International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: 10.7439/ijbar
CODEN: IJBABN

Original Research Article

Role of oxidant stress in rheumatoid arthritis

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Abstract

Oxygen derived free radicals have been implicated in the causation of Rheumatoid arthritis (RA). In this study, evidence of free radical injury and oxidative stress in patients with RA is compared with healthy subjects by estimating superoxide dismutase (SOD) and catalase, which are anti-oxidant enzymes in RBCs, Glucose 6 Phosphate Dehydrogenase (G_6PD) in RBCs and serum Malon-di-aldehyde (MDA) levels. Serum MDA levels in RA could be used as a biochemical marker of disease activity and for monitoring the response to treatment. There was no definite correlation between enzyme levels of SOD, catalase and G_6PD and disease activity.

Keywords: Rheumatoid arthritis (RA), oxidative stress, anti-oxidants

1. Introduction

RA is a collagen disease affecting connective tissue of the whole body with focalised involvement of musculo-skeletal system [1]. It is the most common inflammatory arthropathy worldwide and affects 0.75% of Indian population [2].

In RA there is inflammation of synovial membrane which becomes oedematous and thickened with inflammatory exudates. The inflammatory process spreads into the capsule and peri-articular tissue, forming a pannus (eroded surface covered by soft membrane of inflammatory tissue) Later articular cartilage and sub-chondral bone gradually soften and erodes [3].

During healing process, the granular pannus becomes fibrous, involves the joint surfaces and causes fibrous ankylosis. The muscles around the joint also undergo inflammatory changes and get atrophied [4].

The disease follows a chronic course and the outcome may be unsatisfactory despite treatment. This is because, the aetiology of RA is not known and the pathogenesis remains to be fully elucidated.

1.1 Oxidative stress in pathogenesis of Rheumatoid arthritis

Oxygen derived free radicals have been implicated in the causation of RA [5]. Synovial fluid has chemo-attractant property. The polymorphonuclear leukocytes will accumulate within the synovial fluid rather than the synovium. These cells, during phagocytosis, trigger a respiratory burst characterised by increased oxygen consumption and increased anaerobic glycolysis leading to generation of oxygen radicals including superoxide, hydroxyl, hypochlorite radicals, etc. [6,7,8].

Using chemiluminescence assays, it has been demonstrated that activation of neutrophilic myeloperoxidase-hydrogen peroxide system takes place at a vigorous rate in the synovial fluid of patients with RA [9]. This oxidative stress may contribute to the cyclic self-perpetuating nature of rheumatoid inflammation [10].

It is important that, the predominant reaction in RA is enormous cellular proliferation rather than cellular destruction. Cytotoxicity is directed only

against specific isolated cell types. Luxuriant growth of cells and not necrosis is the characteristic feature in RA. Nevertheless, there could be an important role of oxygen radicals, especially, when considering possible alterations in matrix and enzymes that degrade the matrix. In vitro studies demonstrated that enzymatically generated superoxide radicals produce hypochlorite ions. It has been focussed that this hypochlorite can depolymerise purified hyaluronic acid and damage protease inhibitors, resulting in uncontrolled activity of proteases [1,11,12].

In RA, physical movements induce hypoxia in the synovial joints. Later reperfusion of the synovial joints trigger generation of reactive oxygen species and oxygen derived free radicals. It has been proposed that persistence of synovitis in RA can be explained by the occurrence of hypoxia-reperfusion injury in the joint [2,13,14].

1.2 Aims & objectives

This study is aimed at evaluating whether oxidative stress has a role in the pathogenesis of RA. Any correlation of MDA, SOD and catalase, which are direct parameters, are estimated. G_6PD, an indirect parameter is also estimated. Their levels are correlated with disease activity and compared with levels in normal subjects.

2. Materials & methods

Patients of the present study were from the outpatients attending Rheumatology clinic of a tertiary care teaching hospital. The study was conducted on fifty cases of RA and fifty healthy controls, taken from samples of blood taken from volunteers for blood donation in the blood bank of the above hospital.

Patients were selected irrespective of age, sex, disease duration, disease activity and treatment received. Patients having RA overlapping with other connective tissue disease like Systemic lupus erythematosis, systemic sclerosis, polymyositis were excluded from the present study. In addition, RA patients with acute infection like malaria or co-existing systemic diseases like coronary artery disease, hypertension, diabetes mellitus, chronic renal failure which can alter the MDA levels were not included.

Blood samples for MDA were collected by venipuncture using disposable syringes and needle and transferred to clean dry plain glass tubes. Blood was allowed to clot and serum separated by centrifugation at 3000rpm for 15 minutes.

2.1 Preparation of haemolysate

For estimation of SOD and catalase, blood was collected in heparin tubes. For G_6PD estimation, blood was collected in Ethylenediaminetetraacetic acid (EDTA) tube and plasma was separated from each of these.

The supernatant plasma is removed and the remnant centrifuged. White blood cells (WBCs) were removed from the top layer and the Red blood cells (RBCs) were washed with saline twice. The RBCs were haemolysed by adding 1.5 volumes of distilled water. Haemoglobin concentration was measured by cyanmethaemoglobin method of Drabkin [15] and adjusted to 10gm%.

Chemicals used for estimation were of analar quality, obtained from reputed chemical suppliers. Analysis was done in UV-Vis spectrophotometer 115 (Systronics).

Parameters done to assess oxidative stress were

1. Serum MDA level based on Valipasha & Sadasivudu procedure [16].
2. Anti-oxidant enzymes, SOD based on method by Christine C, Winterbourn *et al* [17]
3. Catalase activity in RBC was measured by method developed by Beauchamp & Fridovich [15].
4. G_6PD activity in RBC was measured Glock and Maclean assay method [18].

2.2 **Statistical analysis**

Results were expressed as mean and standard deviation (SD). Comparison between two variables were done using Student's *t* test. Correlations between the variables were examined using the Pearson's correlation coefficient.

3. Results & analysis

The study was conducted on 50 cases of RA and 50 normal controls. Out of the 50 cases of RA, there were six cases with active disease and the rest were in remission. So, the study was evaluated by grouping into three – control, cases active and cases inactive.

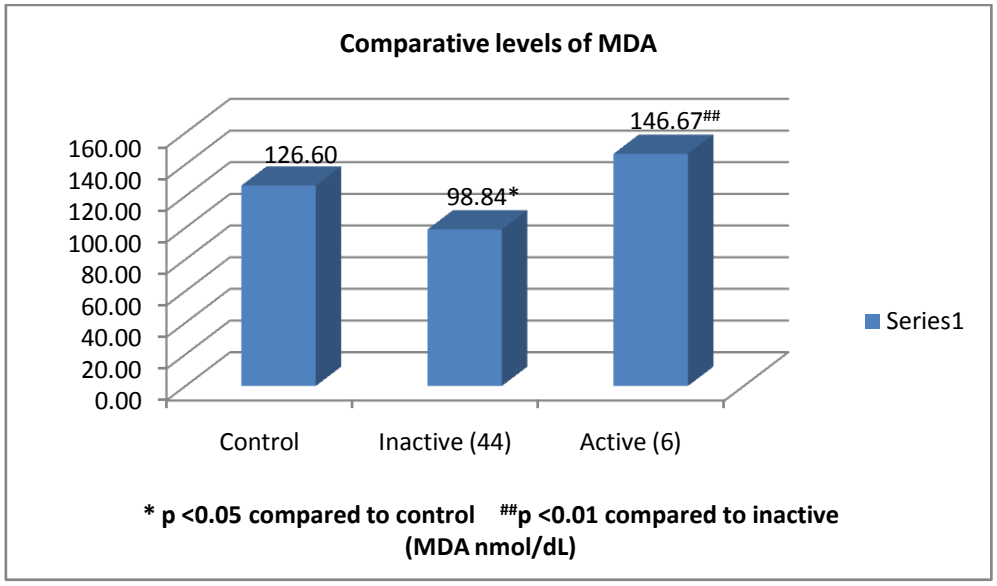
3.1 Comparison of mean levels of MDA, G_6PD, SOD and Catalase

Table 1: Comparison of mean levels of MDA, G_6PD, SOD and Catalase

MDA nmol/dL	**Control (n=50)**	**Inactive (n=44)**	**Active (n=6)**
	126.6 ± 3.20	98.84 ± 3.20*	146.67 ± 7.75##
Enzymes			
G_6PD U/gm of Hb	12.1 ± 2.09	27.06 ± 2.23**	25.8 ± 2.18*
SOD U/gm of Hb	2286.78 ± 65.25	2248.39 ± 119.88	2777.7 ± 469.75
Catalase k/gm of Hb	6.53 ± 0.49	12.28 ± 0.78**	7.82 ± 1.71

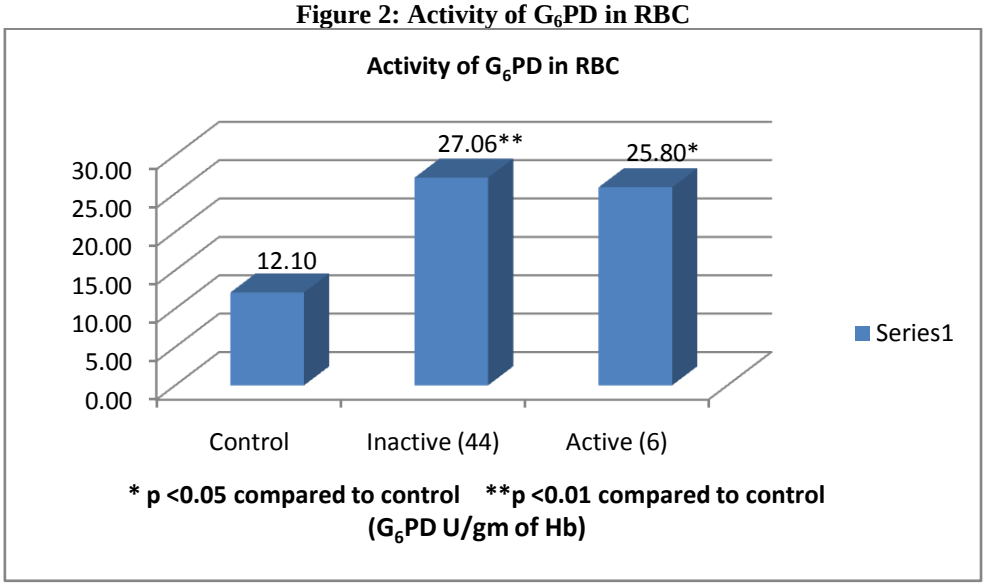
Significance: *p <0.05 values compared to control; **p < 0.01 values compared to control; # p <0.05 values compared to inactive; ## p <0.01 values compared to inactive

Figure 1: Comparative levels of MDA



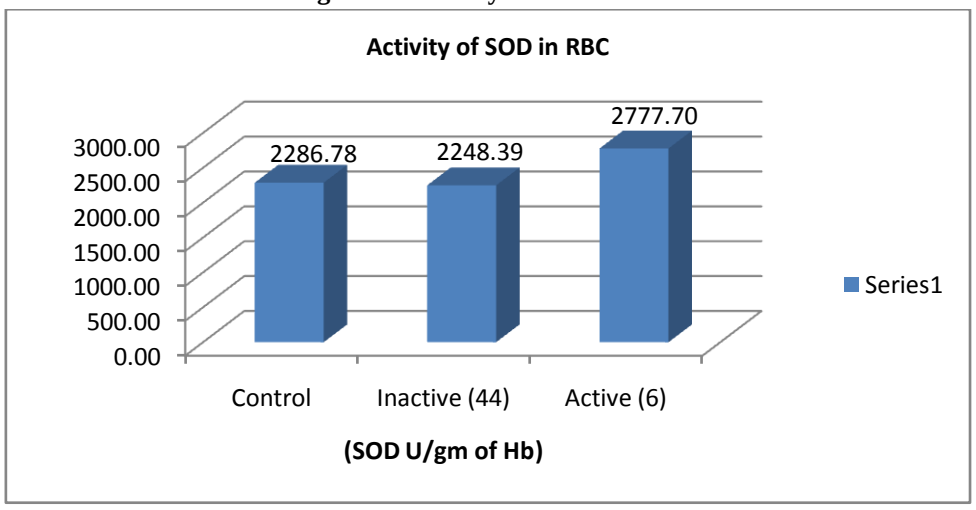
Control 126.6 ± 3.20 Inactive 98.84 ± 3.20* Active 146.67 ± 7.75##

Figure 2: Activity of G_6PD in RBC



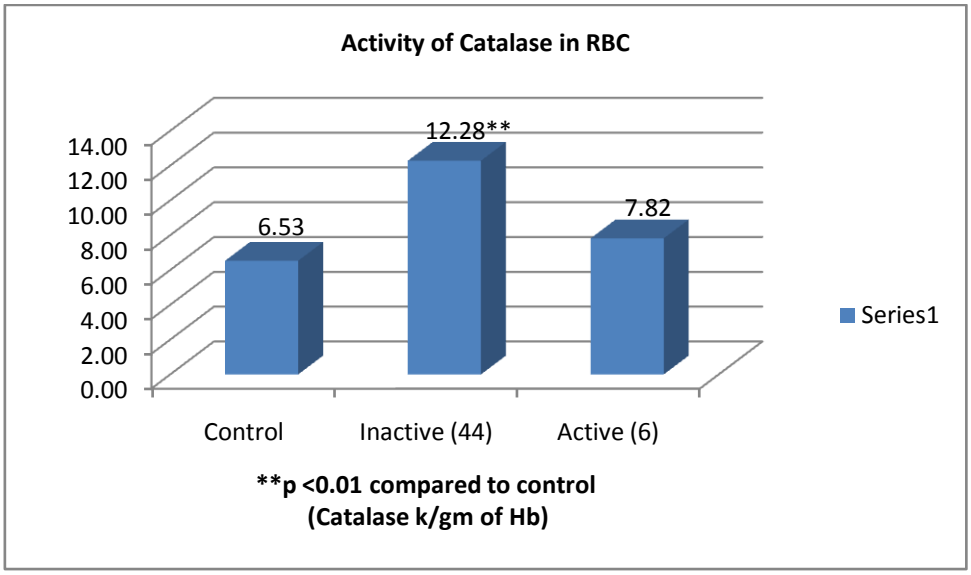
Control 12.1 ± 2.09 Inactive 27.06 ± 2.23** Active 25.8 ± 2.18*

Figure 3: Activity of SOD in RBC



Control 2286.78 ± 65.25 Inactive 2248.39 ± 119.88 Active 2777.7 ± 469.75

Figure 4: Activity of Catalase in RBC



Control 6.53 ± 0.49 Inactive 12.28 ± 0.78** Active 7.82 ± 1.71

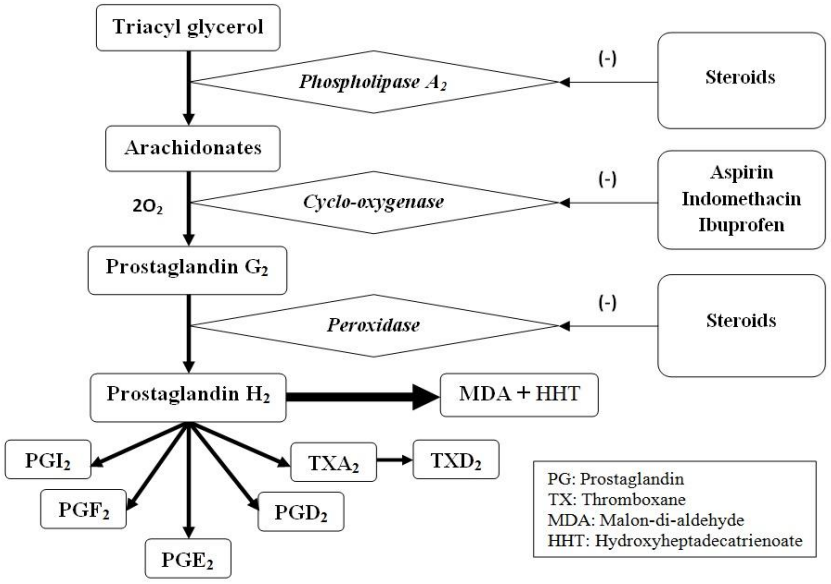
4. Discussion

In active cases, compared to the control, MDA levels were significantly high ($p < 0.01$) and in inactive cases, significantly low ($p < 0.05$) [Figure 1]. There is remarkable elevation of MDA levels in patients with RA compared to inactive and control. A similar study was conducted by the Departments of Medicine and Biochemistry at All India Institute of Medical Sciences, New Delhi, where the compared the levels of MDA in RA with healthy controls and patients with Osteoarthritis (OA). Their study showed that serum MDA levels in RA were significantly higher than healthy controls as well as patients with OA. [19] Similar observations are also noted in many other published studies [20-24].

Low serum levels of MDA found in inactive cases may be due to influence of drugs like non-steroidal anti-inflammatory drugs (NSAIDS), steroids, and disease modifying anti-rheumatoid drugs (DMARDS) as part of treatment, which cause remission of disease. This suggests the usefulness of MDA levels as a biochemical parameter to predict whether the disease is going for remission with treatment or not. [16,25,26]

Steroids and NSAIDS used in the treatment of RA have free radical inhibiting activity. The levels of action of various anti-inflammatory drugs in the lipid peroxidation pathway are shown below [Figure 5].

Figure 5: Lipid peroxidation and level of action of anti-inflammatory drugs [27]



MDA is formed as a product of lipid peroxidation during eicosanoid metabolism. Prostanoid synthesis involves the consumption of two molecules of oxygen, catalysed by prostaglandin H synthase (PGHS). PGHS has two separate enzyme activities namely cyclo-oxygenase and peroxidase. PGHS has two isoenzymes, PGHS-1 and PGHS-2, both possessing cyclo-oxygenase and peroxidase activity. The product of cyclo-oxygenase pathway, an endoperoxide, prostaglandin H (PGH), is converted to prostaglandins D, E and F as well as Thromboxane (TXA) and prostacycline (PGI).

Aspirin, an NSAID inhibits cyclo-oxygenase of both PGHS-1 and PGHS-2 by acetylation. Most other NSAIDs such as Indomethacin and ibuprofen inhibit cyclo-oxygenase by competing with arachidonates. Transcription of PGHS-2, but not PGHS-1 is completely inhibited by anti-inflammatory corticosteroids [27]. Steroids also act at a higher level of the pathway by inhibiting Phospholipase A_2, thereby preventing cleavage of arachidonic acid.

Platelet activating factor (PAF) causes oxidative burst and stimulates the synthesis of eicosanoids, which is one of the pathways of production of free radicals in the body [28]. Likewise, Tumour necrosis factor (TNF) increases the production of eicosanoids and consequently, free radicals in the body [28]. Thus by inhibiting PAF and TNF by specific drugs, the production of free radicals is reduced, as evidenced by reduced level of MDA in inactive cases our study [29].

G_6PD activities of both active and inactive cases in RBCs were elevated compared to controls ($p < 0.05$ & $p < 0.01$ respectively) [Figure 2]. This could be due to disinhibition of G_6PD, a regulatory enzyme of HMP pathway. Antimalarials used in the treatment of RA have superoxide radical scavenging effect. The superoxide is dismutated by SOD to form H_2O_2, which is then acted upon by glutathione peroxidase to form inert water molecules. In this, coenzyme NADPH generated mainly by G_6PD of HMP shunt pathway is consumed and its oxidised form, NADP is released. This causes disinhibition of G_6PD, the regulatory enzyme of HMP shunt pathway. This is reflected as raised activity of G_6PD in the study. Further studies are needed for confirmation of this. Gheita *et al* [30] in their study found that in RA, the activity of G_6PD in RBCs was reduced. This difference may be due to the difference in phases of activity of RA.

SOD and catalase, though elevated, did not show statistically significant difference in activity between cases and control [21,22,31]. [Figure 3 & 4] Thus G_6PD, SOD and catalase levels did not show consistent correlation for active, inactive and control sub-groups and are poor predictors of oxidant stress in RA patients receiving treatment.

The elevated levels of enzymes in cases may be due to increased synthesis of SOD in response to hyper-oxidant stress [32]. Enzyme elevation, though not statistically significant, has practical significance. The drugs used in treatment of RA may be causing scavenging of free radicals by promoting activity of enzymes. Further studies are needed to establish this. In some studies, lowered activity of RBC catalase and SOD in RA, have been reported [33,34].

5. Conclusion

Overproduction of free radicals by inflammatory processes in RA causes oxidative injury and damage antioxidant defence system in RA patients.

The elevated lipid peroxidation in plasma in the present study, indicated by elevated MDA can be related to a compensatory defence system in RA. Thus, MDA levels in RA could be used as biochemical marker of disease activity and monitor treatment response.

There is no definite correlation between enzyme levels of SOD, G_6PD and catalase and disease activity in the present study. The drugs used in treatment may be causing scavenging of free radicals by promoting the activity of enzymes. Free radical overproduction in the course of disease and interference by the drugs taken for treatment may be affecting enzyme activity. Irregular pattern of drug intake could also be a factor that contributed to the lack of correlation of enzyme levels and disease activity. To establish this, further elaborate study is required.

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