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# EVALUATION OF SERUM BUTRYLCHOLINESTERASE ACTIVITY AS BIOMARKER FOR PREDICTING RISK OF ISCHEMIC HEART DISEASE

**GIRISH M.DESAI[1]*, RAGHUNANDANA .R[2], ANAND K.BOYAPATI[3] AND ARCHANA C.NANDYAL[1]**

*[1]Department of Biochemistry, Mahadevappa Rampure Medical College, Gulbarga-585105 (Karnataka-India)*
*[2]Department of Biochemistry,Sri Siddhartha Medical College, Tumkur (Karnataka-India)*

## ABSTRACT

The incidence, prevalence, mortality and morbidity of ischemic heart disease is increasing allover the world. Early detection and prevention of ischemic heart disease is important. Hence, identification of biomarkers for cardiovascular disease and its risk factors is needed.Aims and objectives of this study was to evaluate the association of butrylcholinesterase activity with lipid risk ratios in ischemic heart disease. Materials & Methods: The cross sectional comprises 50 healthy subjects and equal number of myocardial infarction cases. Serum butrylcholinesterase activity, lipid profile was estimated and lipid ratios were calculated. Data was statistically analyzed.In our study , serum butrylcholinesterase activity was increased in myocardial infarction cases and correlated with lipid ratios in them . Serum butrylcholinesterase activity may be used in clinical practice as an tool for prediction of ischemic heart disease.

**KEYWORDS:** **Butyrylcholinesterase; Atherosclerosis; Ischemic heart disease; Lipid profile; Biomarkers.**

**GIRISH M.DESAI**
**Department of Biochemistry, Mahadevappa Rampure Medical College, Gulbarga-585105 (Karnataka-India)**

***Corresponding author**

# INTRODUCTION

WHO has declared coronary artery disease as our modern epidemic. It is a very important public health problem. Cardiovascular disease accounts for 30% of deaths worldwide[1]. Epidemological trends indicate that there would be an increase in incidence of cardiovascular diseases all over the world particularly developing countries. WHO predicts 11.1 million deaths globally and 71% death in developing countries due to coronary heart disease by 2020 AD[2]. The prevalence of coronary artery disease is constantly rising. The estimated global cost of cardiovascular disease was USD 863 million in 2010 and is expected to increase by 22% in next 20 years due to population ageing. India is in epidemiological transition. In addition to the burden of endemic infections , emerging threat of non-communicable diseases are a matter of concern. The incidence of cardiovascular disease has increased from 7% in 1970 to 32% in 2011. Ischemic heart disease is predicted to rise in India by 30-60% between 1990 and 2020. The current prevalence is 65.9 per 1000 males and 47.8 per 1000 females. Research has shown that treatment of cardiovascular disease is difficult. Hence, the ability to prevent the development of cardiovascular disease is important and serum markers of atherosclerosis for early detection and prediction of risk has gained much attention in years ( 3 ). Ischemic heart disease is a condition in which there is an inadequate supply of blood and oxygen to a portion of myocardium. It typically occurs when there is an imbalance between myocardial oxygen supply and demand (4). The most common cause of myocardial infarction is atherosclerotic disease of epicedial artery. Atherosclerosis is thickening of coronary arterial walls due to deposition of lipids with reduction in the lumen. The risk factors for above conditions are age, sex, family history, diabetes mellitus, hypertension, hyperlipidemia, cigarette smoothing, etc.[4].

Adverse lipid profile is one of the major risk factors of coronary artery disease and myocardial infarction. Dylipidemia is a prominent and modifiable risk factor for cardiovascular diseases[5]. Dyslipidemia is manifested by elevation or attenuation of plasma concentrations of lipoproteins[6]. A traditional atherosclerotic lipid profile is characterized by high serum levels of cholesterol, triglycerides, low density lipoproteins and low levels of high density lipoproteins. The casual association between plasma lipid level and risk of atherosclerosis is well established[7]. Serum lipid levels are strongly correlated with coronary artery disease. Currently there is a clear perception of interrelationship between serum lipids, atherosclerosis and ischemic heart disease[7]. The need for identifying risk factors and serum markers of atherosclerosis for early detection and prediction of coronary artery disease has gained importance . The lipoproteins contribute to development of ischemic heart disease through lipid accumulation leading to vascular injury, atheroma and thrombus formation ( 8 ). The variations of serum lipids from their normal levels can predict the development of coronary artery disease[9]. Intensive treatment of dyslipidemia stabilizes atherosclerosis and promotes its regression and reduces cardiovascular mortality in patients with coronary artery disease ( 10 ).

In the absence of abnormal lipid profile the possibility of coronary artery disease cannot be ruled out[11]. Risk of coronary artery disease may be under estimated in some patients without typical dyslipidemia. Several indices have been derived from lipid profile to establish an index for predicting the risk of coronary heart disease. These lipid indices optimize the predictive capacity of lipid profile[12]. They reflect a balance between atherogenic and anti-atherogenic lipids[13]. They have a greater correlation with coronary artery disease. Castelli's risk index, atherogenic index of plasma, atherogenic coefficient, cholindex are some of the lipid ratios used for predicting the risk of coronary artery disease in clinical practice[14]. Lipid ratios indicate dyslipidemia and risk of coronary artery disease. They are better predictors of coronary artery disease than lipids alone[15]. The enzyme butrylcholinesterase is synthesized by liver and present in serum. It is a glycoprotein with esterase activity[16]. It has a role in lipid and lipoprotein metabolism[17-20]. Its activity has

shown a correlation with low density lipoprotein, high density lipoprotein and triglycerides. It shows positive correlation with coronary artery disease and has been implicated in its development[21]. It has been proposed for use as complementary risk factor for coronary artery disease[22]. Butrylcholinesterase has a role in lipid and lipoprotein metabolism and is associated with coronary artery diseases. It is hypothesized that butryl cholinesterase has a role in causing serum lipid changes leading to atherogenesis and ischemic heart disease. The present study was undertaken to evaluate the relationship of serum butrylcholinesterase activity with lipid risk indicators in patients with ischemic heart disease.

### *OBJECTIVES*

1. To compare the serum pseudocholinesterase activity, and lipid ratios in myocardial infarction patients and healthy subjects.
2. To correlate the serum butrylcholinesterase activity with lipid ratios in myocardial infarction patients and healthy subjects.

## MATERIALS AND METHODS

The research protocol was approved by M.R.Medical College's Ethical Committee. The study design was analytical observational cross sectional study. . It was carried out for a period of one year from 01.11.2012 to 31.10.2012 at Basaveshwar Teaching & General Hospital, Gulbarga, Karnataka . The study subjects were selected by simple random sampling. They comprised of 100 subjects divided into 2 groups – cases and controls. The case group included myocardial infarction patients and healthy volunteers were included in the control group. The study subjects were selected by the following inclusion and exclusion criteria. The inclusion criteria were patients (1) between 30 to 60 years of age (2) of both male and female sex (3) having myocardial infarction. and exclusion criteria were hPatients with (1) history of cardiac risk factors ,(2)any systemic illness, (3) recent surgery (4) trauma, (5) endocrinal and nutritional disorders, (6) on treatment with drugs affecting lipid metabolism . The equipment used for the study are BD vacutainers, syringes, Biohit Micropippettes, Remi Centrifuge and auto-analyzer Roche CIII auto analyzer. The reagent kits for biochemical estimations were obtained from Roche Diagnostics, Gmbh, Mannheim. The material used for this study consists of a well structured questionnaire and blood samples. Before participation, the volunteers were informed of the nature and purpose of the study and written consent was obtained from them expressing their voluntary willingness to participate in this study. A detailed history was obtained by the physicians and a complete physical examination was done with special emphasis on cardiovascular disease. Diagnosis of myocardial infarction was done based on ECG changes or rise in cardiac biomarkers. With a disposable plastic syringe with needle gauge No. 20 , 2 ml of fasting blood sample was collected aseptically from median cubital vein of each individual into a plain vacutainer. Blood was allowed to clot and then centrifuged at 4000 RPM for 15 minutes to obtain serum. The serum sample was subjected to the following biochemical estimations,

Butrylcholinesterase activity by Butrylthiocholine method ,
CKMB by Immunoinhibition IFCC method ,
Troponin I by Immunochromatography card method ,
Total Cholesterol by CHOD-PAP method ,
Triglycerides by GPO PAP ESPAS method ,
HDL by Immunoinhibition method and
LDL by Homogeneous enzymatic assay .

The reference ranges of above parameter were ,

| | |
|---|---|
| CKMB | 0-24 IU/L |
| Butrylcholinesterase | 4659-14443 IU/L |

| | |
|---|---|
| Total cholesterol | 150-200 mg/dL |
| Triglycerides | 50-150 mg/dL |
| HDL | 35-70 mg/dL |
| LDL | 80-130 mg/dL |

The atherogenic lipid ratios were calculated as follows:

Castelli's Risk Index-I (CRI) = $TC/ HDL_C$

Castelli's risk index-II (CRII) = $LDL_C/ HDL_C$

Atherogenic index of plasma (AIP) = $\text{Log } TG/ HDL_C$

Atherogenic coefficient (AC) = $\frac{TC - HDL_C}{HDL_C}$

Cholindex = $LDL_c - HDL_C$ (TG<400)

= $LDL_c - HDL_C$ (TG>400)

The descriptive analysis of the data was done by mean and standard deviation. The comparison of parameters between cases and controls were done by unpaired 2-tailed t-test. $p<0.05$ was considered statistically significant. The association between dependent and independent variables was analyzed by Pearson correlation coefficient test. Statistical analysis of the data by using statistical data analysis software SPSS Version-1.7.

## RESULTS

The data from the above analysis were compiled into two tables i.e., Table-1 and Table-2.

Table-1 compares the mean value of the variables in healthy volunteers and myocardial infarction patients. The difference in age and sex were not statistically significant. Statistically significant differences were seen for butryl cholinesterase activity ( Fig 1 ), Castelli's risk index, atherogenic index of plasma, atherogenic coefficient and cholindex between the 2-groups.

**Table-1**

***Comparison of pseudocholinesterase and lipid ratios of the subjects***

| Parameters | Healthy subjects (mean±SD) | MI patients (mean±SD) | t-value | p-value | Significance |
|---|---|---|---|---|---|
| Age (years) | 46.82±8.62 | 51.62±16.82 | 1.76 | >0.05 | Not significant |
| Sex | M=50; F=0 | M=44; F=6 | 0.52 | >0.05 | Not significant |
| Buche (IU/L) | 7981±697.99 | 9661.15±2564.81 | 3.16 | <0.05 | Significant |
| CR-I | 3.54±0.74 | 4.69±1.90 | 2.85 | <0.05 | Significant |
| CR-II | 1.92±0.65 | 2.90±7.44 | 4.02 | <0.05 | Significant |
| AIP | 0.44±0.12 | 0.57±0.14 | 2.31 | <0.05 | Significant |
| AC | 2.39±0.92 | 3.88±0.19 | 3.24 | <0.05 | Significant |
| CI | 43.8±8.45<br>62.9±12.24 | 71.08±10.23<br>95.98±14.44 | 2.60<br>2.45 | <0.05<br><0.05 | Significant |

***Buche = Butrylcholinesterase***
***CI = Cholindex***
***CR = Castelli's risk index***
***AIP = Atherogenic index of plasma***
***AC = Atherogenic coefficient***

Table-2 shows the association of serum pseudocholinesterase activity with Castelli's risk index ( Fig 3 ), atherogenic index of plasma, atherogenic coefficient and cholindex. The enzyme activity showed statistically significant correlation with the atherogenic lipid ratios.

**Figure-1**
***Comparison of Cholinesterase activity in cases and controls***

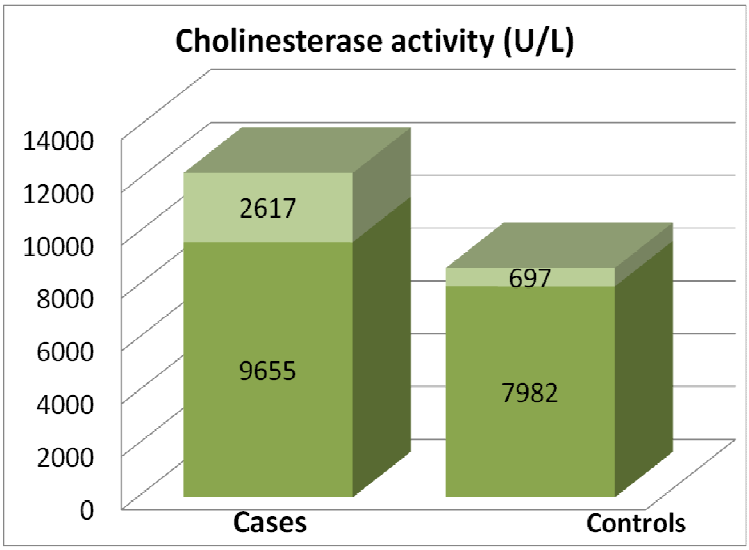
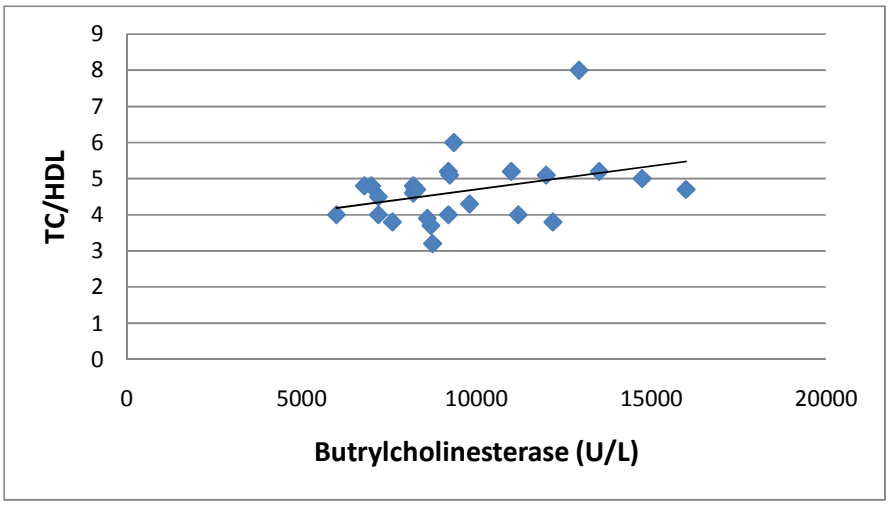



**Table 2**
***Correlation of Butrylcholinesterase with lipid ratios***

| Lipid ratios | r-value | |
|---|---|---|
| | Healthy subjects | Myocardial infarction patients |
| CR-I | 0.34 | 0.60* |
| CR-II | 0.33 | 0.55* |
| AIP | 0.45 | 0.68** |
| AC | 0.38 | 0.52* |
| CI | 0.35<br>0.39 | 0.59*<br>0.62** |

**** Significant; ** High significant***
***CI = Cholindex***
***CR = Castelli's risk index***
***AIP = Atherogenic index of plasma***
***AC = Atherogenic coefficient***

**Figure 2**
***Correlation of Butrylcholinesterase with Castelli's Index in Myocardial infraction .***



## DISCUSSION

The study was done to evaluate the relationship of serum butryl-cholinesterase activity with lipid risk indicators in ischemic heart disease. In the present study, we have compared the values of butrylcholinesterase activity and lipid ratios in healthy subjects and myocardial infarction patients. Also we correlated the butrylcholinesterase activity with lipid ratios. We found increase in pseudocholinesterase and lipid ratios in myocardial infarction patients and pseudocholinesterase activity was associated with the lipid ratios. Increased butrylcholinesterase increases triglyceride synthesis increasing total cholesterol, LDL in blood[24,25]. Increase in triglycerides decreases HDL levels. Increased triglycerides, cholesterol, LDL and decreased HDL cause increase in values of lipid ratios. Increased low density lipoprotein and triglycerides cause atherosclerosis. The findings of our study were similar to other studies done[26-28]. The limitations of the study, where the cross sectional study design, , subjects not sex matched , single measurements and small sample size. Standardized research protocol, examination by experienced physician and biochemist t were the strength of this research work . The demographic and clinical data are lacking. Markers of inflammation oxidative stress, endothelial dysfunction and coagulation were not done. No conflict of interest was declared. All authors contributed in designing of study, collection, analysis, interpretation of study and manuscript preparation. We conclude that elevated butrylcholinesterase activity though in normal range may increase risk of ischemic heart disease through lipid mediated atherogenesis. Further studies need to be done on large to validate the test to use of butrylcholinesterase in clinical practice for screening, prediction and early diagnosis of ischemic heart disease.

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