

The Effect of Type of Delivery on the Nitric Oxide Metabolites and Endothelial Dysfunction in Pregnant Women

Abstract

Background: Since endothelial dysfunction is related to atherosclerosis, this study was planned to determine the effect of type of delivery on Nitric Oxide (NO) metabolites and endothelial function. **Materials and Methods:** This Cohort study was conducted in 2015 in selected hospitals of Isfahan. 88 nulliparous women with gestational age of 39 weeks and above were enrolled in this study using convenience sampling method and finally, after giving birth, 51 mothers with vaginal delivery, 21 with urgent C-section and 13 with elective C-section were considered for data analysis. The serum levels of NO metabolites were measured in the laboratory with standard kits and data was analyzed using student and paired *t*-test, one-way ANOVA, and Chi-square test. The significance level was considered less than 0.05 for all tests. **Results:** The NO metabolites levels in mothers who had vaginal delivery or urgent C-section showed a significant difference before and after delivery (normal vaginal delivery (NVD): $t_{50} = 5.61$, $p < 0.001$, Urgent C-section: $t_{23} = 5.38$, $p < 0.001$). But those with elective C-section showed no significant difference in the nitrate and total nitrite levels before and after delivery ($p > 0.05$). **Conclusions:** Since reduction in serum levels of NO metabolites may possibly indicate endothelial dysfunction and predict cardiovascular disease, especially atherosclerosis in the future, it could be concluded that, childbirth, regardless of the type of delivery, could damage the endothelial cells but C-section (urgent or elective) could cause more disruption than vaginal delivery.

Keywords: Cesarean section, natural childbirth, nitrates, nitric oxide, nitrites

Introduction

One of the major causes of mortality in the world is cardiovascular diseases. They are projected to cause 6 million deaths in developed countries in 2020 and 9 million in developing countries.^[1] The most common and most important cardiovascular disease is atherosclerosis that is a chronic vascular disease which can persist for many years and cause myocardial infarction, unstable angina and sudden death.^[2] One of the causes of vascular plaque formation and atherosclerosis is impaired endothelial function. Endothelial dysfunction is a pathological condition characterized by the loss of ability in the vascular endothelial layer cells to induce vasodilatory response by production of endothelium-derived messengers which disrupts the dilation of vessels and regulation of blood flow and vascular tone.^[3]

Researchers have linked endothelial dysfunction, known as nitric oxide (NO)

deficiency, to the pathology of many other diseases such as septic shock,^[4] hypertension,^[5] diabetes,^[6] chronic kidney disease,^[7] and Alzheimer.^[8] Vascular endothelial function is evaluated using different physical and biochemical methods. One way to evaluate endothelial function is to investigate plasma soluble endothelial markers such as NO.^[9] Due to the very short half-life of NO, it is difficult to measure it directly. As a result, it can be measured using its stable metabolites, nitrite, and nitrate.^[10]

The effect of type of delivery on endothelial function has been evaluated in few previous studies concluding controversial results. A cohort study on 48 vaginal delivery, 20 elective C-section, and 11 urgent C-section showed that flow mediated dilation (FMD) levels increased after vaginal delivery and emergency C-section but decreased after elective C-section.^[11] Also studies showed that increased plasma concentration of homocysteine, which prevents the

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production of NO and leads to endothelial dysfunction, have been observed in women after caesarean section in comparison to normal vaginal delivery.^[12,13] It is also reported that maternal RBC glutathione was increased significantly after vaginal delivery whereas the elective C-section had no similar effects.^[14]

Contrary to the above mentioned results, in 2015, a study found that oxidative stress that causes NO reduction through production of oxygen free radicals, and ultimately leads to endothelial dysfunction, was increased during labor due to various causes including pain, fear, anxiety, and hypoxia.^[15] Other studies also confirmed an increase in oxidative stress in normal vaginal delivery.^[16,17]

However, results of the studies are contradictory in this regard, and considering the importance of vascular endothelial role in development of atherosclerosis, as well as the fact that endothelial dysfunction can be prevented and treated,^[18] long-term maternal cardiovascular health prognosis can be improved by providing the necessary prenatal education to reduce maternal cardiovascular risk factors. This study was aimed to evaluate the serum concentrations of NO metabolites to compare the endothelial function in vaginal delivery and C-section in pregnant women.

Materials and Methods

This quantitative cohort study was conducted on 88 nulliparous women with gestational age of 39 weeks and above who were recruited when they presented for their last prenatal visit at the obstetric clinics of selected hospitals in Isfahan (Iran) [private hospital (level 2) and governmental hospital], from March 2015 to September 2016. In this study, samples were selected using convenience sampling method.

Inclusion criteria were having nulliparous singleton pregnancy with no history of cardiovascular diseases, diabetes, hypertension, and venous thrombosis, having no history of smoking, as well as absence of any pregnancy complications such as preeclampsia, gestational diabetes, and fetal growth restriction.^[11] Also mothers who declined or withdrew participation, had vacuum assisted vaginal deliveries, had deliveries that took long more than 18 h after the rupture of membrane and also had deliveries that encountered problems such as preeclampsia during the course of study, were excluded. According to the sample size $N = (z_1 + z_2)^2 (2s^2) / d^2$, z_1 : with 95% confidence coefficient: 1.96, z_2 : with the test power coefficient of 80% is 0.84, s : An estimate of the standard deviation of serum nitrite or nitrate levels in three groups and d : Minimum difference in mean serum levels of nitrite and nitrate between three groups that is significant and considered to be 0.6s, the number of samples was calculated to be at least 44 in each group. But after reaching the required number of natural delivery samples, due to the health

Ministry's policies regarding the reduction in the number of cesarean delivery, we faced a sharp reduction in C-sections, and despite doubling the predicted timeframe, it was not possible to collect more samples; and continuing with sampling would have led to the loss of the prenatal normal delivery group samples and was not ethically correct. So finally, data from 88 participants that included 51 mothers with normal vaginal delivery, 24 with urgent C-section and 13 with elective C-section were considered for data analysis.

After presenting the introduction letter and explaining the goals of the study and obtaining the consent of the research units, aseptically, 5cc venous blood sample was taken from the samples and then, the subjects were followed up daily through phone calls to find out about the exact time of delivery. Postpartum mothers were divided into three groups of vaginal delivery, emergency cesarean and elective cesarean section, depending on the type of delivery. Between 6 and 48 h postpartum, second blood sample was taken at maternity ward and characteristics were recorded from subject's medical file. The blood samples, were centrifuged at 3,000 rpm for 10–15 min to separate the plasma and were kept at -20°C until measurements. The serum levels of nitrite, nitrate, and total nitrite were determined using R&D Systems™ Total Nitric Oxide and Nitrate/Nitrite Parameter Assay Kit. This diagnostic kit has two assay steps which were developed by Griess reaction and using an enzyme-linked immunosorbent assay (ELISA) reader device. In the first step, the serum level of nitrite was measured according to the kit procedures and standard curve, and in the second step, nitrate was converted to nitrite using nitrate reductase and total nitrite was determined. In order to obtain the nitrate concentration, the nitrite value was subtracted from the total nitrite value. After collecting the data, they were logged into SPSS Version 18 (SPSS Inc., Chicago, IL, USA, SPSS) software and were analyzed using descriptive and inferential statistical methods; Independent *t*-test, Mann–Whitney, and Chi-square tests to compare the demographic features, and paired *t*-test to compare before and after delivery nitrite and nitrate serum levels within three groups. The significance level of the tests was less than 0.05.

Ethical considerations

This research was conducted after receiving informed consent from the research units and receiving the Code of Ethics: IR.MUI.REC1394.3.468 from the Office of Vice President for Research at Isfahan University of Medical Sciences in 2015.

Results

At the end of the recruitment period, we examined data from 88 participants that included 51 mothers with normal vaginal delivery, 24 with urgent C-section and 13 with elective C-section. One-way ANOVA, Kruskal–

Table 1: Demographic, obstetrics and delivery characteristics in vaginal delivery, elective and urgent caesarean section groups

Variables/Group	Vaginal Delivery n=51 Mean (SD)	Elective Caesarean n=13 Mean (SD)	Urgent Caesarean n=24 Mean (SD)	t	df	p		
						One-way ANOVA	Kruskal Wallis	Chi square
Age (Year)	26.42 (5.51)	27.93 (5.24)	27.96 (4.96)	1.02	2,85	0.53	-	-
Education(<high school/high school/academic)	5-29-17	3-5-5	2-11-11	0.22	4	0.41	0.879	-
Gestational age (week)	39.27 (1.56)	39.55 (0.68)	38.90 (0.54)	0.90	2,85	0.85	-	-
Apgar 1 min	8.73 (1.14)	8.50 (1.65)	8.97 (0.31)	0.16	2,85	0.32	-	-
Apgar 5 min	9.94 (0.34)	9.85 (0.55)	10 (0.00)	1.14	2,85	0.09	-	-
Birth weight (Kg)*	3.21 (0.34)	3.36 (0.38)	3.15 (0.28)	3.16	2,85	-	-	-
Baby sex (female/male)	28-23	7-6	16-8	0.44	2	-	-	0.813

*Kilogram

Wallis and Chi-square tests showed that demographic and clinical variables were not significantly different in the three groups. ($p > 0.05$) [Table 1]. Paired t -test showed that serum levels of nitrate, nitrite and total nitrite were decreased significantly before and after delivery in the vaginal delivery group as well as in the emergency caesarean section ($p < 0.001$). In the elective caesarean section, postnatal serum nitrite levels were significantly decreased ($t_{12} = 2.39$ $p = 0.034$), but the nitrate and total nitrite levels were slightly altered, which was not statistically significant ($p > 0.05$) [Table 2].

Furthermore, the nitrite/nitrate ratio in urgent caesarean section group was increased in comparison with the vaginal delivery group ($p = 0.080$) [Figure 1]. In addition, this ratio was different before and after delivery in urgent caesarean section mothers ($p = 0.080$) [Figure 1].

Discussion

In this study, which was conducted to determine the relation between the type of delivery and endothelial dysfunction, results indicated that serum levels of nitrate and nitrite and total nitrite in vaginal delivery and urgent caesarean section have significantly decreased after delivery. In the case of elective caesarean, postpartum nitrate levels remained unchanged compared to pre-delivery. Although nitrite levels decreased significantly after elective caesarean section, total nitrite did not significantly decrease. Previous study reported that the rate of FMD was increased after normal vaginal delivery and urgent caesarean section compared to before delivery. However, in elective caesarean section, this rate had decreased after delivery.^[11] The findings of our study are not consistent with the findings of this study but in relation to the elective caesarean, the findings are consistent with the current one. One of the reasons might be the difference between the methods of evaluation of endothelial dysfunction in the two studies. Also in this study details of normal vaginal delivery, including receiving any type of analgesia (intravenous, epidural, spinal or Entonox gas),

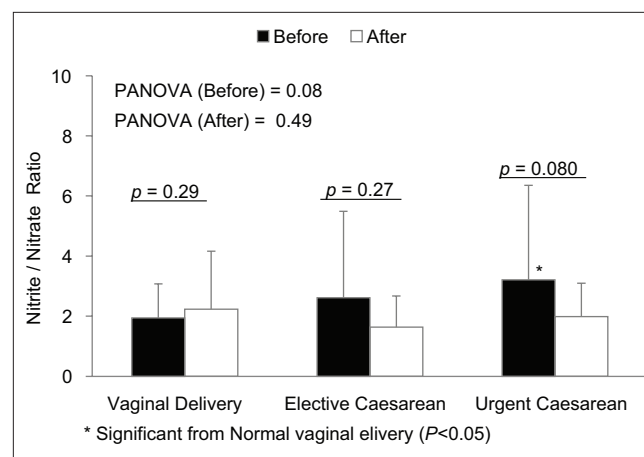
**Figure 1: Nitrite/Nitrate Ratio In vaginal delivery, elective caesarean and urgent caesarean**

Table 2: Serum levels of nitrite, nitrate and total nitrate before and after delivery in vaginal delivery, elective and urgent caesarean section groups

Group	Nitrite ($\mu\text{mole/l}$)			Nitrate ($\mu\text{mole/l}$)			Total Nitrite ($\mu\text{mole/l}$)		
	Before	After	t	Before	After	t	Before	After	t
Vaginal Delivery n=51	92.81 (28.42)	64.72 (25.83)	5.01	53.55 (19.68)	36.02 (7.78)	4.71	146.25 (2.22)	101.69 (37.97)	5.61
Elective caesarean n=13	86.19 (26.54)	58.37 (24.01)	2.39	45.24 (19.05)	45.1 (23.87)	0.003	131.37 (37.46)	103.53 (37.89)	1.57
Urgent Caesarean n=24	91.95 (23.22)	50.69 (17.64)	4.52	45.23 (28.20)	30.71 (14.43)	3.20	137.18 (38.85)	81.26 (27.80)	5.38
F	0.43	1.73		0.91	1.74		0.84	1.74	
p	0.72	0.08		0.26	0.07		0.43	0.07	
ANOVA test									

water birth, presence of doula, etc. which all reduce stress and pain and subsequently, reduce oxidative stress and improves endothelial function, were not mentioned. In the present study, normal vaginal delivery was not physiologic delivery and the cause of NO metabolites depletion may be pain and anxiety due to vaginal delivery. In a study in 2015, Chitra *et al.* concluded that oxidative stress during labor would increase due to various causes including pain, fear, anxiety caused by unawareness of labor conditions and Hypoxia and can disrupt the maternal antioxidant system.^[15] The results of other studies also confirmed the increase in oxidative stress in normal vaginal delivery.^[16,17] The present results are in line with these studies; the decrease in nitrite and nitrate concentrations were the same as the increase in oxidative stress indices and subsequently reduction in antioxidant defense could decrease NO bioavailability and impair endothelial function.

It states that the rate of pain and anxiety and fear in the routine delivery is significantly higher than in the physiological delivery.^[19] In the present study, normal vaginal delivery is a routine type and interventions such as induction, amniotomy, episiotomy, etc. have been performed during the delivery process. According to the findings of the above study, it can be said that oxidative stress in physiological vaginal delivery is probably lower than in routine normal vaginal delivery and it seems that physiological vaginal delivery may decrease oxidative stress and subsequently decrease endothelial dysfunction and eventually predict future maternal cardiovascular health. This study may justify the reduction of postnatal nitrite and nitrate levels in vaginal delivery in the present study.

Also, the results of studies examining the effects of oxidative stress and vaginal delivery with epidural anesthesia showed that epidural anesthesia had reduced oxidative stress markers.^[20,21] It can be concluded from this finding that reduction of pain during labor can reduce oxidative stress and ultimately decrease endothelial dysfunction resulting from normal vaginal delivery. Also acute stress such as labor stress would decrease L-Arginine and NO metabolites.^[22] Considering the above factors, deduction in postpartum NO metabolites is expected; also, the rate of S-Cortisol in normal birth is higher than cesarean section,^[23] indicating that the labor is a stressful process and that would be the reason that NO metabolites are decreased significantly after vaginal delivery.

In case of urgent cesarean section, the study of Hu *et al.*, showed that Serum proinflammatory cytokines after emergency cesarean were significantly higher than vaginal delivery and elective C-section. Also, interleukin-8, was higher in postoperative cesarean section than in elective cesarean section.^[24] According to the results of this study and the close and synergistic relationship between inflammation and oxidative stress as well as emergent labor

conditions and the possibility of increased fear and anxiety, it can be concluded that oxidative stress after emergency C-section will activate vascular endothelium. The results of our study are in line with these results. Also, in the study of Schulpis *et al.* after emergency cesarean section, the rate of 8-Hydroxy-2-deoxy Guanosine (8-OHdG), one of the indicators of oxidative stress, was significantly increased compared to vaginal delivery and elective C-section. The results also showed that the total antioxidant status (TAS) after emergency C-section was significantly lower than the other two groups.^[25]

Finally, the results of the present study and above studies confirmed the possibility of increased endothelial dysfunction after emergency cesarean section. About elective C-section, the results of previous studies showed that NO metabolites was decreased after surgery and blood transfusion.^[26] Also, the use of anesthetic drugs in the operating room such as Halothane and Enflurane and Isoflurane, which are vascular relaxants, can reduce the serum NO level after surgery.^[27] Other causes of decrease in the serum level of nitric oxide metabolites after elective C-section might be disturbances in the salivation pathway of nitrite to nitrate due to the abnormal swallowing reflex caused by intubation during surgery. Also, surgical trauma with reduction of NOS synthesis prerequisites such as ornithine, arginine, and citrulline can decrease the NO metabolites,^[28] which were in line with the results of the present study.

The risk of venous thromboembolism (VTE) was fourfold greater following C-section than following vaginal delivery; and was greater following emergency C-section than following elective C-section^[29]; our finding is clinically consistent with this result that C-section may alter endothelial function.

Antioxidants such as vitamin E and C could attenuate endothelial dysfunction^[30] and they were recommended to mothers after delivery to prevent its development and later cardiovascular disease.

One of the limitations of this study was that the results could not be generalized to the whole country because this study was conducted only in Isfahan, however; the results of this study can provide a basis for further researches on vascular endothelial function in midwifery. Given that many factors affect vascular endothelial function and blood nitrite and nitrate levels, controlling these factors was the other limitation and we tried to control them via including primary gravid mothers with no history of previous diseases.

Conclusion

Since reduction of NO metabolites may possibly indicate endothelial dysfunction and predict cardiovascular disease, especially atherosclerosis in the future, it can be concluded that, childbirth, regardless of the type of delivery could

damage the endothelial cells but C-section (urgent or elective) could cause more disruption than normal delivery. To reduce the endothelial dysfunction of vaginal delivery, it seems that normal physiological delivery without unnecessary interventions to reduce birth pain through non-pharmacological pain relievers such as massage, Aromatherapy, Thermotherapy, music therapy, reflexology, relaxation, breathing techniques, etc. will reduce oxidative stress and thereby reduce endothelial dysfunction and can improve future maternal cardiovascular health.

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Conflicts of interest

Nothing to declare.

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