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# Selected oxidative stress biomarkers in antenatal diagnosis as 11–14 gestational weeks

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ABSTRACT

The primary objective in modern obstetrics and prenatal diagnosis is to predict risks of congenital abnormalities. The aim of the research was to assess the correlation between selected oxidative stress biomarkers with the risk of foetal chromosomal aberration evaluated at the first trimester screening.

A series of studies show that balanced free radical activity and oxidative homeostasis are essential for proper bodily growth and function. Reactive oxygen species (ROS) may be one of the factors associated with disruption of cell cycle and tissue development, thus leading to developmental abnormalities. That's why it's so important to examine connection between level of oxidative stress and congenital abnormalities.

Using ultrasonography examinations between 11–13 + 6d gestational weeks combined with serum levels of pregnancy associated plasma protein A and human chorionic gonadotropin and spectrophotometric analysis of oxidative stress markers such as glutathione (GSH), S-transferase, S-nitrosothiols (RSNO), trolox equivalent antioxidant capacity (TEAC), protein and nitrites we tried to find correlation between birth defects and oxidative stress status.

In conclusion, our analysis suggests that elevated maternal serum levels of protein, S-transferase and TEAC as well as decreased maternal serum levels of GSH and protein correlated with the risk of chromosomal aberrations and congenital developmental defects in a foetus.

## 1. Introduction

The risk assessment for foetal chromosomal aberrations is carried out routinely as a part of the first antenatal ultrasound scan at 11–13 + 6d gestational weeks. It includes ultrasound assessment of foetal anatomy, nuchal translucency (NT) and foetal heart rate (FHR), as established markers of chromosomal aberrations as well as biochemical assays such as serum levels of pregnancy associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG). A search for substances which disrupt protein synthesis and mechanisms which regulate biological homeostasis is still pending. Reactive oxygen species (ROS) may be one of the factors associated with disruption of cell cycle and tissue development, thus leading to developmental abnormalities.

The balance of metabolic and oxidation processes is essential for normal bodily growth and function. ROS overproduction disturbs this homeostasis. ROS include free radicals and peroxides, that is, chemical molecules containing oxygen atoms with an unpaired electron, which makes them exceptionally reactive. ROS also include less reactive

*Abbreviations:* BMI, body mass index; NT, nuchal translucency; FHR, foetal heart rate; PAPP-A, pregnancy associated plasma protein A; hCG, human chorionic gonadotropin; ROS, reactive oxygen species; PxG, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; GST, glutathione *S*-transferase; RSNO, S-nitrosothiols; GSH, glutathione; TAC, total antioxidant capacity; TEAC, trolox equivalent antioxidant capacity; CRL, crown-rump length; BPD, biparietal diameter; OR, odds ratio; CI, confidence intervals; CSF, cerebrospinal fluid

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molecules (e.g. superoxides), which - when reacting with reductors - change into more aggressive forms, likely to disrupt cellular metabolism. Preliminary research in several centres demonstrated the effect of these processes on embryonic and foetal development. Their effect on organogenesis cannot be excluded [1,2].

Oxidative damage affects cell-building compounds such as lipids, proteins, nucleic acids and hydrocarbons. Biological effect of the discussed damage includes changes in physical properties of cellular membranes, which inhibit transmembrane transporters and transport proteins [3,4] and alter the activity and biological function of proteins [5,6]. Their contribution to abnormalities of embryonic and foetal development cannot be excluded.

Balanced free radical activity and oxidative homeostasis are essential for proper bodily growth and function. Oxidative stability can be assessed using different markers such as antioxidant enzymes: glutathione peroxidase (PxG), superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), vitamin C, vitamin A, vitamin E, lycopene or glutathione (GSH), or by determining the total antioxidant capacity (TAC) and nitrite levels [7–10].

## 2. Objective

The aim of the research was to assess the correlation between selected oxidative stress biomarkers (glutathione – GSH, S-transferase, S-nitrosothiols - RSNO, trolox equivalent antioxidant capacity – TEAC, protein and nitrites) at 11–13 + 6 gestational weeks in patients with the risk of foetal chromosomal aberration ranging between 1:2 and 1:20,000.

## 3. Material

Our material were 45 of 1925 patients undergoing standard antenatal screening at 11–13 gestational weeks, whose risk of chromosomal aberrations assessed with combined ultrasound and biochemistry exceeded 1:50. Amniotic fluid test confirmed abnormal karyotype in 17 cases (trisomy 21n = 9, trisomy 18n = 4, trisomy 13n = 3, Klinefelter's Syndrome n = 1). The following developmental defects were demonstrated: ectopiacordis (n = 1), atrioventricular septal defect (n = 2), tricuspid regurgitation (n = 7), suspected ventricular septal defect (n = 3), encephalocele (n = 1), foetal hydrops (n = 8), cystic lymphangioma (n = 5), hydrothorax (n = 2), echogenic bowel (n = 1), reduced blood flow volume in choroid plexus (n = 1), limb deformity (n = 3), megacystis (n = 1), craniofacial defects (n = 1), chorionic cyst (n = 1), eventeration and significant dysmorphia (n = 2) and omphalocoele (n = 3). Increased nuchal translucency (> 95th percentile) occurred in 10 patients as an isolated abnormality. The control group consisted of 31 pregnant women without any known complications, whose risk of chromosomal aberrations was lower than 1:20,000.

## 4. Methods

The risk assessment for foetal chromosomal aberrations is carried out routinely as a part of the first antenatal ultrasound scan at 11–14 gestational weeks. It includes ultrasound assessment of foetal anatomy, nuchal translucency (NT) and foetal heart rate (FHR), as established markers of chromosomal aberrations as well as biochemical assays such as serum levels of pregnancy associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG) [11].

The following anatomical parameters were assessed during the antenatal ultrasound scan at 11–13 + 6d gestational weeks: skull – shape, cerebral falx, lateral ventricles and their choroid plexi, abdominal wall – imaging umbilical base and stomach location, heart – location, axis, rate (FHR), urinary bladder, spine, upper and lower extremities, chorion (in single pregnancy). Biometry measurements included the crown-rump length (CRL) and biparietal diameter (BPD) [12–14]. The risk assessment of the most common chromosomal aberrations (trisomy 21, 18, 13) in foetuses with CRL of 45–84 mm included nuchal translucency (NT) assessment in line with the standards of Foetal Medicine Foundation [15] and recommendations of Polish Society of Gynaecology, Ultrasonography Division [12].

Serum levels of pregnancy associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG) were determined on DELFIA Xpress analyser using the time-delayed fluorescence immunoassay (TRFIA) (Perkin Elmer Life and Analytical Sciences, Waltham, USA). The surface of the reaction cup is coated with the specific IgG capture antibody targeting the respective determinant of the PAPP-A/proMBP /β-hCG complex present in maternal serum. The fluorescence is measured at 612 nm.

The risk of aberration was computed based on results of ultrasound and biochemical testing using Astraia software (Astraia Software Gmbh, Munich, Germany).

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 PAPP-A level [16]. GST activity was measured using the method described by Habig et al., using 1-chloro-2,4-dinitrobenzene substrate [17]. The level of reduced glutathione was determined by modified Ellman method, using 5,5′-dithiobis(2-nitrobenzoic acid) substrate (DTNB, Ellman's reagent) [18]. S-nitrosothiols was assessed using the method by Bonin et al., based on colorimetric reaction at the presence of sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride [19]. Nitrites were detected using Griess method modified by Kleinbongard et al. in 2002 [20]. The trolox equivalent antioxidant capacity was determined using a method comparing the antioxidant capacity of a substance to reduce the ABTS stable radical (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) with the antioxidant capacity of trolox, which is a reference compound [21].

Statistical analyses were carried out using the Statistica 10 software bundle (StatSoft, Kraków, Poland). Statistical significance level of $p < 0.05$ was assumed for all comparisons. 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, in order to assess the correlation between the variables, Spearmans's rank correlation coefficient (RSRS) was calculated. In order to determine factors which significantly predicted chromosomal aberrations logistic regression analysis was carried out. In order to assess which predictor variables affected the outcome variable and what the size of their contribution was, Wald's chi-squared test was carried out. For statistically significant variables, odds ratio (OR) and confidence intervals (CI) were calculated.

Each patient signed consent to participate in a research study. The Medical University Bioethics Committee in Poznań has granted its consent to the project examinations (Resolution No.537/14; 12th July 2014).

Inclusion criteria:

a. Pregnant women screened at the Outpatient Ultrasound and Prenatal Care Center at 10–13 + 6d gestational week.

Exclusion criteria:

a. Twin pregnancy
b. Preeclampsia
c. Diabetes
d. Hypertension
e. Smoking (active/passive/e-cigarettes) and drinking alcohol.

## 5. Results

Means body mass index (BMI) and maternal age were significantly