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The Effect of Examination Stress on the Levels of Zn^{2+}, Total Protein, Albumin in Serum and Carbonic Anhydrase Isoenzymes in Erythrocytes

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In this study, the levels of Zn^{2+}, total protein and albumin in the serums of students with temporarily examination stress without any physical illnes were determined and the relationship between these values was evaluated.

Furthermore, the change in the activity of carbonic anhydrase metaloenzyme with Zn^{2+} and in the rates of carbonic anhydrase I and carbonic anhydrase II were also investigated.

With this aim, blood samples were taken from 100 students just before examination and from 20 control subjects. Zn^{2+} levels were determined enzymatically, while total protein and albumin assays were done with an autoanalyzer. Carbonic anhydrase activity was measured by Maren's method.

The levels of Zn^{2+} in the serum of students experiencing examination stress were significantly lower than those of the control group, $(t = 3.958, P < 0.001)$ whereas there was no significant difference between the levels of total protein in the two groups (t=0.95, P>0.05) or in the albumin amounts. In the stress group, the level of correlation between Zn^{2+} and total protein was significant $(r = 0.720, P < 0.05)$.

Carbonic anhydrase activity in the stress group was significantly lower than it was in the control group $(t = 6.82, P < 0.001)$, and variation in rate of carbonic anhydrase-I and carbonic anhydrase-II was highly significant as well $(t = 4.84, P < 0.001)$.

Key words: Carbonic Anhydrase, Stress, Zinc

Introduction

Zinc is an important element in the growth and development of living organisms[1]. Some diseases that occur due to changes (increase or decrease) in the levels of brain and spinal cord fluid and in urine have been identified[2]. Zinc is carried by proteins in serum in which about 25% of zinc is bound strongly to α_2-macroglobuline and 75% is bound weakly to albumin[3,4]. However, it has been reported that there is an another protein that also carries zinc[5].

Carbonic anhydrase (CA) is firstly identified as a metaloenzyme that contains Zn^{2+}. This enzyme catalyses the reactions of CO_2 by hydration and dehydration of H_2CO_3 in cells. Carbonic anhydrase is found most abundantly in the erythrocytes (CA-I and CA-II), muscles and liver in mammalian tissues. There is about 15-17 g haemoglobin in 100 ml blood, and almost 1% of this is CA[6].

Stres can be described as occurring in response to physical and psychic situations which the human organism can not adapt to and which abolish the balance of the organism. Stress is not only physiological depression; it is a situation that appears following physical illness and emotional depression. In brief, every situation which imbalances human physiology and demolishes regulation of the organism and can subsequently lead even to differentiation of tissue can be desribed as stress.

There is a marked loss of tissue protein in patients under the effect of serious physical stress, such as surgery, injury, trauma, burn and infection. Patients under physical stress lose about 25% to 30% of skeletal muscles within seven days. As protein synthesis is sensitive to stress, it ceases in serious stress situations. In the case of moderate stress, the sending out of zinc, copper, magnesium, potassium and calcium into the urine increases; however, the levels of vitamin A, ascorbic acid, zinc and ferrous in the blood increase while the levels of sodium and water change in moderate stress[7].

In physical stress situations, the levels of zinc, total protein and albumin have been studied as well as some other parameters[8]. However, there is so far no report on the effect of short term stress, such as examination stress, on the levels of zinc, total protein, and albumin in serum. Furthermore in the case of examination stress, the relationship between above parameters has not been determined. Therefore in this study both of the above were investigated in students experiencing examination stress. In addition, the effect of this short-term stress on the activity of carbonic anhydrase, which is a metalloenzyme with zinc and is found in the blood, has been studied in this report. As it is known that in the case of some chronic diseases, the rates of carbonic anhydrase-I and carbonic anhydrase-II (CA-II/CA-I) isoenzymes change in favour of CA-II in erythrocyte[9], the effect of examination stress on the rate of these isoenzymes were studied as well.

Experimental

Preparation of Blood Samples and Determination of Haemoglobin Level

Determinations of the levels of Zn^{2+}, total protein and albumin were conducted on serum samples, while haemoglobin assays and studies related to CA were performed on blood. Blood samples from the control group and from student group just before examination were taken in two different ways.

In one approach, the blood samples were collected in tubes without anticoagulant, and these were left for 30 minutes at room temperature to allow the blood to coagulate. Coagulated blood was centrifiged at 1500 rpm for 15 minutes, and then serum was separated and kept in a freezer until Zn^{2+}, total protein and albumin assays could be performed.

The blood samples on which activity of CA and haemoglobin assay were performed on the other

hands were collected in heparin containg tubes. In half of the blood samples, the amount of haemoglobin was measured by cyanomethaemoglobin method[9,10]. The rest of the sample was centrifuged at 1500 rpm for 30 minutes and then layers of plasma and leukocyte were separated. Afterwards, the erythrocytes obtained were washed twice with saline and centrifuged. Subsequently, erythrocytes were hemolyzed with distilled water that had 1.5 volume of erythrocytes. The hemolysate used for determination of total CA activity was diluted 100 times with distilled water, while the hemolysate used in the inactivation of brompruvic acid was diluted with distilled water 50 times.

Determination of Zn^{2+}

The level of Zn^{2+} in serum was measured by a new method, the basis of which was regaining of the activity of apocarbonic anhydrase by the zinc present in the sample[11].

Determination of Total Protein and Albumin in Serum

Total protein and albumin assays in the serums of both control group and students under examination stress were performed by autoanalyzer[12].

Measurement of Carbonic Anhydrase Activity in Erythrocytes

CA activity was measured by the Maren method, which is principally based on the determination of time required lowering of pH from pH 10.0 to pH 7.4, due to CO_2 hydration H^+, which causes pH reduction. In the assays, phenol red indicator, sensitive to the change of pH at the range of pH 6.4-8.2, was used and the buffer was 0.5 M Na_2CO_3-0.1 M $NaHCO_3$, (pH 10)[13]. Temperatures of all solutions were reduced to 0° C before their use. In this method, one unit of CA activity is decribed as that the amount of enzymes that reduces by 50% the time of CO_2 hydration without enzyme. That is,

$1U = (T_\circ - T_c)/T_\circ$

In which

$T_\circ$ represents time of pH change in the absence of enzyme,

T_c represents time of pH change in the presence of enzyme.

From this equation, the enzyme unit in hemolysate can be found, and then this value is divided by the amount of haemoglobin previously determined. CA enzyme activity per gram haemoglobin was determined[14].

The Determination of Rates of Erythrocytes CA Isoenzymes

To be able to determine the shares of both CA-I and CA-II activites in total activities, the features of selective inhibition of isoenzymes were used, namely, the fact that brompruvic acid belonged solely to CA-II. Hence, the difference between total activity and the activity measured in the presence of the above inhibitor is equal to the share of CA-I activity in total activity.

Inhibition experiments were performed as follows: 1 ml of hemolysate diluted 50 times was mixed with 1 ml of acid solition (10 mM brompruvic acid, 50 mM $NaHPO_4$, pH 7.5). This solution was shaken very well and was incubated at room temperature for 50 minutes. Finally activity was determined as outlined before[15,16].

Results and Discussion

All results were tested statistically, and the results of these statistic calculations are given in following the tables.

Although stress can cause led to certain diseases, it is not a disease in itself. Stress is different from physiological illness. As can be seen from Table 1, in the case of stress the level of Zn^{2+} was sfgnificantly lower than in the control group ($t = 3.958, P < 0.001$) while change in the total protein was not significant. It has been previously reported that in the case of serious physical stress, the levels of Zn^{2+} and total protein decrease in serum[7]. Thus our results related to Zn^{2+} support this reference. However, there was a slight reduction in the level of total protein, which was not statistically significant, while there was no change in albumin level. Therefore, it may be suggested that a short period of physiologic stress does not effect protein synthesis, perhaps particularly albumin synthesis. The lowering of the Zn^{2+} level could be via the urine and sweat as happens during physiologic stress[7].

Table 1. "t" Test between control group and examination stress group for the values of Zn^{2+}, total protein and albumin

	Control group $(X \pm SD), N = 20$	Stress group $(X \pm SD), N = 100$	t	P
Zn^{2+} (μg /100 mL)	123.4±20.8	94.9±13.7	3.958	<0.001
Total protein (g/100 mL)	7.3±0.7	6.9±0.2	0.95	>0.05
Albumin (g/100 mL)	4.9±0.5	4.9±0.15	0	>0.05

There was a significant correlation between Zn^{2+} and total protein in the stress group ($r = 0.720, P < 0.05$) (Table 2) while there was no correlation between Zn^{2+} and albumin in the same group (Table 3). Hence, one way think that there is a loss of protein, although it may be slight in short-term physiologic stress.

Table 2. Correlation test for control group and stress group between Zn^{2+}, and total protein values

Zn^{2+}, total protein correlation pair	N	r	t	P
Control group	20	0.385	1.790	>0.05
Stress group	100	0.720	3.000	<0.05

Table 3. Correlation test between Zn^{2+}, and serum albumin values

Zn^{2+}, Albumin correlation pair	N	r	t	P
Control group	20	0.490	2.420	<0.05
Stress group	100	0.070	0.641	>0.05

Briefly, perhaps it is possible to say that the level of Zn^{2+} is markedly effected by short term physiologic stress, while there is only a slight effect on the total protein level, and albumin synthesis is not effected at all.

In addition, erythrocytes, total CA activity and the rates of CA-II/CA-I isoenzymes were determined in the stress group. Total CA activity per gram of haemoglobin can change even within the same age and the same sexual groups, while the rate of isoenzyme does not change often[15,17]. This was observed in our results as well. The mean of total CA activity in the healthy control group was 1402.7±389.3 U/g Haemoglobin (Table 4), while the rate of CA-II/CA-II was 1.250±0.11. Thus the high variation in total CA activity and low variation in isoenzymes rate support the above literature (Table 5).

There was a statistically significant reduction in total CA activity (t=6.82, P<0.0001) while there was a statistically highly significant increase (t=4.76, P<0.0001) in the case of examination stress.

Table 4. Correlation test for Carbonic anhydrase values

	N	X±SD	t	P
Control group	20	1402.7±389.3	6.820	<0.0001
Stress group	100	643.9±67.4		

Table 5. Correlation test between control and stress group for the rate of CA-II/CA-I

	N	X±SD	t	P
Control group	20	1.25±0.11	4.760	<0.0001
Stress group	100	4.99±3.47		

In conclusion, it was found that there are changes in total erythrocyte activity and in the rates of CA-II/CA-I isoenzymes during examinaton stress. These results support previous work[9]. As studied, the reduction in CA activity due to stress for short period may be due to some chemical molecules secreted. These molecules inhibit enzyme activity. Another explanation is that examination stress simply reduces synthesis of enzymes. This could be further evaluated more clearly by radioimmunological methods.

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