SERUM AND SYNOVIAL FLUID OXIDANTS AND ANTIOXIDANTS AS RISK FACTORS IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disorder of unknown etiology. Studies indicate that RA is related to the oxidative damage caused by reactive oxygen species, but results have been inconsistent. The aim of this study is to measure the oxidant levels (xanthine oxidase and malondialdehyde) and antioxidant levels (enzymatic superoxide dismutase, catalase and non-enzymatic vitamin E) in the serum and synovial fluid of RA patients. Serum and synovial fluid samples from 146 patients (68 males and 78 females) with active RA and serum samples from 136 healthy volunteers (65 males and 71 females) set as control were collected. The level of oxidants and antioxidants were measured. RA patients had significantly higher mean serum xanthine oxidase and malonaldehyde levels, while levels of superoxide dismutase, catalase and vitamin E were significantly lower when compared to control. Female RA patients had

significantly higher serum and synovial fluid xanthine oxidase and malonaldehyde levels when compared to their male counterparts but significantly lower serum and synovial fluid SOD levels. Moreover, the serum catalase levels were lower whereas the serum vitamin E levels were higher in female RA patients when compared to male, but differences were not significant. There was also a significant decrease in the synovial fluid catalase levels in female RA patients when compared to male counterparts, but no significant differences were observed in the synovial fluid levels of vitamin E. This suggests that there is an increase in oxidative stress and a decrease in antioxidant defense in RA patients indicating oxidative damage.

Keywords: Rheumatoid arthritis, Xanthine oxidase, Malondialdehyde, Superoxide dismutase, Catalase, Vitamin E

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive inflammatory autoimmune disorder that primarily affects the joints causing joint swelling, stiffness, pain, increased morbidity, and increased mortality with multi-systemic manifestation [1, 2]. RA is classified as a type III hypersensitivity disorder of unknown etiology affecting 1-2% of the population, predominantly women of all age groups and hormonal statuses [3]. Despite the controversy around its etiology, it is well recognized that RA principally affects the synovium causing chronic hypertrophic synovitis and synovial fluid (SF) inflammation, triggering connective tissue destruction and the functional damage of cartilage and bone structures [4,5].

The pathogenesis of RA starts with an unknown antigen attacking the synovial membrane and initiating a local inflammatory response, which quickly develops into chronic inflammation with cellular infiltrate recruitment and cytokine release. Eventually, pannus develops and irreversible deformities and damage to the joints occur [6,7]. However, the chronic inflammatory process does not occur in isolation. Contemporaneously, an acute inflammatory process occurs in the SF attracting activated phagocytes predominantly polymorphonuclear leukocytes into the area [6]. Activated monocytes, macrophages and neutrophils phagocytose the immune complexes in their attempt to resolve the inflammation, only to initiate the 'respiratory burst' phenomenon wherein reactive oxygen species (ROS) and free radicals (FRs) are produced via the NADPH oxidase system. Both ROS and FRs are considered oxidants and play a protective

role under physiological conditions, such as cell signaling and apoptosis, but can also be toxic in large quantities [8].

Oxidants and antioxidants occur in a state of redox; their levels are tightly controlled to maintain a normal biochemical balance. It has been suggested that an imbalance in the oxidant-antioxidant redox system, favoring the former, could be an important pathological phenomenon that plays a role in the development of chronic and autoimmune diseases such as RA [9]. Superoxide anion ($O_2^{\bullet-}$) is one of the main ROS produced during the pathogenesis process, and is ultimately converted into hydrogen peroxide (H_2O_2) and the highly toxic hydroxyl radicals (OH$\bullet$) [7]. The superoxide anion acts as the chief inflammatory agent that damages the endothelium of blood vessels, allowing the emigration of neutrophils into areas of inflammation [8]. Collectively, the cytotoxic ROS (also known as lipid peroxidation-inducing agents) target and degrade various biological molecules including membrane lipids, proteins, lipoproteins, DNA, hyaluronic acid, and cartilage [9-11]. The breakdown of membrane lipids yield several end-products, one being malondialdehyde (MDA) whose elevated levels have been reported in the serum and SF of RA patients.

This makes MDA a good indicator for lipid peroxidation (LPO) [11-15].

Several mechanisms have been suggested that account for the high levels of oxygen free radicals found in the SF of RA patients [11,16]. In their studies, Miesel and Zuber 1993 and Edmonds et al 1993 demonstrated that RA patients have significantly elevated XO levels when compared to healthy controls, proposing that the excess free radicals primarily stem from the xanthine oxidase system rather than a defect in the antioxidant system [17,18]. It has been shown that in patients with RA, the movement of the inflamed joints creates sufficient pressure to trigger transient ischemia in the superficial synovial membrane. This ischemia leads to articular cartilage damage and a gradual loss of function. In addition, the ischemia triggers the conversion of xanthine dehydrogenase (XOD), which normally utilizes NAD+ as an electron acceptor, into XO by proteolytic cleavage. XO then goes on to produce $O_2^{\bullet-}$ by converting hypoxanthine into xanthine, and eventually xanthine into uric acid. Moreover, during the ischemic period, RA joints consume excessive amounts of ATP which leads to the production of large amounts of purine metabolites. Subsequent reperfusion and oxygenation causes the conversion of the

purine metabolites into huge amounts $O_2^{\bullet-}$ and H_2O_2 [11, 17-21].

The accumulation of ROS leads to oxidative stress, which is defined as the imbalance between the production of FRs and the body's anti-oxidant response that aimto neutralize and detoxify the harmful effects generated by the FRs. Therefore, an antioxidant is any agent that can prevent the oxidation process or is capable to scavenge FRs. Physiologically, the harmful chain reactions generated by endogenous ROS such as XO, NADPH oxidase, and cytochrome P450 are balanced by enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants such as glutathione, vitamin E (tocopherol), and retinolby converting them into harmless derivatives. Enzymatically, SODis the first-line antioxidant defense agent that catalysis the dismutation of $O_2^{\bullet-}$into O_2, and CAT works by detoxifying H_2O_2 by converting it into water and oxygen[22, 23-25]. Non-enzymatically, antioxidants such as vitamin E (α-tocopherol), a lipid soluble vitamin predominantly found in the inner hydrophobic cell membrane, principally protects against oxidant induced membrane injury. Dietary intake of vitamin E has been shown to modulate the activity of the antioxidant defense system by directly

impacting the oxidative reactions occurring in specific cells and tissues [26].

Several researchers have studied the relationship between oxidative stress, the initiation of the inflammatory response, and the structural damage caused by ROS in RA but their results have been inconsistent [27-29]. The degree of enzymatic and non-enzymatic antioxidant defense in RA patients has also been studied but demonstrates inconsistent results as well. This study aims to investigate the activity of XO and MDA as sources of oxidative stress, as well as the enzymatic and non-enzymatic antioxidant activity by measuring SOD, CAT and vitamin E and comparing their levels in the serum and SF of RA patients.This provides important data which can be used to develop novel therapeutic agents and biochemical markers for RA.

MATERIALS AND METHODS

The aim is to measure the levels of XO, MDA, SOD, CAT and vitamin E in serum and SF of RA patients. In total, 146 RA patients (68 males and 78 females) and 136 healthy volunteers (65 males and 71 females) were recruited into the study after focused histories and physical examinations were taken in various out-patient rheumatology clinics in Amman, Jordan. The healthy volunteers were set as control. All controls

and RA patients were matched for age, sex, and body mass index (BMI). Informed consent was obtained from the participants after the aims and objectives of the study were explained. Blood and SF samples were obtained. The study was conducted in accordance to the standards of ethics outlined in the Declaration of Helsinki.

All RA patients had to fulfill the revised American Rheumatism Association 1987 criteria for rheumatoid arthritis [30], had to be between the ages of 30 and 50 years, have a BMI between 18.5 and 39.9, and have a positive rheumatoid factor (RF) to be included in the study. Female patients included in the study had not been taking any form of oral contraception for at least 6 months prior to the collection of samples.

RA patients excluded from the study were patients with negative RF, patients receiving disease modifying anti-rheumatic drugs, patients receiving corticosteroid treatment, patients consuming any antioxidant agents, patients with gout or osteoarthritis, patients with history of chronic disease such as diabetes mellitus and liver diseases, and patients that are current smokers or alcohol drinkers.

Sample collection and preparation

From the RA patients and controls, 5-10ml of blood was collected in anticoagulant free test-tubes and left to clot for 30 minutes at room temperature. Blood samples were then centrifuged at 3000 G for 15 minutes at 4°C. The sera were then separated and stored at -70°C until needed for analysis but for no longer than one month. From RA patients, SF samples were collected in anticoagulant free test-tubes using needle aspiration and immediately cooled using ice. The SF samples were then centrifuged at 3000 G for 30 minutes. The supernatants were then separated and stored at -70°C until needed for analysis but for no longer than one month. At the time of analysis, the serum and SF samples were slowly thawed back to room temperature and tested.

It was not possible to obtain SF levels for XO, MDA, CAT, SOD and vitamin E from healthy participants (controls). This is because it was determined unethical to obtain SF samples from healthy controls as the procedure is invasive and carries its own risks.

Measurement of XO

The serum and SF levels of XO were measured using spectrophotometry by calculating the increase in absorbance at 293 nm through the formation of uric acid from xanthine using Prajda N 1975 method [31]. 10-50 mU/ml standard XO solution (Sigma X- 1875) was used to create a calibration

curve.1 μmol of uric acid formed per minute at 37°C and pH 7.5 was considered as one unit of activity. Results were expressed in U/ml.

Measurement of MDA

The serum and SF levels of MDA were measured using the Ohkawa et al 1970 method [32]. Fluorometry was used to measure 2-thiobarbituric acid (TBA). Results were expressed in nmol/ml.

Measurement of CAT

The serum and SF levels of catalase were measured usingthe Abei Kinetic method [33]. The activity of catalase was measured spectrophotometrically by measuring the degradation of H_2O_2. Results were expressed in IU/L.

Measurement of SOD

The serum and SF levels of SOD were measured using the Sun et al 1988method [34]. The reduction of Nitrobluetetrazolium (NBT) was inhibited by oxygen generated from xanthine/ xanthine oxidase. The amount of enzyme causing 50% NBT inhibition reduction rate was considered as one unit of SOD activity. Results were expressed in U/ml.

Measurement of vitamin E

The serum and SF levels of vitamin E (α-tocopherol) were measured using the Baker and Frank 1980 method [35]. Ferrous ions were reduced forming a red complex with α-α1 dipyridyl and measured. Results were expressed in mg/dl.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) was used for statistical analysis in this study. All values represent the mean ± standard deviation (SD) and P<0.05 was considered statistically significant. Pearson Correlation Coefficients were used to calculate the relationships between variables.

RESULTS

As shown in Table 1, the mean age of male and female RA patients and their BMI were comparable with the controls. The duration of disease and DAS28 scores were comparable between male and female RA patients.

As shown in table 2, the serum XO and MDA levels were significantly higher in male and female RA patients when compared to their comparable controls. On the other hand, serum catalase, SOD and vitamin E levels were significantly lower in male and female RA patients when compared to their control.

Serum XO and MDA levels were significantly higher in female RA patients when compared to male RA patients, while serum SOD levels were significantly lower in

female RA patients when compared to their male counterparts.

Serum catalase was lower and serum vitamin E was higher in female RA patients when

compared to male RA patients however, these differences are not significantly different.

Table 1: Demographics and biochemical/DAS28 RA activity of RA patients and controls

Variables	Males		Females	
	Controls	RA patients	Controls	RA patients
	n=65	n=68	n=71	n=78
Age (years)	42.4 ±6.6	40.8 ±8.8	41.8 ±7.5	41.7 ±5.8
BMI (Kg/m2)	28.2 ±7.6	27.9 ±9.1	27.9 ±8.9	27. 6 ±8.8
Duration of disease (years)	-	7.7 ±3.9	-	7.4 ±4.7
DAS 28 score	-	5.8 ±0.9	-	6.1 ±1.1
ESR mm/h	11.2 ±5.6	55.6 ±35.9a,b	15.6±6.2	61.8 ±33.6a,b
CRP mg/L	2.91 ±1.4	13.16 ±4.8a,c	3.21 ±2.6	16.8 ±5.7a,c
Serum rheumatoid factor (IU/ml)	0/0	45/13	0/0	47/13

BMI: Body Mass Index (BMI = weight in Kg / (height in m)^2); DAS 28: Disease Activity Score 28; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein. Values in the table represent the mean ± standard deviation (SD).
a $P<0.0001$: significantly different from corresponding control.
b $P<0.0001$, c $p\leq0.001$: significantly different from opposite gender.
All RA patients were found to be IgM-RF seropositive measured by latex agglutination. Female RA patients had significantly higher ESR and CRP when compared to male RA patients.

Table 2: Level of oxidants and antioxidants in the sera of RA patients and controls

Parameters	Males		Females	
	Controls	RA patients	Controls	RA patients
	n=65	n=68	n=71	n=78
Serum XO U/ml	0.95 ±0.14	2.61 ±0.65a,c	1.09 ±0.23	3.14 ±0.82a,c
Serum MDA nmol/ml	1.96 ±0.70	3.31 ±0.81a,d	2.05 ±0.86	4.10 ±0.92a,d
Serum catalase IU/l	4.31 ±0.65	3.26 ±0.89a	4.27 ±0.91	3.18 ±1.14a
Serum SOD U/ml	5.11 ±1.02	4.21 ±0.81a,d	4.99 ±1.01	3.41 ±0.71a,d
Serum Vitamin E mg/dl	0.91 ±0.11	0.73 ±0.14b	1.05 ±0.16	0.76 ±0.63b

XO: Xanthine Oxidase; MDA: malondialdehyde; SOD: superoxide dismutase. Values in the table represent the mean ± standard deviation (SD)
a $P<0.0001$, b $P<0.001$: significantly different from corresponding control.
c $P<0.001$, d$P<0.01$: significantly different from opposite gender

Table 3: Levels of oxidants and antioxidants in the SF of RA patients

Variables	Male RA patients	Female RA patients	P value
	n=68	n=78	
XO U/ml	3.11 ±0.70	3.94 ±0.86	0.01
MDA nmol/ml	4.11 ±0.83	4.89 ±0.99	0.001
Catalase IU/l	3.00 ±0.81	2.70 ±1.08	0.05
SOD U/ml	3.22 ±1.04	2.50 ±1.00	0.01
Vitamin E mg/dl	0.72 ±0.15	0.71 ±0.14	NS

XO: Xanthine Oxidase; MDA: malondialdehyde; SOD: superoxide dismutase; NS: Not Significant. Values in the table represent the mean ± standard deviation (SD). The P values represent the statistical significance between male and female RA patients
The SF levels of XO and MDA were significantly higher in female RA patients when compared to male RA patients. Conversely, the SF levels of SOD and catalase were significantly lower in female RA patients when compared to male RA patients. There were no significant differences in the levels of vitamin E between male and female RA patients.

Table 4: Levels of oxidants and antioxidants in the serum and SF of RA patients

Variables	Male RA patients n=68		P value	Female RA patients n=78		P value
	Serum	SF		Serum	SF	
XO U/ml	2.61 ±0.65	3.11 ±0.70	0.01	3.14 ±0.82	3.94 ±0.86	0.01
MDA nmol/ml	3.31 ±0.81	4.11 ±0.83	0.001	4.10 ±0.92	4.89 ±0.99	0.001
Catalase IU/l	3.26 ±0.89	3.00±0.81	0.05	3.18 ±1.14	2.70 ±1.08	0.05
SOD U/ml	4.21 ±0.81	3.22 ±1.04	0.001	3.41 ±0.71	2.50 ±1.00	0.01
Vitamin E mg/dl	0.73 ±0.14	0.72 ±0.15	NS	0.76 ±0.63	0.71 ±0.14	NS

XO: Xanthine Oxidase; MDA: malondialdehyde; SOD: superoxide dismutase; SF: Synovial Fluid; NS: Not Significant. Values in the table represent the mean ± standard deviation (SD). The P values represent the statistical significance between serum and SF of RA patients.
The levels of XO and MDA in the SF of male and female RA patients were significantly higher when compared to their serum, whereas catalase and SOD levels were significantly lower in the SF of male and female RA patients when compared to their serum. There were no significant differences in the levels of vitamin E between the serum and SF of male and female RA patients.

DISCUSSION

Rheumatoid arthritis is an autoimmune disorder characterized by chronic hypertrophic synovitis, the hallmark of which is the destruction of connective tissue and the functional damage of cartilage and boney structures [3, 23, 36]. Its etiology remains unknown [17, 37, 38]. This study investigated the changes in the oxidant levels of XO and MDA and the antioxidant levels of SOD, CAT and vitamin E in the serum and SF of RA patients. The study also investigated the differences in the serum and SF levels of oxidants and antioxidants between male and female RA patients and the difference in the levels between the serum and SF for each gender. Male and female RA patients in this study have been confirmed to have active RA disease demonstrated by their DAS28 scores and

their elevated serum ESR, CRP and IgM RF levels [37-39].

It is well recognized that RA is an autoimmune disorder [11], but various studies also report a pathological increase in ROS production and a pathological decrease in the removal of oxidative stress [23]. Consequently, increased oxidative damage has been reported in the SF of RA patients [16, 18,19]. Several ROS have been identified in the SF of RA joints, one of the major ones being $O_2^{\bullet-}$ anion [11, 12, 15, 16, 40-43]. The main enzymatic source of $O_2^{\bullet-}$ anion radical is XO which cleaves $O_2^{\bullet-}$ from the conversion of hypoxanthine into uric acid. Since XO cleaves different ROS and is also part of the purine and adenosine metabolism, it has been identified as a major source of oxidative stress in RA [11]. It is worthy to note, however, that XO activity in healthy subjects does not account for a large

proportion of the total ROS produced naturally [44].

The results of this study show that male and female RA patients have significantly elevated XO levels in their serum when compared to the level in the healthy control. Furthermore, female RA patients had significantly higher XO levels in their serum and SF when compared to the levels in their male counterparts, and there is a significant increase in the SF levels of XO when compared to the serum in both male and female RA patients. This is consistent with other studies [11, 15, 38, 45].The increase in XO levels in the serum and SF of RA patients could be due to an increase in systematic production or a decrease in clearance. XO could also be elevated due to an increase inlocal production through the synovial cells of the inflamed joints, or through the phagocytic cells attacking the synovium [46, 47]. In addition, it has been shown that the disordered mitochondrial oxidative phosphorylation in RA patients leads to a reduction in toxic FR production through the xanthine/ XO system, accumulating adenosine and its breakdown products, xanthine and hypoxanthine. These accumulations then act as substrates for the available XO, increasing the enzyme's activity. As a result, it can be said that the

production of oxygen free radicals in this case is in part due to the actions of XO and that the significantly elevated XO levels in RA patients when compared to control indicate increased purine metabolism [48, 49].Regarding the significant increase in the serum and SF levels of XO in female patients when compared to their male counterparts, this could be explained by the female hormonal effect, notably estrogen, which plays an important role in the female physiology[50].In their study, Hutchinson et al 2001[51]demonstrated that three in four women with RA reported significant improvement in their symptoms when they were pregnant and a recurrence after delivery, suggesting that RA is indeed affected by hormonal status. This could also explain why female sex is a known risk factor for the development of RA.

ROS are unspecific and highly reactive; therefore, they can attack almost all biomolecules including lipid membranes. The oxidation of membrane poly-unsaturated fatty acids generates lipid peroxides which are capable of further lipid peroxidation through a free radical chain reaction [52]. Because MDA is a breakdown product of the peroxidation of long chain fatty acids, MDA accumulates as lipid peroxidation increases. Our study demonstrates that RA patients

have significantly higher serum MDA levels when compared to their control. Furthermore, our study shows a significant increase in the SF levels of MDA when compared to the serum in both male and female RA patients. Similar results have been previously reported [6-8, 11-13, 53-55]. The elevated MDA levels in RA patients represent heightened membrane lipid peroxidation secondary to the disease. This increase in lipid peroxidation can be the result of heightened FR generation pathways or suppressed natural scavenger processes. Since human SF contains small amounts of antioxidants such as catalase and SOD, phagocyte generated superoxide radicals and hydrogen peroxide in the inflamed RA joints would not be efficiently scavengered [6, 53-55]. This leads to elevated MDA levels in the SF representing increased in vivo lipid peroxidation. The high SF levels of MDA are subsequently able to escape into the serum causing an increase in serum MDA, also observed in our study. Similar results have been previously reported [12, 43, 45, 56-58]. Previous studies agree that oxidative stress is increased in the serum and SF of RA patients however, conflicting results were found regarding serum and SF antioxidant levels, with some authors reporting an increase while others reporting a decrease or no

activity [28, 43,59, 60].Despite that, it is widely acknowledged that SOD enzyme acts as the first-line defense molecule against ROS, removing $O_2^{\bullet-}$ by catalyzing the dismutase reaction [20,21,43,61,62].Current literature demonstrates variable levels of SOD in the serum and SF of RA patients when compared to the levels in the healthy control, with some authors reporting no difference [11,53] and others reporting increased levels in RA patients [19,43,45,61]. However, some of the previous studies report significantly lower serum SOD levels in RA patients when compared to healthy control [13, 53, 60, 63-65, 66], which is consistent with our findings. The decrease in the levels of SOD is thought to represent a degradation process in which SOD is degraded due to the detoxification process of free radicals. On the other hand, other studies propose that the decrease in SOD is due to enzyme inhibition by hydrogen peroxide, indicating an elevated superoxide anion production, or due to the disease itself having an inhibitory effect on SOD, suppressing its synthesis [53]. In addition, our study demonstrates significant decrease in the SF levels of SOD when compared to the serum in both male and female RA patients. This perhaps demonstrates the heightened antioxidant response occurring in the SF of RA patients,

reducing the SF levels of SOD lower than the serum.

One important byproduct of SOD activity is hydrogen peroxide which needs to be detoxified by catalase and other enzymes. Catalase protects the cells from the toxic buildup of hydrogen peroxide by converting H_2O_2 into water and oxygen [63]. Our current study demonstrates a significant reduction in the serum CAT levels in RA patients when compared to the levels in the healthy control. Similar results have been previously reported [12, 20, 59,61,64-66]. The decrease in serum levels of CAT in RA patients can be explained by the role H_2O_2 plays in the inactivation of the enzyme itself. This demonstrates that a reduction in CAT plays an important role in the increased oxidative stress and therefore the rheumatic process experienced by RA patients. On the contrary, other studies demonstrated no CAT activity in the serum and SF of RA patients [43, 66, 67]. In addition, our study demonstrates that the SF levels of CAT in female RA patients were significantly lower when compared to their male counterparts, which could also be explained by the female hormonal effect previously mentioned.

The serum and SF levels of vitamin E, a component of the non-enzymatic antioxidant system, were also studied. A significant decrease in the serum level of vitamin E was observed in RA patients when compared to their control, indicating increased oxidative damage. This is consistent with other studies [15, 26, 68]. Furthermore, our study demonstrates that there is no significant reduction in the SF levels of vitamin E in of RA patients when compared to their serum. Vitamin E is well recognized for its antioxidant properties, it works by trapping free radicals and slowing down the chain reaction responsible for cellular damage [12, 22, 25, 68]. When there is increased oxidative stress, vitamin E is recruited to try and reduce the amount and deleterious effects of the excess ROS, thereby reducing their endogenous levels. Helmy et al 2001 [68] demonstrated that high-dose vitamin E supplements taken alongside conventional RA medications decreases disease activity demonstrating the powerful antioxidant properties provided by vitamin E.

Despite that the amount of oxidative stress experienced by RA patients is affected by the degree of inflammation present, results from this study confirm that lipid peroxidation and oxidative stress is increased in patients with active RA and their antioxidant systems are depressed. Therefore, the detection of oxidant and antioxidant levels in the serum may be considered an accurate reflection of

articular SF changes and potential good biomarkers for disease progression in RA patients. However, this argument cannot be fully supported using results from this study as control SF samples were not obtained for ethical reasons. Nonetheless, Chaturvedi et al 1999 [69] demonstrated in his study that MDA is an effective biomarker of tissue oxidative damage in RA patients. This is supported by the suggestion that oxidants can leak from the SF into the blood, making their serum levels a good reflection of the damage occurring in RA joints. Further research is needed to confirm a useful relationship between serum oxidants and antioxidants and articular damage, and their potential use as biomarkers for disease status. Control SF samples will be needed and oxidant and antioxidant levels should be compared to conventional biomarkers such as CRP and RF to assess their importance and value.

Results from our study also suggest that antioxidants, which are capable of initiating and improving the antioxidant defense pathway, could be used as therapeutic agents to reduce peroxidation and thus the severity of disease. Several studies have recognized the therapeutic benefit provided by antioxidants, notably SOD, and several clinical trials recommend using antioxidant supplements alongside conventional treatments, but results remain inconstant. Documented improvement in disease progression using antioxidants demonstrates the importance of antioxidants in reducing oxidative stress [70, 71]. Furthermore, the increase in serum and SF levels of XO observed in this study suggest that XO inhibitors could be used as therapeutic agents in RA patients to reduce inflammation. Further research is needed to reveal the therapeutic potential of oxidants and antioxidants in managing RA.

CONCLUSION

In conclusion, our current study demonstrates that RA patients experience heightened oxidant response and suppressed antioxidant defense in their serum and SF, demonstrated by the elevated XO and MDA levels, and the decrease in SOD, CAT and vitamin E. This provides evidence supporting the involvement of the oxidant-antioxidant system in the pathogenesis of RA that has been previously disregarded. Furthermore, the study demonstrates that female RA patients experience higher levels of oxidative stress when compared to their male counterparts, and changes in the levels of oxidants and antioxidants are greater in the SF when compared to the serum. Our data provides the foundation for further research

needed to unfold novel therapeutic agents and biochemical markers for RA.

ACKNOWLEDGMENTS

ETHICS

Participants were recruited from different rheumatology outpatient clinics in Amman, Jordan. This study conforms to the declaration of Helsinki regarding research involving human subjects and approved by the Ethics Committee of the University of Petra (HNR-7-2015). Written informed consent was obtained from all individuals prior to the participation in the study.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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