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Can redox imbalance predict abnormal foetal development?

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ABSTRACT

Objectives: Based on the current state of knowledge, elevated levels of oxidative stress markers may be considered as risk factors for pregnancy complications. The aim of the research was to assess the correlation between selected oxidative stress biomarkers with the occurrence of foetal chromosomal aberration and congenital malformations.

Material and methods: This retrospective research lasted for two years. The purpose was to determine serum levels of selected oxidative stress markers, including total protein (TP), glutathione (GSH), S-nitrosothiols (RSNO), nitric oxide (NO), trolox equivalent antioxidant capacity (TEAC) and glutathione S-transferase (GST) at 11–13 + 6 gestational weeks in 38 women with confirmed foetal developmental abnormalities and in 34 healthy pregnancies in order to assess their utility as predictors of abnormal foetal development.

Results: Serum concentrations of TP (56.90 ± 5.30 vs 69.1 ± 15.30 mg/mL), TEAC (4.93 ± 0.82 vs 5.64 ± 0.74 μM/mL) and GST (15.94 ± 4.52 vs 21.72 ± 6.81 nM/min/mg) were statistically significantly ($p < 0.05$) lower in the group of patients with developmental abnormalities in the fetus, whereas GSH levels (6.43 ± 1.24 vs 4.98 ± 1.88 nM/mg) were significantly higher, compared to the group of healthy fetuses. There were no differences in the concentration of these markers between chromosomal aberrations and fetal dysmorphia in subjects. A significant difference in odds ratio obtained for GSH (OR = 0.57, 95% CL: 0.40–

0.80) indicates that its higher concentration can relate to reduced risk of developmental abnormalities, whereas odds ratio for TP (OR=1.11, 95% CL: 1.04–1.17), TEAC (OR = 3.54, 95% CL: 1.56–8.05) and GST (OR = 1.18, 95% CL: 1.03–1.17) indicate that their elevation may increase the risk of developmental abnormalities

Conclusions: Elevated levels of TP, GST, TEAC and low GSH level may be relevant to predict congenital defects.

Key words: oxidative stress; congenital malformations; chromosomal abnormalities; prenatal diagnostic

INTRODUCTION

Oxidative stress and oxidative homeostasis in the human body have become the subject of extensive research into the pathophysiology of numerous medical, especially autoimmune, conditions [1–4]. Research shows that balanced activity of reactive oxygen forms (ROS) and the balance of metabolic and oxidation processes are essential for normal bodily growth and function [5].

Oxidative stress is caused by redox imbalance i.e., a type of chemical reaction in which the oxidation states of atoms are changed and defined as excessive activity of ROS and an imbalance between their production and removal in the human body. The main consequences of oxidative stress include inactivation of some proteins, adenine nucleotide catabolism, increased rate of lipid peroxidation, mitochondrial damage, decreased ATP, glutathione levels and DNA damage. ROS production site depends on the conditions the cells have been exposed to. Their excessive production is an established risk factor for malignancies and inflammation. It also regulates body ageing mechanisms [6].

The available literature confirms the effect of oxidative stress on mitochondrial dysfunction and such genetic disorders as Down syndrome, Louis–Bar syndrome, Bloom syndrome or Nijmegen breakage syndrome [7–9].

In pregnancy, the effect of oxidative stress has been established for such abnormalities as placental insufficiency, preterm birth or miscarriage [9]. Previous research indicating the association between the level of oxidative stress and increased risk of congenital foetal abnormalities became a motivation for the authors to continue analysis of oxidative status in pregnancy and its effect on congenital abnormalities *e.g.*, as chromosomal aberrations and dysmorphia [10]. Based on the current state of knowledge, elevated levels of oxidative stress markers may be considered as risk factors for foetal chromosomal aberrations [11].

Objective

The purpose of the current research was to determine serum levels of selected oxidative stress markers, including total protein (TP), glutathione (GSH), S-nitrosothiols (RSNO), nitric oxide (NO), trolox equivalent antioxidant capacity (TEAC) and glutathione S-transferase (GST) at 11–13 + 6 gestational weeks in women with confirmed foetal developmental abnormalities and in healthy pregnancy, in order to assess their utility as predictors of abnormal foetal development.

MATERIAL AND METHODS

Material

The research was carried out in 72 pregnant females, outpatients at Ultrasonography and Prenatal Imaging Clinic at Gynaecology and Obstetrics Hospital of Poznan University of Medical Sciences, attending the routine antenatal scan at 11–13 + 6d gestational weeks.

The inclusion criteria were single pregnancy, no comorbidities, no substance abuse (smoking, alcohol and/or drug abuse).

The study group consisted of 38 subjects with abnormally developing foetuses out of which 11 foetuses had chromosomal aberrations (confirmed with genetic amniocentesis), whereas 27 foetuses had dysmorphia such as heart defects, cystic hygroma or omphalocele. The control group consisted of 34 females with normally developing foetuses (controls).

The study protocol was approved by the Ethics Committee of the Poznan University of Medical Sciences (Resolution No.537/14; July 12, 2014). Research material in subjects and controls included blood serum samples collected from the pregnant participants as a part of routine biochemical testing, which was divided into aliquots and stored at - 80˚C until measurements.

Methods

Foetal congenital malformations were assessed during the antenatal ultrasound scan at 11–13 + 6d gestational weeks in line with the standards of the Foetal Medicine Foundation and recommendations of the Polish Society of Gynaecology, Ultrasonography Division [12–14].

Cytogenetic evaluation of amniocyte karyotype cultured on special growth factor-enriched media included G-banded metaphase analysis assay.

The following ROS-related assays were used: Lowry method was applied to assess TP concentration in serum samples [15]. The level of GSH was determined by modified Ellman

method, using 5,5′-dithiobis (2-nitrobenzoic acid) substrate (DTNB, Ellman's reagent) [16]. RSNO concentration was assessed using the method by Bonin et al., based on a colorimetric reaction at the presence of sulfanilamide and N-(1-naphthyl) ethylenediaminedihydrochloride [17]. NO was detected using Griess method modified by Kleinbongard et al., in 2002 [18]. TEAC was determined using a method comparing the antioxidant capacity of a substance to reduce the ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) stable radical with the antioxidant capacity of trolox, which is a reference compound [19]. GST activity was measured using the method described by Habig et al., [20], using 1-chloro-2,4-dinitrobenzene substrate.

Statistical analyses were performed using PQStat and Statistica v10 bundles (StatSoft, Kraków, Poland). The results were considered statistically significant for $p < \alpha$ ($\alpha = 0.05$). Distribution normality was assessed using Shapiro-Wilk test. In order to compare variables, a non-parametric Mann-Whitney U-test was used due to significant deviation of distribution from normal distribution. For the same reason, correlations between the variables were determined. In order to compare groups, the kernel density estimation (KDE) was carried out using PQstat and the receiver operating characteristic (ROC) curves were plotted.

We confirm that all methods were performed in accordance with the relevant guidelines and regulations from the statement of Ethics Committee of the Poznan University of Medical Sciences.

Data availability

Informed consent to use and publish identifying information was obtained.
The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

RESULTS

There was a significant difference ($p < 0.05$) in patient age between the subjects and controls. The mean age of controls was 27 ± 2.4 (mean ± SD) years, compared to the mean age of 35 ± 5.7 years in the subjects (Tab. 1.).

There was no significant difference in body mass index (BMI), although it should be noted that the BMI values in the subjects showed significant variability (Tab. 1.).

The mean TP level was significant higher ($p < 0.05$) in subjects: 69.1 ± 15.3 mg/mL as compared to 56.9 ± 5.3 mg/mL in controls (Tab. 2).

We observed significant differences in terms of GSH levels ($p < 0.05$). The mean GSH level in subjects (4.98 ± 1.88 nM/mg) was significantly lower than the respective value in controls (6.43 ± 1.24 nM/mg) (Tab. 2).

We observed no significant differences in RSNO (1.47 ± 0.46 nM/mg vs 1.48 ± 0.31 nM/mg) and NO levels (0.27 ± 0.10 nM/mg vs 0.26 ± 0.06 nM/mg) between subjects and controls respectively (Tab. 2).

There was a difference in TEAC ($p < 0.05$). The mean TEAC in subjects (5.64 ± 0.74 μM/mL) was significantly higher than the respective value in controls (4.93 ± 0.82 μM/mL) (Tab. 2).

The mean GST level in subjects (21.72 ± 6.81 nM/min/mg) was significantly higher ($p < 0.05$), than the respective value in controls (15.94 ± 4.52nM/min/mg).

Kernel density estimation (KDE) confirmed variance in both subgroups.

We observed no significant differences in levels of selected oxidative stress markers (TP, GSH, RSNO, NO, TEAC and GST) between subjects with foetal chromosomal aberrations and those with foetal dysmorphia (Tab. 3).

We obtained no significant differences in the values of all following markers: TP, GSH, RSNO, NO, TEAC and GST between younger (< 30 years old) and older (≥ 30 years old) women in patients with detected foetal developmental abnormalities respectively (Tab. 3).

Moreover, there were also a significant differences in TP, GSH, TEAC and GST levels ($p < 0.05$) between subjects and controls in young patients (under 30 years old). The mean values of TP (67.11 ± 13.88 vs 56.94 ± 6.34 mg/mL), TEAC (5.61 ± 0.81 vs 4.93 ± 0.83 μM/mL) and GST (21.19 ± 6.06 vs 15.94 ± 4.52 nM/min/mg) in subjects were higher than the respective values in controls. The mean GSH level in subjects (4.61 ± 1.67 nM/mg) was lower than the respective value in controls (6.43 ± 1.98 nM/mg). There were no significant differences in RSNO and NO levels between these two groups of young women (Tab. 5).

A significant difference in odds ratio obtained for GSH [odds ratio (OR) = 0.57, 95% CL: 0.40–0.80] indicates that its higher concentration can relate to the reduced risk of developmental abnormalities (Tab. 6).

Significant ($p < 0.05$) differences in odds ratio obtained for TP (OR = 1.11, 95% CL: 1.04–1.17), TEAC (OR = 3.54, 95% CL: 1.56–8.05) and GST (OR = 1.18, 95% CL: 1.03–1.17) indicate that their elevation can prove the increases in the risk of developmental abnormalities (Table 6).

In order to identify a classifier to differentiate between pregnant females with normally and abnormally developing foetuses, receiver operating characteristic (ROC) curve analysis was carried out, which confirmed significant prognostic value ($p < 0.05$) of selected oxidative stress parameters (Fig. 1).

Total protein (TP) had a sensitivity of 78.95%, specificity of 79.40%, and area under the curve (AUC) of 0.786. TEAC had a sensitivity of 78.95%, specificity of 61.80 %, and AUC of 0.753; GST had a sensitivity of 71.10%, specificity of 52.90%, and AUC of 0.691. GSH had a sensitivity of 68.40%, specificity of 61.80%, and AUC of 0.728 (Tab. 7).

DISCUSSION

Nowadays, researchers are becoming increasingly interested in oxidative stress and its effect on human body. Redox imbalance may induce damage to a number of biomolecules and other bodily structures. Reactive oxygen species may be one of the factors leading to developmental and functional abnormalities. The pro-apoptotic effect of oxidants also seems to be supported in the literature [21].

It is well known that the patients' age is a risk factor for birth defects, especially chromosome aberrations such as Down syndrome, Edwards syndrome or Patau syndrome [22–24]. Our research confirmed the role of mother age as a risk factor of foetal abnormalities such as chromosomal aberrations, congenital heart disease or other nonchromosomal foetal malformations, demonstrating its significantly higher values in subjects as compared to pregnant females with normally developing foetuses. Whereas, in our analysis we excluded age as a factor influencing oxidative stress level, due to the lack of statistically significant differences in the levels of selected markers in young (up to 30 years old) and older patients (30 years and more) in the group with foetuses with congenital malformations. This is also confirmed by the significant differences in the levels of the studied factors in young patients up to 30 years of age, among patients with healthy and abnormal developing foetuses. The earlier mentioned relationships strengthen the predictive value of selected markers.

Pregnancy is characterized by increased oxidative stress in the foetus and mother. Oxidative stress is induced by placental mitochondrial activity, increased production of ROS, mainly the superoxide radical, and reduced scavenging function of antioxidants. Oxidation in the placenta that is positioned in an oxygen gradient between the mother and foetus is affected by increased ROS production in the maternal circulation. Changes in ROS levels during pregnancy are indispensable to assure normal development of the embryo and foetus [25]. Excessive oxidative stress during pregnancy has been associated with miscarriage and other

various pregnancy complications, such as intrauterine growth retardation, diabetes or pre-eclampsia [26].

Considering the above findings, we attempted to determine the correlation between oxidative stress markers and foetal abnormalities, and to estimate predictive value of these correlations.

Normal foetal development requires certain conditions including maternal biological fitness. The markers we have assessed may indicate disturbance of redox homeostasis. According to previous research it is a crucial factor pointing to the risk of chromosomal aberrations, which supports using these markers in antenatal diagnosis. Since there is just one publication to assess protein and other oxidative stress markers as potential predictors of foetal abnormalities, we have attempted to estimate their predictive value introducing TP in clinical assessment [10].

Our analysis of mean total protein level demonstrated its significantly higher concentrations in females with foetal abnormalities by 17.65% as compared to controls. As elevated TP levels are also present in a number of other pathologies including those where oxidative stress has been implicated as one of possible aetiological factors such as rheumatoid arthritis, type 2 diabetes mellitus or endometriosis, it appears reasonable to suggest that developing and biologically immature systems may demonstrate similar associations [27–29]. It is, therefore, likely that elevated serum TP levels may reflect abnormalities in the environment, in which the foetus develops, as exemplified by elevated TP levels in pregnant smokers [30].

Natural defence mechanisms contribute to maintaining normal intracellular redox potential and to bodily ability to neutralise the harmful effect of ROS. This neutralisation is possible owing to antioxidants and enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, vitamins A, C and E, carotenoids, polyphenols as well as glutathione [6].

The mean levels of GSH, an antioxidant which restores proper redox balance, are significantly lower by 22.5% in pregnant women with abnormally developing foetuses. The decreased GSH levels were demonstrated in amniotic fluid of women with gestational diabetes and pregnancy-induced hypertension (PIH), as compared to healthy controls [20, 21, 25]. Until now, the research demonstrated a significant association between decreased GSH levels and increased risk of chromosomal aberrations in antenatal diagnosis during the first trimester. It confirms the hypothesis that increased oxidative stress caused by insufficient amount of antioxidants may induce cell damage and developmental abnormalities [10].

The analyses did not demonstrate the utility of s-nitrosothiols in predicting foetal abnormalities, as the differences between the groups, albeit present, were not significant. Other covariables, unknown at present, may affect this association. Their identification goes beyond the scope of the current study.

Our analyses did not demonstrate significant differences in serum nitric oxide levels between subjects and controls. The research has so far shown that NO prevents lipid oxidation, inactivates ROS and plays a role in regulating fetoplacental and uteroplacental blood flow as well as that some pregnancy-associated complications, such as IUGR, may be associated with NO deficiency [31–33]. However, the analysis of the current literature did not yield unequivocal conclusions to the nature of the association between NO levels and the course of pregnancy. We did not attempt to analyse this issue further, due to minor and non-significant differences between subjects and controls. It should be noted, though, that high variance of results and small differences between the groups may be associated to different grouping, or increased heterogeneity of the study population.

The total antioxidant capacity (TAC) of the body is expressed as trolox equivalent antioxidant capacity. In the current research, we demonstrated a significantly higher mean TEAC levels by 14.4% in blood serum of the subjects with known foetal abnormalities. It may be associated with compensatory mechanism counteracting the harmful effect of oxidative stress, which leads to developmental abnormalities [10]. Such high TEAC level may also reflect the existing redox imbalance and mobilising the internal antioxidant capacity aiming at restoring the homeostasis.

Our analysis of mean serum S-transferase levels demonstrated its significantly higher concentrations by 26.3% in females with foetal abnormalities as compared to controls. Increased activity of glutathione S-transferase may reflect bodily response to factors enhancing the oxidative stress. The primary role of GSTs is to detoxify xenobiotics by catalyzing the nucleophilic attack by GSH on electrophilic carbon, sulfur, or nitrogen atoms of said nonpolar xenobiotic substrates, thereby preventing their interaction with crucial cellular proteins and nucleic acids. Therefore, it seems likely that this enzyme can be a marker to confirm, at least to some extent, the presence of adverse conditions for the development of reproductive cells and foetal development. Elevated glutathione S-transferase levels demonstrated in women pregnant with abnormally developing foetuses may be associated with a compensatory mechanism counteracting the factors that increase maternal oxidative stress. There are reports which indicate similar association between glutathione and itsmetabolizing enzyme, S-transferase [10].

It seems that chromosomal aberrations are not associated with dysmorphia in foetuses. However, the analysis did not demonstrate significant differences to support different aetiology of those abnormalities. On the other hand, the lack of differences in concentrations or activity of oxidative stress markers do not enable us to reject the hypothesis of different aetiology of foetal abnormalities due to the presence of some, albeit non-significant, differences. Finally, the tendency for chromosomal aberrations to develop in foetuses of mothers with signs of redox imbalance appears to justify further research with more robust selection criteria and well-defined grouping principles.

CONCLUSIONS

Elevated levels of total protein, glutathione S-transferase, high trolox equivalent antioxidant capacity and low reduced glutathione level may be relevant to predict abnormal foetal development and congenital defects.

Author contributions statement

M.P. conceived the experiment. M.P., K.T-W., P.D., M.N., S.R-M., K.Z., J.B. and E.F conducted the experiment. P.D. and K.T-W. analysed the results. P.D. prepared all figures. All authors reviewed the manuscript.

Conflict of interest

All authors: Marek Pietryga, Piotr Dydowicz, Kinga Toboła-Wróbel, Marta Napierała, Sandra Radzicka-Mularczyk, Katarzyna Ziółkowska, Jacek Brązert, Ewa Florek declare no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Table 1. Study sample characteristics: group I — pregnant females with normally developing foetuses (controls); group II — pregnant females with abnormally developing foetuses (subjects)

Variable	**Controls (females with normally developing pregnancy) n = 34**			**Subjects (females with foetal abnormalities) n = 38** Trisomy 21 — 8 cases Trisomy 18 — 2 cases Klinefelter syndrome — 1 case Cystic hygroma — 12 cases Heart defects — 12 cases Omphalocele — 3 cases			**p**
	Mean ± SD	MIN	MAX	Mean ± SD	MIN	MAX	
Age [years]	27 ± 2.4	21	33	35 ± 5.7	19	45	< **0.05**
BMI [kg/m^2]	21.3 ± 3.2	11.1	28.1	23.8 ± 4.3	16.9	40.1	> 0.05

SD — standard deviation; BMI — body mass index

Variable	Controls (females with normally developing pregnancy) n = 34			Subjects (females with foetal abnormalities) n = 38			p
	Mean ± SD	MIN	MAX	Mean ± SD	MIN	MAX	
TP [mg/mL]	56.90 ± 5.30	47.80	81.20	69.1 ± 15.30	35.66	98.85	< **0.05**
GSH [nM/mg]	6.43 ± 1.24	9.95	4.62	4.98 ± 1.88	2.34	10.61	< **0.05**
RSNO [nM/mg]	1.48 ± 0.31	0.79	2.19	1.47 ± 0.46	0.57	2.91	> 0.05
NO [nM/mg]	0.26 ± 0.06	0.14	0.42	0.27 ± 0.10	0.12	0.67	> 0.05
TEAC [µM/mL]	4.93 ± 0.82	2.04	6.20	5.64 ± 0.74	3.53	6.71	< **0.05**
GST [nM/min/mg]	15.94 ± 4.52	4.01	32.85	21.72 ± 6.81	10.35	44.89	< **0.05**

Table 2. The levels of total protein, glutathione, S-nitrosothiols, nitric oxide, trolox equivalent antioxidant capacity and glutathione S-transferase (mean ± SD, minimum and maximum values) in subjects and controls (Mann-Whitney U-test)

SD — standard deviation; TP — total protein; GSH — glutathione; RSNO — S-nitrosothiols; NO — nitric oxide; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase

Table 3. The levels of total protein, glutathione, S-nitrosothiols, nitric oxide, trolox equivalent antioxidant capacity and glutathione S-transferase (mean ± SD, minimum and maximum values) in subject subgroups: foetal chromosomal aberrations vs dysmorphia (Mann-Whitney U-test)

SD — standard deviation; TP — total protein; GSH — glutathione; RSNO — S-nitrosothiols; NO — nitric oxide; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase

Variable	**Foetal chromosomal aberrations n = 11**			**Foetal dysmorphia n = 27**			**p**
	Mean ± SD	MIN	MAX	Mean ± SD	MIN	MAX	
TP [mg/mL]	71.93 ± 14.74	45.83	98.83	62.55 ± 14.88	32.17	97.67	> 0.05
GSH [nM/mg]	5.17 ± 2.01	2.43	7.94	6.21 ± 2.17	2.34	10.61	> 0.05
RSNO [nM/mg]	1.33 ± 0.43	0.84	2.31	1.50 ± 0.46	0.57	2.91	> 0.05
NO [nM/mg]	0.25 ± 0.06	0.18	0.39	0.28 ± 0.10	0.12	0.67	> 0.05
TEAC [µM/mL]	5.82 ± 0.58	4.96	6.71	5.61 ± 0.76	3.53	6.69	> 0.05
GST [nM/min/mg]	19.66 ± 4.75	16.23	33.27	22.00 ± 7.03	10.35	44.85	> 0.05

Table 4. The levels of total protein, glutathione, S-nitrosothiols, nitric oxide, trolox equivalent antioxidant capacity and glutathione S-transferase (mean ± SD, minimum and maximum values) in subject subgroups: under vs over the age of 30 (Mann-Whitney U-test)

Variable	**Subjects (< 30 years old) n = 18**			**Subjects (≥30 years old) n = 20**			**p**
	Mean ± SD	MIN	MAX	Mean ± SD	MIN	MAX	
TP [mg/mL]	67.11 ± 13.88	45,83	97.67	70.00 ± 16.07	35.67	98.83	> 0.05
GSH [nM/mg]	4.61 ± 1.67	2.51	7.66	5.14 ± 1.98	2.43	8.51	> 0.05

RSNO [nM/mg]	1.33 ± 0.42	0.66	2.31	1.39 ± 0.40	0.71	2.17	> 0.05
NO [nM/mg]	0.25 ± 0.08	0.12	0.39	0.25 ± 0.08	0.16	0.49	> 0.05
TEAC [µM/mL]	5.61 ± 0.81	3.53	6.71	5.57 ± 0.67	3.84	6.63	> 0.05
GST [nM/min/mg]	21.19 ± 6.06	14.07	33.27	18.65 ± 6.19	10.35	44.85	> 0.05

SD — standard deviation; TP — total protein; GSH — glutathione; RSNO — S-nitrosothiols; NO — nitric oxide; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase

Table 5. The levels of total protein, glutathione, S-nitrosothiols, nitric oxide, trolox equivalent antioxidant capacity and glutathione S-transferase (mean ± SD, minimum and maximum values) in subjects and controls at the same age (under 30 years old) (Mann-Whitney U-test)

Variable	**Subjects (< 30 years old) n = 18**			**Controls (< 30 years old) n = 34**			**p**
	Mean ± SD	MIN	MAX	Mean ± SD	MIN	MAX	
TP [mg/mL]	67.11 ± 13.88	45.83	97.67	56.94 ± 6.34	47.8	81,2	< **0.05**
GSH [nM/mg]	4.61 ± 1.67	2.51	7.66	6.43 ± 1.24	4.62	9.95	< **0.05**
RSNO [nM/mg]	1.33 ± 0.42	0.66	2.31	1.48 ± 0.31	0,79	2,19	> 0.05
NO [nM/mg]	0.25 ± 0.08	0.12	0.39	0.26 ± 0.06	0.14	0.42	> 0.05
TEAC [µM/mL]	2.61 ± 0.81	3.53	6.71	4.93 ± 0.83	2.04	6,20	< **0.05**
GST [nM/min/mg]	21.19 ± 6.06	14.07	33.27	15.94 ± 4.52	4.01	32.19	< **0.05**

SD — standard deviation; TP — total protein; GSH — glutathione; RSNO — S-nitrosothiols; NO — nitric oxide; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase

Table 6. Logistic regression model including total protein, glutathione, trolox equivalent antioxidant capacity and glutathione S-transferase (Wald chi-square test)

Variable	**OR**	**95% CI**	**p***
TP [mg/mL]	1.11	(1.04; 1.17)	< **0.05**
GSH [nM/mg]	0.57	(0.40; 0.80)	< **0.05**
TEAC [µM/mL]	3.54	(1.56; 8.05)	< **0.05**
GST [nM/min/mg]	1.18	(1.03; 1.35)	< **0.05**

OR — odds ratio; CI — confidence interval; p* — statistical significance; TP — total protein; GSH — glutathione; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase

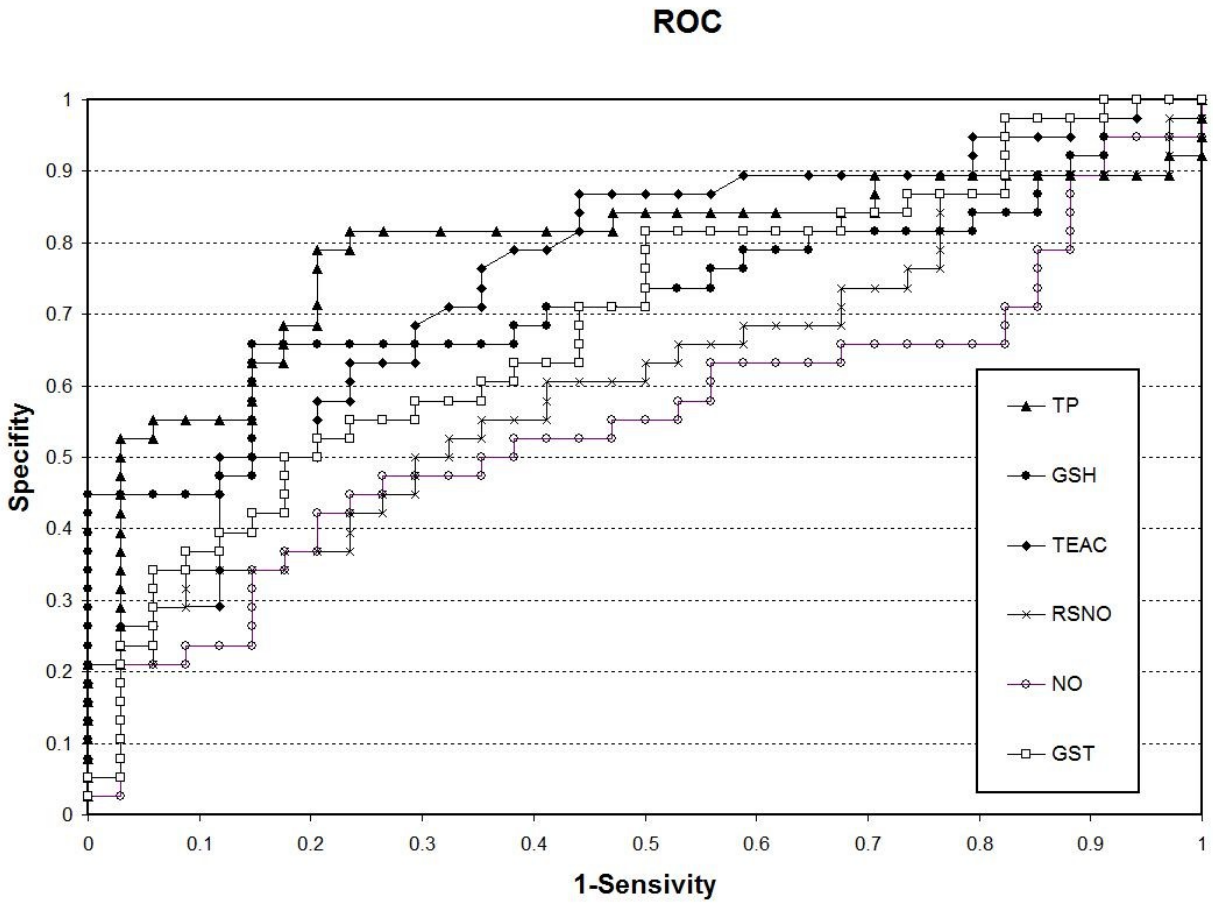
Table 7. Receiver operating characteristic (ROC) curve for total protein, glutathione, trolox equivalent antioxidant capacity and glutathione S-transferase — classifiers differentiating

between subjects (females with foetal congenital abnormalities) and controls (normally developing foetuses). The following parameters were included: cut-off point, sensitivity, specificity, area under the curve, and statistical significance (p)

Variable	**Cut-off point**	**Sensitivity (%)**	**Specificity (%)**	**AUC**	**p**
TP [mg/mL]	60.83	78.95	79.40	0.786	< **0.05**
GSH [nM/mg]	5.37	68.4	61.80	0.728	< **0.05**
TEAC [µM/mL]	5.07	78.95	61.80	0.753	< **0.05**
GST [nM/min/mg]	16.15	71.10	52.90	0691	< **0.05**

AUC — area under the curve;TP — total protein; GSH — glutathione; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase

Figure 1. Receiver operating characteristic (ROC) analysis for total protein, glutathione, trolox equivalent antioxidant capacity and glutathione S-transferase — classifiers differentiating between pregnant females with normally developing foetuses and those with abnormally developing foetuses. The following parameters were included: sensitivity and specificity; TP — total protein; GSH — glutathione; RSNO — S-nitrosothiols; NO — nitric oxide; TEAC — trolox equivalent antioxidant capacity; GST — glutathione S-transferase



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