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RESEARCH ARTICLE

OXIDATIVE STRESS AND ENZYMATIC ANTIOXIDANT STATUS AND SERUM CALCIUM PHOSPHORUS LEVELS IN RHEUMATOID ARTHRITIS

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Abstract

Rheumatoid Arthritis (RA) is a chronic multisystem disease of unknown cause. It is a major cause of morbidity as it affects the joints, causing stiffness and loss of mobility. Enhanced lipid peroxidation may occur in RA as a result of imbalance between scavenging mechanisms and free radical generation process. Objectives of the present study were to determine oxidative stress by estimating blood melondialdehyde (MDA) levels and to assess enzymatic antioxidant status by estimating glutathione peroxidase (GSH-Px) and catalase in RA and also to find out the effect of this disease process on minerals by estimating serum levels of calcium and phosphorus in RA patients and then comparing with healthy individuals. Forty (40) subjects served as controls and 40 RA patients served as cases. The controls and cases were from both sexes and age group of 40-60 years. The parameters were estimated by standard biochemical methods.The blood levels of MDA in RA cases were significantly increased as compared to controls ($p<0.0001$). The plasma activity of GSH-Px ($p<0.0001$) and catalase ($p<0.0001$) in RA cases were significantly decreased as compared to controls. The serum levels of calcium in RA cases were significantly decreased as compared to controls ($p<0.0001$). The serum levels of phosphorus in RA cases were significantly increased as compared to controls ($p<0.0001$). The present study concludes that there is an increased oxidative stress and a decreased antioxidant defense in patients with RA as evidenced by increased lipid peroxidation and low antioxidant levels. Also there is a significant variation in calcium and phosphorus levels indicating altered bone metabolism in RA.

Key words: Catalase, Calcium, Glutathione peroxidase, Melondialdehyde, Phosphorus, Rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis is a persistent inflammatory synovitis usually involving peripheral joints in a symmetric distribution [1]. The potential of the synovial inflammation to cause cartilage damage and bone erosions and subsequent changes in joint integrity is the hallmark of the disease. The important feature of RA is non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues and joint deformities. The characteristic feature of established RA is not only a polyarthritis, and the name 'rheumatoid disease' is preferable since it directs attention to the whole patients and not just the joints [2]. RA

affects approximately 1-2% of the total world population. Annual incidence rate of RA between 0.5% to 1% of total population is reported every year in both developed and developing countries. Women are affected more than men. The onset is more frequent during the fourth and fifth decades of life , with 80% of all patients developing the disease between the ages 35 and 50 years. Actual reason behind bone erosion and joint deformities is not fully understood. Many investigators have focussed on oxidative stress since last few years and suggest that RA patients are more prone to lipid peroxidation. Lipid peroxidation occurs as a result of free radicals generated in the body. Free radicals and free radical derived oxidants play important roles in biological system and have been implicated in the pathology of many diseases. In recent years it has been shown that oxidative stress and antioxidants play an important role in the disease process of RA. So the present study was undertaken to assess oxidative stress by measuring MDA and antioxidant status by estimating GSH-Px and catalase in RA patients. Hypoxic conditions also disrupt an intracellular ionic environment and alter calcium and phosphorus levels. So the present study also aims to find out the effect of RA disease process on calcium and phosphorus levels.

MATERIALS AND METHODS

A total of 80 subjects (40 controls and 40 cases of RA) were studied over a period of one year. Ethical clearance was obtained from the Institutional Ethical Committee, Jawaharlal Nehru Medical College (JNMC), Belgaum. Informed consent was taken from all participants. All the participants were in the age group of 40-60 years, of both sexes and were not on any nutritional supplements. Inclusion criteria comprised of clinically diagnosed cases of RA confirmed by laboratory tests, admitted in KLE Society's Dr. Prabhakar Kore Hospital and Medical Research Centre, attached to JNMC, Belgaum. Exclusion criteria comprised of patients with osteoarthritis, tubercular arthritis, infective arthritis, rheumatic fever, pulmonary tuberculosis, pneumonia, costochondritis (Tietze's syndrome), arthritis other than RA fitting into any syndromes, chronic smokers and alcoholics and any other systemic disorder. Age and sex matched 40 healthy individuals served as controls.

Five ml of blood sample was collected by vena puncture from each participant under strict aseptic precautions. Out of this 1ml of whole blood was used for estimation of MDA by Thiobarbituric acid (TBA) method [3]. This reaction depends on the formation of pink coloured complex between MDA and TBA, having an absorption at 532 nm. Two ml of the sample was processed immediately for hemolysate preparation [4], which was used for estimation of enzymatic antioxidants like GSH-Px and catalase by Beutler's E method respectively [5]. The remaining 2 ml of blood sample was allowed to stand for sometime, the serum was separated and used for estimating calcium and phosphorus [6]. Calcium was estimated by Trinder method which is by precipitation. Trinder has described the use of naphtholhydroxamic acid as a precipitating agent which is so efficient that only a small excess of reagent is required. This means that it is unnecessary to wash the precipitate to remove excess reagent and a very convenient and accurate method results. Phosphorus was estimated by Delsal and manhouri method which is a variant of the phosphomolybdic acid reaction. A substituted phenol is used as a reducing agent and the pH is controlled by an acetate buffer. Copper in the buffer hastens colour development which is complete after 5 minutes. The blue colour is stable for atleast 30 minutes.

Statistical analysis

The above mentioned parameters were recorded as mean and standard deviation (SD). Statistical analysis of all the obtained parameters was done using unpaired student's 't' test. The observations were tabulated and conclusions were obtained from the biochemical data.

RESULTS

The results of the present study are summerised in the table 1 and 2. The blood levels of MDA in RA cases were significantly increased as compared to controls ($p< 0.0001$). There was significant decrease in GSH-Px ($p<0.0001$) and catalase ($p<0.0001$) activity in RA patients as compared to healthy controls. The serum level of calcium was significantly decreased in cases as compared to controls ($p<0.0001$). The serum level of phosphorus was significantly increased in cases as compared to healthy controls ($p<0.0001$).

Table 1: Showing MDA and enzymatic antioxidants in controls and RA cases

Parameters	MDA nmol/ml	GSH-Px IU/g of Hb	Catalase IU/g of Hb
n=40 Controls Mean ± S.D	6.19 ± 0.96	20.52± 1.18	7.2 ± 0.6
n=40 Cases Mean ± S.D	11.48 ± 0.76	14.15±1.34	2.4 ± 0.42
p-values	<0.0001	<0.0001	<0.0001

Table 2: Showing serum calcium and phosphorus levels in controls and RA cases

Parameters	Calcium (mg/dl)	Phosphorus (mg/dl)
n=40 Controls Mean ± S.D	10.21± 0.65	2.89± 0.57
n=40 Cases Mean ± S.D	7.33± 0.93	4.28± 0.81
p-values	<0.0001	<0.0001

DISCUSSION

Rheumatoid arthritis is a major cause of morbidity as it affects the joints, causing stiffness and loss of mobility. The cause of RA is mainly joint inflammation initiated by oxidative stress. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process. A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in the outer orbital [7]. Free radicals are produced in the body due to leak in the electron transport chain, inflammatory conditions , ionizing radiations, drugs, chemical toxins etc. Oxidation reactions ensure that molecular oxygen is completely

reduced to water . The products of partial reduction of oxygen are highly reactive and make havoc in the living systems. Hence they are also called reactive oxygen species (ROS) ex. superoxide anion radical ($O2^{-}$), hydroperoxy radical ($HOO^{\cdot}$), hydroxyl radical ($OH^{\cdot}$) etc. Important characteristics of the ROS are extreme reactivity, short life span, generation of new ROS by chain reaction and damage to various tissues. It is possible that , generation of ROS may be particularly important factor for bone resorption in inflammatory process.
The damage produced by ROS may be prevented by antioxidants. In view of the recent animal studies strongly suggesting anti-inflammatory role of antioxidants like superoxide dismutase [8] and vitamin E [9] in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA [10-16].

MDA is a product of lipid peroxidation and a reliable marker of oxidative stress. In the present study mean level of MDA was increased significantly in cases compared to controls. Our findings are in accordance with the study of Yousef Shaabani et al [17], Walwadkar SD et al [18], Gambhir et al [19], Kartas F [20], etc. MDA is a decomposition product of lipid peroxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. Intracellular defense system is largely dependent on the antioxidant enzymes such as GSH-Px, SOD, and catalase. GSH-Px is a selenoprotein antioxidant enzyme located in the cytosol and mitochondria. It removes hydrogen peroxide and other hydroperoxides [21]. Catalase is major antioxidant defense component present in all mammalian cell types, reacts rapidly with hydrogen peroxide inside of cells and converts it into water and oxygen. Catalase catalyzes the breakdown of toxic hydrogen peroxide directly to water and preventing the secondary generation of toxic intermediates such as hydroxyl radical. High levels of catalase activity are found in liver, kidney, red blood cells, microsomes and also in cytosol. Purified catalase have been shown to consist of four protein subunits, each of which contains Fe^{+3}, protoporphyrin group which is bound to its active site. In the present study the levels of GSH-Px and catalase showed significant decrease in RA cases as compared to controls. Our findings are in accordance with the findings of several other studies [22-24]. GSH-Px and catalase are the first line of defense against oxidative injury, decomposing oxygen and hydrogen peroxide before interacting to form the more reactive hydroxyl radical. Decrease in the levels of GSH-Px and catalase could be due to inactivation of the enzymes by cross-linking or due to exhaustion of the enzymes by increased peroxidation. Serum calcium levels were significantly decreased in cases as compared to controls. The findings of our study are in accordance with the study of Walwadkar SD et al [18]. Decrease in calcitriol level may contribute to a negative calcium balance and acceleration of immunomodulatory effects as observed by studies of Oelenzer.P et al [25] and Kroger.H et al [26]. There is another study by D. L. Scott et al [27] where the authors have noticed that transient hypercalcaemia and hypocalcaemia occurred occasionally. Mean serum calcium levels are lower in disease than health and this occurs in RA as well as other diseases. Our findings are contradictory to the findings of Kennedy A.C et al [28] who showed that hypercalcemia is a common occurrence in RA. Serum phosphorus levels were significantly increased in cases as compared to controls. The findings of our study are in accordance with the study of Walwadkar SD et al [18]. The elevation of phosphorous may be related to tissue hypoxia with an increase in ATP degradation resulting in the release of inorganic phosphorous from cells. Acidosis is another factor that may act to promote shift of phosphate from the intracellular to extracellular pool. The rise in serum

inorganic phosphate may parallel increase in blood lactate levels suggesting that a state of parallel anaerobic metabolism may be contributory factor. As calcium and phosphorous are important constituents of bone, ultimately the bone metabolism is altered in RA.

CONCLUSION

Our study concludes that there is an increased oxidative stress and decreased antioxidant defense in patients of RA as evidenced by increased blood levels of MDA and decrease in the plasma activity of enzymatic antioxidants like GSH-Px and catalase, which is the root cause of joint inflammation causing arthritis. Supplementation of natural antioxidants to the individuals who are prone for RA may help to prevent the morbidity to certain extent. Our study also revealed that there was a significant variation in calcium and phosphorus levels in patients of RA. As calcium and phosphorus are important constituents of bone, ultimately bone metabolism is altered in RA.

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