SALIVARY, PLASMA AND CORD BLOOD OXIDATIVE STRESS BIOMARKERS IN MOTHER AND NEONATE: A COMBINED ANALGESIA CONCERN

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ABSTRACT

Objective: To measure oxidative stress biomarkers in saliva and venous blood of mothers and cord blood of newborns delivered wih and without combined analgesia.

Methodology: In this analytical cross-sectional study, carried out in 2015. 68 parturient mothers and newborns were recruited in random to two groups in Fatemieh Teaching Hospital, Hamadan City, Iran. Thirty four of them were delivered via normal vaginal delivery (NVD) and 34 delivered through combined analgesia (CA). This study was designed to measure total antioxidant capacity (TAC), Total thiol molecules (TTM) and catalase activity (CAT), in blood and saliva of mothers at the second stage of labor and cord blood of newborns delivered through these different delivery modes.

Results: No significant difference could be observed in the mean of first and third labor stages in CA and NVD groups, but the difference was significant during the second stage in CA and NVD groups, respectively. No statistically significant difference was noticed between the means of oxidative stress parameters (TTM, TAC and CAT) in plasma, saliva and umbilical cord samples in two groups (P> 0.05). A significant positive correlation existed between (plasma and umbilical cord TAC) and (plasma and saliva CAT). There was no significant relationship between newborn birth weight and oxidative stress parameters in two groups.

Conclusion: Markers of oxidative stress does not seem to have a major role in the delivery with combined analgesia.

Key Words: Oxidative stress, Combined analgesia, Labor, Newborn

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INTRODUCTION

Oxidative stress is believed to appear in seemingly normal pregnancy, even with lack of complications[1]. Exposure to noticeable oxidative stress in the intrauterine life has been deemed to lead to intrauterine fetal death[2-4] and poor perinatal outcome[5]. There is an increased risk of birth asphyxia for fetuses which are exposed to oxidative stresses in the intrauterine life; this may get worse by labor and stress of delivery. Depending on the pregnancy conditions, from conception through labor, babies are delivered either vaginally or via caesarean delivery. Each of which has its own effects on both the baby and the mother[6].

Oxidative stress is an imbalance between pro-oxidant and antioxidant forces which results in an overall pro-oxidant insult. Reactants derived from oxygen-, collectively called reactive oxygen species (ROS), are normal byproducts of cellular metabolism; per se, cells also include a natural antioxidant defense system[1,2]. The body has endogenous antioxidants as in glutathione peroxidase, superoxide dismutase and bilirubin; however the total antioxidant capacity is specified by the exogenous molecules like vitamin C and vitamin E. The metabolic activation of molecular oxygen often increases ROS; but, when the antioxidant protective capacity level has been transgressed, as these products stimulate lipid peroxidation and protein inactivation[3], they can become toxic to most cellular components. The ensued damage is called oxidative stress.

Oxidative stress can be assessed in vivo using the plasma biomarkers malondialdehyde and protein carbonyl. Lipid peroxide and antioxidant levels are elevated in women with normal, uncomplicated pregnancies as compared to non-pregnant women; the rise is proportional to gestational length[4]. A major source of oxidative stress during pregnancy is placenta. The placenta is rich in polyunsaturated fatty acids, and it is a rich source of lipid peroxides, secreted into the maternal circulation. Placental lipid production is deemed to be kept under control by placental antioxidant enzymes in normal pregnancy due to various factors such as pain, fear, anxiety, and hypoxia[1]. Labor is a very stressful process for mothers and their babies[2]. Additionally, these factors lead to notable oxidative stress and high production of free radicals involved in the pathogenesis of diverse diseases. Pregnancy is a state of oxidative stress resulting from aggregated placental mitochondrial activity and the production of ROS, mostly superoxide anion. The placenta produces other ROS too, such as carbon monoxide, nitric oxide and peroxynitrite which have conspicuous effects on placental function containing trophoblast proliferation and differentiation and vascular reactivity[3].

The lipid peroxidation is significantly higher in the delivering women than pregnant women. Also, there was a positive correlation between lipid peroxidation of labor and thiol groups and total antioxidant capacity in women with their newborns[3,7].

Considering the labor stress, as the fetus navigates the birth canal, it can be assumed that free radical may be generated more in women and babies delivered through normal vaginal delivery than those delivered by combined analgesia before vaginal delivery. However, what may lead to emergency caesarean section may be associated with the generation of more free radical and an enhanced consumption of antioxidants. If the indication does not relate to oxidative stress injury, babies delivered through elective caesarean section may be most free of this injury[4].

An efficient and popular way to relieve the pain of labor is epidural analgesia; but it may interfere with normal mechanism of labor[5]. Since the introduction of combined spinal–epidural analgesia about forty years ago for labor pain relief, its effect on the labor process has been an issue of controversy. Hence, substantial research has been performed and findings have caused changes in practice.

It has been claimed that combined analgesia leads to a prolonged labor; increased oxytocin requirements, instrumental and operative delivery rates, maternal pyrexia and postpartum backache. Meanwhile, there is growing evidence which rejects some of these claims. Despite persistent controversies, combined analgesia rates have increased; 66% of women in the USA and 24% of them in the UK receive epidural analgesia in labor.

Compared with non-epidural analgesia, combined analgesia has consistently been proved to provide superior analgesia for labor pain, though this is not always associated with greater maternal satisfaction. Maternal satisfaction is a significant measure, however it is influenced by many other factors, such as labor outcome, interaction with and support of the staff and pain control rather than its amelioration[6,8-10].

No consistent difference has been noted in neonatal arterial pH or Apgar scores in babies born to mothers with epidurals. Some benefits for the neonate have been reported, such as a reduction in the incidence of low Apgar scores at 5 min and in the demand for naloxone [4].

This study was designed to measure TAC, TTM and KAT in Blood and saliva of mother at the second stage of labor and cord blood of newborns delivered through these different delivery modes.

METHODOLOGY

This was an analytical cross-sectional study, performed in Fatemieh teaching hospital, Hamadan city, Iran, 2015. Subjects were randomly assigned to labor wards and painless delivery wards of this institution. Sixty eight women and new born babies were included in the study, thirty four of whom were delivered via normal vaginal delivery and another thirty four through combined analgesia.

All pregnant women were not known hypertensive; diabetic and pre-eclamptic. Newborns of with clear congenital malformations were excluded. Written consent form was taken from women before collection of saliva, umbilical cord blood and venous blood. The study protocol was approved by the Ethics Committee of the Hamadan University of Medical Sciences, Hamadan, Iran, and it was conducted according to the Declaration of Helsinki, Good Clinical Practice Guidelines. Five ml venous blood and saliva were taken at the labor second stage and 5 ml of newborn cord blood was drawn immediately after delivery in two groups.

Venous and umbilical cord blood were distributed into specimen bottles which contained lithium heparin. However, after centrifuging the sample at 3000 g for 15 minutes, the supernatant (plasma) was extracted into plain specimen bottle. Then, until laboratory analysis, the plasma was kept frozen.

The subjects' unstimulated whole expectorated saliva was gathered into sterile tubes, a single mouth rinse with 15.0 ml of distilled water was performed[11], then they were centrifuged at 3000 rpm and the supernatant

for 15 minutes; the samples were reserved at - 80°C until oxidative stress biomarkers analysis.

This plasma and saliva was applied for measuring the total antioxidant capacity (TAC), total thiol molecules (TTM) and catalase activity (CAT) in the laboratory.

Reagents and Chemicals used in this study were 2 thionitrobenzoic acid (DTNB), ethylenediamine tetracetic acid (EDTA), 2,4,6-tripyridyl-s-triazine (TPTZ) and peroxide hydrogen(H_2O_2). All other chemicals were obtained from the Sigma. Oxidative stress biomarkers in this study were measured as protein oxidation with total thiol group assay, total antioxidant capacity and enzyme antioxidant activities such as catalase assay.

In order to assay catalase activity in the samples, the absorbance reduction at 240 nm in a reaction medium containing H2O2 (10 mM), sodium phosphate buffer (50 mM, pH=7.0) was measured. One unit of the enzyme is 1mol H2O2 as substrate consumed/min, and the specific activity is related to units/ml plasma[13].

Ferric reducing ability of plasma (FRAP) method was used to measure assay of total antioxidant activity (TAC). FRAP is on the basis of the ability of plasma in reducing Fe3+ to Fe2+ in the presence of TPTZ. The result of the reaction of Fe2+ and TPTZ is a complex with blue color and maximum absorbance in 593 nm[4,13].

DTNB was used as a reagent to evaluate the plasma total thiol molecules. It reacts with thiol molecules and produces a yellow complex with good absorbance at 412 nm in spectrophotometer[14,15].

Continuous and categorical variables were displayed as mean and standard deviation values and percentages, respectively. To analyze the differences between variables, regression, student's t-test and chi square test were applied. All data analyses were done via SPSS Version 16.0. P-value <0.05 was regarded as significant. Trial registration code of project was IRCT201502266888N7.

RESULTS

Sixty eight subjects were recruited. As shown in Table 1, the two parturient groups with CA and NVD were comparable in terms of baseline characteristics including age, education, active and passive smoking, exercise activity, mean of parity and gestational age and history of disease. They were similarly observed between both groups (P> 0.05).

The duration of labor and Apgar score of study population are demonstrated in table 2. There was no significant difference in the mean of first (108.1±67.7, 153.8±118.0) and third stages of labor (8.8±5.2, 7.3±4.2) in CA and NVD groups, respectively, but the difference was significant during first stage of labor (83.1±64.3 , 21.5±14.2) in CA and NVD groups, respectively (P=0.001). Mean Apgar score at 1st minute decreased significantly in CA group (P=0.001), while no significant difference existed in the mean of Apgar score at 5th minute (9.6±0.2 and 9.4±0.2) in CA and NVD groups, respectively (p= 0.10).

Table 3 is showning the mean ±SD (95% confidence interval) of oxidative stress parameters in plasma, saliva and umbilical cord samples of the two groups. No statistically significant difference could be noted between the means of oxidative stress parameters (TTM, TAC and CAT) in plasma, saliva and umbilical cord samples in CA and NVD groups (P >0.05). However, a trend was perceived in these parameters. Mean salivary biomarkers were highest than plasma in two groups.

Mean saliva and plasma TTM status, µmol /ml^{-1} showed an increase in mothers in the NVD group at second stage of delivery. Mean saliva and plasma TAC was noticed to be highest in subjects delivered through CA, whereas the lowest was found in subjects delivered through NVD.

A significant positive correlation existed between plasma and umbilical cord TAC (p=0.01). Additionally a significant positive correlation between plasma and saliva CAT was noted (p=0.01) (Table 4). There was no significant relationship between newborn birth weight and oxidative stress parameters in two groups (P>0.05) (Table 5).

DISCUSSION

The present results demonstrated that women delivered through CA have decreased TTM molecules, TAC and increased CAT activity relative to women in the NVD group. There was no significant change in the oxidative stress biomarkers. The present information well indicates that in pregnancy there is decrease in body antioxidant defense and induction of oxidative stress.

Normal pregnancy has been accompanied with oxidative stress injury. Oxidative stress has been associated with poor perinatal outcome and birth asphyxia. The severity of this oxidative stress in mother and newborn may be related to different delivery mode. The present study demonstrated that, no significant relationship exists between oxidative stress markers in saliva and plasma of mothers and umbilical cord blood of neonates in delivery with combined analgesia[16,17].

In biology and medicine, oxidative stress occurs when generation of free radicals (oxidants) overwhelm the available antioxidants[18]. Additionally, it could be due to insufficient antioxidants in the system. This happens even with a minimal generation of free radicals. The majority of these free radicals as in the group reactive oxygen species have a damaging influence on cellular organelles such as polyunsaturated membrane lipids[16].

Table 1: Baseline characteristics and clinical data of study population

Characteristics	Vaginal Delivery with Combined Analgesia (n=34)	Normal Vaginal Delivery (n=34)	P-value
Age (yrs), Mean (SD)	26.85± (6.13)	25.91± (6.14)	0.20
Education (%)			
Primary school	23 (67.7)	27(79.4)	0.60
Under graduate	5 (14.7)	4(11.8)	
Post graduate	6 (17.6)	3(8.8)	
Employed (%)			
Yes	1 (2.9)	-	0.20
No	33 (97.1)	34 (100)	
Active Smoking (%)			
Yes	1 (2.9)	1 (2.9)	0.30
No	33 (97.1)	33 (97.1)	
Passive Smoking (%)			
Yes	3(8.8)	8(23.5)	0.50
No	31 (91.2)	26(76.5)	
Exercise			
Yes	20 (58.8)	17(50.0)	0.30
No	14 (41.2)	17(50.0)	
History of Disease (%)			
Yes	7(20.6)	8(23.5)	0.50
No	27 (79.4)	26(76.5)	
Parity, Mean (SD)	1.8± (1.0)	1.6± (1.1)	0.30
Gestational Age (wks), Mean(SD)	39.0 ± (1.4)	39.1± (1.6)	0.2

Table 2: The Effect of combined analgesia on labor duration and Apgar score of study population

Characteristics	Group	Mean± (SD)	P-value
First stage of labor (minute)	1	108.1 ± (67.7)	0.05
	2	153.8±(118.0)	
Second stage of labor (minute)	1	83.1±(64.3)	0.001
	2	21.5±(14.2)	
Third stage of labor (minute)	1	8.8±(5.2)	0.20
	2	7.3±(4.2)	
Newborn Weight (grs)	1	3370±(470)	0.40
	2	3270±(480)	
Apgar score (1st minute)	1	7.82±(1.4)	0.001
	2	8.8±(0.54)	
Apgar score (5th minute)	1	9.6±(0.20)	0. 10
	2	9.4±(0.20)	

Group 1: Vaginal delivery + combined analgesia; Group 2: Normal vaginal delivery

Table 3: Oxidative stress parameters in plasma, saliva and umbilical cord samples of study population

Biomarker		Group	Mean± SD	P- Value
TTM (µmol/ ml-1)	Plasma	1	0.3788±0.1706	0.10
		2	0.7054±1.1301	
	Saliva	1	1.2907±0.6150	0.30
		2	1.5684±1.6325	
	Umbilical Cord	1	0.9602±1.6485	0/50
		2	0.3815±0.1440	
TAC (nmol/ ml-1)	Plasma	1	0.563±0.2117	0.50
		2	0.5925±0.1822	
	Saliva	1	2.4339±1.3027	0.30
		2	2.1216±1.1907	
	Umbilical Cord	1	0.6833±0.2616	0.40
		2	0.7291±0.2042	
CAT (U/ ml-1)	Plasma	1	0.2690±0.3885	0.52
		2	0.2190±0.2303	
	Saliva	1	1.41±24.75	0.10
		2	7.9418±9.0144	
	Umbilical Cord	1	1.017±3.656	0.20
		2	0.3124±0.2190	

Group 1: Vaginal delivery +Combined analgesia; Group 2: Normal vaginal delivery

Table 4: Regression analysis of oxidative stress parameters in plasma, saliva and umbilical cord samples of study population

Biomarker	Sample	r	P-value
TTM (µmol/ ml-1)	Plasma	-0.06	0.6
	Saliva		
	Plasma	-0.01	0.9
	Umbilical cord		
	Saliva	0.005	0.9
	Umbilical cord		
TAC (nmol/ ml-1)	Plasma	-0.02	0.8
	Saliva		
	Plasma	0.33	0.01
	Umbilical cord		
	Saliva	0.14	0.2
	Umbilical cord		
CAT (U/ ml-1)	Plasma	0.3	0.01
	Saliva		
	Plasma	-0.08	0.5
	Umbilical cord		
	Saliva	-0.08	0.5
	Umbilical cord		

Table 5: Regression analysis of oxidative stress parameters in plasma, saliva and umbilical cord samples on newborn weight

Biomarker	r	P-value
TTM (Plasma)	-0.09	0.4
TTM (Saliva)	-0.05	0.6
TTM (Umbilical Cord)	0.1	0.2
TAC (Plasma)	0.2	0. 8
TAC (Saliva)	-0.003	0.9
TAC (Umbilical Cord)	-0.04	0.7
CAT (Plasma)	0.7	0.5
CAT (Saliva)	0.19	0.1
CAT (Umbilical Cord)	0.15	0.2

Therefore, measurement of biomarkers of oxidative stress, from any biologic sample is utilized[19].

This study showed that no significant noted between the means of oxidative stress parameters (TTM, TAC and CAT) in plasma, saliva and umbilical cord samples among the studied groups. However, a tendency of changes was observed (statistically not significant) in these parameters. This means that free radical generation in mothers and babies have equal significance despite passing through various stressful conditions, by their different modes of delivery. However, the change trend in plasma MDA levels among the study groups, though significant, may predict free generation to be most in babies delivered through spontaneous vaginal delivery and lowest in babies delivered through emergency cesarean section[16].

Another study has demonstrated that increased placental lipid peroxidation and decreased levels of antioxidants may have an important role in the pathogenesis of preeclampsia. These findings point to the involvement of oxidative stress markers in pre-eclamptic patients[20]. Umbilical cord blood stress-associated hormone/oxidative stress markers highly reflect maternal and neonatal conditions at the delivery time[21]. As the infection of *Chlamydia trachomatis* increases oxidative stress, it could be a significant factor in pathogenesis of threatened abortion and preterm delivery[22-26].

Also, a study similar to the present trial was conducted by D,souza et al[11] who evaluated the levels of salivary and serum IMA and IMA: albumin ratio (IMAR) in preeclampsia and its severity. This study observed the involvement of oxidative stress in the pathogenesis of preeclampsia, reflected in serum and saliva. Salivary Ischemia modified albumin could be a better marker for early preeclampsia prediction.

Although some controlled trials could demonstrate alteration in the oxidant/antioxidant balance in saliva during pregnancy. This study has shown variation in the oxidant/antioxidant balance in saliva during pregnancy and after birth, which may be influenced by periodontal health status in the latter case. Whether this is associated with adverse pregnancy outcomes, or not, remains to be clarified. Early recognition of ROS markers in saliva may have clinical value in the periodontal management of pregnant women[27]. Elevated oxidative stress and impairment of anti-oxidative defense mechanisms may lead to disease processes both in preeclampsia and IUGR[20].

The malondialdehyde results are in line with other studies of this marker and could be construed as showing increased oxidative stress associated with prematurity and labour. However, lower protein carbonyls in pre-term infants would end in an opposite interpretation. Before drawing strong conclusions on how they reflect oxidative stress in this and other clinical situations additional information is required on the source and fate of these and other biomarkers[28].

CONCLUSION

There was no statistically significant difference noted between the means of oxidative stress parameters (TTM, TAC and CAT) in plasma, saliva and umbilical cord samples in CA and NVD groups. Function of oxidative stress markers does not seem to have a major role in the delivery with combined analgesia, and can be used combined analgesia in labor and delivery. Detection of oxidative stress parameters in saliva could be easy and its recognition can help better management of mothers exposed to higher oxidative stress during delivery.

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CONTRIBUTORS

FS conceived the idea, planned the study, and drafted the manuscript. AR, FGA and MN helped acquisition of data and did statistical analysis. All authors contributed significantly to the submitted manuscript.