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Evaluation of Some Immunological and DNA Damage Parameters among Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) can cause permanent joint damage and disability through inflammation in the joints. Other organs, such as blood vessels, lungs, heart, eyes and skin may also get affected. This study aimed to evaluate the changes of some oxidative stress markers including oxidative DNA damage and the clinical outcomes that are associated with Iraqi rheumatoid arthritis patients. Commercial enzyme linked immunosorbent assay (ELISA) kits were applied to evaluate malondialdehyde (MDA), DNA damage marker 8-hydroxy-2-deoxyguanosine (8-OHDG), and glutathione peroxidase 3 (GPX3) levels in serum of 60 patients having RA with ages ranging between 20-70 years and 30 age-matched healthy controls. The results showed a significant increase in MDA levels in RA patients compared with the control group. Also, the levels of GPX3 decreased in RA patients with highly significant differences as compared with healthy control. Highly significant serum levels of oxidative DNA damage in RA patients were observed compared to the control group. Body Mass Index (BMI) results indicted significant differences between RA patients and healthy control. It can, therefore, be concluded that **t**here is a potential role if malondialdehyde, oxidative DNA damage and GPX3 are used as biomarkers for progression of in patients with RA.

Keywords: Rheumatoid Arthritis (RA), Glutathione peroxidase 3 (GPX3), Malondialdehyde (MDA), Oxidative DNA Damage (ODD).

تقييم بعض المعلمات المناعية وتلف الدنا بين مرضى التهاب المفاصل الرثياني

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الخلاصة

يمكن ان يسبب التهاب المفاصل الروماتويدي ضرراً واعاقة دائمة للمفاصل، كذلك قد تتأثر أيضًا أعضاء أخرى بالتهاب المفاصل الرثياني ، مثل الرئتين،القلب،الأوعية الدموية، الجلد والعينين. صُممت هذه الدراسة لتقييم التغايرات في بعض علامات الإجهاد التأكسدي وكذلك تلف الدنا التاكسدي والنتائج السريرية المصاحبة لمرضى التهاب المفاصل. تم استخدام تقنية الامتزاز المناعي المرتبط بالانزيم (ELISA) لتقييم المالونديالدهيد، تلف الدنا التاكسدي ومستويات الغلوتاثيون في مصل ستين مريضا مصابا بالتهاب المفاصل الرثياني لفئات عمرية مابين العشرين الى ستين عاما و كذلك ثلاثين عينة للاشخاص الاصحاء كمجموعة سيطرة. اظهرت نتائج الدراسة زيادة معنوية ملحوظة في مستويات(المالونديالدهيد) في مرضى التهاب المفاصل الرثياني مقارنة مع الاشخاص الاصحاء .وايضا انخفاض في مستويات الغلوتاثيون لمرضى مصابين بالتهاب

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المفاصل الرثياني مع فروق معنوية ذات دلالة احصائية مقارنه مع الاشخاص الاصحاء. لوحظ ارتفاع معنوي لمستويات مصل الدم من تلف الدنا التاكسدي في مرضى التهاب المفاصل الرثياني عند مقارنتها بالاشخاص الاصحاء. كذلك اظهرت الدراسة وجود فروق معنوية بمعدل موشر كتلة الجسم بين مجموعة المرضى والاصحاء. بالخلاصة ، هناك دور محتمل لاستخدام المالونديالدهيد ,تلف الدنا التاكسدي ,الغلوتاثيون كمؤشرات حيوية لتطور التهاب المفاصل الرثياني.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease and a systemic autoimmune disorder characterized by inflammation of the synovial joints. Prolonged inflammation has also been related to issues with the skin, eyes, heart, neurological system, and digestive system [1]. The pathophysiology of RA is mediated by autoimmune and inflammatory-related processes that result in an erosion of the bone and cartilage of the joints [2]. Since it is an inflammatory condition, RA effects heart functions via the enlargement of intimal-medial thickness, endothelial disorder, and total increase in spreading atherosclerosis [3]. Rheumatoid arthritis is a chronic inflammatory autoimmune disease that primarily affects small joints that leads to chronic inflammation in the synovial fluid, resulting in the destruction of small joints, deformity and disability [4]. Several immune cells, including macrophages, dendritic cells, lymphocytes, and neutrophils, migrate into the joint tissues during the pathogenesis of RA, activating resident cells like synovial fibroblasts, chondrocytes, osteoblasts, and osteoclasts. This mechanism involves three overlapping mechanism: synovial hyperplasia, modulated immune response and inflammation [5]. RA is a well-known autoimmune disease that is characterized by systemic complications, early mortality and progressive disability. The causative factors are not known and, therefore, a careful diagnosis ought to be made. However, the knowledge of the disease pathogenesis has led to much recent therapeutics with low incomes which can include series of factors such as genotyping, chance, and environment. RA is also characterized by hyperplasia, inflammation of synovia, RA antibody and anti-citrullinated protein/peptide antibody (ACPA) production, cartilage and bone deformity, and many systemic signs such as psychological, skeletal, pulmonary, and cardiovascular disorders [6].

The increased accumulation of immune cells within the synovial membrane which produce a variety of cytokines and inflammatory molecules, causes synovial hyperplasia [7]. It has become clear that a number of cytokines are connected to the pathophysiology of RA several of which might be targets for potential future treatments [8]. Also, various cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), different interleukins including IL-2, 7, 17 are familiar to be energetic from acute to be chronic stages of RA and may have the possibility of getting targeted therapeutically [9]. According to Littlejohn and Monrad [10] the RA patients usually have joint pain and hardness across multiple joints. Most commonly affected are the metacarpophalangeal, proximal interphalangeal, and wrist joints. An inflammatory cause is suggested by morning stiffness that persists for more than an hour [11] .

Malondialdehyde (MDA) is a highly reactive compound that contains 3 carbons atoms of dialdehyde and is created as a result of arachidonic acid metabolism as well as peroxidation of polyunsaturated fatty acids [12]. Study of Salzman *et al.* [13] indicated that MDA can be used as indicator for cancer. Malondialdehyde is widely used as a biomarker for assessing oxidative stress in biomedical fields. Many findings suggest the validity of the MDA assay as a reliable tool in finding out the oxidative stress in different disease pathologies, including RA disease [12, 13]. However, the cells have an efficiency to neutralize excessive reactive oxygen stress (ROS) and this balance is called antioxidant system which includes enzyme-based antioxidants (glutathione peroxidases (GPXs), superoxide dismutase (SOD) and catalase

(CAT) and non-enzyme-based antioxidants. Both act to maintain the oxidative stress [14]. Glutathione peroxidase 3 is the major mechanism anti oxidative stress necessary to keep cellular redox homeostasis. Transforming hydrogen peroxide (H_2O_2) and organic peroxides appropriate alcohols and H2O. GPX3 preserve cells from oxidative stress damage from one side to the other enzymatic mechanism [15].

Deoxyribonucleic acid (DNA) is a specific objective for oxidative stress as damage which can cause persistent alterations to the genome as a response to various external and internal sources such as enzymatic activity, endogenous chemical processes and cell metabolism. However, iIt has been suggested that oxidative DNA damage derived by ROS may involve rise in mutation rates, genome modification, cell growth, apoptosis as well as tissue regeneration. Also, it has been proposed that oxidative DNA damage plays a significant role in the pathogenesis of inflammatory disorders including rheumatoid arthritis. More information about the role of oxidative DNA damage in RA development is needed [16]. This study aimed to evaluate the changes of some oxidative stress markers, including oxidative DNA damage and clinical outcomes that are associated with Iraqi rheumatoid arthritis patients.

2.Materials and Methods

2.1 Subjects

A case-control study was performed on 60 RA patients with ages ranging between 20-70 years, and 30 healthy controls with ages ranging between 20-60 years. Patients were admitted to AL-Yarmouk Teaching Hospital, Baghdad, Iraq, from October to December 2022. The diagnosis was made by the consultant medical staff. It was based on clinical examinations, X-ray results and laboratory tests, according to the revised diagnostic criteria.

2.2 Blood Collection

Using disposable plastic syringes 5ml each venous blood samples were collected from the RA patients and the control. The serum was then obtained and preserved in 1.5 ml Eppendorf tubes and kept at -20ºC for serological tests.

2.3 Measurements of MDA and GPX3

The GPX3 and MDA analysis were performed following the manufacturer's instructions (MyBioSource Sunlong, China) using the enzyme linked immunosorbent assay (ELISA). In brief,a total of 100 µl of standards working solution and 100 µL of samples were loaded into the appropriate wells and incubated for 80 minutes at 37°C. Then, the solution was aspirated and washed with 200 µL of 1× wash solution. After that, 100 µL of biotinylated antibody working solution was added to each well and incubated for 50 minutes at 37°C which was then washed 3 times as conducted in step 2 and then 100 µL of streptavidin-HRP working solution was added and incubated for 50 minutes at 37°C. Afterwards, the wells were washed 5 times and then 90 µL of TMB substrate solution was added to each well and incubated for 20 minutes at 37°C. Then, 50 µL of stop reagent was added to each well. The absorbance in wells was read in an ELISA reader at 450 nm, immediately.

2.4 Measurements of Oxidative DNA Damage (8-OHdG)

The human oxidative DNA damage marker 8-OHdG analysis was performed following the kit manufacturer's instructions (MyBioSource Sunlong, China) using ELISA technique. First, a volume of 50 µL of the standards working solution and 100 µL of samples into the appropriate wells were added and incubated for 80 minutes at 37°C, the microtiterplate then was washed 3 times with 250 µL 1X wash buffer. Then, 100 µL of the diluted secondary antibody-enzyme conjugate was added to all wells which was followed by incubation at room

temperature for 1 hour on an orbital shaker. After that, 100 µL of substrate solution was added to each well and incubated at room temperature on an orbital shaker. Immediately after that 100 µl of stop solution was added and the absorbance in wells was read on the ELISA reader at 450 nm.

3. Ethical Approval

This study was authorized by the Department of Biology Ethical committee, College of Science, University of Baghdad (Ref.: CSEC/0922/0111 September 29, 2022). The Declaration of Helsinki on the code of ethics adopted by the World Medical Association for studies involving humans, guided the conduct of this study.

4. Statistical Analysis

Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS), Version 23. A difference was deemed statistically significant if the *p*-value was equal or less than 0.05. The data was presented as mean ± SE and t-test was used to conduct statistical comparisons between the study groups.

5. Results

The results showed that age was significantly higher in RA patients (47.06 ±1.60) than the control group (34.76 ±2.69). Also, the results of the body mass index (BMI) indicted significant differences between RA patients and the healthy control (28.27 ±0.61and 23.54 ±1.06 respectively) (Table 1).

Table 1: Comparison between RA patients and control group according to age and BMI.

Group	Mean ± SE	
	Age (years)	BMI (kg/m^2)
RA Patients	47.06 ±1.60	28.27 ±0.61
Control	34.76 ±2.69	23.54 ±1.06
T-test	5.888 **	2.284 *
P-value	0.0001	0.0165
* (P≤0.05), ** (P≤0.01). NS= Non-significant		

The results showed that 40-60 years age group was significantly higher compared with the control group. Other age groups, however, showed non-significant differences (Table 2).

Table 2: Distribution of the study samples according to age groups.

Group	20-40 yr.	40-60 yr.	>60 yr.	Total	*P*-value
RA Patients	15 (25.00%)	40 (66.7%)	5 (8.3%)	60	0.0001 **
Control	19 (63.33%)	9 (30.00%)	2 (6.67%)	30	0.0074 **
P-value	0.217 NS	0.0061 **	1.00 NS	90	---
** (P≤0.01), NS= Non-significant					

The results of gender showed significant differences between RA patients and the control group in the females group (Table 3).

Table 3: Distribution of the study samples according to gender groups.

Group	No.	Male No. (%)	Female No. (%)	P-value
RA Patients	60	20 (33.3%)	40 (66.7%)	0.0089 **
Control	30	13 (43.33%)	17 (56.67%)	0.466 NS
p-value	--	0.872 NS	0.0219 *	---
* (P≤0.05), NS= Non-significant				

The statistical analysis of MDA indicated a highly significant value ($p \leq 0.05$) in RA patients (43.32 ±1.48) than the control group (9.95 ±0.12). The results of GPX3 showed that GPX3 levels in the RA patients were lower than the control group (19.61 ±0.73 and 37.69 ±2.16 respectively). However, there were highly significant differences as shown in Table 4. The level of oxidative DNA damage marker 8-OHdG was significantly ($p \leq 0.05$) higher in RA patients (37.69 ±2.16) than in the control group (1.234 ±0.09) (Table 4).

Table 4: Comparison between RA patients and the control group in respect to MDA, GPX3 and oxidative DNA damage marker 8-OHdG.

Group	Mean ± SE		
	MDA (nmol/ml)	GPX3 (ng/ml)	8-OHdG (µg /ml)
RA Patients	43.32 ±1.48	19.61 ±0.73	37.69 ±2.16
Control	9.95 ±0.12	37.69 ±2.16	1.234 ±0.09
T-test	3.988 **	4.026 **	0.712 **
P-value	0.0001	0.0001	0.0001
** (P≤0.01) NS=Non-significant			

According to the statistical analysis, MDA levels did not differ significantly when compared between 20-40 yr. and 40-60 yr. groups. However, MDA levels were significant in 60 yr. group in RA patients (57.55 ±1.94). The levels of GPX3 did not differ significantly between 20-40 yr. and 40-60 yr. groups. GPX3 levels showed significant increase with >60 yr. group (12.54 ±0.99 yr.). The level of oxidative DNA damage was non-significant ($p \leq 0.05$) among all age groups of the studied RA patients (Table 5).

Table 5: Effects of age groups on parameters of the study samples.

Age Groups (Year)	Mean ± SE		
	MDA (nmol/ml)	GPX3 (ng/ml)	8-OHdG (µg /ml)
20-40	42.38 ±3.09 b	19.34 ±1.43 a	6.29 ±0.39
40-60	42.85 ±1.70 b	20.47 ±0.78 a	7.44 ±0.37
>60	57.55 ±1.94 a	12.54 ±0.99 b	7.57 ±1.82
P-value	0.045 *	0.0345 *	0.768 NS
Means having the different letters in same column differed significantly. * (P≤0.05). NS=Non-Significant.			

6. Discussion

RAis the almost usual exclusive reason of chronic synovitis, influencing numerous diarthrodial joints in dispensation leading to pain, malformation, and decreased quality of life. The exact etiology of RA disease is not clear but many epidemiologic and genomic studies suggest a complicated interaction between hereditary and environmental components having role in development of RA disease [17]. Also, the results showed that women more likely to achieve RA compared to men. Many studies have shown that RA increases significantly in older people. Study of Otsa *et al*.[18] showed that RA was significantly higher in women than men in all age groups [19]. The study by Chalan *et al*. [20] suggested that as a result of early immune-senescence of the immune system in RA patients, chances of RA may increase with age. Also, the inflammatory state has been suggested as another alternative process for premature aging in RA patients. However, similar demographic parameters were reported in a recent Iraqi study conducted by Mathkhor *et al*. [21] who evaluated about 470 patients with RA in Basra city.

The study by Kumar *et al*. [22] proved that prevalence of RA was more in the females (nearly three-fourth) than males. A strong correlation was found between development of RA and sex hormones and the genes that regulates the sexual chromosomes have an impartment role in supporting the prevalence of RA among female [23]. The causative factors are not yet known, however a careful diagnosis has to be made. However, the knowledge of the disease pathogenesis has only led to many recent therapeutics with low impacts. The disease affects women 2 to 3 times more often than men and may occur at any age. The peak prevalence is in the sixth decade. RA of pathophysiology involves chronic inflammation of the synovial membrane which can destroy the articular cartilage and articular bone [24]. Also, the results of this study showed that most of RA patients were overweight and therefore an increase in BMI could contribute to higher risk for RA. Study by Lu *et al*. [25] showed that the risk of RA increased significantly in serological parameters of women who were overweight and obese, particularly younger women.

In this study the level of the malondialdehyde (MDA) revealed a significant increase in sera of the RA patients compared with those in the healthy group. Also, the results showed a decrease in glutathione peroxidase 3 (GPX3) levels. These results are in line with another study that showed a significant increase in MDA level in RA patients and confirmed that the total antioxidant levels significantly decreased [26]. It has been pointed out that articular chondrocytes play a role in oxidative stress production during sore of the synovial membrane and this process can involve cartilage damage [27]. It has been suggested that induced glutathione reductase (GR) might be predictable in RA patients with chronic synovial inflammation in response to oxidative stress. Also, raised glycolysis and hypoxia in RA joints are highly related with neutrophil activation and ROS production [28].

The results of this study showed an increase in oxidative DNA damage in RA patients. These results are in line with another study that indicated that DNA damage level increases in patients with RA [29]. It has been reported that development of RA disease may be linked with increase of oxidative DNA damage as well as increase in ROS production where they both play critical role in pathogenesis of RA disease [30]. DNA damage includes nucleic acids (base and sugar modifications) covalent crosslinks, and DNA strand breaks that can be caused by oxidative stress. The increased development of oxidative DNA damage in RA patients may be a result of compromised antioxidant system performance and an imbalance between free radicals and endogenous antioxidant forces [31].
According to the results of this study there is a potential role of MDA, oxidative DNA damage and GPX3 usage as a biomarker of the progression of RA.

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