



## EFFECT OF MODE OF DELIVERY ON THE LEVEL OF ZINC COPPER SUPEROXIDE DISMUTASE IN UMBILICAL CORD BLOOD

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### ABSTRACT

Normal pregnancy is associated with high metabolic demand and elevated requirements for tissue oxygen which results in increased oxidative stress as well as increased metabolic demands and deficiency of one or more of the trace elements (Zinc and Copper) which will lead to adverse maternal and perinatal outcomes. These two elements are essential for metalloproteinase enzymes such as superoxide dismutase. Babies were delivered either vaginally or through caesarean delivery depending on the circumstances surrounding the pregnancy from conception through labour. The current study was aimed to investigate umbilical cord blood levels of zinc, copper and superoxide dismutase in normal vaginal delivery and elective cesarean section. So, a case-control study was conducted in Baghdad Hospitals from January 2015 to May 2016. Sixty four pregnant women were involved to determined umbilical cord blood levels of zinc copper and superoxide dismutase in two mode of delivery; normal vaginal and elective cesarean section. A significant increase in the level of endogenous antioxidant enzyme superoxide dismutase ( $p < 0.001$ ) was found in cord blood of cesarean section versus normal vaginal delivery. Both zinc and copper shows a significant decrease in cord blood of cesarean section versus normal vaginal delivery ( $p < 0.035$  and  $0.05$  respectively). No significant correlation was found between trace elements and the antioxidant enzyme in cord blood of the two groups. So from all results above, it can be concluded that elective caesarean section could be a more safety mode of delivery than the normal vaginal as it will minimize the risks which directly involves fetal outcome.

**KEYWORDS:** mode of delivery, trace elements, elective cesarean section, normal vaginal delivery, superoxide dismutase



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## INTRODUCTION

Pregnancy is a physiological stress due to hormonal, neuronal and metabolic changes occurring at various levels to meet the growing demands of the fetus. More commonly, the pregnancy-stress gets overwhelmed in the form of oxidative stress at the time of labour. Oxidative stress is known to occur in apparently normal pregnancy, even in the absence of complications.<sup>1</sup> Oxidants like reactive oxygen species (ROS) are normal products of aerobic metabolism. They are called free radicals and include superoxide radical, hydroxyl radical and hydrogen peroxide. When produced in excess, they can be harmful.<sup>2</sup> The level of the oxidants is controlled by antioxidant enzymes e.g. superoxide dismutase (SOD) and small molecule antioxidants where either inhibits or delays the activity of oxidant.<sup>3</sup> There are several enzymes have evolved in aerobic cells to overcome the damaging effects of ROS. They are significantly used to maintain the redox balance during oxidative stress and are collectively called as endogenous anti-oxidative enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems of all aerobic cells. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species.<sup>4</sup> In women with normal, uncomplicated pregnancies, antioxidant levels like (superoxide dismutase) are elevated as compared to non-pregnant women; this increase is proportional to gestational length.<sup>5</sup> In normal pregnancy, placental oxidative stress is believed to be kept under control by placental antioxidant enzymes. When compared to women with normal pregnancies, women with complicating factors such as diabetes mellitus, preeclampsia, and preterm labor experience ever increasing levels of oxidative stress and reduced antioxidant capacity.<sup>6</sup> During parturition, oxidative stress increases more profoundly. Increasing energy demand and metabolic activity by the contraction of skeletal muscle during any type of exercise, combined with a rise in using oxygen, is known to result in increased levels of ROS. As labor involves a series of contractions involving both skeletal muscle and uterine smooth muscle, Toescu *et al* 2002 expects that oxidative stress will increase during vaginal delivery (VD) as compared to an elective cesarean section (CS).<sup>4</sup> Despite the well-known adverse effects of oxidative stress on the mother, fetus, and newborn, the effects of the type of delivery on the oxidative stress experienced by both mother and child are still not clear.<sup>6</sup> Trace elements are minerals required in minute quantities to maintain proper physical functioning. The role of trace elements in the process of parturition is poorly understood.<sup>7</sup> Deficiencies of zinc, copper, and magnesium during pregnancy have been implicated in various reproductive events like infertility, pregnancy wastage, congenital anomalies pregnancy-induced hypertension, placental abruption, premature rupture of membranes, stillbirth, and low birth weight.<sup>8</sup> This study aimed to investigate the effects of the mode of delivery on the antioxidant system represented by endogenous SOD enzyme via umbilical cord blood analysis, and the association of trace elements levels represented by Zn and Cu on this enzyme.

## MATERIALS AND METHODS

A case-control study was conducted between the 1<sup>st</sup> of January 2015 and 30<sup>th</sup> of May 2016. Sixty four pregnant women at term gestation (37–41 completed weeks) were involved. They were approached from Al-Hakeem and Al-Imamayn Al-Khademiyian Medical City Hospitals in Baghdad. All women were within reproductive age. They were divided into two groups; about thirty women in each group based on mode of delivery. The first group contains 33 women with normal vaginal delivery (NVD) at age mean (26.44±1.1) years while the second group involved 31 women with caesarean section delivery (C/S) at age mean 29.1 ±1.21 years. Ethical approval was obtained from the Institutional Review Board (I.R.B.) at Al-Nahrain College of Medicine together with written informed patient consent. Mothers with any medical disorder (e.g. diabetes mellitus, hypertension, thyroid disease, smoking, preeclampsia, renal disease) were excluded from both groups. Anthropometric characteristic of the mothers (gestational age and age) were collected from the medical records. All women involved in this study did not receive any nutrient supplement or any other medication that could have influenced the concentration of Zn and Cu or any other trace elements. The mothers were interviewed using a structured questionnaire to determine smoking during pregnancy. A bout 10 mls of umbilical cord blood was taken during delivery; allowed to clot, and then centrifuged at 2500 rpm for 15 min at room temperature to collect serum. All serum samples were stored at -20°C till the time of assay. Zinc and copper concentrations were determined using colorimetric enzymatic method (Biomagreb, France kits) while superoxide dismutase concentration (SOD) was determined using Enzyme Liked immune-sorbent assay (ELISA) technique (Elabsience Biotechnology Co. USA, kit No. :E-EL-H1113).

### Statistical analysis

All data analyses were done using the Statistical Program for Social Sciences (SPSS) version 16.0 (SPSS Chicago Inc., IL, and U.S.A.). P-value<0.05 was considered as significant. Mean ± Standard Error (S.E.) was used to describe the continuous variables while frequencies and percentages were used to describe categorical variables. Moreover, correlation and Receiver Operating Characteristics (ROC) curve were analyzed. ROS was calculated to assess the sensitivity and specificity of the used kits in classifying the true positive versus false positive cases in this study. The ROC curve plots were presented as well.

## RESULTS

Sixty-four women were involved in this study. Thirty one women were delivered by cesarean section while the rest 33 women were all deliver normally. Women's age was nearly comparable as well as their gestational age with no significance differences ( $p>0.05$ ) as shown in table-1. A highly significant increase in SOD concentration was found in umbilical cord blood (UCB) sera from C/S *vis.* UCB from NVD, while trace elements concentrations (Zn and Cu) were both significantly lower in UCB sera from C/S *vis.* NVD ( $p<0.035$  and  $0.05$  respectively)(table-1).

**Table 1**  
**Mean ± standard error for anthropometric and biochemical parameters in study groups**

Anthropometric parameters	C/S (n= 31)	NVD (n= 33)	P value
Age (years)	29.1±1.21	26.44±1.1	NS
Gestational age (wks)	37.26±0.1	37.42±0.1	NS
Biochemical parameters			
Serum SOD (pg/dl)	1851.03±153.68	947.94±45.5	<0.001
Serum Zn (µg/dl)	197.39±3.29	205.27±1.71	0.035
Serum Cu (µg/dl)	89.97±6.78	113.21±9.34	0.05

C/S= cesarean section, NVD= normal vaginal delivery, n= number, wks=weeks, SOD= supper oxide dismutase, Zn= zinc, Cu= cupper, pg= pictogram, µg= microgram and NS=not significant.

By using ROC analysis for SOD, Zn and Cu concentrations in both women groups, a significant area under curve (AUC) for SOD with 0.847 was found indicating a threshold of 1200 pg/dl with sensitivity of 83% and specificity 88% and more level of UCB serum SOD concentration was associated with increased rate of C/S . While ROC analysis for Zn and Cu revealed a

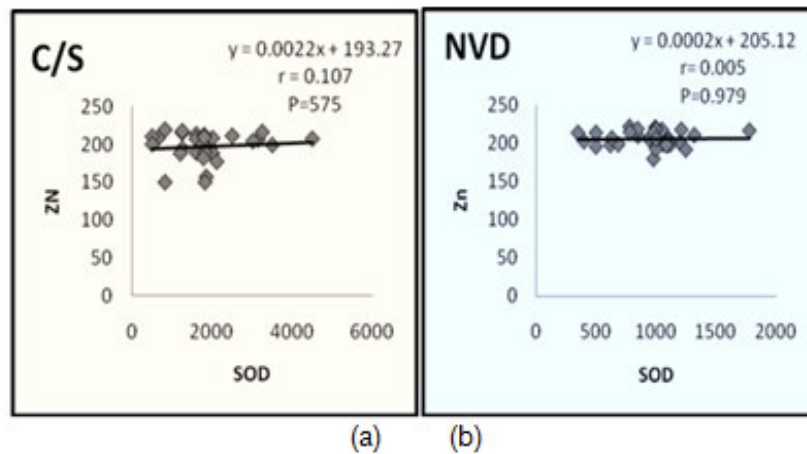
significant area under curves (AUC) 0.610 and 0.607 respectively, indicating that a threshold of 205 µg/dl for Zn and 100µg/dl for Cu. Their sensitivity was 55% and 58% respectively while specificity was 54% and 52% respectively. Less level of both UCB serum for Zn and Cu concentrations were associated with increased rate of C/S (table-2).

**4Table 2**  
**Estimated area under curve and predictive cut-off point values for serum SOD, Zn and Cu concentrations in cesarean section and normal vaginal delivery women**

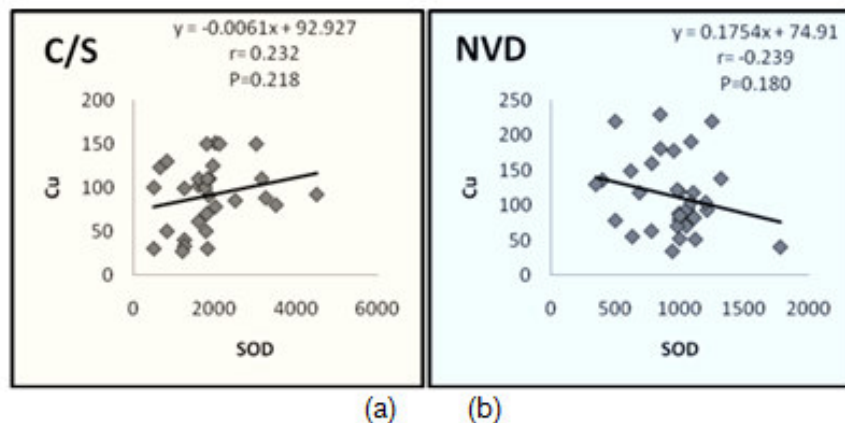
Parameters	Area	Cutoff point value	Sensitivity	Specificity	P value	95%CI	
						Lower Bound	Upper Bound
SOD (pg/dl)	0.847	1200	83%	88%	0.001	0.736	0.959
Zn (µg/dl)	0.610	205	55%	54%	0.129	0.471	0.750
Cu (µg/dl)	0.607	100	58%	52%	0.143	0.468	0.745

SOD= supper oxide dismutase, Zn= zinc, Cu= cupper, pg= pictogram, µg= microgram and CI= confidence interval.

Regarding this inverse results shown in table-2, no significant correlation was found between UCB serum SOD with both trace elements Zn and Cu as shown in figure-1 and figure-2 in both delivery groups.



**Figure 1**  
**Correlation between Zn and SOD concentrations in umbilical cord blood of (a): cesarean section delivery and (b): normal vaginal delivery.**



**Figure 2**  
**Correlation between Cu and SOD concentrations in umbilical cord blood of**  
**(a): cesarean section delivery and (b): normal vaginal delivery**

## DISCUSSION

The role of oxidative stress in various significant pathological processes has recently become a subject of research.<sup>9</sup> During pregnancy; the antioxidative defence system is reorganized to restrict oxidative stress, while in normal pregnancies oxidative stress parameters and antioxidant protection are known to increase.<sup>10</sup> In the present study, serum SOD of cord blood from babies born by NVD was significantly lower than those babies born by caesarean section. During fetal development, antioxidant defense mechanisms have generally been considered less active than those of adults.<sup>11</sup> This may be due to a lower requirement for cellular antioxidant activity as the fetus is in an environment with a low partial pressure of oxygen.<sup>12</sup> Soumika *et al.* 2014 results were in agreement with present study; they have found mean cord blood plasma superoxide dismutase values lower in cord blood of normal delivery born babies than that of caesarean section born babies.<sup>13</sup> Also, other studies such as S.Gulbayzar *et al.*<sup>14</sup>, Greco A *et al.*<sup>15</sup>, U. Mehmetoulu *et al.*<sup>16</sup> were all in agreement with our results. The importance of trace elements due to the fact that they are present in metalloprotein (Zn), ceruloplasmin (Cur), superoxide dismutases (Se and Zn) and glutathione peroxidase (Se). Thus, metalloprotein (Zn) is an important trace element in metabolism, growth, development and reproduction. It is a constituent of many enzymes (*e.g.* superoxide dismutases SOD) as an efficient antioxidant.<sup>17</sup> It plays important roles in nucleic acid metabolism and protein synthesis, as well as membrane structure and function.<sup>18</sup> Many studies were comparing between trace elements concentrations in maternal serum and cord blood serum despite the mode of delivery. The present study compare between trace elements (Zn and Cu) as they are the most elements related to the SOD antioxidant enzyme in cord blood serum in C/S and NVD. The significantly increase in Zn concentration in cord blood serum of NVD vs. C/S ( $p < 0.035$ ) reflects the decrease in cord blood serum SOD concentration in NVD vs. C/S ( $p < 0.001$ ) (table-1). This may explain as following; a decline in circulating Zn is found during pregnancy, and this increases as the pregnancy progresses possibly due to decrease in Zn binding and increased transfer of Zn from the mother to

the fetus.<sup>19</sup> Zinc is essential for proper growth of fetus and the fall in Zn during pregnancy could also be a physiological response to expanded maternal blood volume.<sup>20</sup> In addition to Zn, copper (Cu) is known to be a component of a number of copper metallo enzymes such as catalase, superoxide dismutase, and cytochrome oxidase and its deficiency can lead to variety of nutritional and vascular disorders.<sup>21</sup> Tamura *et al* 2000 studied Zn and Cu in cord blood serum and compare them with their concentration also in maternal serum. Regarding Zn concentration in cord blood serum, they have found it much higher than it was seen in our study (mean cord serum for Zn was  $819 \pm 224$  vs. our result  $205.27 \pm 1.71$   $\mu\text{g/dl}$ ) despite the mode of delivery. This increase may due to the same explanation mentioned above.<sup>19</sup> Also, physiological conditions, nutritional status in addition to mode of delivery ( NVD or C/S) and ethnics may affect Zn concentration. Awadallah *et al* 2004 also reported that physiologic, metabolic, and hormonal changes during pregnancy affect the metabolism and body needs for micronutrients and minerals.<sup>22</sup> The reduction in maternal zinc levels observed during the third trimester is attributed to several factors, such as increased zinc uptake by the fetus and placenta, increased transfer of plasma zinc to maternal erythrocytes, expanded plasma volume, and decreased serum albumin availability for zinc binding during pregnancy.<sup>23</sup> Furthermore, zinc transporters, such as ZnT, that are localized in placenta<sup>24</sup> may play an important role in accelerating zinc uptake from the mother's blood to the developing fetus. Thus, the level of zinc would be expected to be higher in cord blood than that in maternal blood. Regarding the other trace element involved in this study, Cu concentration in UCB sera from NVD group was significantly higher than C/S group but still in general its concentration was lower than Zn concentration (table-1) and didn't show any correlation with the SOD enzyme as shown in figure-1 & 2. These results were agrees with a previous studies done by Elhadi *et al.* 2015 and Lazer *et al.* 2012; they found that both elements zinc and copper concentrations in UCB sera were significantly elevated in normal vaginal delivery group compared to cesarean section group.<sup>25-26</sup> Recent researches although not study the effect of oxidative stress on mode of delivery or their levels in umbilical cord blood but have been

concludes that there was an imbalance between oxidants and antioxidants levels resulting in an increased oxidative stress in preterm infants and their mothers than in term infants and their mothers.<sup>27</sup>

## CONCLUSION

From all results above, it can be concluded that elective caesarean section could be a more safety mode of delivery than the normal vaginal as it will minimize the risks which directly involves fetal outcome.

### Recommendations

It should be mentioned that there are other studies which show a totally opposite our findings. So, this part

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## CONFLICT OF INTEREST

Conflict of interest declared as none.

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