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Title: Comparison between the levels of Pentraxin-3 and soluble lectin like oxidized LDL receptor-1 in rheumatoid arthritis patients

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Abstract: Rheumatoid arthritis can defined as autoimmune disease that characterized by inflammatory synovitis, joint pain and functional disability and this can lead to destruction in the articular cartilage and bone. Our objective is to assess serum level of sLOX-1 and PTX-3 in patient with rheumatoid arthritis (Study design: Case control study)**.** Eighty four people were included in this study, all from Al-Yarmook Teaching Hospital during period from December 2019 to February 2020 Subjects were divided into three groups:1- Control group (non- patient group): involve 28 healthy subjects 2-Patient study group 1: involve 28 subjects suffering from mild sign of Rheumatoid arthritis.3- Patient study group 2: involve 28 subjects suffering from severe sign of Rheumatoid arthritis. All patients were diagnosed with Rheumatoid arthritis have tender, warm, swollen joints with joint stiffness usually in the morning or after period of inactivity with fatigue, fever and loss of appetite. Mild cases is tend to be affect the smaller joints like fingers , hand and toes of feet and severe cases the sign spread to wrist, knee, elbow, hips and shoulders.. Serum samples were analyzed by using (ELISA). There was significantly increasing in mean level of sLOX-1and PTX-3, it was significantly higher in severe RA patient group than mild RA patient group and healthy control group ($P<0.001$)**.** We can conclude that we can use sLOX-1 and PTX-3 as diagnostic biomarkers of RA also it can be markers for severe RA disease.

Key words: sLOX-1, pentraxin-3, Rheumatoid arthritis, inflammatory biomarkers, Joint pain

I. Introduction

Rheumatoid arthritis can defined as autoimmune disease that characterized by inflammatory synovitis, joint pain and functional disability and this can lead to destruction in the articular cartilage and bone , the main cause of RA remain unknown; it may be genetic, environmental or immunological factors. [1,2,3] Because rheumatoid arthritis is inflammatory interaction so it can be diagnosed by evaluation of many

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inflammatory biomarkers like erythrocyte sedimentation rate ESR or CPR C-reactive protein that reported as clinical data.

Recently there are new and noval biomarkers can be used in the early diagnosis in RA and this will be useful for treatment and suppress inflammation before joint destruction occurs irreversibly. [4, 5]

Soluble lectin like oxidized LDL receptor-1, it synthesized in variety of cells like macrophage, chondrocyte and endothelial cells, its production enhanced by pro-inflammatory cytokines ; it act as receptors for oxidized-LDL that result from oxidative stress or inflammation like in case of RA.[6, 7] Interaction occur between ox-LDL and sLOX-1 play an important role in the pathogenesis of joint inflammation and accelerate cartilage destruction so we can use it as biomarker in early diagnosis of RA.[8, 9]

Pentraxin is considered as member of acute phase protein that synthesized according to the presence of inflammatory conditions and play an important role in innate immune system.[10] Pentraxin-3 (PTX-3) is synthesized by many cells like phagocyte, fibroblast, endothelial cell act as member of long pentraxin super family that involve amyloid P and C-reactive protein and also it act as inflammatory marker because it involved in many biological processes like inflammation and innate immunity, autoimmunity and also it has important role in the regulation of self-antigen presentation, clearance of apoptotic cell and it result in modifications of angiogenesis and atherosclerotic lesion development.[11, 12]

Many studies showed that serum level of PTX-3 I autoimmune disease like rheumatoid arthritis RA, systemic lupus erythematous SLE and ankylosing spondylitis AS play an important role in the inflammatory reactions, so it can be used as biomarker for early diagnosis of disease.[13,14,15, 16]

Aim of this study is to evaluating serum level of sLOX-1 and PTX-3 in patient with rheumatoid arthritis and if we can use it as biomarker for early diagnosis of disease by compare their levels with healthy persons.

II. Method

Eighty four people included in this study, all from Al-Yarmook Teaching Hospital during period from December 2019 to February 2020, study design (case –control study).

Subjects were divided into three groups:

1- Control group (non- patient group): involve 28 healthy subjects

2-Patient study group 1: involve 28 subjects suffering from mild sign of Rheumatoid arthritis.

3- Patient study group 2: involve 28 subjects suffering from severe sign of Rheumatoid arthritis.

All patients were diagnosed with Rheumatoid arthritis have tender, warm, swollen joints with joint stiffness usually in the morning or after period of inactivity with fatigue, fever and loss of appetite. Mild cases is tend to be affect the smaller joints like fingers , hand and toes of feet and severe cases the sign spread to wrist, knee, elbow, hips and shoulders.

Five milliliters of venous blood was obtained from all groups (patient and control group) then blood samples were collected in plain tubes let them at room temperature for 10-20 minutes for clotting then

centrifuged at 2000-3000 rpm for approximately 20 minutes after that the supernatant collected carefully, transferred to another tube and frozen at -20°C to be analyzed, any hemolyzed samples were rejected.

Enzyme-linked Immunosorbent assay ELISA kit was used that involve antigen antibody reaction , the principle of this kit is double antibody sandwich one step ELISA this process was used to assay the levels of sLOX-1 and pentraxin-3 in all samples collected.

The principle of sLOX-1 involve presence of specific antibodies pre-coated on a micro plate; the standard and the samples pitted into the wells that contain specific antibody; so it bound by the immobilized antibody, after washing conjugated Horseradish Peroxidase (HRP) is added to each well then washes the wells to remove any excess unbound reagent and substrate solution is added to wells. The color will developed according to the amount of sLOX-1 that bound to specific antibody; the development of the colors is stopped and the intensity of the color measured, the concentrations determined by comparing the optical density of the samples in standard curve.

The kit for PTX-3 assay, use Purified Human Pentraxin 3 antibody to coat the micro plate wells that make solid-phase antibody, then add the samples to the wells combined antibody with HRP , antigen antibody complex formed , after washing TMB substrate solution will added,blue colored will developed and this reaction will terminated by addition of sulphuric acid solution then the color changed ,the concentration of PTX-3 in samples determined by comparing optical density of the samples with standard curve.

Statistical package for social sciences version 24 (SPSS v24) used to analyze data. Continuous variables presented as means with standard deviation and discrete variables presented as numbers and percentages.

ANOVA and Kruskal Wallis tests were used as appropriate to test the significance of observed difference in means of more than two independent samples.

T test for two independent samples and Mann-Whitney test were used as appropriate to test the significance of observed difference in means of two independent samples.

Findings with P value less than 0.05 were considered significant.

III. Result

Result found in table (1) showed that the mean value of sLOX-1 in severe rheumatoid arthritis patient group (2538.90 ± 969.36 pg/ml) was higher than the mean value in mild rheumatoid arthritis patient group (946.82 ± 61.00 pg/ml) and higher than the mean value of healthy control group(713.92 ± 86.17 pg/ml).

Mean value of sLOX-1 was significantly higher in severe rheumatoid arthritis patient group than mild rheumatoid arthritis patient group and healthy control group ($P<0.001$) as showed in figure (1).

Table (1) sLOX-1 levels in three study groups

Marker	Study Group	Mean ±	SD	P value*
sLOX-1 (pg/ml)	Control Group	713.92	±86.17	< 0.001
	Mild RA disease	946.82	±61.00	
	Severe RA disease	2538.90	±969.36	

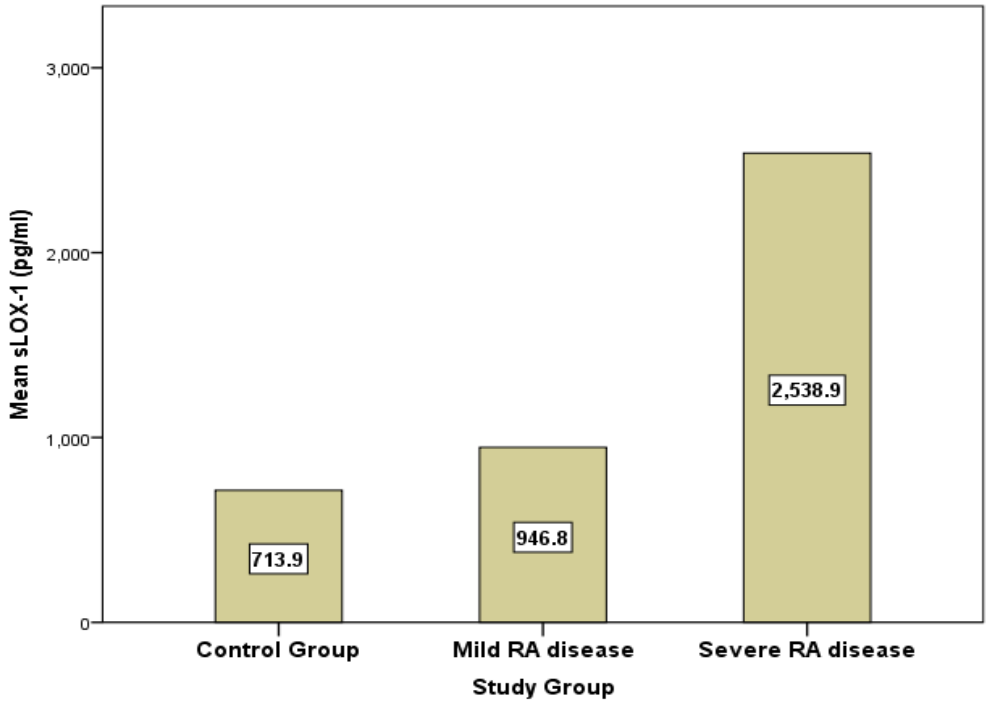

Figure (1): Mean sLOX-1 level according to study group

Result found in table (2) showed that the mean value of pentraxin-3 in severe rheumatoid arthritis patient group (6.31 ± 2.41ng/ml) was higher than the mean value in mild rheumatoid arthritis patient group (2.24 ± 0.98 ng/ml) and higher than the mean value of healthy control group(1.88 ± 1.12 ng/ml).

Mean value of pentraxin-3 was significantly higher in severe rheumatoid arthritis patient group than mild rheumatoid arthritis patient group and healthy control group (P<0.001) as showed in figure (2).

Table (2) pentraxin-3 levels in three study groups

Marker	Study Group	Mean	±SD	P value*
pentraxin3 (ng/ml)	Control Group	1.88	±1.12	< .001
	Mild RA disease	2.24	±0.98	
	Severe RA disease	6.31	±2.41	

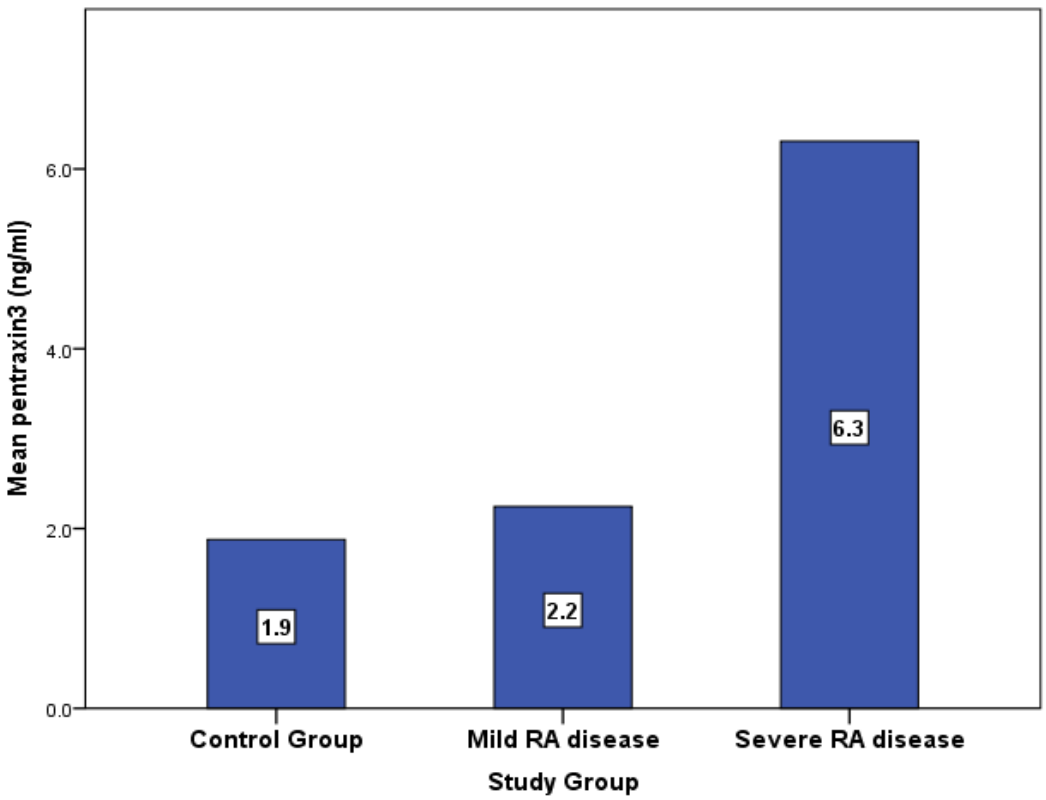

Figure (2): Mean pentraxin3 according to study group.

IV. Discussion

In present study it was found that mean level of sLOX-1 in severe RA (2538.90 ± 969.36 pg/ml) was higher than the mean value in mild RA patient group (946.82 ± 61.00 pg/ml) and higher than the mean

value of healthy control group(713.92 ± 86.17 pg/ml)(p value <0.001). This significant increasing in the level of sLOX-1 is in agreement with previous studies.

Many studies proves that sLOx-1 level in plasma of RA patient were markedly increase compare with healthy control group; this finding suggested that the synthesis of sLOX-1 indicate the reflection of entire inflammatory process that play an important role in the pathogenesis of RA.[17,18,19]

This result may lead us to suggest that sLOX-1 may compete with LOX-1 for the uptake of OX-LDL that located on the cell surface and lead to neutralize the inflammation and reduced join destruction, so its level in serum reflect disease activity.

According to this study we can use sLOX-1 as powerful biomarker to differentiate between severe, mild and healthy control according to their level in serum of inflamed joints patients.[20,21,22,23]

In this study it was found that the mean value of pentraxin-3 in severe rheumatoid arthritis patient group (6.31 ± 2.41ng/ml) was higher than the mean value in mild rheumatoid arthritis patient group (2.24 ± 0.98 ng/ml) and higher than the mean value of healthy control group (1.88 ± 1.12 ng/ml)(P value <0.001).This significant increasing in level of PTX-3 is in agreement with previous studies.

It was found that PTX-3 produced highly in site of inflammation and travel to remote sites; so it released in inflamed tissue in response to pro-inflammatory factors in another wards it produced by various tissues after exposure to inflammatory stimuli and this in agreement with previous studies that showed the serum level of PTX-3 was significantly higher in severe RA patients than mild RA and healthy control; so there is significantly correlated with disease activity and this made PTX-3 potent diagnostic biomarker for early diagnosis of RA.[24,25,26,27,28,29]

V. Conclusion

According to these findings we can use sLOX-1 and PTX-3 as diagnostic biomarkers of RA also it can be markers for severe RA disease.

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