتملت الدراسة على عشرين مريضا تراوحت اعمارهم بين (٢٠-٨٠) سنة (٥ ذكور و ١٥ اناث)

الذين يزورون عيادة الروماتيزم الإستشارية في محافظة بابل (مدينة مرجان) - العراق للتشخيص و / او

العلاج ايضا لغرض الإشراف على المرض ، وعشرة متطوعين أصحاء (٥ ذكور و ٥ إناث) كمجموعات

ضابطة..

تنقسم الدراسة الحالية إلى جزأين رئيسيين: المعلمات الدموية (عدد كرات الدم الحمراء ، عدد كرات الدم

البيضاء (الإجمالي والتفاضلي) ، عدد الصفائح الدموية ، مستوى الهيموغلوبين ، و (PCV) والعلامات

الحيوية الالتهابية للبروتين التفاعلي سي (CRP) و ESR. أظهرت نتائج الدراسة زيادة في مستوى

المؤشرات الحيوية الالتهابية (CRP) و (ESR)

كما أظهرت النتائج انخفاضاً في معايير الدم ، باستثناء النسبة المئوية للخلايا الليمفاوية ، والتي كانت

عالية مقارنة بنسبتها لدى الأشخاص الأصحاء.