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Oxidative Stress Status In Patients With Rheumatoid Arthritis

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Abstract

Background:
Rheumatoid arthritis (RA) is the most common form of inflammatory arthritis, which is an autoimmune disease characterized by chronic inflammation of synovial joints, ultimately leading to joint destructions and permanent disability.
In RA oxidative stress are impaired (which caused by free radicals) might have an essential role in the etiology of RA.

Objectives:
The objective of this study was to determine oxidative stress by measuring malondialdehyde and enzymatic status by estimating superoxide dismutase, catalase and glutathione peroxidase in patients of RA and then comparing with healthy individuals.

Setting and design:
A total 42 patients with Rheumatoid Arthritis (20 females, 22 males) in the age of 30-50 years were included, as a control, 50 matched healthy volunteers (20 females and 30 males) were involve, non of the subjects smokes nor receiving any form of drugs. Subjects with any acute infections or with coexisting system disease such as coronary artery disease, hypertension or chronic renal failure were excluded.

Methods:
Antioxidant enzymes in erythrocyte , superoxide dismutase (SOD), glutathione peroxidase (Gpx) and Cayalase (CAT) was measured.
Malondialdehyde (MDA) along with Copper, Zink and Iron are measured by using the serum of the patients and control group.

Results:
The serum level of MDL was higher in RA patients compared with the control group, the differences among both groups was statistically significant ($P < 0.001$)

Conclusion
There is oxidative stress in RA patients evidenced by increased serum MDA and decreased antioxidant enzymes activity.

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Key Words: Rheumatoid arthritis, MDA, catalase, superoxide dismutase and glutathione peroxidase

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Introduction:

Rheumatoid arthritis (RA) is a chronic multisystem disease of unknown cause which affects about 1-2% of the total world population.. Women are affected more than men. The onset is more frequent during the fourth and fifth decades of life , with 80% of all patients developing the disease between the ages 35 and 50 years. The characteristic feature of RA is non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues and joint deformities .The characteristic feature of established RA is a persistent inflammatory synovitis usually involving peripheral joints in a symmetric distribution [1,2] . A free radical is "any species capable of independent existence that contains one or more unpaired electrons" [٣].

ROS and RNS are formed during normal physiological processes that occur when the cell is not under stress. For example, this occurs within the electron transport chain Of respired oxygen, 98% is utilized by mitochondria to generate adenosine triphosphate (ATP) [٤].

Lipid oxidation is a free–radical chain reaction, and reactive oxygen species can accelerate lipid oxidation [٥]. Cell membranes are phospholipids bilayers with extrinsic proteins and are the direct target of lipid oxidation [٦].

To protect themselves from the harmful effects of ROS and RNS, cells have several antioxidant enzymes and other antioxidant mechanism. The later include glutathione (GSH) and numerous GSH-dependent enzymes, metal binding protein, and vitamins. The three main types of antioxidant enzymes are the superoxide dismutase (SOD), catalase (CAT) and peroxidase of which glutathione (GPx) are thought to be the most important [٧].

The state of balance between ROS generation and the protection capacity of endogenic antioxidant defense of biological system could be specified as ecological oxidative stress [٨] .

Oxidative stress is related to an imbalance between the production of reactive species and the antioxidant defenses. In essence, oxidative stress has been defined as a disturbance in the pro-oxidant/antioxidant balance, leading to potential damage. the antioxidant defenses include nonenzymatic (especially dietary antioxidants) and antioxidant enzymes. Vitamins, minerals and phytochemicals (polyphenols and carotenoids) are among the major dietary antioxidants. The assessment of oxidative stress status though specific biomarkers has acquired great importance. The major biomarkers include the products of the attack of free radicals and reactive species to various substrates [2&٩]. Oxidative stress has been related in the pathogenesis of rheumatoid arthritis..RA is not generally recognized as a disease of oxidative stress but it has been suggested that the level of reactive oxygen species(ROS) in patients with RA is higher than in healthy subjects. Oxidative stress in RA is due to the fact that the antioxidant systems are impaired [10, 11] . Thus,the objectives of the study was to determine oxidative stress by measuring malondialdehyde and enzymatic antioxidant status by estimating

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Biochemical parameters.

Lipid peroxidation product (MDA) in serum was measured by the method of Guidet and Shah (1989) [12]. Under the acid and heating condition of the reaction, the peroxides break down to form MDA, which complexes with thiobarbituric acid (TBA) to form a colored red compound that can be measured spectrophotometerically at 535 nm . The superoxide dismutase activity in erythrocytes was carried out by the method of Winterbourn et al(1975) [13]. It depends on the ability of SOD enzymes to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide, which is generated by the reaction of photo reduced riboflavin and oxygen. SOD activity was expressed in unit per gram hemoglobin. GPx activity levels were determined by the method of Paglia and Valentine[14] using a commercially available kit (Randox, UK) and the activity levels were expressed as U/g Hb. The activity of catalase in erythrocytes was carried out by the method of Aibe (1984) [15], which is based on determination of the rate constant (S^{-1}, k) of the hydrogen peroxide decomposition rate

Statistical analysis:

All results were expressed as mean ± SD. The data were analyzed statistically by one–way analysis of variance (ANOVA) while the correlation between the data was tested statistically by simple linear regression test by using computer SPSS program. The criterion for significance was $p<0.05$.

superoxide dismutase , catalase and glutathione peroxidase in patients of RA and then comparing with healthy individuals.

Materials and methods

Subjects:

A total of 42 diagnosed rheumatoid arthritis patients (20 females and 22 males) in the age group of 30-50 years were included in the present study, In addition subjects with any acute infections or with co-existing systemic diseases such as coronary artery disease, hypertension or chronic renal failure were excluded. As control, 50 age matched healthy volunteers (20 females and 30 males) were recruited. None of the subjects were receiving any form of drugs and smoking.

Methods:

About 5 ml venous blood samples were obtained from all patients and control subjects. About 3 ml was added to EDTA anticoagulant tubes for antioxidant enzymes in erythrocytes superoxide dismutase (SOD) glutathione perooxidase (GPx) and catalase (CAT), which was centrifuged and washed twice in 0.9% NaCl. The reminder was allowed to clot in a clean plain tube for 20-30 minutes at room temperature. The serum was recovered by centrifugation and divided into 2 parts, the first part of serum was transferred into plain tubes which was used for measuring malondialdehyde (MDA) within 1-3 hours. The rest of serum was transferred into another plastic plain tube for measuring copper, zinc and iron . The tubes were stored at -20°C until analysis

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Results

The biochemical characteristics which were investigated in this study for (RA) patients and control groups are presented in Table-1. The serum levels of MDA was higher in RA patients compared with the control group. The differences among both groups were statistically significant ($p<0.001$) .

The same table shows that erythrocytes SOD, CAT and GPx activities were significantly lower in RA patients compared with the control group ($p< 0.001$).

Table (2) describes correlation between MDA and antioxidant enzymes in RA patients and control groups. In RA patients group, there was a significant negative correlation as compared SOD, CAT and GPx with MDA ($p< 0.001$

Table 1. Biochemical parameters among RA patients and control groups.

Parameter	Control group	RA group	P-value
	No. 50	No. 42	
S.MDA µmole/L	o.67 0.12	1.24 ± 0.12	0.001
Erythrocytes SOD U/g Hb	1800±313	1158 ± 254	0.001
Erythrocytes CAT k/g Hb	275 ± 37.7	218 ± 30.3	0.001
GPx U/gHb	45.9±15.3	36.4±9.8	0.001

Values were expressed as mean ± SD .
P values < 0.001

Table 2. Correlation coefficient (r) between MDA and selected biochemical parameters in RA patients and control groups.

Parameters	MDA	
	RA	Control
SOD	-0.546**	-.239
CAT	-0.5633**	-0.029
GPx	-0.487**	-0.052

Values were expressed as correlation coefficient (r).
*,** correlation is significant at the 0.05and 0.01 levels respectively.

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Discussion

patients when compared with healthy control group was found, Similar reports of decreasing SOD activity have been reported in patients with rheumatoid arthritis [2.,23,24, 25]. Results are controversial Our findings are contradictory to the findings of Surapneni and Gopan (2006) [28] and Vijayakumar et al (2006) [29] who showed significant increase in SOD levels in RA patients. While, Shaabani et al (2009) [19] , Ozkan et al (2007) [20], and Akyol (2007) [30] noticed no change in erythrocytes SOD activity.. Decreased SOD activity levels in patients with RA may indicate a degradation of this antioxidant enzyme by free radicals during detoxification processes, also dismutate the excess superoxide radicals that a regenerated and diffused from the inflammatory sites into plasma.

DiSilvestro et al (1992)[31] reports that treatment with anti-inflammatory drugs increases SOD activity, indicating the inflammation process produces free radicals, which decreasing SOD activity.

Disease itself may inhibit the activity of SOD and decrease the synthesis of SOD.

In the present study, significant decreasing differences in CAT and GSH-Px activities were noticed and it is in agreement with most of studies [23,25, 28,32], but also disagreement while, Jacobson (33) demonstrated that GSH-Px activity was markedly elevated in the rheumatoid arthritis sufferers. Samia. and Tamer (2011) [34] reported that GSH and GSH Px were remarkably altered in RA and SLE patients compared with a healthy individuals . Markers of increased oxidative stress and impaired antioxidant capacity were profound in RA and

The oxidative stress, manifested by unbalanced ROS production and/or impaired endogenic antioxidant defense is related to aging as well as to several diseases such as cancer, atherosclerosis, rheumatoid arthritis, renal diseases, uraemia, diabetes, Alzheimer's. Parkinson's diseases, as well as the acquired immunodeficiency syndrome (AIDS) and pulmonary inflammations etc. [16 و17 &18].

Rheumatoid arthritis (RA) is a chronic multisystem disease of unknown cause and it is an autoimmune disease characterized by chronic inflammation. leading to joints' destruction [2]..

Evidence of oxygen free radicals generation in patients with RA has been observed by measuring the product of lipid peroxidation malondialdehyde (MDA). In the present study mean level of MDA was increased significantly in RA patients compared to controls. This is in agreement with most studies [2,19,20,21,22,23,24,25,26, 27] . Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process. Pathogenic mechanism of chronic

inflammation is associated with increased production of ROS [18]. Elevated free radical generations in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis (RA). This could be due to excessive generation and diffusion of lipid peroxides from the inflammed or injured joints of rheumatoid arthritis. Antioxidant enzymes are responsible for defence against free radicals. There are some reports on erythrocyte SOD, CAT and GSH-Px activities in patients with RA. In the present study, a significant reduction in enzymatic anti-oxidants (SOD) in RA

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significant correlation between oxidative stress and MDA levels in patients with

RA, and claimed it would be useful in predicting disease activity
In conclusion, there is oxidative stress in RA patients evidenced by increased serum MDA and decreased antioxidant enzymes activity. These findings confirm the role in the tissue damage and inflammation process of this disease and may be a useful marker for disease activity in patients with RA

significantly reflected disease activity in RA.

. The finding of significant negative correlation between high MDA concentrations and low activities of SOD, CAT and GPx (Table 2), suggest increased utilization by ROS as an important contributing factor to the lower concentrations of anti-oxidants and further support a link between oxidative stress and RA diseases. Our findings agree with studies [23&27] who demonstrated a

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حالة الاكسدة في مرضى التهاب المفاصل الرثوي

أ. د. جواد كاظم مهدي

د. عبدالكريم محمد جواد د محمد ناجي قاسم

الخلاصة

الخلفيه:

التهاب المفاصل الرثوي هو اهم انواع التهابات المفاصل شيوعا . وهو مرض مناعي ذاتي يمتاز بالتهاب مزمن لغشاء المفصل والذي ينتهي بتخريب وعوق دائم للمفصل

في مرض التهاب المفاصل الرثوي هناك اضطراب في شد الاكسده (الذي ينتج من تكون الجذور الحرة) واذي من الممكن ان يكون له الدور المهم في حدوث هذا المرض .

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الهدف :

هدف هذة الدرلسة ايجاد شد الاكسدة بقياس مالون داي الدهايد وقياس حالة الانزيمات بقياس السوبراوكساید دسميوتيز والكاتاليز والكلوكوثايون بيروكسديز في مرضى التهاب المفاصل الرثوي ومقارنتها بالاناس المعافين .

الاعداد والتصميم:

شملت الدراسة مجموع ٤٢ مريضا بالتهاب المفاصل الرثوي (٢٠ اناث،٢٢ ذكور) في عمر مابين ٣٠ – ٥٠ سنة . مجموعة المقارنة شملت ٥٠ متطوعا بصحة جيدة (٢٠ اناث ، ٣٠ ذكور) . لا يوجد مدخن في كلا المجموعتين وكذلك لايستلم اي شخص منهم اي دواء .

لا تشمل الدراسة اي شخص مصاب بمرض التهابي او اي مرض يخص اي جهاز حيوي كامراض الشرايين القلبية او ارتفاع الضغط الدموي او العجز الكلوي المزمن .

طرق العمل:

تم قياس الانزيمات المضادة للاكسدة في الخلايا الحمراء وهي السوبراوكساید دسميوتيز و الكلوكوثايون بيروكسديز والكاتاليز .

مالون داي الديهايد مع النحاس والخارصين والحديد تم قياسها باستعمال سيرم المرضى بالتهاب المفاصل الرثوي وكذلك مجموعة المقارنة .

لوحظ ارتفاع مستوى المالون داي الديهايد في مرضى التهاب المفاصل الرثوي مقارنة بمجموعة المقارنة . الفروقات بين المجموعتين كان مفيد احصائيا .