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DIFFERENTIAL EXPRESSION OF PREDICTIVE VARIABLES OF MEDICAL IMPORTANCE AND THEIR INTERPLAY TO DEVELOP RHEUMATOID ARTHRITIS

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ABSTRACT

INTRODUCTION: Rheumatoid arthritis (RA) is an inflammatory disorder that may be characterized by an autoimmune multisystem disease. Other important characteristic features include inflammation of synovial membrane of joints followed by the pain and swelling. Primary aim of the current study is to elucidate the effects of various predictive variables in the development of the rheumatoid arthritis and their differential expression in blood, saliva and synovial fluid of the subjects.

MATERIALS AND METHODS: For the current study fifty (n=50) patients of clinically diagnosed (RA) along with fifty (n=50) age and sex matched individuals were included. blood, saliva and synovial fluid samples were collected from Jinnah hospital Lahore and were stored at -70^{o}C until assayed. All of the experimental protocols were approved by research ethical committee of Institute of Molecular biology and Biotechnology (IMBB) University of Lahore. Levels of Malondialdehyde (MDA), Isoprostanes, 8-Hydroxy-2-Deoxyguanosine (8-OHdG) and 4-Hydroxynonenal (4-HNE) were estimated by their respective spectrophotometric and ELISA methods.

RESULTS: Findings of the study stated elevated levels of the said markers in the diseased group as compared to the healthy individuals. Levels remained sensitive in the synovial fluid as compared to the serum and saliva. MDA levels remained elevated in serum, saliva and synovial fluids in subjects (0.95±0.019, 0.056±0.0056, 0.019±0.0016) as compared to the controls (1.95±0.094, 0.012±0.0034, 3.26±0.65). Similarly, the levels of Isoprostanes, 8-OHdG and 4-HNE also remained higher in the subjects when compared in controls.

CONCLUSION: Findings of the study show that levels of the said markers remained higher among the patients of rheumatoid arthritis when compared with the healthy controls. Findings show that the levels remained more sensitive in the synovial fluid as compared to the serum and saliva samples thus, it may be concluded that estimation of the said variables in the synovial fluid may remain as promising variable as the indicator of early onset of the rheumatoid arthritis.

KEYWORDS: Rheumatoid Arthritis, Autoimmune Disease, MDA, 8-OHdG, 4-HNE

INTRODUCTION

Rheumatoid arthritis is an autoimmune and inflammatory disease characterized by hyperplasia, systemic chronic and erosive inflammation of synovial joints characterized by proliferation of cells associated with synovial joints, activation and infiltration of macrophages, memory T cells and plasma cells and permanent destruction of bone and cartilage[1, 2]. A number of secretory products such as leukotrienes, proteases, prostaglandins, hydrolases components of complement system and free reactive oxygen radicals are present in rheumatoid synovium [3]. The extra articular manifestations associated with RA including inflammation in lungs and heart, vasculitis and peripheral neuropathy. RA cause a significant increase risk of other diseases including renal disorders, cardiovascular disease, intestinal abnormalities, pulmonary dysfunction and a major cause of premature deaths[4]. 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[5]. Other risk factors include smoking, obesity, vitamin D deficiency, menstrual disorders, periodontal diseases and many others[6]. 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[7].

A number of studies have indicated inverse relation between dietary intake of antioxidants and RA occurrence[8]. 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[9, 10]. 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 drived DNA damage has been reported in rheumatoid arthritis patients[11]. Reactive oxygen and nitrogen species cause either DNA strand breakage or damage to individual nucleotide base damage. Hydroxyl redical 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[12]. Lipid peroxidation induces a number of end products including malondialdehyde (MDA), isoprostane and 4-hydroxynonenal (HNE) and its increase level has been demonstrated into synovial fluid and sera of RA patients[13].

The study presented (as shown in table 01) here demonstrates that the levels of MDA, isoprostane, 8-oHdG, HNE in serum, saliva and synovial fluid of patients suffering from RA were significantly higher as compared to control. Previous investigations suggest that the concentration of thiol were significantly lower in the serum of RA patients that indicates impaired antioxidant defense and considerable risk of ROS induced tissue injury[14]. 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[15]. In our study a significant high level of thiobarbituric acid reactive substances (TBRS) were recorded in serum, saliva and synovial fluid of RA patients (as shown in and table: 01 and figure 01).

A significant increase in plasma and synovial fluid level of malondialdehyde (MDA) was observed in rheumatoid arthritis patients as indicated by Lunec et al[16]. A significant high level of thiobarbituric acid reactive substances remained in the plasma of children suffering from juvenile rheumatoid arthritis in comparison with control group[17]. In another study a significant increase concentration of MDA and decrease vitamin E level were observed in the plasma of rheumatoid arthritis patients[18]. 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 among the reported patients[19]. Environmental toxins, smoking and several infectious agents cause DNA modification and formation of DNA adducts that result in impaired DNA replication and gene activation[20]. 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. A 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 [21]. A heightened level of oxidized low-density lipoprotein and lipid peroxidation will be 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- hydroxyguonosine (8-oxo-dG) in synovial fluid of RA patients[21, 22]. Local and systemic increase in inflammatory cytokines results in increased production of ROS and elevated level of lipid, proteins and DNA products including MDA, HNE, isoprostanes and 8-oxo-dG[23]. Heightened level inflammatory cytokines, neutrophils, macrophages and lymphocytes present in synovial fluid cause increase ROS products that leads towards the development of RA[24]. Results of these studies indicate oxidation of arachidonic acid by both enzymatically and non-enzymatically in RA patients[25, 26]. Selley et al.[27] worked on RA and osteoarthritis patients and he concluded that markedly higher level of 4-HNE were recorded in RA patients as compared to osteoarthritis patients.

In the lieu of previous studies, a remarkably high levels of lipid peroxidation end products have been documented in RA patients in comparison with healthy population[27, 28, 29]. A significant correlation were observed between modified Larsen scores and serum RA levels that indicates the association of 4-HNE with joint damage[30]. 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[31]. Our study shed light on association of oxidative stress and inflammatory diseases. The results of present shows that the production of aldehydes such as MDA and HNE, isoprostanes and 8-OHdGis in relation with progression of RA that points towards the importance of lipid peroxidation in implications and pathophysiology of RA. The monitoring of lipid peroxidation biomarkers in serum, saliva and synovial fluid could be useful for monitoring of RA.

AIMS AND OBJECTIVES

Primary aim of the current study is to elucidate the effects of various predictive variables in the development of the rheumatoid arthritis and their differential expression in blood, saliva and synovial fluid of the subjects.

MATERIALS AND METHODS

Present study was designed to find an association of oxidative stress and RA. Fifty (n=50) patients diagnosed with rheumatoid Arthritis and fifty (n=50) age and sex matched individuals were taken as control. Blood, saliva and synovial fluid samples were obtained from Jinnah hospital Lahore and collected samples were then stored at -70^{o}C until assayed. All of the experimental protocols were approved by research ethical committee of Institute of Molecular biology and Biotechnology (IMBB) University of Lahore.

BIOCHEMICAL ANALYSIS

Levels of MDA in serum, saliva and synovial fluid were estimated spectrophotometrically by using the methods of Ohkawa *et al*.[32] 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 was 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 obtained at 532 nm against blank. Whereas, levels of 8-OHdG, Isoprostanes and 4-HNE were determined by the help of their commercially available ELISA kits.

STATISTICAL ANALYSIS

Data management and statistical analysis was performed by using Statistical Package for the Sociological Sciences (SPSS, version 21.0). Depending on data distribution ANOVA test was applied to check the comparison among ACS patient group and control group. Followed by their sensitivity and specificity analysis. Difference was considered statistically significant when the p value was <0.05. To determine statistically significant correlation between variables Pearson correlation was done.

RESULTS

Results of the study stated elevated levels of the said markers in the diseased group as compared to the healthy individuals. Levels remained sensitive in the synovial fluid as compared to the serum and saliva. MDA levels remained elevated in serum, saliva and synovial fluids in subjects (1.95±0.094, 0.012±0.0034, 3.26±0.65) as compared to the controls (0.95±0.019, 0.056±0.0056, 0.019±0.0016). Likewise, the levels of Isoprostanes were elevated in the patient group (12.26±5.26, 2.16±0.019, 34.26±4.26) as compared to the controls (1.26±0.015, 0.816±0.017, 0.136±0.019) respectively, levels of 8-OHdG and 4-HNE were also reported to be higher in the subjects (0.945±0.014, 0.0024±0.0003, 1.33±0.451 and 4.265±1.25, 1.26±0.15,6.35±1.16) when compared in controls (0.019±0.0035, 0.0029±0.00017, 0.055±0.0016 and 1.99±0.016, 0.19±0.0091, 0.094±0.00165) as shown in Figure 1,2,3,4 and Table 1, in serum saliva and synovial fluid respectively.

DISCUSSION

Autoimmune disease can be defined as the initiation of immune response against own tissues of the body. Patients with autoimmune multi-disease defects are reported to have increased oxidative stress if are compared with the healthy controls as shown in the current study the levels of MDA were significantly higher in the rheumatoid arthritis patients when compared with the healthy individuals. Several evidences of oxidative damage to extracellular collagen, cartilage and DNA have been established earlier as reported in the current study significant higher level of thiobarbituric acid reactive substances (TBARS) were recorded in serum, saliva and synovial fluid of RA patients (as shown in and table: 01 and figure 01). A significant increase in plasma and synovial fluid level of malondialdehyde (MDA) were observed in rheumatoid arthritis patients as indicated by Lunec et al[16]. Similar, findings were reported in another study a significant increase concentration of MDA and decreased vitamin E level were observed in the plasma of rheumatoid arthritis patients. Current study reports levels of 8-OHdG a DNA damage marker in serum, saliva

and synovial fluid of rheumatoid arthritis were significantly higher as compared to the controls. Current study shows significantly high level of isoprostanes and 4-HNE in serum, saliva and synovial fluid were recorded (as shown in table: 01 and figure: B and D). The results of current study remained 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. Moreover, current study tends to rule out more specific and sensitive markers involved in the onset of disease in all the three reported mediums and it shows more sensitive findings in the synovial fluid as compared to the serum and saliva.

CONCLUSION

Findings of the study show that levels of the said markers remained higher among the patients of rheumatoid arthritis when compared with the healthy controls. Findings show that the levels remained more sensitive in the synovial fluid as compared to the serum and saliva samples thus, it may be concluded that estimation of the said variables in the synovial fluid may remain as promising variable as the indicator of early onset of the rheumatoid arthritis

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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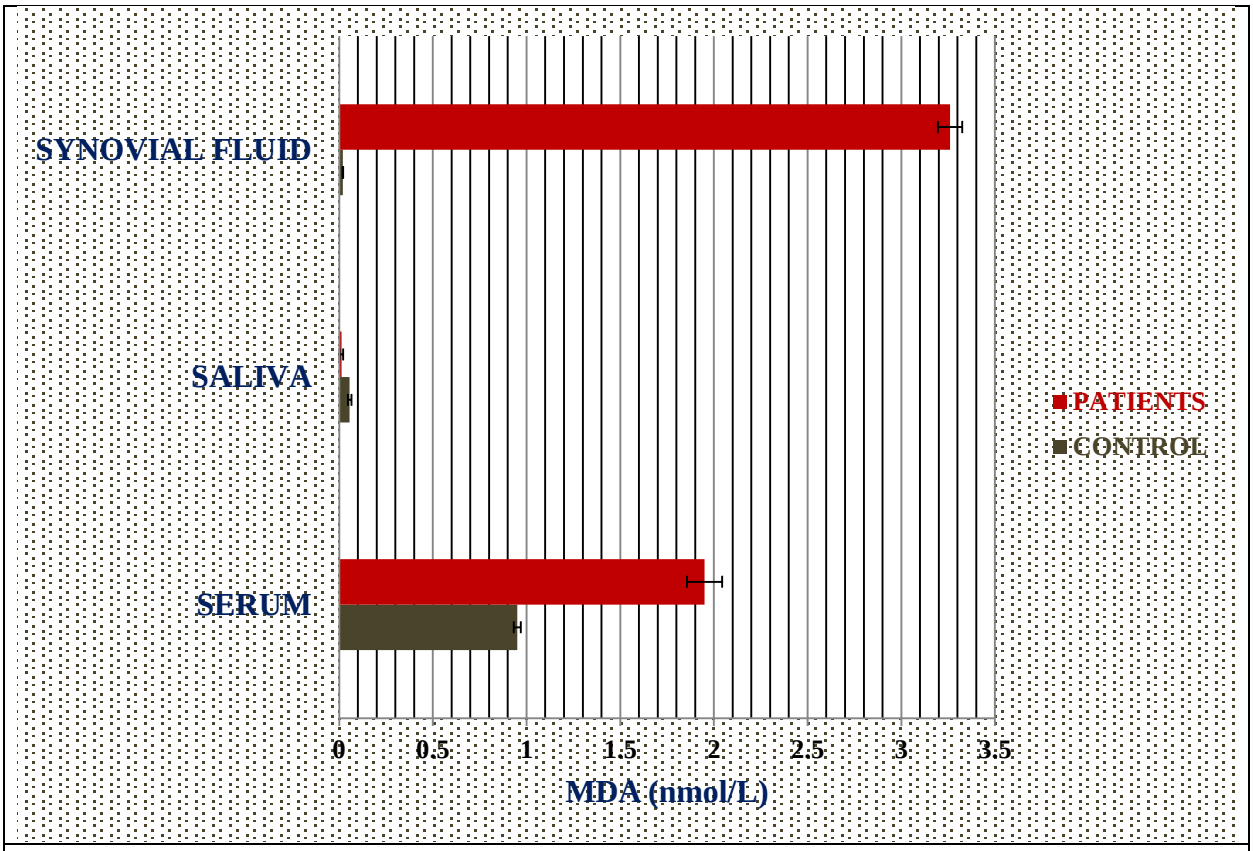

FIGURE:01 LEVELS OF MALONDIALDEHYDE (MDA) nmol/ml [P=0.015]

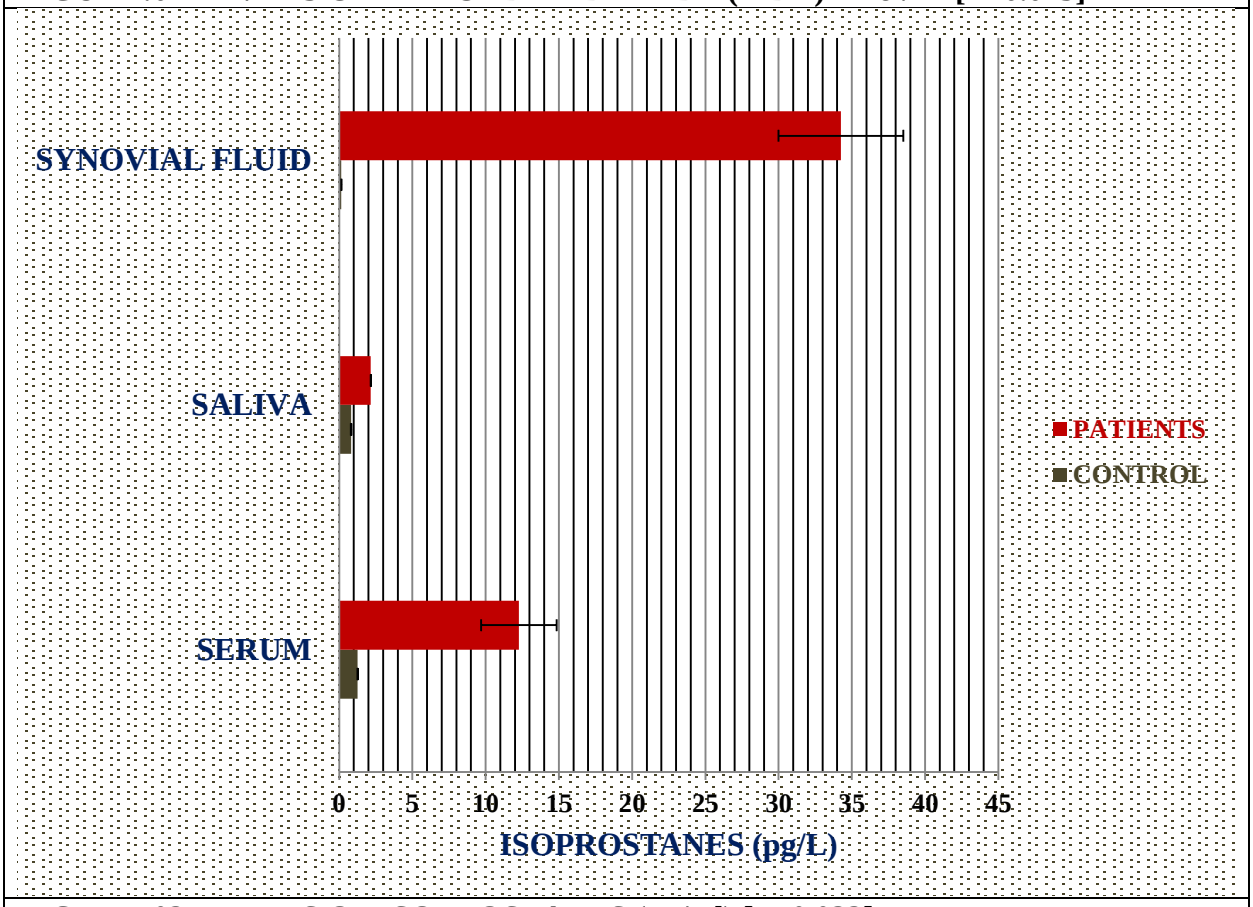

FIGURE:02 LEVELS OF ISOPROSTANES (pg/ml) [P=0.022]

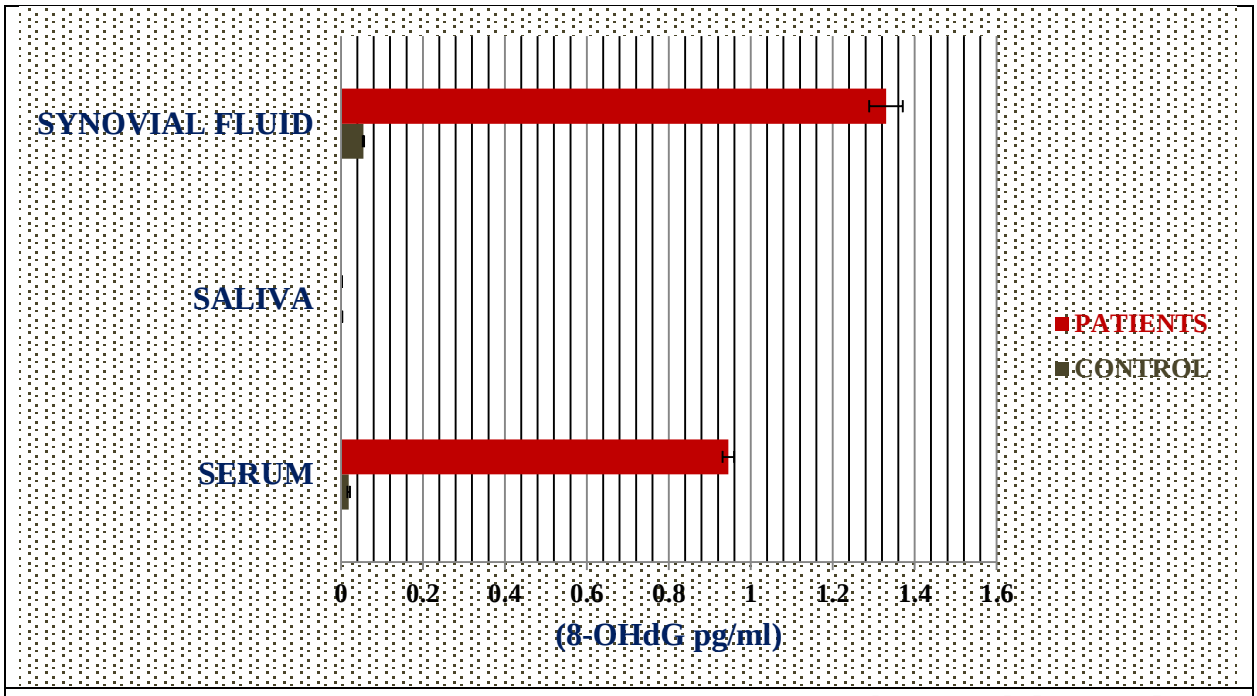

FIGURE:03 LEVELS OF 8-HYDROXYGUANOSINE (8-OHdG) (pg/ml) [P=0.036]

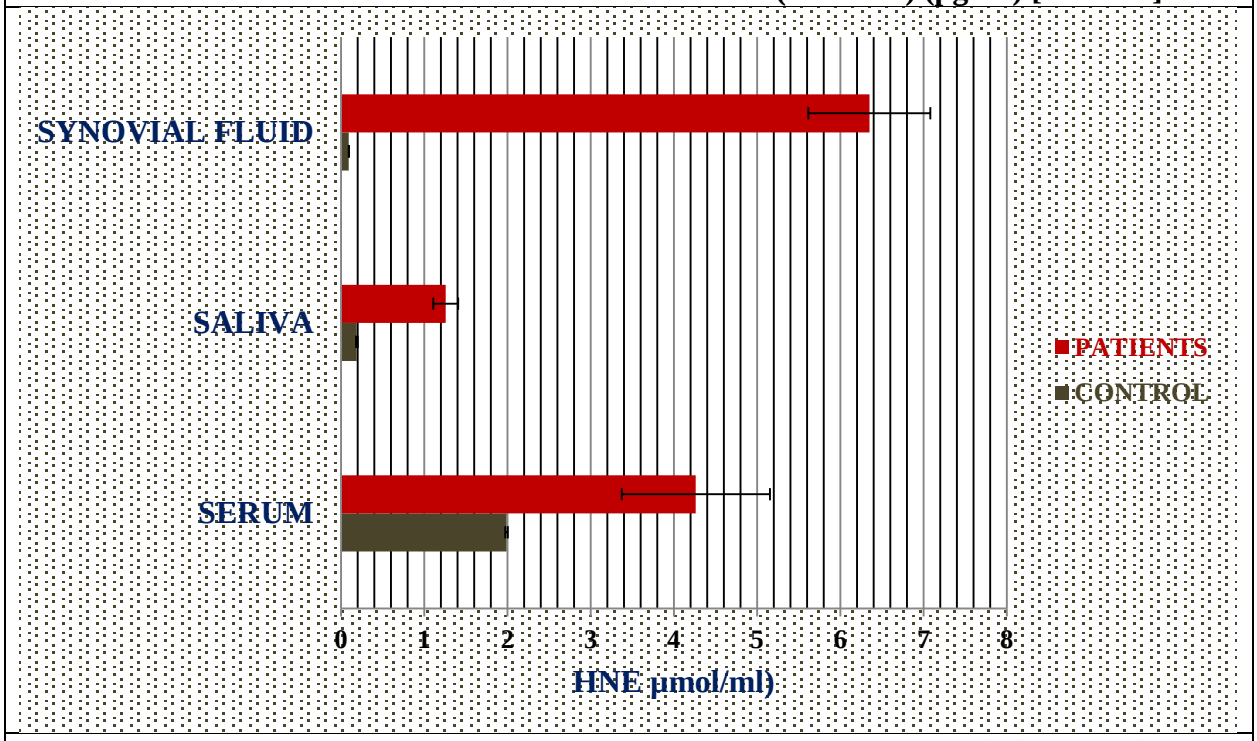

FIGURE:04 LEVELS OF 4-HYDROXY-2-NONENAL(HNE) (µmol/ml) [P=0.004]

TABLE:01 **INCREASED LEVELS OF LIPID PEROXIDATION AND EXPRESSION OF PROPHETIC VARIABLES OF DIAGNOSTIC IMPORTANCE AND THEIR INTERPLAY IN RHEUMATOID ARTHRITIS**

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