A Study of Erythrocyte Cholinesterase in Comparison with Serum Cholinesterase Activity in Organophosphorous Poisoning Patients

Gagandeep Sidhu¹*, Shubhra Jandial², Asha Khubchandani³

¹Associate Professor, Department of Biochemistry, GMERS Medical College, Sola, Ahmedabad.
²Associate Professor, Department of Biochemistry, Medical College, Baroda
³Associate Professor, Department of Biochemistry, B.J. Medical College, Ahmedabad

ABSTRACT
BACKGROUND: To investigate the relative values of erythrocyte and serum cholinesterase as biochemical markers in clinical assessment of Organophosphorous poisoning patients related to severity grade at presentation, requirement of ventilator support and follow-up of values of patients who required ventilator support.

MATERIALS AND METHODS: Serum cholinesterase and Erythrocyte cholinesterase were estimated in 35 patients with history of Organophosphorous poisoning. Estimation was done at the time of presentation/day 1, day 2 and day 4. Grading of severity was done according to Modified Point System. For baseline values, 30 individuals were included as controls.

RESULTS: The Erythrocyte cholinesterase activity at the time of presentation was significant in all grades of severity (p-value up to 5% in grade I, 2% in grade II and up to 0.1% in grade III, IV and V). The serum cholinesterase values were not significant up to 10% in grade I, II, III; however, the values were significant up to 1% in grade IV and 0.1% in grade V. In relation to requirement of ventilator support, erythrocyte cholinesterase values showed higher significance (t-value = 4.72) than serum cholinesterase values (t-value = 3.23). In follow-up values it was observed that erythrocyte cholinesterase values remained suppressed and showed no statistical significance difference in day 1 and day 2 or day 4 values whereas serum cholinesterase showed statistical significance difference in day 1 and day 4 values (p < 0.05).

CONCLUSION: The erythrocyte cholinesterase values could more significantly relate to the clinical toxicity profile in organophosphorous poisoning patients and reflect toxicity in a linear manner whereas the wide variation about the mean limits the diagnostic and prognostic value of serum cholinesterase.

Keywords: Erythrocyte cholinesterase, Serum cholinesterase, Organophosphorous poisoning

INTRODUCTION
Organophosphates are one of most widely used insecticides world over. Acute poisoning with these compounds is dangerous and necessitates emergency medical support. The primary mechanism of action of organophosphate pesticides is inhibition of carboxyl ester hydrolases, particularly acetylcholinesterase. Acetylcholine esterase is an enzyme that degrades the neurotransmitter acetylcholine into choline and acetic acid. Acetylcholine is found in the central and peripheral nervous system, neuromuscular junctions, and red blood cells (RBC). Organophosphates inactivateacetylcholinesterase by phosphorylating the serine hydroxyl group located at the active site of acetylcholinesterase. The phosphorylation occurs by loss of an organophosphate leaving group and establishment of a covalent bond with acetylcholinesterase. Once acetylcholinesterase has been inactivated, acetylcholine accumulates throughout the nervous system, resulting in overstimulation of muscarinic and nicotinic receptors. Clinical effects are manifested via activation of the autonomic and central nervous systems and at nicotinic receptors on skeletal muscle. Confirmation of organophosphate poisoning is based on the measurement of cholinesterase activity. Although RBC and plasma(pseudo) cholinesterase levels can both be used, RBC cholinesterase correlates better with CNS acetylcholinesterase since it represents the acetylcholinesterase found on RBC membranes, similar to that found in neuronal tissue. Therefore, the values more accurately reflect nervous system acetylcholinesterase inhibition. Plasma cholinesterase is a liver acute-phase protein that circulates in the blood plasma. It is found in CNS white matter, the pancreas, and the heart. It can be affected by many factors, including pregnancy, infection, and medical illness. RBC cholinesterase is the more accurate of the 2 measurements, but plasma cholinesterase is easier to assay and is more readily available. This study compares the clinical profile of organophosphorous poisoning in relation to erythrocyte and serum cholinesterase values.

*Corresponding Author
Dr. Gagandeep Sidhu,
Asso. Professor, Department of Biochemistry,
GMERS Medical College,
Sola, Ahmedabad
Email: drgagandeepsidhu@rediffmail.com
Erythrocyte Cholinesterase in Comparison with Serum Cholinesterase Activity

MATERIALS AND METHODS
The study was conducted in Medical College, Baroda. The inclusion criteria was history of organophosphorous poisoning and patients having clinical features suggestive of anticholinesterase poisoning. The pediatric patients and patients with other life threatening conditions or consumption of other drugs like opiates, barbiturates, benzodiazepines with poisoning were excluded. 35 patients from Emergency Medicine Ward, Sri Sayaji General Hospital, Baroda were included in the study. 30 individuals were taken as controls for baseline values. Estimation of serum cholinesterase (millimol/min/ml) was estimated by Ellman method. The mean activities in the control group including both males and females were 4848.56 +/- 782.74 millimol/min/ml blood ranging from 4065.82 to 5631.30 millimol/ml/min of blood. The patients’ clinical condition was graded according to Modified Point System score (Table 1).

Severity score is the sum of all scores of different symptoms and signs present in the patient as according to the Modified Point System. Grade Severity score
I < 6 - mild
II 7 – 8 - moderate
III 9 – 16 - moderate to severe
IV 17 – 24 - severe
V 24 - very severe/life threatening

RESULTS
The results obtained showed that the difference between serum cholinesterase values and their lower normal means was not significant upto 10 % in Ist, IInd and IIIrd grade at time of presentation according to the Modified Point System. The values were significant upto 1 % in grade IV and upto 0.1% in grade V. However, the values for erythrocyte cholinesterase were significant from its lower normal mean in all the grades of patients showing toxicity. The p-value showed significance upto 5% in grade I; 2% in grade II and upto 0.1% in grades III, IV and V implying that erythrocyte cholinesterase activity showed highly significant correlation with toxicity.

Table1: Relation between severity grade at presentation and serum and erythrocyte cholinesterase values

<table>
<thead>
<tr>
<th>Grade at presentation</th>
<th>Serum Cholinesterase</th>
<th>Erythrocyte Cholinesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>MEAN +/- S.D (units/l)</td>
</tr>
<tr>
<td>I</td>
<td>9</td>
<td>5091.3 +/- 3424.77</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>2405 +/- 1605</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>3049.8 +/- 1979.74</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>1901.66 +/- 1100.8</td>
</tr>
<tr>
<td>V</td>
<td>13</td>
<td>1642.69 +/- 1044.18</td>
</tr>
</tbody>
</table>

Table 2: Comparison of serum and erythrocyte cholinesterase in relation to requirement of ventilator support

<table>
<thead>
<tr>
<th>Required Ventilation</th>
<th>Serum Cholinesterase</th>
<th>Erythrocyte Cholinesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>MEAN +/- S.D (units/l)</td>
</tr>
<tr>
<td>Required Ventilation</td>
<td>15</td>
<td>1541.93 +/- 994.73</td>
</tr>
<tr>
<td>Did not require ventilation</td>
<td>20</td>
<td>3775.85 +/- 2865.83</td>
</tr>
</tbody>
</table>

Table 3: Follow up values of serum and erythrocyte cholinesterase activity on day 2 and day 4 were as

(A) Follow up values for serum cholinesterase activity on day 2 and day 4 were as

<table>
<thead>
<tr>
<th>N =14</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>1558.07</td>
<td>1854.14</td>
<td>2760.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>1031.22</td>
<td>1091.29</td>
<td>1795.75</td>
</tr>
</tbody>
</table>

(B). Follow up values for erythrocyte cholinesterase activity on day 2 and day 4 were as

<table>
<thead>
<tr>
<th>N =14</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>701.85</td>
<td>836.92</td>
<td>1024.85</td>
</tr>
<tr>
<td>S.D.</td>
<td>465.96</td>
<td>546.59</td>
<td>566.21</td>
</tr>
</tbody>
</table>

Combined standard error between:
Mean values of day 1 and day 2 – 191.95 +/- 0.70,
Difference – not significant.
Mean values of day 1 and day 4 – 195.97 +/- 1.64,
Difference- not significant.
The comparison by taking the combined standard error showed that serum cholinesterase values showed no statistical significant difference on day 1 and day 2 but the difference was significant in day 1 and day 4 (p<0.05). The mean values of serum cholinesterase on day 4 reach the mean values in the second grade of severity. This could be due to the early reactivation of serum cholinesterase. The erythrocyte cholinesterase values on day 2 and also day 4 remained suppressed and showed no
statistically significant difference from mean of values on day 1.

**DISCUSSION**

Both the biochemical markers have proved to reflect clinical toxicity profile and can be considered as valid surrogate markers of toxicity. Being readily available, serum cholinesterase can prove as an auxiliary test but it has value only when combined with appropriate clinical judgement. This contrasts with erythrocyte cholinesterase in that its value per se can prove as a reliable judge for clinical prediction in organophosphorous poisoning. Serum cholinesterase levels are altered in many conditions, it is decreased in organophosphorous poisoning, hepatitis, liver failure, eczema, epilepsy, kwashiorkor, carcinoma, early pregnancy, genetic variation, renal ischemia. The levels are increased in nephrotic syndrome, Diabetes, asthma, hyperthyroidism, toxemia in later pregnancy. In organophosphorous poisoning, serum cholinesterase is estimated as a surrogate of the inhibition by the compound of acetylcholinesterase in synapses and neuromuscular junctions. There are reports of occurrence of severe toxicity to development of intermediate syndrome in all patients with serum cholinesterase less than 10% of normal values at the time of admission. The values are useful in monitoring of organophosphorous poisoning. Serum cholinesterase recovery of more than 10% at 72 hours correlate with good prognosis. However if the value is more than 10%, the situation may be less clear due to issue of other conditions affecting the values and also wide variation within and between populations. Erythrocyte cholinesterase has inhibitor and substrate susceptibility profiles of that the acetylcholinesterase in the synapses and neuromuscular junctions. So, it carries the toxicity profile much closer than that provided by serum cholinesterase. Erythrocyte cholinesterase also excludes the problems with inter and intra population variations seen with butryl cholinesterase and discounts conditions in which it may be falsely low or high. So considering its identity with synaptic acetyl cholinesterase it is a better biochemical parameter for severity of toxicity. The study of follow-up values showed that serum cholinesterase regenerates at an exponential rate with a half life of about 12 days and recovery being complete after 50 days. The recovery of erythrocyte cholinesterase is more linear over time, attaining baseline value by about 82 days. The serum cholinesterase restoration rate is commensurate with the de novo synthesis rate of serum cholinesterase while erythrocyte cholinesterase restoration rate is slightly less than the life span of red blood cells, probably due to some degree of spontaneous reactivation. The estimated recovery rate for serum cholinesterase is 25% in the first week, while that for erythrocyte cholinesterase is 1% per day.

However, there are drawbacks regarding erythrocyte cholinesterase. It is a membrane bound enzyme and its estimation involves serial washing of red blood cells and precautions are required to avoid the serum cholinesterase activity from confounding the results. Also, its levels may be seen higher in younger red blood cells rich in reticulocytes.

**REFERENCES**