IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 3, Issue 12, Dec 2015, 45-54

THE ROLE OF OXIDATIVE STRESS IN INFLAMMATION IN PATIENTS WITH RHEUMATOID ARTHRITIS IN THI-QAR PROVINCE

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ABSTRACT

The aim of this study isto determine the level of blood markers of cellular oxidative stress and then to establish the oxidative profile in 145 patients with rheumatoid arthritis (RA).The samples of the study include patients with RA. They were achieved four or more of the criteria of the 2010 American College of Rheumatology, the sample of the study was conducted to fifty persons apparently healthy volunteered to perform the test of this study. We determined the plasmatic levels of malondialdehyde, compared with the inflammatory parameters.

Results: in comparison to controls, patients with juvenile rheumatoid arthritis presented high concentrations of lipid per oxidation products (determined by plasmatic levels of malondialdehyde). concluded our results which indicate the presence of molecular damage determined by oxygen free radicals in patients with rheumatoid arthritis.

KEYWORDS: Free Radicals, Inflammation, Malondialdehyde (MDA), Rheumatoid Arthritis, ROS

INTRODUCTION

Rheumatoid Arthritis (RA) is chronic systemic autoimmune disease primarily affecting peripheral joints and leading ultimately to joint deformation. in RA ,multiple joints are usually inflamed in a symmetrical pattern and symptoms in the course of disease include fever, fatigue, pain, stiffness in joints and weight-loss (Lipsky,2005).

Oxidative stress is imposed on cells as a result of one of three factors the first is an increase in oxidant generation, the second is a decrease in antioxidant protection, and the third which is a failure to repair oxidative damage. Cell damage is induced by reactive oxygen species (ROS). The main source of ROS in vivo is aerobic respiration, although ROS are also produced by peroxisomal β-oxidation of fatty acids.

The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential protein, and DNA. Additionally, oxidative stress and ROS have been implicated in disease states, such as rheumatoid arthritis (Hitchon, & El-Gabalawy 2004.),Free radicals are chemical species that have single unpaired electron in an outer orbit. Energy created by this unstable configuration is released through reactions with adjacent molecules, such as inorganic or organic chemical –proteins, lipids, carbohydrate, nucleic acids- many of which are key components of cell membranes and nuclei(Rice-Evans, 1995).

Malondialdehyde is an aldehyde (3 carbon molecules with two aldehyde group)(Cighetti*et al.*,2001). It is considered to be the terminal compound and the most important marker for monitoring lipid peroxidation and oxidative

damage induced by reactive oxygen species (ROS) (Kose*et al*, 2001), oxygen free radicals may be related to, for example, the pathogenesis of synovitis. Cells found in inflamed joints (macrophages, neutrophils, lymphocytes, and endothelial cells), are capable of producing oxygen free radicals. These radicals, in the presence of lipid, DNA, protein, carbohydrate, or proteoglycan molecules can cause oxidative injury In this sense, it is also well-known that there is a relation between oxygen free radicals and damage of articular cartilage (Sevanian, &Hochstein 1985).

Neutrophils killing of microorganism occurs through combination of oxidative and non-oxidative killing ,oxidative killing also known as the respiratory burst ,is mediate by the NADPH oxidase enzyme complex ,with convert oxygen into reactive oxygen species such as hydrogen peroxide and superoxide that are lethal to microorganisms.By free radicals, macrophages and cytotoxic lymphocytes Killing of micro-organisms and cancer cells. But overproduction of free radical from Phagocytes by consistent of inflammation, action of cytokines and acute phase protein can cause occurrence of cells damage. In addition pro-inflammatory cytokines produced at the site of injury have profound systemic effect IL-1,IL6-TNF, act on hypothalamus to raise the temperature set-point stimulate the production of acute phase proteins by the liver such as C reactive protein (Davidson 2007; Robbins *at el* ., 2010).Early infiltration of phagocytic cells and increase in enzymes within the inflamed tissue such as cyclo-oxygenase, the MDA can be generated during cyclo-oxygenase (COX) catalysis in human platelets, forming from prostaglandin end operoxide (PGH_2) catalyzed by thromboxane synthase(and in liver cell(Plastaras*etal* ,2000)by break down of (PGH_2).

Aims of the Study

- Determining the level of hematological markers of cellular oxidative stress.
- Evaluating the role of MDA as a parameter of inflammatory with RA.
- Establishing the oxidative profile in patients with rheumatoid arthritis.

MATERIALS AND METHODS

Patients Group

This study was performed on 145 Iraqi patients with RA patients, who attended the consultant clinic for Rheumatology in Al- Husain Teaching General Hospital in the period from beginning September 2013 to end March 2014. The committee of rheumatologists performed the clinical examination under the supervision of staff in rheumatology unite.

Healthy Control Group

This study included sixty person apparently healthy individuals as a control group. , who have no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities,

The assessment of oxidative stress is achieved via the determination of the by product,Malondialdehyde was determined by a modified procedure described by (Guidet and Shah ,1989)

The test is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA_2product that absorbs strongly at 532 nm as follows:

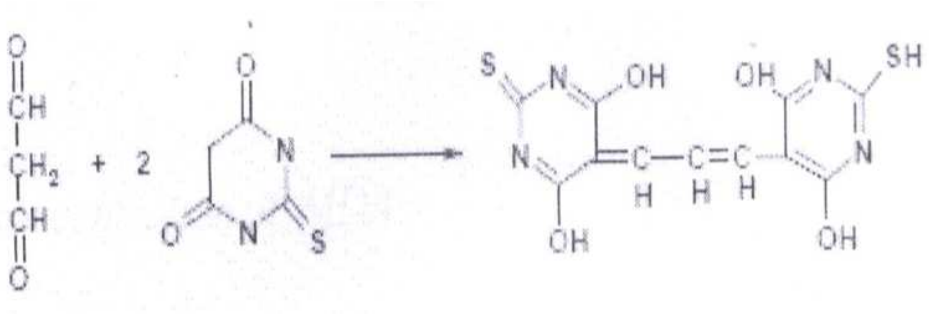

Figure 1

RESULTS

Table 1: Shown Distribution of the Studied Groups (Smoking Ra Patients, Non-Smoker Ra Patients and Apparently Healthy Control) According to Gender

Gender	Studied Groups		
	Smoking RA	Non-Smoking RA	Healthy Group
N Male %	33	15	24
	66%	16%	40%
N Female %	17	80	36
	34%	84%	60%
N Total%	50	95	60
	100.0%	100.0%	100.0%
F/ M ratio	0.5:1	5.3:1	1.5:1
Chi-Square	Value=29.37	df = 2	P-value0.00 HS

HS= Highly Significant difference (P≤0.001).df: degree freedom

NS= Non Significant difference (P>0.05).

Table 2: Hematological Parameters in RA Patients and Control Group

parameter	subject	No of cases	Mean	F	df	P-value
ESR mm/hr	Smokerspateints	50	47.162±11.105	139.73	2 1 202 2 204 3	0.00 HS
	Smokersnon pateints	95	36.61±13.19			
	Control	60	14.75±1.68			
WBC Total count	Smokerspateints	50	8.27±.867	23.42	2 1 202 2 204 3	0.00 HS
	nonmokers pateints	95	7.8017± .99171			
	Control	60	6.9992±1.11678			
No. of neutrophils	Smokerspateints	50	70.31±17.88	99.32	2 1 202 2 204 3	0.00 HS
	nosmokers pateints	95	69.89±6.19			
	Control	60	44.89±11.66			
No. of monocytes	Smokerspateints	50	4.96±1.158	10.27	2 1 202 2 204 3	0.00 HS
	oSmokers pateints	95	4.54±.818			
	Control	60	4.18±.75			
No. of lymphocytes	Smokerspateints	50	27.4238±3.3	54.58	2 1 202 2 204 3	0.00 HS
	Non Smokers pateints	95	28.94±3.7			
	Control	60	22.42±4.28			
No. of platelets	Smokerspateints	50	292.63±38.79	92.30	2 1 202 2 204 3	0.00 HS
	Non smokers pateints	95	285.53±36.99			
	Control	60	211.65±34.44			

df; degree of freedom[1]dfBetween Groups ,[2]df Within Groups ,[3]dfTotal

HS= High Significant difference (P≤0.001). NS= Non Significant difference(P>0.05).

Table 3: Shown Concentration of MDA* in Subject Group Smoking, no Smoking, Healthy Group

Groups	N0.	% of No.	Mean of MDA Concentration	Minimum	Maximum	P Value
**patients smokers	50	24.4%	1247.17±400.58	105.1280	1499.9867	HS .000
**patients non-smokers	95	46.3%	1226.42±191.10	105.1280	1467.3345	
Healthy	60	29.3%	667.10±202.78	281.4106	990.3846	
Total	**205**	**100.0%**	**1067.77±366.18**	**105.1280**	**1499.9867**	

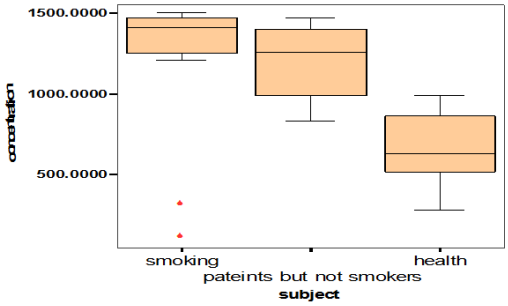

Figure 2: Shown the Comparison between Studied Group to Detection Concentration of MDA in Sera of RA by Box Plot Graph

Table 4: Shown Concentrations of MDA Comparison between Studied Group According the Ages and the Gender

Group	No	Percentage	Mean of MDA Concentration	Result
male	48	33.1%	1185.21±.406.60	Sig .144
female	97	66.9%	1257.50±187.41	df143
Total	145	100.0	1233.57±280.25	T -1.468
(1-20) age group	23	15.9%	1035.41±155.79	
(21-40) age group	71	49.0%	1235.33±286.73	Sig .000
(41-60) age group	35	24.1%	1398.69±82.17	df57 87 144
(>60) age group	16	11.0%	1149.44±433.62	F 24.521
Total	145	100.0%	1233.57±280.25	

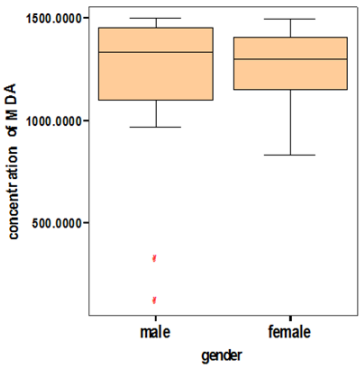

Figure 3: Shown the Comparison between Studied Group to Detection Concentration of MDA in Sera of RA by Box Plot Graph According the Sex

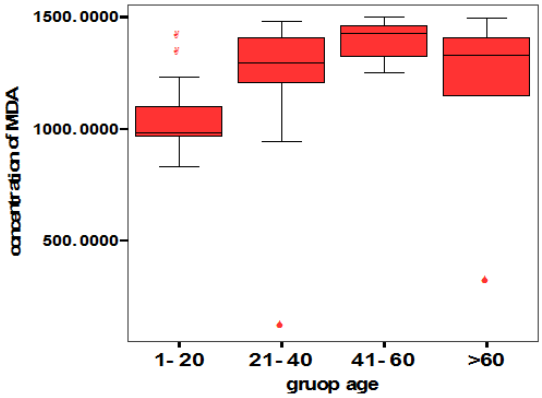

Figure 4: Shown the Comparison between Studied Group to Detection

Concentration of MDA in Sera of RA by Box Plot Graph According Age Groups

Table 5: Correlation Coefficient between Some Parameters (Pearson)

Parameters	MDA	ESR	WBC	No .of Neutrophils	No. of Monocytes	No. of Lymphocyte	No. of Platelets
MDA coefficient Significance(2-tailed) N	1 205	.581** .000 205	.224** .001 205	.496** .000 205	.183** .009 205	.356** .000 205	.487** .000 205
ESR Correlation coefficient Significance(2tailed) N	.581** .000 205	1 205	.423** .000 205	.603** .000 205	.325** .000 205	.443** .000 205	.576** .000 205
WBC Correlation coefficient Significance(2-tailed) N	.224** .001 205	.423** .000 205	1 205	.430** .000 205	.296** .000 205	.212** .002 205	.187** .009 205
No of neutrophils coefficient Significance(2-tailed) N	.496** .000 205	.603** .000 205	.430** .000 205	1 205	.118 .092 205	.303** .000 205	.474** .000 205
No of monocytes coefficient Significance(2-tailed) N	.183** .009 205	.325** .000 205	.296** .000 205	.118 .092 205	1 205	.195** .005 205	.057 .416 205
No of lymphocytes coefficient Significance(2-tailed) N	.356** .000 205	.443** .000 205	.212** .000 205	.303** .000 205	.195** .005 205	1 205	.430** .000 205
No of palettes coefficient Significance(2-tailed) N	.487** .000 205	.576** .000 205	.187** .007 205	.474** .000 205	.057 .000 205	.430** .000 205	1 205

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

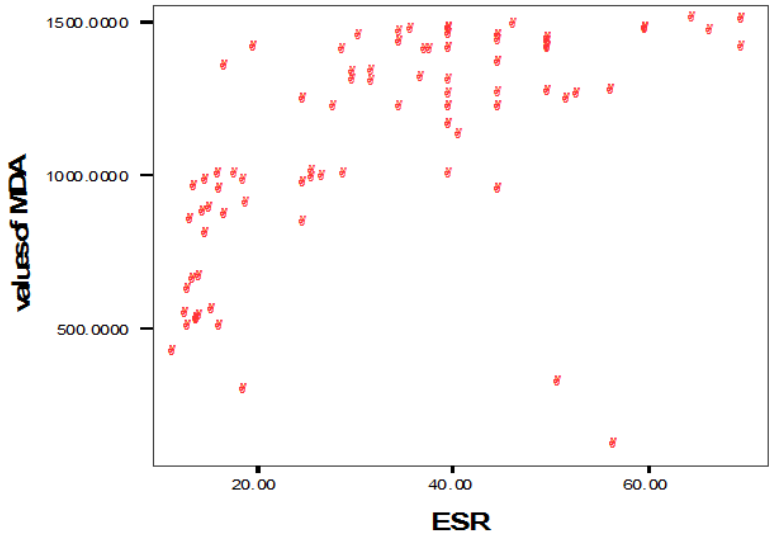

Figure 5: Box Plot Showing the Correlation between MDA Concentration and ESR Value

DISCUSSIONS

By analyzing the distribution, the results of studied group according to gender showed the majority of RA patients are females (66.9%) with a ratio of (5.2: 1) while the frequency of male group is (33.1**%**) as in table 1, the level of ESR is higher in RA patients than control group, though it cannot be given absolute conclusion for this result, since ESR is not specific test and it may increase significantly in so many pathological disorder (Davidson 2007) .

As well as we can show in result which described in table 2 were explain increase in total number of WBC , monocytes , neurophils, lymphocytes and platelets .This in correspondence goes with Phagocytes (eating cell) are specialized cell which ingest and kill microorganisms .scavenge cellular and infectious debris and produce inflammatory molecules which regulate other component of immune system .they include neutrophils ,monocytes and macrophage ,and are crucial for defense against invading organism , cellular debris and injury damage of collagen tissue.

The results also shown that mean ± SD of serum MDA are described in Table3 There was a statistically significant increase in serum MDA ($p \leq 0.001$) level in patients with rheumatoid arthritis in comparison with the control . In the present study the lipid per oxidation product MDA level has been increased significantly in sera of the patients with rheumatoid arthritis. The rise in MDA could be attributed to the increased generation of reactive oxygen species (ROS) owing to the excessive oxidative damage generated in these patients.

These oxygen species in turn have the ability to oxidize many other important biomolecules including membrane lipids. Data present in table4 show that the mean $\pm$SD of MDA concentration in serum of males group and females group of RA patient were (1185.21±.406.60nM) and (1257.50±187.41nM) respectively.

The results in table 4 showed that there is a significant elevation ($p \leq 0.001$) in serum MDA concentration of RA patients is highest level in (41-60) age group when we compare according the ages group,also shown in figure 3. These results of present study indicate higher oxidative stress in Rheumatoid arthritis patients, either due to increased extent of lipid per oxidation or due to decreased levels of antioxidants (Hooiveld, *etal* 2001). On the other hand the results in this

study showed no found a significant elevation ($p \leq 0.001$) in the concentration of MDA in serum of male RA patients as compared with female patients, but the female is higher than male group ,as shown in Fig 2. The increase of MDA concentration in female may be due to the difference in age. There were positive correlations between MDA levels and age. This findings show that peroxidative damage increases with the aging processes. Specific content of MDA product of lipid per oxidation was increasing with age and in male patient with RA and control. As well as the figure 1 shown the comparison between studied group to detection concentration of MDA in sera of RA is highest in smokers groups more than other groups.

In the present study the per oxidation product (MDA) level has been increased significantly in sera of the patients with rheumatoid arthritis this goes in correspondence with (Humad, *et al*, 1985; Ozkan*et al* 2006; Al-Saddi, 2007; Al-Maamory, 2008). The rise in MDA could be attributed to the increased generation of reactive oxygen species (ROS) owing to the excessive oxidative damage generated in these patients (Hitchon, & El-Gabalawy 2004.). These oxygen species in turn have the ability to oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA level have been reported in patients with rheumatic disease. In contrast to these results**,** reported that there is insignificant changes in MDA levels in patients with rheumatoid arthritis when compared to control group. Similar reports of elevated MDA level have given results similar to the results of the current study (Kose*et al* .,2001;Subdhi*et al* .,2001;Ozkan*et al* 2006;Al-Saddi,2007;Al-Maamory 2008;).

Deficiency as well as excess of MDAconcentrationhas been associated with neutrophil activation , and that will affect modulation of immune responses and susceptibility to infection, which considered as a cause in generation of free radicals (Cighetti*et al* 2001)., stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolismThe rate of sedimentation of erythrocytes was measured vertically during 1 hour. An increased tendency toward sedimentation of erythrocytes in shedding blood is observed in certain pathological conditions, particularly chronic inflammatory such as rheumatoid arthritis .To demonstrate the correspondence of ESR with, MDA, and serum lipid profile in rheumatoid arthritic patients. The positive correlation with high significantly difference regression analysis was used to evaluate the data which described in table 5.

This analysis indicated significant positive correlation for MDA values with ESR of rheumatoid arthritic patients ($r = +.581(**)$ $P \leq 0.001$) table 5, Figure 5 shows the increase in both concentrations of MDA and ESR, through the positive apparent correlation between them. These results could be explained depending on the fact that the activated inflammatory cells process lead to ROS production in RA a systemic autoimmune disease.

Acute inflammatory is the result of rapid and complex interplay between the cells and soluble molecules of the innate immune system .the classical external sign include heat .pain ,swelling .inflammatory processes is initiated by local tissue injury or infection , with early infiltration of phagocytic cells and in increase in enzymes with in the inflamed tissue such as cyclo- oxygenase and inducible (ROS .as result there is release of leukocytes ,prostaglandin histamine ,kinins ,anaphlyotoxins ,and nitric oxide the effect is vasodilation and increasing local vascular permeability thereby increasing flow of fluid and cells to the affected tissue .in in addition pro-inflammatory cytokines produced at the site of injury have profound systemic effect IL-1,IL6-TNF .act on hypothalamus to raise the temperature set-point stimulate the production of acute phase proteins by the liver. Increase activated inflammatory cells production of ROS in RA patients has been suggested by raised level of lipid peroxidation products degradation of hyaluronic acid by free radical mechanisms

oxidized low- density lipoproteins and increased carbonyl groups reflective of oxidation damage to proteins (Dai *et al.*, 2000). Oxidative damage to cartilage, extracellular collagen, , intracellular DNA. and destroy mitochondria .

The result in table 5 also demonstrated that there was found out appositive correlationbetween MDA and platelets r= .487(**).Significance(2-tailed) sig=0.00 .(P ≤0.001), this result goes correspondence with Subdhi*et al* 2002 whereas indicate that the MDA can be generated during cyclooxygenase (COX) catalysis in human platelets, forming from prostaglandin endoperoxide (PGH_2) catalyzed by thromboxane synthaseand in liver cell, by break down of (PGH_2) (Plastaras*et al* 2000) .

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