

Blood cholinesterase levels in a group of Malaysian blood donors

Lucy CHAN FANZCA, S BALABASKARAN BSc MSc(Victoria) PhD,* AE DELILKAN FFARCS, FFARACS and Leong Huat ONG.**

Departments of Anaesthesiology, Biochemistry and Social and Preventive Medicine,** Faculty of Medicine, University of Malaya, Kuala Lumpur.*

Abstract

Data on blood cholinesterase levels in the Malaysian population is lacking. The spectrophotometric method of Ellman was used to determine the red cell, plasma and whole blood cholinesterase (ChE) levels in 407 Malaysian blood donors. The mean±SD for plasma ChE in females (n=48) was 2.37±0.70 umol/min/ml and 2.76±0.75 umol/min/ml in males (n=359). The mean plasma ChE in males was higher than in females (p<0.001). The mean±SD for red cell ChE in females was 9.01±1.20 umol/min/ml whereas in males it was 7.69±1.30 umol/min/ml (the mean red cell ChE in females was higher than in males, p<0.0001). The mean±SD for whole blood ChE for females was 4.31±0.58 umol/min/ml and for males it was 4.95±0.71 umol/min/ml. The mean whole blood ChE in males was higher than in females (p<0.0001). Sex influenced the plasma, red cell and whole blood ChE.

In males the plasma ChE was affected by the race factor. The mean±SD plasma ChE for the Malay, Chinese and Indian were 2.92±0.80, 2.73±0.71 and 2.61±0.73 respectively (p<0.002). The age factor in males affected the red cell ChE with 7.88±1.32 in the (30-69) age group and 7.47±1.23 in the (15-29) age group (p<0.005).

The whole blood ChE in females was affected by blood groups. The mean±SD whole blood ChE for blood groups A,B and O were 4.19±0.42, 3.93±0.46 and 4.49±0.62 respectively (p<0.03). The significant difference is between the ChE of group B and O, but the ChE of group A could not be determined to be different from group B or O.

These results serve as guidelines for our local population in the evaluation of cholinesterase levels with regard to pesticide poisoning, liver biosynthetic capacity and unusual sensitivity to succinylcholine.

Key words: Cholinesterase, blood donors, reference values, Ellman method.

INTRODUCTION

Physiologically, the most important choline ester in the human body is acetylcholine, which is hydrolysed by acetylcholinesterase. Acetylcholine, a chemical stimulant, is released from nerve endings and its hydrolysis enables the regulation of nervous transmission between preganglionic fibres and autonomic ganglia, postganglionic cholinergic nerves and muscles, and the transmission of nervous control to the adrenal medulla. The red blood cell is rich in this enzyme and its level of activity reflects tissue activity presumably. However, on a practical note, whole blood acetylcholinesterase is also useful because procedures using whole blood are more practical than those using separated erythrocytes.

The other cholinesterase (ChE) found in the plasma, serum ChE or pseudocholinesterase, has no known biological function. Clinically, its activity is useful to detect exposure or toxicity to

pesticides, loss of biosynthetic capacity of the liver and unusual sensitivity to succinylcholine, a muscle relaxant, at the time of surgery.

Substantial variations in enzyme levels exist among individuals in different geographical areas and reported data on the distribution of ChE are predominantly Western.^{1,2} This paper was undertaken to contribute some observations on ChE levels in a group of Malaysian blood donors.

MATERIALS AND METHODS

Sampling process

This study was carried out on fit adult Malaysian blood donors who donated blood at the University Hospital Kuala Lumpur. A total of 407 samples were collected: 2ml were dispensed into EDTA treated sterile containers (Vacutainer). Without regard to age, sex or race (Malay, Chinese, Indian), the first six donors were selected

into the sample on every clinic day (Monday to Friday). Data on sex, age, race and blood group were recorded.

The red blood cell, whole blood and plasma ChE levels were analysed on the day of collection of blood samples in the laboratory, Department of Anaesthesiology, University of Malaya.

Laboratory

Acetylthiocholine (ATCh) iodide, butyrylthiocholine (BTCh) iodide, di-sodium hydrogen orthophosphate, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and saponin were obtained from Sigma Chemical Company, St. Louis, Mo., USA. Enzyme reaction was recorded on a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer (double beam). A Beckman pH meter was used for pH measurements and haematocrit was estimated by Coulter Electronic, INC, Hialeach, Fl. (Model S-Plus IV).

Separation of erythrocytes and plasma

Whole blood was centrifuged in a Sorvall GLC-2B general laboratory centrifuge (Du Pont) at 200 rpm for 15 minutes. The plasma was removed and the erythrocytes washed twice in physiological saline adjusted to pH 7.4.

Assay method

A modification of the Ellman spectrophotometric method³ was employed. It involved the liberation of thiocholine from the substrate molecules (i.e. ATCh and BTCh) by the action of the enzyme and the reaction of thiocholine with DTNB to produce a complex and a free yellow coloured 5-thio-2-nitrobenzoate anion. This anion absorbs maximally at 412nm with a molar extinction coefficient of 14140 at pH8.0.

The modifications of the Ellman method^{4,5} were:

1. Enzyme assays were conducted at pH7.4 and 37° C
2. ATCh and BTCh were prepared in 10mM phosphate buffer at pH6.0 as these substrates were more stable at this pH than at alkaline values.
3. Haemolysed whole blood or packed cells in a dilution of 1:1800 in 0.1M phosphate buffer was used in preference to suspension of RBC in 0.1M phosphate buffer at pH 8.0 at a dilution of 1:600.

Preliminary kinetic studies with the above dilutions of the enzyme showed it to be stable at the above pH and temperature and the Michaelis

constant not affected by variations in enzyme concentrations.

ATCh was the substrate for red blood cell and whole blood ChE while BTCh served as the substrate for plasma ChE. Enzyme activity was expressed in $\mu\text{mol}/\text{min}/\text{ml}$.

Statistical methods

The following methods were used to analyse the data:

1. The t-test for two independent samples
2. The one-way ANOVA for (R) independent samples.
3. The Newman-Keuls test to test significant difference between individual pairs of means.
4. Chi-square test (χ^2) to compare differences in proportion of qualitative data.

All tests were based on the 5% level of significance.

RESULTS

Table 1 shows the distribution of the samples (n=407) by sex, age group, race and blood group. There was no difference in the mean age between males and females (t=1.84, p=0.063) but there was a difference between males and females in the distribution of age when grouped into 15-29 yr and 30-69 yr ($\chi^2 = 6.84$, p=0.009). There was a bigger percent of age group 15-29 yr among females compared to males. The ethnic

TABLE 1: Distribution of 407 blood donors by age group, sex, race and blood group

	Male (n=359) No. (%)	Female (n=48) No. (%)
Age group in yr.		
15-29	163(45.4%)	32(66.6%)
30-69	196(54.6%)	16(33.4%)
(mean+1SD)	(31.9+8.3)	(29.5+9.7)
(min-max)	(18-59)	(18-56)
Race		
Malay	105(29.2%)	11(22.9%)
Chinese	166(46.2%)	31(64.6%)
Indian	88(24.6%)	6(12.5%)
Blood group		
A	107(29.8%)	11(22.9%)
B	91(25.3%)	10(20.8%)
O	161(44.9%)	27(56.3%)

TABLE 2: Plasma, whole blood (WB) and red cell (RC) ChE by sex

	Plasma ChE	WB ChE	RC ChE
Male=359	2.76+0.75 (1.02-4.95)	4.95+0.71 (3.06-7.53)	7.69+1.30 (3.50-13.94)
Female=48	2.37+0.70 (1.18-4.02)	4.31+0.58 (3.06-5.98)	9.01+1.20 (6.67-13.16)
t-value	3.41	6.02	6.68
p-value	0.0008	0.0000	0.0000

ChE = Cholinesterase; Enzyme activity in $\mu\text{mol}/\text{min}/\text{ml}$; Data are mean+1SD, with range in parenthesis.

distribution by sex was significant ($\chi^2=6.18$, $p=0.045$). There was no difference in the blood group distribution between males and females ($\chi^2=2.23$, $p=0.327$).

Statistical analysis on the following parameters were significant:

- plasma, whole blood and red cell ChE by sex (Table 2)
- plasma ChE by race in males (Fig. 1)
- red cell ChE by age groups 15-29 yr and 30-69 yr in males (Fig. 2)
- whole blood ChE by blood group in females (Fig. 3)

Table 3 presents the ChE levels that were not affected by race, age or blood group.

DISCUSSION

Various methods such as gasometric, titrimetric, spectrophotometric and radiometric have been used for quantitative assay of ChE activity. The most often used among these are the spectrophotometric and radiometric methods which enable

TABLE 3: ChE levels that are unaffected by race, age group or blood group

Enzyme	Sex	Mean+1SD
Plasma ChE	male	2.76+0.75 (n=359)
	female	2.37+0.70 (n=48)
Whole blood ChE	male	4.95+0.71 (n=359)
Red cell ChE	female	9.01+1.20 (n=48)

ChE = cholinesterase; ChE activity in $\mu\text{mol}/\text{min}/\text{ml}$

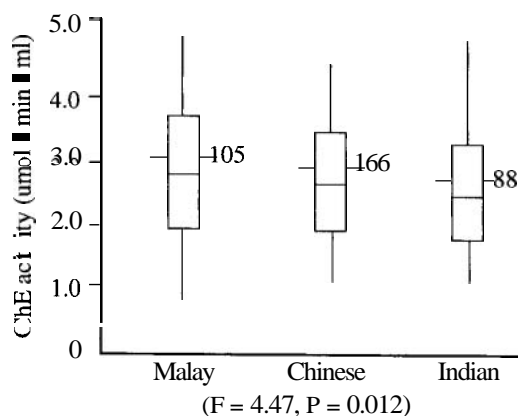


FIG. 1: Plasma ChE by race in males. (Mean \pm 1SD, range and sample size). (F = 4.47, P = 0.012)

rapid measurements. The Ellman spectrophotometric method requires thiocholine esters instead of natural choline esters as substrates and it fulfills the criteria of speed, accuracy and reproducibility. However, spectrophotometric methods often show substantial variations in parallel runs.

Unfortunately, ChE activity is expressed in different units when measured by different methods. These units are not readily or strictly convertible because the kinetics are not identical. One such unit is the International Unit (U) which is used as a measure of enzyme activity in many commercial kits (e.g. Monotest Cholinesterase, Cat. no. 124125, Boehringer-Mannheim,

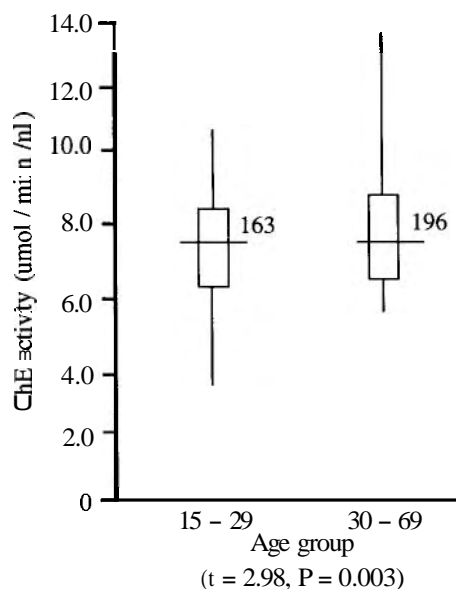


FIG. 2: Red cell ChE by age group in males. (Mean \pm 1SD, range and sample size). (t = 2.98, P = 0.003)

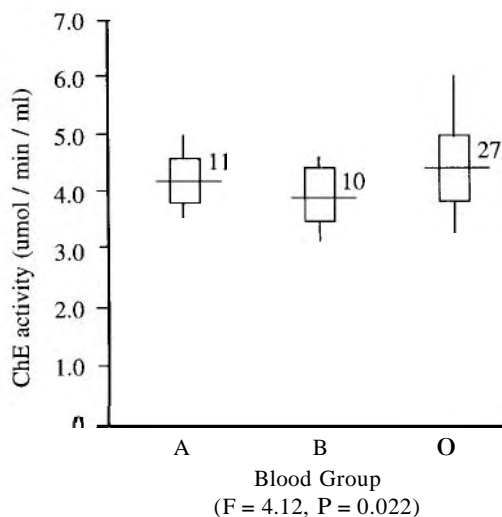


FIG. 3: Whole blood ChE by blood group in females. (Mean \pm 1SD, range and sample size).

Mannheim, West Germany). However, results of any method can be expressed as a percentage of normal and compared with values determined by any other method. Even these conversions must be interpreted with caution. Ideally, the same laboratory should be used for all enzyme measurements on the same individual.

This study indicated a relationship between sex and the level of ChE activity in plasma, whole blood and red blood cell. Some reports claim that sex was important in plasma ChE^{6,7} but others found no such correlation⁸.

The age factor in males affected the red cell ChE. It was higher in the age group 30-69 yr than the age group 15-29 yr, although other workers have observed the influence of age only in the newborn when compared to adults⁹.

Ethnicity affected the mean plasma ChE in males (highest in the Malay and lowest in the Indian), while blood group affected the mean whole blood ChE in females (highest for group O and lowest for group B) although the mean whole blood ChE in females for group A was not significantly different from groups O or B. Until a larger sample is studied, an attempt to interpret these findings would not seem profitable.

When baseline levels are established locally, ChE activity becomes a sensitive indicator of pesticide intoxication, loss of hepatic biosynthetic capacity and unusual sensitivity to succinylcholine. Nevertheless, the ChE activities in this preliminary work serve as guidelines.

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