



## CHOLINESTERASE AS MARKER OF CONGENITAL ANOMALIES

## Biochemistry

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## ABSTRACT

Increase incidence of congenital abnormalities in north-west India suggests the role of some environmental factor. As N-W India is predominantly an agricultural community and pesticides exposure are hypothesized to be one of the causative agents of it. The present study was planned to analyse the levels of cord blood cholinesterase in newborns with congenital anomalies. Thirty healthy newborns and thirty newborns with visible congenital anomalies are selected as control and cases respectively. Detailed history of present pregnancy were taken and detailed physical examination of babies were done. Five ml cord blood was collected from placental end of umbilical cord after delivery of baby and serum was separated and serum cholinesterase were estimated enzymatically. Serum cholinesterase levels were significantly decreased in babies with congenital anomalies as compared to healthy counterparts. Low cholinesterase levels can be caused by various agricultural and industrial pollutant in this region and might result in congenital anomalies in this region.

## KEYWORDS

Cholinesterase, congenital anomalies, cord blood, newborns, pesticides

## INTRODUCTION

Organophosphate (OP) or carbamate esters are used as pesticides, insecticides, chemical warfare agent and drugs for treatment of medical disorders such as glaucoma, parasite infections and Alzheimer's disease. These compounds are potent inhibitors of cholinesterase. Serum cholinesterase levels have been reported to be reduced in acute hepatitis, cirrhosis of liver, organophosphorous poisoning and in some malignant tumors. Acetylcholine (ACh) and other neurotransmitters play unique trophic roles in the development of the CNS and inhibition of acetylcholinesterase (AChE) by organophosphates (OPs) and the resulting accumulation of acetylcholine may then conceivably disturb this development. Maternal serum acetylcholinesterase levels have been reported to be low and independent of gestational age between 14-20 weeks in a sample of normal pregnancies with normal alpha fetoprotein levels. Raised levels of maternal serum secretory acetylcholinesterase were found in 100% of pregnancies involving spina bifida and three of four anencephalics.<sup>1</sup>

The prevalence of congenital abnormalities have increased manifold.<sup>2-4</sup> Increase incidence of congenital abnormalities in north-west India suggests the role of some environmental factor as N-W India is predominantly an agricultural community and pesticides exposure are hypothesized to be one of the causative agents.

However, no such studies are available in Indian context especially in Haryana. Hence the present study was planned to evaluate cholinesterase levels in cord blood as a marker for congenital anomalies in Indian population to collect baseline data so that preventive and diagnostic measures can be planned in environmental health studies for Indian children.

## MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry in collaboration with Department of Obstetrics & Gynecology, Pandit Bhagwat Dayal Sharma PGIMS, Rohtak. Sixty babies delivered in the labour room were selected for the study and grouped as: Group I: Newborn infants with visible congenital anomalies (n=30); Group II: Healthy newborn infants without congenital anomalies (n=30). All the patients were subjected to detailed history and clinical examination. Also, examination of parents for nutritional deficiency, pesticide/heavy metal exposure was done. Examination of baby was carried out for the site of defect, neurological deficit if any, detailed radiological investigation.

To analyse the association with cholinesterase variables such as occupation, age, sex, type of deformity, any use of medications prior to conception or during pregnancy, medical history of parents; history of smoking, alcohol intake, passive smoking history, pesticide exposure were also noted.

Five mL cord blood was collected from placental end of umbilical cord (vein) after delivery of the baby. The serum was separated by centrifugation and routine investigations were done as per standard methods by autoanalyzer. Serum (cord blood) cholinesterase were measured by Ellman's reaction using standard kit in autoanalyzer.<sup>5</sup> The data so obtained was subjected to appropriate statistical analysis namely Student's t-test.

## RESULTS

Cleft lip (n=7) was the most common congenital anomaly in the present study, while CTEV was second most common (n=6). Hypospadias, polydactyly, spina bifida showed similar distribution with 3 cases each; limb defect (DDH) was seen only in two cases. Omphalocele, anencephaly, gastroschisis, encephalocele, cleft palate and imperforate anus was seen in 1 case each.

• **Table 1 – Serum Cholinesterase in both groups Mean ± SD**

Parameter	Group I (n=30)	Group II (n=30)
Serum cholinesterase (U/L)	3575.90 ± 1200.65*	4784.33 ± 1265.04
Range	2089 – 6786	1203 – 7403

\* $p < 0.001$  as compared to group II (Controls)

In the present study, serum cholinesterase levels were significantly decreased in babies with congenital anomalies as compared to healthy counterparts ( $p=0.000$ , Table 1).

On analysing cord blood cholinesterase levels according to occupation, it was observed that there was significant decrease in serum cholinesterase levels in babies born to the mothers whose occupation were farmers when compared to housewives and service women (Table 2).

**Table 2 – Serum Cholinesterase levels according to occupation in Group I Mean ± SD**

Parameters	Farmers (n=7)	Housewives (n=16)	Service women (n=7)
Serum cholinesterase levels (U/L)	2657.14 ± 487.36**	3723.0 ± 1261.85	4158.43 ± 1145.02

\*\* $p < 0.05$  as compared to group housewives and service women

**Table 3 - District wise distribution of serum cholinesterase levels in cases (Mean ± SD)**

District (No. of cases)	Serum Cholinesterase (U/L)
Sonipat (7)	3081.71 ± 795.48
Panipat (6)	2815.33 ± 293.53
Bhiwani (5)	3851.40 ± 1896.24
Jind (5)	3696.40 ± 1389.06

Rohtak (3)	4135.33 ± 111.51
Hisar (3)	5369.37 ± 1583.55
Jhajjar (1)	3569.00 ± 0.000

Serum cholinesterase levels were lowest in babies from Panipat district and highest in Hisar (Table 3).

Babies with encephalocele (n=1), anencephaly (n=1), omphalocele (n=1) and hypospadias (n=3) had lower serum cholinesterase levels than other congenital anomalies in group I (2089 ± 0.000, 2126 ± 0.000, 2496 ± 0.000 and 2916.33 ± 375.30 U/L respectively though other congenital anomalies also have lower serum cholinesterase levels compared to control group.

## DISCUSSION

Serum cholinesterase found in the liver, pancreas, heart, white matter of the brain, and serum. Plasma ChE is produced by the liver in man and animals.

No data is available in literature regarding serum (of cord blood) cholinesterase levels in newborns with congenital anomalies. In a study by Elejalde et al. a group of 27 abnormal pregnancies demonstrated that fetal vomiting and regurgitation, fetal demise, multiple cysts syndrome, idiopathic IUGR, arthrogryposis multiplex, hydrocephaly (stenosis of aqueductus), trisomy 21, trisomy 18, hydronephrosis, pyloric stenosis, heart malformation, ectopia cordis and multiple gestation produced elevated levels of pseudocholinesterase (PChE) in amniotic fluid.<sup>6</sup> Venkataraman et al. also reported that there is a decrease in plasma cholinesterase in normal pregnant woman during the 2<sup>nd</sup> and 3<sup>rd</sup> trimester.<sup>7</sup> But, unlike plasma cholinesterase, acetylcholinesterase levels are significantly increased during pregnancy.<sup>8,9</sup>

In the present study, serum cholinesterase levels were significantly decreased in babies with congenital anomalies as compared to healthy counterparts (p=0.000, Table 1).

On analyzing cord blood cholinesterase levels according to occupation in the present study, it was observed that there was significant decrease in serum cholinesterase levels in babies born to the mothers whose occupation were farmers when compared to housewives and service women. (Table – 2).

Farahat et al. reported that organophosphorous exposure causes decrease in serum cholinesterase level and that causes adverse perinatal outcome in terms of reduced gestational age, low birth weight and small head circumference.<sup>10</sup> Willis et al. on the other hand found no association of pesticides exposure and adverse birth outcome.<sup>11</sup>

Workers in agriculture and in organic chemical industries are subject to poisoning by inhalation or by direct contact with various OP compounds. In addition to OPs and carbamate insecticides, pesticides from different chemical family, heavy metals, polycyclic aromatic hydrocarbons and nanoparticles have been reported to decrease ChE activity. Thus, mixed exposure of pesticides and toxic elements can cause a significant reduction in ChE activity.

Exposure to higher but sub-toxic level of pesticides causes increase in BuChE level. This paradoxical effect was attributed to enhanced BuChE synthesis as a natural protective mechanism against sub-toxic pesticides exposure.<sup>12</sup>

It has been postulated that organophosphorus pesticides indirectly generate oxidant molecules that attack cell membranes resulting in lipid peroxidation and further interference with membrane dependent process. AChE is also known to be particularly sensitive to this oxidative/nitrosative stress. In particular, pyrethroids caused a decrease in erythrocytes and brain AChE which was related to increased lipid peroxidation.

It has been reported that rivers flowing through Haryana have been contaminated by domestic and industrial wastes along with agricultural runoff and excessive amount of pesticides were detected in the river water.<sup>13</sup> As river water is one of the major source of drinking water in Haryana, its contamination with pesticides can cause various harmful effects! Lowest ChE level in babies from Panipat district can be appreciated by maximum number of factories and industries in this district (Table – 3).

With respect to developmental neurotoxicity of organophosphates (OPs) in humans, knowledge is still relatively sparse, and most studies reflect exposures to more than one pesticide.

Findings of low cord blood cholinesterase is possibly due to contamination of drinking water by toxic chemicals of industrial waste or pesticide used in the fields. It is evident in the present study that babies of farmers with congenital anomalies has lower levels of cholinesterase, which may attribute to the causation of congenital anomalies in the newborns.

## CONCLUSION

Findings of the present study suggest that low cord blood cholinesterase levels can be caused by various agricultural and industrial pollutant in this region and its effect on development might result in congenital anomalies in this region. The data presented may serve as baseline in planning precautionary approaches to reduce community exposure to environmental pollution.

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