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8-Hydroxy-2-deoxyguanosine, a product of oxidative DNA degradation, is increased in the amniotic fluid of preterm births

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Abstract. – OBJECTIVE: 8-Hydroxy-2-deoxyguanosine (8-OH-2dG) is a measurable biomarker of oxidative DNA damage. This study was designed to determine amniotic fluid 8-OH-2dG levels in healthy full-term pregnant women and preterm pregnant women. To reveal the effect of reactive oxygen species on 8-OH-2dG levels, amniotic fluid total oxidant capacity (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) were also measured.

PATIENTS AND METHODS: A total of 60 patients, 35 patients with full-term pregnancy and 25 patients with preterm pregnancy, participated in the study. Labor occurring before 37 weeks of gestation was considered as spontaneous preterm birth. Amniotic fluid samples were collected from full-term patients during cesarean section or normal vaginal delivery. 8-OH-2dG concentrations in amniotic fluid samples were measured quantitatively by Enzyme-Linked Immunosorbent Assay (ELISA). Amniotic fluid total antioxidant capacity (TAC) and total oxidant capacity (TOC) was determined in amniotic samples.

RESULTS: The amniotic fluid 8-OH-2dG levels of the preterm group were significantly higher than the full-term group (60.8±7.02 ng/mL *vs.* 33.6±4.11 ng/mL, $p<0.01$). Similarly, TOC levels of the preterm group were significantly higher than the full-term group (89.7±4.80 µmol/L *vs.* 54.3±6.60 µmol, $p<0.02$). TAC was significantly higher in the full-term group compared to the preterm group (1.87±0.10 mmol/L *vs.* 0.97±0.44 mmol/L, $p<0.01$). The OSI values of the preterm group were significantly higher than the full-term group. A negative and significant correlation was found between gestational age and amniotic fluid 8-OH-2dG levels in the full-term pregnancy group (r=-0.78, $p<0.01$). A negative and significant correlation was observed between TAC and amniotic fluid 8-OH-2dG levels in the full-term group (r=-0.60, $p<0.02$). A positive and significant correlation was also detected between TOC, OSI and amniotic fluid 8-OH-2dG levels in the full-term group. There was a negative but insignificant correlation between fetal weight and amniotic fluid 8-OH-2dG levels. The correlation analysis results of the preterm pregnancy group were similar to the full-term group.

CONCLUSIONS: Increased reactive oxygen derivatives in preterm birth increase amniotic fluid levels of DNA degradation product 8-OH-2dG and may lead to premature rupture of fetal membranes. This is the first clinical study investigating 8-OH-2dG levels in amniotic fluid of preterm birth.

Key Words:
Preterm birth, 8-OH-2dG, TAC, TOC, OSI, Fetal membranes.

Introduction

Fetal membranes are key tissues for the fetus and its appendages to complete their development in protected amniotic fluid throughout pregnancy. Its phenotypic and plasticity properties allow fetal membranes to adapt to each stage of pregnancy to compensate for the enlarged feto-placental volume. Its strong collagen content and ability to respond to hormonal and local cytokines also allow the membranes to continue their development without tearing until term[1,2]. With the advancing weeks of gestation, amniotic fluid proinflammatory cytokines, reactive oxygen derivatives, and matrix metalloproteinase enzyme levels begin to increase exponentially[3-5]. The developing fetus and its appendages, as well as the maternal endocrine system, are the main determinants of amniotic fluid cytokine and hormone profile changes. The combined effect of all these changes is to initiate labor by causing fetal membranes to lose their phenotypic character and subsequently to rupture.

Physiological changes in the chorioamniotic membrane during pregnancy lead to the emer-

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gence of reactive oxygen derivatives (ROS). Increasing ROS causes damage to DNA, lipid cell membranes and proteins, leading to rupture of fetal membranes. ROS emerging during the mitochondrial respiratory chain reaction cause damage to the genomic content of the dynamic structures of amnion and chorion cells[6,7]. The increase in superoxide anions towards the end of pregnancy leads to breakage in the DNA of the cells that make up the fetal membranes, causing the membrane damage to become evident and the membrane to rupture[7-10]. Amniotic fluid is an important and easily accessible biological fluid for detecting damage to the DNA of fetal membranes by ROS[11]. The main product that emerges after oxidative damage of fetal membrane DNA is 8-Hydroxy-2-deoxyguanosine (8-OH-2dG), which is used as a biomarker in the evaluation of oxidative stress[9,10]. It is known that levels of 8-OH-2dG increase in the presence of oxidative stress and pair with adenine and cytosine[12].

Increased DNA damage due to increased ROS production in term pregnancies may lead to accumulation of 8-OH-2dG levels in amniotic fluid and genomic instability in fetal membranes[6,7]. However, there is no study on how amniotic fluid 8-OH-2dG levels change in term and preterm pregnancies. If the increase in 8-OH-2dG is responsible for the rupture of fetal membranes, the levels of this oxidative product should be increased in both full-term and preterm cases. If preterm birth is the forefront of full-term birth, 8-OH-2dG levels may contribute to rupture of membranes in both term and preterm cases. This study was designed to determine the changes in amniotic fluid 8-OH-2dG levels in healthy full-term pregnant women and preterm pregnant women. In addition, to reveal the effect of ROS derivatives on 8-OH-2dG levels, amniotic fluid total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) were also measured.

Patients and Methods

A total of sixty patients, 35 patients with full-term pregnancy and 25 patients with preterm pregnancy, were included in the study. Whether the pregnancies were natural spontaneous conception or ICSI pregnancy was not taken into account. Labor occurring before 37 weeks of gestation was considered as spontaneous preterm birth. The gestational week was calculated according to the last menstrual history and the first registered USG examination. Patients diagnosed with multiple pregnancy were not included in the study. Since amniotic fluid cytokine and ROS dynamics increased in proportion to the week of gestation, preterm deliveries before the 32nd week of gestation were not included in the study. The patients included in the preterm delivery group were older than 32 weeks and less than 36 weeks and 6 days. Medical histories and demographic characteristics of full-term and preterm births were collected from data obtained from face-to-face interviews or from their medical records.

Amniotic fluid samples were collected from full-term patients during cesarean section or normal vaginal delivery. Approximately, 10 cc of amniotic fluid was sampled as soon as the fetal membranes were cut during cesarean delivery. The fetus and its appendages were delivered after fluid collection. In cases of vaginal delivery, samples were collected from amniotic fluid accumulating in the vagina or speculum following artificial or spontaneous rupture of the fetal membranes. Amniotic fluid was collected in preterm cases, similar to term cases. If the amniotic fluid was not fully discharged in patients presenting with premature rupture of the fetal membranes, amniotic fluid was collected during cesarean section or normal vaginal delivery. Cases whose amniotic fluid was completely emptied or cases contaminated with dense blood were not included in the study. Amniotic fluids collected from each patient were immediately sent to the laboratory and centrifuged at 4000 rpm for 5 minutes. While blood and vernix accumulated at the bottom of the centrifuge tube, clean supernatants were collected in a separate tube and stored frozen until analysis.

Amniotic Fluid 8-OH-2dG Measurement

After the frozen amniotic fluid samples were thawed under appropriate laboratory conditions, they were subjected to Enzyme-Linked Immunosorbent Assay (ELISA) for the measurement of 8-OH-2dG, following the manufacturer's recommendations. 8-OH-2dG concentration in amniotic fluid samples were measured quantitatively by ELISA using human 8-OH-2dG ELISA kit (CusabioBiotechCo., Ltd., WUHAN, CHINA). The detection range of the 8-OH-2dG kit (assay range) was 3.12 ng/mL 800 ng/mL and the minimum measurable level (sensitivity) was 3.12 ng/mL. The intra- and inter-assay coefficients of variation

were <8% and <10%, respectively.

Amniotic Fluid TAC and TOC Measurement

Amniotic fluid total antioxidant capacity (TAC) was measured using an automated method[13]. Results were presented as mmolTrolox equivalents/L. Total oxidant capacity (TOC) was determined in amniotic samples using a commercial kit (Rel Assay, Gaziantep, Turkey). TOC results were expressed as the micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L). By dividing TOC by TAC, the OSI value was calculated (TOC/TAC=OSI). The results of OSI were presented as arbitrary unit.

Statistical Analysis

Analyzes of laboratory and demographic data were performed on SPSS 21 (SPSS Inc., Armonk, NY, USA). Whether the data showed normal distribution was checked with the Shapiro-Wilk test. Mann-Whitney U test was used for comparison of intergroup parameters. The Sperman's correlation analysis was used to determine the relationship between 8-OH-2dG values and TAC, TOC, OSI and other parameters. Results are given as mean ± standard deviation. $p<0.05$ was considered significant for all values.

Results

Maternal age, parity, gestational age at birth, fetal birth weights of the full-term group were significantly higher than preterm birth group (Table I). The amniotic fluid 8-OH-2dG levels of the preterm group were significantly higher than the full-term group (60.8±7.02 ng/mL *vs.* 33.6±4.11 ng/mL, $p<0.01$). Similarly, TOC levels of the preterm group were significantly higher than the full-term group (89.7±4.80 μmol/L *vs.* 54.3±6.60 μmol, $p<0.02$). TAC was significantly higher in the full-term group compared to the preterm group (1.87±0.10 mmol/L *vs.* 0.97±0.44 mmol/L, $p<0.01$). The OSI values of the preterm group were significantly higher than the full-term group (92.4±7.88 au *vs.* 39.7±3.09 au, $p<0.01$).

We found a negative and significant correlation between gestational age and amniotic fluid 8-OH-2dG levels in the full-term pregnancy group (r=-0.78, $p<0.01$). Similarly, we found a negative and significant correlation between TAC values and amniotic fluid 8-OH-2dG levels in the full-term pregnancy group (r=-0.60, $p<0.02$). We found a positive and significant correlation between TOC and OSI values and amniotic fluid 8-OH-2dG levels in the full-term group. There was a negative but insignificant correlation between fetal weight and amniotic fluid 8-OH-2dG levels. The correlation analysis results of the preterm pregnancy group were similar to the full-term pregnancy group. In the preterm birth group, amniotic fluid 8-OH-2dG levels were negatively correlated with gestational age and TAC levels, while positively and significantly correlated with TOC and OSI levels. Amniotic fluid 8-OH-2dG levels were not correlated with the weight (Table II).

Discussion

Preterm labor is a major health problem, affecting approximately 10% of all pregnancies[6]. Although it is a condition with multifactorial etiology, the most common cause is premature rupture of membranes (PPROM)[14]. The cause of premature rupture of fetal membranes depends on the conditions created by the underlying etiological cause. Infections, trauma, history of in-

Table I. Comparison of amniotic fluid 8-OH-2dG, TAC, TOC and OSI values of groups with full term and preterm deliveries.

	Full term birth (> 37 w) N = 35	Preterm birth (< 37 w) N = 25	*p*-values
Maternal age (y)	29.2 ± 2.04	25.9± 3.09	0.03
Gestational age at birth (week)	38.7 ± 3.08	34.9± 3.22	0.02
Parity	1.64	1.20	0.01
Fetal birth weight (gr)	2980.7 ± 243.5	2370.5 ± 113.2	0.03
8-OH-2dG (ng/mL)	33.6 ± 4.11	60.8 ± 7.02	0.01
TOC (μmol H_2O_2 equivalent/L)	54.3 ± 6.60	89.7 ± 4.80	0.02
TAC (mmolTrolox equivalents/L)	1.87 ± 0.10	0.97 ± 0.44	0.01
OSI (TOC/TAC) (arbitrary unit)	39.7 ± 3.09	92.4 ± 7.88	0.01

The results were given as mean ± SD. Mann Whitney U test was used for comparisons between two groups. $p < 0.05$ was accepted as statistically significant.

Table II. Comparison of amniotic fluid 8-OH-2dG levels of full term and preterm birth groups with TAC, TOC and other parameters.

	Full term (> 37 gw)		Preterm birth (< 37 gw)	
	Amniotic fluid 8-OH-2dG			
	R	p	r	p
Gestationel age	r = -0.78	0.01	r = -0.53	0.04
TAC	r = -0.60	0.02	r = -0.63	0.01
TOC	r = 0.69	0.01	r = 0.44	0.03
OSI	r = 0.50	0.04	r = 0.49	0.02
Fetal weight	r = -0.52	0.22	r = -0.39	0.33
TAC: Total antioxidant capacity TOC: total oxidant status (TOC) OSI: TOC/TAC 8-OH-2dG: 8-Hydroxy-2-deoxyguanosine				

fertility, lesions located in the endometrium or myometrium may cause premature rupture of the membranes and initiate preterm labor. In the last decade, the relationship between genomic analysis of fetal membranes and preterm birth has begun to be investigated[6]. The role of DNA damage in preterm birth cases was investigated by genomic analysis of fetal membranes[15]. Nuclear DNA is a structure that contributes to cell membrane integrity apart from its genomic effect. Since the DNA content of erythrocytes is destroyed over time, their membrane structures are quite different from other cells. For these reasons, the emergence of DNA damage in the amnion and chorion cells that form the fetal membranes may lead to preterm premature rupture of the membranes.

By measuring the levels of 8-Hydroxy-2-deoxyguanosine, which is an indicator of DNA damage, we can have information about the integrity of the genomic structure[9,10]. 8-OH-2dG, which is an indicator of DNA damage, may be increased in the amniotic fluid of this patient group because preterm labor often presents with premature rupture of the membranes. In the light of these data, we measured the 8-OH-2dG levels in the amniotic fluid of patients who presented with preterm labor. As the control group, we included patients with full-term pregnancy. We found that amniotic fluid 8-OH-2dG levels in preterm delivery patients were approximately two times higher than in healthy term pregnancies. This finding is the first clinical evidence showing that DNA damage plays an important role in premature rupture of fetal membranes. In line with our results, changes in the genes expressed in the genome-wide expression analysis of second trimester and full-term fetal membranes are an indication of the change in fetal membrane gene profile in cases close to birth or in preterm births[16]. The change in fetal membrane gene profile may be possible by the destruction of existing genes by damage and replacement by new genes. Lim et al[7] reported that when labor begins, amniotic membranes activate the cell death and apoptosis gene network and initiate the synthesis of prostaglandin and proinflammatory cytokines.

Oxidative stress products and an increase in proinflammatory cytokines may be responsible for the basic mechanism that initiates DNA damage in fetal membranes[6,7]. Proinflammatory cytokines and ROS, which increase exponentially towards term, can induce structural disruptions in the DNA of fetal membranes. Labor starting at full-term and preterm labor are similar in terms of molecular mechanism. The only difference is that genomic and non-genomic changes in fetal membranes start earlier in patients with preterm birth. Changes in the mechanical dynamics of the myometrium, an increase in feto-maternal originating hormone secretion stimulates mitochondrial respiratory chain reactions, leading to the emergence of more ROS[8]. Increased ROS can cause DNA damage, leading to premature rupture of membranes. The increase in TOC, which is a marker of ROS increase in the preterm birth group, suggests that oxidative stress is responsible for DNA damage compared to the term group. Similarly, the higher OSI values in the preterm group compared to the full-term group supports that ROS is a product responsible for DNA damage and preterm labor. The significant decrease

in TAC, a component of the scavenging enzymes of ROS, in preterm women also suggests that membranes are unprotected against DNA damage. The positive correlation of amniotic fluid 8-OH-2dG levels with TOC and OSI in both term and preterm groups is an indicator of the role of oxidative stress in DNA damage. On the contrary, the negative correlation between TAC and amniotic fluid 8-OH-2dG levels is an indication that the antioxidant defense system is preventive against DNA damage.

Damage to adenine and cytosine bonds of DNA due to increased ROS and decreased TAC in the presence of oxidative stress may allow premature rupture of fetal membranes[12]. As a result of DNA damage due to ROS, 8-OH-2dG levels increase and this increase is reflected in the amniotic fluid. The fact that amniotic fluid 8-OH-2dG levels are twice as high in preterm cases compared to term cases (33.6±4.11 ng/mL *vs.* 33.6±4.11 ng/mL) is an important indicator that both ROS production and DNA damage increase significantly in preterm cases compared to term cases. The sudden and rapid increase in ROS and proinflammatory cytokines, which increase exponentially in physiological conditions during term pregnancy, may lead to premature rupture of membranes in preterm cases.

Conclusions

Despite the small number of participants, our study is the first clinical study to measure amniotic fluid 8-OH-2dG, TAC and TOC levels in both term and preterm patients. The increase in amniotic fluid 8-OH-2dG, which is a product of DNA damage in preterm birth, is significantly higher than in full-term cases. Considering our findings and literature data, we can say that oxidative stress causes premature rupture of fetal membranes by causing DNA damage.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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None.

Authors' Contribution
All authors contributed to the study conception and design. Material preparation was performed by Cevat Rifat Cundubey and Mustafa Bertan Demir. Data were collected by CRC and MBC. All authors contributed to statistical analysis. The first draft was written by CRC. All authors approved the final version of the manuscript.

Ethics Approval
All interventional procedures in this study were performed in accordance with both ethical and Helsinki Declaration standards, with the consent of the patients and the Ethics Committee of the Kayseri City Training and Research Hospital (Decision Number: 2023/771).

Informed Consent
Informed consent was obtained from all participants.

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