Evaluation of Oxidative Stress (MDA) and some Immunological Markers in Immune Response in Patients with Rheumatoid Arthritis at Al Nasiriya City, Iraq

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***Abstract*-** Objective: This study aimed to determine the level of serumindicators of cellular oxidative stress and the antioxidant the relevance with inflammations parameters, establishing the inflammatory profile in patients with rheumatoid arthritis.

Method: The sample of the study take in 50 patients with RA. They were confirming the dealings of the 2007 American College of Rheumatology,also the sample of the study include fifty person seemingly healthy volunteers were included in this study. We determined the plasmatic levels of malondialdehyde, compare with the inflammatory parameters such as CRP, ESR, RF,calculation of total WBCand diffraction numerous of WBC. In addition, the phagocytosis processes.

Results: in comparison to controls, patients with rheumatoid arthritis presented high concentrations of lipid peroxidation products (determined by plasmatic levels of malondialdehyde, as well as the study shown high signification between the studied groups according the immunological markers

Conclusion: our results indicate the presence of molecular damage determined by oxygen free radicals in patients with rheumatoid arthritis and this is play main role in immune response of patients with RA.

***Index Terms*-** C-reactive protein, free radicals ROS, MDA, neutrophil, phagocytosis, rheumatoid arthritis.

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic persistent systemic inflammatory polyarthritis of unknown cause, mainly affected synovial membrane of multiple joints, principally the peripheral joints in a symmetrical fashion such as those of fingers, resulting in cartilage destruction, bone erosion and joints deformities,Rheumatoid arthritis (RA)that causes inflammation and deformity of the joints. Other problems throughout the body (systemic problems) may also develop, including inflammation of blood vessels (**vacuities**), the development of bumps (called rheumatoid nodules) in various parts of the body, lung disease, blood disorders, and weakening of thebones (**osteoporosis**) (Christen *et al*., 2007)

Moreover, RA is a common chronic inflammatory and destructive arthropathy that cannot be cured and that has substantial personal, social, and economic costs. The long- term prognosis is poor: 20 percent of affected patients are disabled after 20 years (Pany G.1996). The median life expectancy of persons with RA is shortened by 3 to 7 years (Braunwald*et al.*,2001).

Diagnosis of rheumatoid arthritis

The diagnosis of RA is based on recognition of certain clinical and laboratory features which have been set out as revised by American Rheumatoid Association criteria. These criteria are developed by the AmericanCollege of Rheumatology in2007, (Geirson A.J.&Sturfelt G1987). Diagnosis of RA is made with four or more of the following:
Morning stiffness (> 1 hour)
Arthritis of three or more joint areas.
Arthritis of hand joints.
Symmetrical arthritis.
Rheumatoid nodules.
Rheumatoid factor.
Radiological changes.

Prognosis

About 15% of all RA patients will have symptoms for a short period of time and will ultimately get better, leaving them with no long-term problems. A number of factors are considered to suggest the likelihood of a worse prognosis. These include:
• race and gender (female and Caucasian).
• more than 20 joints involved.
• extremely high erythrocyte sedimentation rate.
• extremely high levels of rheumatoid factor.
• consistent, lasting inflammation.
• evidence of erosion of bone, joint, or cartilage on xrays.
• poverty.
• older age at diagnosis.
• rheumatoid nodules.
• other coexisting diseases.

Oxidative stress

Oxidative stress is carry out 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, microsomal cytochrome P_{450} metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. (Fiers W.*et*

al.,1999: Schafer, Buettner 2001). Under normal condition, ROS are cleared from the cell by the action of superoxide dismutase (SOD), catalase, or glutathione peroxidase (Hayes *et al.*, 1999).

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 CA, El-Gabalawy HS.) Alzheimer's disease (Christen Y.20000, Parkinson'sdisease)and thepathologies caused by diabetes.

Malondialdehyde MDA

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 K, Yazici, &Assioglu, 2001) It is also considered as a thio- barbituric acid reactive substance – Hong, Y.Yeh S.& Hu M. (2000).

Accumulation of oxygen-derived free radicals (oxidative stress)

Cell injury induced by free radicals, particularly reactive oxygen species, is in important mechanism of call damage in many pathologic conditions, such as chemical and radiation injury, ischemia-reperfusion injury (induced by restoration of blood flow in ischemic tissue), cellular aging, and microbial killing by phagocytes. 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. Moreover, free radicals initiate autocatalytic reactions, whereby molecules with which they react are themselves converted into free radicals, thus propagating the chain of damage. Reactive oxygen species (ROS) are a type of oxygen –derived free radical whose role in cell injury is well established. ROS are produced normally in cells during mitochondrial respiration and energy generation, but they are degraded and removed by cellular defense systems. Thus, cells are able to maintain a steady state in which free radicals may present transiently at low concentrations but do not cause damage. When the production of ROS increases or the scavenging systems are ineffective, the result is an excess of these free radicals, leading to a conditions called oxidation stress. Oxidative stress has been implicated in a wide variety of pathologic processes, including cell injury, cancer, aging, and some degenerative diseases such as Alzheimer disease. ROS are also produced in large amounts by leukocytes, particularly neutrophils and macrophages, as mediators for destroying microbes, dead tissue and other unwanted substances. Therefore, injury caused by these reactive compounds often accompanies inflammatory reactions, during which leukocytes are recruited and activated. **(Davidson 2007)**

In the following section, we discuss the generation and removal of ROS, and how they contribute to cell injury. The properties of some of the most important free radicals.

Generation of Free Radicals. Free radicals may be may be generated within cells in several ways. The reduction- oxidation reaction that occur during normal metabolic processes. During normal respiration, molecular O2 is reduced by the transfer of four electrons to H2 to generate two water molecules. This conversion is catalyzed by oxidative enzymes in the ER, cytosol, mitochondria, peroxisomes, and lysosomes. During this process small amounts of partially reduced intermediates are produced in which different number of electrons have been transferred from O2, these include superoxide anion (O2, one electron), hydrogen peroxide (H2O2, two electrons), and hydroxyl ions (OH, there electrons).

Absorption of radiant energy (e.g., ultraviolet light, x-rays). For example, ionizing radiation can hydrolyze water into OH and hydrogen (H) free radicals.Rapid bursts of ROS are produced in activated leukocytes during inflammation. This occurs by a precisely controlled reaction in a plasma membrane multiprotein complex that uses NADPH oxidase for tha redox reaction. In addition, some intracellular oxidases generate O2.

Enzymatic metabolism of exogenous chemicals or drugs can generate free radicals that are not ROS but have similar effects (e.g., CCI4 can generate CCL3.Transition metals such as iron and copper donte or accept free electrons during intracellular reactions and catalyze free radical formation, as in the Fenton reaction (H2O2+Fe2+---->Fe3+ +OH + OH-). Because most of the intracellular free icon is in the ferric (Fe3+) state, it must be reduced to the ferrous (Fe2+) form to participate in the Fenton reaction. This reduction can be enhanced by O2, and thus sources of iron and O2 may cooperate in oxidative cell damage.

Nitric oxide (NO), an important chemical mediator generated by endothelial cells, macrophages, neurons, and other cell types, can act as a free radical and can also be converted to highly reactive peroxynitrite anion (ONOO-) as well as NO2 and NO3

Removal of Free Radicals. Free radicals are inherently unstable and generally decay spontaneously. O2, for example is unstable and decays (dismutatse) spontaneously into O2 and H2O2 in the presence of water. In addition, cells have developed multiple non enzymatic and enzymatic mechanisms to remove free radicals and thereby minimize injury.

Antioxidants either block the initiation of free radicals. Examples are the lipid-soluble vitamins E and A as well as ascorbic acid and glutathione in the cytosol.

As we have seen, iron and copper can catalyze the formation of ROS. The levels of these reactive metals are minimized by binding of the ions to storage and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin), thereby minimizing the formation of ROS.

A series of enzymes acts as free radical-scavenging systems and breaks down H@O@ andO@. These enzymes and include the following:

1- Catalase, present in peroxisomes, decomposes H2O2 (2H2O2--> O2+2H2O.

2- Superoxide dismutases (SODs) are found in many cell types and convert O2 to H2O2 (2O2+2H--> H2O2+O2). this group includes both manganese –SOD, which is localized in mitochondria, and copper-zinc-SOD, which is found in the cytosol.

3- Glutathione peroxidase also protects against injury by catalyzing free radical breakdown (H2O2+2GSH-->GSSG (glutathione homodimer) + 2H2O, or 2OH+ 2GSH-->

GSSG+2H2O). the intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of the oxidative state of the cell and is an important indicator of the cell s ability to detoxify ROS.

Pathologic Effect of Free Radicals. The effects of ROS and other free radicals are wide-ranging, but three reactions are particularly relevant to cell injury:

Lipid peroxidation in membranes. In the presence of O2, free radicals may cause peroxidation of lipids within plasma and organelle membrane. Oxidative damage is onitated when the double bods inunaturated fatty acids of membrane lipids are attacked by O2- derived free radicals, particularly by OH. The lipid- free radical interactions, yield peroxides, which are themselves unstable and reactive, and an autocatalytic chain reaction ensues (called propagation), which can result in extensive membrane damage.Oxidative modification of proteins. Free radicals promote oxidation of amino acid side chains, formation of protein-protein cross-linkages (e.g. Disulfide bonds), and oxidation of the protein backbone. Oxidative modification of proteins may damage the active sites of enzymes, disrupt the confirmation of structural protein, and enhance proteasomal degradation of unfolded or misfolded proteins, raising havoc throughout the cell.

Lesions inDNA. Free radicals are capable of causing single and double strand breaks in DNA, cross –linking of DNA strands and formation of adducts. Oxidative DNA damage has been implicated in cell aging (discussed later in this chapter) and in malignant transformation of cells

The traditional thinking about free radicals was that they cause cell injury and death by necrosis, and in fact, the production of ROS is a frequent prelude to necrosis. However, it now clear that free radicals can trigger apoptosis as well. recent studies have also revealed a role of ROS in signaling by a variety of cellular receptors and biochemical intermediates. In fact, according to one hypothesis, the major actions of O2 stem from its ability to stimulate the production of degradative enzymesrather than direct damage of macromolecules. It is also possible jet these potentially deadly molecules serve important physiologic functions. (Robbin *et al*., 2011)

Malondialdehyde is a naturally occurring product of lipid peroxidations,itis a highly reactive three carbon dialdehyde product as byproduct of poly unsaturated fatty acid peroxidation (Janero,1990) and also during arashidonicacid metabolism for the synthesis of prostaglandin (Marnette ,1999). MDA can be generated during cyclooxygenase (COX) catalysis in human platelets, forming from prostaglandin endoperoxide (PGH_2) catalyzed by thromboxane synthase(Diczfalusy*etal*,, 1977) and in liver cell(Plastaras*etal* ,2000)by break down of (PGH_2).

Phagocytes. 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 bacterial and fungal infections.

Phagocyte express wide range of surface receptors that allow them to identify microorganisms. these pattern recognition receptors include the toll like receptors and mannose receptors. they recognize generic motif not present on mammalian cell. such as bacterial cell walls component s

Bacterial DNA and viral double –stranded RNA while phagocytes can recognise microorganism through pattern recognition receptors alone

Engulfment of microorganisms is greatly enhanced by opsonisation. Opsonins include acute phase proteins such as C-reactive proteins. antibodies and complement. theybind both to the pathogen and to phagocyte receptors. Acting as bridge between the two and facilitating phagocytosis**. (Davidson 2007)**

Neutrophils

Neutrophils. also known as polymorphonuclears leucocytes are derived from the bone marrow and circulate freely in the blood. they are short lived cells with a half-life at 6 hours .and are produced at rate of 10 cells daily.There function are to kill microorganism directly .facilitate they rapid transit of cell through tissue ,and nonspecifically amplify the immune receptors .this is mediate by enzymes contained in granules which also provide an intracellular milieu for infected cells trigger the local production of inflammatory molecules and cytokines .these stimulate the production and maturation of neutrophils in bone marrow .the the neutrophils are recruited to the site by chemo tactic agent and by change in the activated local endothelium .the transit of neutrophil through the blood stream is responsible for the rise in leucocyte count that occurs in early infection . once with in infected tissues, activated neutrophils through seek out and engulfinvading microorganism s

These are initially enclosed with in membrane bound vesicle whichfuse withcytoplasmic granules to from the phagolysosome. with this protected compartment, killing of organism 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 thatare lethal to microorganisms. Combined withmyeloperoxidase, hypochlorous ions (HOCl), analogous to bleach – are produced which are highly effective oxidants and antimicrobial agent **(Davidson 2007)**

Non-oxidative (oxygen- -independent killing occurs through the
Release of bacterial enzyme and lactoferrin. each enzyme has a unique antimicrobial spectrum. providing broad coverage against bacterial and fungi.

The process of phagocytosis depletes neutrophil glycogen reverse, and is followed by neutrophil cell death .as the cell die, their contents are released and lysosomal enzymes degrade collagen and other components of the interstitium, causing liquefaction of closely adjacent tissue. the accumulation of death and dying neutrophil result in the formation of pus which, ifextensive, may result in abscess formation. (Robbin *et al*., 2011)

Monocyte and macrophage

Monocyte are precursor of tissue macrophage. they are produced in the bone marrow and exported into the circulation. where they constitute about 5% of leucocyte. after 7-10 hours in the blood stream, they migrate to peripheral tissues where they differentiate into tissue macrophage and reside for long periods. specialized population of tissue macrophage include Kuffer cells in the liver. alveolar macrophage in the lung. meningeal cells in

the kidney, and microbial cells in brain. macro-killing of microorganism but also play important role in the amplification and regulation role of in inflammatory response unlike neutrophils macrophage do not die after killing pathogens. **(Davidson 2007)**

Acute inflammatory

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 nitric oxide synthase .as result there is release of leucocyte ,prostaglandin histamin,kinins,anaphlyo toxins,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 **(Davidson 2007)**

Acute phase protein

Acute phase proteins are product by liver in response to inflammatory stimuli and have a wide range of activities, C-reactive proteins (CRP)and serum amyloid A may be increase 1000-fold, contributing to host defense and stimulating repair and re generation.some of the chemical components role play in pro-inflammatory cascade to control of inflammation.by neutralizing the enzyme produced by activity neutrophils preventing widespread tissue destruction .in addition antioxidant, superoxide dismutase scavenges for oxygen free radicals, while increase level of iron –binding proteins such as transferrin and ferritin andlactoferrin which represented decrease in iron. at last the acute phase proteins increase in often the chronic phase.(Rubbin*etal*., 2011)

Material and methods

Patients Group: This group includes 50 patients with RA, who fulfilled four or more of the 2007 ACR criteria. These patients attended the rheumatology Department of Al- Hussein Teaching Hospital during the study period. The diagnoses of those patients were performed by the rheumatic disease consultant staff.

Their ages ranged between (17-68) years, 15 males and 25 females. Subjects suffered from any disease such as diabetes mellitus, hypertension, hypothyroidism, cardiovascular disease, oral contraceptive use, and liver disease which interferes with the data obtained, were excluded.

Control Group

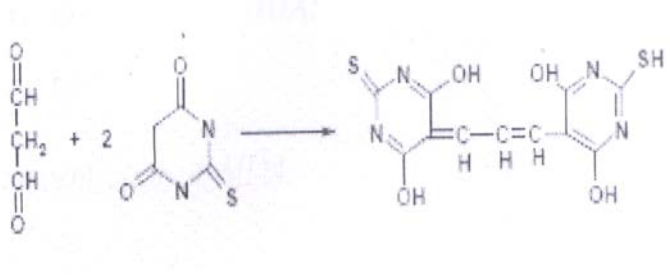
Fifty person apparently healthy volunteers were included in this study. They were matched in their sex and age with the patients groupinduded 50 33female and 17 male

malondialdehyde concentration in serum

The level ofmalondialdehydewas determined by modified procedure described by (Guidet,B.andShah, S.V.,1989)

Principle:

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



Determination of C-reactive protein(CRP)

The latex reagent is a suspension of polystyrene latex particles of uniform size with the IgG fraction of an anti-human CRP specific serum according to Ward and cooper,1975.

Biostatistician analysis

The results were expressed as mean ± standard deviation (M±SD) of mean. Statistical analyses were achieved by using Student′s t-test. Significant variation was considered when p-value was less than 0.05. The correlation coefficient was used to study various analyses

Result

Table 1:Demographic and some clinical and laboratory characteristics of RA patients

parameter	treatment	Control	p-value
Age (years)	45.8_+9.2	42.7_+4.8	NS
Gender female/male	38/12	33/17	NS
Body max index	24.5±1.4	25.3±1.9	NS
Duration of disease (years)	9.6 ± 6.3	8.1 ±7.5	NS
Morning stiffness (minutes)	34.7 ± 33.6	17.8 ± 23.5	0.004*
Tender joint count	5.7 ± 5.2	2.8± 3.3	0.007*

*p<0.01, NS: No significant.
Body mass index (BMI) kg/m

Table 2:parameters and inflammatory characteristics of RA patients and control group.

parameter	treatment	Control	P value
Hemoglobin(gm/dl)	11.35±1.57	14	HS
ESR mm/hr	56.7	20±0.006	HS
WBC total count	7.21±3.35	4.65± 1.76	HS
differential lymph	26.6± 4,55	19.07±1.4	HS
neutrophil	69.7±4.55	59.25±9.87	S
Monocyte	4.11±2.26	2.94± .45	S
Platelet	313.3±73	219±.12.75	S
RF	86.4±4,6	2.1±0	HS
CRP	26.4±6.6	1.4±o.6	HS
MDA concentration	1 .72±0.05	1.228 ± 0.0259	HS

Table 3: Malondialdehyde (MDA) concentration (µM) in serum of patients with RA and healthy control

Control	Patient with RA	Group	
17	15	n	**Male**
1.28016 ± 0.0201	1.9092 ± 0.0397	Mean ± SD	
33	35	n	**Female**
1.2023 ± 0.0252	1.6813 ± 0.0408 **	Mean ± SD	
50	50	n	**Total**
1.228 ± 0.0259	1.7952 ± 0.0402 **	Mean ± SD	

ANOVA: Patientsvs control; **= p<0.001, Femalevs male; ** = p<0.001

Table 4: CRP percent as $a(+/-)$ value concentration $(\rangle\langle 6mg/L)$ in serum of patients with RA and normal control

Control	Patient with RA	Group		
2	14	n	+Ve	**Male**
11.1%	93.3%	%		
15	1	n	-Ve	
88.9%	607%	%		
4	33	n	+Ve	**Female**
12.5%	91.7%	%		
19	3	n	-Ve	
87.5%	8.3%	%		
6	47	n	+Ve	**Total**
12.2%	92.2%*	%		
29	4	n	-Ve	
87.8%	7.8%	%		

ANOVA: patients Vs. control; * = p<0.01, +Ve ≥ 6 mg/L-Ve< 6 mg/L

II. Discussion

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease. The sample of the study includes 50 patients with RA. They wereachieved the criteria of the 2007 American College of Rheumatology. These patients entered the Al Husain Teaching Hospital at al Nasiriya city during the period between march 2016 and mid-April 2016. The diagnosis of these patients were performed under supervision of group of rheumatologists. The sample of the study include fifty person apparently healthy volunteers were included in this study.The objective of the study is the evaluation of Malondiladehade (MDA)concentration change. level of significant at (0.001>P at revealed a significant decrement level in sera of the patients compare with those of the malondialdehyde in the control group, and we show increase of total count of WBC especially

neutrophil and macrophage .as result of damage cell of oxidative B beta fatty acid and the formation of free radicals, which is secondary to the production of reactive oxygen species, is part of the process of aerobic metabolism. In this manner, cellular metabolism produces free radicals in physiological conditions. These active radicals, in turn, can be very useful.

More over the level of ESR is higher in RA patients than control group, (table 1) 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. As well as we can show in result which described in table 1 were explain increase in total number of WBC, monocytes, neutrophils, lymphocytes and platelets. This result goes in correspondence 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.

According to the table provided was demonstrated that high level of RF with high difference of significant. The group of RFs is among the only autoantibodies clearly shown to be involved in disease pathogenesis. RF can be detected in 60-80% of RA patients and in up to 15% of healthy individuals this results goes with Al-Salih 2014and Abdullah 2010.

Regarding both groups, the prevalence of RA among females (patients group) was (70%) while in male group of RA is (30%). This frequency is higher to some extent than that of local previous studies in Iraq mentioned by Tofiq (2007) (80.85%). While Al-Haidary (2003) and Abdul-Abbas (2007) noticed the lower percentage which reached to (70.7%) and (79.7%) respectively. However, it is clear that there is a gradual elevation in this frequency in the last years. This elevation may be due to environmental conditions beside the psychological situation of Iraqi people which result in high stress that enhance RA development. The prevalence of disease varies from one person to person and from time to time (Abdullah 2010).

The mean ± SD of serum MDA are described in (Table 3) There was a statistically significant increase in serum MDA in female gruop (p< 0.001) level in patients with rheumatoid arthritis in comparison with the control group with percent of (59.86%), while the level of serumof male (p< 0.001) significantly decreased in these patients compared to the control group with percent of (58.3%).

In the present study the lipid peroxidation 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 otherimportant biomolecules including membrane lipids (Surapneni&ChandrasadaGopan, 2008) Similar reports of elevated MDA level have been reported in patients with rheumatic disease. In contrast to these resultsKajanachumpol*et al.*,2000 reported that there are insignificant changes in MDA levels in patients with rheumatoid arthritis when compared to control group (Ozkan*et al.*,2006).

That is indicated a significant decrease in the levels of serum GSH (non enzymaticanti-oxidant) in patients with rheumatoid arthritis can be observed when compared to the control group. The decrease in the levels of this non enzymatic antioxidant parameter may be related to the increased turnover, for preventing oxidative damage in these patients suggesting an increase defense against oxidant damage in rheumatoid arthritis (Surapneni&ChandrasadaGopan, 2008). Similar reports of elevated MDA level have been reported which were consistent with the current result (Ozkan*et al*., 2006).

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 (Naeem*et al*., 2015)., stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. (Fiers,W.*et al.*,1999). Under normal condition, ROS are cleared from the cell by the action of superoxide (Hayes *et al.*,1999). Data present in table(3) show that the mean $\pm$ SD of MDA concentration in serum of males andfemalesRApatient were ($1.9092 \pm 0.0397\mu$ M) and ($1.6813 \pm 0.0408\mu$ M) respectively, while the Mean $\pm$ SD of MDA concentration in serum of control males and Females were ($1.28016 \pm 0.0201\mu$ M) and ($1.2023 \pm 0.0252\mu$ M) respectively.

The results in table (3.2) showed that there is a significant elevation (p<0.001) in serum MDA concentration of RA patients($1.7952 \pm 0.0402\,\mu$ M) compared with healthy controls ($1.228 \pm 0.0259\mu$ M).These results of present study indicate higher oxidative stress in Rheumatoid arthritis patients,either due to increased extent of lipid peroxidation or due to decreased levels of antioxidants (Paredes *et al.,* (2002). On the other hand, the results in this study showed a significant elevation (p<0.001) in the concentration of MDA in serum of male RA patients as compared with female patients, and also a significant elevation in serum MDA concentration (P<0.001) in male control as compared with femalecontrol. Theincrease of MDA concentration in male may be due to the difference in age. There were positive correlations between MDA levels and age. This finding show thatperoxidativedamage increases with the aging processes (Naeem et al.,2015).Specific content of MDA product of lipid peroxidation was increasing with age (Naeem*et al.*, 2015) and in male patient with RA and control.

Relevance of oxidative stress to rheumatoid arthritis

Oxidative stress has a role in the pathogenesis of RA. Epidemiologic studies have shown an inverse association between dietary intake of antioxidants and RA incidence (Cerhan*et al*.,2000), and inverse associations between antioxidant levels and inflammation have been found (Paredes *et al.,* 2002). Tissue injury releases iron, which is catalytic for hydroxyl radical production from hydrogen peroxide, is present in RA synovial tissue and is associated with poorer prognosis [66%-86%]. And increased oxidative enzyme activity along with decreased antioxidant levels in RA sera and synovial fluids

[66%-87%-66%-91%]. Because of the highly reactive nature of ROS, it is difficult to directly demonstrate their presence in vivo. It is considerably more practical to measure the 'footprints' of ROS and RNS, such as their effects on various lipids, proteins, and nucleic acid [66%-92%-66%-95%]Increase 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 []. Oxidative damage to cartilage, extracellular collagen, and intracellular DNA. Oxidative stress has been shown to induce T cell hypo responsiveness in RA through effects on proteins and proteosomal degradation

Data in (table 4) showed the negative and positive values of CRP between RA patients and healthy control.

The result showed a significant elevation ($p<0.01$) in concentration of CRP in patients RA. These results are in agreement with (PooleRobin ,2000)and contrary with (Elisteigefest ,2005). The high concentration of CRP in patients with RA compared with healthy control may be due to the systemic inflammatory feature in RA which demonstrated by small but significant elevation of serum CRP (Sharif*etal*,1997: Al-Salih 2014)

III. Conclusions

From the present study can conclude that:

1. High levels of MDA in sera of male with RA, as compared with female of RA.
2. High levels of MDA in sera of RA patient, while remaining within normal level IN control group.
3. A significant elevation in the activity of neutrophil and total WBC OF RA patients.
4. A significant decrease in HP and increase in ESR value andRF concentration of RA serum
5. A great increase in concentration of CRP in RA serum. These results are
6. in contrast to plasma total protein as main factor in inflammatoryof RA

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