

ADIPONECTIN LEVEL AND ITS RELATION TO OSTEOPOROSIS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

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Background: Adiponectin possesses anti-inflammatory properties and also has pro-inflammatory aspects in some diseases. Adiponectin plays an important role in bone metabolism and osteoporosis (OP) pathophysiology, modulating bone formation and remodeling by selectively enhancing stem cell development between adipocyte and bone precursors in the bone marrow in response to reduced energy storage and metabolic needs.

Aim of work: The aim of our study was to assess serum adiponectin level in rheumatoid arthritis (RA) patients and to study the possible role of adiponectin as a marker of osteoporosis in rheumatoid arthritis patients.

Patient and Methods: The study included 30 premenopausal rheumatoid arthritis female patients. The age ranged from 26 to 46 years old and body mass index (BMI) ranged from 23 to 37.34 compared to 15 healthy controls. They were subjected to full history taking. Laboratory investigation was done to exclude other causes of osteoporosis. Serum level of adiponectin was measured by ELISA. They were also assessed radiologically by DEXA scan of lumbar spine to confirm osteoporosis.

Results: In our study, we found that RA patients had statistically significant higher adiponectin level than healthy controls. Moreover, serum adiponectin was significantly higher in patients with both RA and OP compared to RA female patients only. Moreover, our results demonstrated that a serum adiponectin of 1400 µg/L or higher is capable of detecting osteoporosis among RA patients with a sensitivity of 100% and a specificity of 100%. Regarding predictive ability of adiponectin in detecting RA with osteoporosis, our findings showed that there was an inverse association between adiponectin level and bone density, and there was a significant negative correlation between adiponectin level and DEXA scores in RA group.

Conclusion: There was a clinical usefulness of measuring serum adiponectin in predicting OP among RA premenopausal female patients. This strongly suggests the possible utility of serum adiponectin as a marker to detect OP in patients with RA hand in hand with conventional parameters.

Keywords: Adiponectin, Osteoporosis, Rheumatoid Arthritis.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized

by synovial inflammation often followed by cartilage and bone erosion. The patients suffer from reduced functional ability, pain,

and stiffness that often lead to impaired quality of life. The prevalence of RA is ~0.5–1% of the western population and the disease is more common among women and elderly ⁽¹⁾.

Osteoporosis (OP) is one of the most common comorbidities associated with RA, a risk factor for osteoporosis, is accompanied by both periarticular and systemic osteoporosis. The mechanisms of osteoporosis associated with RA involve the influence of inflammatory cytokines, such as interleukin IL-1, IL-6, and tumor necrosis factor (TNF); glucocorticoid treatment; and immobility ⁽²⁾.

Adiponectin possesses anti-inflammatory properties and also has pro-inflammatory aspects in some diseases. Adiponectin functions as an insulin sensitizer and exhibits anti-diabetic, anti-inflammatory, and anti-atherogenic effects. Its multifunctional aspects render it a highly favorable target for metabolic disorders. The central role of adiponectin is energy homeostasis with a newly proposed role as a “starvation gene” ^(3,4).

Adiponectin plays an important role in bone metabolism and osteoporosis pathophysiology, modulating bone formation and remodeling by selectively enhancing stem cell development between adipocyte and bone precursors in the bone marrow in response to reduced energy storage and metabolic needs ⁽⁵⁾.

AIM OF THE WORK:

The aim of this study is to assess serum adiponectin level in RA patients and to study the possible role of adiponectin as a marker of osteoporosis in RA patients.

PATIENTS AND METHODS:

The present study included 30 premenopausal RA female patients fulfilled

the ACR/ EULAR 2010 classification criteria for RA patients and 15 healthy females age and BMI matched as a control group.

Inclusion criteria:

Pre-menopausal female RA patients (ranged from 26 to 46 years) diagnosed according to the 2010 American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) RA classification criteria (ACR/ EULAR, 2010).

Exclusion criteria:

Pregnancy, breast feeding female, patients with other causes of secondary osteoporosis as smoking, bed ridden, hyperthyroidism, primary hyperparathyroidism, malignancy, malabsorption, osteomalacia and cushing’s syndrome, patients on treatment of osteoporosis (Anti resorptive drugs and bone forming “anabolic” drugs), morbid obesity (BMI more than or equal 40) and patients on drugs that may influence bone metabolism (estrogen, glucocorticoids, anticonvulsants, vitamin D, calcium);all of these cases were excluded from our study.

- Written consent was taken from all patients, the study was approved by Medical Ethics Committee.

All patients will undergo the following:

1. Full medical history taking with special attention to history of previous fracture, calcium, vitamin D or anti-resorptive medications
2. Full Clinical examination
3. Laboratory investigation including complete blood count, Erythrocyte sedimentation rate, rheumatoid factor, Anti cyclic citrullinated peptide, Liver function tests, Kidney function tests and Serum adiponectin level (µg/ml) using Human Adiponectin ELISA commercial test system.
4. Radiological assessment: DUAL Energy X-ray Absorptiometry (DEXA).

Both patients and controls are subjected to:

- A- Measurement of Serum adiponectin level ($\mu\text{g/ml}$) using Human Adiponectin ELISA commercial test system.
- B- Radiological assessment of Bone Mineral Density in lumbar spine (L2-L4) by using DEXA which categorizes the osteoporosis according to T-score: Normal (-1.0 and above), osteopenia (-1.0 to -2.5), osteoporosis (-2.5 and below), Severe "established" osteoporosis (-2.5 and below plus one or more osteoporotic fracture(s)).

Statistical Analysis:

1. **Student T test** was used to assess the statistical significance of the difference between two study group means.
2. **Chi-Square test** was used to examine the relationship between two qualitative variables
3. **The ROC Curve (Receiver Operating Characteristic)** provides a useful way to evaluate the Sensitivity and specificity for quantitative Diagnostic measures that categorize cases into one of two groups
4. **Correlation analysis (using Pearson's method):** To assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables.
 - $r = 0-0.19$ is regarded as very weak correlation
 - $r = 0.2-0.39$ as weak correlation
 - $r = 0.40-0.59$ as moderate correlation
 - $r = 0.6-0.79$ as strong correlation.
 - $r = 0.8-1$ as very strong correlation.

RESULTS:

The present study included 30 premenopausal RA female patients and 15 healthy female. There was no statistically significant difference between RA patients and controls regarding age and BMI as shown in **table (1)**.

There was statistically highly significant increase in the level of adiponectin in RA patients compared to controls (P-value <0.001) as shown in **table (2) & diagram (1)**.

As regard DEXA T-score at lumbar spine (L1-L4) there was statistically highly significant difference (P-value <0.001) between RA patients and controls as shown in **table (3)**.

RA patients were classified as regard the presence of OP to group A (RA patients without OP) and group B (RA patients with OP)

There was no statistical significant difference between group A and group B as regards age (P-value = 0.455).

There was statistically significant increase in BMI in group (A) patients compared to group B (P-value <0.001) and there was no statistical significant difference between both groups as regards disease duration (P-value = 0.898) as in **table (4)**.

There was statistically highly significant increase in serum adiponectin in group B (P-value <0.001) as in **table (5) & diagram (2)**.

As regards T-score at lumbar spine; there was statistically highly significant increase in T score in group B compared to group A (P-value <0.001) as in **table (6) & diagram (3)**.

There was a significant negative correlation between adiponectin level and DEXA in group A and B patients (RA patients) ($r = -0.635$ & $r = -0.606$ respectively) with a statistically significant

p-value (p=0.011 & p=0.017 respectively) as in **table (7) & diagram (4).**

The ROC curve was used to define the best cut-off value of serum adiponectin between group A and group B.

The cut-off value of serum adiponectin level >**600** to detect rheumatoid patient with area under curve (AUC) 1 (95%confidence Interval (CI) = 0.921 to 1.000, p

value<0.001), sensitivity 100% and specificity 100% as shown in **table (8).**

The cut-off value of serum adiponectin level >**1400** to detect osteoporosis in patients with RA with area under curve (AUC) 1 (95%confidence Interval (CI) =0.841 to 1.000, p value<0.001), sensitivity of 100% and specificity of 100%, as shown in **table (9).**

Table (1): Comparison between RA patients and controls as regard age and BMI.

	RA patients (n=30)		Controls (n=15)		t test	
	Range	Mean±SD	Range	Mean ±SD	p value	sig.
Age(y)	26-46	37.2±6.9	26-46	35.9±6.8	0.554	NS
BMI(kg/m2)	23-37.34	29.85±4.06	23-37.79	28.79±3.11	0.338	NS

BMI: Body mass index

Table (2): Comparison between RA patients and controls groups in serum adiponectin level.

Adiponectin level (microgm/L)	RA patients(n:30)		Control(n:15)		t test	
	Range	Mean ±SD	Range	Mean ±SD	p value	sig.
	750-3000	1574.0±565.4	175-300	345.0±153.6	<0.001	HS

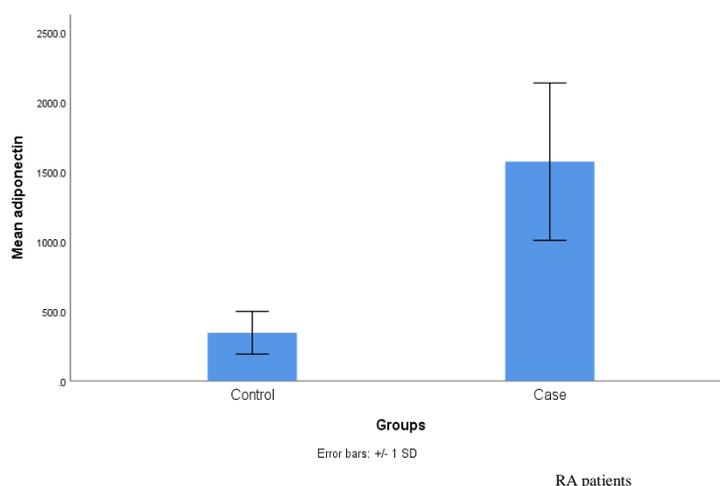


Diagram (1) Comparison between RA patients and controls groups in serum adiponectin level

Table (3): Comparison between RA patients and controls as regards T-score at the lumbar spine.

DEXA	RA patients (n:30)		Control (n:15)		t test	
	Range	Mean±SD	Range	Mean±SD	p value	sig.
	(-3.9) -2.1	-1.1±2.2	0.8-2.5	1.5±0.5	<0.001	HS

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Table (4): Comparison between group A and group B as regards descriptive data

	RA patients without OP		RA patients with OP		t test	
	Range	Mean±SD	Range	Mean±SD	p value	sig.
Age (y)	27-46	38.2±6.5	26-45	36.3±7.5	0.455	NS
BMI(kg/m ²)	24.8-37.97	31.54±3.96	23-37	28.16±3.51	0.020	S
DD (Y)	1-13	7.47±3.02	4-12	7.60±2.61	0.898	NS

BMI: Body mass index. DD: Disease duration.

Table (5): Comparison between group A and B patients as regards serum adiponectin level.

	RA patients without OP		RA patients with OP		t test	
	Range	Mean±SD	Range	Mean±SD	p value	sig.
adiponectin	750-1400	1155.0±215.3	1500-3000	1993.0±489.6	<0.001	HS

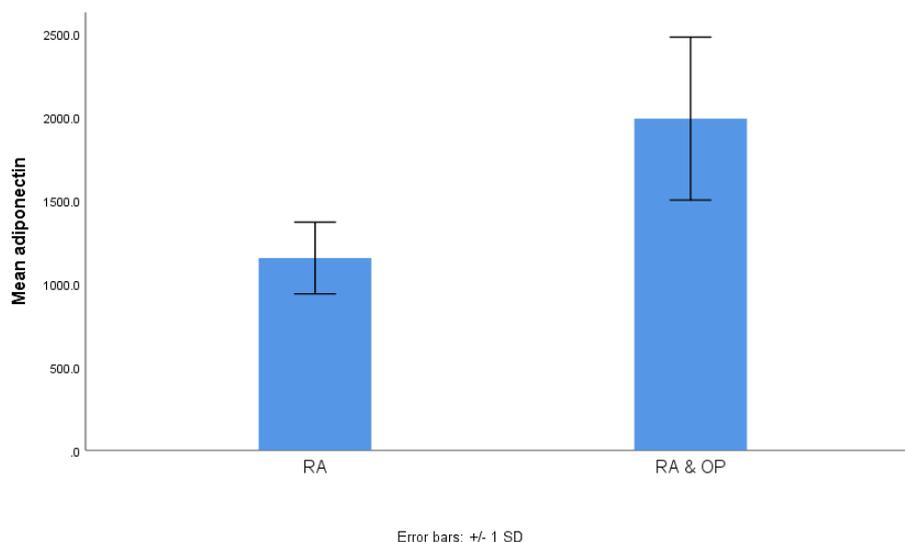


Diagram (2): Comparison between group A and B patients as regards serum adiponectin level.

Table (6): Comparison between group A and group B as regards T-score at the lumbar spine.

	RA patients without OP		RA patients with OP		t test	
	Range	Mean±SD	Range	Mean±SD	p value	sig.
DEXA	(0.1) - (2.1)	1.0±0.7	(-3.9) - (-2.5)	-3.1±0.5	<0.001	HS

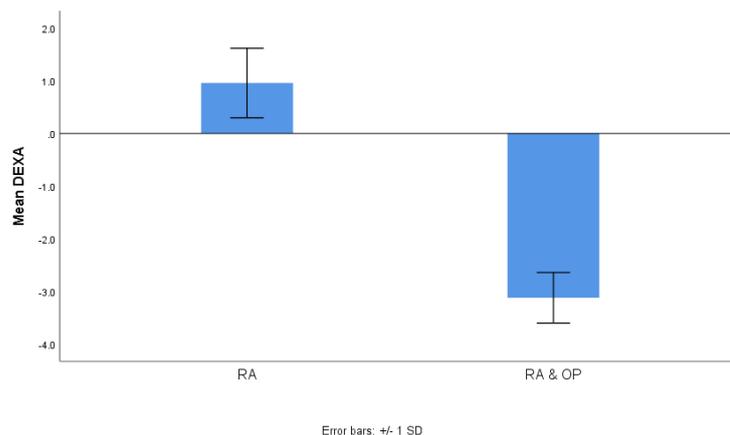
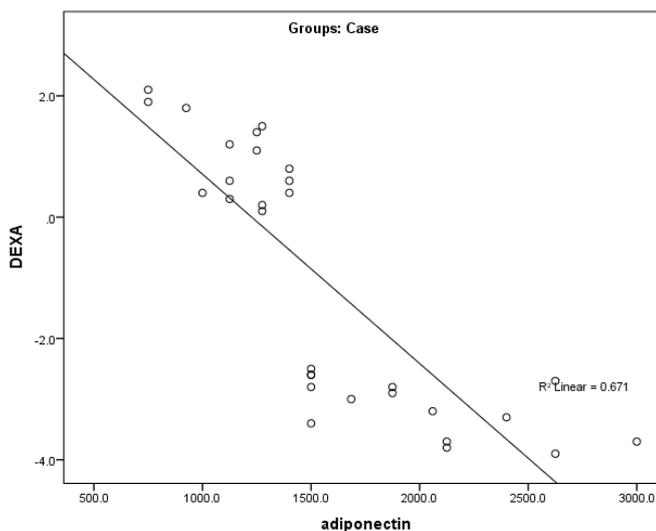


Diagram (3): Comparison between group A and group B as regards T-score at the lumbar spine.

Table (7): Correlation between serum adiponectine and T-score in groups (A&B)

Groups	RA patients without OP		RA patients with OP	
	T-score		T-score	
	r	p value	r	p value
adiponectin	-0.635	0.011	-0.606	0.017



Diagram(4): Correlation between serum adiponectine and T-score in groups (A&B).

Table (8): The area under the ROC curve (AUC) for serum adiponectin in Rheumatoid patients.

AUC	95% CI	p value	sig.	Cut off point	Sensitivity	Specificity	+PV	-PV
1	0.921 to 1.000	<0.001	S	>600	100	100	100	100

AUC: Area under curve, CI: confidence Interval, PV: predictive value

Table (9): The area under the ROC curve (AUC) for serum adiponectin level to detect patients with RA & OP.

AUC	95% CI	p value	sig.	Criterion	Sensitiviy	Specifity	+PV	-PV
1	0.841 to 1.000	<0.001	S	>1400	100	100	100	100

AUC: Area under curve, CI: confidence Interval, PV: predictive value.

DISCUSSION:

Osteoporosis is a condition characterized by low bone mass and decreased bone strength, resulting in an increased propensity for fracture. OP is one of the major comorbidities of RA and is caused by a number of complex pathophysiologic processes⁽⁶⁾. Adiponectin is predominantly produced by adipose tissue and is adipocyte-specific adipocytokine. It is a specific protein secreted by adipocytes with presumptive anti-atherogenic and anti-inflammatory characteristics. It is considered to have multiple beneficial effects on metabolism including insulin-sensitizing, anti-inflammatory, and antiapoptotic effects⁽⁷⁾.

In our study we had 30 RA patients, they were divided into 2 groups according to DEXA results group (A) RA without OP and group (B) patients RA&OP. In the present study, the mean of serum adiponectin level in the RA patients was (1574± 565.4) while in the controls was (345± 153.6) with statistically highly significant increase in RA patients compared to controls (p value <0.001).

In consistent with our findings, a study conducted by Polyakova et al., 2017⁽⁸⁾ on 88 RA women and 45 healthy females, serum adiponectin level was statistically significant higher in RA females compared to healthy controls (p<0.001). Likewise, Qian and his colleagues⁽⁹⁾ in 2018 studied the serum levels of adiponectin in 38 patients with RA, 40 patients with osteoarthritis (OA), and 20

healthy controls and they found that adiponectin level was significantly higher in RA group compared to healthy controls.

The same result was recorded by Nicolaou et al.,2018⁽¹⁰⁾ who found that serum adiponectin was significantly higher in RA patients at baseline; however, its level tends to reach normal levels as DAS -28 score improved over time.

Also, A recent meta-analysis investigated 813 RA patients and 684 healthy controls in 11 studies and indicated that circulating adiponectin level of RA patients was significantly higher than that in control groups⁽¹¹⁾.

All these studies recorded the higher adipoonectin level in RA patients as adiponectin possesses pleiotropic effects on chronic inflammatory diseases. Also, increase adiponectin in the synovium promotes higher yield secretion of chemical mediators (IL1, IL6, IL8 ,MCPand MMPs) from RA synovial fibroblasts and induces lymphocytes to synthesize TNFα,IL6 and IL8⁽¹²⁾.

In contrast with our study Li et al., 2015⁽¹³⁾ who studied the serum adiponectin level in established 180 RA Chinese female patients and 160 age- and sex-matched healthy volunteers ,they reported lower adiponectin levels in RA patients compared to healthy controls. Also Veluri and his colleagues⁽¹⁴⁾ in 2019 studied forty RA patients (newly diagnosed n = 20, RA in clinical remission n = 20) and thirty healthy controls and found that serum adiponectin

levels were significantly decreased in groups of RA patients .

A recent study conducted by Zhao et al.,⁽¹⁵⁾ in 2020 measured serum adiponectin in 617 patients with RA and 639 healthy controls they found that patients with RA had considerably lower plasma level of adiponectin.

The difference in these results suggests that the actual relationship of adiponectin with inflammatory milieu of RA is quite complex. Physiologically, adiponectin inhibits the release of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines from activated inflammatory cells. Conversely, pro-inflammatory mediators such as TNF- α and IL-6 are known to suppress adiponectin production⁽¹⁶⁾.

Also, this difference can be explained by the presence of different adiponectin isoforms, there are 3 isoforms (HMW, MMW, gAPN). Many studies recorded higher HMW and gAPN in patients with RA while MMW showed no difference in RA patients compared to controls , so different isoforms of adiponectin may be the cause of different results recorded in these studies⁽⁴⁾.

Other reasons why adiponectin concentrations had shown variable results between studies include variable disease duration, disease activity, sample size, and ethnicity of the study population⁽¹¹⁾.

Lumbar spine bone densitometry assessed by DEXA is the standard technique to determine secondary osteoporosis in RA, In our study the T score at lumbar spine (L1-L4) for RA patients ranged between -3.9 to 2.1 with mean (-1.1 ± 2.2) , and for the controls T-score at lumbar spine (L1-L4) was ranging between 0.8 to 2.5 with mean (1.5 ± 0.5) which was highly significant and 50% of our patients had OP.

However our results differ from a study conducted by Fassio et al ., 2016⁽¹⁷⁾ who reported that, among women with RA, the

proportion of pre-menopausal patients with OP was 13% compared with healthy women, This difference may be due to difference in sample size, disease duration and disease activity and ethnicity of the study population.

In our study the serum level of adiponectin was significantly higher in patients with both RA and OP compared to RA patients only ($p < 0.001$). In agreement with our result, Polyakova et al., 2017⁽⁸⁾ reported that RA patients with overlying OP disease had significantly higher serum adiponectin than patients who suffers RA with normal bone density ($p < 0.001$). The same result was recorded by Mpalaris and his colleagues⁽²¹⁾ in 2016 who studied the relationship between adiponectin and OP and found that women with OP had higher adiponectin concentrations compared to those without OP.

One of our strengths was identifying a new predictive cut-off value of serum adiponectin level $> 600 \mu\text{g/L}$ to detect RA patient, with a sensitivity of 100% and a specificity of 100%. To our knowledge no other studies reported cut-off value of serum adiponectin to diagnose RA.

Our results confirm that a serum adiponectin of $1400 \mu\text{g/L}$ or higher is capable to detect osteoporosis among RA patients with a sensitivity of 100% and a specificity of 100%.Also Polyakova et al.,⁽⁸⁾ reported the first cut-off value for predicting OP among RA patients which was $\geq 44 \mu\text{g/ml}$.

However, our study faced some limitations,first the patients number were small,second we couldn't judge the effect of different RA treatment on serum adiponectin level. Third,we didn't monitor serum adiponectin level serially to detect the changes in its level with the course of the disease.Nevertheless, these limitations didn't detract from a potentially valuable role of serum adiponectin in detection OP in

patients with RA as suggested by the results of our study.

In conclusion we demonstrated that there was clinical usefulness of measuring serum adiponectin in predicting osteoporosis among premenopause RA female patients which strongly suggest the possible utility of serum adiponectin as a marker to detect OP in patients with RA hand in hand with conventional parameters.

We recommend RA patients should be routinely screened for the presence of osteoporosis, Serum adiponectin can be used as a marker of osteoporosis in rheumatoid patients together with DEXA. Further longitudinal studies with larger numbers of patients are recommended to detect changes in serum adiponectin level during the course of the disease. Further studies are recommended to detect the effect of treatment of RA patients on serum adiponectin level and further studies are recommended to detect the relation between DEXA on head of the femur in RA patients and serum adiponectin level.

Declaration of interests:

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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مستوى مادة الأديبونكتين في الدم وعلاقته بهشاشة العظام في المرضى الذين يعانون من مرض الرثيان المفصلي

المقدمة: الأديبونكتين يصنع ويفرز من الأنسجة الدهنية و أظهرت الدراسات العلمية الأساسية أن مادة الأديبونكتين لديها حساسية للأنسولين و خصائص مضادة للتصلب ومضادة للالتهابات. لذلك من المهم للباحثين أن يكون لديهم فهم شامل لمادة الأديبونكتين. قد تؤدي هذه المعرفة إلى طرق علاجية جديدة للأمراض مثل مرض السكري من النوع الثاني ومتلازمة التمثيل الغذائي وأمراض القلب والأوعية الدموية والسمنة و تم توثيق أن مادة الأديبونكتين تحفز تكوين العظام وتعيد تشكيلها كما تمنع تآكل العظم و أظهرت معظم الدراسات أن الأديبونكتين يحفز التمايز والتعدن لكتلة العظم وكذلك التعبير عن الأوستيوكالسين الذي يعمل كهرمون ينظم عملية أيض الجلوكوز وكتلة الدهون.

الهدف من الدراسة: - تهدف الدراسة الى دراسة تأثير مستوى الأديبونكتين في الدم في مرضى الرثيان المفصلي ودراسة القيمة المحتملة لمستوى الأديبونكتين في الدم كعلامة تشخيصية ممكنة لمرض هشاشة العظام في مرضى الرثيان المفصلي .

الحالات: تم اخذ ٣٠ سيدة يعانون من الروماتويد المفصلي ثم تم تقسيمهم بناء علي مقياس هشاشه العظام الي **المجموعه أ :** سيدات تعاني من مرض الرثيان المفصلي (١٥ سيده) و **المجموعه ب:** سيدات تعاني من مرض الرثيان المفصلي مع هشاشه العظام (١٥ سيده) و**المجموعه ج (مجموعه ضابطه):** سيدات لا تعاني من مرض الرثيان المفصلي ولا من اي الالم في المفاصل (١٥ سيده).

نتائج الدراسة: فيما يتعلق بنتائجنا الأولية ، وجدنا أن مرضى الرثيان المفصلي لديهم مستوى أديبونكتين أعلى من الاصحاء علاوة على ذلك ، كان أديبونكتين في الدم أعلى بشكل ملحوظ في المرضى الذين يعانون من كل من الرثيان المفصلي وهشاشة العظام مقارنة بمرضى الرثيان المفصلي فقط ثانيًا ، فيما يتعلق بالقدرة التنبؤية للأديبونكتين في الكشف عن الرثيان المفصلي مع هشاشة العظام ، أظهرت نتائجنا أن أديبونكتين في الدم قادر على اكتشاف هشاشة العظام بين مرضى الرثيان المفصلي بحساسية ١٠٠٪ وخصوصية ١٠٠٪. علاوة على ذلك ، أظهرت النتائج التي توصلنا إليها أن هناك ارتباطًا عكسيًا بين مستوى الأديبونكتين وكثافة العظام ، حيث كان هناك ارتباط سلبي كبير بين درجات أديبونكتين ونتائج فحص أشعة سينية ثنائية الطاقة في المرضى الذين يعانون من مرض الرثيان المفصلي فقط والمرضى الذين يعانون من مرض الرثيان المفصلي ومرض هشاشه العظام.