



Research Article

Correlation between Biochemical and Anti-Oxidative status in Rheumatoid Arthritis Patients Update from Lahore, Pakistan

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HMA and MKAK supervised the research. HMA wrote the manuscript. MA and ZSA collected the data. MA, HM, IM, NG, SN and AS perform experiments. MKAK analysed the data.

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Arthritis, Nitric Oxide, SOD, MDA, CAT

Abstract | Bones determinedly altered through integration body matrix osteoblast, resorption bone via osteoclasts. Rheumatoid arthritis allied with considerable levels pain, tiredness, disability, reduced distinction life. Precise pathogenesis job rheumatoid factor not known, through formation immune complexes engross establishment harmonize. To determine the correlation between Anti-Oxidants and Biochemical status in Rheumatoid Arthritis patients. 60 clinically diagnosed Rheumatoid Arthritis (RA) patients and 50 healthy persons were included in this study. 5 ml Blood was drawn and serum was separated. Anti-Oxidant Biomarkers, Serum micronutrients, serum Electrolyte balance was measured through spectrophotometric procedure. Serum MDA level jump high in patients (14.19) as compared to normal subjects (3.26). Serum Glutathione declined in disease persons (0.56) from healthy subjects (6.39). Serum Micronutrients level was also declined in RA patients as compared to healthy persons and substantial statistically (P=0.000). Serum Nitric Oxide jump high in patients (32.11) than normal persons (15.2). Abnormal concentration of reactive oxygen species can disturb the body's defense mechanism which cause potential oxidative injury to tissues and lead to cartilage degradation in RA patients.

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Introduction

Bones determinedly altered through integration body matrix osteoblast, resorption bone via osteoclasts. Biological alterations inflammatory cytokines, growth factors, hormones sources imbalance between osteoclast, osteoblast behaviors consequence skeletal irregularities, like osteoporosis (Zhao *et al.*, 2014). Osteoporosis overpowering diseases distinguished lower bone density, often found older people particularly women paralyzed

patients even astronauts result understanding zero gravity ultimately results bone fractures (Teiji *et al.*, 2006). Rheumatoid Arthritis (RA) is classical inflammatory joint disease defined inflammatory tissues, lining joints (synovium). It researched adult population developed regions RA affects ~0.5–1% (Carbonell *et al.*, 2008; Symmons *et al.*, 2002). Even though few patient gentle-self-limited diseases, some them practice joint damage, ruthless physical disorder, many multiple co-morbidities (Plenge, 2009). Mortality rates rheumatoid arthritis patients higher (twice) than general population this difference seems widening (Gonzalez *et al.*, 2007).

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Rheumatoid Arthritis (RA) clinical course highly

erratic random, subtle, hostile (with no period relative remission). Rheumatoid arthritis allied with considerable levels pain, tiredness, disability, reduced distinction life. Synovial fluid inflamed rheumatoid joint over run inflammatory cells comprise activated neutrophils, involved in production of hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), reactive hydroxyl ion (OH). Neutrophils RA patient's synovial fluid exhibit greater production superoxide radical their exposure cytokines present synovial fluid (Robbinson *et al.*, 1993). When inflamed joint move Ischemia, reperfusion involved production free radical oxygen species (Black *et al.*, 1989). Not forage, then these reactive species damage lipid, protein, DNA. Pathogenesis RA reactive oxidants important mediators related oxidant damage, phagocyte function RA patients (Babior, 2000). Protect themselves against free radical attacks, cells various arrangements counting squat molecular heaviness antioxidants glutathione. Outside resistance aliened oxygen free radicals, SOD accelerate dismutation superoxide anion O_2^- through catalase. Glutathione peroxidase selenoprotein, while oxidizing glutathione diminish lipidic, non-lipidic hydroperoxidase H_2O_2 . Taking place outcell antioxidants Ceruloplasmin helps loading iron transferring (TF) (Gutteridge and Stocks, 1989).

Precise pathogenesis job rheumatoid factor is not known, through formation immune complexes engross establishment harmonize. NF- κ B vital role demarcation, activation, survival, mammalian cell's defense. It involved autoimmune disorders like RA different behaviors. Firstly, NF- κ B necessary DC endurance normal lymphocytes, their expansion activation (positive, negative options B and T- cells), morphogenesis lymphoid organ (Cimen *et al.*, 2000). NF- κ B imperfection control sanction survival discharge periphery auto-reactive T-cells from thymus, whereas following antigen stimuli elicit autoimmune disease. Various analysis autoimmune disorders proved that NF- κ B contribute inflammatory cytokines induction other regulators inflammation compel pathology. NF- κ B turned diverse pathogenic stimuli, bacterial products, cytokines, viral proteins, reperfusion/ischemia, growth hormones, rays oxidative pressure. harmonized NF- κ B activation occur every cell type alarmed inflammatory reaction, together neutrophils, macrophages, lymphocytes, epithelial mesenchymal cells, key parts self-protective reply pathogens anxiety. Outset NF- κ B needed multiple terminologies inflammatory immune response manager (Gambhir *et al.*, 1997). Regulated NF- κ B originated human synovial tissues early stage joint inflammation (Kerimova *et al.*, 2000), extra specimens achieved early phase of disease. Nuclear extracts study from synovial explants uncover occurrence augmented NF- κ B DNA binding activity RA patients (Lotz, 1999). Immunohistochemically scanned sensed nuclear Re1A (p65), NF- κ B (p50) rheumatoid endothelium, synovial lining, principally CD14-positive cells no staining typical synovium (Tiku *et al.*, 1999). Immunostaining antibodies excluding active (disconnected

from I κ B) NF- κ B revealed active NF- κ B incidence macrophage like nuclei within vascular endothelium synovial lining. Without being affected assorted mold active NF- κ B found both RA, OA. Acute rheumatoid arthritis individuals explain vessel staining, uniformly showed less repeated staining synovial lining distinguish Osteoarthritis (OA) patients. TNF family molecule RANKL relates activation persuaded (TRANSE), TNSF11, ODF, (TNFRSF11A) main organizer fillet redesigning, viral extension commencement osteoclasts. Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) stimulates T-cells/dendrites interactions dendritic cells existence (Fletcher *et al.*, 1998), lymph node organogenesis. Furthermore, RANKL assembly via T-cells openly command osteoclastenogenesis, bone modification, elucidate why autoimmune disarray like leukemia, asthma, cancer, chronic viral contagions periodontal ailment fallout systematic narrow fillet beating (Clancy *et al.*, 1998). Thoroughly, RANKL expose pathogenic that lead bone cartilage breakdown. RANKL unwillingness during natural persuaded receptor (OPG, TNFRSF11B) keep away from fillet thrashing, cancer metastasis, totally block crippling different arthritis mock-up. Fascinatingly, RANKL, RANK performs imperative role organization lactating mammary gland pregnancy (Moshage *et al.*, 1995). Existence of mammalian species relies RANKL pathways which provides changeable molecular pattern that links bone morphogenesis, T-cells origination, group lymphoid tissues mammary gland ordering. Inactivation RANKL occupation throughout accepted entrap receptor Orthopantomogram (OPG) little molecular upcoming treatment alternative close down, tooth loss immobilizes harm arthritis. Active CD+ T-cells covey osteoprotegerin ligands motivate osteoclastenogenesis. Such activated T-cells involved RA joint destruction. These activated lymphocytes; macrophages, fibroblast, goods regulate angiogenesis; make clear high vascularity synovium RA. Activated stratum synovial communicate adhesion molecules that enhance conscription inflammatory cells into joint. Liberation chemokines improved system like interleukin-8 through inflammatory cells joints (Rowley *et al.*, 1984).

The aim of present study was to determine the correlation between anti-oxidants and biochemical status in Rheumatoid Arthritis patients.

Materials and Methods

Source of data

60 clinically diagnosed Rheumatoid Arthritis (RA) patients and 50 healthy persons were included in this study. 5ml blood Sample was collected in EDTA vial from Mayo and Jinnah Hospital Lahore. Detailed patient's history, clinical complications, particular smoking and tobacco chewing were collected from subjects of the study, by providing them a questionnaire. Clinical analysis of the

patient was also being taken into consideration.

Inclusion criteria

Patient should be victim of Rheumatoid Arthritis (RA) and having age from 30–60 years. Vitamin D and calcium deficient patients.

Exclusion criteria

Individuals having age below 30 and above 60 is excluded from present study.

Following parameters were estimated

Anti-Oxidant Biomarkers (Glutathione, Catalase, Superoxide Dismutase, Malondialdehyde, Nitric oxide), Serum micronutrients (Vitamin A, C and E) and serum Electrolytes (Sodium, Potassium) balance were measured through spectrophotometric procedure.

Estimation of superoxide dismutase (SOD)

SOD was measured through spectrophotometric procedure of [Kakkar et al. \(1984\)](#).

Determination of malondialdehyde (MDA) in tissues

MDA was checked by spectrophotometric procedure of [Ohkawa et al. \(1979\)](#).

Estimation of catalase (CAT)

CAT was observed by the procedure of [Aebi \(1984\)](#).

Determination of GSH

GSH was determine by the process of [Moron et al. \(1979\)](#).

Determination of nitric oxide (NO)

Nitrite concentration was typically measured by a well-known method such as colorimetric Griess assay ([Moshage et al., 1995](#)).

Estimation of vitamin C (VIT C)

Ascorbic acid (VIT C) was analyzed by the method described by [Roe and Keuther \(1943\)](#) by spectrophotometrically.

Estimation of vitamin A (VIT A)

Vitamin A (Tocopherol) was estimated in the plant samples by the Emmutir-Engel reaction as reported by [Rosenberg \(1992\)](#).

Statistical analysis

Statistical Analysis was done by using Statistical Package for Social Sciences (SPSS) (Independent T-test and Bivariate Pearson's Correlation).

Results

[Table 1](#) and [Figure 1](#) shows the high serum level of MDA (14.19) in RA patients as compared to control

(3.26). Whereas there was statistically high significant plasma MDA activity in RA patients ($p < 0.000$). Reduction of GSH (0.56) observed in RA patients in contrast to healthy persons (6.39). Statistically GSH was highly significant ($p < 0.000$). Activity of catalase was higher (6.92) than in healthy individuals (4.8). Statistically catalase was highly significant ($p < 0.000$). SOD quietly increased in RA patients (12.04) as compared to control (2.15). Superoxide dismutase significantly showed the increased actions ($p < 0.000$).

Table 1: Antioxidative status profile of rheumatoid arthritis patients.

Biomarkers	CONTROL (n=50) Mean±S.D	SUBJECTS (n=60) Mean±S.D	P<0.05
MDA	3.26±0.27	14.19±0.16	0.000
GSH	6.39±0.20	0.56±0.20	0.000
CATALASE	4.8±1.02	6.92±0.11	0.000
SOD	2.15±0.33	2.04±1.03	0.000

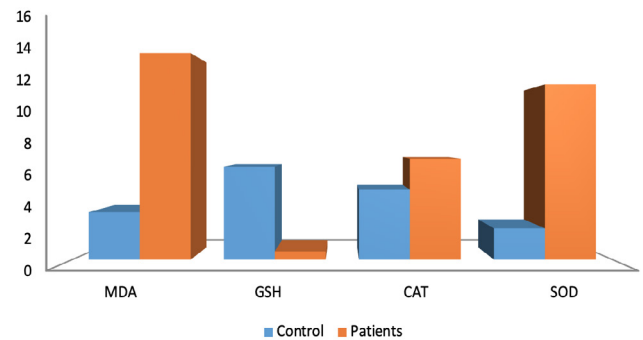


Figure 1: Graphical representation of antioxidative status between normal and RA patients.

[Table 2](#) and [Figure 2](#) depicts the Micronutrients level (Vitamin A, C, and E) in plasma of RA patients and control subjects. The level of vitamin A critically low in RA patients (0.15) as compared to healthy subjects (7.21). Statistically vitamin A was significant in RA patients ($p < 0.000$). Vitamin C remarkably reduced in rheumatoid patients (0.12) as contrast to control (6.21). Statistical analysis shows increased significance ($p < 0.000$). Vitamin E in plasma of RA patients confirm drastically low (0.54) whereas in healthy patients (4.41). Reduced concentration of vitamin A, C, and E showed low anti-oxidant activities in diseased patients. Vitamins E low level is highly significant ($p < 0.000$) as contrast to control.

[Table 3](#) and [Figure 3](#) illustrates advance oxidation protein products (AOPP) as biomarker of protein oxidation. Level of AOPP is amazingly high (11.23) in RA patients against control (2.04). AOPP was extremely significant ($p < 0.000$) in plasma of RA patients as compare to healthy individuals. While Nitric Oxide (NO) linked as mediator of inflammatory arthritis and noticed high level in RA patients (32.11) alongside healthy persons (15.2).

Nitric Oxide (NO) concentration from RA patients was over twice ($p < 0.000$) than that of healthy persons.

Table 2: Micronutrients profile of rheumatoid arthritis patients.

Biomarkers	Control (n=50) Mean ± S.D	Subjects (n=60) Mean ± S.D	P<0.05
Vit. A	7.21±0.046	0.15±0.21	0.000
Vit. C	6.21±1.06	0.12±0.15	0.000
Vit. E	4.41±0.92	0.54±0.50	0.000

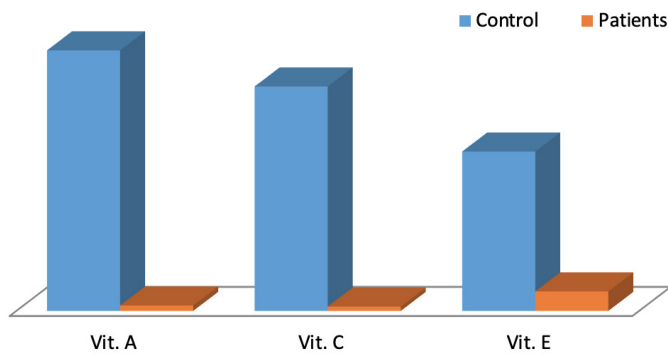


Figure 2: Graphical representation of micronutrients in normal and RA patients.

Table 3: Different biomarker profile of Rheumatoid arthritis.

Biomarkers	Control (n=50) Mean ± S.D	Subjects (n=60) Mean ± S.D	P<0.05
AOPP	2.04±0.34	11.23±2.42	0.000
Nitric Oxide (NO)	15.2±2.04	32.11±0.72	0.000

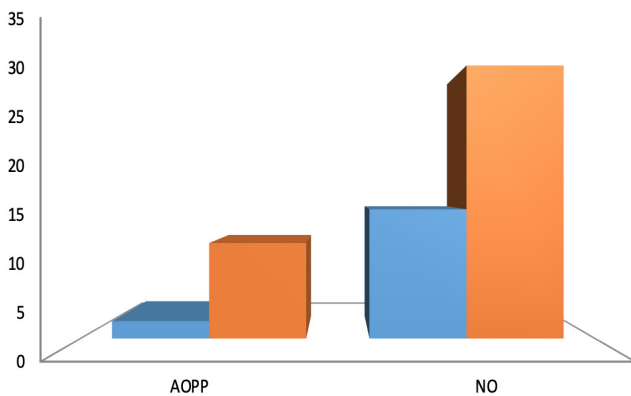


Figure 3: Graphical representation of AOPP and NO in normal and RA patients.

Electrolytes profile of RA patients established in [Table 4](#), sodium (Na^+) level was significantly enlarged in RA patients (171.31) as compared to control (132.26). Statistically Na^+ significant ($p < 0.00$) which was distinguish to normal. Potassium (K^+) was five times less in RA patients (1.21) against normal individuals (6.24) and statistically significant ($p < 0.00$).

Table 4: Electrolytes status of rheumatoid arthritis patients.

Biomarkers	Control (n=50) Mean ± S.D	Subjects (n=60) Mean ± S.D	P<0.05
Sodium (Na^+)	132.26±10.28	171.31±9.11	0.000
Potassium (K^+)	6.24±0.12	1.21±1.03	0.000

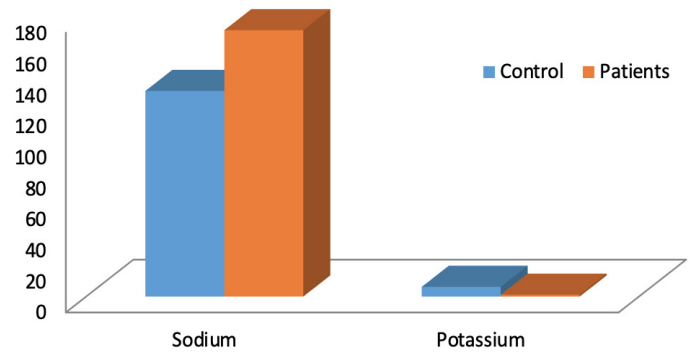


Figure 4: Electrolyte profile in normal and RA patients.

Discussion

Rheumatoid arthritis is an autoimmune chronic inflammatory joint disease and one of the most common disorders worldwide. In present study enzymatic and non- enzymatic anti-oxidants status were measured in rheumatoid arthritis patients in contrast to healthy patients. Free radicals are constantly produce in our body; these free radicals play pivotal role in our body but in greater amount are toxic for body. Imbalacement of free radicals cause oxidative stress and induced lipid per oxidation in various pathological conditions including chronic inflammation ([Gutteridge, 1995](#)).

The toxic effects of Reactive Oxygen Species (ROS) are neutralized by enzymatic (SOD, Catalase) and non-enzymatic antioxidants (Vitamin A, E, C, and reduced glutathione) protecting the lipids of lipoproteins and other bio membranes against per oxidative injure by stopping oxidants before they can attack the tissues. Lipid per-oxidation process occurred at the site of inflammation and subtle into blood and can be estimated in serum which in result tells us about the severity of the damaged tissues. Thus the elevated level of plasma lipid pr-oxidation observed in rheumatoid arthritis.

An inverse relationship between lipid per oxidation and non-enzymatic antioxidants has been well recognized. Hence, the lessen in plasma non enzymatic antioxidants can be correlated to destruction in the antioxidant defense mechanism, due to glut utilization by the inflamed tissues to forage the too much lipid peroxides that are breed at inflammatory sites, or to hunt accumulated lipid peroxides in plasma ([Gutteridge, 1995](#)).

Data Presented in Table 5 predict the Correlation amongst multidirectional parameters of RA patients. Correlation ($r = 0.268^*$), between vitamin C and catalase was found to be positive, which depicts enhanced activity of catalase leads to high production of vitamin C and vice versa, statistically significant ($p < 0.03$). Vitamin E and Nitric Oxide (NO) showed inverse correlation $r (-0.297^*)$, high production of NO generates free radicals which weakens the anti-oxidant activity, statistically highly valuable ($p < 0.021$). Correlation between vitamin C and A was found to be very important ($r = 0.336^{**}$), less production of vitamin C also effect the vitamin A production and vice versa, these have anti-oxidants properties, statistically highly significant ($p < 0.009$). Vitamin E and A had direct relationship ($r = 0.323^*$). High concentration of vitamin E and A, manipulate each other, statistically found significant ($p < 0.012$).

Table 5: Pearson's correlation among different parameters in RA patients.

Parameters	Correlation (r) n=60	P-value
Vitamin C vs. Catalase	0.268*	0.038
Vitamin E vs Nitric Oxide	-0.297*	0.021
Vitamin C vs Vitamin A	0.336**	0.009
Vitamin E vs Vitamin A	0.323*	0.012

*: Correlation is the significant at the 0.01 level (2-tailed); **: Correlation is the significant the 0.05 level (2-tailed).

Conclusion

With the escalating approval of ROS as common place in pathology and clinical biochemistry, we understood that the excessive production of free radical species have major role in inflammatory events in rheumatoid arthritis. Abnormal concentration of ROS can disturb the body's defense mechanism, which cause potential oxidative injury to tissues and lead to cartilage degradation in RA patients. High MDA level can be connected to a compensatory defense system in RA. Therefore, MDA levels in RA could be used as biochemical marker of disease activity and observe treatment reaction.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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