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ComparisonofMalondialdehyde Level in the Cord Blood of Newborn Infants of vaginal and cesarean deliveries

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ABSTRACT

The oxidative stress injury that has been linked to poor perinatal outcome and birth asphyxia may be found even with normal pregnancy, and it is severity in a newborn may be related to modes of delivery for which our study aimed to identify. Furthermore, this study was aimed to study the effect of both related- maternal, and related neonatal characteristics on baby's oxidative stress marker level (Malondialdehyde). Fifty newborn children were selected in both labor ward and operating theater of Al-Sadder Teaching Hospital, Misan, Iraq. They were divided into two groups. The first group comprised 28 newborns, who were born by a vaginal delivery; the second group consisted of 22 newborns who delivered by elective cesarean section. The laboratory measurement of levels of an important antioxidant factor [malondialdehyde (MDA)] in baby's cord blood has been extracted and used as an indicator of stress. We compared the two samples of different malondialdehyde levels in relation to variables as the delivery type, some maternal, fetal and neonatal characteristics. The results of this study revealed that MDA level was higher among neonates delivered through Elective Cesareans Section than those delivered through Vaginal Delivery with significant statistical value (*p>0.0001).* The previous delivery mood has a statistically significant value of (*p> 0.02*) among mothers who had no previous deliveries. While there were no significant statistical values regarding maternal characteristics as (ages, parity, residency, antenatal care, history of previous abortion, and body mass index) as well as fetal and neonatal characteristics as (sex, birth weight, fetal presentation, and Apgar score) and high MDA level. The current study was concluded that babies delivered by Elective Cesarean Section had been exposed to more oxidative stress compared to the normal Vaginal Deliveries especially for the Primigravidas.

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INTRODUCTION

For optimal growth of fetuses, the pregnant women have high-energy demand to achieve appropriate metabolic functions throughout pregnancy. Therefore, they need a lot of oxygen (Kinalski *et al.*, 2001). Frequently, the molecular oxygen metabolic acti-

vation gives rise to reactive oxygen species (ROS) like free radicals Yaacobi *et al.* (1999). Under normal conditions, these (ROS) usually cleared from the cell by the action of antioxidants (Yaacobi *et al.*, 1999) (that is a molecule, which can inhibit the oxidation of other molecules, thus protecting the body from the free radicals damaging effect (Yaacobi *et al.*, 1999). Most living organisms have developed well-integrated antioxidant defenses to scavenge free radicals. These mechanisms include enzymes, e.g., superoxide dismutases (SODs), catalase (CAT), glutathione peroxidases (GPs), and molecules, e.g. glutathione (GSH), vitamins C and E, and betacarotene (Gyurkovits *et al.*, 2013), free oxygen radicals are neutralized by the antioxidant system, and a balance is maintained. When this balance is impaired, however, tissue damage may result. Malondialdehyde (MDA) is one of the reactive metabolic products resulting from the effect of free oxygen radicals on tissues and from a series of reactions during lipid peroxidation. (Gülbayzar *et al.*, 2011a) MDA is a widely used indicator of oxidative stress, bearing in mind the stress of labor as the fetus navigates the birth canal, an assumption can be made that free radical may be generated more in women and babies delivered through spontaneous vertex delivery (SVD) than those delivered by cesarean section (CS), especially planned CS (Adekanle *et al.*, 2013). Babies delivered through elective cesarean section (ECS) if the indicator does not relate to oxidative stress injury may be freest of this injury (Gülbayzar *et al.*, 2011a). The oxidative stress has a role in the normal development of the placenta as well as in the complications such as pathophysiology of miscarriage, preeclampsia, intrauterine growth restriction (IUGR), and premature rupture of the membranes (Hracsko *et al.*, 2008; Gülbayzar *et al.*, 2011a; Mert *et al.*, 2012; Kressig *et al.*, 2008).

Babies delivered either vaginally or through cesarean delivery, depending on the circumstances surrounding the pregnancy from conception through labor (Penn and Ghaem-Maghami, 2001). Each of these modes of delivery has its own effects on both the baby and the mother. During spontaneous vaginal delivery labor, because of the repeated uterine contractions leading to ischemia, the oxidative stress increases several folds; this is followed by reperfusion, resulting in increased ROS production (Penn and Ghaem-Maghami, 2001). The resultant stress is influenced by neural and hormonal factors also by anxiety, pain, fear, and labor duration (Alehagen *et al.*, 2005). Moreover, throughout intrauterine life fetuses might exposed to oxidative stresses leading to increased risk of perinatal asphyxia and hypoxic-ischemic encephalopathy, as well risk of bronchopulmonary dysplasia, retinopathy, necrotizing enterocolitis, and intraventricular hemorrhage, & complications risk of pregnancy like preterm labor, preeclampsia, fetal growth restriction, and miscarriage (Myatt and Cui, 2004; Burton and Jauniaux, 2004; Mondal *et al.*, 2010; Jauniaux *et al.*, 2006). Supplementation of antioxidants like vitamins (A, C and E), folic acid, selenium, and flavonoids could be an effective option to oxidative stress prevention Diplock (1991). As some authors claimed, CS is advantageous in order to avoid oxidative stress. Therefore, we expect more increasing oxidative stress levels during vaginal delivery as compared to a planned cesarean section, while some authors declared that CS might cause a deficiency of antioxidant defense in the human newborn (Adekanle *et al.*, 2013), which may increase the risk of fetal stresses, and poor outcome. Hence, we aimed to study and compare MDA levels in baby's cord blood immediately after delivery (as a stress marker) of both NVD and Elective C\S deliveries in responding to some maternal related and newborn-related factors, which may increase these levels, to demonstrate which delivery type is less stressful. So, this study was conducted to evaluate the effect of delivery mode (Vaginal versus Elective Cesarean Section) on the neonatal oxidant system via analysis of umbilical's cord blood MDA level as a stress marker.

MATERIALS AND METHODS

Overall, fifty women were recruited randomly in both labor room and operating theater of this institution between 38 and 42 weeks of gestation. The samples were divided into two groups, including vaginal delivery (n=27) and non-emergency cesarean section delivery under spinal anesthesia (n=23). Babies delivered by both groups were analyzed for MAD levels in their Umbilical cord blood samples.

A special form had been designated to collect data about the following variables:

Vaginal delivery, elective cesarean section (CS). For the category delivered by SC, planned cesarean delivery was chosen for women with breech presentation or previous CS delivery or for maternal desire. Maternal age, residence, occupation, BMI, number of abortions, gravidity, parity, gestational age, mode of the previous deliveries, chronic diseases, pregnancy complications, antenatal care.

The study was excluded Mothers who delivered via emergency CS, surgery after prolonged labor, or had gestational problems such as oligohydramnios, eclampsia/preeclampsia, diabetes mellitus, or

preterm labor might have increased levels of oxidative stress due to reasons beyond the mode of delivery and thus were excluded. The sampling from the umbilical vein was provided immediately after birth while the placenta is still in situ. Four Howard Kelly forceps were placed on the cord to isolate a 20cm segment in the middle. Cut between the two sets of clamps so that the isolated segment is independent, and both the baby and the placenta still have a clamp in place. 3 ml of cord blood was collected 1ml into EDTA tube & 2ml in a serum separating tube. The sample was, however, centrifuged at 3000 g for 10 minutes, and the supernatant (plasma) was extracted into the plain specimen bottle. The plasma was, therefore, kept frozen until laboratory analysis.

Lipid peroxidation is determined by using the thiobarbituric acid method. In this method, Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids were identified as the product of LPO that react with thiobarbituric acid (TBA), in coexisting trichloroacetic acid (TCA), to give a pink chromophore absorbing at 535 nm. MDA concentrations were calculated, using the molar extinction coefficient of MDA (MDA ε) & equal to 1.56 x10^5 mol^{-1}. Cm^{-1} Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction. The concentration of MDA calculated as follows:

The data were entered, compiled, tabulated & Excel 2013, and SPSS was used. The data were presented in tables. Significance level was sought by performing a Chi-square test.

RESULTS AND DISCUSSION

In this study, Out of 50 cases, 28 delivered vaginally constitute (56%) of the total cases, the mean plasma MDA level was (3.8±1.17 mol/l); the elevated level was found in 11 cases (39%). While the 22 delivered by elective cesarean section constitute (44%) of total cases, the mean level was (5.36±1.2 mol/l), the elevated level seen in 20 cases (90.9%). Plasma level was found to be higher in subject delivered through ECS (5.36±1.2 mol/l) than those delivered through VD (3.8±1.17 mol/l) these differences in mean plasma MAD levels were statistically significant (*p>0.0001)*. Shown in Table 1.

In our study, the mean age of women delivered vaginally was 23.5±5.7 y and of those delivered by ECS was (28±4.5y) which is apparently higher. Moreover, they found to have higher mean MDA level. (Figure 1). The MDA level found to be elevated in 57% of mothers aged >20y, 60% of those aged 20-30y, 75% of those aged 30-40y and none of those aged < 40y. However, no statistically significant value. (Table 2)

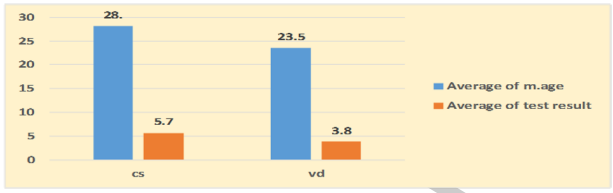

Figure 1: MDA distribution according to maternal age

In addition, the study shows no statistically significant difference found between the NVD and elective C/S group regarding maternal parity. However, the level found to be elevated more in those with parity of >5. (Table 2)

Furthermore, this study showed that nine mothers were living in a rural area, (66.7%) of W had elevated level of MDA. While (61%) of those living in the urban area had an elevated level, with no statistical significance values identified for living in a rural or urban area. The mean level found to be the highest in those who live in rural areas and delivered by CS, and lowest in those living in the urban area deliver by VD. (Table 2), (Figure 2)

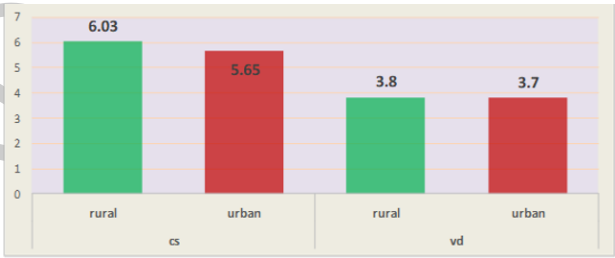

Figure 2: MDA distribution according to maternal residency

The average gestational age for those delivered vaginally was 39±0.37wks. While for those delivered by ECS was 40.2±0.86. The level was higher (83% had elevated MDA) for GA of 41-42 weeks compared to GA of 38-40 weeks, but it was not statistically significant. (Table 2)

No statistically significant difference was found between the NVD, elective C/S groups regarding maternal BMI. However, the mean level found to be elevated more in mothers who were thin (71.4%). (Table 2)

Antenatal care was good in 36 cases 21 of them (58.4%) had an elevated MDA level while was poor in 14 cases, 10 of them (71.4%) had an elevated level. However, no statistical significance identified. (Table 2). Regarding the history of abortion, this study found that about 7 mothers had a history of <

Table 1: MDA distribution according to the delivery type

		Normal		Elevated		MDA Total		Mean level (SD)	P value
		no	%	no	%	no	%	mmol/l	
Delivery type	CS	2	9.1	20	90.9	22	44	5.36 (1.2)	0.0001 Sig.
	NVD	17	60.7	11	39	28	56	3.8 (1.17)	

2 abortions (71.5%) of them had elevated MDA level. However, no statistical significance value was identified. (Table 2)

MDA level was elevated in (92.3%) of those who previously had delivered by CS, while only elevated in (53.6%) in those delivered previously vaginally, and elevated in (44.5%) of mothers with no previous deliveries and this was found to be statistically significant ($p > 0.02$). (Table 2).

Regarding fetal and neonatal characteristics MDA distribution: the present study showed that 29 of the studied neonates were male babies, (55.2%) of them had elevated MDA, 12 of distressed males delivered by CS, while 21 of studied babies were females, (71.4%) of them had elevated MDA (10), so the percentage was higher in females delivered by CS, but that was not statistically significant. (Table 3) The highest mean MDA was found in females delivered by CS. (Figure 3)

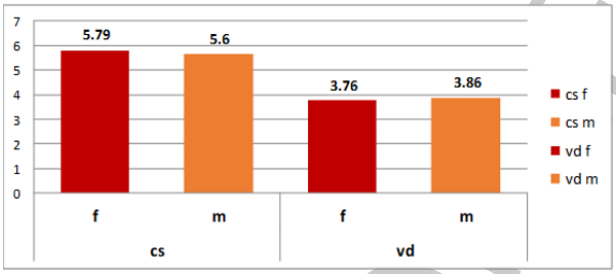

Figure 3: MDA distribution by sex of the baby

In our study, four of the babies had breech presentation all delivered by CS, 75% of them have high MDA level, while 60.9% of those with cephalic presentation had high MDA level. However, with no statistically significant association. (Table 3)

Regarding the babies birth weight, 70% of those weighted < 3.5kg. 61.5% of those weighted 2.5-3.5kg. 50% of those weighted > 2.5kg. Had an elevated MDA level and it was not statistically significant. (Table 3)

Comparing babies of vaginal delivery with those of CS delivery regarding ANC and body weight at birth, it is found that the lowest mean level observed among those who delivered vaginally with good antenatal care and their birth weight was > 3kg. (Figure 4)

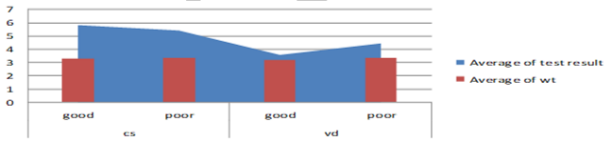

Figure 4: Distribution of MDA according to ANC and babies' average birth weigh

Regarding Apgar score: at (1) min. (Apgar score mean 1 min). Apgar score was 8.6 of those who delivered vaginally and 8.9 of those delivered by CS. However, no statistical significance identified, while at (5) min. Apgar score: the mean 5 min. Apgar score was 9 of those who delivered vaginally and 9.2 of those delivered by CS. Of those who had 4-6, Apgar scores, 25% had elevated MDA and 65.2% of those who had ≥7 Apgar score. However, no statistical significance identified. (Table 3, Figure 5). The lowest mean MDA level was observed in females delivered vaginally with good ANC and mean Apgar score of 9.5. However, in general, the highest mean MDA observed in those who have a higher Apgar score.

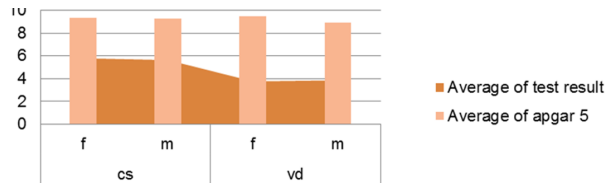

Figure 5: Distribution of MDA according to sex and 5 min Apgar score of babies

Most of the free radical's species can damage cellular organelles like polyunsaturated membrane lipids (Gülbayzar *et al.*, 2011a). As one of the intermediate products of these dangerous reactions is Malondialdehyde (MDA) which produces from Free radical attack on membrane lipid (Gülbayzar

Table 2: MDA distribution according to maternal characteristics

		MDA						
		Normal		elevated		Total		P-value
		no	%	no	%	no	%	
M. age	<20	3	42.85	4	57.15	7	22	0.4
	20-30	12	40	18	60	30	60	
	30-40	3	25	9	75	12	16	
	>40	1	100	0	0	1	18	
M. parity	<5	15	39.5	23	60.5	38	76	0.7
	>5	4	33.35	8	66.65	12	24	
Residency	Rural	3	33.35	6	66.7	9	18	0.7
	urban	16	39	25	61	41	82	
G. Age (weeks)	38-40	18	41	26	59	44	88	0.2
	41-42	1	16.6	5	83.4	6	12	
BMI	Normal	16	39.1	25	60.9	41	82	0.8
	Obese	1	50	1	50	2	4	
	thin	2	28.6	5	71.4	7	14	
ANC	Good	15	41.6	21	58.4	36	72	0.3
	poor	4	28.6	10	71.4	14	28	
Previous delivery	CS	1	7.7	12	92.3	13	26	0.02
	NVD	13	46.4	15	53.6	28	56	Sig.
	Primigravida	5	55.5	4	44.5	9	18	
Previous Abortion	0	17	39.5	26	60.5	43	86	0.5
	1-3	2	28.6	5	71.5	7	14	

Table 3: MDA distribution regarding fetal and neonatal characteristics

		MDA						
		Normal		elevated		Total		P-value
		no	%	no	%	no	%	
Gender	Male	13	44.8	16	55.2	29	58	0.2
	Female	6	28.6	15	71.4	21	42	
	41-42	1	16.6	5	83.4	6	12	
Birth wt.	<2.5 kg	1	50	1	50	2	4	0.8
	2.5-3.5 kg	15	39.5	23	61.5	38	76	
	>3.5 kg	3	30	7	70	10	20	
Fetal presentation	Cephalic	18	39.1	28	60.9	46	92	0.5
	breech	1	25	3	75	4	8	
Apgar score	4-6	3	75	1	25	4	8	0.5
	>7	16	34.8	30	65.2	46	92	

et al., 2011a). Therefore, MDA measurement used to measure the oxidative capacity of these free radicals (Adekanle *et al.*, 2013). Kaya *et al.* (2000) found that the MDA level was a more sensitive indicator than blood gases by compared cord blood MDA level and blood gas, after conducting an evaluation of the presence of oxidative stress in babies with presentation abnormality (Kaya *et al.*, 2000).

Our study found a significant difference of MDA level in plasma of cord blood in babies of the studied groups. MDA level was higher among those delivered by ECS. This means that free radical generation and oxidative stress in babies delivered by CS were higher than those delivered vaginally were. A study carried out by Adekanle *et al.* (2013). in Nigeria show that the mean plasma level of MDA was higher in subjects delivered through VD (5.78 ± 1.56. mol/l) than in those delivered through ECS

(5.01 ± 1.21. mol/l). With no statistically significant; p>0.05 (Adekanle *et al.*, 2013).

Our study results agree with the results of Jain *et al.* (2015). That carried out in India, the mean of cord blood MDA level in the NVD was (4.38 ± 0.28), whereas in C/S it was (6.47 ± 0.51) which was highly significant (P < 0.0001). (Jain *et al.*, 2015). Another study carried out by Siddiqui *et al.* (2014) In India the mean ± SD of MDA level in vaginal delivery, maternal plasma was (4.8± 0.8 mol/l) while that of elective cesarean section was 7.8±1.2 mol/l. The MDA levels were significantly higher (p <0.001) in maternal plasma of ECS as compared to VD group (Gülbayzar *et al.*, 2011a).

In a study carried out by *Gülbayzar S.* in Turkey, it was found that the mean of MDA values in cord blood in the NVD group was higher (statistically and significantly) than in the elective cesarean group (Gülbayzar *et al.*, 2011a). (Mocatta *et al.*, 2004) estimated that the MDA levels of cord blood in elective C/S were lower than in those of NVD (Mocatta *et al.*, 2004). A study by Yigit *et al.* (1998) was found that the MDA levels of cord blood in neonates born by spontaneous vaginal delivery were higher than in those born by C/S. (Yigit *et al.*, 1998). We may explain our results of higher mean MDA levels in ECS deliveries by their exposure to surgical trauma that is a catabolic condition, accompanied by an increment of oxidative stress as well as a reduction in skeletal muscle antioxidant Glutathione (GSH) pool, which plays a major role in recycling ascorbate from dehydroascorbate. Hence, pronounced impairment of the intracellular antioxidant system and increased free radical's production. (Sankhla *et al.*, 2012).In this study, the mean age of women delivered by CS was (28y) which was higher than those delivered vaginally (23.5y), the higher mean age may be accompanied with more medical and pregnancy complications which may explain the higher MDA level in those delivered by CS to decreases their risks & poor outcome, but this result not correlate with a study by Buonocore *et al.* (2002) in which the mean age of those delivered by CS was higher, but the mean MDA level was lower for CS group. However, no statistical significant found in both studies. (Siddiqui *et al.*, 2014). The highest MDA level was in women lived in rural areas and delivered by CS, and the lowest was in those lived in urban areas and delivered by VD. This may be explained by their social & cultural environment that necessitate daily hardworking at home or farms which aggravated stresses, complications and C\S delivery. Our result correlates with Siddiqui *et al.* (2014) Study but of no statistical significance regarding residency Siddiqui *et al.* (2014).The level found to be elevated in more percentage of those who had GA < 40wks which may be attributed to more occurrence of placental insufficiencies. Agreed with Gülbayzar *S study.* However, both showed no statistical significant regarding GA. Gülbayzar *et al.* (2011a). study as our study showed no statistical significant regarding parity and oxidative stress. Siddiqui *et al.* (2014) . In this study, thin mothers had cord blood with higher MDA mean. This is not correlated with a study carried by (Sankhla *et al.*, 2012). On obese people, he found that obese subjects exhibit increased systemic oxidative stress, which is enhanced when obesity is associated with abdominal adiposity (Sankhla *et al.*, 2012).The highest mean MDA found to be associated with poor antenatal care mothers that may be explained by decrease supplements of Folic acid and other vitamins that act as antioxidant agents to reduce oxidative stress (Bolisetty *et al.*, 2002).MDA level was high among babies of mothers with a history of abortion which is a psychological trauma that probably aggravated stress and carried a negative impact on the delivery environment, but was not statistically significant. No other study has considered this variable to compare with.A statistical significance identified regarding the mode of the previous delivery. The highest MDA level found in babies of those previously delivered by CS probably because of a bad previous experience and outcome or by the already existing pathology necessitates previous C\S or any previous complications. While the lowest among those who had no previous deliveries. No other study has considered this variable to compare with.

Regarding fetal and neonatal characteristics: The highest mean MDA found in females delivered by CS. This correlates with Siddiqui *et al.* (2014). Showed the following results: the male child with VD had mean values as 4.35 and in female as 4.38, whereas in C/S it was 6.19 and 6.90 in a male and female child. In addition, females delivered by CS had the highest mean, but in both studies, this was not statistically significant. (Siddiqui *et al.*, 2014). The level found to be more elevated in those who had a breech presentation explained by a higher stress exposure during the delivery process but with no statistical significance. Babies with higher birth weight had a higher percentage of MDA elevation because of difficult labor and a higher risk of hypoxia, but it was not statistically significant as the study done by Gülbayzar *et al.* (2011b). In this study, the highest mean MDA observed in those who have a higher Apgar score. In the study by Gülbayzar *et al.* (2011a). Apgar score at the (1) minute (6.27±0.88, 6.87±0.92, 6.00±1.65; P>0.05) and Apgar score at the (5) minute (8.93±0.26, 8.93±0.26,

8.60±0.51; P>0.05).As in our study, this was not statistically significant. Buonocore *et al.* (2002). found that a significant association between Apgar score and of cord blood stress marker (Buonocore *et al.*, 2002). While Yigit *et al.* (1998) were found no correlation between plasma MDA level in the first hour and Apgar score (Yigit *et al.*, 1998) . Bilgili *et al.* (2005) determined that the cord blood MDA level was higher in cases where the Apgar scores at the first and fifth minutes were lower than 7 (Bilgili *et al.*, 2005). These differences between studies may be associated with the limitations of the Apgar score in predicting birth asphyxia and neurological morbidity (Moster *et al.*, 2001; Papile, 2001).

CONCLUSIONS

MDA in cord blood of newborns is a sensitive indicator of fetal distress which carries serious complication; the highest levels among non-emergency C\S deliveries may be decreased by delivering through NVD if appropriate to improve neonatal outcomes.

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