

Correlation of acetylcholinesterase activity inhibition with ageing in organophosphate-exposed farm workers at Ibadan, South-Western Nigeria

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Abstract

Background/Aim: In an attempt to destroy pests and enhance food production, Nigerian local farmers are occupationally exposed to organophosphate pesticides (OP). During exposure, OP affect the nervous system by impairing the activity of acetylcholinesterase enzyme (AChE) thus leading to accumulation of acetylcholine at the neuromuscular junction. This defect in the cholinergic pathway has been consistently reported for over two decades in studies on the brains of those with advanced age. This study is aimed at determining the relationship between the levels of AChE activity with ageing in apparently healthy OP-exposed farmers as well as in unexposed controls in Nigeria due to paucity of data in this environment.

Method: This case-control study comprises a total of 104 apparently healthy participants. Fifty-two farmers (41 males, 11 females; aged 30-62 years) occupationally exposed to OP and 52 sex-matched unexposed controls (aged 38-60 years) were recruited from Idi-Ayunre farm settlements on the outskirts of Ibadan and University College Hospital, Ibadan respectively.

Results: Mean AChE level of OP-exposed farmers (7.22 ± 1.99 kIU/L) was significantly lower ($p < 0.05$) than the controls (12.23 ± 1.67 kIU/L). Correlation of AChE activity with age was 0.189, $p = 0.179$ and -0.325, $p = 0.019$ for the farmers and controls respectively.

Conclusion: Low AChE activity level of the farmers indicates a persistent accumulation of acetylcholine at the neuromuscular junction, which may predispose them to intermediate syndrome. Significant negative correlation between AChE activity and age of the controls probably indicates that the normal ageing process is associated with decreased AChE activity.

Keywords: *Acetylcholinesterase, Organophosphate, Ageing, Farmers, Cholinergic Pathway*

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Résumé

Contexte / But: Dans le but de détruire les ravageurs et d'améliorer la production alimentaire, les fermiers locaux nigériens sont exposés professionnellement aux pesticides organophosphorés (PO). Au cours de l'exposition, PO affecte le système nerveux en altérant l'activité de l'enzyme acétylcholinestérase (AChE) conduisant ainsi à l'accumulation d'acétylcholine à la jonction neuromusculaire. Ce défaut dans la voie cholinergique a été régulièrement signalé depuis plus de deux décennies dans des études sur le cerveau des personnes ayant un âge avancé. Cette étude vise à déterminer la relation entre les niveaux d'activité de l'AChE avec le vieillissement chez des fermiers apparemment sains exposés au PO ainsi que chez des témoins non exposés au Nigeria en raison du manque de données dans cet environnement.

Méthode: Cette étude de cas-contrôle comprend un total de 104 participants apparemment en bonne santé. Cinquante-deux fermiers (41 hommes, 11 femmes, âgés de 30 à 62 ans) exposés professionnellement à l'OP et 52 témoins non exposés genre-appariés (âgés de 38 à 60 ans) ont été recrutés respectivement dans les colonies fermières d'Idi-Ayunre à la périphérie d'Ibadan et au Collège Hospitalier Universitaire, Ibadan.

Résultats: Le niveau moyen d'AChE des fermiers exposés au PO ($7,22 \pm 1,99$ kIU / L) était significativement plus bas ($p < 0,05$) que celui des témoins ($12,23 \pm 1,67$ kIU / L). La corrélation de l'activité d'AChE avec l'âge était de 0,189, $p = 0,179$ et -0,325, $p = 0,019$ pour les fermiers et les témoins respectivement.

Conclusion: Le faible niveau d'activité d'AChE chez les fermiers indique une accumulation persistante d'acétylcholine à la jonction neuromusculaire, ce qui peut les prédisposer au syndrome intermédiaire. Une corrélation négative significative entre l'activité de l'AChE et l'âge des témoins indique probablement que le processus de vieillissement normal est associé à une diminution de l'activité de l'AChE.

Mots-clés: *Acétylcholinestérase, Organophosphoré, Vieillesse, Fermiers, Voie Cholinergique*

Introduction

The enzyme acetylcholinesterase (AChE; E.C. 3.1.1.7) found in the cholinergic terminal is a specific choline esterase, hydrolyzing predominantly choline esters (acetylcholine- ACh) [1]. It plays a key role in cholinergic metabolism as it hydrolyzes the neurotransmitter acetylcholine into acetate and choline, thus terminating nerve impulse transmission [1]. These enzymes are highly distributed in the brain, nerves and red blood cells [2] as well as in the central and peripheral nerve tissues of different vertebrates, hence, demonstrating a high range of variation [3, 4].

The importance of acetylcholinesterase in the body homeostasis is underscored by the fact that they are the targets of some of the most potent toxins including insecticides or pesticides, snake venom and chemical weapons [5]. Inhibition of AChE by these compounds leads to accumulation of acetylcholine in the synaptic cleft and results in impeded neurotransmission [6]. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions [7]. Its accumulation within the nervous system leads to continuous stimulation of cholinergic receptors, resulting in symptoms of toxicity such as salivation, tremors, and miosis and in severe cases, respiratory paralysis and death [8].

Organophosphates, esters of phosphoric acid, are a class of irreversible AChE inhibitors [6]. Thus, the inhibition of acetylcholinesterase (AChE) activity has been used widely as a biomarker of exposure to organophosphate pesticides (OPs) [9]. In our environment today, farm workers are the ones directly at the receiving end of the hazards associated with chronic exposure to organophosphate pesticides. These farmers are therefore vulnerable to defects in the cholinergic system attendant on accumulation of acetylcholine at the neuromuscular junction. Similarly, a marked decrease in AChE activity with increasing age has been reported by [10]. These researchers also suggest the vulnerability of those with advanced age to impaired neurotransmission [10]. Normal ageing is reported to be associated with a slow decline in brain functions such as sensory and motor performance. At times, this decline is accompanied by progressive memory loss, dementia and cognitive dysfunctions, ultimately resulting in limited functionality [11].

A neurochemical hypothesis has been proposed in which brain ageing is related to changes in cerebral neurotransmission, and the initial focus has been on cholinergic neurotransmission [12]. A body of animal literatures further suggested that

disruption to the cholinergic system could be one possible mediating factor in age-related cognitive change in humans [12-19]. Changes in cholinergic function have been characterized and a strong correlation has been observed with cognitive decline associated with ageing [10].

Extensive evidence has substantiated the general hypothesis that cortical cholinergic inputs primarily mediate attention processes and capacities [20-24]. The aim of this study therefore, was to determine the plasma acetylcholinesterase (AChE) activity in apparently healthy organophosphate (OP)-exposed farmers and unexposed controls, and thereafter, correlate the levels of AChE activity with age in these participants.

Materials and methods

Selection and description of participants

A total of 104 apparently healthy participants were recruited for this case-control study. Fifty-two occupationally exposed farmers consisting of 41 (78.85%) males and 11 (21.15%) females between the ages of 30 and 62 years were recruited from Idi-Ayunre farm settlements, Oluyole Local Government Area located on the outskirts of Ibadan Metropolis, South-western Nigeria. All farmers studied used the pesticide parathion (*paraphos*). Similarly, 52 control participants comprising 41 (78.85%) males and 11 (21.15%) females between the ages of 38 and 60 years were recruited from the University College Hospital (UCH) Ibadan and environs. All participants were not on any medication. The study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee and informed consent was duly obtained from each of the participants prior to specimen collection.

Questionnaire administration

At enrolment, the participants completed a short self-structured questionnaire designed to obtain information on their demographic characteristics, life style, number of years in the farming profession, duration of exposure to organophosphate pesticides per day, medical histories and dietary habits.

Blood sample collection

Five millilitres of blood was collected into lithium heparin bottles, and centrifugation of the blood samples was done at 3,000 rpm for five minutes, followed by freezing of the plasma samples at -20°C until the samples were analyzed.

Assay Methodology for Acetylcholinesterase Activity in Plasma

Using the method of Ellman *et al.* [25], AChE activity in plasma samples was measured at the wavelength of 412 nm using a double beam spectrophotometer.

Statistical analysis

The statistical analysis of the data was carried out using Software Package for Social Sciences (SPSS) version 20. Results were presented as mean \pm standard deviation (SD). Student t-test was used to examine the differences in mean AchE activity between the farmers and controls. Pearson correlation coefficient (r) was used to test the relationship between variables and the level of significance was set at $p < 0.05$.

Results

Some demographic and clinical indices of the subjects are shown in Table 1. As depicted in this table, there was a significantly higher systolic blood pressure in the farmers compared with the controls ($p < 0.05$). Table 2 depicts age grouping of the farmers and the controls with AchE showing that there is a significant depletion of AchE activity with increasing age in the controls but not in the exposed farmers. Table 3 depicts the correlation of AchE activity with age of the farmers. Inversely and positive association was found between these parameters in control group. Figure 1 shows a graphical representation of the mean plasma AchE activity observed in the participants. A significantly

Table 1: Comparison of Some Demographic and Clinical Indices of the Subjects

Index	Farmers (n = 52)	Controls (n = 52)	P-Value
Years of farming experience	19.79 \pm 13.97	N/A	
Duration of exposure/day (Hours)	2.53 \pm 1.30	N/A	
Systolic blood pressure (mmHg)	132.88 \pm 8.25	123.37 \pm 10.88	0.000*
Diastolic blood pressure (mmHg)	82.12 \pm 7.76	95.2 \pm 8.30	0.102

*significant at $p < 0.05$

n= number of-subjects

N/A = Not applicable

Table 2: Acetylcholinesterase (AchE) per age groups

Participants/ Age (yrs)	30-40 years	41-50 years	51-60 years	61 years and above	Total No. (N)	Chi- Square	p-values
Exposed Groups (n)	6.20 \pm 1.05 6	6.94 \pm 0.74 10	7.25 \pm 2.04 26	8.06 \pm 2.87 10	7.22 \pm 1.99 52		
Unexposed Control Group (n)	12.93 \pm 2.41 7	12.67 \pm 1.61 25	11.51 \pm 1.23 17	11.08 \pm 0.13 3	12.23 \pm 1.67 52	12.16*	0.007
Total No. (N)	13	35	43	13	104		

*0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.50.

Table 3: Correlation of AchE Activity with Age and Years of Farming Experience of the Participants

Index	Index	Farmers (n = 52) r (p-value)	Controls (n = 52) r (p-value)
AChE	Age	0.189 (0.179)	-0.325 (0.019**)

**significant at $p < 0.05$

n = number of subjects

r = Pearson Correlation Coefficient

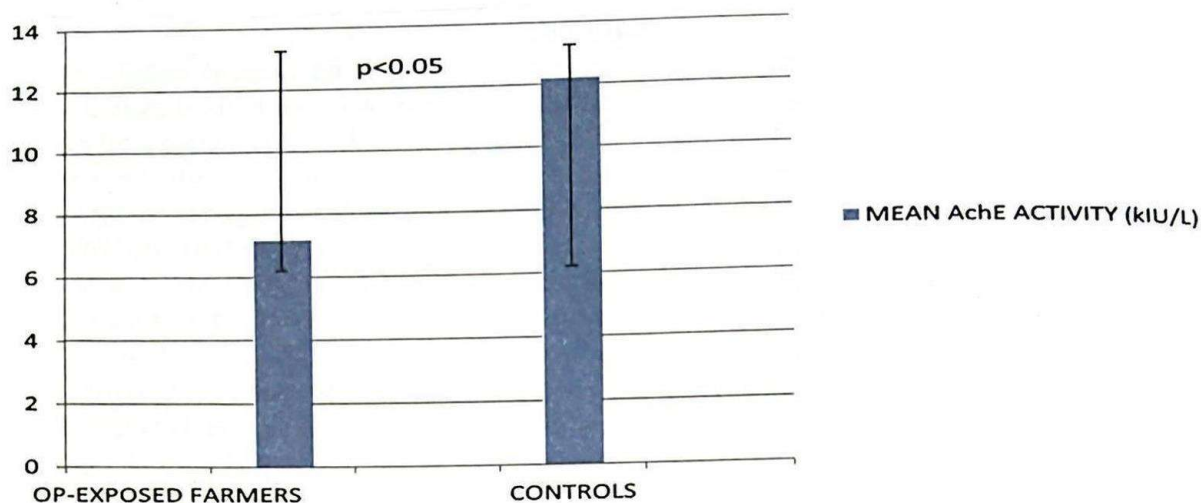


Fig. 1: Mean AChE activity (kIU/L) of the participants

lower value was found in the OP-exposed farmers compared with the controls.

Discussion

Occupational exposure to organophosphate pesticides has been shown to be characterized by a marked inhibition of acetylcholinesterase activity. In this study, the results indicated a clearly high level of exposure to organophosphate pesticide; *paraphos*, among the local farmers around Ibadan, Southwest Nigeria. This corroborates reports from the previous studies [26-27] in which significantly inhibited AChE activity was found in organophosphate-exposed workers. Inhibition of AChE may lead to accumulation of the neurotransmitter, acetylcholine at the neuromuscular junction which may render these farmers vulnerable to defects in cholinergic neurotransmission as indicated by the work of Shaked, Meerson, and Wolf [6].

This study also reveals that the normal ageing process is associated with a progressive decline in AChE activity as indicated by the depletion of AChE activity with increasing age in the controls but not in the exposed farmers and significant negative correlation observed between age and AChE activity in the controls ($p < 0.05$). This finding supports the previous reports of Jha and Rizvi [10] in which there was a marked decrease in AChE activity with increasing age in normal healthy participants. Similar findings were also reported separately by Das *et al* [28] and Skau and Triplett [29] in their studies in which the relationship between ageing and AChE activity in different brain regions were examined.

Decline in cholinergic indices (choline acetyltransferase, AChE, and muscarinic acetylcholine receptors) has already been reported

during normal ageing process [30]. Ageing is an inevitable biological process. It has been defined as the progressive accumulation of diverse deleterious changes with time that increases the chance of disease and death [10]. McNeil and Valenzuela [12] have suggested that disruption to the cholinergic system could be one possible mediating factor in age-related cognitive change in humans. Therefore, it is presumed that increasing cholinergic transmission may enhance cognitive function in aged individuals [31]. Cells in all regions of the nervous system are affected by ageing, as indicated by the decline in sensory, motor and cognitive functions with time [32]. Data emanating from studies conducted in Egypt have indicated that blurred vision, dizziness, numbness, paraesthesia, headache, vertigo, asthenia, superficial sensory loss, trophic and vasomotor changes and decrease ankle and deep reflexes were more prevalent among pesticide applicators than controls especially those with longer duration of exposure [33]. It has also been indicated that moderate exposure to organophosphates over several years may also be associated with deficit in verbal abstraction, attention and memory [34]. However, there is considerable variability among individuals in the apparent rate of ageing, the neural systems most affected, and whether and how age-related deficits are compensated [35]. In contrast to the above, Salvi *et al* [36] found normal acetylcholinesterase activity in patients aged 60 years and below chronically exposed to organophosphates who also presented with psychomotor alterations, cognitive slowing, memory and attention deficits, and other psychiatric symptoms, alterations which are expected to be associated with inhibited acetylcholinesterase activity.

Conclusion

The marked inhibition of AChE activity by organophosphates is well established and further demonstrated by this study. Ageing-induced decline in AChE activity is also well documented in other parts of the world but has not been established in our environment due to paucity of data; which this study has shown to be so in our environment also. However, the correlation of changes in cholinergic function occasioned by a marked decrease in AChE activity with cognitive decline associated with ageing is needed, thus necessitating follow-up studies.

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