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Role of Free Radicals on Retinitis Pigmentosa

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Retinitis pigmentosa is a retinal disease that leads to blindness. It has an unclear etiology and no known cure available in literature. The aim of this study is to study the role of free radicals on retinitis pigmentosa. This study was conducted on a test group composed of subjects who have been diagnosed with retinitis pigmentosa for ten years; and a control group of healthy individuals. To analyze the role of reactive oxygen products on retinal cell apoptosis, malondialdehyde (MDA) as a product of lipid peroxidation was measured. Also, to study the protectiveness of antioxidant enzymes over the retinal cells from apoptosis; activities pertaining to superoxide dismutase, catalase and glutathione peroxidase have been analyzed. The malondialdehyde values at the patient group have been found to be significantly higher than those at the control group. Antioxidant enzyme activities were found to be significantly lower in patient groups. Similar pattern of change was observed between patient and control group for all parameters in females and males. The malondialdehyde value was significantly higher and other parameters were lower in patient group according to controls. Results obtained from this research indicate that radical damage was high and antioxidant enzymes were low in retinitis pigmentosa. We may interpret these results to mean that exposure to free radical or antioxidant enzyme insufficiency may be some of the factors that trigger retinitis pigmentosa.

Key Words: Retinitis pigmentosa, Apoptosis, Free radical, Antioxidant.

INTRODUCTION

Retinitis pigmentosa is a group of diseases caused by a large number of mutations resulting in rod photoreceptor cell death followed by gradual death of cones[1,2]. Depletion of rods is responsible for night blindness, the first symptom of retinitis pigmentosa[1], but patients are still able to function well if illumination is adequate. Upon rod loss, gradual loss of cones start, accompanied by constriction of the visual field and eventually blindness. If cone death could be prevented in patients with retinitis pigmentosa, blindness could be averted[3]. The mechanism of cone cell death is uncertain[1]. Rod photoreceptors are the most numerous and metabolically active cell type in the retina. Rods consume the most oxygen. After rods die, oxygen level in the outer retina is elevated resulting in oxidative damage [4].

In several model of retinitis pigmentosa, exogenous antioxidant slow cone cell death, indicating a potential therapeutic approach in all retinitis pigmentosa patients despite tremendous heterogeneity in pathogenic mutations[5]. There is a very little number of studies that research the mediator role of reactive oxygen substrate for death of photoreceptor cells[2-4]. Considering that blindness can be prevented by searching the causes of this disease, of which etiology is not fully known yet and there is no therapy, it is aimed to study the role of free radicals in this disease.

EXPERIMENTAL

Total of 75 individuals-30 patients and 45 controls- were included in the study. The patient group was formed amongst patients who received an retinitis pigmentosa diagnosis between the years 1999 and 2009 at the Mersin University Medical Research Hospital, Clinic of Ophthalmology. Control group was composed of healthy individuals. All participants gave written informed consent as a part of the research protocol that was fully approved by the Mersin University Ethics Committee.

For both groups individuals not having chronic diseases (diabetes mellitus and hypertension) leading to loss of vision were included in the study. Presence of errors of refraction, along with cigarette and alcohol consumption was not taken into consideration. Patients with developed full blindness due to retinitis pigmentosa were included in the study.

Venous blood samples into tubes with EDTA were taken, centrifuged and stored by freezing for analysis with the purpose of conducting plasma measurements from the individuals

formed from adequate number of patient and control group with similar age group and sex. Studies were conducted on 30 blood samples from the patient group and 45 blood samples from the control group. In this study plasma malondialdehyde (MDA) level was examined to determine lipid peroxidation; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) levels in plasma were examined to determine antioxidant activity.

Measurement of plasma malondialdehyde concentration: Malondialdehyde level, as an index of lipid peroxidation, was determined by thiobarbituric acid (TBA) reaction according to the Yagi method[6]. This method is based on the measurement of the pink colour produced by interaction of thiobarbituric acid with malondialdehyde elaborated as a result of lipid peroxidation. The coloured reaction of 1,1,3,3-tetraethoxypropane was used as the primary standard.

Measurement of superoxide dismutase activity: Superoxide dismutase activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction by O_2^- generated by the xanthine/xanthine oxidase system. One unit of superoxide dismutase activity was defined as the amount of protein causing 50 % inhibition of the NBT reduction rate[7].

Measurement of catalase activity: Catalase activity of tissues was determined according to Aebi[8]. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm, resulting from enzymatic decomposition of H_2O_2. The difference in absorbance per unit time was a measure of catalase activity.

Measurement of glutathione peroxidase activity: Activity of glutathione peroxidase was measured spectrophotometrically at 340 nm. The method was based on the changes in absorbance resulting from the conversion of NADPH into NADP[9].

Statistical analysis: Statistical analysis was performed using SPSS version 11.5 statistical packet program. Shapiro-Wilk test was used to determine whether all parameters were normally distributed or not and it was found that all parameters were normally distributed. Descriptive statistics (count, %, mean ± standard deviation) were calculated in each group for all parameters. Student *t*-test was used to test the differences between the groups and sex for each parameter. Pearson chi-square and Fisher exact tests were used to determine relation between the groups and age, sex parameters. Graphs were obtained in STATISTICA 6.0 packet program. The results were considered statistically significant if *p* values were less than 0.05.

RESULTS AND DISCUSSION

In the current study, age variant was classified and variations between the groups were examined. Furthermore, variations between the groups were examined for the sex variant too. Average age of the individuals in the control group was identified as 28; average age of the individuals in the patient group was identified as 27. There is no statistically significant difference between the groups as to age and sex (*p* values consecutively 0.214 and 0.697). In other words, age distribution and distribution of sex are similar in the control and retinitis pigmentosa groups (Table-1).

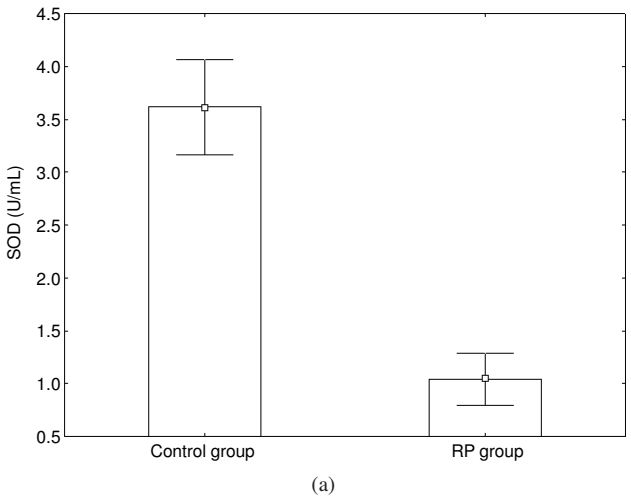
TABLE-1
AGE AND SEX VALUES OF EACH GROUP

Parameters		Control n (%)	RP n (%)	P
Age	20 -	7 (15.6)	11 (36.7)	0.214
	20-30	22 (48.9)	11 (36.7)	
	30-40	9 (20.0)	4 (13.3)	
	41 +	7 (15.6)	4 (13.3)	
Sex	Female	29 (64.4)	18 (60.0)	0.697
	Male	16 (35.6)	12 (40.0)	

n: Number of individuals.

Variations were identified between the groups for all parameters. For malondialdehyde values for the patient group are significantly greater than the values of the control group ($p < 0.0001$). Superoxide dismutase, catalase, glutathione peroxidase enzyme activity values for the retinitis pigmentosa group were lower than the values in the control group and these variances are considered statistically significant ($p < 0.0001$) (Fig. 1).

When variances for sex (women and men) at superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde parameters in control group were examined, variance for none of the parameters were seen statistically significant. Likewise, this was examined for also retinitis pigmentosa group and no variance was found in these parameters in men and women (Fig. 2).



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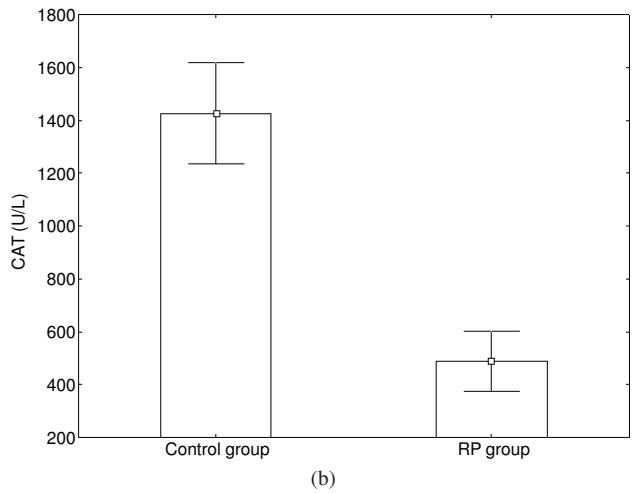

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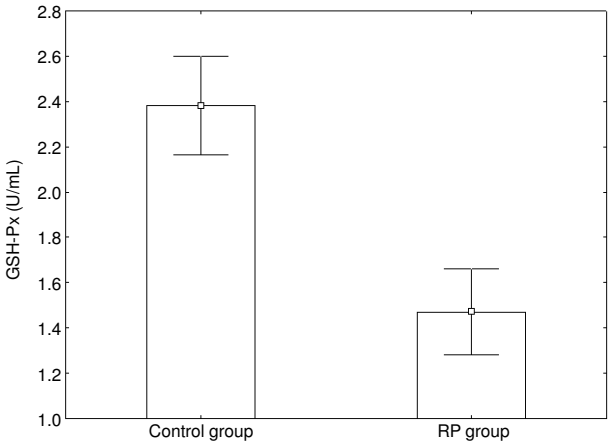

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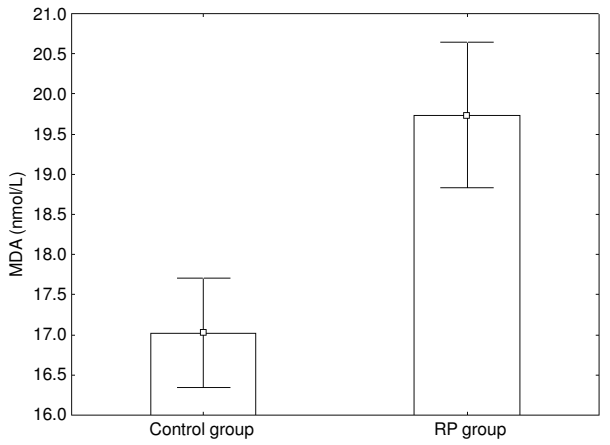
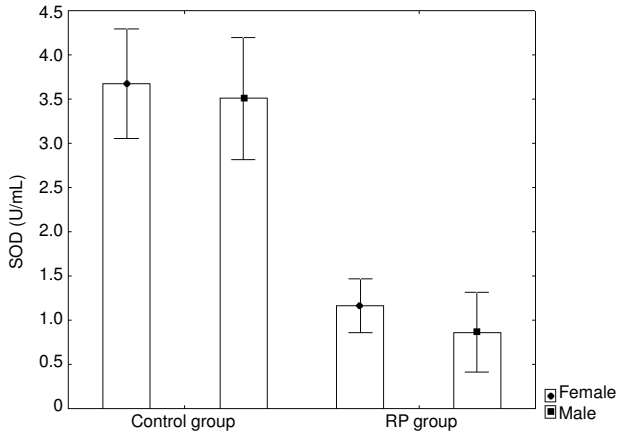


(d)

Fig. 1. (a) Superoxide dismutase activity, (b) catalase activity, (c) glutathione peroxidase activity and (d) malondialdehyde level in control and retinitis pigmentosa groups

When variance between the groups for parameters of women examined, variance for all parameters was found significant. For example, values of the retinitis pigmentosa group for superoxide dismutase, catalase and glutathione peroxidase were lower than the value in control group. When malondialdehyde was examined value for the retinitis pigmentosa group is greater than the control group value. For men also, similarly, variance between the retinitis pigmentosa and the control group were found to be statistically significant (Fig. 2).



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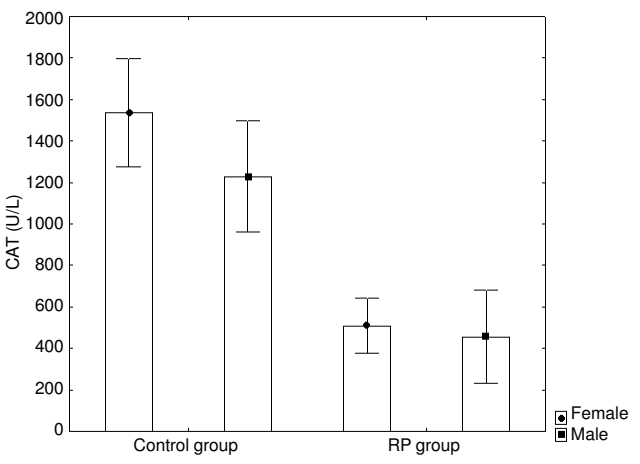

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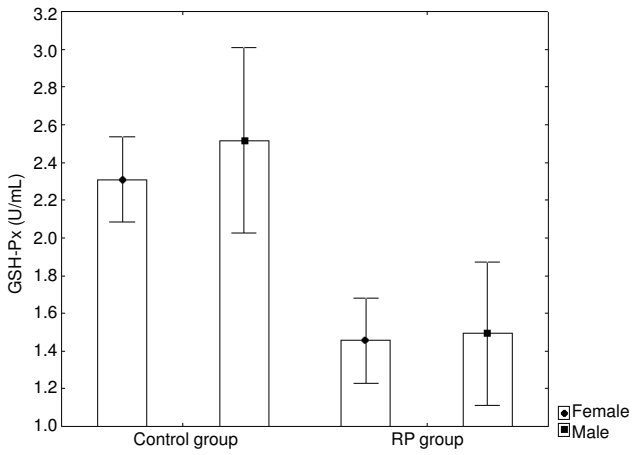

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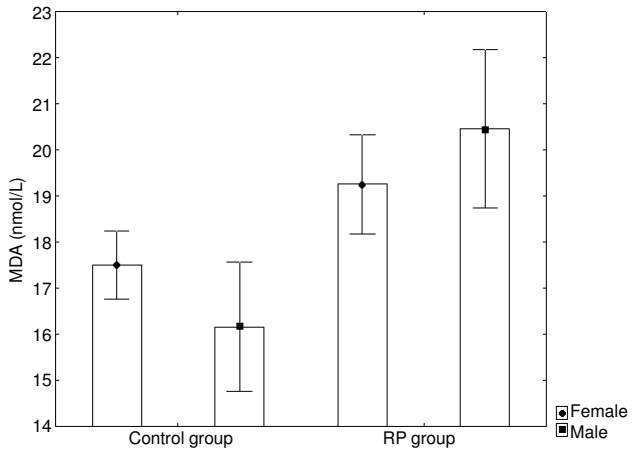

(d)

Fig. 2. (a) Superoxide dismutase activity, (b) catalase activity, (c) glutathione peroxidase activity and (d) malondialdehyde level in female and male individuals of control and retinitis pigmentosa groups

Retinitis pigmentosa is an incurable retinal disease that leads to bilateral vision loss and blindness. One puzzling aspect concerns the progression of the disease. Although most mutations that cause retinitis pigmentosa are in rod photoreceptor-specific genes, cone photoreceptors also die as a result of such mutations[10]. Matter to be clarified at retinitis pigmentosa is; why first the

rods and later the cones die and the mechanism for this is not fully known. And second; why the cone cells face apoptosis through being affected by genetic defect encountered by the rod cells[11,12]. Additionally, emphasis is placed on many factors other than gens. However, information on these factors is not definite yet.

There are some theories of why cones depend upon rods for their survival. One of the possible explanation for the slowly progressive death of cones after the death of rods is oxidative damage. Rods are more numerous than cones and are metabolically active cells with a high level of oxygen consumption. Choroidal vessels are not subject to autoregulation by tissue oxygen levels and as death of rods occurs, the level of oxygen in the retina increases[4].

Furthermore, it is known that reactive oxygen metabolites produced by active neutrophils lead to tissue damage[13]. Consequently, even an inflammation at micro level which may affect photoreceptor cells in which oxidative metabolism is intensive, can lead to cell death caused by the genetic structure[14]. Retina is a cell having the highest metabolic rate, in comparison to the unit weight. Damage caused by free oxygen radicals formed as the result of metabolic activity occurring in the cell may cause photoreceptor cell death. Information on oxidative and metabolic variances in the retinitis pigmentosa patients is rather little. In the previous study, it was reported that retina degeneration triggered by light is mitigated by antioxidants. This, too, shows that oxidative stress is responsible for the death of the photoreceptor cells[15]. However, number of studies on this issue is little.

Purpose of this study is to search the role of free radicals in retinitis pigmentosa. Some findings have been seen leading to the consideration that free radicals can be effective. The most important free radicals are superoxide and hydroxyl radicals[16]. Identification of free radical reactions is determined with lipid peroxidation, especially, with malondialdehyde. Photoreceptor cell membrane is quite rich in polyunsaturated fatty acid. As the result of cell degeneration which is primer pathology in retinitis pigmentosa, toxic products very easily lead to peroxidation in the membranes of these cells, leading to malondialdehyde level rises[17].

In the malondialdehyde findings in our study, when plasma malondialdehyde levels of the patients with retinitis pigmentosa are compared with those of the control group, patient plasma malondialdehyde level was found to be significantly higher than the control group ($p < 0.0001$). Study of Wu and his colleagues[18] corroborate our findings too. They have shown that oxidative damage plays a big role in cone cell death in retinitis pigmentosa[18]. In studies conducted with various animal models, too, relation of retinitis pigmentosa with apoptosis was mentioned as occurring as the result of oxidative stress. Also in the same studies, benefits gained from antioxidants in therapies were also reported[19].

Besides, examination of the components of protective antioxidant defense system are indicative of free radicals and cell damage they create: superoxide dismutase, which is an enzyme containing Cu^{2+} and Zn^{2+}, causes detoxification of superoxide radical and protecting the cell from free radical damage. Superoxide anions constantly generated by mitochondria is transformed into H_2O_2 in the organism through superoxide dismutase[16]. H_2O_2 is harmful for biologic systems and increases formation of $OH^{\bullet}$. One of the enzymes destroying H_2O_2 in the cell is catalase[20].

In this study, when plasma superoxide dismutase, catalase and GPx antioxidant enzyme values of the patients with retinitis pigmentosa diagnosis are compared with those of the control groups, each enzyme value was found significantly lower than the control group ($p < 0.0001$). A research with supporting features of these findings, conducted in John Hopkins University[21], have shown that increased expression of catalase and superoxide dismutase, in mouse model of retinitis pigmentosa, have significantly decreased cone cell death. As a different finding, Geromel and colleagues[22], have shown that superoxide radical was excessively high in the retinitis pigmentosa patient and this triggered the superoxide dismutase activity. Increased superoxide dismutase activity associated with cell death brings forth the interpretation that it is indicator of oxidative stress.

In addition, some studies showing reactive oxygen types in photoreceptors apoptosis play a mediator role, it was reported that antioxidants reduce the retina degeneration. This fact makes one think that oxidative stress is responsible for the death of photoreceptor cells[1-3,15].

It is suggested that, through following this and similar studies, free radicals play an important role within the mechanisms leading the cell to apoptosis and antioxidant therapy may provide benefit. This issue will be clarified by studying and identifying other factors playing role in etiology.

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