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Original Article

Cholinesterase Levels in Cord Blood in Preeclamptics

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ABSTRACT

Objective: Human placenta, a non-neural tissue, contains cholinergic system and high affinity muscarinic receptors. Role of Cholinesterases (CE) in trophoblast function and pregnancy is not clear. The present study was planned to analyse cholinesterase (CE) levels in cord blood in preeclamptic women. **Materials and Methods:** Cholinesterase levels were analyzed in maternal and cord blood in women with preeclampsia (n=25) and compared with those of normotensive pregnant women (n=25) and normal, healthy controls (n=25) by kinetic method (new DGKC method) using auto analyzer. **Results:** Cholinesterase levels were lower in maternal blood of preeclamptics as compared to normotensive controls. Cord blood cholinesterase levels were lowered in babies of normotensives and cord blood CE levels were 88.65% of the maternal levels. Cord cholinesterase levels were significantly lowered in preeclamptic as compared to normotensive pregnant. On comparing CE levels with normal control (Group III) it was observed that CE levels were significantly raised both in normotensive as well as preeclamptic women. **Conclusion:** Findings of the present study indicate that decrease in cholinesterase levels in preeclampsia via loss of muscarinic cholinergic receptors occurring in preeclampsia.

Key words: Cholinergic system, Cholinesterases, Pregnancy, Preeclamptics, Cord blood.

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INTRODUCTION

Hypertensive disorders are the most common medical complications of pregnancy, occurring in approximately 7-10% of all pregnancies.[1] The basic pathology of pregnancy induced hypertension is intense vasospasm affecting whole of vascular system, especially renal, uterine and cerebral vessels. This vasospasm probably occurs due to an increase in vasopressor substances like angiotensin II, thromboxane A_2, endothelin-1 and a decrease in vasodilator substances such as nitric oxide and prostacyclin due to endothelial cell dysfunction.[2]

The initiating event in preeclampsia appears to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles. Placental ischemia leads to widespread activation/dysfunction of the maternal vascular endothelium resulting in enhanced formation of endothelin and thromboxane; increased vascular sensitivity to angiotensin II and decreased formation of vasodilators such as nitric oxide and prostacyclin.[2]

Cholinesterases (CE) are widespread enzymes present in cholinergic and non-cholinergic tissues as well as in their plasma and other body fluids.[3-6] Based on their substrate specificity, behaviour in excess substrate and susceptibility to inhibitors, they are divided into two classes: acetylcholinesterase or "true cholinesterase" and butyrylcholinesterase (BChE, also known as pseudocholinesterase, non-specific cholinesterase or simply cholinesterase). They are hydrolytic enzymes catalysing break down of acetylcholine to choline and acetate. Acetylcholinesterase (ACh) hydrolyzes acetylcholine (ACh) faster than other cholinesterases and is much less active on butyrylcholine. On the contrary, BChE preferentially acts on butyrylcholine, but also hydrolyzes acetylcholine.[5,7] AChE is abundant in brain, muscle and erythrocyte membrane, whereas BChE has higher activity in liver, intestine, heart, kidney and lung.[8,9]

Non-neuronal cholinergic system has been identified in several tissues including keratocytes, cancer cells, immune cells, urinary bladder, airway epithelial cells, vascular endothelial cells, reproductive organs.[10] Non-neuronal cholinergic components act locally via paracrine and autocrine mechanisms to control basic cellular functions such as proliferation, differentiation, cell-cell interaction and response to various insults including stress. This non-neuronal cholinergic system is known to be involved in the regulation of function and that cholinergic dysfunction is related to path physiology of certain diseases. Dysfunction of the non-neuronal cholinergic system is involved in the pathogenesis of diseases.[10]

Human placenta, a non-neural tissue, contains cholinergic system and high affinity muscarinic receptors. Role of AChE in trophoblast function is not clear and placental (ChAT choline acetyl transferase) gets localized to cytotrophoblast and mesenchymal cells in human placenta.[11]

Placental acetylcholine has been reported to vary with gestational age, reaching a peak at 20-22 weeks of gestation and declining toward term and this pattern paralleled by the activity of choline acetyltransferase (ChAT), suggesting that the placental cholinergic system may be involved in regulating the developmental processes relevant to placental growth.[12] Multiple muscarinic acetylcholine receptor (mAChR) subtypes and all subtypes of nicotinic acetylcholine receptor (nAChR) alfa subunit are present in the placenta.[13]

ACh is an important placental signaling molecule. It stimulates nAChR, controls nutrients uptake, blood flow and fluid volume in the placental vessels and vascularisation during placental development. As placental ChAT expression overlaps that of eNOS (endothelial nitric oxide synthase) and locally produced ACh may stimulate eNOS vía a Ca^{2+} -dependent mechanism.[14] Kambam *et al* reported that both normal and pre-eclamptic pregnancies are associated with a significant decrease in plasma cholinesterase activity and pre-eclamptic pregnant women showed significant decrease in plasma cholinesterase activity when compared with normal pregnant women.[15]

Conflicting data are available regarding cholinesterase in preeclamptic and status in cord blood is also not known. Hence the present study was planned to analyse cholinesterase levels in cord blood in preeclamptic women.

MATERIALS AND METHODS

The present randomized controlled trial was conducted in the Department of Biochemistry in collaboration with Department of Obstetrics and Gynaecology, Pt. B.D. Sharma, PGIMS, Rohtak from July 2011 to Dec 2012. Cholinesterase levels were analyzed in maternal and cord

blood in women with preeclampsia and compare with those of normotensive pregnant women. An informed consent was taken from all the patients and the research protocol was approved by the Institutional Review Board. Women with history of smoking, chronic hypertension, any metabolic disorder before or during pregnancy or presence of high risk factors like anemia, heart disease, diabetes, renal disease were excluded. Also, only those women who delivered by normal vaginal delivery were included in the study.

Fifty pregnant women attending the Outpatient Department of Obstetrics and Gynaecology were enrolled.

GROUP I (Control): Twenty five normotensive women with singleton pregnancy at the time of delivery. GROUP II (study): Twenty five age and gestation matched women with singleton pregnancy and systolic blood pressure reading ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg with or without proteinuria at the time of delivery. GROUP III (healthy controls): Twenty five age matched healthy volunteers.

Five ml blood was drawn aseptically and serum was separated by centrifugation. 10 mL umbilical cord blood was drawn and serum was separated. Routine investigations and serum cholinesterase levels were analyzed in maternal blood, cord blood and healthy controls and healthy controls. Quantitative *in vitro* cholinesterase activity is measured in serum by kinetic method (new DGKC method) using autoanalyser.[16]

RESULTS

Cholinesterase levels were lowered in maternal blood of preeclamptics as compared to normotensive controls (p>0.05, Table 1). Cord blood cholinesterase levels were lowered in babies of normotensives (7067.8±1087.38 U/L *vs.* 7972.2±1544.15 U/L respectively) and cord blood CE levels were 88.65% of the maternal levels. In group II, cholinesterase levels were lowered in cord blood as compared to maternal blood (6281.8±1482.03 U/L *vs.* 7011.8±1073.12 U/L; p>0.05) and cord blood CE levels were 89.58% of the maternal levels. Cord cholinesterase levels were significantly lowered in preeclamptic as compared to normotensive pregnant (6281.8±1482.03 U/L *vs.* 7067.8±1087.38 U/L; p<0.05). On comparing CE levels with normal control (Group III) it was observed that CE levels were significantly raised both in normotensive as well as preeclamptic women (2167.028+ 533.988 U/L *vs*. 7972.2±1544.15U/L, 7011.8±1073.12 U/L respectively; p<0.001 in both cases, Figure 1, Table1, Table 2).

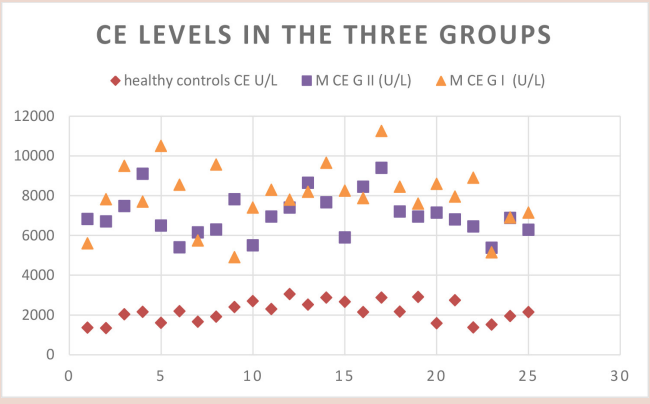

Figure1: CE levels in various groups

Table 1: Maternal and cord blood CHOLINESTERASE levels (Mean±SD)

Cholinesterase (U/L)	Maternal levels (n=25)	Cord blood levels (n=25)
Group I	7972.2±1544.15	7067.8±1087.38
Group II	7011.8±1073.12	6281.8±1482.03

Table 2: Mean cholinesterase levels in all three groups.

Parameter	Group I	Group II	Group III
Mean Cholinesterase	7972.2 + 1544.15	7011.8 + 1073.12	2167.02 + 533.98

DISCUSSION

The mean age of Group I and Group II were 25.68±4.46 and 25.48±3.96 years respectively. Mean gestational age of group I and group II at time of delivery was 37.86±0.99 and 37.89±0.93 weeks respectively. Mean birth weight of babies born in Group I and Group II was 2.59±0.46 kg and 2.48±0.53 kg respectively. Mean systolic and diastolic pressure in the study group was 159.60±12.28 and 99.44±6.59 respectively, while in the control group mean systolic and diastolic pressure was 116.32±6.02 and 74.56±5.08 respectively.

Conflicting reports are available regarding cholinesterase levels in pregnancy. In the present study cholinesterase levels of preeclampsia mothers (Group II) were decreased as compared to normotensive mothers (7011.8±1073.12 vs 7972.2±1544.15) but it was not statistically significant. (p >0.05 Table-1, Figure-1). Data from several investigators agree that plasma cholinesterase activity declines during normal pregnancy.[17-19] While, some studies have reported further decline in plasma cholinesterase activity. While others have reported that there is no difference in plasma cholinesterase activity between preeclamptic and normal pregnancy.[15,20-22]

The decreased activity of cholinesterase levels in preeclamptic mothers may be attributed to hemodilution and hypoalbuminemia. Preeclampsia induced hepatic dysfunction may be another cause for this decline of cholinesterase activity. in cord sera which might indicate lower liver function in this period of life.[23,24] Lowered mean values of CE activity in cord blood may reflect smaller liver cell mass in newborn infants. Investigators had reported low serum CE and albumin concentrations.

Endothelial cells contribute to the regulation of perfusion. In vascular tissue, ACh via activation of mAChRs (M3 and M1 subtypes) is a well-known mediator for the release of nitric oxide (NO), endothelium-derived hyperpolarizing factor and prostanoids. Blood flow, shear stress, body temperature and local blood pressure may affect endothelial ACh synthesis and release and as a consequence may modulate the release of vasoactive mediators. Plasma cholinesterase is a mucoprotein produced in the liver. Pre-eclamptic and eclamptic pregnancies are associated with significant hepatic dysfunction.[23] Release of non-neuronal acetylcholine from the human placenta is mediated via organic cation transporters (OCTs) of the OCT1 and OCT3 subtype. The activity of OCTs is modulated by a variety of drugs and endogenous compounds, like monoamines and steroids which can target the release of non-neuronal acetylcholine under physiological conditions. Particularly, circulating catecholamines may affect the release of non-neuronal acetylcholine.[25]

Placental ChAT localizes to the cytotrophoblast and some mesenchymal cells in human placenta. It further suggests that ACh acts via muscarinic receptors on the trophoblast cell membrane to modulate NO in an estrogen-dependent manner. Expression of ChAT overlaps the expression of eNOS in the human placenta suggesting that these signaling interactions are likely to be physiologically relevant at the maternal-fetal interface. Collectively, these findings support the hypothesis that the placental cholinergic system interacts with nitric oxide and estrogen signaling pathways to regulate placental cell growth and/or function. Cholinergic recognition sites in human placenta have been demonstrated in placenta and there is a possible significant role of placental cholinergic system in pathophysiology of PE.

On comparing CE levels with normal control (Group III) it was observed that CE levels were significantly raised both in normotensive as well as preeclamptic women (Figure 1; $p<0.001$ in both cases).

Plasma cholinesterase has been reported to be significantly decreased during pregnancy and they attributed this to altered haemodynamics and or other inter-related changes occurring in pregnancy.[26] In the present study, cord blood cholinesterase levels of preeclampsia mothers were decreased as compared to normotensive mothers (6281.8±1482.03 vs 7067.8±1087.38 U/L) but it was not statistically significant (p>0.05, Table 1). This study also showed decreased cord blood cholinesterase activity in babies born to normotensive mothers as compared to maternal levels.

The human placenta is not innervated by extrinsic or intrinsic cholinergic neurons.[15] Thus, released acetylcholine is not contaminated by neuronal acetylcholine. AchE, an organic cation (with a permanent positive net charge at physiological pH), is widely expressed in non-neuronal cells in humans, may be a substrate for organic cation transporters (OCT). Non-neuronal acetylcholine leaves placental cells via OCTs (ubiquitously expressed in humans) and acts in an auto-/paracrine manner. The activity of OCTs is modulated by a variety of drugs and endogenous compounds, like monoamines and steroids which can target the release of non-neuronal acetylcholine under physiological conditions. Also, adrenergic-cholinergic interactions may also occur at the level of non-neuronal acetylcholine. There is an up regulation of placental BChE as the first line of defense against poisons and drugs is associated to environmental OP (organophosphorus) exposure.[27]

It has been reported that in preeclampsia, muscarinic receptor are decreased more so in the umbilical artery than in vein. Thus, preeclampsia is characterized by a loss of muscarinic cholinergic receptors in the umbilical circulation not accompanied by change of acetylcholine metabolizing enzyme AChE. This might contribute to increased resistance of umbilical circulation occurring in preeclampsia.[28]

CONCLUSION

The findings of the present study indicates that decreased in cholinesterase levels in preeclampsia occurs via loss of muscrainic cholinergic receptors in preeclampsia. Further studies on muscrainic cholinergic receptors in preeclampsia would clarify this.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CE: Cholinerserase; **AChE:** Acetyl cholinesterase; **ACh:** Acetylcholine ; **BChE:** Butrylcholinersterase; **mAChR:** Muscarinic acetylchominer receptor; **nAChR:** nicotinic acetylcholinesterase receptor.

SUMMARY

The present study was conducted in the Department of Biochemistry, in collaboration with the Department of Obstetric and Gynecology, Pt. B.D. Sharma, Post Graduate Institute of Medical Sciences, Rohtak. Fifty pregnant women attending the outpatient department of Obsetric and Gynecology were enrolled. Also, 25 age matched healthy volunteers were enrolled as healthy control. These were divided into three groups:

- Group I: 25 normotensive women with singleton pregnancy at the time of delivery
- Group II: 25 age and gestation matched women with systolic blood pressure > 140 mmHg and diastolic blood pressure > 90 mmHg with or without proteinuria at the time of delivery
- Group III: age matched healthy volunteers

Serum cholinesterase levels were analyzed in maternal blood, cord blood and healthy volunteers.

The results and findings are summarized:

Cord blood cholinesterase levels were lowered in babies of normotensive (7067.8 + 1087.38 U/L vs. 7972.2 + 1544.15 U/L respectively; $p>0.05$) and cord blood CE levels were 88.65% of the maternal levels.

In Group II, cholinesterase levels were lowered in cord blood as compared to maternal blood (6281.8 + 1482.03 U/L vs. 7011.8 + 1073.12 U/L respectively; $p>0.05$) and cord blood CE levels were 89.58% of the maternal levels.

Cord CE levels were significantly lowered in preeclamptic as compared to normotensive pregnant (6281.8 + 1482.03 U/L vs. 7067.8 + 10.87.38 U/L; $p<0.058$)

On comparing CE levels with heathy control (Group III) it was observe that CE levels were significantly raised both in Group I and Group II (2167.02 + 533.98 U/L vs. 7972.2 + 1544.15 U/L and 7011.8 + 1073.12 U/L respectively; $p< 0.001$).

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