

SHORT REPORT

Salivary acetylcholinesterase as a biomarker for organophosphate exposure

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Background	Workers exposed to organophosphate (OP) pesticides are required to undergo periodic statutory medical surveillance in several countries.
Aim	To study the relationship between serum, erythrocyte and saliva acetylcholinesterase (AChE) levels and to explore the use of salivary AChE as potential biomarker for OP exposure.
Methods	A cross-sectional study was conducted on 19 healthy adult male lead-exposed workers who were undergoing six monthly statutory medical examination. Passive drool saliva samples were collected from each worker. Each blood sample was tested for serum and erythrocyte AChE, and each saliva sample was tested for AChE.
Results	Among the 19 subjects, the mean (\pm standard deviation) of salivary, erythrocyte and serum AChE/cholinesterase were 22.7 (\pm 17.4), 17171 (\pm 1467), 8861 (\pm 1876) U/l, respectively. There was a moderate correlation between salivary and erythrocyte AChE ($r = 0.42$, $P = 0.071$), but not salivary and serum AChE ($r = -0.17$, $P = 0.48$). The level of AChE in saliva was \sim 1820 times lower than AChE in erythrocytes.
Conclusion	It is probably not feasible to use saliva as a replacement for blood for the measurement of AChE levels. This is because of the much lower levels of AChE in saliva relative to erythrocytes, the weak correlation between the two measurements and the previously reported high intra-individual variation of salivary AChE.
Key words	Acetylcholinesterase; blood; organophosphate exposure; saliva.

Introduction

Workers exposed to organophosphate (OP) pesticides are required to undergo periodic statutory medical surveillance in several countries. Measurement of cholinesterase (ChE) levels in blood is the conventional method of assessing the degree of occupational exposure to OP pesticides.

Two principal types of ChE measured in blood are erythrocyte acetylcholinesterase (AChE) and plasma or serum ChE. The latter is also named as 'pseudo-', 'butyryl-' and 'non-specific' ChE [1]. The determination of erythrocyte AChE is an indicator of acute intoxication with anticholinesterase pesticides [2]. However, the interpretation of ChE inhibition in either serum or erythrocytes is complex [3].

AChE catalytic activity has recently been shown to be detectable in saliva, where its catalytic activity is stable at

room temperature for up to 6 h [4]. As collection of saliva is less invasive than collection of blood samples, such a procedure may be more readily acceptable for medical surveillance and biological monitoring of OP-exposed workers. There is, however, limited information available on the relationship between plasma, erythrocyte and saliva AChE levels.

In this study, we examined the relationship between serum erythrocyte and saliva AChE levels among healthy workers who were not exposed to OP pesticides.

Methods

The study was conducted in conjunction with a routine six monthly statutory medical examination of a group of workers employed in a factory manufacturing lead (Pb)-based polyvinyl chloride stabilizer. These workers had no occupational or other history of OP exposure. The medical examination involved sampling of venous blood for measurement of haemoglobin and lead and was carried out in the late morning. In this study, the blood samples were also tested for serum and erythrocyte AChE. Subjects were also asked to gently drool their saliva into a sterile

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conical tube and ~2 ml of saliva was collected from each subject immediately before venepuncture.

Erythrocyte AChE was measured using the established calorimetric method described by Ellman *et al.* [5]. Serum AChE was analysed using the method of Ellman *et al.* with some modifications. Salivary AChE was determined using the Molecular Probes Amplex® Red Acetylcholine/Acetylcholinesterase Assay kits (Invitrogen Ltd, Carlsbad, CA) with some modifications. All the samples were analysed on the same day within 6 h of collection.

Approval to conduct this study was obtained from the Institutional Review Board of the National University of Singapore. All workers were briefed about the study and provided written informed consent before participation.

Data were analysed using SPSS version 15. All *P* values are two sided and the level of statistical significance is 0.05.

Results

Nineteen healthy adult workers participated in the study. All of them were males, with a mean age [\pm standard deviation (SD)] of 35 ± 13 years. The duration of employment ranged from 3 months to 27 years. None of the workers had any significant current or past history of medical conditions.

The blood lead levels of the 19 workers were all <35.0 $\mu\text{g/dl}$ (mean \pm SD: 20.6 ± 8.15 $\mu\text{g/dl}$ and ranged from 7.6 to 34.6 $\mu\text{g/dl}$).

Among the 19 subjects, the mean (\pm SD) of salivary and erythrocyte AChE were $22.7 (\pm 17.4)$ U/l and $17171 (\pm 1467)$ U/l, respectively. The mean (\pm SD) of serum ChE was $8861 (\pm 1876)$ U/l. There was a moderate correlation between salivary and erythrocyte AChE ($r = 0.42$, $P = 0.071$), but not with serum ChE ($r = -0.17$, $P = 0.48$) (Figure 1). The level of AChE in saliva was ~1820 times lower than AChE in erythrocytes. There was no correlation between serum ChE and erythrocyte AChE ($r = 0.019$, $P = 0.94$) (Figure 2).

Discussion

In our study of 19 healthy adults, the range of salivary AChE was 1.1–62.5 U/l (mean = 22.7 ± 17.4 U/l), demonstrating that the AChE activity in saliva is low; ~1820 times lower than in erythrocytes.

Measurement of AChE in human saliva as an index of organophosphorus pesticide exposure has recently been explored in research [1,6]. Saliva as a diagnostic fluid has benefits such as ease and low cost of collection, the possibility of collecting multiple samples and of the lack of any need for a phlebotomist.

Based on the study findings of Ueda and Yamaguchi [7], AChE in human saliva is derived from salivary glandular cells, while pseudo ChE in whole saliva may be derived from microorganisms in the oral cavity. Our findings

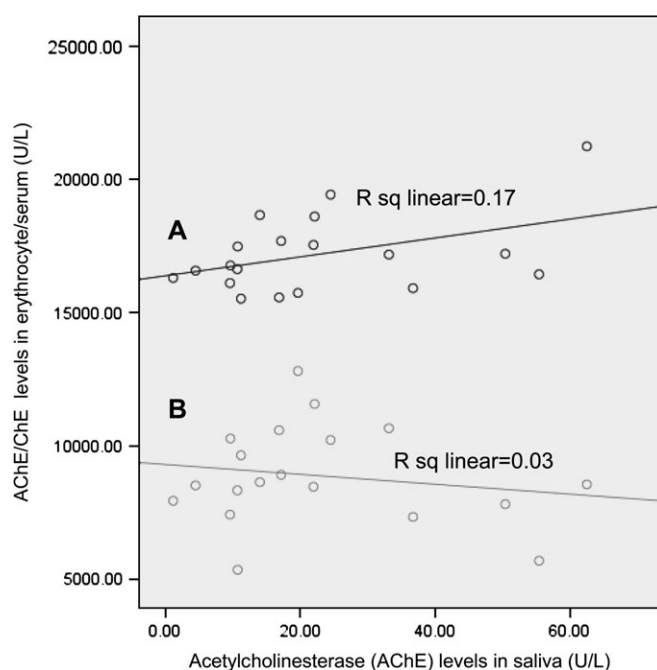


Figure 1. Bivariate scattergram of salivary acetylcholinesterase levels in saliva (U/L) with (A) erythrocyte acetylcholinesterase levels (U/L): $r = 0.42$, $P = 0.071$, $n = 19$; (B) serum cholinesterase levels (U/L): $r = -0.17$, $P = 0.48$, $n = 19$.

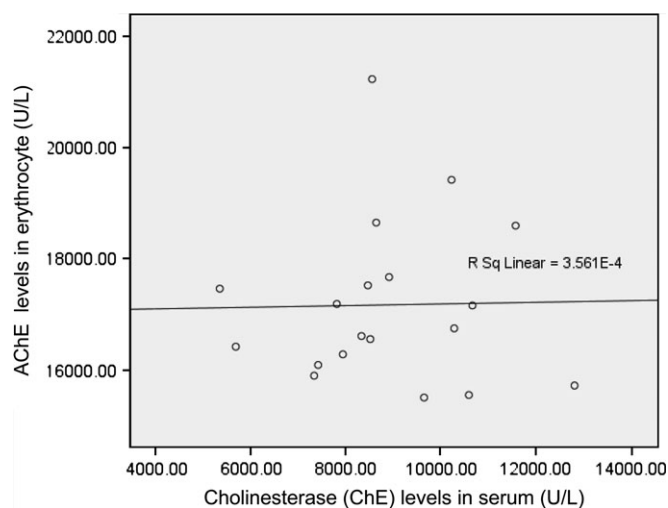


Figure 2. Bivariate scattergram of acetylcholinesterase levels in erythrocyte (U/L) with serum cholinesterase levels (U/L): $r = 0.019$, $P = 0.939$, $n = 19$.

of correlation between salivary and erythrocyte AChE but limited correlation between salivary and serum ChE are in accordance with Ueda and Yamaguchi's findings that no correlation was found between erythrocyte AChE and plasma AChE levels [7].

A recent study using radiometric assay for determination of the ChE activity in saliva in a group of healthy

adults suggested that salivary ChE may be a useful indicator of potential neurotoxic effects from exposure to organophosphorus and carbamate pesticides. The range of salivary total cholinesterase activity detected in that study was 0.051–4.5 U/l [6].

The relatively low level of AChE in saliva may well result in high variability in level detected with reference to the true level of activity present in blood.

The study findings of Claus Henn *et al.* indicated that the intra-individual coefficient of variance (CV) of saliva ChE was 35% [6]. Lefkowitz *et al.* [8] demonstrated that the mean intra-individual CV for red blood cell ChE in a group of 46 workers was ~3.9%. This indicates that a single measurement of erythrocyte ChE can be a reliable method for assessment of exposure to OP pesticides.

A recent report described a carbon nanotube-based electrochemical sensor method which appears to be a sensitive and quantitative tool for biomonitoring of salivary biomarkers of exposure to OP pesticides [9].

However, at present, it is probably not feasible to use saliva as a biological fluid to replace blood for the measurement of AChE levels, in view of (i) the much lower levels of AChE in saliva relative to erythrocytes, (ii) the weak correlation between the two measurements and (iii) the previously reported high intra-individual variation of salivary AChE.

Key points

- AChE activity in saliva is over a thousand times lower than that in erythrocytes.
- Salivary AChE does not correlate closely with either erythrocyte ($r = 0.42$) or serum ($r = -0.17$) AChE.
- It is therefore not feasible to use saliva as a replacement for blood for the measurement of AChE levels.

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Conflicts of interest

None declared.

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