IMMUNOLOGICAL, PHYSIOLOGICAL, AND BIOCHEMICAL STUDY OF SOME PATIENTS WITH RHEUMATOID ARTHRITIS IN KIRKUK CITY

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ABSTRACT

This study was designed in order to estimate some immunological (IL-6 and C-Reactive Protein –CRP–), physiological (Resistin) and biochemical (Glutathione and Malondialdehyde) parameters of some patients with rheumatoid arthritis in Kirkuk city. The study involved (60) blood samples were divided into 2 groups: group one: adult normal healthy individuals as control group, group two: adult patients with rheumatoid arthritis. The results of this study found significant increase in pro-inflammatory cytokine IL-6 concentrations at levels (P≤ 0.05) of second group in comparison with control group; and there are significant increases in adipokine resistin concentrations at levels (P≤ 0.05) of second group in comparison with control group; and there are significant increases in CRP concentrations at levels (P≤ 0.05) of second group in comparison with control group and there are significant decreases in glutathione (GSH) concentrations at levels (P≤ 0.05) of second group in comparison with control group, and there are significant increases in malondialdehyde (MDA) concentrations at levels (P≤ 0.05) of second group in comparison with control group.

Keywords: Rheumatoid arthritis, IL-6, Resistin, CRP, Glutathione


INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronicsynovial inflammation. RA occurs when autoimmune response leads to tissue damage which is mostly caused by type III hypersensitivity, with the participation of antibodies, complement, antigen-antibody complex, macrophages, T and B cells; leading to chronic progressive joint destruction causing a decline in quality of life, physical function, and working ability. Small joints of the hands and feet are more frequently affected, by which the main characteristics of RA are stiffness and swelling of the joints as a result of inflammation of the synovium.

IL-6 is 26 kDa, four-helical glycopeptide pleiotropic cytokine mediates several biological functions, including regulation of the immune system, regenerative processes, metabolism, bone homeostasis, cardiovascular protection and neural function. IL-6 plays an important role in the development and activation of the innate and adaptive immune system. IL-6 considered an important pro-inflammatory cytokine in conditions of
inflammation, infection and autoimmunity by which many inflammatory responses in tissues are initiated by IL-6, which promotes the infiltration and activation of mononuclear leucocytes while suppressing neutrophil infiltration. Furthermore, IL-6 signaling is associated with the upregulation of anti-apoptotic factors that promote T cell survival. Ig synthesis is also regulated by IL-6, which induces the differentiation of activated B cells into Ig-producing plasma cells and promotes the differentiation of T follicular helper cells. During autoimmunity (including RA) and inflammatory disorders, IL-6 stimulates hepatocytes in the liver, resulting in the production of acute phase proteins such as CRP, serum amyloid A, fibrinogen; as a result, acute phase proteins such as CRP are often used as biomarkers of inflammation. Thus interleukin 6 (IL-6) is one of the cytokines which play a significant role in RA pathogenesis.

Resistin is adipocyte-derived signaling cysteine-rich molecule made up of 114 amino acids secreted from the white adipose tissue, and is involved in various inflammatory processes. In addition, human resistin has also been detected in tissues like placenta, skeletal muscle, small intestine, spleen, stomach, thymus, thyroid gland and uterus. Resistin is predominantly expressed in macrophages in humans and considered a pro-inflammatory molecule, which plays an important role in the pathogenesis of many medical conditions. The release of resistin is often stimulated by the inflammatory process, IL-6, hyperglycemia and hormones such as growth hormone and gonadal hormones. Resistin is engaged in inflammatory conditions in humans by means of its secretion in substantial quantities by mononuclear cells. It has been found in plasma and synovial fluid of RA patients.

Malondialdehyde (MDA) is a highly toxic by-product formed by lipid oxidation induced free radicals. MDA is the major metabolite of arachidonic acid reacting both irreversibly and reversibly with proteins and phospholipids. Malondialdehyde is increase in the serum of rheumatoid arthritis patients due to lipid peroxidation take place in this disease because of oxidative stress which characterize RA.

Glutathione (γ-L-glutamyl-L-cysteinylglycine-GSH-), is a water soluble endogenous tripeptide, from three amino acids glycine, cysteine, and glutamic acid. GSH is potent antioxidant protecting the cell from the oxidative stress, and act as cofactor for Glutathione peroxidase (GPx) which is a defense mechanism against peroxides, preventing the accumulation of reactive oxygen species (ROS) and so preventing cellular injury. Glutathione levels decrease in RA because of oxidative stress condition in this disease which deplete the defensive antioxidant system components inside human body including glutathione.

MATERIAL AND METHODS

Patients and Blood Collection

This study was done in the period from April 2019 to August 2019 involving 60 blood samples with 30 blood samples from adult patients diagnosed with rheumatoid arthritis with age range (51-72) year. The blood samples are collected from external laboratories in Kirkuk city and divided into two groups: First group include 30 blood samples of rheumatoid arthritis patients, second group include 30 blood samples of healthy normal adult individuals as control group. Collection of blood samples was prepared from (5ml) venous blood obtained by
using disposable syringe and clean dry plain tubes without any anticoagulants and left it at room temperature to coagulate. After that centrifuged for ten minutes at 4000 rpm to get serum without any hemolysis.

**Determination of parameters**

Serum malondialdehyde was measured according to modified method used by (Guidet and Shah, 1989)\(^{(15)}\). Glutathione was estimated by the modified method used by (16)\(^{(16)}\). C-Reactive Protein (CRP) concentration was estimated in serum using slide test from Vitroscient Company (Germany), and IL-6, resistin were determined by using its kit from (MyBioScourse) company (USA) of ELISA technique.

**Statistical Analysis**

The data were analyzed by (SAS, 2001) software according to one way ANOVA followed by Duncan range test used at a statistical level of (p 0.05).

**RESULTS AND DISCUSSION**

This study showed high significance increase in blood serum pro-inflammatory cytokine IL-6 concentrations (p≤0.05) in rheumatoid arthritis group during the experiment period Figure (1) (398.40±176.20pg/ml) as compared with control group.

![Figure 1: Concentrations of blood serum IL-6 (pg/ml) in the study groups](image)

These results coincide with the studies of (7,17); they found significant increase of blood serum IL-6in rheumatoid arthritis patients in comparison with control group. Interleukin-6 (IL-6) is a pleiotropic cytokine with multiple functions in different pathophysiologic systems, including rheumatoid arthritis (RA)\(^{(18)}\). IL-6 can promote synovitis and joint destruction by stimulating neutrophil migration, osteoclast maturation, and pannus formation. It may also mediate numerous systemic manifestations of RA, including joint erosions development as a result of IL-6 action toward osteoclasts and osteoblasts differentiation\(^{(19)}\).
This study showed significance increase in blood serum adipokine resistin concentrations (p≤ 0.05) in rheumatoid arthritis group during the experiment period Figure (2) (8.22 ± 3.14ng/ml) as compared with control group.

Figure 2: Concentrations of blood serum resistin (ng/ml) in the study groups

These results agree with the studies of\(^\text{20,21}\); they found significant increase of blood serum resisting in rheumatoid arthritis patients in comparison with control group.

Resistin is expressed by different cells in the rheumatoid synovium such as macrophages, B cells, and plasma cells; resisting also induced the production of pro-inflammatory cytokines such as IL-6 and TNF-α and IL-1- β in peripheral blood mononuclear cells (PBMCs) by the activation of NF- κ B-dependent pathways to produce IL-6,TNF- α, and IL-1 β in human peripheral blood mononuclear cells :resistin, in turn, is inducible by TNFα, IL-6, and IL-1β in PBMCs, resulting in a positive feedback mechanism of inflammation\(^\text{22,23}\). The increased serum levels of resistinin rheumatoid arthritis patients correlated with markers of inflammation, such as CRP, ESR, IL-1Ra, and total leukocyte count, disease activity, and joint destruction\(^\text{24}\).

This study showed significance increase in blood serum C-Reactive Protein (CRP) concentrations (p≤ 0.05) in rheumatoid arthritis group during the experiment period Figure (3) (7.13±1.25mg/dl) as compared with control group.
These results coincide with the studies of (25,26); they found significant increase of blood serum CRP in rheumatoid arthritis patients in comparison with control group.

CRP is one of the many acute phase reactants produced from hepatocytes that is elaborated in response to inflammation and/or tissue injury, and its rise is commensurate with inflammatory mediators (cytokines) produced by cells actively participating in the milieu of tissue injury (27).

In RA, high serum CRP levels correlate to rapid and severe progression of joint damage because it reflects both systemic and local inflammatory responses. CRP production increase in RA by stimulation of pro-inflammatory cytokines on hepatocytes such as IL-6, TNF-α and IL-1- β(6,28).

The study revealed significance decrease in blood serum glutathione (GSH) concentrations (p≤ 0.05) in rheumatoid arthritis group during the experiment period Figure (4) (3.18±1.26 μmol/L) as compared with control group.
These results agree with the study of (14) which found significant decrease of blood serum glutathione in rheumatoid arthritis patients in comparison with control group.

Glutathione (GSH) is non-protein sulfhydryl molecule considered as an important antioxidant defense system in the body. It functions as an intracellular reductant in redox reactions taking place in the human body. It protects cellular components from damage caused by reactive oxygen species (ROS). Low concentration of GSH found in the plasma of RA due to its depletion because of oxidative stress which cause deficiencies and impairment in body antioxidant defensive systems including GSH (29,30).

This study showed significance increase in blood serum Malondialdehyde (MDA) concentrations (p≤ 0.05) in rheumatoid arthritis group during the experiment period Figure (5) (4.17±0.89 μmol/L) as compared with control group.

![Figure 5: Concentrations of blood serum MDA (μmol/L) in the study groups](image)

These results coincide with the studies of (31, 32); they found significant increase of blood serum MDA in rheumatoid arthritis patients in comparison with control group.

MDA produced in RA due to lipid peroxidation occurs by the oxidation of lipids via free radicals of oxygen; by which in the inflamed joint, hypoxia-reperfusion take place which result in increased lipolysis, during this process, some lipids get oxidatively modified by free radicals and removed by macrophages; MDA may also be produced in high levels as a byproduct of thromboxane synthesis, degradation of endoperoxides and cyclooxygenase reactions (33,29); thus in RA patients activated macrophages and neutrophils release oxidants in high concentrations that lead to oxidative stress this will cause damage to lipids, proteins, carbohydrates and DNA. The unsaturated fatty acids of cell membranes undergo lipid peroxidation and MDA is released which acts as a oxidative marker; additionally MDA reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation (34).
CONCLUSION

This study showed significance increase in blood serum adipokineresistin, blood serum C-Reactive Protein (CRP) and Malondialdehyde (MDA) concentrations but there is a significance decrease in blood serum glutathione (GSH) concentrations in rheumatoid arthritis group during the experiment period.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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