The effect of intraarticular glucocorticoid injection on the serum level of resistin in Osteoarthritis of the knees. Correlation of the level of resisitin to ultrasonographic findings

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ABSTRACT

Background: Osteoarthritis (OA) is one of the ten disabling diseases affecting 9.6% of men and 18 % of women aged over 60 worldwide. OA characterized by articular cartilage loss, subchondral bone remodeling, soft tissue damage and low grade synovitis It is the most common form of arthritis and major cause of disability in the adult population. The main source of resistin in humans is mononuclear cells. Evidence has shown that higher serum levels of resistin in patients with severe OA compared to controls with no OA and resistin are detected in both serum and synovial fluid, proving its systematical and local involvement in inflammatory changes of OA.

Aim of the Work: The aim of this work is to study the effect of local GC intra articular injection on the level of serum resistin in OA knees. Also, to study the relation between different serum levels of resistin and US findings of the knee.

Patients and Methods: The ethical approval was obtained from the hospital ethical research committee and each patient entering the study will sign an informed consent. Thirty patients with primary knee OA will be recruited for the study from the Physical medicine, 'Rheumatology and Rehabilitation Outpatient Clinic of Al-Azhar University Hospitals starting from November 2017 till may 2018. In this study we measure Serum Resistin level and Musculoskeletal ultrasound examination of the knee before and three months after steroid injection

Results: Serum resistin level is markedly decreased after intra articular steroid injection into OA knee joint.

Conclusion: Our study revealed that: Resistin could be considered as an important severity marker of knee OA. Serum resistin level is markedly decreased after intra articular steroid injection into OA knee joint. The longer the duration of illness the higher the resistin level. The older the age the higher the resistin level. The longer the duration of illness, the higher the radiological grade. Serum resistin level should be equal to or higher than 2.8 ng / ml to diagnose a case of primary knee OA

Keywords: Osteoarthritis, Resistin.

INTRODUCTION

Osteoarthritis (OA) is one of the ten disabling diseases affecting 9.6% of men and 18 % of women aged over 60 worldwide [1]. OA characterized by articular cartilage loss, subchondral bone remodeling, soft tissue damage and low-grade synovitis. It is the most common form of arthritis and major cause of disability in the adult population[2].

There are proinflammatory agents including classical cytokines (interleukin-6 (IL-6), interleukin- 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) as well as adipokines such as leptin, visfatin, resistin which play a critical role in immune and inflammatory responses [3].

The main source of resistin in humans is mononuclear cells [4]. Evidence has shown that higher serum levels of resistin in patients with severe OA compared to controls with no OA and resistin are detected in both serum and synovial fluid, proving its systematical and local involvement in inflammatory changes of OA [5].

OA is one of the rheumatic disorders in which advances in high resolution Ultrasound (US) have greatly enhanced our ability to observe the detailed changes in the pathological joint potentially providing insight into the causes of pain, the role of inflammation and the progression of the disease process such as joint effusions and popliteal cysts are common findings in OA. Synovitis has been confirmed in knee OA especially in patients with early disease [6].

Intra-articular (IA) Glucocorticoid (GC) injections are frequently used to treat acute and chronic inflammatory conditions. Especially during the OA flare, when there is evidence of inflammation and joint effusion, GC injections decrease acute episodes of pain and increase joint mobility [7].

AIM OF THE WORK

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PATIENTS AND METHODS

The ethical approval was obtained from the hospital ethical research committee and each patient entering the study will sign an informed consent.

Thirty patients with primary knee OA will be recruited for the study from the Physical medicine, 'Rheumatology and Rehabilitation Outpatient Clinic of Al Azhar University Hospitals starting from November 2017 till may 2018.

In this study we measure serum resistin level and musculoskeletal ultrasound examination of the knee before and three months after intraarticular steroid injection

Patient's selection

A) Inclusion criteria (group A): This group will comprise OA Patients Grade 2-3 Kellgren Lawrence score (8) in acute flare according to ACR criteria with or without effusion

B) Exclusion criteria: All cases of secondary OA. All systemic autoimmune diseases. Previous local GC injection within 3 months preoperative evaluation:

Control group: (group B) Twenty healthy individuals matched for age, sex and BMI with patients were also enrolled in this study as a control group.

All patients were subjected to full medical history taking with special emphasis on: Personal history name, age, sex, residency, occupation, special habits. Complaint: onset, course and duration.

History of present illness: Pain (onset, course, duration, site, radiation, character, what increases, what decreases). Swelling (onset, course, duration, diffuse or localized). Symptoms of inflammation as hotness or redness.

Past history: Drug history, knee trauma, previous surgical operation, rheumatologic diseases, DM or other metabolic diseases, cardiac diseases, kidney or liver diseases.

Family history: Similar condition, rheumatic diseases, DM.

General examination: Vital data (pulse, blood pressure, respiratory rate and temperature). Weight and height (BMI =Weight (kg) / Height (m)2).

Local examination; Inspection.

In standing position: To see the alignment of the lower limb and the presence of deformities on weight bearing. For observation from behind to see if there are any obvious popliteal swellings. From in front for genu valgus genu varus From the side for genu recurvatum

In supine position: Skin changes such as discoloration, scar or rashes. Muscle wasting in particular atrophy of the medial aspect of the quadriceps muscle - vastus medialis (to be confirmed by measurement) Swelling: either generalized or localized.

Palpation: Warmth. Swelling either generalized or localized, site and type: Detection of knee effusion with its three degrees:Fluid displacement or bulge test for minimal effusion. Fluctuation test for moderate effusion. Ballottement test for tense effusion. Synovial hypertrophy. Bony enlargement. Crepitus: clicking of the joint with motion. Quadriceps muscle examination with comparing thigh circumferences 10 cm above the upper pole of patella for each side to detect if there is wasting,

IV Ligament stability and meniscial testing: Varus stress test: for lateral collateral ligament laxity. Valgus stress test: for medial collateral ligament laxity. Anterior drawer test: for anterior cruciate ligament laxity. Posterior drawer test: for post cruciate ligament laxity. McMurray test for meniscial tear.

V- Other joint examination. Investigations: Routine laboratory investigations; 1. Complete blood count. 2. Erythrocyte sedimentation rate: by Westergren method. 3. C-Reactive Protein. 4. Fasting and 2hr post prandial blood glucose to exclude DM. 5. Serum uric acid and serum creatinine. 6. Serum resistin level by ELISA technique before and three months after injection.

The procedure: Serum resistin level measurement using: Kit name is Assay Max Human Resistin ELISA. Catalog No.E 0338 Hu. Blood samples and serum preparation: Blood samples were collected under complete aseptic conditions from cubital vein of participants (30 patients and 20 controls) on the same day of WOMAC assessment and doing the knee x-ray. Blood samples were collected in a serum separator tube, after clot formation centrifuged to remove cells and debris and stored at - 80 °C until used.

Measuring of resistin level in serum: 1- The assay was performed at room temperature (20-30). 2- Briefly, 50 ul of standards of human resistin and serum samples were added to 96 well microtiter plates precoated with- a murine monoclonal antibody specific for resistin and incubated for 2 hours. 3- The wells were then washed five times with washing buffer then 50 ul of Biotinylated Antibody (polyclonal antibody against human resistin) to each well was added and then incubated for 2 hours. 4- The wells were then washed five times with washing 'buffer then 50 ul of Streptavidin Peroxidase Conjugate to each well was added and then incubated for 30 minutes., 5- The wells were then washed five times with washing buffer then 50 ul of Chromogen Substrate to each well was added and then incubated for l0 minutes 6- 50 ul of stop solution to each well was added to stop the reaction. 7- Then absorbance was measured at 450 nm using automated microtiter plate reader. 8- The resistin concentration was calculated by the standard curve.

Technique of injection: Patient fulfilling the criteria will be injected with single intra articular betamethasone guided by ultrasonography.

Musculoskeletal ultrasound examination of the knee: US Examination has done by TOSHIBA APILO 400. inear probe frequency 12L5 I-In case of effusion it is better to examine the knee is 30 degrees of flexion. II-The transducer is placed longitudinally on the knee above the patella and the suprapatellar space is depicted. III-The transducer is also moved both laterally and medially to scan the respective compartments. The infrapatellar space scanned for joint effusion. IV -Fluid appears on ultrasound as anechoic or echogenic material, which can be replaced by compression of the transducer. V-In healthy patients some fluid (less than 3 mm in sagittal diameter) can be detected in the suprapatellar recess in most cases. VI-Depicting proliferative synovitis of the knee joint with ultrasound scanning is performed exactly as described above for depicting joint effusion. VII-Normally the thin synovium can be hardly seen with ultrasound and does not contain Doppler signal. VIII-In OA and chronic rheumatic diseases the synovium becomes thickened and may show more or less Doppler signal in or around the area [9].

Follow-up: Post injection examination was done after three months by measuring serum resistin level and musculoskeletal examination of knee joint.

Statistical analysis: Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done: Independent-samples t-test of significance: was used when comparing between two means. Chi-square test: was used when comparing between non-parametric data. Pearson's correlation coefficient (r): test was used for correlating data. Receiver operating characteristics (ROC) curve: was used to detect sensitivity, specificity, cut-off point, PPV and NPV P- value: level of significance: Probability (P-value) P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant. P-value >0.05 was considered insignificant. The diagnostic sensitivity: It is the percentage of diseased cases truly diagnosed (TP) among total diseased cases (TP+FN). The diagnostic specificity: It is the percentage of non diseased truly excluded by the test (TN) among total non diseased cases (TN+FP). The Positive predictive value:-It is the percentage of cases truly diagnosed among total positive cases. The Negative predictive value: It is the percentage of cases truly negative among total negative cases.

RESULTS

Table (1): Comparison between patients & control as regard demographic data (age, gender, weight, height & BMI).

Variables	Groups	Patients (N = 30)	Control (N = 20)	p-value
Age (years)	Mean	52.2	51.8	0.7
	±SD	3.4	3.6	
Gender	Male	3 (10%)	4 (20%)	0.3
	Female	27 (90%)	16 (80%)	
Weight (kg)	Mean	79.8	83.3	0.1
	±SD	8.3	6.7	
Height (cm)	Mean	158.1	162.4	0.05
	±SD	6.9	8.1	
BMI	Mean	31.8	31.6	0.8
	±SD	2.7	2.7	

This table shows no statistical significant difference (p-value > 0.05) between patients and control as regard demographic data (age, gender, weight, height & BMI).

As regard age, the mean age of patients was 52.2 years while that of control was 51.8 years. As regard sex, patients were 10% males and 90% females while control were 20% male and 80% female. As regard weight, the mean weight of patients was 79.8 Kg while that of control was 83.3 Kg. As regard height, the mean height of patients was 158.1 cm while that of control was 162.4 cm. As regard BMI, the mean BMI of patients was 31.8 while that of control was 31.6

Table (2): Comparison between patients (before injection) and control as regard serum resistin.

Groups Variables		Patients (N = 30)	Control (N = 20)	p-value
Resistin (ng/ml)	Mean	7.6	2.2	< 0.001*
	±SD	2.8	0.9	

*: p-value < 0.001 is considered highly significant.

This table shows highly statistical significant difference (p-value < 0.001) between patients and control as regard serum resistin. Serum resistin was 7.6 ng/ml in patients and 2.2 ng/ml in control.

Table (3): Comparison between serum resistin before and after injection in studied patients.

Groups Variables		Before (N = 30)	After (N = 27)	p-value
Resistin (ng/ml)	Mean	7.6	5.1	< 0.05
	±SD	2.8	2.6	

This table shows statistical significant difference (p-value < 0.05) before and after injection as regard serum resistin in studied patients. Serum resistin was 7.6 ng/ml in patients before injection and 5.1 ng/ml in patients after injection.

Table (4): Comparison between WOMAC score before and after injection.

Groups Variables		Before (N = 30)	After (N = 27)	p-value
WOMAC score	Mean	53.03	38	< 0.001*
	±SD	6.1	9.5	

*: p-value < 0.001 is considered highly significant.

This table shows highly statistical significant difference (p-value < 0.001) WOMAC score before and after injection. WOMAC score was 53.03 in patients before injection and 38 in patients after injection.

Table (5): Comparison between KOFUS score before and after injection.

Groups Variables		Before (N = 30)	After (N = 27)	p-value
KOFUS score	Mean	8.9	5.5	< 0.001*
	±SD	0.7	1.5	

*: p-value < 0.001 is considered highly significant.

This table shows highly statistical significant difference (p-value < 0.001) KOFUS score before and after injection. KOFUS score was 8.9 in patients before injection and 5.5 in patients after injection.

Table (6): Comparison between US before and after injection as regard effusion.

Groups Variables		Before (N = 30)	After (N = 27)	p-value
US	No effusion	0 (0%)	14 (52%)	< 0.001*
	Mild effusion	11 (37%)	10 (37%)	
	Moderate effusion	19 (63%)	3 (11%)	

*: p-value < 0.001 is considered highly significant.

This table shows highly statistical significant difference (p-value < 0.001) US before and after injection.

Table (7): Correlation study between serum resistin and other studied parameters before injection.

Before injection	(r)	p-value
Serum resistin vs age	0.5	0.004*
Serum resistin vs duration	0.5	< 0.001**
Serum resistin vs K.L score	0.5	0.002*
Serum resistin vs WOMAC score	0.06	<0.001*
Serum resistin vs KOFUS score	- 0.1	0.5
Serum resistin vs US findings	0.2	0.2
Serum resistin vs synovitis	0.2	0.2
Serum resistin vs BMI	- 0.1	0.3

(r): Pearson correlation coefficient.

*: p-value < 0.05 is considered significant.

**: p-value < 0.001 is considered highly significant.

This table shows:

Highly statistical significant (p-value < 0.001) positive correlation (r = 0.5) between serum resistin and (WOMAC and duration of disease).

Statistically significant (p-value < 0.05) positive correlation (r = 0.5) between serum resistin and (age and K.L score).

Non-statistically significant (p-value > 0.05) correlation between serum resistin and (KOFUS score, US, synovitis and BMI).

Table (8): Correlation study between duration of the disease and K/L score of knee x-ray.

	(r)	p-value
K/L score of knee x-ray vs duration	0.7	< 0.001*

(r): Pearson correlation coefficient.

*: p-value < 0.001 is considered highly significant.

This table shows highly statistical significant (p-value < 0.001) positive correlation (r = 0.7) between duration of disease and K/L score of knee x-ray.

Table (9): Correlation study between serum resistin and other studied parameters after injection.

After injection	(r)	p-value
Serum resistin vs WOMAC score	- 0.008	0.9
Serum resistin vs KOFUS score	0.07	0.7
Serum resistin vs US findings	0.1	0.5
Serum resistin vs synovitis	0.1	0.5

(r): Pearson correlation coefficient.

*: p-value < 0.05 is considered significant.

This table shows:

Non-statistically significant (p-value > 0.05) correlation between serum resistin and (WOMAC score, KOFUS score, US findings and synovitis).

DISCUSSION

In our study no statistical significant difference between patients and control as regard demographic data (age, gender, weight, height & BMI) (p-value > 0.05).

In our study, the comparison between patients before injection and control as regard serum level of resistin showed highly significant difference (p-value< 0.001). Serum resistin was 7.6 ng/ml ±SD 2.8 in patients and 2.2 ng/ml ±SD.9 in control.

Our results were also confirmed previously by *Filkova* in 2009 [10], *De Boer* in 2012 [11] & *Perruccio* in 2014 [5] who found that serum resistin levels were significantly higher in OA patients than those in control subjects.

In our study, comparison between serum resistin before and after injection in patients showed significant difference (p-value <0.05). Serum resistin was 7.6 ng/ml±SD 2.8 in patients before injection and 5.1 ng/ml ±SD 2.6 in patients after injection.

What was interesting from the results in our work was that the median level of serum resistin from patients who have entered remission was higher than level in normal control, by research we found no positive or negative support for this data. NO studies measures serum resistin before and after IA GC injection.

WOMAC score shows highly significant difference before and after injection (p-value < 0.001).

WOMAC score was 53.03 ±SD 6.1 in patients before injection and 38 ±SD 9.5 in patients after injection

KOFUS score shows highly significant difference before and after injection (p-value < 0.001).

KOFUS score was 8.9 ±SD 0.7 in patients before injection and 5.5 ±SD 1.5 in patients after injection.

In our study, the comparison between US before and after injection showed highly significant difference as regard effusion (p-value < 0.001). This results agree with those of Loeuille in 2011 who found reduction in the effusion after injection of steroid in knee OA [12].

In our study there is significant (p-value < 0.05) positive correlation (r = 0.5) between serum resistin in patients before injection and age.

This indicates the older the age the higher the resistin level. This result agrees with that of Gharibeh in 2010 who found positive correlation between plasma resistin and age [13] and disagrees with that of De Boer in 2012 who found that resistin showed negative relation with age [11].

In our study there is highly significant (p-value < 0.001) positive correlation (r=0.5) between serum resistin level in patients before injection and disease duration. This means the longer duration of illness the higher the resistin level. This result agrees with that of Cubukcu in 2012 who found positive correlation between plasma resistin and disease duration [14].

In our study, we found that there is highly significant (p-value < 0.001) positive correlation (r = 0.5) between serum resistin level in patients before injection and (WOMAC score, K.L score).

These results suggest that resistin could be considered as an important severity marker of OA.

This is supported by *Choe* in 2012 [15] and *Pullerits* in 2008 [16] who found that the serum resistin was significantly correlated with radiological joint destruction in patients with OA.

In our study we found that there is no significant correlation between serum resistin and BMI. (p-value > 0.05).

This come in accordance with *De Boer* in 2012 [11] & *Koskinen* in 2014 [17] who found also resistin did not correlate with BMI

Our finding is supported by the idea that resistin is the link of inflammatory process rather than to obesity or insulin resistance in humans [18].

Our study shows highly significant (p-value < 0.001) positive correlation (r = 0.7) between duration of disease and K/L score of knee x-ray and this agrees with a study by *Cubukcu* in 2012 [14].

This means the longer the duration of illnes, the higher the radiological grade.

In our study there is no-statistically significant (p-value > 0.05) correlation between serum resistin and (WOMAC score, KOFUS score, US findings and synovitis) After injection.

According to our results, we could hypothesize that serum resistin level should be equal to or higher than 2.8 ng / ml to diagnose a case of primary knee OA.

This value was reported through Roc curve and determining the cutoff point serum resistin levels were higher than cut off point in all our patients, suggesting that the contribution of resistin to the pathogenesis of primary knee OA. However there was three values that was higher than cut off point in the control group and this may be attributed to a trivial joint trauma that passed unnoticed or subclinical infection [19].

CONCLUSION

Our study revealed that: Resistin could be considered as an important severity marker of knee OA. Serum resistin level is markedly decreased after intra articular steroid injection into OA knee joint. The longer the duration of illness the higher the resistin level. The older the age the higher the resistin level. The longer the duration of illness, the higher the radiological grade. Serum resistin level should be equal to or higher than 2.8 ng / ml to diagnose a case of primary knee OA

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