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CJOG CLINICAL JOURNAL OF OBSTETRICS AND GYNECOLOGY
ISSN 2640-2890

Research Article

Expression of Cholinesterase in Bone Tumors, Blood and Cord Blood

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Abstract

The present study aimed to analyze Cholinesterase (CE) levels in cord blood from preeclamptic women and to evaluate cholinesterase status in patients with osteosarcoma. Serum cholinesterase levels were assessed in 30 cases of osteosarcoma and 30 controls suffering from musculoskeletal pain. Additionally, maternal and cord blood samples were collected from 25 women with preeclampsia and compared with those from 25 normotensive pregnant women and 25 normal, healthy controls. The results indicated that serum cholinesterase levels were significantly lower in osteosarcoma patients (Group I) compared to those with musculoskeletal pain (Group II, $p < 0.05$). Similarly, cholinesterase levels were reduced in the maternal blood of women with preeclampsia when compared to normotensive controls. Cord blood cholinesterase levels were lower in the infants of normotensive mothers, with levels reaching 88.65% of the maternal levels. Furthermore, cord blood cholinesterase levels were significantly lower in preeclamptic women compared to normotensive pregnant women. When comparing cholinesterase levels to those of normal controls, it was observed that CE levels were significantly elevated in both normotensive and preeclamptic women. The findings of low serum cholinesterase levels in this study suggest that cholinesterase secreted by osteoblasts is utilized in bone formation and tumorigenesis. Additionally, the decrease in cholinesterase levels associated with preeclampsia may be linked to the loss of muscarinic cholinergic receptors that occur in this condition.

More Information

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Submitted: November 20, 2024
Approved: November 26, 2024
Published: November 27, 2024

How to cite this article: Kharb S, Nanda S, Kumar S, Kundu ZS. Expression of Cholinesterase in Bone Tumors, Blood and Cord Blood. Clin J Obstet Gynecol. 2024; 7(4): 120-124. Available from: https://dx.doi.org/10.29328/journal.cjog.1001178

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Keywords: Cholinesterase; Bone tumours; Blood; Cord blood; Pregnancy

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Introduction

Cholinesterase (ChE) enzymes catalyse the hydrolysis of choline esters, and acetylcholine (ACh) being the most important substrate. Also, AChE has The non-catalytic/ structural functions of AChE have a role in the proliferation and development of blood, retinal, and neuronal cells [1,2].

In acute hepatitis, cirrhosis of the liver, organophosphate poisoning, and some malignant tumours, levels of serum ChE are reduced. Recent studies have revealed the role of serum cholinesterase as a biomarker of various diseases in humans [3,4].

Low levels of cholinesterase have been demonstrated in cases of multiple myeloma and serum cholinesterase levels are reliable indicators for monitoring changes in behaviour and remission of multiple myeloma [3]. In the differentiating human osteosarcoma Saos-2 cells, Grisaru, et al. showed that morphogenic ally active 3′ alternative splicing variant of acetylcholinesterase increased and after midgestation in normal differentiating and post proliferative, fetal chondrocytes, the E6-AChE mRNA levels were raised however, this was not the case in the osteogenically impaired chondrocytes of dwarf fetuses with thanatophoric dysplasia. They suggested the morphogenic involvement of E6-AChE in maintaining the balance between proliferation and differentiation in human osteogenesis [5].

It has been reported that osteoblast-derived acetylcholinesterase is a novel mediator of cell-matrix interactions in bone. Osteosarcoma cell lines and primary cultures of osteoblasts have been reported to express AChE mRNA, indicating that AChE is a novel bone matrix protein that mediates cell-matrix interactions and bone formation and regulates remodeling [6].

A non-neural cholinergic system thus exists, along with the ubiquitous presence of cholinesterase and cholinergic receptors (nicotinic and muscarinic) [7]. Also, the cholinergic system has been demonstrated to exist in human endothelium and acetylcholine has multiple effects on the vascular endothelium-derived relaxing factor and cholinergic dysfunction is implicated in the pathophysiology of certain

diseases. The human placenta, being a non-neuronal tissue, contains an active cholinergic containing acetylcholine (ACh), choline acetyltransferase (ChAT), acetylcholinesterase (AChE), and high-affinity muscarinic receptors. In the human placenta, anti-ChAT (choline acetyltransferase) immunoreactivity in multiple sub-cellular compartments is found, and enhanced levels of acetylcholine have been detected in various inflammatory diseases [8]. Placental concentration of Ach varies with gestational age and reaches a peak at approximately 20-22 weeks gestation and declines toward term [9].

Conflicting reports regarding plasma cholinesterase activity in pre-eclamptic pregnancies are available in the literature [10]. Decreased plasma cholinesterase activity in pre-eclamptic pregnancy when compared to normal pregnancy has been documented in the literature [11]. On the other hand, others have reported no significant difference in plasma cholinesterase activity between normal pregnant and preeclamptic women [12]. The exact mechanisms of reduction in plasma cholinesterase activity in normal and eclamptic pregnancies are not known.

Given the scanty and conflicting literature on cholinesterase levels in bone, placenta, and blood, the present study was designed to assess serum cholinesterase levels in healthy adults, osteosarcoma patients, and normotensive pregnancy and preeclamptic.

Data and methods

Method

This study was done in the Departments of Biochemistry, Obstetrics and Gynaecology, and Orthopaedics Pt. B.D. Sharma, PGIMS, Rohtak. In this study, ninety subjects were included and sub-grouped as Group I (healthy Controls, n = 30); Group II (histologically confirmed cases of osteosarcoma with localised without metastasis, n = 30); Group III (pregnant women, n = 30 who were further sub-grouped as Group III A (healthy pregnant, n = 15) and group III B (preeclamptic women, n = 15). Women with a history of smoking, metabolic disorders, anaemia, heart disease, diabetes, or renal disease were excluded from the study. Women who delivered by normal vaginal delivery were included in the study after taking informed consent. Four mL of venous blood was drawn aseptically and serum was separated by centrifugation. Routine investigations were done by standard methods. Quantitative in vitro cholinesterase activity was measured in serum by kinetic method [12].

Statistical analysis

Data so obtained was analyzed statistically using Excel 2010 (14.0) and computed as mean ± SD, and Student's t-test was applied, and $p < 0.05$ was accepted as statistically significant.

Results

In the present study, serum calcium levels were significantly raised in Group I as compared to Group II ($p < 0.01$). Serum phosphorus levels were lower in group I as compared to group II and the results were comparable ($p > 0.05$). Serum alkaline phosphatase levels were significantly raised in Group I as compared to Group II ($p < 0.001$, Table 1).

Low serum cholinesterase levels were observed in group II (121.30 ± 40.80 µkat/L) when compared to group I (124.34 ± 27.87 µkat/L; p = 0.737, Table 2). In males, serum ChE levels were higher in group I compared to females (132.21 ± 30.78 µkat/L and 116.47 ± 23.0 µkat/L respectively, p = 0.124). In group II, serum ChE levels were significantly higher in males as compared to females (139.43 ± 39.21 µkat/L and 103.17 ± 37.74 µkat/L respectively, p = 0.012). No statistically significant difference could be found between males and females of groups I and II (p = 0.579 and p = 0.227, respectively).

Significantly higher serum cholinesterase levels were noted in Group I when compared to Group II (137.77 ± 29.93 µkat/L *vs.* 116.47 ± 23.0 µkat/L respectively, p = 0.007, Table 3). In preeclamptics, maternal serum cholinesterase levels were lowered as compared to healthy pregnant, although not statistically significant (121.09 ± 21.83 µkat/L *vs.* 137.77 ± 29.93 µkat/L; p = 0.092). Serum cholinesterase levels were higher in preeclamptic women when compared to osteosarcoma females, although non-significant statistically (121.09 ± 21.83 µkat/L and 103.17 ± 37.74 µkat/L respectively, p = 0.102).

Discussion

In several tissues, including keratocytes, cancer cells, immune cells, urinary bladder, airway epithelial cells, vascular endothelial cells, and reproductive organs, a non-neuronal cholinergic system has been reported. Cholinergic dysfunction has been implicated in the pathophysiology of certain diseases. The non-neuronal cholinergic components have local actions and act *via* paracrine and autocrine mechanisms to control basic cellular functions such as proliferation, differentiation, cell-cell interaction, and response to various insults including stress [13].

In human osteoblasts and chondrocytes, AChE is expressed depending on their proliferation and differentiation states. In osteoblast cells, nicotinic modulation of gene expression of MG-63 has been reported [14]. Also, acetylcholinesterase

Table 1: Serum Calcium, Phosphorus, and Alkaline Phosphatase Concentrations in Group I and Group II (MEAN ± S.D.).

Parameter	Group I	Group II
Serum calcium (mg/dL)	10.01 ± 0.58*	9.46 ± 0.70
Serum phosphorous (mg/dL)	4.41 ± 0.55	4.58 ± 0.51
Serum alkaline phosphatase (U/L)	331.73 ± 173.69**	120.23 ± 31.78

*$p < 0.01$ as compared to group II
**$p < 0.001$ as compared to group II

Table 2: CE (μkat/L) Levels in Osteosarcoma and Controls (Mean ± SD).

Control Male (n = 15)	Control Female (n = 15)	Group I (n = 30)	Cases Male (n = 15)	Cases Female (n = 15)	Group II (n = 30)
132.21 ± 30.78	116.47 ± 23.0	124.34 ± 27.87	139.43 ± 39.21*	103.17 ± 37.74	121.30 ± 40.80

*p < 0.01 as compared to group II females.

Table 3: CE (μkat/L) Levels in Osteosarcoma, Healthy Pregnant and Preeclamptics (Mean ± SD).

Control female (n = 15)	Osteosarcoma female (n = 15)	Healthy Pregnant (n = 15)	Preeclamptics (n = 15)
116.47 ± 23.0	103.17 ± 37.74	137.77 ± 29.93*	121.09 ± 21.83

*p < 0.01 as compared to group I females.

derived from osteoblasts has been reported to mediate bone cell-matrix interactions. Osteosarcoma cell lines and primary cultures of osteoblast express AChE mRNA, and tissue immunohistochemistry localization confirmed this, indicating that it may be a principal participant in organized bone formation and regulates remodeling [15].

In the present study, decreased serum cholinesterase levels were observed in osteosarcoma patients when compared to group I (p > 0.05, Table 1). Therapeutic benefits of serum cholinesterase levels have been reported in multiple myeloma patients, and the initial low value of cholinesterase detected in these patients indicated that cholinesterase has a role in the pathology of tumours [16]. Acetylcholinesterase expression occurs at the sites of new bone formation, being regulated by osteogenic stimuli. Findings of low levels of serum cholinesterase levels in the present study demonstrate that AChE secreted by osteoblasts is consumed in bone formation and tumorigenesis. Studies have reported that AChE inhibitors decrease osteoblastic adhesions in MC3T3-EI cells and HOBs cultures [17].

Significantly decreased levels of serum cholinesterase levels were noted in Group II when compared to healthy pregnant. Serum cholinesterase levels were comparable in both groups and slightly higher in preeclamptic than in osteosarcoma females.

Significant changes in maternal bone metabolism occur during pregnancy, and the mechanism of maternal placental–fetal mineral homeostasis and skeletal development during pregnancy are still unclear. Cytokines and hormones derived from maternal sources possibly influence the placental calcium transport to the fetus and have a direct effect on the placental function. During early fetal haematopoiesis, non-neuronal cholinergic system components are expressed, and the colonization of the fetal bone with haematopoietic stem/progenitor cells is affected by nicotine [18]. Nicotine and endothelial ACh are proangiogenic factors, and nicotine has been reported to promote atherosclerotic plaque growth, potentiate endothelial–monocyte interactions, and endothelial progenitor cells incorporation into newly established vessels. Nicotine has also been demonstrated to stimulate angiogenesis during inflammation, ischemia, tumour, or atherosclerosis and promotes the growth of atherosclerotic plaques, tumours, and pathological angiogenesis [19].

Conflicting reports are available regarding the status of cholinesterase levels in pregnancy. In the present study, serum cholinesterase levels of preeclampsia mothers were comparable and slightly lower when compared to normotensive mothers. Several workers have shown that plasma cholinesterase activity declines during normal pregnancy [17,20,21]. While others have reported no difference in plasma cholinesterase activity between preeclamptic and normal pregnancy [22-24].

In preeclamptic, decreased serum cholinesterase activity might be due to hemodilution and hypoalbuminemia. Also, preeclampsia-induced hepatic dysfunction may be another cause for this decline [25]. Lowered CE activity in cord blood may be due to smaller liver cell mass in newborns. Also, low serum CE and albumin concentrations in cord sera have been reported in the literature, indicating a lower liver function during this period of life [26].

Perfusion is regulated by endothelial cells and in vascular tissue via activation of mAChRs (M3 and M1 subtypes), ACh acts as a mediator for releasing Nitric Oxide (NO), endothelium-derived hyperpolarizing factor, and prostanoids. Non-neuronal acetylcholine release from the human placenta is mediated by organic cation transporters (OCTs: OCT1 and OCT3 subtypes). In cytotrophoblast and some mesenchymal cells in the human placenta, ChAT is localised. ACh modulates NO by acting on trophoblast cell membrane muscarinic receptors, and these signalling interactions may have a physiological relevance at the maternal-fetal interface. It has been demonstrated that in the placenta, cholinergic recognition sites exist, and the placental cholinergic system may have a possible significant role in the pathophysiology of preeclampsia. In the present study, CE levels were significantly raised both in normotensive as well as preeclamptic women (p < 0.001 in both cases).

Nicotinic acetylcholine receptors have been reported to mediate angiogenesis in response to diverse stimuli, and an endogenous cholinergic pathway exists for angiogenesis [27] that might play a role in pathological and therapeutic angiogenesis. In human cytotrophoblast cells and immune cells, the non-neuronal cholinergic system is expressed as having homeostatic regulatory functions [28-30]. The results of the present study support a novel role of acetylcholine derived from trophoblast cells for modulating antigen-presenting cell migration and activation, favoring an immunosuppressant profile at the maternal-fetal interface during pregnancy for the maintenance of immune homeostasis.

Endogenous acetylcholine in the normal human maternal–placental interaction has a boosting effect. In microarray studies of term placenta from women treated with choline, it was shown that placental and circulating levels of the anti-angiogenic factor fms-like tyrosine kinase-1 (sFLT1) were decreased, and they proposed it as a pre-eclampsia risk biomarker [28]. High maternal choline intake among third-trimester pregnant women has been reported to lower placental and circulating concentrations of the antiangiogenic factor fms-like tyrosine kinase-1 (sFLT1) that was further confirmed in human trophoblast cell lines, and this was found to be associated with enhanced signalling of acetylcholine. In contrast, another study reported that pregnancies complicated by preeclampsia have very low ACh synthesis in the trophoblast cells that are undergoing advanced cell degeneration.

The findings of the present study lend support to the studies suggesting that cholinesterase secreted by osteoblasts is utilized in bone formation and tumorigenesis [31-33].

Limitations and, future research directions

The sample size was small, and it was conducted at a single institution. A larger cohort of multi-centric settings could provide more statistically robust results. Bone tumors comprise a range of histological subtypes having potentially different cholinesterase expression patterns. In the present study, only the osteosarcoma cases without metastasis were collected, and limited stratification of data by tumor type may obscure subtype-specific trends.

No follow-up was possible in this study, and incorporating follow-up data in larger, multi-centric cohorts to observe cholinesterase expression changes over time and during treatment can offer insights into its potential as a prognostic marker.

Future studies investigating the relevance of cord blood cholinesterase expression are required in predicting future risk or susceptibility to bone tumors or other malignancies.

Conclusion

The findings of the present study suggest that dysregulation of the non-cholinergic system occurs in various diseases and this system can be a promising target for developing newer therapeutic strategies. Integration of cholinesterase activity analysis in future research with other biomarkers, along with transcriptomics or proteomics, would elucidate the clinical relevance of cholinesterase in bone tumors and cholinergic dysfunction during pregnancy and its potential as a diagnostic, prognostic, or therapeutic biomarker.

Declaration

Ethical declaration: Informed patient consent was obtained for the data publication from all the groups involved in the study (Groups I-IV), and the study was approved by the Institutional Review Board for the study. The study was conducted adhering to the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Pt. BDS PGIMS, Rohtak, for studies involving humans.

Informed consent statement: Written Informed consent was obtained from all subjects involved in the study.

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