

## Comparison of Red Cell Cholinesterase and Plasma Cholinesterase Activities in Early Detection of Organo-Phosphorus Toxicity in Exposed Industrial Workers in Port Harcourt, Nigeria

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### ABSTRACT

**Background:** The Niger Delta region of Nigeria has a high concentration of companies using organo-phosphorus compounds with workers who have been exposed to these compounds for varying lengths of time resulting in toxic manifestations in some of the workers. Studies have revealed that plasma cholinesterase and red cell cholinesterase levels are reduced following organo-phosphorus toxicity. This study looks at cholinesterase levels in plasma and red cell in the occupationally exposed groups and control with a view to determining which will be more useful in the early detection of organo-phosphorus toxicity.

**Method:** Forty seven subjects with early symptoms of organo-phosphorus toxicity who have worked for more than 4 years with organo-phosphorus compounds were selected from five companies localized around the same area in Port Harcourt. Fifty subjects who do not work directly with organo-phosphorus compounds from the same companies as the 47 workers above but not working directly in the organo-phosphorus units were also studied while 50 persons working in the civil service were recruited as controls. The kinetic colorimetric method was used for measuring plasma and red cell cholinesterase activity.

**Result:** The results showed significant reduction in the mean plasma cholinesterase activity in the chronically exposed subjects ( $4614. \pm 532$ ) who showed symptoms of toxicity, when compared with that of the control group ( $8095 \pm 575$ ). There was no statistical difference in the mean red cell cholinesterase activity of the chronically exposed group ( $7998. \pm 948$ ) when compared with that of the control ( $8115. \pm 712$ )  $P > 0.05$

**Conclusion:** The effect of organo-phosphorus poisoning occurs early and is more marked in plasma cholinesterase than in the red cell cholinesterase. Plasma cholinesterase is thus more useful in early detection of organo-phosphorus toxicity than red cell cholinesterase.

**KEYWORDS:** Comparison; Plasma and red cell; Cholinesterase; Organo-phosphorus toxicity.

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### INTRODUCTION

Cholinesterases (Pseudocholinesterase which occurs in plasma EC.3.1.1.8 and red cell or "true" or acetylcholinesterase EC. 3.11.7) are enzymes that break down acetylcholine into choline and acetate. Succinyl choline a neuromuscular blocking agent that is widely used for producing muscle relaxation during surgery is also broken down by these enzymes.<sup>1-4</sup> The absence or reduced concentration/activity of cholinesterase leads to the uninhibited action of acetylcholine at the myoneural junctions. The effects of this is the development of symptoms and signs like miosis, blurred vision, muscle weakness, twitching, fasciculation, bradycardia, nausea, diarrhoea, vomiting, salivation, sweating, lacrimation, running nose, pulmonary oedema, arrhythmias, convulsion and in more severe cases, death<sup>5</sup>.

Organo-phosphorus compounds are chemical agents that inhibit the activity of cholinesterases in red cell and therefore, lead to the development of the above symptoms and signs<sup>6-8</sup>.

The organo-phosphorus compounds are used widely in making chemical weapons, insecticides and also by some oil companies in their chemical plants in the process of drilling for crude oil. These compounds can be absorbed into the body through the skin, by inhalation or accidental ingestion<sup>5,8</sup>. When absorbed in excessive quantities, they are toxic to the body and produce symptoms and signs described previously<sup>5</sup>. In Port Harcourt, there are a number of oil/oil service companies, and Agro-chemical industries in the Trans-Amadi industrial Area. These companies utilize organo-phosphorus compounds for components of certain drilling process and for the production of insecticides and pesticides respectively. Staff's working in these companies are therefore exposed to these chemicals. Studies carried out in other parts of the world have shown a reduction in plasma and red cell cholinesterase levels in individuals with prolonged occupational exposure to organo-phosphorus compounds<sup>5,9</sup>. This study examines and compares the usefulness of Red cell or plasma cholinesterase for the early detection of organo-phosphorus toxicity before the onset of largely non-specific symptoms.

## MATERIALS AND METHODS

A total of 147 subjects were used for this study. At the start of the study, questionnaires with specific questions aimed at elucidating the presence and frequency of symptoms suggestive of organo-phosphorus toxicity were distributed to all staff of the following companies: Baker Hughes, Agricultural development company (ADP), West African Oil Services (WAOS), African Oilfield Services (AOS), Pest Control Chemical Company (PCCC) all situated at the Trans-Amadi area of Port Harcourt. Ninety percent of the field staff in these companies and indeed all that took part in this study are from the Niger Delta area of Nigeria.

Fourty seven subjects who manifested symptoms suggestive of organophosphorus toxicity all of whom had worked in the organo-phosphorus units of the companies for more than four consecutive years were selected following a screening process using a questionnaire and individual interview. Fifty other subjects were selected (10 from each of the five companies) of staff who work in the companies but not directly in the organo-phosphorus units and who have no contact with organo-phosphorus compounds. 50 subjects were also selected from the state secretariat complex (Rivers State Secretariat) to act as control.

Subjects were aged between 30 and 45 years. An informed consent was obtained from all participants in this study while a corporate consent was also obtained from each of the five companies involved in this study. Approval was also obtained from the ethical committee of the University of Port Harcourt Teaching Hospital.

Workers in all the companies involved agreed to adequate provision and use of personal protective equipment. No subject used in this study had hemolytic anemia or chronic liver disease.

Samples were collected from all subjects over a period of one month. All samples were collected in the field (i.e. at the companies' operational sites and at the Rivers State secretariat complex-for controls) for convenience of subjects and for effective compliance.

Subjects were in sitting position and made to relax comfortably during sample collection. About 5ml of venous blood was collected from each subject into an EDTA (Ethylene Diamine Tetraacetic Acid) bottle<sup>4</sup>.

Samples were centrifuged at 2500g within 1 hour of collection. Plasma extracts were separated into plain tubes and stored at 2-8°C for 24-48hr. Red Cells were treated and re-centrifuged and stored at 2-8°C for 24-48hr<sup>4</sup>.

Samples were batch analyzed for plasma and red cell cholinesterase using kinetic colorimetric methods

for pseudo-and true (Red cell) cholinesterases<sup>4</sup>.

Data obtained were analyzed using the statistical software EPI info version 6.04c. The student t-test was used to compare means of variables. Results are expressed as means (SD). P values <0.05 were considered significant.

## RESULTS

Ninety seven (97) subjects selected from five companies formed the study population. Of this number, forty-seven (48.5%) had symptoms of organo-phosphorus toxicity and all the 47 (100%) had worked in the organo-phosphorus units for more than four years. Of the 97 subjects selected from the companies, 50 (51.5%) did not have symptoms of organo-phosphorus toxicity and have never worked in those unit of the companies. Fifty (50) subjects were recruited as controls.

The mean age of the subjects used was 386 while that of control was 377. There was no difference between the mean ages of the subjects and controls (P>0.05).

All the 47 "exposed" subjects were males and so were the 50 "non-exposed" selected from the companies. All the 50 controls were therefore males.

All staff of the five companies used for this study agreed to adequate provision and use of personal protective equipment (PPE). None of the workers however had a pre-employment plasma or red cell cholinesterase assessment (Base line value), as none of the five companies offered this test as one of the pre-employment prerequisite investigations. Subjects were distributed into three major groupings of:

- A- Those with symptoms of organo-phosphorus toxicity who had worked in the organo-phosphorus units for more than four consecutive years.
- B- Those that had no exposure to organo-phosphorus compounds but works in the companies involved in the study.
- C- The control group.

Table I shows subject distribution. Table II shows the plasma and red cell cholinesterase levels in the three groups.

There was a statistically significant difference when the mean plasma cholinesterase level of group A (4614±532) was compared with that of the control group Group C (8095±875) P<0.05.

There was however no statistically significant difference when the mean plasma cholinesterase level of Group B (8042±1011) was compared with the mean plasma cholinesterase level of the control group C

(8095±875) P>0.05. There was a statistically significant difference between the mean plasma cholinesterase level of Group A (4614±532) and that of Group B (8042±1011) P<0.05.

There was no statistically significant difference when the mean red cell cholinesterase levels of Groups A (7998±948) and B (8100±648) were compared with that of the control Group C (8115±712) P>0.05. There was a statistically significant difference when the mean plasma cholinesterase level of Group A (4614±532) was compared with the mean Red cholinesterase level of Group A (7998±948).

Table I. Subject Distribution and Major Groupings

GROUPS	BAKER	ADP	WAOS	AOS	PCCC	TOTAL
Subjects with symptoms of organo-phosphorus toxicity (PROLONGED EXPOSURE TO ORGANO-PHOSPHORUS COMPOUNDS >4yrs (GROUP A)	7	11	8	9	12	47
Subjects who work in companies but not in organo-phosphorus units (GROUP B)	10	10	10	10	10	50
TOTAL	10	21	18	19	22	97

CONTROL: n=50 (GROUP C)

MEAN AGE OF SUBJECTS: 38±6

MEAN AGE OF CONTROL: 37±7

BAKER Baker Hughes Nig. LTD

ADP: Agricultural Development Project

WAOS: West African Oil Services

AOS: African Oilfield Services

PCCC: Pest Control Chemical Company.

Table II. Showing Comparison of Mean (Sd) Plasma and Red Cell Cholinesterase between the Groups

GROUPS	A SUBJECTS WITH SYMPTOMS OF TOXICITY	B SUBJECTS WITHOUT SYMPTOMS OF TOXICITY	C CONTROL
Number involved (n)	47	50	50
Mean plasma cholinesterase (SD) □/L	4614 (532)	8042 (1011)	8095 (875)
Mean red cell cholinesterase (SD) □/L	7998 (948)	8100 (648)	8115 (712)

**P-values**

**Plasma Cholinesterase**

Group A & C	<0.05	Significant
Group A & B	<0.05	Significant
Group B & C	>0.05	not significant

**Red Cell Cholinesterase**

Group A & C	>0.05	not Significant
Group A & B	>0.05	not Significant
Group B & C	>0.05	not significant

**GROUP A**

Plasma cholinesterase & Red cell cholinesterase P<0.05 Significant.

**DISCUSSION**

All the subjects involved in the study were males working in the company operational sites and especially in the organo-phosphorus units. This is due to the male preponderance attributable to the physical nature of these jobs. This explains the male dominance in this study. The selection of fifty subjects who are all males to serve as control for this study is considered appropriate given the number involved in this study and the male dominance in subjects from the five companies. In other studies carried out in similar industries in other parts of the world, this male dominance has constantly been observed<sup>1,-3,6,7,10</sup>.

The mean age of subjects used in this study is 38.6years. This represents the productive age group. This was taken into consideration in the selection of control group.

Fourty seven subjects from the five companies who where exposed to organo-phosphorus compounds for more than four years showed minor symptoms of toxicity and a significant reduction in their mean plasma cholinesterase level irrespective of adequate use of personal protective equipment. This finding corroborates the findings in other studies that the longer the duration of exposure to organo-phosphorus compounds, the more the incidence and frequency of symptoms of toxicity arising from insidious accumulation of organo-phosphorus compounds through inhalation and penetration through the skin<sup>2,5,10</sup>.

A striking finding in this study is that whereas the mean plasma cholinesterase activity of the 47 workers who displayed symptoms of toxicity was significantly reduced (4614±532) when compared with that of the "non-exposed" group (8042±1011) and the control group (8095±875), the mean red cell cholinesterase activity of this group "the exposed and symptomatic group" (7998±948) was not significantly reduced when compared with those of the "non-exposed" company workers (8100±648) and the control group (8115±712). This finding is clinically significant especially in the early detection, monitoring and periodic assessment of organo-phosphorus toxicity among any given population at risk (e.g. industrial workers in organo-phosphorus units) and in patients. It also implies by extension that significant reduction in red cell cholinesterase activity may be synonymous with very severe organo-phosphorus toxicity, which is in keeping with report from previous studies<sup>1,2,5,8</sup>.

**CONCLUSION**

Plasma and red cell cholinesterase activities are useful indicators of organo-phosphorus poisoning.

Organo-phosphorus toxicity causes reduction in the activities of the cholinesterase enzyme system.

Plasma cholinesterase reduction occurs earlier in organo-phosphorus toxicity than red cell cholinesterase. Decrease in red cell cholinesterase activity may be associated with very severe toxicity which may be life threatening.

Plasma cholinesterase is thus more useful in early detection of organo-phosphorus toxicity than red cell (true) cholinesterase.

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