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RESEARCH ARTICLE

The relationship between blood antioxidant enzyme levels and genotype in athletes training at high altitudes.

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Abstract

Objectives: Although acute exhaustive exercise increases oxidative stress, regular exercise programs strengthen oxidant protection. The aim of this study was to investigate the effects of high altitude training on malondialdehyde (MDA) as index of lipid peroxidation. We also aimed to investigate the incidence of gene polymorphism of the manganese superoxidodismutase (Mn-SOD), glutathione peroxidase-1 (GSH-Px1) and catalase (CAT) enzymes.

Materials and Methods: Thirty male subjects between the ages of 20 to 25 trained at a high altitude camp at 2750 meters for 10 days. The subjects were divided into 3 groups: athletic (n=10), sedentary (n=10), and sedentary control (n=10). While the athletic and sedentary groups performed aerobic exercise at high altitude, the sedentary control group did not partake in any physical exercise. Blood samples were obtained pre-exercise, post-exercise, before ascent, on the 1st, 5th and 10th days at high altitude, and after descent.

Results: There were significant statistical difference in CAT and GSH-Px1 activity prior to ascent, at high altitude and on the 1st, 5th, and 10th days at high altitude and after descent ($p < 0.05$). However, there were no statistically significant differences between groups with respect to plasma MDA and SOD levels ($p > 0.05$). There was a statistically significant difference in terms of CAT genotype between groups ($p < 0.05$). However, there were no statistically significant differences for Mn-SOD and GSH-Px1 genotype among the groups of before ascent, at high altitude, on the 1st, 5th and 10th days at high altitude and after descent ($p > 0.05$). For both genes, we did observe a statistically significant difference for CAT allele ($p < 0.01$). However, there were no differences between the groups in terms of allele frequency.

Conclusion: We concluded that high altitude might play a role in the gene activity of oxidative stress and antioxidant enzymes.

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Introduction:-

The human metabolism when undergoing aerobic stress produces a number of reactive oxygen metabolites and radicals during metabolic processes. A number of studies have suggested that athletic activities performed at high intensity cause an increase in the production of free radicals in skeletal muscles and the myocardia (1).

These damaging molecules lead to a number of diseases, including cancer, lung diseases, brain and kidney dysfunctions, blood diseases and premature aging (2-4).

One of the measures taken against these diseases is to develop an antioxidant defense system against free radicals. Due to this and a number of similar effects, it has been found that activities, which are performed regularly, can protect against and even help treat many diseases (5-9).

Free radicals, which are continually produced in the human body, exist in balance with the antioxidant defense system which itself is a mechanism to defend against said free radicals. If, for whatever reason, production of these reactive oxygen derivatives increases and outpaces the antioxidant defense system, then oxidative stress occurs. The antioxidant defense system tends to adapt to a situation by developing itself in these kinds of situations. However, during acute increases to which the antioxidant defense system is not habituated, tissue damage may occur. The antioxidant defense system, which occurs naturally in the human body in some cases, may not be sufficient to impede free radical damage. In recent years, researchers have made quite intensive studies on methods to develop the antioxidant defense system. Because of this, this study investigates how athletes who train regularly and sedentary subjects are affected by oxidative stress levels against acute athletic strain. There are two basic antioxidant systems, which defend against this oxidative stress in cells. These are enzymatic defense systems such as superoxide dismutase, glutathione peroxide, and catalase, and non-enzymatic antioxidant defense systems such as vitamin E, vitamin A, and glutathione (10,11).

The athlete, while ascending to high altitudes not previously experienced, faces a number of ecological factors with which he/she is not familiar, such as oxygen deficiency, decreased air pressure, sun rays and different types of aerosols.

For this reason, the adoption of a training program for athletes at high altitudes will contribute to studies on athletic performance and sport science, by examining the effects of high altitude training on performance, the antioxidant defense system and genotype relations.

Materials and Method:-

A total of 30 students in the Department of Physical Education and Athletics at YuzuncuYil University, between the ages of 20 and 25, participated in the study, comprising 3 groups: 10 athletes who exercised regularly, 10 sedentary subjects who exercised on a sporadic basis, and 10 sedentary controls subjects who never exercised. Subjects in the athletic group had been exercising regularly for at least 5 years.

The sedentary group consisted of subjects who had not participated in any training program on a regular basis for the last 2 years. The subjects, who had no clinical complaints or diagnoses, were given information about the study, and were asked to sign forms prepared according to the Helsinki Report and containing information about the study indicating the voluntary status of their participation.

The average age of the athletic group was 24.23 ± 4.50 , while that of the sedentary group was 22.17 ± 1.77 . Non-athletic group none of the subjects used antioxidants on a regular basis.

In this study MDA, GSH-Px1, CAT and Mn-SOD values were examined both prior to and following strenuous athletic activity performed at high elevations, in order to determine the connection between oxidative stress and exercise. The athletes avoided active and regular training for approximately 1-2 months during the rest season. In order to determine the extent of the affect of exercise on oxidative stress in athletes returning to intensive activity following this rest period, the acute reaction to exercise after the rest period was examined. MDA, GSH-Px1, CAT and Mn-SOD values were used to measure oxidative stress. It is put forth that during aerobic exercise oxidative stress increases in connection with activity level with the increase in electron seepage in the mitochondria. Because of this, for our study we chose athletic activity that includes aerobic exercise. To carry this out, 20 students in the Department of Physical Education and Sports at Yuzunu Yil University, ranging in age from 20 to 25, camped for 10 days at high-altitude (at Mt. Nimrod, at an elevation of 2.800 m). The subjects were divided into three groups: 10 athletes who trained regularly and 10 sedentary who did not train on a regular basis, all on Mt. Nimrod at an elevation of 2.800 m, and 10 people in the sedentary control group. The athletes followed an intensive endurance training program aimed at strength and stamina, consisting of two 60-minute sessions twice a day for 10 days at 2.800 m. The sedentary control group was not allowed to exercise at all.

Blood samples of the subjects were taken immediately prior to and after exercise, before ascending to high altitude (in the city of Van, at an elevation of 1.727 m), and at high altitude (at Mt. Nimrod, 2.800 m), 6 hours after training on the first, fifth and tenth days.

Our study comprised two stages: in the first, blood antioxidant enzyme levels (GSH-Px1, Mn-SOD and CAT) and MDA levels were biochemically analyzed, and afterwards, genotype activities of the GSH-Px1, Mn-SOD and CAT enzymes were examined at the molecular level.

The analyses were carried out in the biochemistry laboratory of YuzuncuYil Medical School, with evaluations made by experts in the relevant areas. For the biochemical analyses, blood samples with heparin were taken from the antecubital veins of the subjects in the athletic, sedentary and sedentary control groups, and the test tubes with EDTA were brought to the laboratory as quickly as possible. Whole blood in the test tubes with EDTA was stored at -20°C. In the test tubes with heparin, after 10 minutes in the centrifuge at 2,500 rpm, blood plasma was separated out. Blood samples with the plasma separated out were then cleaned three times with physiological serum and preserved at -20°C

In addition, for molecular analysis, 2 ml of blood the test tubes with EDTA prepared earlier taken from the subjects' antecubital veins. DNA samples were isolated from the whole blood samples taken from each group stored at +4°C.

In the statistical analysis, the Kolmogorov-Smirnow test was performed with the goal of determining whether or not the data exhibited normal distribution, while Leven's Bartlet test was applied to determine the homogeneity of the data. It was found that the data did not conform to a normal distribution pattern. As a result, the Mann Whitney U test, a non-parametric method used in paired comparisons, was performed, while the Kruskal Wallis H test was applied for the comparison of more than two groups of data. In order to determine statistically significant difference between the groups, the Mann Whitney U test was used for paired comparison with the goal of determining which groups the difference originated from. A *p* value of <0.01, <0.05 was accepted as statistically significant. The SPSS (Statistical Packages of Social Sciences, SPSS for Windows, Version 15.0, Inc, Chicago, IL, USA) statistical package program was used for all statistical tests.

Results:-

Oxidant (MDA) and Antioxidant (GSH-Px, CAT ve SOD) levels:-

MDA levels, GSH-Px1, CAT and Mn-SOD enzyme activities in the high-altitude regular exercise group, sporadic exercise group and control group are presented in **Table 1**.

Table 1. MDA levels, GSH-Px1, CAT and Mn-SOD enzyme activities in the high-altitude regular exercise group, sporadic exercise group and control group.

	Before ascent and at elevation	Before and after ascent	At elevation and after descent	<i>P</i>
MDA (nmol/ml)	1.331±3.518	1.673±2.849	0.341±2.142	<i>P</i> < 0.05
GSH-Px1 (U/gHb)	0.554±3.698	1.481±3.751	0.926±1.666	<i>P</i> < 0.05
CAT (k/gHb)	0.009±0.030	0.010±0.027	0.000±0.020	<i>P</i> < 0.05
Mn-SOD (U/gHb)	6.661±26.122	5.193±30.822	1.467±31.807	<i>P</i> > 0.05

MDA: Malondialdehyde

Mn-SOD: Manganese superoxide dismutase

GSH-Px1: Glutathione peroxidase-1

CAT: Catalase

Whole blood malondialdehyde levels:-

In the results of the study, in the analysis of the blood samples collected, the difference between measurements taken before and after ascent of the test subjects was found to possibly be statistically significant (*p*<0.05). For the variable MDA, the difference between the three groups in measurements taken prior to ascent, at elevation and after descent was found not to be statistically significant (*p*>0.05).

Blood Glutathione peroxidase-1 activity:-

In analyses performed on the erythrocyte package, the differences between measurements taken at elevation and after descent were determined to be statistically significant (*p*<0.05). For the variable GSH-Px1, the differences

between the three groups in measurements made prior to ascent, at elevation and following descent were not found to be statistically significant ($p>0.05$).

Blood catalase activity:-

Analyses performed on the erythrocyte package found the difference between measurements taken at elevation and after descent were to be statistically significant ($p<0.05$). For the variable CAT, the differences between the three groups in measurements made prior to ascent, at elevation and following descent were determined to be statistically significant ($p>0.05$).

Blood manganese superoxidizedismutase activity:-

In analyses of the erythrocyte package, the differences between measurements taken at elevation and after descent were not found to be statistically significant ($p>0.05$). For the variable Mn-SOD, the differences between the three groups in measurements made prior to ascent, at elevation and following descent were also determined not to be statistically significant ($p>0.05$).

The results of multiple paired comparisons based on the data are shown in **Table 2**.

Table 2. Multiple paired comparison in athletic, sporadic exercise and sedentary control groups at high altitude

Group type	Group type	Average difference	Standard error	P
Athletic	Sporadic	,0016300	,0080825	,842
	Control	,0253000(*)	,0080825	,004
Sporadic	Athletic	-,0016300	,0080825	,842
	Control	,0236700(*)	,0080825	,007
Control	Athletic	-,0253000(*)	,0080825	,004
	Sporadic	-,0236700(*)	,0080825	,007

According to the multiple paired comparison results shown in Table 2, there are statistically significant differences between the athletes and the control group and between the sporadic exercise group and the control group ($p<0.01$).

At high altitude, in the athletic, sporadic exercise and control groups, examination of the polymorphic region of the genotype and allele incidences of Mn-SOD, GSH-Px1 and CAT RFLP was planned. These regions are Mn-SOD, GSH-Px1 and CAT RFLP. These gene regions were digested in order by the MroN I, Dde I and Sma I restriction enzymes.

Mn-SOD, GSH-Px1 and CAT RFLP were analyzed according to these fragments: for Mn-SOD, MroN I; base pairs 107, 89 and 18; for GSH-Px1, Dde I, base pairs 359, 299, and 60; for CAT, Sma I, base pairs 340, 185 and 155. Observation of parts of the restriction enzymes in gel solution indicated that one individual was heterozygote, only base pair 89 and base pair 18; base pair 259 and base pair 60; as for base pair 185 and base pair 155, one individual was shown to be mutant. The base pair restriction enzyme products 107, 359 and 340, observed in normal individuals, were true gene products.

According to the results, in the athletic group, the Mn-SOD VV and Mn-SOD AA genotypes could not be found, while the Mn-SOD VA genotype was found in 10 individuals (100%). When examined in regards to the control group, the Mn-SOD VV genotype was found in 3 individuals (30%) and the Mn-SOD VA genotype in 7 individuals (70%), whereas the Mn-SOD AA genotype was not found at all.

In the athletic group, the GSH-Px1 CC genotype was found in 6 individuals (60%), GSH-Px1 TC genotype in 4 individuals (40%) and GSH-Px1 TT genotype was not observed. In the sporadic activity group, GSH-Px1 CC genotype occurred in 4 individuals (40%), GSH-Px1 TC genotype in 6 individuals (60%), while GSH-Px1 TT was again not found. When genotypes were investigated in the control group, GSH-Px1 CC genotype was found in 9 individuals (90%), GSH-Px1 TC genotype in 1 individual (10%) and GSH-Px1 TT genotype was not observed.

In the athletic group, the CAT TT genotype was found in 2 individuals (20%), CAT TC genotype in 8 individuals (80%) and CAT CC genotype could not be established. In the sporadic activity group, CAT TT genotype occurred in 7 individuals (70%), CAT TC genotype in 3 individuals (30%), while CAT CC was again not found. When

genotypes were investigated in the control group, CAT TT genotype was found in 1 individual (10%), CAT TC genotype in 9 individuals (90%) and CAT CC genotype was not observed.

When comparing each genotype for Mn-SOD, CAT and GSH-Px1 between the athletic, sporadic activity, and control groups, a statistically significant difference based on exercise regime for Mn-SOD and GSH-Px1 genotypes was not found, however it was established that CAT genotypes exhibited a statistically significant difference according to type of exercise regime.

Discussion:-

At present, it is known that exercise, along with its beneficial effects, causes oxidative stress. Thus the main goal in research on exercise physiology, together with exercise done for health benefits, is to better understand the changes that occur in the human body during athletic activity and the adaptation of the organism to exercise. With this aim in mind, studies on free radicals and antioxidants have proliferated (11–13).

In sedentary subjects, MDA levels gradually decreased at a statistically significant rate dependent on the exercise regime. This decline was observed in both groups, both within the groups and between the groups, during a 75-day period of exercise. In addition, MDA levels in individuals exercising in a disciplined manner were significantly lower than those in sedentary individuals at every stage of the program ($p < 0.05$). In the literature, as noted, there is a connection between exercise and MDA response (11, 14–16). During exercise metabolic rate is determined to increase, in various ways, according to exercise type and intensity (17).

In our study, in the results of the examinations of plasma samples, it was found that the difference in measurements taken before and after ascent could be statistically significant ($p < 0.05$). At high altitude, an increase in MDA levels is expected due to the decrease in oxygen levels. Consequently, the findings of this research mirror those found in other studies.

A statistically significant difference was not found between the three groups for the MDA variable based on measurements taken before ascent, at high altitude and after descent ($p > 0.05$). In our study, it was observed that the athletic subjects had a more developed defense system than sedentary subjects and were exposed to less lipid peroxidation during exercise performed over the same period. This can be accepted as a result of adaptation to physical training.

For GSH-Px1, in analyses made on erythrocyte packages, differences in measurements of individual subjects at high altitude versus prior to ascent and after descent, in our study were observed to be statistically significant ($p < 0.05$). For the GSH-Px variable, in measurements taken before reaching high altitude, at high elevation, and after descent, the difference between the three groups was determined not to be statistically significant ($p > 0.05$).

For the GSH-Px1 variable, the difference between values taken on the 5th and 10th days compared with the first day of aerobic exercise in sedentary subjects at elevations prior to ascent, at high altitude and after ascent was found to be significant ($p < 0.05$), while those between the athletic group, sporadic exercise and control groups were not. However, in mathematical terms the difference in these values is under consideration. Although the differences within groups were found not to be statistically significant, the difference between the sedentary and athletic groups was ($p < 0.05$). This can be interpreted as a clear indication of how much exercise done on a continual basis strengthens the antioxidant defense system. Powers et al. (11) state that endurance activities lead to an increase in musculo skeletal GSH-Px1 activity. Most of this increase is in the mitochondrial GSH-Px1. This adaptation provides a benefit in eliminating hydroperoxides in mitochondria and cytosol. Similar to Mn-SOD, the amount of the increase in GSH-Px1 caused by training is affected by the intensity and duration of exercise. When compared with exercise at low and medium intensity, exercise at high intensity causes a greater increase in GSH-Px1 activity.

In analyses performed on the erythrocyte packages, it was determined that the difference in individuals' measurements taken at high altitude and after descent were statistically significant ($p < 0.05$). For the CAT variable, the difference between the three groups taken at three different times, before ascent, at altitude, and after descent were found to be statistically significant ($p < 0.05$).

In this study, analyses carried out with the aim of examining differences between athletic, sporadic activity and control groups concerning the variables Mn-SOD, GSH-Px1 and CAT showed no change ($p > 0.05$) in Mn-SOD and

GSH-Px1 based on exercise regime, whereas the CAT variable did exhibit a difference ($p < 0.05$) according to exercise regime.

Liu (18) found that under normal conditions, the body has a sufficient enzymatic and no enzymatic antioxidant reserve to handle an increase in the production of free radicals. Endurance training increases the consumption of oxygen, while lipid per oxidation decreases following exercise. Miyazaki et al. (15) state that lipid per oxidation decreases following endurance training. Wozniak et al. (19) found significant decreases in TBARS concentrations following the tenth day of exercise in a group, which they trained. This situation can be explained by the fact that exercise, by increasing antioxidant levels, leads to a protective effect.

In our study, in the analyses performed on the erythrocyte packages, the difference between individuals' measurements at high altitude and after descent were shown to be statistically insignificant ($p > 0.05$). For the Mn-SOD variable, in measurements taken at three different times, prior to ascent, at high elevation, and following descent, the difference between the athletic, sporadic activity and control groups was also found to be statistically insignificant ($p > 0.05$). According to the results of multiple paired comparisons between the three groups, it was determined that the differences between the athletic and control groups, and between the sporadic activity and control groups, could be statistically significant ($p < 0.01$). However much of a rise is seen in the increase in GSH-Px1 and CAT enzyme levels, the increase occurring in the MDA level is not at a level considered normal. A lack of any kind of change in the Mn-SOD enzyme level, which functions in the first step in the clean-up of free radicals in the body, is tied to an increase in MDA levels. In the literature, the reason for this difference may originate from the characteristics of the group used in the studies made, differences in methods of measurement, or any number of differences in the exercise programs used. In studies that noted an increase caused by exercise in muscle antioxidant enzyme activity, generally intensive training programs are employed. According to this, training programs in which intensity is well regulated lead to an unavoidable rise in antioxidant enzyme activity (11). In other studies, in exercise programs in which intensity and duration are gradually increased, this causes an increase in SOD activity. In addition, as stated in the literature (11,20), the increase in total Mn-SOD level does not exhibit parallelism with the results of our study. The lack of any change in Mn-SOD enzyme activity in our study is connected to the limiting of the intensity and duration of the exercise program resulting from the limited timeframe and unfavorable weather conditions.

There are a number studies on various diseases relating to antioxidant enzyme-gene polymorphism. However, polymorphisms pertaining to antioxidant enzyme genes of athletic, sporadically active and sedentary control groups before, at, and after high altitude have not yet been studied.

In our study, in the athletic group, the Mn-SOD VV genotype was not found, the Mn-SOD VA genotype was found in 9 individuals (90%) and the Mn-SOD AA genotype in 1 individual (10%). In the sporadic activity group, the Mn-SOD VV genotype was not found, while the Mn-SOD VA genotype was found in 10 individuals (100%). When the control group genotypes were examined, the Mn-SOD VV genotype was found in 3 individuals (30%), the Mn-SOD VA genotype was found in 7 individuals (70%) and the Mn-SOD AA was not found.

In the athletic group, the GSH-Px1 CC genotype was found in 6 individuals (60%), GSH-Px1 TC genotype in 4 individuals (40%) and the GSH-Px1 TT genotype was not found. In the sporadic activity group, the GSH-Px1 CC genotype was found in 4 individuals (40%), GSH-Px1 TC genotype in 6 individuals (60%) and the GSH-Px1 TT genotype was not found.

When the control group genotypes were analyzed, it was found that 9 individuals had the GSH-Px1 CC genotype (90%), 1 individual had GSH-Px1 TC genotype (10%) and none had GSH-Px1 TT genotype.

In the athletic group, the CAT TT genotype was found in 2 individuals (20%), CAT TC genotype in 8 individuals (80%) and the CAT CC genotype was not found.

In the sporadic activity group, the CAT TT genotype was found in 7 individuals (70%), CAT TC genotype in 3 individuals (30%) and the CAT CC genotype was not found.

When genotypes for the control group were examined, the CAT TT genotype was found in 1 individual (10%), the CAT TC genotype in 9 individuals (90%), and the CAT CC genotype was not found.

When statistical comparisons were made between the three groups for Mn-SOD, CAT and GSH-Px1, it was determined that the Mn-SOD and GSH-Px1 genotypes exhibited no change according to exercise regime ($p > 0.05$), while CAT genotypes did show a statistically significant difference according to exercise regime ($p < 0.05$).

Examining allele frequencies in Mn-SOD, GSH-Px1 and CAT polymorphisms, a statistically significant difference in allele frequencies was not observed between groups ($p < 0.01$). However, allele frequency in the Mn-SOD region was found to be 0.35 in the control group, 0.5 in the sporadic activity group and 0.55 in the athletic group. Regarding frequency of the valine allele, although a numeric difference was found between the groups it was not a statistically significant difference.

In the athletic, sporadic activity and control groups at high altitude, the polymorphism of Mn-Sod, CAT and GSH-Px1 in the study, which we have done at the molecular level leads us to believe that high altitude, may play a role in the gene activity of these antioxidant enzymes. Regarding statistical data, the major limiting factor in our study in the section on genetic analyses was the number of test subjects, which was kept low due to limited funding; new studies with wider sampling and larger test and control groups can shed more light on this topic. The resulting biochemical analyses suggested to us that exercise, by increasing antioxidant levels, causes a protective effect.

In conclusion, although there is a limited number of sources in the literature relating to biochemical analyses, no sources oriented towards genetic investigation could be found. Thus, this topic requires the support of additional scientific studies. This study makes an important contribution at a scientific level in bringing to light the relationship of exercise at high altitude with oxidative stress and antioxidant enzymes, and its molecular genetic dimension.

Conflict of Interest:-

The authors declare no conflict of interest.

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