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SELENIUM SATURATION OF HEMOGLOBIN AS ANTIOXIDATIVE FACTOR AT HIGH TENSION ELECTRIC FIELDS ACTION

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Abstract- High tension electrical fields represent a physical environmental factor influencing on oxidative-destructive processes rate in human organism. Resistance to this electrical field depends mainly on multi-component system of natural antioxidants. Among them trace element selenium and enzyme glutathione peroxides are of great importance. However the mechanism of anti-oxidative action of selenium has not been investigated completely. Creating selenium-deficit conditions is essential for study of the action mechanism. Hence, both the erythrocytes of pregnant women and the erythrocytes at glucose-6-phosphatedehydrogenase enzyme deficiency are appropriate natural models. The results of investigations have shown that the erythrocytes of pregnant women (initial stage of pregnancy) proved to be more resistant to electrical field effect than the erythrocytes with glucose-6-phosphatedehydrogenase pathology. Due to the intensive consumption of Se at late stages of pregnancy, erythrocytes of pregnant women are more labile to oxidation than the glucose-6-phosphatedehydrogenase deficiency erythrocytes, thus that glutathione peroxides activity of erythrocytes of pregnant women remains close to norm.

Keywords: Hemoglobin, Meta-hemoglobin, Hemolysis, Lipid Per-Oxidation, Antioxidants, Selenium, Glutathione Peroxides, Pregnancy, Glucose-6-Phosphatedehydrogenase, High Tension Electric Fields.

I. INTRODUCTION

Among the environmental physical factors affecting people health, electromagnetic fields of industrial frequency take an important place. Like the other environmental factors, electrical field (EF) can be an initial reason of some pathologies (or can complicate the course of already expressed diseases) associated with oxidation process acceleration [1, 2].

Natural resistance of organism to adverse factors, first of all to oxidative stress, is adjusted by the homeostasis system, which includes anti-oxidative (AO) protective complex. Study of natural antioxidant metabolism and oxidation promoters under the physical and chemical environmental factors influence is necessary for understanding of the primary mechanisms of the damage of bio-objects that, in any case, are connected with population health. One of the vital biological molecules is hemoglobin, of which oxidation condition is extremely important for the development of free radical oxidation of cellular structures.

A special interest represents the action of adverse environmental factors on people health at weak AO system. In particular, deficiency of glucose-6-phosphatedehydrogenase (G-6-PhD) enzyme, which is frequently, associated with the reduction of glutathione, peroxides (GPx) enzyme activity [3]. The gene of (G-6-PhD) enzyme deficit is widely distributed among the Azerbaijan population [4]. Besides, as a natural model of AO deficiency, the erythrocytes of pregnant women are of special interest (especially in the second half of pregnancy). Considering that during the pregnancy GPx activity of erythrocytes changes ambiguously, and the concentration of selenium in blood and erythrocytes essentially falls, that research of this phenomenon represents scientifically-practical interest [5].

Selenium and related AO proteins (GPx, P-protein, some cellular intermediates, etc.) are the most important components of natural AO system. It is necessary to note, that the territory of our republic is a region characterized with low and average selenium supply that raises the urgency of study of the pathologies related to selenium deficiency and activation of oxidative-destructive processes [6].

Considering all the above mentioned, we have undertaken a study of oxidation process development induced by industrial frequency high tension EF in erythrocytes at glucose-6-phosphatedehydrogenase enzyme deficiency and erythrocytes of late stages of pregnancy with selenium deficit symptoms.

II. MATERIALS AND METHODS

A. Creation of High Tension EF

For creation of high tension EF an installation was assembled using cylindrical cored cables. The scheme of installation is presented in Figure 1. The tension of electric field was calculated according to the Equation (1). The tension of electric field affecting and the objects changes are depending on the distance according to Equation (1).

$E_x = U_o/[x*\ln 2H/r_o]$, $r_o << x$ (1)

where

U_o - an initial tension (voltage) of conductor

x - distance from conductor to object

H - distance from conductor to "0" potential

r_o - radius of conductor

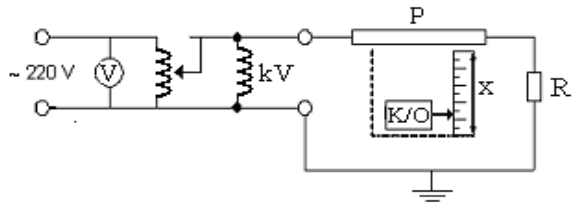

Figure 1. Creation of high tension EF installation using cylindrical cored cables - K/O: the cell, P: cored cylindrical cable

GPx activity in suspension of erythrocytes and lisate are defined spectrophotometrically to decrease GSH on Moin method [7]. The concentration of selenium in blood, erythrocytes, lisate and hemoglobin fractions is defined extraction by flour metrical method with application selenium sensitive reactant of 2.3 diaminonaphtalin [8].

B. Erythrocytes of Persons

The carriers of the gene of G-6-PhD enzyme deficiency and erythrocytes of the women at normal proceeding pregnancy in various stages are served as materials of research. Erythrocytes were extracted by standard method: centrifugalized, washed three times and diluted by the phosphate buffer (0.1 M, pH 7.4) 1:50 (hematocryte ≈ 1), erythrocyte shadows were obtained by Dodge's method [9]. Suspensions of erythrocytes were placed in glass flasks at 25°C temperature and exposed to electric field, 50-150 kV/m. Aliquots were taken in each 3 hours. The condition of hemoglobin oxidation was determined in suspension and buffer solution. The rate of oxidative hemolysis (λ_{670}) was studied in supernatant by spectrophotometrically method. The rate of oxidative-destructive processes was evaluated on the accumulation of products reacting with thiobarbituric acid (TBA) in suspension and in erythrocyte shadows [10]. The hemoglobin oxidation conditions were determined by the following formulas [11]:

$[HbO_2] = 29.8A_{577} - 9.8A_{630} - 22.2A_{560}$ (2)

$[MetHb] = 7.0A_{577} + 76.8A_{630} - 13.8A_{560}$ (3)

$[Hi] = -33.2A_{577} - 36.0A_{630} + 58.2A_{560}$ (4)

where A_{577}, A_{560}, A_{630} are extinction of samples on corresponding wave lengths. Statistical processing of measurement results was carried out using student's criterion [12].

III. RESULTS AND DISCUSSIONS

It is well known that increased tendency to oxidative hemolysis is one of the consequences of G-6-PhD deficiency [13]. In many respects, it is associated with the fact that selenium containing glutathione peroxidase (GPx) connected with G-6-PhD in pentosephosphate shunt is also low active. As a result, hemolysis is accelerated during the promoter action of various oxidizers including medicals such as hydrazine and premixing, and also at the use of beans in meals (Vicia Fava) [13, 14]. From modern point of view, it is associated with the fact that beans contain high concentration of lipoxigenase enzyme, accelerating lipid peroxidation (LPO) membranes of erythrocytes that cause hemolysis under low concentration of a natural antioxidant - selenium and low GPx activity [15].

Selenium plays an important role during reproductive period. Due to double consumption, the demand for selenium in pregnant women considerably increases. Additional content of selenium in the diet of pregnant women will help prevent progress of some diseases [16]. However, the necessary and safe level of selenium receipt in the organism of pregnant woman, which is based on the regional features of selenium supply, is not established up to now.

We established that the level of selenium during pregnancy falls from 0.080±0.012 mkg/ml to 0.040±0.002 mkg/ml, and GPx activity in the first 2-4 months tends to growth then it is stabilised and is close to initial level (Figure 2).

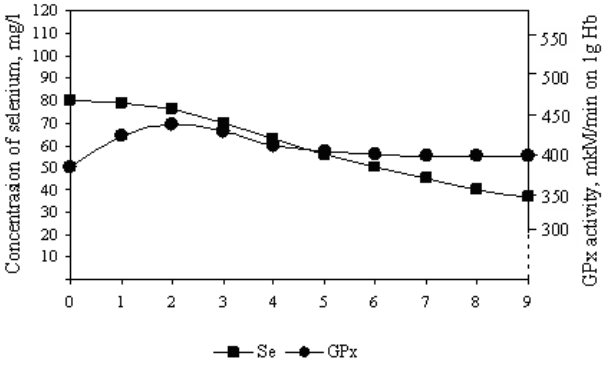

Figure 2. Concentration of selenium and GPx activity in erythrocytes of women (n=14) at normal pregenancy

For glucose-6-phosphatedehydrogenase enzyme deficiency takes place decrease of the selenium status (0.062±0.011 mkg/ml (n=12) against norm 0.085±0.012 mkg/ml (n=15), and GPx activity makes 230±30 mkM/min on 1 g Hb.

As can be seen from Figure 3 there are threshold values of EF intensity (at fixed 5 hour expositions), under which marked acceleration of oxidative processes is observed. The threshold for control erythrocytes is E ≈ 83 kV/m, for initial stages of pregnancy E ≈ 74 kV/m, for G-6-PhD deficiency E ≈ 67 kV/m and for late stages of pregnancy E ≈ 55 kV/m. Under the influence of high tension EF in erythrocytes at G-6-PhD pathology the accumulation of TBA of active products proceeds faster than in erythrocytes of pregnant women (the first trimester). But at the late stages of pregnancy in conditions of selenium deficiency the accumulation of TBA of active products proceeds faster than in erythrocytes at G-6-PhD pathology.

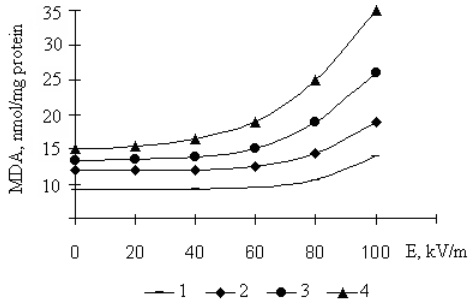

Figure 3. Kinetics of TBA active products (MDA) accumulation in erythrocytes under electrical field effect
1- control, 2- initial stage of pregnancy,
3- erythrocytes with G-6-PhD deficiency, 4- late stage of pregnancy

We examined the influence of high electrical field 10-50 kV/m on human erythrocyte. The results of these experiments showed that the electrical field in this range does not significantly affect the development of oxidative processes in erythrocytes. A single indicator at 50 kV/m was some tendency to increase in GPx activity in donor's erythrocytes. In these communications for clarity, we used 100 kV/m and over and time exposition 5 hour. GPx activity in health persons increased by 20%, activity does not change for G-6-PhD pathology erythrocytes, for erythrocytes of initial stage of pregnancy GPx activity increased by 20% and 5% increase in GPx activity was observed for erythrocytes of late stage of pregnancy.

Erythrocytes contain at least two significant "targets" for oxidative influence: hemoglobin itself (MetHb and other oxidative forms of hemoglobin) and membrane structures (LPO). These two oxidative processes are interconnected. However, their sequence is unknown. Therefore, kinetics of these two processes is considered simultaneously. The curves of meta-hemoglobin accumulation and hemolysis under electric field effect are presented in Figure 4 (a, b). As can be seen from the figure, the curve of MetHb accumulation has more acute shape that may indicate that oxidative degradation of erythrocytes is induced by hemoglobin oxidation. Interestingly, erythrocytes of the women at late stages of pregnancy show greatest tendency to oxidation (both hemoglobin and membrane structures).

Comparative examination of the kinetics of accumulation of active products' TBA in suspension and in erythrocyte shadows (Figure 5) shows significant difference both in initial level of LPO and in oxidative process intensity. It is explained by the presence of antioxidative factors in cytosine of erythrocytes (GPx, super oxide dismutase, catalyses, etc.). It should be noted that in erythrocyte shadows oxidative process proceeds more faster in late terms of pregnancy that indicates considerable reduction of antioxidants.

We also consider a question on a condition of saturation by hemoglobin selenium. Experiments show that in lisate of erythrocytes of pregnant women it continuously decreases in parallel its reduction in hemoglobin. This reduced concentration of selenium in hemoglobin in many respects also defines stability of hemoglobin to oxidation and in a final case in erythrocytes that puts degree loading of hemoglobin with selenium abreast the important factors of AO protection along with others (super oxide dismutase, catalyses, GPx).

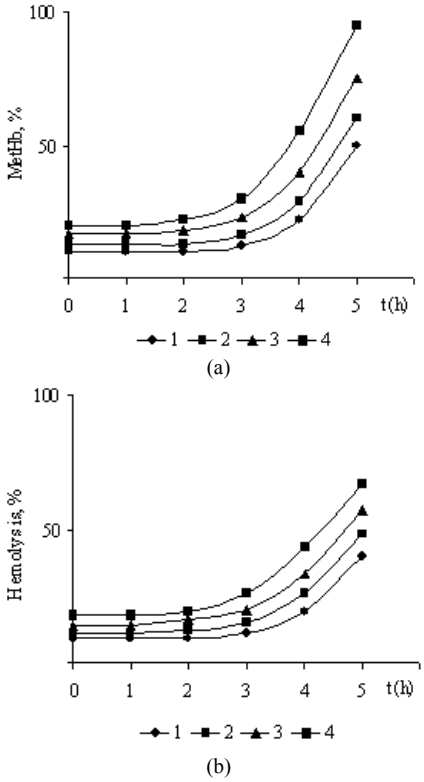

Figure 4. Accumulation MetHb and Hemolysis in erythrocytes under electrical field effect - (a) Accumulation MetHb (b) Hemolysis
1- control, 2- initial stage of pregnancy,
3- erythrocytes with G-6-PhD deficiency, 4- late stage of pregnancy

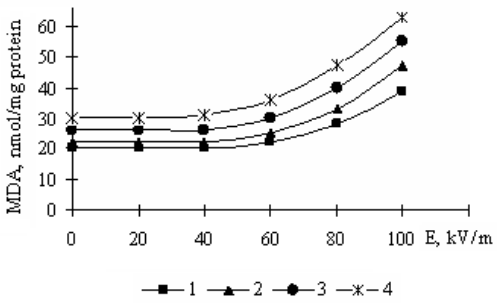

Figure 5. Kinetics of TBA active products (MDA) accumulation in shadows of erythrocytes under electrical field effect
1- control, 2- initial stage of pregnancy,
3- erythrocytes with G-6-PhD deficiency, 4- late stage of pregnancy

IV. CONCLUSIONS

Electromagnetic fields and radiation accelerates the oxidative destructive processes in isolated biological objects with a relatively high tension. Changing the GPx activity is an early indicator of oxidative damage to erythrocytes. Erythrocytes of pregnant women and erythroenzimophaty G-6-PhD having a weak status of selenium are more affected by the electromagnetic fields. The greater stage of pregnancy and the greater lack of selenium cause the greater propensity to oxidative damage. Selenium is an antioxidant may participate in protection from oxidative processes caused by electromagnetic fields.

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