

# Cord Blood Butyrylcholinesterase Activities in Normal Pregnant and Preeclamptic Women

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## Abstract

**Background:** Human placenta, a non-neural tissue, contains cholinergic system and high-affinity muscarinic receptors. The role of cholinesterases (CE) in trophoblast function and pregnancy is not clear.

**Objectives:** The present study aimed to analyze cholinesterase (CE) levels in cord blood in preeclamptic women.

**Methods:** In the present study, maternal and cord blood butyrylcholinesterase levels were analyzed in women with preeclampsia (n = 25) and compared to those of normotensive pregnant women (n = 25) and normal, non-pregnant healthy controls (n = 25) by a kinetic method (the new DGKC method) using the AutoAnalyzer.

**Results:** In the present study, maternal butyrylcholinesterase levels were lower in preeclamptics as compared to normotensive controls. Butyrylcholinesterase levels were lower in the cord blood of the babies of normotensives, amounting to 88.65% of the maternal levels. Cord blood butyrylcholinesterase levels were significantly lower in preeclamptic pregnant women as compared to normotensive pregnant women. On comparing these butyrylcholinesterase levels to the normal, non-pregnant control (Group III), it was observed that CE levels were significantly lower in both normotensive and preeclamptic women.

**Conclusions:** The findings of the present study indicate that butyrylcholinesterase levels are lower in preeclamptics and that this might be due to the loss of muscarinic cholinergic receptors that occurs in preeclampsia.

**Keywords:** Cholinergic System, Cholinesterases, Pregnancy, Preeclamptics, Cord Blood

## 1. Background

Hypertensive disorders occur in around 7 - 10% of pregnancies and are the most common medical complications of pregnancy (1). The basic pathology of pregnancy-induced hypertension is intense vasospasm affecting the entire vascular system, especially renal, uterine, and cerebral vessels. This vasospasm probably occurs due to an increase in vasopressor agents, like angiotensin II, thromboxane A<sub>2</sub>, and endothelin-1, and a decrease in vasodilator agents, such as nitric oxide and prostacyclin, due to endothelial cell dysfunction (2).

In preeclampsia, the initiating event is reduced uteroplacental perfusion that occurs due to an abnormal cytotrophoblast invasion of spiral arterioles. Widespread activation/dysfunction of the maternal vascular endothelium occurs following placental ischemia, resulting in increased endothelin and thromboxane generation, decreased production of vasodilators (nitric oxide and prostacyclin), and increased vascular sensitivity to angiotensin II (2).

Cholinesterases (CE) are widely present in cholinergic and non-cholinergic tissues, plasma, and other body fluids (3-6).

Based on their substrate specificity, behavior in excess substrate, and susceptibility to inhibitors, they can be grouped as acetylcholinesterase, or "true cholinesterase", and butyrylcholinesterase (BChE or pseudocholinesterase, non-specific cholinesterase, or simply cholinesterase). AChE (acetylcholinesterase) hydrolyzes acetylcholine much faster than any other cholinesterase; however, it is much less active on butyrylcholine. In contrast, BChE preferentially acts on butyrylcholine and it also hydrolyzes acetylcholine (5, 7). AChE is found abundantly in brain, muscle, and erythrocyte membranes, whereas the higher activity of BChE occurs in the liver, intestines, heart, kidneys, and lungs (8, 9).

The non-neuronal cholinergic system is found in several tissues, such as keratocytes, cancer cells, immune cells, urinary bladder, airway epithelial cells, vascular endothelial cells, and reproductive organs (10). Components of the non-neuronal cholinergic system act via paracrine and autocrine mechanisms for controlling cell proliferation, differentiation, cell-cell interaction, and response to vari-

ous insults, including stress. The non-neuronal cholinergic system is known to be involved in the regulation of function, and cholinergic dysfunction is reported to be involved in the pathogenesis of various diseases (10).

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

Placental acetylcholine (ACh) varies with gestational age and reaches its peak at 20 - 22 weeks of gestation and then declines at term. This pattern is paralleled by ChAT activity, thereby pointing towards the involvement of the placental cholinergic system in the regulation of the developmental processes responsible for placental growth (12). Multiple muscarinic receptor (mAChR) subtypes and all subtypes of the nicotinic receptor (nAChR) alpha subunit are also present in the placenta (11).

ACh is an important placental signaling molecule. It stimulates nAChR, controls nutrient uptake, blood flow, and fluid volume in the placental vessels and vascularisation during placental development. Since the placental ChAT expression overlaps that of eNOS (endothelial nitric oxide synthase), the locally-produced ACh may stimulate eNOS via a  $Ca^{2+}$ -dependent mechanism (12). Kambam et al. reported that both normal and preeclamptic pregnancies are associated with decreased plasma cholinesterase activity and that preeclamptic pregnant women show a significant decrease in plasma cholinesterase activity as compared to normal pregnant women values (13).

## 2. Objectives

Conflicting data are available regarding cholinesterase in preeclampsia and its status in cord blood is unknown. Thus, the present study aimed to analyze cord blood cholinesterase levels in preeclamptic women.

## 3. Methods

The present study was carried out in the department of biochemistry in collaboration with the department of obstetrics and gynaecology, Pt. B.D. Sharma, PGIMS, Rohtak, India, from July 2011 to December 2012. Maternal and cord blood butyrylcholinesterase levels were analyzed in preeclampsia and compared with normotensive pregnant women values. Written informed consent was obtained from all the patients and this research protocol was approved by the institutional review board. Women with normal vaginal delivery were included in the study.

Women with a history of smoking, chronic hypertension, any metabolic disorder before or during pregnancy, or the presence of high-risk factors like anemia, heart disease, diabetes, and renal disease, were excluded.

Fifty pregnant women attending the Outpatient Department of Obstetrics and Gynecology were enrolled and were grouped as follows: GROUP I (normotensive pregnant control, n = 25); normotensive women with singleton pregnancy at the time of delivery. GROUP II (study, n = 25); age- and gestation-matched women with singleton pregnancy (systolic blood pressure reading  $\geq$  140 mmHg or diastolic blood pressure  $\geq$  90 mmHg with or without proteinuria at the time of delivery). GROUP III (healthy, non-pregnant controls, n = 25); age-matched, healthy volunteers.

Five ml of maternal blood was drawn from the subjects aseptically and serum was separated by centrifugation. Ten ml of umbilical cord blood was drawn and serum was separated. In the maternal and cord blood, routine investigations and serum cholinesterase levels were analyzed. Butyrylcholinesterase activity was measured in serum by a kinetic method (the new DGKC method) using the Auto-Analyzer (14). Butyrylcholinesterase catalyzes the hydrolysis of butyrylthiocholine substrate forming butyrate and thiocholine. Thiocholine reduces hexacyanoferrate (3) to hexacyanoferrate (2). The decrease of absorbance is read at 405 nm and is proportional to the activity of butyrylcholinesterase. The data so obtained was subjected to appropriate statistical analysis, namely Student's t-test and regression analysis.

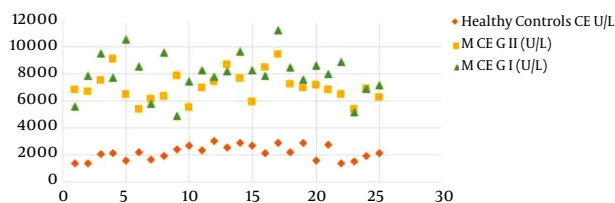
## 4. Results

Butyrylcholinesterase levels were lower in the maternal blood of preeclampsia when compared with the normotensive controls ( $P < 0.05$ , Table 1).

Cord blood butyrylcholinesterase levels were lower in the babies of normotensives ( $7067.8 \pm 1087.38$  U/L vs.  $7972.2 \pm 1544.15$  U/L respectively) as well, and cord blood butyrylcholinesterase levels were 88.65% of the maternal levels. In Group II, cholinesterase levels were lower in cord blood as compared to maternal blood ( $6281.8 \pm 1482.03$  U/L vs.  $7011.8 \pm 1073.12$  U/L;  $P < 0.05$ ), and cord blood CE levels were 89.58% of the maternal levels. Cord cholinesterase levels were significantly lower in preeclamptic, as compared to normotensive, pregnant women ( $6281.8 \pm 1482.03$  U/L vs.  $7067.8 \pm 1087.38$  U/L;  $P < 0.05$ ). On comparing CE levels with the normal, non-pregnant control (Group III), it was observed that CE levels were significantly lower, both in normotensive and preeclamptic women ( $8167.028 \pm 533.988$  U/L vs.  $7972.2 \pm 1544.15$  U/L,  $7011.8 \pm 1073.12$  U/L, respectively;  $P < 0.001$  in both cases, Figure 1 and Table 1).

**Table 1.** Maternal and Cord Blood Cholinesterase Levels (N=25)<sup>a</sup>

Cholinesterase, (U/L)	Maternal Levels	Cord Blood Levels
<b>Group I</b>	7972.2 ± 1544.15	7067.8 ± 1087.38
<b>Group II</b>	7011.8 ± 1073.12 <sup>b</sup>	6281.8 ± 1482.03 <sup>b</sup>
<b>Group III</b>	8167.028 ± 533.988	-

<sup>a</sup>Values are expressed as mean ± SD.<sup>b</sup>P < 0.05 as compared to Group I.**Figure 1.** CE Levels in Various Groups

## 5. Discussion

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

Conflicting reports are available regarding cholinesterase levels in pregnancy. In the present study, the cholinesterase levels of preeclamptic mothers (Group II) were lower than normotensive mothers ( $7011.8 \pm 1073.12$  vs.  $7972.2 \pm 1544.15$ ,  $P < 0.05$ , Table 1 and Figure 1).

Data produced by several investigators show that plasma cholinesterase activity declines during normal pregnancy (15-17), while others have reported that there is no change in plasma cholinesterase activity between preeclamptic and normal pregnancy (13, 18-20).

Plasma cholinesterase is a mucoprotein produced in the liver and preeclamptic and eclamptic pregnancies are associated with significant hepatic dysfunction (21). The decreased activity of cholinesterase levels in preeclamptic mothers may be attributed to hemodilution and hypoalbuminemia. Another cause for the decline in cholinesterase activity in cord sera may be preeclampsia-induced hepatic dysfunction (21, 22). Thus, low CE activity in cord blood may reflect smaller liver cell mass in newborns. Furthermore, investigators have reported low

serum CE and albumin concentrations in newborns (22).

Endothelial cells regulate placental perfusion. In vascular tissue, ACh is a well-known mediator for the release of nitric oxide (NO) and prostanoids and it acts via the activation of mAChRs (M3 and M1 subtypes). Moreover, blood flow, shear stress, body temperature, and local blood pressure may affect the synthesis and release of endothelial ACh with consequent modulation of the release of vasoactive mediators. AChE is widely expressed in non-neuronal cells in humans, and 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 release of non-neuronal acetylcholine from the human placenta is mediated via OCTs (of the OCT1 and OCT3 subtypes). OCT activity is modulated by a variety of drugs and endogenous compounds, such as monoamines and steroids. These agents can target the release of non-neuronal acetylcholine under physiological conditions and circulating catecholamines may also affect the release of non-neuronal acetylcholine (23).

Placental ChAT is localized to cytotrophoblast and some mesenchymal cells in human placenta. It may further be added that via muscarinic receptors ACh acts on trophoblastic cell membranes to modulate NO in an estrogen-dependent manner. It seems that the placental cholinergic system interacts with NO and estrogen-signaling pathways to regulate placental cell growth and/or function. In human placenta, cholinergic recognition sites, and possibly the placental cholinergic system, plays a significant role in the pathophysiology of preeclampsia. Since there is no nerve innervation in human placenta by extrinsic or intrinsic cholinergic neurons (15) the released acetylcholine is not contaminated by neuronal acetylcholine. The placental BChE is upregulated as the first line of defense against poisons and drugs and is associated with environmental OP (organophosphorus) exposure (24).

On comparing the CE levels with the normal, non-pregnant control group (Group III), it was observed that CE levels were significantly lower both in normotensive and preeclamptic women (Table 1,  $P < 0.001$  in both cases).

Plasma cholinesterase has been reported to significantly decrease during pregnancy and researchers have attributed this to altered hemodynamics in pregnancy (25). In the present study, cord blood cholinesterase levels of preeclamptics were lower when compared to those of normotensive pregnant women ( $6281.8 \pm 1482.03$  vs.  $7067.8 \pm 1087.38$  U/L,  $P < 0.05$ , Table 1).

It has been reported that muscarinic receptors are lower in the umbilical artery than they are in the vein in preeclampsia. Since preeclampsia is characterized by a loss of muscarinic cholinergic receptors in the umbilical circulation, it is not accompanied by changes in AChE. The loss of muscarinic cholinergic receptors might contribute to the increased resistance of umbilical circulation that occurs in preeclampsia (26). The findings of the present study support this, indicating that a decrease in via the loss of muscarinic cholinergic receptors occur in preeclampsia. Further studies on muscarinic cholinergic receptors in preeclampsia would clarify this. A limitation of the present study was that muscarinic cholinesterase receptors, anticholinesterase immunoreactivity in placenta, and cytokines could not be measured. Systemic treatment with muscarinic and/or nicotinic agonists may be helpful in women experiencing preeclampsia in future.

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