

VALIDITY OF SERUM CHOLINESTERASE ESTIMATION IN HEALTHY AND ORGANOPHOSPHATE INTOXICATED PATIENTS

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ABSTRACT

This study was conducted on 120 subjects in Dakahlia governorate 60 of them were healthy persons (control group I) while the other 60 were patients suffering from acute organophosphate toxicity attended toxicology unit in Mansoura Emergency Hospital (group II). All selected subjects in both groups had no hepatic dysfunction evaluated by normal levels of AST and ALT. Both control and acute organophosphate intoxicated groups were subdivided into 3 subgroups according to age, subgroup A (less than 15 years) i.e. children, subgroup B (20 - 50 years) and subgroup C (more than 60 years). In each subgroup 10 persons were from urban areas (5 males and 5 females) and 10 persons were from rural areas (5 males and 5 females). A venous blood sample was obtained from every subject in both groups and was divided into two halves: the first half was used for determination of AST and ALT activity and the second half used for estimation of serum cholinesterase (ChE) level. Serum cholinesterase (ChE) levels of both males and females from rural and urban areas in each subgroup were estimated. In acute organophosphate intoxicated group estimation of ChE was done within 2 - 4 hours post ingestion. It was found that serum cholinesterase values were significantly lower in organophosphate exposed group compared with normal control group of either sex from rural or urban areas. It has been also found that serum ChE levels in subjects of subgroup (B) from rural areas were significantly lower than those from urban areas of both control and organophosphate exposed group. In addition, serum ChE values of subgroup (B) were significantly lower than those of subgroup (A) and (C) in control group from rural areas only. On the other hand serum ChE values in subjects of subgroup (A) were significantly lower than those in subgroup (B) and (C) in both urban and rural areas in organophosphate exposed group. Sex variation has no significant effect on serum ChE values in both control and organophosphate exposed groups whether in rural or urban areas.

INTRODUCTION

Organophosphorus pesticides are used widely for agriculture, vector control, and domestic purposes. Despite the apparent

benefits of these uses acute organophosphorus pesticide poisoning is an increasing worldwide problem, particularly in rural areas. They are the most important cause of severe toxicity and death from

acute poisoning worldwide (Eddleston, 2000). In developing countries, more than 200000 deaths occur in each year as a result of occupational or deliberate exposure to this group of pesticides (Wesseling et al., 2000).

The main toxic action of these pesticides is inhibition of both erythrocyte acetylcholinesterase (AChE) and serum cholinesterase (ChE) activities (Namba et al., 1971). Erythrocyte AChE is present in erythrocyte, lung, spleen, nerve endings and in the gray matter of brain and serum ChE which exists in plasma, liver, intestine, smooth muscles, heart, pancreas and white matter of brain (Moss and Henderson, 1999).

The measurement of serum ChE cholinesterase and erythrocyte AChE activities is used widely to investigate organophosphorus pesticides over-exposure, or as part of health surveillance in workers at risk of organophosphorus exposure (Lotti, 1995). Although organophosphate poisoning is more accurately assessed by measurement of erythrocyte AChE, serum ChE measurement are technically simpler to run and are more readily available in hospitals (Mason, 2000). There is wide inter-individual variation in ChE activity, and to define a significant depression in ChE, a baseline activity measurement is needed (Mason and Lewis, 1989).

Organophosphate poisoning occurs in various settings, including occupational exposure (Adamis et al., 1985) as well as accidental exposure of children to these agents in the treatment of pets for fleas and other pests (Zwiener and Ginsburg, 1988). Occupational exposure occurs in farm workers involved in the application of these agents and those engaged in their manufacture (Haddad, 1998).

The purpose of the present study is to determine the validity of serum cholinesterase estimation in subjects acutely exposed to organophosphate insecticides and to determine the magnitude of the changes of the circulating levels of this enzyme in relation to age, sex and locality in both healthy and acute organophosphate exposed subjects.

MATERIAL AND METHODS

This study was conducted on 120 subjects, 60 of them were patients attended toxicology unit in Mansoura Emergency Hospital suffering from acute organophosphate toxicity while the other 60 were healthy (control) subjects gave no history of liver disease.

Subjects were grouped into group I (Healthy or Control) and group II (acute organophosphate intoxicated) subjects. Each group was subdivided into 3 subgroups according to age, subgroup A (less

than 15 years i.e. children), subgroup B (20 - 50 years) and subgroup C (more than 60 years). In each subgroup 10 persons were from urban areas (5 males and 5 females) and 10 persons were from rural areas (5 males and 5 females).

A venous blood sample (10 ml.) was obtained from every subject in both groups and was divided into two halves. The first half (5 ml.) was used for determination of AST and ALT activity according to method of Reitman and Frankel (1957) using transaminases Kit manufactured by bio-Merieux Vitex, Inc. in USA. AST and ALT activity was measured to evaluate liver function and any subject with liver dysfunction was excluded from this study. The second half (5 ml.) was used for estimation of serum cholinesterase (ChE) level after separation of the serum from the blood sample. Blood samples were obtained after taking informed oral consent from each subject tested during the study.

Colorimetric assay for serum cholinesterase : was done according to method of Ellman et al ., (1961) using Shimadzu spectrophotometer UV-120 and Kits for cholinesterase produced by Boehringer Mannheim GmbH Diagnostica and was purchased from B.M.Egypt Co. in Cairo.

Statistical analyses: Data were analyzed statistically using Mann-Whitney test for groups comparison and a signifi-

cant level was considered at $P < 0.05$.

RESULTS

It was found that serum ChE values were significantly lower in organophosphate exposed group compared with normal control group of either sex from rural or urban areas (Tables 1 and 2).

Effect of residency difference on serum ChE values:

In control healthy group and organophosphate exposed group; the mean serum ChE values in both subgroup (A) and subgroup (C) showed no significant difference in both males and females from rural areas compared with those from urban areas. On the other hand the mean serum ChE values in subgroups (B) were significantly lower in both males and females from rural areas compared with those from urban areas (Table 3).

Effect of age difference on serum ChE values:

In control healthy group: There was no significant difference of mean serum ChE values of males and females in urban areas between all subgroups. In rural areas the mean serum ChE values of males and females were significantly lower in subgroup (B) compared with both subgroups (A) and (C), while there was no significant difference between subgroup (A) and subgroup (C) (Table 4).

In organophosphate exposed group: In both urban and rural areas the mean serum ChE values of males and females were significantly lower in subgroup (A) compared with subgroups (B) and (C) while there was no significant difference between subgroup (B) when compared with subgroup (C) (Table 4).

Effect of sex variation on serum ChE values:

In control healthy group and organophosphate exposed group in both urban and rural areas, there was no significant difference of mean serum ChE values between males and females in all subgroups (Figures 1 and 2).

DISCUSSION

The significantly lower serum ChE values ($P > 0.05$) in organophosphate exposed group compared with normal control group of either sex from rural or urban areas in this study is in agreement with findings of Cunha et al. (1995). They found that serum ChE assay was useful in diagnosis of organophosphate poisoning and also in monitoring the clinical course.

Concerning residency variations, it was found that the mean serum ChE values in subgroups (B) were significantly lower ($P > 0.05$) in both males and females from rural areas compared with those from urban

areas. This finding coincide with the results obtained by Abdollahi et al. (1995) who observed that serum ChE values of workers and farmers occupationally exposed to organophosphate insecticides were significantly lower than non exposed subjects.

As regard age variations, there was no significant difference of mean serum ChE values between all control subgroups in urban areas. On the other hand, in organophosphate exposed subjects of urban areas as well as both control and organophosphate exposed subjects of rural areas, the mean serum ChE values of males and females were significantly lower ($P > 0.05$) in subgroup (B) compared with subgroups (A) and (C).

This significantly lower serum ChE value in age group between 20 - 50 years (subgroup B) in rural areas can be explained on the basis that they are more exposed to organophosphate insecticides whether from surrounding polluted environment or during handling these agents in fields. In addition the significantly lower serum ChE values in organophosphate exposed individuals from urban areas in the same age group (subgroup B) indicate that they are more exposed to these agents than other age groups (subgroups A and C). These results coincide with those obtained by Abdollahi et al. (1995) who observed that serum ChE values in workers

employed at the production of organophosphate compounds were significantly lower than accepted standard normal limits.

In addition, the mean serum ChE values of males and females in both urban and rural areas were significantly lower ($P > 0.05$) in subgroup (A) compared with both subgroups (B) and (C) while there was no significant difference ($P < 0.05$) between subgroup (B) and subgroup (C). These results coincide with the study of Atterberry et al. (1997) who demonstrated experimentally that young rats were more sensitive to acute toxicity of organophosphate compound than adult rats. The magnitude of age related difference decreased as the rats proceeded towards maturity.

Sheets (2000) supposed that the greater susceptibility of children appears to be due to limited metabolic capacity rather than an inherent difference in the sensitivity of target sites.

Sex variation has no significant effect

on serum ChE values in both control and organophosphate exposed groups whether in rural or urban areas in the present study. In this respect Moser et al. (1998) found also that sex variation had no significant influence on the mean serum ChE values whatever may be their environment polluted by organophosphate or not.

It can be concluded from this work that children are more sensitive to acute organophosphate exposure. In addition middle aged individuals in rural areas constitute a higher risk group as compared with those in urban areas. Preventive measures should be taken to protect these age groups from accidental and occupational exposure to organophosphorus compounds.

It can be concluded also that serum ChE estimation can be more helpful in confirming the diagnosis of organophosphate exposure in presence of baseline levels of exposed subjects, because of some age and residency related variations detected in healthy subjects.

Table (1): Mean serum cholinesterase values (IU/L) of all subgroups in urban areas.

Groups		Urban ♂ < 15 years	Urban ♀ < 15 years	Urban ♂ 20-50 years	Urban ♀ 20-50 years	Urban ♂ > 60 years	Urban ♀ > 60 years
Control	mean	2400	2420	2440	2500	2480	2420
	S.D.	± 158.11	± 106.07	± 138.74	± 79.06	± 103.68	± 83.67
O.P. exposed	mean	472	468	860	880	780	800
	S.D.	± 16.43	± 17.89	41.83	± 57.01	± 57.01	± 79.06

Table (2): Mean serum cholinesterase values (IU/L) of all subgroups in rural areas.

Groups		Rural ♂ < 15 years	Rural ♀ < 15 years	Rural ♂ 20-50 years	Rural ♀ 20-50 years	Rural ♂ > 60 years	Rural ♀ > 60 years
Control	mean	2380	2360	2200	2240	2390	2410
	S.D.	± 158.11	± 119.37	± 119.37	± 96.18	± 134.16	± 96.18
O.P. exposed	mean	402	404	700	730	800	750
	S.D.	± 37.01	± 27.01	± 79.06	± 75.83	± 97.06	± 79.06

Table (3): Comparison of mean serum cholinesterase values in both control and organophosphorus exposed groups regarding residency difference.

Age group	Control Group		Organophosphorus Exposed Group	
	Urban male versus Rural male	Urban female versus Rural female	Urban male versus Rural male	Urban female versus Rural female
< 15 years	0.056	0.841	0.056	0.841
20 - 50 years	0.008*	0.008*	0.008*	0.008*
> 60 years	0.548	0.690	0.548	0.690

*Significant at $P < 0.05$

Insignificant at $P > 0.05$

Table (4): Comparison of mean serum cholinesterase values in both control and organophosphorus exposed groups regarding age difference.

Groups		Subgroup A versus Subgroup B	Subgroup A versus Subgroup C	Subgroup B versus Subgroup C
Control Group	Urban males	0.690	0.548	0.421
	Urban females	0.421	0.660	0.841
	Rural males	0.008*	0.548	0.008*
	Rural females	0.008*	0.841	0.008*
Organophosphorus Exposed Group	Urban males	0.008*	0.008*	0.056
	Urban females	0.008*	0.008*	0.151
	Rural males	0.008*	0.008*	0.095
	Rural females	0.008*	0.008*	0.690

*Significant at $P < 0.05$

Insignificant at $P > 0.05$

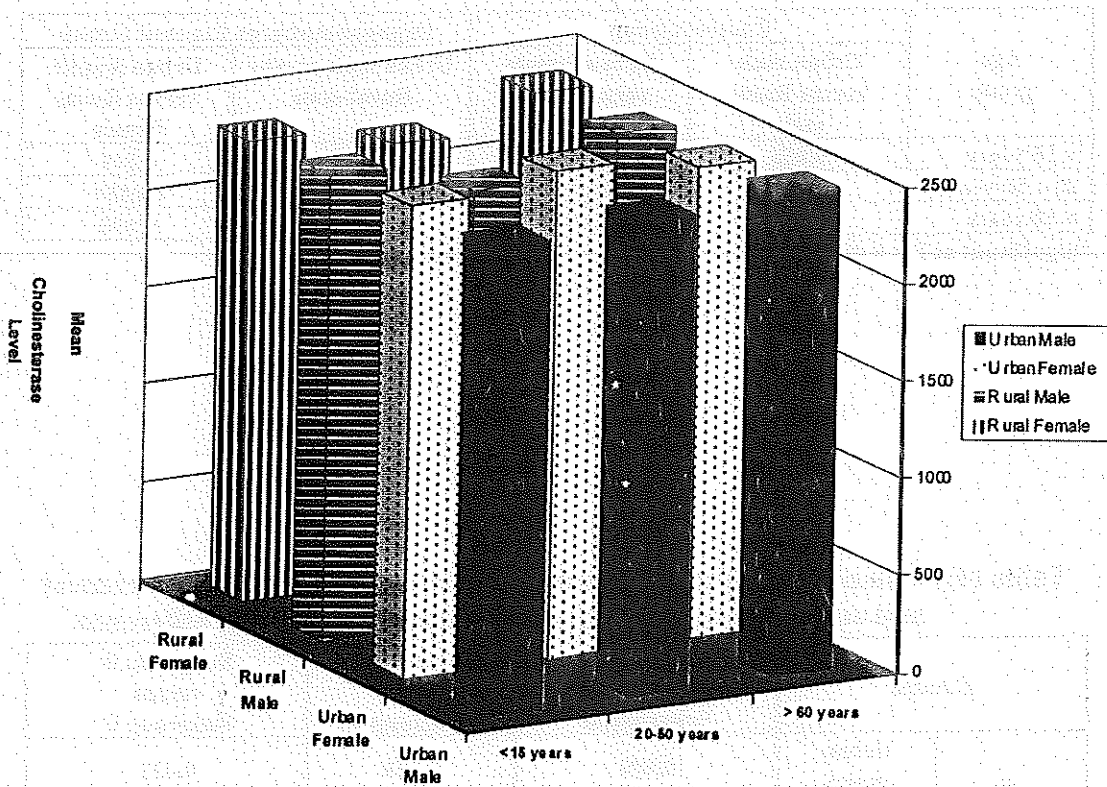


Fig. (1) : Differences between mean serum cholinesterase values (IU/L) in males and females of control subgroups.

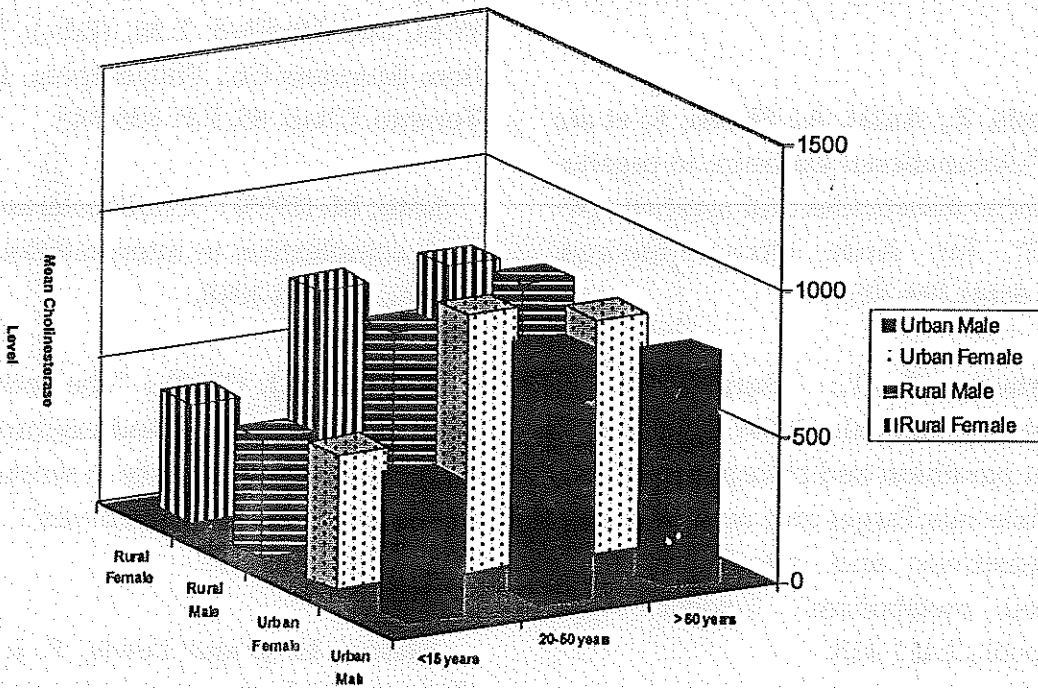


Fig. (2) : Differences between mean serum cholinesterase values (IU/L) in males and females of organophosphorus exposed subgroups.

REFERENCES

- Abdollahi, M.; Kebriaeezadeh, A.; Akkhgari, M.; et al.; (1995): "Cholinesterase levels in saliva, plasma and erythrocytes of Iranian population". *Tox. Lett.*, 78:1-88.
- Adamis, Z.; Antal, A.; Füzesi, I.; et al.; (1985): "Occupational exposure to organophosphorus insecticides and synthetic pyrethroid". *Int. Arch. Occup. Environ. Health*, 66(4):299-305.
- Atterberry, T. T.; Burnett, W. T.; Chambers, J. E.; et al.; (1997) : "Age differences in parathion and chlorpyrifos toxicity in male rats: Target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism". *Toxicol. Appl. Pharmacol.*, 2:411-418.
- Cunha, J.; Povoia, P.; Mourao, A. J.; et al.; (1995) : "An analysis of mortality and the value of serum cholinesterase in monitoring clinical course". *Acta Med. Portuguesa*, 8:469-475.
- Eddleston, M. (2000) : "Patterns and problems of deliberate self-poisoning in the developing world". *Q. J. Med.*, 93:715-731.
- Ellman, G. L.; Courtney, K. D.; Andres, V.; et al.; (1961) : "A new and rapid colorimetric determination of acetylcholinesterase activity". *Biochem. Pharmacol.*, 7:88-95.
- Haddad, L. M. (1998) : Pesticides. In: *Clinical Management of Poisoning and Drug Overdose*. Carlton, F.B.; Simpson, W.M. and Haddad, L.M. (Eds.), 3rd edition, Saunders Co., Philadelphia, London, Toronto, Chap. 65, P.P. 836-845.
- Lotti, M. (1995) : "Cholinesterase inhibition: complexities in interpretation". *Clin. Chem.*, 41:1814-1818.
- Mason, H. J. (2000) : "The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos". *Occup. Med.*, 50(5):343-347.
- Mason, H. J. and Lewis, P. J. (1989) : "Intra-individual variation in plasma and erythrocyte cholinesterase activities and the monitoring of uptake of organophosphorus pesticides". *J. Soc. Occup. Med.*, 39:121-124.
- Moser, V. C.; Chanda, S. M.; Mortensen, S. R.; et al.; (1998) : "Age and gender related differences in sensitivity to chlorpyrifos in the rat reflect developmental profiles of esterase activities". *Toxicol. Sci.*, 46:211-222.
- Moss, D. W. and Henderson, R. A. (1999) : Enzymes. In *Tietz Textbook of*

Clinical Chemistry. Carl, A.B. and Edward, R.A. (Eds.), 3rd edition, Saunders Co., Philadelphia, London, Toronto, Chap. 20, P.P. 382-386.

Namba, T.; Nolte, C. T.; Jackrel, J.; et al.; (1971) : "Poisoning due to organophosphate insecticides: Acute and chronic manifestations". Am. J. Med., 50:475-492.

Reitman, S. and Frankel, S. (1957) : "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases". Am. J. Clin. Pathol., 28(1):56-63.

Sheets, P. (2000) : "A consideration of age dependent differences in susceptibility to organophosphorus and pyrethroid insecticides". J. Neurotoxicol., 1:57-63.

Wesseling, C.; McConnell, R.; Partanen, T.; et al.; (1997) : "Agricultural pesticide use in developing countries: health effects and research needs". Int. J. Health Serv., 27:273-308.

Zwiener, R. J. and Ginsburg, C. M. (1988) : "Organophosphate and carbamate poisoning in infants and children". Pediatr., 81(1):121-126.

صلاحية قياس مستوى إنزيم الكولين إستيريز في مصل الأشخاص الأصحاء وحالات التسمم بالهوكبات الفوسفورية العضوية

المشركون في البحث

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أجريت هذه الدراسة على عدد ١٢٠ شخصاً من محافظة الدقهلية، تم إختيار نصف هؤلاء الأشخاص (٦٠ شخصاً) من الأصحاء كمجموعة ضابطة (مجموعة ١) أما النصف الآخر (٦٠ شخصاً) تم إختيارهم من المرضى المتسممين بالمبيدات الفوسفورية العضوية بوحدة السموم بمستشفى الطوارئ بالمنصورة (مجموعة ٢)، كان كل الأشخاص الذين تم إختيارهم في المجموعتين لا يعانون من اضطراب في وظائف الكبد، تم تقسيم كل مجموعة إلى ثلاثة مجموعات تبعاً للسن يضم كل منها ٢٠ شخصاً : كمجموعة (أ) تضم أشخاص سنهم أقل من ١٥ عام (أطفال) ومجموعة (ب) تضم أشخاص يتراوح سنهم من ٢٠-٥٠ عام ومجموعة (ج) تضم أشخاص سنهم أكثر من ٦٠ عام، تضم كل مجموعة فرعية ١٠ أشخاص من الحضر نصفهم من الذكور ونصفهم من الإناث و ١٠ أشخاص من الريف نصفهم من الذكور ونصفهم من الإناث. تم أخذ عينة دم من كل شخص في المجموعتين أستخدم نصفها الأول لاختبارات وظائف الكبد (AST و ALT) وأستخدم نصفها الثاني لقياس مستوى إنزيم الكولين إستيريز في المصل، لوحظ إنخفاض ذو دلالة إحصائية في مستوى إنزيم الكولين إستيريز بمصل الأشخاص المتسممين بالمبيدات الفوسفورية العضوية عنه في مصل الأشخاص الأصحاء. كما لوحظ إنخفاض ذو دلالة إحصائية في مستوى إنزيم الكولين إستيريز بمصل الأشخاص المتسممين بالمبيدات الفوسفورية العضوية عنه في مصل الأشخاص الأصحاء، كما لوحظ إنخفاض ذو دلالة إحصائية في مستوى إنزيم الكولين إستيريز بمصل الأشخاص المتسممين بالمبيدات الفوسفورية العضوية (ب) أي الأشخاص من ٢٠-٥٠ عام بالريف عنه بالمجموعة الفرعية (ب) بالحضر سواء كانوا أصحاء أو متسممين بالمبيدات الفوسفورية العضوية، وكان هناك إنخفاض ذو دلالة إحصائية في مستوى إنزيم الكولين إستيريز بالمصل في الأشخاص الأصحاء (المجموعة الضابطة) من الريف فقط بالمجموعة الفرعية (ب) عنه بالمجموعتين (أ) و (ج). كما كان هناك إنخفاض ذو دلالة إحصائية في مستوى إنزيم الكولين إستيريز بمصل الأشخاص المتسممين بالمبيدات الفوسفورية بكل من الريف والحضر بالمجموعة الفرعية (أ) أي الأطفال عنه بالمجموعتين الفرعيتين (ب) و (ج)، ولم يوجد إختلافات ذات دلالة إحصائية في مستوى إنزيم الكولين إستيريز بالمصل بين الذكور والإناث في الريف أو الحضر سواء كانوا أصحاء أو متسممين بالمبيدات الفوسفورية العضوية.