Oxidative stress and antioxidant status in patients with rheumatoid arthritis

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1. Introduction

Rheumatoid arthritis (RA) is a multifactorial autoimmune disease affecting around 1% of the world's population leading to autoimmune arthritis. Though the exact etiology of RA remains unknown, various environmental and biological triggers have been suspected. The most important factor which is implicated in the pathogenesis of RA is oxidative stress. However the exact relationship between antioxidants and lipid peroxidation is not yet known in patients of RA. Hence the case control study was conducted in 50 patients of rheumatoid arthritis (Group II) diagnosed by criteria's recommended by American Rheumatology Association were compared with age and sex matched 50 normal healthy controls (Group I). Malondialdehyde (MDA) a marker of oxidative stress, antioxidant enzymes Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) were measured by enzymatic spectrophotometric method. MDA levels was significantly increased (p< 0.01) in RA patients as compared to controls. The concentrations of GPx and SOD were significantly lower in RA patients as compared to normal healthy adults. Our findings indicate that oxidative stress does occur in patients RA patients. Treatment with antioxidant therapy to limit the disease process of RA patients needs to be assessed.
(PUFA) in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function. The harmful effect of reactive oxygen species is neutralized by a broad class of protective agents termed antioxidants which prevents oxidative damage by reacting with free radicals before any other molecules can become a target and antioxidant enzymes super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) play an important role in the protection of cells and tissues against free radical mediated tissue damage [5,6].

Numerous studies have indicated discordant results in patients with RA. Hence, the aim of the present study was to assess the lipid peroxidation and effect of enzymatic antioxidants in patients with RA.

2. MATERIALS AND METHODS

Present study was designed as a case control study conducted in the Department of Biochemistry, NKP Salve Institute of Medical Sciences. 50 patients of RA and 50 normal healthy controls matched for age, sex were included in this study. Patients were diagnosed by the criteria’s recommended by the American Rheumatism Association [7]. Patients suffering from diabetes mellitus, abnormal renal and hepatic function, history of myocardial infarction patients on long term medications, other causes of arthritis like osteoarthritis, gout were excluded as these diseases directly or indirectly lead to free radical production.

The research protocol was approved by ethics committee of the institution. 5 ml of venous blood samples was collected in EDTA bottles and plain bulbs from patients with rheumatoid arthritis and normal healthy individuals. Blood samples was centrifuged at 3000g for 10 minutes.

Malondialdehyde was measured by the method of randoxy laboratory. This method was based on the fact that lipid peroxide condense with 1 methyl -2 phenyl indole (MPI) under acidic conditions resulting in the formation of a red chromophore. To determine specifically lipid peroxide in plasma, proteins are precipitated to remove water soluble MPI reactive substance. The level of lipid peroxide is expressed in term of malondialdehyde. Tetramethoxypropane, which is converted quantitatively to MDA was used as standard.

The erythrocytic GPx was estimated by spectrophotometric kit method [8]. The principle being that GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance was measured at 340nm.

SOD was measured from hemolysate by spectrophotometric enzymatic kit method [9]. The principle employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The statistical analysis was done by student t-test.

3. Results & Discussion

Table 1 shows comparison of MDA, SOD, GPx levels in RA with control subjects. MDA levels were found to be significantly elevated in patients with RA compared to control. F. Karatas et al [10], S. D. Walwadkar et al [11], Cimen MY et al [12], Desai PB et al [13], Sarban S et al [14], Shivani Jaswal et al [15], Kamanli A et al [16] reported higher MDA levels in patients with RA in their studies [10-16]. It indicates increased oxidative stress because of increased lipid peroxidation. MDA, the product of lipid per oxidation can exacerbate inflammation by producing immunogenic molecule and causing cell damage. The polysaturated fatty acids are more prone for lipid peroxidation and loss of lubrication of synovial fluid is very important consequence of exposure of synovial fluid to superoxide and hydrogen peroxide. Our results differed from other studies which found that MDA levels are high when patients are in active stage of the disease and our patients were not in the active stage [17-18]. Olevieri et al [19] reported no change in lipid peroxidation in RA patients but Saowanee et al [20] and Ozkan et al [21] observed increased MDA levels but not statistically significant to conclude that there was an increased oxidative stress in RA patients because supporting results were not obtained from total oxidative status and SOD measurement. SOD, an antioxidant enzyme, catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide anion, which is believed to be one of the initiators of free radical production reactions plays an important role in the determination of the levels of antioxidant enzyme SOD. At physiological rates of hydrogen peroxide generation, the glutathione system is important in detoxifying hydrogen peroxide. GPx reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

Decreased activities of antioxidant enzymes (SOD & GPx) were found to be statistically significant in RA patients compared to controls in our study. The results of present study are similar to that observed by F. Karatas et al [10], N Aryaeian et al [22], Bae SC et al [23], Desai PB et al [13]. The decreased antioxidant enzymes levels might be due to utilization of these enzymes for detoxification of free radicals.

Our findings are contradictory to the findings of Alpay et al [24] & Ozkan et al [21] who reported no change in SOD levels of RA patients & controls.

However, Cimen MY et al [12] observed higher SOD levels and unchanged GPx activity in RA patients suggesting that excessive free radical production may be through xanthine – xanthine oxidase system rather than an impaired antioxidant system and therapeutic use of XO enzyme inhibitors can be beneficial. Similarly D Vijay Kumar et al [25] reported increased levels of SOD and GPx in their study which could be due to dismutate the excess superoxide radicals that are generated and diffused from the inflammatory sites and due to over expression of antioxidant defence system of RA patients.
Thus in RA patients overproduction of free radicals lead to imbalance in the oxidant antioxidant system. This imbalance might play a role in the tissue damage and inflammation process in this disease.

Hence with regards to this, further studies regarding the administration of antioxidant therapy along with conventional drugs and safety and efficacy of such therapy in limiting the disease process needs to be carried out.

Table I. Levels of MDA and Enzymatic Antioxidants in healthy subjects and in patients with RA

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<tr>
<th></th>
<th>Group I (n=50)</th>
<th>Group II (n=50)</th>
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<tbody>
<tr>
<td>MDA (nmol/litre)</td>
<td>1.09±0.26</td>
<td>3.48±0.50*</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>357.63±82.24</td>
<td>200.07±25.71*</td>
</tr>
<tr>
<td>GPx (IU/L)</td>
<td>10 188.36±843.62</td>
<td>4692.37±463.74*</td>
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4. References


