Amniotic fluid oxidant–antioxidant status in foetal congenital nervous system anomalies

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Abstract
 Objective: This study aimed to evaluate the oxidant–antioxidant status of amniotic fluid in pregnant women with foetal congenital malformations of the central nervous system.
 Methods: We studied pregnant women with foetal congenital nervous system anomalies at 16–22 weeks' gestation (n = 36). The control group (n = 30) consisted of pregnant women at the same gestational age who underwent amniocentesis, resulting in a normal karyotype. We analysed glutathione, catalase, and malondialdehyde levels in amniotic fluid. Enzyme activation was measured by spectrophotometry.
 Results: The demographic features of the groups were similar in terms of age, parity, body mass index, and gestational weeks. We detected lower glutathione and catalase levels in the foetal congenital anomaly group than in the control group. We detected higher malondialdehyde levels in the foetal congenital anomaly group than in the control group.
 Conclusion: In the organism, the rate of formation of free radicals and their rate of removal are balanced, and this is called oxidative balance. As long as oxidative stability is achieved, the organism is not affected by free radicals. This fact should be kept in mind to avoid any type of teratogenic agent that could lead to congenital disorders.

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Keywords
Amniotic fluid, foetal congenital nervous system anomalies, oxidant status, antioxidant, pregnancy, oxygen free radicals

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Introduction
Free radicals are reactive molecules that occur in metabolic processes during the conversion of nutrients into energy using oxygen. Oxygen molecules are indispensable for life, but products that are sources of free radicals are formed during the metabolic processes. These molecules, known as reactive oxygen metabolites, damage lipids, protein, and cell DNA. Antioxidant defence systems keep free radical formation under control. The balance between oxidant and antioxidant mechanisms is important in biological organisms. Deterioration of the balance between oxidant and antioxidant systems is called oxidative stress, and it occurs in situations in which excessive oxygen radicals are produced in large quantities or when antioxidant mechanisms are inadequate.\(^1,2\) In an organism, the rate of formation of free radicals and their rate of removal are in balance, and this is called oxidative balance. As long as oxidative stability is achieved, the organism is not affected by free radicals. Oxidant–antioxidant balance systems play important roles in carcinogenesis, aging, metabolic events, immune disorders, neurodegenerative cases, and embryogenesis. Weakening of the antioxidant defence systems in the period of embryogenesis is related to congenital malformations. An increase in the levels of oxygen free radicals has a major role in causing foetal anomalies in early embryological development.\(^3,4\)

Although there are various factors leading to congenital foetal anomalies, including genetics, environmental factors, drugs, impaired glucose concentrations, hyperthermia, radiation, oxidative stress, and metabolic disorders, the cause of many of these anomalies is unknown.\(^5\) Therefore, this study aimed to investigate the oxidant–antioxidant status of amniotic fluid (AF) of pregnant women whose foetuses had anomalies of the central nervous system.

Materials and methods

Patients
Pregnant women who were admitted to a tertiary university hospital in the east region of Turkey between 4 January and 1 June 2017 were included in the study. The study was conducted in accordance with the principles of the Helsinki Declaration. Before the study commenced, approval was received from the Ethics Committee and all of the patients provided written informed consent before enrolling. A total of 66 patients initially participated in the study. The study group consisted of pregnant women with foetal congenital nervous system anomalies (e.g., anencephaly, acrania, neural tube defects, encephalocele, and iniencephaly), which were detected by ultrasound between 16 and 22 weeks of gestation (n = 36). Pregnant women at the same weeks of gestation as those with foetal congenital nervous system anomalies who had undergone amniocentesis (because of a high risk in the triple test), resulting in a normal karyotype, were included in the control group (n = 30). In both groups, amniocentesis was conducted in the presence of appropriate indications. Pregnant women with systemic diseases, such as
hypertension and diabetes, were not included in the study.

**Collection of materials**

AF samples were obtained through amniocentesis. AF samples were sent for genetic examination. Small amounts of AF that were not sent to the genetics laboratory were collected in empty tubes and immediately stored at 4°C. The fluid was centrifuged at 3000 rpm for 10 minutes to separate it from cells and precipitate. AF samples that were used for measuring malondialdehyde (MDA), catalase (CAT), and glutathione (GSH) levels were stored at −40°C until later use.

**Biochemical analysis**

Lipid peroxidation was measured by estimating MDA levels as previously described.6 The results are expressed as nmol/mL for AF. Reduced GSH levels in AF were measured using a spectrophotometer (Unicam Ltd., Cambridge, UK) at 412 nm.7 Quantification is based on the reaction between free sulphydryl groups of reduced GSH with Ellman reagent (5,50-dithiobis-[2-nitrobenzoic acid]) when yellow adduct (2-nitro-5-mercaptopentanoic acid) is formed.

CAT activity was measured using H₂O₂ as a substrate.8 Degradation of H₂O₂ was monitored at 240 nm for 5 minutes using a spectrophotometer (Unicam Ltd.), and enzyme activity was expressed in U/L of serum for AF.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. The Shapiro–Wilk test was used to test the distribution of normality. The Mann–Whitney U-test was performed to determine differences between the groups. A p value < 0.05 was considered statistically significant.

**Results**

Age, parity, body mass index, and gestational weeks were similar between the groups (p > 0.05) (Table 1). Lower levels of GSH and CAT were detected in the foetal congenital anomaly group, indicating antioxidant status, than in the control group (p < 0.01). Higher levels of MDA, indicating an increased oxidant status, were found in the foetal congenital anomaly group than in the control group (p < 0.01) (Table 2).

**Discussion**

This study aimed to evaluate whether there is a relationship between oxidant–antioxidant status and congenital nervous system anomalies in the foetus. Some studies have investigated the oxidant and antioxidant status of AF of pregnant women with foetal aneuploidy and congenital anomalies.9 However, to the best of our knowledge, no studies have investigated CAT,

![](https://example.com/table1.png)

|                      | Foetal congenital anomaly group, median (range) (n = 36) | Control group, median (range) (n = 30) | p value |
|----------------------|------------------------------------------------------|--------------------------------------|---------|
| Age (years)          | 27.7 (20–40)                                         | 27.3 (20–40)                         | 0.846   |
| Parity               | 2.02 (1–4)                                           | 2.03 (1–4)                           | 1.000   |
| Haemoglobin (mg/dL)  | 11.88 (9–15)                                         | 11.83 (9–15)                         | 0.791   |
| Body mass index (kg/m²) | 22.9 (19–28)                                     | 22.7 (19–28)                         | 0.461   |
MDA, and GSH levels in AF of pregnant women whose foetuses have congenital nervous system anomalies. An important aspect of our study is the variety of anomalies (anencephaly, acrania, iniencephaly, neural tube defects, encephalocele, agenesis of the corpus callosum, holoprosencephaly, and Dandy–Walker syndrome) that we reported. We found lower levels of antioxidants and increased levels of oxidants in the AF of pregnant women with foetal congenital nervous system anomalies compared with pregnant women with no foetal anomalies. In our previous study, we also found low levels of antioxidants in the blood of pregnant women whose foetuses had neural tube defects.10

The body produces a variety of enzymatic and non-enzymatic mechanisms to protect cells against oxidative stress. CAT and GSF are among these mechanisms. CAT is an intracellular antioxidant enzyme and is found primarily in peroxisomes. CAT catalyses a reaction between two H₂O₂ molecules, resulting in formation of water and O₂. Additionally, CAT can promote the interaction of H₂O₂ with hydrogen donors so that H₂O₂ can be converted to one molecule of water and the reduced donor is oxidized (peroxidatic activity of CAT).11 GSH, which is present in all cells of organisms and constitutes most of the content of the sulphhydryl group outside the protein structure of the cells, plays important roles in making harmful compounds inactive. GSH acts as a counterpart to radical-induced damage and antioxidant enzymes. GSH acts as a radical receptor. GSH is especially important for the activity of peroxidase and reductase enzymes. Levels of GSH are reduced in oxidative stress.12

Free radicals are unable to pass through the cell membrane because of its selective permeability, but need to pass through this membrane to interact with cells. Therefore, free radicals initiate lipid peroxidation by eliminating hydrogen atoms from alpha-methylene groups of polyunsaturated fatty acids in the cell membrane. Finally, polyunsaturated fatty acids are hydrolysed into biologically active compounds. The most important of these compounds is MDA, which reflects lipid peroxidation in the body.2

The foetus is surrounded by AF. AF plays an important role in foetal development and growth. AF originates from maternal and foetal tissues. The mother, placenta, and foetus play role in the production of AF, but nearly all AF is produced by the foetus. Therefore, AF reflects the foetal situation.13 Deterioration in AF is related to poor obstetric outcomes.14,15 An imbalance in antioxidant defence mechanisms in the placental and foetal membranes of cows can cause illness in newborns, reproductive problems, and complications in pregnancy.16 Cellular and oxidative stress are thought to activate the nuclear factor-kappa B-mediated inflammatory pathway in foetal membranes.17

|                      | Foetal congenital anomaly group, median (range) (n = 36) | Control group median, (range) (n = 30) | p value |
|----------------------|---------------------------------------------------------|----------------------------------------|---------|
| MDA (nmol/mL)        | 1.62 (1.42–1.89)                                         | 1.43 (1.35–1.56)                        | <0.01   |
| CAT (U/L)            | 0.14 (0.01–0.40)                                         | 0.26 (0.10–1.11)                        | <0.01   |
| GSH (mmol/g)         | 0.06 (0.01–0.19)                                         | 0.10 (0.03–0.19)                        | <0.01   |

MDA, malondialdehyde; CAT, catalase; GSH, glutathione.
Oxidative DNA damage occurs because of increased formation of reactive oxygen species, decreased levels of antioxidant enzymes, and defects in DNA repair mechanisms. Single- and double-stranded fractures, base modifications (base participation, rearrangement in some cases), and nucleoside damage may occur in DNA. There may also be crosslinking between DNA and protein depending on oxidative damage.\textsuperscript{18,19}

Foetal development occurs under the influence of genetic, metabolic, and environmental factors. Deterioration of antioxidant defence mechanisms leads to defects in DNA synthesis. This could play a role in congenital malformation and cell death.\textsuperscript{20}

Oxidative stress might be one of the main causes of molecular damage to cell and tissue structures.\textsuperscript{21} Early embryonic development is vulnerable to oxidative stress because of the immaturity of free radical scavenging mechanisms.\textsuperscript{5} The paired box 3 (\textit{Pax3}) gene plays a major role in the development of neuroepithelium of embryos. In the absence of \textit{Pax3}, neural tube defects occur.\textsuperscript{22} Oxidative stress occurring before \textit{Pax3} expression leads to an increased risk of neural tube defects and diminished gene expression.\textsuperscript{23}

Diabetes and high glucose levels are increased oxidative situations and are associated with birth defects, particularly neural tube defects.\textsuperscript{24} Additionally, neurotrophic factors play an important role in neuronal life during the developmental process and after injury. Brain-derived neurotrophic factor modulates neurogenesis and synaptic plasticity.\textsuperscript{25} However, cAMP/\textit{Ca}\textsuperscript{2+} response element-binding protein is required for this activation. Previous studies have shown that increased oxidative damage reduces cAMP/\textit{Ca}\textsuperscript{2+} response element-binding protein levels, increases nuclear factor-kappa B expression, decreases brain-derived neurotrophic factor levels, and damages cognitive function.\textsuperscript{26}

Brain and nervous tissues are predisposed to oxidative stress because of the rapidity of \textit{Ca}\textsuperscript{2+} trafficking among neuronal membranes, high consumption of oxygen, and the presence of excitotoxic amino acids (glutamate and aspartate). Additionally, these tissues are predisposed to oxidative stress because of easy auto-oxidation of some neurotransmitters, low levels of antioxidant enzymes of lipids in neuronal membranes, the presence of cytochrome P450 in some brain regions, brain metabolism of H\textsubscript{2}O\textsubscript{2}, and high polyunsaturated fatty acid levels. Permanent damage to the brain might be caused by oxidative damage due to impaired oxidant/antioxidant balance in the foetal period and early childhood.\textsuperscript{27}

Our study showed that oxidative stress was present in AF of foetuses with congenital nervous system anomalies, and that this was reflected by an increase in serum MDA levels. Similarly, the activities of antioxidant enzymes (CAT and GSH) were notably decreased in pregnant women whose foetuses had congenital nervous system anomalies. These findings indicate that there may be an association between congenital nervous system anomalies in the foetus, inflammation, and increased production of oxygen free radicals. As previously mentioned, tissue damage due to lipid peroxidation and failure of antioxidant defence mechanisms may play a major role in the pathogenesis of neurogenesis. The main limitation of this study is the low number of patients.

Increased oxidative stress markers in amniotic fluid are associated with neural tube defects.\textsuperscript{28} The oxidant–antioxidant status of AF could serve as a diagnostic marker in foetal congenital nervous system anomalies. Our study shows the importance of the oxidant–antioxidant status of AF. There is a strong association between the oxidative process and foetal congenital anomalies. Stress, teratogens, radiation,
and infection could be considered as oxidative states and an antioxidant status has a positive effect on foetal development.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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