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International Journal of Current Research
Vol. 7, Issue, 12, pp.23667-23670, December, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

A STUDY TO ASSESS THE USEFULNESS OF PLASMA CHOLINESTERASE (PSEUDOCHOLINESTERASE) AS A MARKER IN ACUTE INFLAMMATORY CONDITIONS : A CASE CONTROL STUDY

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ARTICLE INFO

Article History:
Received 25th September, 2015
Received in revised form
07th October, 2015
Accepted 07th November, 2015
Published online 21st December, 2015

Key words:

Total leucocytes count (TLC),
Acute inflammation,
and Plasma cholinesterase,
ROC(receiver operator) curve,
AUC(area under curve).

ABSTRACT

Background: Acute inflammatory conditions are one of the most common problems faced in the clinical setting. Plasma cholinesterase has been shown to have variable correlation with chronic inflammatory conditions like Diabetes, Alzheimer's etc. This study aims to assess its correlation in acute inflammatory conditions like sepsis
Objective: To assess the usefulness of Cholinesterase as a marker in Acute Inflammatory conditions.
Methodology: The correlational case control study involved 30 patients admitted in the surgery & medicine departments with complaints of fever, abdominal pain & cough. Total leucocytic count (TLC) and plasma cholinesterase were determined and the results were compared with 30 ages and sex matched healthy people taken as controls.
Results: There was a moderate negative correlation between CHE & TLC in cases ($r= -0.567$, $p< 0.05$). Unpaired students t test ($T= 3.194$, $p< 0.05$) showed a significant difference in CHE values in cases in comparison to controls. Finally the ROC analysis showed that AUC for CHE in cases was 0.7, indicating a fair test with sensitivity of 50% and a specificity of 100%.
Conclusion: In our study it was found that the correlation of plasma cholinesterase was more with the cases than for the controls. Because the correlation is not very strong, doing cholinesterase in isolation for sepsis is not recommended. Also it was found that even though CHE levels had moderate sensitivity but had high specificity enabling us to at least rule out acute inflammation.

***Citation*:** Arun Sinha, Vibha C. and Vishwanth, H. L. 2015. A study to assess the usefulness of cholinesterase as a marker in acute inflammatory conditions (sepsis) : A case control study", *International Journal of Current Research*, 7, (12), 23667-23670.

INTRODUCTION

Cholinesterase represents a group of enzymes that hydrolyze acetylcholine and other choline esters. There are two main types of cholinesterase with different biochemical properties: true or specific cholinesterase or acetyl cholinesterase (EC 3.1.1.7) found in all excitable tissues (central and peripheral nervous system and muscles) and in erythrocytes and pseudo cholinesterase also known as plasma cholinesterase, butyrylcholinesterase (EC 3.1.1.8) present in the plasma and produced by the liver (Santarpia *et al*., 2013). Butyrylcholinesterase (BChE) is an α-glycoprotein found in the central and peripheral nervous system, in most tissues, and in the liver. It has lower affinity for acetylcholine and is not inhibited by high concentrations of acetylcholine (Santarpia *et al*., 2013; Davis *et al*., 1997). BChE half-life is about 12days. BChE is synthesized in the liver: hepatocellular impairment will reflect a decreased enzyme activity. In fact, plasma levels fall in acute and chronic liver damage, cirrhosis, and liver metastases, being a biochemical marker of organ damage. Low plasma BChE levels have also been found in protein-energy malnutrition, during stress and (chronic and acute) inflammation, and in other clinical conditions (Santarpia *et al*., 2013; Lampón *et al*., 2012). An increased activity of this enzyme has been reported in obesity, diabetes, uraemia, hyperthyroidism, and in hyperlipidemic subjects. (Santarpia *et al*., 2013; Paes *et al*., 2006; Cucuianu *et al*., 2002; Kutty and Payne, 1994) Serum concentrations and BChE activity seem to accurately reflect the availability of amino acidic substrates and/or derangement in protein synthesis due to hepatocellular damage. In cancer, with or without liver impairment, serum BChE levels serve as an accurate functional and prognostic indicator, useful for monitoring clinical and therapeutic interventions according to patients' prognosis. In the absence of inflammation, BChE could also serve as an index of the effectiveness of nutritional support.

Inflammation is fundamentally a protective response whose ultimate goal is to eliminate the injury-inducing agent (that could be a microorganism, physical stimuli, chemical agent, etc.), prevent tissue damage and/or initiate the repair process. In the absence of adequate inflammation cell/tissue injury would go unchecked and the damage done to the cells/tissues/organs would never heal, and ultimately this may lead to the death of the organism itself. Thus, inflammation is

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both beneficial and potentially harmful. (Das, 2007; Das, 2006) Some of the important mediators of inflammation include: histamine, serotonin, lysosomal enzymes, prostaglandins (PGs), leukotrienes (LTs), platelet activating factors (PAFs), reactive oxygen species (ROS), nitric oxide (NO), hypochlorite (HOCL), various cytokines, kinin system, coagulation/ fibrinolysis system, and complement system. The major cellular sources of these mediators are platelets, neutrophils, mast cells and monocytes/macrophages, but mesenchymal cells such as endothelium, smooth muscle, fibroblasts, and most epithelia can also elaborate some of these mediators. In majority of the instances, one mediator triggers the release of another mediator that acts on the target tissue. These secondary mediators either potentiate the action of the initial mediator or paradoxically abrogate its action. Thus, the ultimate degree of inflammation depends on the balance between such pro- and anti-inflammatory mediators. In several low-grade systemic inflammatory conditions plasma and RBC activities of acetyl cholinesterase and butyrylcholinesterase enzymes are increased. Hence, when the activities of enzymes acetyl cholinesterase and butyrylcholinesterase are increased it will lead to reduced levels of acetylcholine. This leads to a reduction in the anti-inflammatory actions exerted by Ach. (Ach has been found to have anti-inflammatory action) Thus, increased plasma, CSF, and RBC activities of acetyl cholinesterase and butyrylcholinesterase enzymes indirectly reflect reduced levels of ACh that, in turn, will enhance local and systemic inflammatory events due to the absence of the negative feedback control exerted by Ach (Santarpia *et al*., 2013).

Objectives

1. To study the correlation of plasma cholinesterase with total leucocytic counts in sepsis patients.
2. To assess the usefulness of plasma cholinesterase in diagnosing acute inflammation (sepsis).

METHADOLOGY

The correlational case control study involved 30 patients admitted in the surgery & medicine departments with complaints of fever, abdominal pain & cough. Total leucocytic count (TLC) was ordered in each of the patients and was found to be elevated. The patient samples were then run for plasma cholinesterase and the results were compared with 30 age and sex matched healthy people taken as controls. Plasma CHE was estimated in Beckman Coulter AU480 by photometric analysis and TLC was estimated using Beckman coulter haematology analyzer LS 780. The patients having documented evidence of liver disorders, alcoholics were excluded from the study.

Statistical analysis (Bernard Rosner, 2000; Robert H Riffenburg, 2005; Sunder RAO and Richard, 2006) & Results

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data are made,

Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, and Cases and control samples should be independent. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Sensitivity, Specificity and Area under Curve (AUC) is obtained using the ROC curve analysis

Diagnostic values based on Area under curve
0.9-1.0: Excellent test
0.8-0.9:Good test
0.7-0.8: Fair test
0.6-0.7: Poor test

RESULTS

The mean age of the cases was found to be 28.33±15.5 yrs in comparison to controls whose mean age was 41.20±16.87 yr, p< 0.05.Of the cases 50% were males and 50% females where as in controls 43% cases were females and 57% males.

Table 1. Age distribution of patients studied

Age in years	Cases		Controls	
	No	%	No	%
1-10	4	13.3	1	3.3
11-20	3	10.0	2	6.7
21-30	16	53.3	8	26.7
31-40	2	6.7	4	13.3
41-50	1	3.3	3	10.0
51-60	2	6.7	9	30.0
>60	2	6.7	3	10.0
Total	30	100.0	30	100.0
Mean ± SD	28.33±15.52		41.20±16.87	

P=0.003**

Table 2. Gender distribution of patients studied

Gender	Cases		Controls	
	No	%	No	%
Female	15	50.0	13	43.3
Male	15	50.0	17	56.7
Total	30	100.0	30	100.0

P=0.605, Not significant, Chi-Square test

Table 3. plasma CHE (u/l) levels in two groups studied

CHE (u/l)	Cases		Controls	
	No	%	No	%
<5320	15	50.0	1	3.3
5320-12920	15	50.0	29	96.7
>12920	0	0.0	0	0.0
Total	30	100.0	30	100.0

P<0.001**, Significant, Fisher Exact test

Table 4. TLC (/cumm) levels in two groups studied

TLC (/cumm)	Cases		Controls	
	No	%	No	%
<5500	0	0.0	0	0.0
5500-11000	0	0.0	30	100.0
>11000	30	100.0	0	0.0
Total	30	100.0	30	100.0

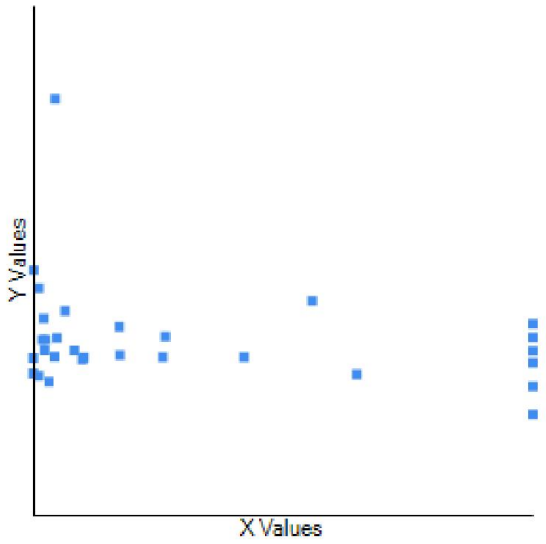
P<0.001**, Significant, Fisher Exact test

Table 5. Comparison of plasma CHE and TLC in two groups studied

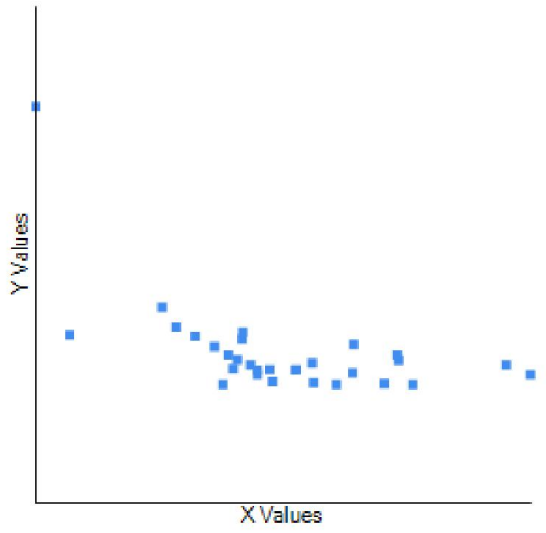
	Cases	Controls	P value
CHE (u/l)	5715.04±2127.31	7208.87±1426.66	0.002**
TLC (/cumm)	15264.93±5072.06	8321.90±1608.10	<0.001**

Comparison of plasma CHE values amongst cases and controls showed that in cases 50% patients had plasma CHE values < 5320 u/l (normal laboratory range 5320- 12000u/l) and 50% patients had values in the normal range. The mean TLC in cases was 15264± 5072 / cumm in comparison to controls where mean TLC was found to be 8321± 1608.

When the correlation coefficient for plasma CHE was calculated, r= -0.5668,p< 0.05.This showed a moderately negative correlation with TLC. In controls r = -0.4731,p<0.05 ,indicating a weak negative correlation.



Scatter dig b/w Plasma CHE y axis v/s TLC in controls



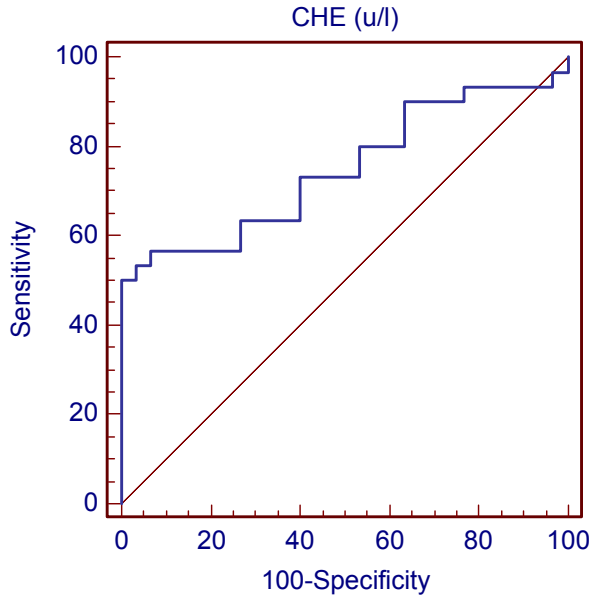
Scatter dig of cases showing moderately negative correlation b/w

Plasma CHE V/S controls

Unpaired student t test was conducted on the sample giving a T value of 3.194, p< 0.05, showing a significant difference in plasma CHE value in cases as compared to controls. Finally ROC analysis was conducted to assess the sensitivity and specificity of plasma CHE in high TLC patients.

Table 6. ROC curve analysis to Comparison of CHE and TLC

	Cut-off	Sensitivity	Specificity	AUC	SE	P value
CHE (u/l)	≤5173	50.00	100.00	0.749	0.063	<0.001**



The ROC analysis showed that plasma CHE had a sensitivity of 50% and a specificity of 100% with AUC being 0.7, p< 0.05.

DISCUSSION

From the above data it can be inferred that there is no age and gender bias in plasma CHE levels in high TLC patients. Also there is statistically significant difference between diseased and healthy patients. From the ROC analysis it found that plasma CHE had a high specificity which would enable it to be used to rule out acute inflammations like sepsis. But the correlation of plasma CHE with high TLC was found to be only moderately negative thus indicating its limitations as a single use marker and hence must be used along with other established markers and clinical judgment. This statement was further supported by the fact that the sensitivity of plasma CHE was only 50% with AUC value showing a fair test.

Conclusion

There have been several studies which have shown that there is an elevation in plasma CHE in low grade systemic inflammatory conditions like diabetes. This study is an attempt to see its levels in acute inflammatory conditions. From the study we may conclude that even though there was a moderate correlation of plasma CHE with high TLC as found commonly in sepsis the sensitivity is its biggest drawback.

Limitations

1. Small sample size
2. The correlation between plasma CHE and high TLC was only moderate, even though it was statistically significant.
3. Cost of estimation of Plasma CHE
4. Plasma CHE is a highly non specific parameter with regards to sepsis as a condition.
5. Nutritional status of patients was not evaluated even though it is thought that plasma CHE has a direct link with it.

REFERENCES

Abou-Hatab K, O'Mahony MS, Patel S, Woodhouse K. Relationship between age and plasma esterases. Age Ageing. 2001;30:41–5.

Bernard Rosner 2000. Fundamentals of Biostatistics, 5th Edition, Duxbury, page 80-240

Buchanan JB, Johnson RW. Regulation of food intake by inflammatory cytokines in the brain. *Neuroendocrinology*. 2007;86:183–90.

Camarero González E, *et al*. Protein-energy malnutrition: its effects on 4 metabolic parameters. *Nutr Hosp*., 1995; 10:158–60.

Cucuianu M, Nistor T, Hâncu N, Orbai P, Muscurel C, Stoian I. Serum cholinesterase activity correlates with serum insulin, C peptide and free fatty acids levels in patients with type 2 diabetes. *Rom J Intern Med*., 2002;40:43–51.

Dabke AT, Pohowalla JN, Inamdar S, Singh SD, Mathur PS. Serum cholinesterase and histopathology of the liver in severe protein calorie malnutrition. *Indian J Pediatr*., 1972;39:151–7.

Das U N. Acetyl cholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. *Med Sci Monit*, 2007; 13(12): RA214-221

Das UN. Acetyl cholinesterase and butyrylcholinesterase as markers of low-grade systemic inflammation. *Ann Hepatol*., 2012;11:409–11.

Das UN: Clinical laboratory tools to diagnose inflammation. *Adv Clin Chemistry*, 2006; 41: 189–229

Davis L, Britten JJ, Morgan M. Cholinesterase. Its significance in anaesthetic practice. *Anaesthesia*. 1997;52:244–60.

Fuhrman MP, Charney P, Mueller CM. Hepatic proteins and nutrition assessment. *J Am Diet Assoc*., 2004;104:1258–64.

Kutty KM, Payne RH. Serum pseudo cholinesterase and very-low-density lipoprotein metabolism. *J Clin Lab Anal*., 1994;8:247–50.

Lampón N, Hermida-Cadahia EF, Riveiro A, Tutor JC. Association between butyrylcholinesterase activity and low-grade systemic inflammation. *Ann Hepatol*., 2012;11:356–63.

Montgomery RD. The relation of oedema to serum protein and pseudo cholinesterase levels in the malnourished infant. *Arch Dis Childr*., 1963; 38:343.

Paes AM, Carniatto SR, Francisco FA, Brito NA, Mathias PC. Acetyl cholinesterase activity changes on visceral organs of VMH lesion-induced obese rats. *Int J Neurosci*., 2006;116:1295–302.

Robert H Riffenburg 2005. Statistics in Medicine, second edition, Academic press. 85-125.

Santarpia L, Grandone F.I, Fabrizio C.P. Butyrylcholinesterase as a prognostic marker: a review. *J Cachexia Sarcopenia Muscle,* (2013) 4:31–39

Soeters PB, Schols AM. Advances in understanding and assessing malnutrition. Curr Opin Clin Nutr Metab Care. 2009;12:487–94. 15. Hubbard RE, O'Mahony MS, Calver BL, Woodhouse KW. Plasma esterases and inflammation in ageing and frailty. *Eur J Clin Pharmacol*., 2008;64:895–900.

Sunder Rao P S S, Richard J 2006. An Introduction to Biostatistics, A manual for students in health sciences , New Delhi: Prentice hall of India. 4th edition, 86-160

Suresh K.P. and Chandrasekhar S. 2012. Sample Size estimation and Power analysis for Clinical research studies. *Journal Human Reproduction Science*, 5(1), 7-13.

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