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RHEUMATOID ARTHRITIS;

INCREASED LEVELS OF LIPID PEROXIDATION AND EXPRESSION OF PROPHETIC VARIABLES AND THEIR INTERPLAY TO DEVELOP RHEUMATOID ARTHRITIS.

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ABSRTACT… Objectives: Assessment of upstream levels of lipid peroxidation and DNA damage regulating the development of rheumatoid arthritis. **Data Source:** All the samples were collected from Jinnah Hospital Lahore. **Study Design:** Comparative cross sectional study. **Period:** Two years from 18-09-2014 to 24-10-2016. **Setting:** The Institute of Molecular Biology and Biotechnology (IMBB) in The University of Lahore-Pakistan. **Material and Methods:** Blood, saliva and synovial fluid of fifty (n=50) individuals diagnosed with Rheumatoid Arthritis and fifty (n=50) age-sex matched controls were added in current study. Levels of MDA were determined spectrophotometrically while the concentrations of isoprostanes, 8-OHdG and 4-HNE were measured by the help of commercially available Elisa Kit. **Results:** Levels of lipid peroxidation products including MDA (µmol/ml), isoprostanes (pg/ml), 4-HNE (µmol/ml) and DNA damage in the form of 8-OHdG (pg/ml) were significantly high ($p<0.015$, $p<0.022$, $p<0.004$ and $p<0.036$) in patients as relative to normal individuals. Levels of MDA in serum (1.95±0.094 vs. 0.95±0.019), saliva (0.012±0.0034 vs. 0.056±0.0056) and synovial fluid (3.26±0.65 vs. 0.019±0.0016) were differed significantly in each groups. Level of isoprostanes in serum (12.26±5.26 vs. 1.26±0.015), saliva (2.16±0.019 vs. 0.816±0.017) and synovial fluid (34.26±4.26 vs. 0.136±0.019) were recorded higher in RA patients as compared to control. Concentrations of 8-OHdG in serum (0.945±0.014 vs. 0.019±0.0035) saliva (0.0024±0.0003/ 0.0029±0.00017) and synovial fluid (1.33±0.451 vs. 0.055±0.0016) were recorded high in RA patients. Significantly higher concentration of 4-HNE in serum (4.265±1.25 vs. 1.99±0.016), saliva (1.26±0.15 vs. 0.191±0.0091) and synovial fluid (6.35±1.16 vs. 0.094±0.00165) were recorded in patients with RA. **Conclusion:** Present study concluded the role of oxidative biomarkers and their differential expression in the onset of autoimmunity in patients with RA. Increased stress is involved in the DNA damage and increased lipid peroxidation in the synovial fluid. Therefore, antioxidant therapy may have some prognostic role in the patients with RA by decreasing the intensity of oxidative stress and DNA damage.

Key word: Isoprostane, Lipid Peroxidation, MDA, Oxidative Stress, 8-OHdG, 4-HNE, RA.

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INTRODUCTION

Rheumatoid arthritis is an autoimmune disease characterized by hyperplasia, systemic chronic and erosive inflammation of synovial joints identified by proliferation of cells associated with synovial joints. Activation and infiltration of macrophages, memory T cells, plasma cells leads to permanent destruction of bone and cartilage.[1,2] Number of secretory products such as leukotrienes, proteases, prostaglandins, hydrolases components of complement system and free reactive oxygen redicals are present in rheumatoid synovium. The extra articular manifestations associated with RA including inflammation in lungs and heart, vasculitis and peripheral neuropathy. RA cause an significant increase risk of other diseases including renal disorders, cardiovascular disease, intestinal abnormalities, pulmonary dysfunction and a major cause of premature deaths.[3] In world population, RA has prevalence of about 1%.

Interplay of genetic and environmental factors is usually associated with implications of RA.

Identification of genetic and environmental factors associated with the development of RA may allow for early diagnosis of patients and implications of preventive measures. Other risk factors include smoking, obesity, vitamin D deficiency, menstrual disorders, periodontal diseases and many others.[4] Imbalance between pro-oxidant and antioxidant results in increase oxidative stress that is usually involved in the pathogenesis of different human diseases. Several lines of biochemical evidences indicate oxidative damage mediated by reactive oxygen species in RA. A number of studies have indicated inverse relation between dietary intake of antioxidants and RA occurrence.[5] Several studies on RA synovial fluid and sera have demonstrated decreased antioxidant level and increased oxidative enzyme activity. Reactive oxygen species (ROS) are highly reactive so their presence in vivo is difficult to demonstrate. So the measure of effects of ROS and RNS on lipids, proteins and DNA is more practical.

Oxidation of lipids cause accelerated atherosclerosis in RA patients. Elevated level of local and systemic inflammatory response and production of cytokines mediate lipolysis and release of free fatty acids that result in dyslipidemia. Lipid peroxidation derived DNA damage has been reported in rheumatoid arthritis patients.[6] Reactive oxygen and nitrogen species cause either DNA strand breakage or damage to individual nucleotide base damage. Hydroxyl radical react with deoxyguanosine and DNA adducts are formed in the form of 8-oxo-7-hydro-deoxyguanosine and its elevated level is reported in the sera of RA suffering patients. Lipid peroxidation induces a number of end products including malondialdehyde (MDA), isoprostane and 4-hydroxynonenal (4-HNE) and its increase level has been demonstrated into synovial fluid and sera of RA patients.[7]

MATERIAL AND METHODS

Current study determined an association in between oxidative stress and RA. Fifty (n=50) patients diagnosed with rheumatoid arthritis and fifty (n=50) age-sex matched controls were selected for the study. Blood, saliva and synovial fluid samples were collected at Jinnah hospital Lahore from anti-cubital vein of each participant. 5ml blood sample was centrifuged for the separation of serum and stored at -70□C for future assays. All experimental protocols were approved by Institutional Review Board (IRB) of The University of Lahore.

Biochemical Analysis

Levels of MDA in serum, saliva and synovial fluid was estimated spectrophotometrically by using the methods of Ohkawa et al.[8] in each test tube, 200µl sample was taken then 200µl of 8.1% SDS, 1.5ml of 20% acetic acid, 1.5ml of 0.8% TBA were added and heated the solution for 1 hour. 4ml n-butanol was added in each test tube after cooling and centrifuged at 3000rpm for 10 minutes. The upper organic layer was separated and absorbance was taken at 532 nm against blank. Levels of isoprostanes and 8-OHdG, 4-HNE and isoprostane were determined by using commercially available Elisa Kits.

RESULTS

Results of the current study are shown in Table-I that represents a clear image regarding their role in development and progress of rheumatoid arthritis. Concentrations of different variables were measured in the serum, saliva and synovial fluid of RA and they remained statistically significant in all groups. The serum, saliva and synovial fluid concentration ($p<0.015$, Table-I and Figure-1) of MDA (µmol/ml) remained higher in RA patients (1.95±0.094, 0.012±0.0034 and 3.26±0.65) as compared to controls (0.95±0.019, 0.056±0.0056 and 0.019±0.0016). Isoprostanes (pg/ml) was estimated in the patients with RA and remained significant ($p<0.022$, Table-I and Figure-2) (12.26±5.26, 2.16±0.019 and 34.26±4.26) while in controls were recorded as (1.26±0.015, 0.816±0.017 and 0.136±0.019) respectively. The levels of 8-OHdG (pg/ml) and 4-HNE (µmol/ml) also differed statistically and were recorded as follow (0.945±0.014, 0.0024±0.0003 and 1.33±0.451, $p=0.036$ as in Table-I and Figure-3) and (4.265±1.25, 1.26±0.15 and 6.35±1.16, $p=0.004$ as in Table-I and Figure-4) whereas, in controls it remained (0.019±0.0035, 0.002±0.000 and 0.055±0.001) and (1.99±0.016, 0.191±0.0091 and 0.094±0.001) respectively.

Variables	Control (n=100)			Subject (n=100)			P- Value
	Serum	Saliva	Synovial Fluid	Serum	Saliva	Synovial Fluid	
MDA (nmol/ml)	0.95±0.019	0.056±0.0056	0.019±0.0016	1.95±0.094	0.012±0.0034	3.26±0.65	0.015
Isoprostanes (pg/ml)	1.26±0.015	0.816±0.017	0.136±0.019	12.26±5.26	2.16±0.019	34.26±4.26	0.022
8-OHdG (pg/ml)	0.019±0.0035	0.0029±0.00017	0.055±0.0016	0.945±0.014	0.0024±0.0003	1.33±0.451	0.036
4-HNE (μmol/ml)	1.99±0.016	0.191±0.0091	0.094±0.00165	4.265±1.25	1.26±0.15	6.35±1.16	0.004

Table-I. Increased levels of lipid peroxidation and expression of prophetic variables of diagnostic importance and their interplay in rheumatoid arthritis

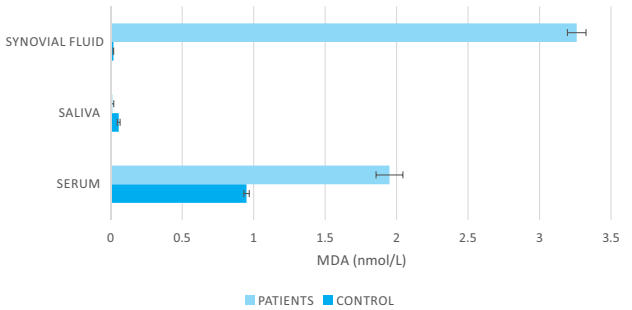

Figure-1. Levels of malondialdehyde (MDA) nmol/ml [P=0.015]

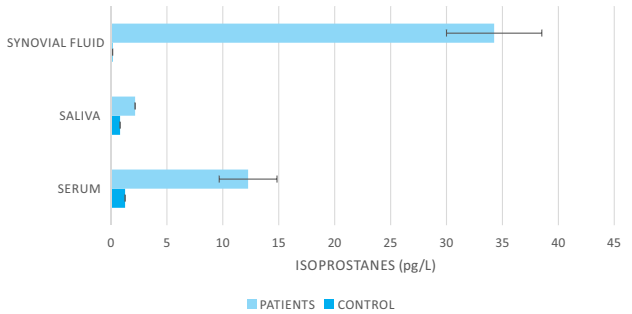

Figure-2. Levels of isoprostanes (pg/ml) [P=0.022]

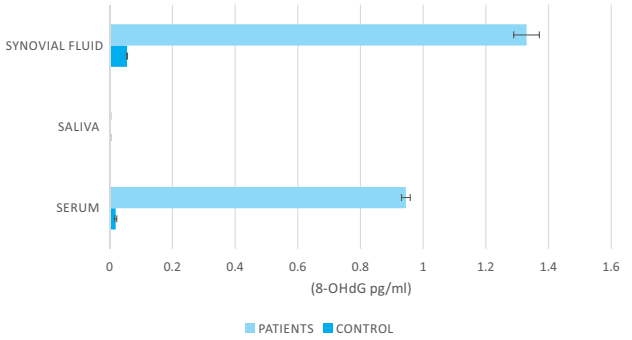

Figure-3. Levels of 8-hydroxyguanosine (8-OHdG) (pg/ml) [P=0.036]

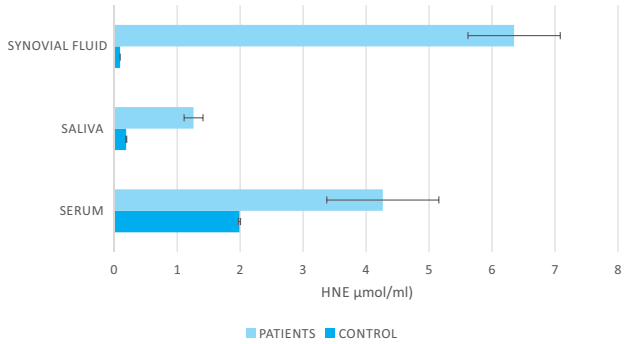

Figure-4. Levels of 4-hydroxy-2-Nonenal (4-HNE) (μmol/ml) [P=0.004]

DISCUSSION

The study demonstrates that the levels of MDA, isoprostane, 8-OHdG, 4-HNE in serum, saliva and synovial fluid of patients suffering from RA were significantly higher as compared to control. Previous investigations suggests that the concentration of thiol was observed significantly lower in the serum of RA patients that indicates impaired antioxidant defense and considerable risk of ROS induced tissue injury.[9] Low antioxidant concentrations and increased oxidative stress detected in the plasma of RA patients and primary osteoarthritis patients suggest the role of free reactive oxygen and nitrogen species in inflammatory response. Findings indicate that increased production of reactive oxygen and nitrogen species could damage lipids, proteins, matrix components and DNA. Autoimmune disease is defined as the initiation of immune response against the own tissues of the body. Patients suffering from autoimmune disease are more vulnerable to oxidative damage as compared to normal individuals. Several evidences of oxidative damage to extracellular collagen, cartilage and DNA have also been established.

In current study significant higher levels of

thiobarbituric acid reactive substances (TBRS) were recorded in serum, saliva and synovial fluid of RA patients. Similar levels were observed in the plasma of children suffering from juvenile rheumatoid arthritis in comparison with control group. In another study a significant increase concentration of MDA and decrease vitamin E level was observed in the plasma of rheumatoid arthritis patients.[10] Increased degradation of hyaluronic acid and elevated level of lipid peroxidation in the plasma and synovial fluid of RA patients is an evidence of increased production of free radicals in these patients.[11] Environmental toxins, smoking and several infectious agents cause DNA modification and formation of DNA adducts that result in impaired DNA replication and gene activation.[12] These factors are also seemed to be involved in RA development. These factors are believed to have significant influence on redox status and production of ROS. Study on blood, synovial fluid and urine of RA patients indicates marked elevation of oxidative stress markers including DNA damage. In RA patients, ROS produced by neutrophils infiltrate into the synovial fluid that is thought to be involved in the pathogenesis of disease. ROS attack on lipids and proteins and cause damage to membranes and DNA. The DNA damage derived by lipid peroxidation has been implicated in the pathogenesis of inflammatory diseases.[13] A heightened level of oxidized low density lipoprotein and lipid peroxidation was observed in plasma of RA patients. ROS attack on polyunsaturated fatty acids and a series of products are formed including α, β-unsaturated aldehydes such as malondialdehyde, carotonaldehyde that are highly protein and DNA reactive. Lines of previous studies demonstrate an increased in the concentration of 8-hydroxy-2-deoxyguonosine (8-OHdG) in synovial fluid of RA patients.[13,14]

Local and systemic increase in inflammatory cytokines results in increased production of ROS and elevated level of lipid, proteins and DNA products including MDA, 4-HNE, isoprostanes and 8-OHdG.[15] Heightened level inflammatory cytokines, neutrophils, macrophages and lymphocytes present in synovial fluid cause increase ROS products that leads towards the development of RA.[16] Present study a significantly high level of isoprostanes and 4-HNE in serum, saliva and synovial fluid were recorded (as shown in Table-I and Figure-B and D).

The results of study are consistent with a number of previous studies that investigate the local and systemic oxidative injury and inflammatory response by measuring the levels of isoprostanes and prostaglandins in blood and synovial fluid of RA patients. Results of these studies indicate oxidation of arachidonic acid by both enzymatically and non-enzymatically in RA patients. Selley et al.[17] worked on RA and osteoarthritis patients and he concluded that markedly higher level of 4-HNE was recorded in RA patients as compared to osteoarthritis patients. In a lines of previous studies a remarkably high levels of lipid peroxidation end products have been documented in RA patients in comparison with healthy population.[18] A significant correlation was observed between modified Larsen scores and serum RA levels that indicates the association of 4-HNE with joint damage.[19] Lipid protein interactions in biological system are important for normal function of biological membranes. ROS induce changes in the protein and lipid structure that results in the change of membrane fluidity and disruption of biological membranes that cause the release of cellular enzymes into the extracellular space and disruption of cellular matrix in as seen in RA patients.[20]

CONCLUSION

Present study concluded the role of oxidative markers and their differential expression in the onset of autoimmunity in patients with RA. Increased stress is involved in the DNA damage and increased lipid peroxidation in the synovial fluid. Therefore, monitoring of lipid peroxidation biomarkers in serum, saliva and synovial fluid and antioxidant therapy may have some prognostic role in the patients with RA by decreasing the intensity of oxidative stress and DNA damage.

CONFLICT OF INTERSEST

Authors declares no conflict of interest.

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I never dreamed about success.
I worked for it.

– Estee Lauder –

AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Umar Saeed Ansari	Reading, Editing.	
3	Bushra Aslam	Reading, Editing.	
4	Nighat Aslam	Reviewing, Reading	
5	Hassan Shafique	Writing, Editing	
6	Sulayman Waquar	Methodology, Writing	