Experimental
&
Clinical Article

DNA Damage and Oxidative Status of Full Term Babies Delivered by Spontaneous Vaginal Delivery and Caesarean Section

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ABSTRACT

OBJECTIVES: At this study, it is aimed to research DNA damage and oxidative stress in infants with born timely normal spontaneous vaginal delivery and elective caesarean.

STUDY DESIGN: Healthy term babies born with normal spontaneous vaginal delivery (n=36) and elective caesarean section (n = 36) were included in the study. Determination of DNA damage was studied in fresh heparinized blood by the Comet Assay (mononuclear cell alkaline electrophoresis) method. Total oxidant capacity and total antioxidant capacity values were measured by using Erel method (colorimetric) on study day by autoanalysers and oxidative stress index values were calculated.

RESULTS: Mean total oxidant capacity, oxidative stress index and DNA damage values were significantly higher in babies born with normal spontaneous vaginal delivery compared to those born with elective cesarean section (p<0.001, p<0.001, p<0.001, respectively). Serum total antioxidant capacity values were not statistically significant (p=0.127).

CONCLUSION: In this study, oxidative stress and DNA damage values of babies born with normal spontaneous vaginal delivery were found to be higher than those born by elective cesarean section. This suggests that there may be a relationship between the mode of delivery and oxidative stress, and that increased oxidative stress may also lead to DNA damage.

Keywords: Delivery, DNA damage, Newborn, Oxidative stress

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Introduction

In biological systems, a molecule that gains an electron is called anti-oxidant or free radical. Reduction in anti-oxidants against oxidants and/or increase in oxidants can be defined as oxidative stress. Free radicals destroy the structure and function of the target molecules by taking an electron away (1,2). Increase in generation of reactive oxygen metabolites, decrease in anti-oxidant enzyme levels and/or defect DNA repair mechanisms lead to an increase in oxidative stress and ultimately a DNA damage (3,4). Changes in molecular integrity of genetic material caused by endogenic and exogenic factors are called DNA damage (5). The cell responds to DNA damage via various metabolic ways (6). Serious DNA damages activate the apoptosis mechanism of the cell and eventually kill it. The cell is able to repair this damage by "DNA repair mechanism". If the DNA damage remains unrepaired, it leads to mutations and genomic instability, malignancy, and aging.

Today, many researches are conducted in the fields of DNA damage and oxidative stress in the neonatal period. It is a known fact that DNA damage is correlated to various complications occurring in the neonatal period, such as prematurity, hypoxic injuries, and necrotizing enterocolitis (7-10). Babies born via normal spontaneous vaginal delivery (NSVD) are exposed to a gradually-increasing level of stress and they adapt to the situation. In this study, it is stated that the metabolisms of babies delivered by caesarean section (c-section) may not respond to stress as quickly, and/or DNA damage may occur due to anesthetic substances are given to the mother during delivery (11,12).

This study aims to compare the DNA damage and oxidative status of full-term babies delivered by c-section and NSVD and investigate the correlation further.

Material and Method

In this study 72 newborns, 36 delivered by NSVD and 36

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delivered by c-section under anesthesia, of mothers who were on pregnancy follow-up in 2013-2014 in obstetrics and gynecology clinic were studied. Mothers had no diseases, had similar socio-cultural statuses, aged 20-30, delivered less than three babies, and completed 38-42 weeks of gestation. Babies who had an Apgar score of 8 or higher, had no congenital anomalies, had no hypoxia in perinatal and natal periods, weighing 2.5-4 kilograms, and found to be completely healthy after a series of examinations and laboratory evaluations were included in this study. Approval of the local ethics committee was granted for this study in Turkey. Mothers of the studied babies were given information about the project and have signed a voluntary participation consent form. This study confirmed to the principles of the 2008 Declaration of Helsinki and was approved by the local ethics committee of Harran University, Medical Faculty, Turkey (Approval date and number: 06.02.2013, Session 13068).

Exclusion criteria

Babies with congenital anomalies, asphyxia during prenatal and neonatal periods and an Apgar score below 8 were excluded from this study. Mothers who didn't want to participate and who had chronic diseases, diabetes mellitus, high blood pressure, oligohydramnios, polyhydramnios, serious infections during pregnancy, anemia, and habit of smoking and alcohol consumption were also excluded.

Blood samples

Complete blood counts were performed on each baby via automatic hematology analyzer (Abbot Celldyn 3700 III, USA) at the beginning of the study. Umbilical venous blood samples (3 mL) drawn via heparin-cleaned tubes were processed to be studied via mono-nuclear leukocyte separation method. One milliliter of blood was pipetted into another tube immediately to measure DNA damage. Blood samples drawn for oxidants were centrifuged at 4000 rpm for five minutes for plasma separation. Part of the plasma samples above were preserved at -80 ^{o}C until the day of study. Electrolytes, kidney and liver function tests, were performed with a spectrophotometric chemistry analyzer (Architect C16000, Abbott Diagnostics, Abbott Park, IL) on the remaining plasma samples within the same day. Total antioxidant status (TAS) and total oxidant status (TOS) levels of the preserved (-80 ^{o}C) plasma samples were measured colorimetrically on auto-analyzer (Abbott Aeroset, Abbott Diagnostics, Abbott Park, IL, USA) with Erel method on the day of study.

Mono-nuclear leukocyte separation

Analysis of DNA damage: 1mL of fresh heparinized blood was added slowly on 1 mL of histopaque-1077 and centrifuged at 2100 rpm at 25 ^{o}C for 30 minutes for the separation of mono-nuclear leukocytes. Leukocytes collected in mid-level of the tube were taken with a pipette, mixed with 1ml phosphate buffered saline (pH=7.4) and centrifuged at 1600 rpm at 25 ^{o}C for 10 minutes. The supernatant above was discarded and the pellet was diluted with phosphate buffered saline (pH=7.4) to the point of 10^6 mononuclear leukocyte/µL. Slides were prepared as 1% normal melting point (NMP) agarose gel. 80 µL gel was dripped on slides with frosted sides. Slides were covered with coverglass, kept in refrigerator (at 4 ^{o}C) for five minutes and uncovered. Prepared slides were kept in moist boxes. Phosphate buffered saline (PBS) and 10 µL of diluted mononuclear cells, mm^3 consists 10^4 cells, were mixed with 80 µL 0.5% low melting point (LMP) agarose gel (37 ^{o}C). The mixture was put on the first layer, covered with coverglass again and left in the refrigerator to freeze for five minutes. At the third step, LMP agarose gel with same concentration as before was prepared and laid thinly on the second layer, and the preparation of slides was completed (13). The slides were left in cold lysis solution with a high concentration of salt and detergent (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris-HCl, pH10-10.5, 1% Triton X-100 and 10% dimethyl sulfoxide (DMSO) added just before use) for approximately one hour after the agarose gel was dry. Nuclear membrane was lysed with lysis buffer (13,14). Before electrophoresis, the slides were left to incubate in alkali electrophoresis buffer (0.3 mol/L NaOH and 1 mmol/L EDTA-2Na, pH >13) at 4 ^{o}C for 40 minutes for the separation of DNA strands. DNAs were later run in this buffer solution (25V/300 mA, 25 min) (13,14). The slides were washed with neutralization buffer (0.4 M Tris-HCI, pH 7.5) for three minutes to remove the alkali buffer solution after the electrophoresis. Fluorescent ethidium bromide dye (5 µg/mL) was used for dyeing (13,14). 80 µL dye was dripped on each slide and the slides were covered with coverglass. 50 out of 100 cells (50 cells from each slide) were randomly selected and visually examined with 20x magnification microscope (Nikon, Tokyo, Japan) (Excitation DB: 546 nm, Emission DB: 580 nm).

DNA migration was visually evaluated according to the fluorescent density in this method. DNA was evaluated in five categories for different levels of DNA damage. As seen in figure 1, no damage DNAs were qualified as 0 and maximum damage DNAs were qualified as 4 (13). According to this technique, scoring system was in between 0 and 400 arbitrary units (AU).

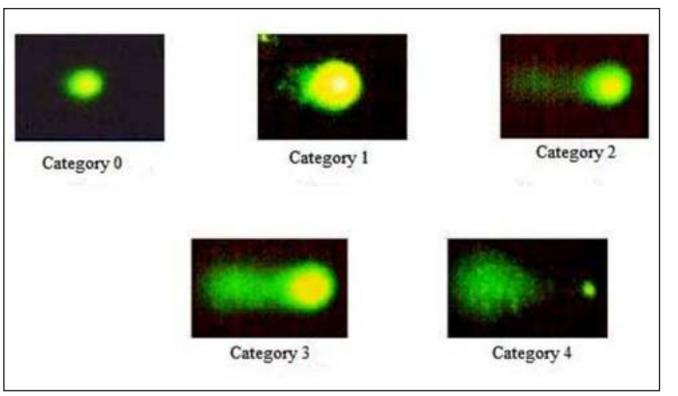

Figure 1: *Visual categories of DNA damage in fluorescent density*

Total anti-oxidant status measurement

TAS measurement was carried out via a fully automatic method (Rel Assay Diagnostics) developed by Erel. With this method, total anti-oxidant capacity of body is measured against strong free radicals (15). Antioxidative effect of the sample is measured against strong radical reactions started by the produced hydroxyl radical. Hydroxyl radical, the strongest radical, is produced through Fenton Reaction in this method. This method of measurement is based on the decolorization of colored radicals, proportional to the total concentration of anti-oxidant molecules, due to the reduction of colored 3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) cationic radical by all anti-oxidants in the samples. Trolox, a water soluble analog of Vitamin E, is used as calibrator. The results are given as mmol Trolox Eqiv/L.

Total oxidant status measurement

TOS measurement was carried out via a fully automatic method (Rel Assay Diagnostics) developed by Erel. As stated in the working principle of the test, colorimetric method was used, which is a method based on the cumulative oxidation of ferrous ion to ferric ion by the oxidant molecules in the samples (16). Color density, measured via spectrophotometry, was proportional to total oxidant molecule count. Hydrogen peroxide was used as calibrator and results are given as μmol H_2O_2 Eqiv/L.

Oxidative stress index measurement

Oxidative Stress Index (OSI) of the samples is the ratio percentage of TOS to TAS. (OSI (arbitrary unit)=TOS (μmolH_2O_2 equivalent/L)/TAS (μmol Trolox equivalent/L)*)* (11).

Statistical analysis

SPSS (Statistical Package for the Social Sciences, version 24 for Windows, SPSS Inc, Chicago, IL) was used for analysis. Distribution of parameters was found to be normal via one-sample Kolmogorov-Smirnov test. The results were given as Mean±standard deviation (SD). Independent Samples *t*-test and Pearson Chi-Square Test were used for inter-group parameter comparison. Pearson correlation analysis was performed to evaluate the correlation between parameters. *P* values smaller than 0.05 were accepted as statistically significant.

Results

Babies who participated in this study were as follows: babies delivered by NSVD were 50% (n=18) male and 50% (n=18) female; and babies delivered by elective c-section were 47.2% male (n=17) and 52.8% (n=19) female. The gender distribution characteristics of the groups were similar (Pearson chi-square test, p=0.814). The average body weight of babies delivered by NSVD was calculated to be 3243.3±160.7 gram; and the average body weight of babies delivered by elective c-section was calculated to be 3316.7±157.9 gram. The average height of babies delivered by NSVD was calculated as 49.7±1.2 cm; and the average height of babies delivered by elective c-section was calculated as 49.9±1.1 cm. Average head circumference of babies delivered by NSVD was calculated as 35.5±0.4 cm; and average head circumference of babies delivered by elective c-section was calculated as 35.4±0.4 cm. Average birth week of babies delivered by NSVD was calculated as 38.3±0.9 weeks; and the average birth week of babies delivered by elective c-section was calculated as 38.1±0.4 weeks. There weren't any statistically significant differences in Independent sample t test regarding body weight, head circumference, and birth week between the groups (p>0.05) (Table 1).

The average age of mothers who delivered by NSVD was calculated as 24.8 ± 2.7 years; and the average age of mothers who delivered by elective c-section was calculated as 25.3±2.7 years. The average pregnancy count of mothers who delivered by NSVD was calculated as 2±0.7 and average pregnancy count of mothers who delivered by elective c-section was calculated as 2±0.6. There weren't any statistically significant differences in Independent sample t test regarding the average age and pregnancy counts between the groups (p>0.05) (Table 1).

The average DNA damage of babies delivered by NSVD was calculated as 5.97±4.3 AU; and the average DNA damage of babies delivered by elective c-section was calculated as 1.11±1.19 AU. The average DNA damage of babies delivered by NSVD was significantly higher than the average DNA damage of babies delivered by selective c-section (p<0.001) (Table 2, Figure 2).

Table 1: *Sociodemographic characteristics of babies and mothers included in the study*

Parameters	NSVD group (n=36) (mean±SD)/(min-max)	C/S group (n=36) (mean±SD)/(min-max)	*p** value
Birth week	38.3±0.9/(38-41)	38.1±0.4(38-40)	0.530
Length (cm)	49.7±1.2/(48-52)	49.9±1.1/(48-52)	0.560
Weight (gr)	3243.3±160.7/(2.90-3.56)	3316.7±157.9/(3-3.64)	0.216
Maternal age (year)	24.8±2.7/(19-30)	25.3±2.7/(21-30)	0.418
Head circumference (cm)	35.5±0.5/(35-36)	35.4±0.4/(35-36)	0.680
Pregnancy count of mothers	2±0.7/(1-3)	2±0.6/(1-3)	0.980

*SD: Standart deviation, NSVD: Normal spontaneous vaginal delivery, C/S: Elective cesarean, *: Independent sample t-test*

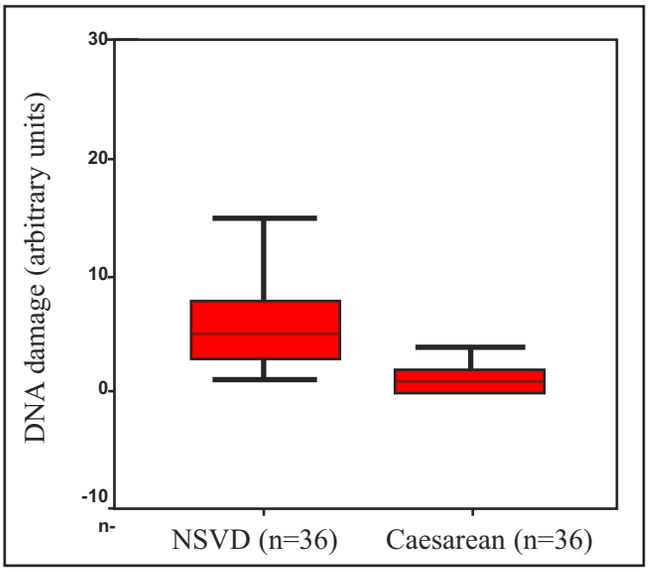

Figure 2: *Comparison of DNA damage between normal spontaneous vaginal delivery and cesarean groups.*

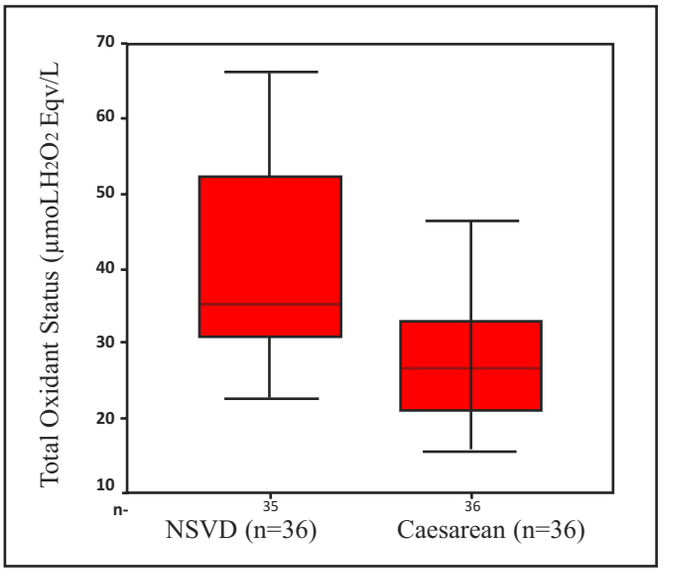

Figure 3: *Comparison of total oxidant status levels between normal spontaneous vaginal deliveries and cesarean groups.*

Average TAS value of babies delivered by NSVD was calculated as 1.22±0.2 mmol Trolox Eqv./L; and average TAS value of babies delivered by elective c-section was calculated as 1.15±0.18 mmol Trolox Eqv./L There weren't significant differences between the average TAS value of the groups (Independent sample *t* - test, *p*=0.127) (Table 2).

Average TOS value of babies delivered by NSVD was calculated as 40.55±13.16 µmol H_2O_2 Eqv. /L; and average TOS value of babies delivered by elective c-section was calculated as 28.27±8.95 µmol H_2O_2 Eqv./L. Average TOS value of babies delivered by NSVD was significantly higher than the average TOS value of babies delivered by selective c-section (Independent sample t test, *p*<0.001). (Table 2, Figure 3).

Average OSI value of babies delivered by NSVD was calculated as 3.38±1.02 AU; and average OSI value of babies delivered by elective c-section was calculated as 2.53±0.94 AU. Average OSI value of babies delivered by NSVD was significantly higher than the average OSI value of babies delivered by selective c-section (Independent sample *t*-test, *p*=0.001) (Table 2).

There weren't any significant correlations between DNA damage and TAS (Pearson correlation test, r=0.042, *p*=0.727). A weak, positive correlation was found between DNA damage and TOS (Pearson correlation test, r=0.292, *p*=0.02) and OSI (Pearson correlation test, r=0.301, *p*=0.017) values.

Discussion

The role free radicals play in tissue damages and etiopathogenesis of various diseases has recently been widely investigated (17). Free radicals disrupt the structure and function of the target molecules by taking an electron away (18). They are constantly produced by biological systems, and they can lead to lipid, protein or DNA oxidation in body (19). In this study, average oxidative stress level of babies delivered by NSVD was found to be higher than the average oxidative stress level of babies delivered by elective c-section. It is believed the results were as such because mother and baby were exposed to more stress due to the length of NSVD period, which is shorter in babies delivered by elective c-section.

In a study, TAS values of peripheral blood and postnatal cord blood of mothers, who were planned to deliver by c-section, were compared. The study showed no significant correlation between two samples (20). In another study comparing

Table 2: *Comparison of DNA damage, TAS, TOS, OSI values of infants born with NSVD and C/S section*

Parameters	NSVD group (n=36) (mean±SD)/(min-max	C/S group (n:36) (mean±SD)/(min-max)	*p** value
DNA damage (AU)	5.97±4.3/(1-20)	1.11±1.19/(0-4)	<0.001
TOS (µmoL H_2O_2 Eqv/L)	40.55±13.16/(22.5-66.27)	28.27±8.95/(15.47-56.59)	<0.001
TAS (mmoL Trolox Eqv/L)	1.22±0.2/(0.79-1.70)	1.15±0.18/(0.62-1.53)	=0.127
OSI (AU)	3.38±1.02/(1.34-5)	2.53±0.94/(1.30-5.33)	=0.001

*SD: Standart deviation, NSVD: Normal spontaneous vaginal delivery, C/S: Elective cesarean, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative Stress Index, *: Independent sample t test*

babies delivered by NSVD to babies delivered by elective c-section, there was no significant difference between the delivery method and TAS (21). In addition to this, there are studies showing that babies delivered by NSVD are exposed to less oxidative stress compared to babies delivered by c-section (11). Correlated with the study above, this study also showed no significant difference between TAS values of babies delivered by NSVD and babies delivered by elective c-section. Such results may indicate that time required for the production of anti-oxidants against oxidative stress may be more after the start of labor where the uterus muscles aren't involved in the delivery.

Increase in reactive oxygen molecules production; decrease in anti-oxidant enzyme levels, and/or defect DNA repair mechanisms increase the oxidative DNA damage (22-24). In a healthy body, DNA damage can be repaired because the oxidative and anti-oxidative balance is maintained (25). If the oxidative DNA damage reaches a level where it doesn't comply with life anymore, cell death (apoptosis) or genotoxic damages occur (25,26). In this study, the effect of birth method on newborn babies in terms of DNA damage was investigated. DNA damage in babies delivered by NSVD was found to be significantly higher than DNA damage in babies delivered by elective c-section. The results were opposite of what we expected. Such results may be due to the length of delivery period and uterus' active role in the delivery; factors that expose the baby to more stress. Nevertheless, it is a known fact that prenatal stress affects cortisol levels of cord blood during delivery (27). As a result of chosen birth method, when babies delivered by c-section are exposed to less stress, cortisol levels of their cord blood may change (28). Increased cortisol levels of cord blood affect the activities of baby positively in babies exposed to stress during NSVY, whereas its effects on DNA damage and oxidative stress were not clearly shown (27,28).

In conclusion DNA damage and oxidative stress levels of babies delivered by NSVD were found to be higher compared to babies delivered by elective c-section. Active participation of uterus muscles during delivery prolongs the duration of NSVD. This may have led to an increase in oxidative stress due to increased cord blood stress hormones levels of newborn and therefore may have caused DNA damage to occur. In addition to this; the effect of local and/or general anesthetic substances, which were used during delivery, on cord blood stress hormones was partially clarified. Nevertheless, how they affect the occurrence of DNA damage and oxidative stress was not completely brought to light yet (12,29). In light of the findings of the study, it can be concluded that c-section delivery does not cause DNA damage and oxidative stress in infants.

: All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work. (Conception-Halil Kazanasmaz, design-Mahmut Abuhandan, analysis and interpretation of data-Halil Kazanasmaz)

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