Clinical Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(3) 1146–1152

DOI: 10.1177/0300060517734443
journals.sagepub.com/home/imr
SAGE

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

Numan Cim[1], Harun Egemen Tolunay[1], Erbil Karaman[1], Barıs Boza[1], Mustafa Bilici[2], Orkun Çetin[1], Recep Yıldızhan[1] and Hanım Guler Sahin[1]

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.

[1]Yuzuncu Yil University Medical Faculty, Department of Obstetrics and Gynecology, Van, Turkey
[2]Yuzuncu Yil University, Van Security Collage, Department of Biochemistry, Van, Turkey

Corresponding author:
Numan Cim, Department of Obstetrics and Gynecology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey.
Email: numancim@yahoo.com

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

Date received: 13 June 2017; accepted: 7 September 2017

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 sulfhydryl groups of reduced GSH with Ellman reagent (5,50-dithiobis-[2-nitrobenzoic acid]) when yellow adduct (2-nitro-5-mercaptobenzoate) is formed.

CAT activity was measured using H_2O_2 as a substrate.[8] Degradation of H_2O_2 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,

Table 1. Demographic variables of the foetal congenital anomaly and control groups

	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/m^2)	22.9 (19–28)	22.7 (19–28)	0.461

Table 2. Comparison of oxidant and antioxidant status between the groups

	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.

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 H_2O_2 molecules, resulting in formation of water and O_2. Additionally, CAT can promote the interaction of H_2O_2 with hydrogen donors so that H_2O_2 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 sulfhydryl 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]

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.[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.[20]

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

Diabetes and high glucose levels are increased oxidative situations and are associated with birth defects, particularly neural tube defects.[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.[25] However, cAMP/Ca^{+2} response element-binding protein is required for this activation. Previous studies have shown that increased oxidative damage reduces cAMP/Ca^{+2} response element-binding protein levels, increases nuclear factor-kappa B expression, decreases brain-derived neurotrophic factor levels, and damages cognitive function.[26]

Brain and nervous tissues are predisposed to oxidative stress because of the rapidity of Ca^{+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 autooxidation 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_2O_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.[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.[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.

Acknowledgements

This document has been professionally proofread by an English native speaker.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Agarwal A, Gupta S, Sekhon L, et al. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. *Antioxid Redox Signal* 2008; 10: 1375–1403.
2. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; 39: 44–84.
3. Zangen SW, Ryu S, and Ornoy A. Alterations in the expression of antioxidant genes and the levels of transcription factor NF-kappa B in relation to diabetic embryopathy in the Cohen diabetic rat model. *Birth Defects Res A Clin Mol Teratol* 2006; 76: 107–114.
4. Perrone S, Longini M, Bellieni CV, et al. Early oxidative stress in amniotic fluid of pregnancies with Down syndrome. *Clin Biochem* 2007; 40: 177–180.
5. Loeken MR. Free radicals and birth defects. *J Matern Fetal Neonatal Med* 2004; 15: 6–14.
6. Yoshioka T, Kawada K, Shimada T, et al. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol* 1979; 135: 372–376.
7. Kapetanović IM and Mieyal JJ. Inhibition of acetaminophen- induced hepatotoxicity by phenacetin and its alkoxy analogs. *J Pharmacol Exp Ther* 1979; 209: 25–30.
8. Aebi H. Catalase. In: Bergmayer HU (ed) *Methods of enzymatic analysis*. 2nd ed. New York: Academic Press Inc., 1974. pp.673–677.
9. Aksin M, Incebiyik A, Vural M, et al. Does a risky outcome of antenatal screening test indicate oxidative stres? *J Matern Fetal Neonatal Med* 2014; 27: 1033–1037.
10. Cetin O, Karaman E, Boza B, et al. Relationship between maternal blood ceruloplasmin level, catalase and myeloperoxidase activity and neural tube defects. *Ginekol Pol* 2017; 88: 156–160.
11. Góth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta* 1991; 196: 143–151.
12. Fang YZ, Yang S and Wu G. Free radicals, antioxidants and nutrition. *Nutrition* 2002; 18: 872–879.
13. Brace RA. Physiology of amniotic fluid volume regulation. *Clin Obstet Gynecol* 1997; 40: 280–289.
14. Ott WJ. Reevaluation of the relationship between amniotic fluid volume and perinatal outcome. *Am J Obstet Gynecol* 2005; 192: 1803–1809.
15. Underwood MA, Gilbert WM and Sherman MP. Amniotic fluid: not just fetal urine anymore. *J Perinatol* 2005; 25: 341–348.
16. Kankofer M. Antioxidative defence mechanisms against reactive oxygen species in bovine retained and not-retained placenta: activity of glutathione peroxidase, glutathione transferase, catalase and superoxide dismutase. *Placenta* 2001; 22: 466–472.
17. Henkler F, Brinkmann J and Luch A. The role of oxidative stressin carcinogenesis induced by metals and xenobiotics. *Cancers (Basel)* 2010; 2: 376–396.
18. Cooke MS, Evans MD, Dizdaroglu M, et al. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* 2003; 17: 1195–1214.
19. Evans MD and Cooke MS. Factors contributing to the outcome of oxidative damage to nucleic acids. *Bioessays* 2004; 26: 533–542.
20. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased

accumulation and DNA gyrase protection. *J Antimicrob Chemother* 2003; 51: 1109–1117.
21. Behrman HR, Kodaman PH, Preston SL, et al. Oxidative stress and the ovary. *J Soc Gynecol Investig* 2001; 8(1 Suppl proceedings): S40–S42.
22. Phelan SA, Ito M and Loeken MR. Neural tube defects in embryos of diabetic mice: role of the Pax3 gene and apoptosis. *Diabetes* 1997; 46: 1189–1197.
23. Chang TI, Horal M, Jain SK, et al. Oxidant regulation of gene expression and neural tube development: Insights gained from diabetic pregnancy on molecular causes of neural tube defects. *Diabetologia* 2003; 46: 538–545.
24. Correa A, Gilboa SM, Besser LM, et al. Diabetes mellitus and birth defects. *Am J Obstet Gynecol* 2008; 199: 237.e1–9.
25. Branchi I, D'Andrea I, Fiore M, et al. Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. *Biol Psychiatry* 2006; 60: 690–696.
26. Kapczinski F, Frey BN, Andreazza AC, et al. Increased oxidative stress as a mechanism for decreased BDNF levels in acute manic episodes. *Rev Bras Psiquiatr* 2008; 30: 243–245.
27. Wu A, Ying Z and Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *Eur J Neurosci* 2004; 19: 1699–1707.
28. Vural M, Camuzcuoglu H, Toy H, et al. Amniotic fluid prolidase activity and oxidative status in neural tube defects. *Fetal Diagn Ther* 2010; 28: 34–39.