Superoxide dismutase and glutathione peroxidase activity in pregnancy complicated by diabetes

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Abstract
The objective of the study was determination of the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood and placental tissues of pregnant women with pregnancy complicated by diabetes, pregnant women with physiological pregnancy, and non-pregnant women, as well as a comparative analysis of blood and placental tissue parameters in the groups of women examined. The material for the study was blood and placental tissue from 50 pregnant women who received treatment due to insulin-dependent diabetes (PD). For the control group, 50 pregnant women without diabetes (HP), and 30 non-pregnant women (NP) were selected. SOD activity in erythrocytes was evaluated by the method of spectrophotometry with the use of RANSOD kit (RANDOX Laboratories Ltd., UK). The activity of GPx in erythrocytes was determined according to the method by Paglia and Valentine using RANSEL kit (RANDOX Laboratories Ltd). The results were subject to statistical analysis. Insulin-dependent diabetes in pregnancy affects the activity of anti-oxidative enzymes. In the blood of women with pregnancy complicated by diabetes, the activity of anti-oxidative enzymes – SOD and GPx is higher than in the blood of women with physiological pregnancy and the control group. In the placental tissue from pregnancy complicated by diabetes, the activity of SOD significantly decreases, while the activity of GPx increases, compared to women in physiological pregnancy.

Key words
superoxide dismutase, glutathione peroxidase, pregnant women, diabetes

INTRODUCTION
Statistical data show that pregnancy complicated by diabetes is not a frequent phenomenon; however, considering the close interaction between diabetes and pregnancy, it is a serious problem in perinatal care [1, 2].

Pregnancy changes the course of diabetes, while diabetes deteriorates obstetric results [1]. The effects of foetal hyperglycaemia depend on its value, duration, and advancement of pregnancy. Diabetes is conducive for the occurrence of complications in the foetus and newborn. In extreme cases, non-treated diabetes in pregnancy may lead to intrauterine death. Early detection of disorders of hydrocarbon metabolism shortens the time of exposure of the foetus to hyperglycaemia and, in consequence, decreases the frequency and intensity of complications in the course of pregnancy and disorders in the early neonatal period [3]. Non-diagnosed and non-treated diabetes in pregnancy causes an increase in perinatal mortality by even 3 times [4, 5]. Metabolic disorders (hyperglycaemia, hyperlipidaemia, acidosis), and vascular complications in the course of diabetes in the mother exert a tremendous effect on the development and fate of the foetus and newborn. The number of miscarriages is approximately twice as high, compared to the group of women without diabetes. Poor metabolic control is primarily reported as a cause [4, 5, 6, 7]. Congenital defects in the foetuses of pregnant women with diabetes occur 2–3 times more frequently, compared to the total population. Their development is confined to the period of organogenesis. In pregnancy complicated by diabetes, foetal macrosomia occurs 10 times more often than in the total population [8, 9].

According to many researchers, in physiological conditions, a relative balance is maintained in a cell between the level of oxygen radicals produced and the activity of substances called the ‘sweepers’ of free oxygen radicals – antioxidants. According to one of the definitions, oxidative stress results in the disturbance of the balance between the production of reactive oxygen species (ROS), and their elimination in enzymatic and non-enzymatic reactions of neutralization and sweeping-up, as well as through the effect of exogenous antioxidants supplied with food [10].

The effect of free radicals leads to the development of lesions in the structures of living organisms. This is due to non-specificity of such reactions with the cell-building particles. Non-specificity means that each encountered particle is a potential target for free radicals. Such a reaction usually leads to the loss of properties defined as biochemical or biological activity.

Diabetes is the state of increased oxidative stress for the organisms [11, 12]. In diabetic patients, ROS in combination with glycation end-products, lead to changes in vascular function as a result of increase in the level of lipid peroxidation, changes in intercellular matrix, as well as an inhibition of nitric oxide synthesis and activity.

Cellular defence against the effects caused by reactive oxygen species is based on enzymatic reactions. Glutathione...
peroxidase and superoxide dismutase are components of the enzymatic system protecting against ROS [13, 14].

Glutathione peroxidase is induced by H₂O₂ and organic peroxides. This enzyme contains selenium and catalyzes the reaction of glutathione disulfide production. Glutathione reductase is the enzyme co-operating with peroxidase and does not directly participate in the protection of a cell against ROS; however, by reproducing the reduced form of glutathione, its effect increases the protective potential and prevents the development of protein damage. The enzyme, which is directly related with GSH, is glutathione transfrase catalyzing the conjunction of glutathione with various electrophilic compounds. The products of its activity are glutathione S-conjugates [13, 14].

The subsequent protein entering into the composition of the enzymatic system of protection against ROS is SOD. SOD are typical of all organisms using oxygen in their metabolism. They have a different structure, but the same functions. Several types of SOD have been described, which differ primarily by the type of metal ion in the active site. However, two forms of SOD exist in eucaryonts – manganese and copper-zinc [13, 14].

OBJECTIVE

The objective of the study was determination of the activity of SOD and GPx in the blood and placental tissues of pregnant women with pregnancy complicated by diabetes, women with physiological pregnancy, and non-pregnant women, as well as a comparative analysis of the examined parameters of blood and placental tissue in the groups of women in the study.

MATERIALS AND METHOD

Material for the study was blood and placental tissues of 50 pregnant women who received treatment due to insulin-dependent diabetes (PD). The control group selected for the study were 50 pregnant women without diabetes (HP), and 30 non-pregnant women (NP). Permission for the study was obtained from the Bioethics Committee at the Medical University in Lublin.

The mean age of pregnant women who received treatment for diabetes was 26.8±4.6, and in the control group – 27.3±6.1 in pregnant women with physiological pregnancy, and 27.8±5.3 in non-pregnant women.

Blood and tissues for the study were collected with the consent of the patients during hospitalization in the Clinic for Obstetrics and Pregnancy Pathology at the Independent Public Clinical Hospital No. 1 in Lublin, the Clinic for Pregnancy Pathology at the Duchess Anna Mazowiecka Public Clinical Hospital in Warsaw, and Diabetology Outpatient Department at Independent Public Clinical Hospital No. 1 in Lublin. The diagnosis of diabetes was based on the clinical symptoms of the disease and based on laboratory tests which covered the evaluation of the level of glucose in blood plasma while fasting, oral glucose tolerance test, evaluation of plasma C-peptide level, and the level of glucose and ketone bodies in urine.

The samples of placental tissue (1g) were homogenized in 10 ml 0.01 M Tris-HCl buffer of pH 7.4 using a mechanical homogenizer MPW-120 (Med. Instruments, Poland). The homogenate obtained was centrifuged for 15 min., at rotation speed 10,000/min., with the use of a centrifuge MPW-312 (Med. Instruments, Poland). The supernatant obtained in this way was used for the determination of SOD and GPx.

The activity of SOD in erythrocytes and in supernatant obtained from the placenta was evaluated by the spectrophotometric method [15] using the RANSOD kit (RANDOX Laboratories, Ltd.). GPx activity in erythrocytes and in placental supernatant was determined by the spectrophotometric method according to Paglia and Valentine [16] using the RANSEL kit (RANDOX Laboratories, Ltd.).

The activity of GPx and SOD in placental tissue was then calculated per gram of placental tissue and expressed in international units of placental tissue activity U/g. In each case, the activity of GPx and SOD was determined in 5 independent, simultaneously conducted trials. The variation of the results of the study obtained for each sample examined did not exceed 10%. The mean values of GPx and SOD activity calculated, based on 5 determinations performed for each sample, were subject to further analysis.

Statistical analysis was performed by computer software STATISTICA Version 6.0 (Stat Soft, Poland). The results of the study obtained were described using the arithmetic mean, median, standard deviation, range of variation, lower and upper quartile. The conformity of distribution of the characteristics examined with the normal distribution was investigated by Sharpio-Wilk test. Considering the lack of conformity of the distribution of the examined characteristics with the normal distribution, U Mann-Whitney test was applied to detect the differences between the groups analysed.

For comparing more than 2 groups, the Kruskal-Wallis ANOVA test by ranks was used. In the case of statistically significant differences, multiple comparisons of individual groups were performed. The relationships between the characteristics examined were investigated by means of Spearman rank correlation test for skew distributions. The p values p<0.05 were considered statistically significant.

RESULTS

SOD activity in erythrocytes in the examined group was the lowest in the control group – 1375.33±60.31 U/gHb (Tab. 1). It slightly increased in erythrocytes of healthy pregnant women, while a considerable increase in the activity of this enzyme was observed in erythrocytes of pregnant women burdened with diabetes. An increase in SOD activity in erythrocytes of pregnant women with diabetes was statistically significant, compared to the group of healthy pregnant women and the control group (p<0.001).

Table 1. Superoxide dismutase (SOD) activity in erythrocytes and placental tissue in the control group (CG), healthy pregnant women (HP) and pregnant women with diabetes (PD)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Value</th>
<th>Groups examined</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CG       HP     PD</td>
<td></td>
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<tr>
<td>Erythrocytes (U/gHb)</td>
<td>Mean and SD</td>
<td>1415.8± 128.85</td>
<td>2283.66±97.96</td>
</tr>
<tr>
<td>Placenta (U/g tissues)</td>
<td>Mean and SD</td>
<td>613.67±23.98</td>
<td>401.25±30.47</td>
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</table>
Differences in SOD activity were also found in placental tissue (Tab. 1). In the group of healthy pregnant women (HP), SOD activity was significantly higher (p<0.001), compared to pregnant women with diabetes.

GPx activity in erythrocytes was considerably higher in the group of patients with diabetes, compared to the 2 remaining groups (Tab. 2). The differences in GPx activity between the group of women with diabetes (PD) and the group of healthy pregnant women (HP), as well as between the control group (CG), and the group of pregnant women with diabetes (PD) the differences in GPx activity were statistically significant.

<table>
<thead>
<tr>
<th>Tissue Value</th>
<th>n = 30</th>
<th>n = 50</th>
<th>n = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>HP</td>
<td>PD</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes (U/gHb) Mean±SD</td>
<td>18.51±4.48</td>
<td>24.09±2.19</td>
<td>70.46±10.98</td>
</tr>
<tr>
<td>Placenta (U/g tissues) Mean±SD</td>
<td>26.3±3.29</td>
<td>31.9±2.66</td>
<td>31.9±2.66</td>
</tr>
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</table>

CG – control group; HP – healthy pregnant women; PD – pregnant women with diabetes.

Similarly, in the placental tissue of pregnant women with diabetes a higher glutathione peroxidase activity was observed, compared to the placenta of health pregnant women (Tab. 2). These differences were statistically significant.

**DISCUSSION**

Diabetes is a social disease which is an important medical problem due to its prevalence, treatment, and accompanying complications. Studies to-date have allowed the explanation of the pathomechanisms of many important unfavourable changes in the organism during the course of diabetes; however, changes in the activity of enzymes of the antioxidant system still remain the subject for discussion and evoke much controversy [11, 12, 13]. Both diabetes and pregnancy may exert an effect on the production of free radicals. The antioxidant barrier, which consists of antioxidant enzymes and fine-particle size antioxidants, counteracts the hazardous effect of free radicals. With the high production of free radicals, the antioxidant system increases its activity which, in time, may lead to its insufficiency or exhaustion. An increased production of free radicals is considered as one of the key factors leading to damage in the course of uncontrolled diabetes [11]. This is emphasized in the reports by Aagrwal et al. [17] who considered free radicals produced during pregnancy as one of the most important factors affecting the development of the foetus.

Many researchers emphasize the unfavourable effect of oxidative stress in a pregnant woman [11, 18, 19]. Among the most important pathologies which develop in the course of oxidative stress are mentioned: miscarriage, premature delivery, preeclampsia, IUGR. PIH, as well as metabolic disorders, such as gestational diabetes. Changes in the antioxidant system vary in the degrees of intensity, but they always cause an intensification of the primary disease, thus leading to the development of complications [20].

Diabetes is associated with the state of increased oxidative stress. In conditions of hyperglycaemia there occurs an intensification of glucose metabolism in the cells of the endothelium, granulocytes, monocytes and blood platelets, which is accompanied by an increased production of free oxygen species. The studies by Nishikawa showed that irrespective of the route of changes dominant in a given type of cells, an increase in the glycolytic activity leads to an increased synthesis of pyruvate [21]. Pyruvate, in turn, is transported to the mitochondria and subject to further transformations in the tricarboxylic acid cycle. This results in an increased formation of dinucleotides NADH and FADH₂ which participate in oxidative phosphorylation. During the intensive processes in the course of the respiratory chain, a part of the electrons may leave the main reaction chain, originating the formation of the superoxide anion radical (O₂⁻

In patients ill with diabetes, ROS, together with glycation end-products, lead to changes in vascular function and disturbance of the cell homeostasis as a result of an increase in the level of lipid peroxidation, changes in intercellular matrix, as well as an inhibition of nitric oxide synthesis and activity [22].

The secondary biochemical exponents of the production of free oxygen radicals are qualitative and quantitative changes in the group of antioxidants.

A relationship is observed between an elevated level of glucose and oxidative stress. The studies showed a significant increase in the synthesis of free oxygen radicals, and an increase in the concentration of products of lipid peroxidation. A considerably intensified expression was observed of mRNA, superoxide dismutase enzymes (Cu,Zn-SOD), catalase (CAT), and glutathione peroxidase (GPx) [11, 12].

The above-mentioned studies confirm the results obtained in the presented study, in which an increase was confirmed in the activity of superoxide dismutase and glutathione peroxidase in the blood of women with pregnancy complicated by diabetes, compared to the women with physiological pregnancy. In the group of women with physiological pregnancy, the activity of these enzymes was similar to that in the control group of non-pregnant women.

Also, a chronic, intensified production of toxic oxygen derivatives most probably leads to the exhaustion of the compensatory capabilities on the part of superoxide dismutase and catalase. It is suggested that the intensity of oxidative stress depends on both metabolic disorders in diabetes, and the duration of the disease [23]. Hence, in order to avoid such transformations, possibly the earliest metabolic control of diabetes is important as the main causative agent of these disorders.

Many researchers tackle problems related with free radical processes and activity of antioxidants in the course of physiological pregnancy.

In the presented study, an increase was observed in the activity of superoxide dismutase and glutathione peroxidase in the blood of women with pregnancy complicated by diabetes. In pregnant women with physiological pregnancy, superoxide dismutase activity increased slightly, while a statistically significant increase was noted in the activity of glutathione peroxidase, compared to the control group.

In the placental tissues after pregnancies complicated by diabetes, also in the presented study, an increase was
found in glutathione peroxidase activity, and a decrease in superoxide dismutase activity. Similarly, Takahara et al. [24], while analyzing antioxidant processes in the placenta, found significant differences in the activity of the enzymatic system. They confirmed a significantly higher activity of catalase (CAT), whereas glutathione peroxidase activity (GPx) did not significantly change in the course of pregnancy.

The placenta possesses a competent and very effectively functioning protective system, the task of which is the 'neutralization' of free radicals and, therefore, the protection of the foetus against their effects [17]. This system is supported by antioxidants of the mother’s organism, and as a whole, they constitute an oxidative barrier capable of the control of ROS [25].

In the studies by Twardowska-Saucha et al., GPx activity considerably decreased in pregnancy in patients with diabetes, compared with the groups of healthy pregnant women and non-pregnant women. The CuZn SOD activity was significantly lower in the group of pregnant women with diabetes, compared with the groups of healthy pregnant women and non-pregnant women. The CuZn SOD activity doi not significantly change in the course of pregnancy.

CONCLUSIONS

Insulin-dependent diabetes in pregnancy affects the activity of antioxidative enzymes:

1. In the blood of women with pregnancy complicated by diabetes the activity of antioxidative enzymes – superoxide dismutase and glutathione peroxidase is higher than in the blood of women with physiological pregnancy and the control group.

2. In placental tissue from pregnancy complicated by diabetes, superoxide dismutase activity significantly decreases, while an increase is observed in the activity of glutathione peroxidase, compared to women with physiological pregnancy.

REFERENCES


