

Acetylcholinesterase and its Kinetics in the Diagnosis of Myocardial Infarction



Madhavi Gaur, Rajeev Gupta* and Santosh Kumar**

Sant Hirdaram Girls College, Hirdaram Nagar, Bhopal (M.P.)

* Associate Professor, Department of Cardiology, Gandhi Medical College, Bhopal (M.P.)

** Former Vice Chancellor, Dr. H.S. Gour University, Sagar (M.P.)

Abstract : Myocardial Infarction (MI) has been diagnosed on the basis of enzyme assay such as CK-MB, SGOT, LDH, and troponin I & troponin T in addition to clinical findings, ECG changes and Echocardiographic changes. In the present study another enzyme AChE has been taken to estimate the damage of myocardium because heart is richly innervated by cholinergic nerves. Along with the study of AChE enzyme kinetics will help to ascertain the salvage of myocardium, which will affect the contractility of myocardium. The CK-MB & SGOT levels were found to be increased in correlation with ST- elevation in ECG tracings. In the present study the K_m was increased from 2.76×10^{-3} M to 4.5×10^{-3} M. However, the AChE activity was inhibited as the activity is inversely proportion to K_m .

Introduction

Myocardial Infarction is one of the major causes of mortality among the population of both developed and developing countries. The disease is also not sparing the rural population. Myocardial infarction (MI/ heart attack) occurs due to a critical obstruction in a coronary artery due to blood thrombus superimposed on a patch of fatty deposit (atheroma) cutting of the blood supply to the heart muscle. In acute myocardial infarction the ischemic muscles begin to necrose due to injury and ultimately the heart muscles stop functioning (Rasmussen *et al.* 1979; Weiss 1980; Willerson and Buja 1980; Pilhstrom *et al.*, 2005, Thygesen K *et al.* 2012).

The diagnosis of myocardial infarction normally depends upon the characteristic electrocardiographic changes, the pathological Q-wave is absent if infarction is not transmural (Ambos *et al.*, 1978; Lee *et al.* 1987; Goldman *et al.* 1988 Gama & Swain, 1990). Since the middle of last millennium the enzyme assays were used for the diagnosis of MI to avoid the misinterpretation of ECG tracing if the infarction is not trans-mural. Due to injury of heart muscles, large numbers of cardiac enzymes are released from injured heart muscles such as LDH, AST (SGOT) and CK-MB, in addition to these enzyme, Cardiac proteins, troponin I (cTnI) and troponin T (cTnT) have also been released in the serum. In cTnI, residues of amino acid sequences 41-49 and 83-93 of the molecule are stable. These enzymes and these proteins in the serum are used as markers in the diagnosis of MI in human beings (Roberts *et al.* 1975, Lee and Goldman 1986, Main *et al.* 1992, Adams *et al.* 1993 and 1994, Marimuthu *et al.*, 2003; Ruseva, 2005, Shah & Haridas 2007, Neumeir, 1996, Lewandrowski *et al.* 2002, Mercer 1997, Baheti *et al.* 2002, Christenson & Azzazy 1998, Conti 1999, Nagapriyatharsini and Venkata lakshmi, 2010 & Mehta *et al.* 2014). The reliability of enzyme assay has been more

efficient with the development of new technique, called immobilization of enzymes, which has made possible the study of enzyme reaction under conditions more similar to those in living cells.

Since the heart of vertebrates are richly innervated by cholinergic, adrenergic nerve fibres and postganglionic nerve fibers of intracardiac ganglia (Abraham, 1969, Kumar 1971,1973, 1974,1975,1976 and 1978, Qayyum and Fatani, 1987, Kumar & Tembhre, 2010, Srivastava & Kumar 2011), during necrosis of myocardium the AChE may also be released by the injury of cholinergic nerves and it is presumed that AChE released by the cholinergic nerves may be taken as a parameter for the diagnosis of MI (Bhargava 1976, Gaur and Kumar 1993 Parveen and Kumar, 1994, Gaur *et al.*,1999]. Kumar and Gaur (1998) produced artificial MI by isoproterenol hydrochloride in the heart of *Rattus norveicus* and noticed that the K_m of the AChE is increased. Wexler and Greenberg (1978) stated that isoproterenol hydrochloride is β agonist and is found to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscle. Gaur and Kumar (1993) studied the effect of organophosphorus pesticide (dimethoate) on AChE enzyme kinetics in normal and injured myocardium of the heart of fish, *Channa punctatus*. They produced injury in the ventricular myocardium by giving incision. According to them, the K_m of the AChE of normal heart was 1.87×10^{-3} M while it is increased in injured myocardium, it is further increased by the action of dimethoate and maximum increase of K_m was found in incised and dimethoate treated heart. Praveen and Kumar (1994) used isoprenaline hydrochloride for producing MI while Gaur *et al.* (1999) used isoproterenol hydrochloride for producing MI in frog heart. Gaur *et al.* (1999) reported inhibition of acetylcholinesterase in heart of *Rana tigrina*. Gaur and Kumar (2003a) studied AChE activity and enzyme kinetics in isoproterenol

hydrochloride induced myocardial infarction in *Calotes versicolor*. Gaur and Kumar (2003b) studied comparative account of certain enzyme in the serum of homeothermal vertebrates subjected to the production of MI by isoproterenol hydrochloride. The present study is an attempt to study that AChE can be considered as biomarker enzyme for the diagnosis of myocardial infarction.

Materials and Methods

The enzyme reports was orobysed from patients was collected of clinical presentation of myocardial infarction and age and sex matched control printing for admission in intensive coronary care unit. The CK- MB, SGOT and enzyme kinetics of AChE in serum of MI patients have been studied in addition to ECG tracings. The CK-MB value was determined by Autopak Kinetic Immunoinhibition Method.

The SGOT (AST) was determined according to the recommendations of the Scandinavian Committee of Enzymes (SCE). For biochemical and enzyme kinetic study of AChE , methods given by Augustinsson (1951), Chernecky and Berger (2013) and Fischbac & Dunning (2009) have been adopted, using AChI as a substrate. The AChE enzyme kinetic parameters, Km and Vmax of normal and MI patients serum have been computed from Line weavers & Burk plot (Line weaver & Burk,1934) according to Michaelis & Menten equation (1913).

Results

The ECG of patients of Myocardial Infarction showed the significant changes in 12 Leads varying from ST –Elevation, Hyperacute T waves, T inversion, Q wave, poor R wave progression, reciprocal ST depression and various arrhythmias. (Fig 1) The preponderance of changes in various group of leads suggested the localization of infarct territory

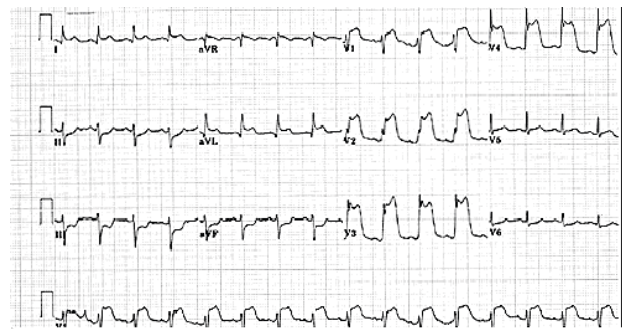
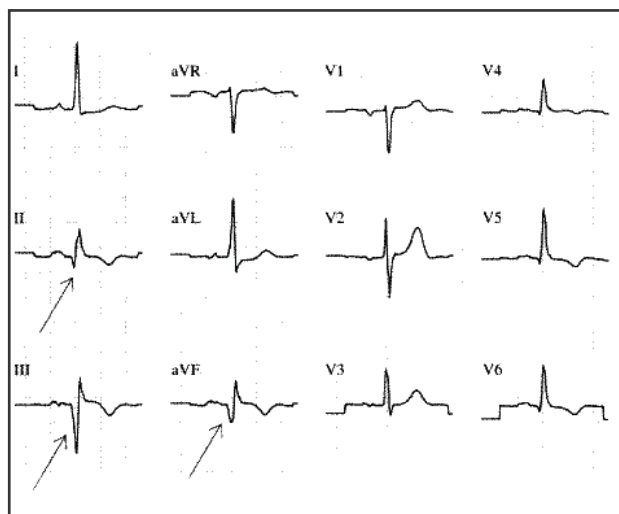


Fig.1. Showing ST elevation in all ECG leads.

The CK-MB value in normal human beings ranged from 0-25 IU/L, however, in MI patients CK-MB value was 158.86 ± 153.97 . There was great elevation of CK-MB level in the serum of MI patients which showed damage of cardiac muscles. The SGOT value in normal human beings ranged from 0-40 IU/L (37°C), whereas in MI patients the SGOT value was increased 136.87 ± 74.35 (Table- 1).

Table 1. CK- MB and SGOT values in control and MI patients serum. Value is the mean \pm S.D. of ten individual observations.

Groups	CK-MB(IU/L)	SGOT (IU/L)
Control (n=10)	0-25	0-40(37° c)
MI(n=10)	158.86 ± 153.97	136.87 ± 74.35

Table 2. AChE enzyme kinetics in normal and MI patients serum. Value is the mean \pm S.D. of ten individual observations.

Groups	Km x 10 ³ M	Vmax (A/mg protein/30/min)
Control (n=10)	2.76 ± 0.664	1.0
MI(n=10)	4.5 ± 1.005	1.0

The inhibitory constants of AChE, Km and Vmax were also determined. In MI patient's serum the Km value was $4.5 \times 10^{-3} M$ against the value of $2.76 \times 10^{-3} M$. The Vmax value remained constant i.e. 1.0 A/mg protein /30min (Table 2 and Fig.2) showing that the inhibition is competitive in nature.

Discussion

The ECG is very sensitive for detecting myocardial ischemia and infarction but frequently is not powerful in differentiating ischemia from necrosis [Lee *et al.*, 1987 & Goldman *et al.*, 1988]. CK-MB has become routine and is regarded as the most sensitive specific and cost- effective means of diagnosing myocardial infarction [Roberts *et al.*, 1975, Grand *et al.*, 1980 and Lee and Goldman, 1986]. Quantitative assay for detecting myocardial injury and measurement of cTnT or cTnI has been proposed as a new

diagnostic criterion for MI [Main *et al.* 1992, Adams *et al.* 1993, 1994, Newby *et al.* 1995].

The LDH and its isoenzyme, SGOT and SGPT are important routine clinical enzymes in the diagnosis of MI in human beings. A number of investigators reported increase of SGOT and SGPT in artificially produced MI by ISO-HCl in rats and in MI patients [Singh *et al.* 1988., Remla *et al.* 1991, Aghi *et al.* 1992, Kaur *et al.* 1995].

The AChE activity during the process of myocardial necrosis has not been studied in detail. The literature concerned the AChE activity and its inhibition deals with pesticide toxicity as pesticide inhibits AChE at nerve terminals [Rao *et al.* 1984, Rao & Rao 1989, Nemcsok *et al.* 1990, Satyadevan *et al.* 1993, Tembhe and Kumar 1994, 1995, 1997, Kumar *et al.* 2000, Gupta *et al.* 2011].

As the heart of vertebrates is richly innervated by cholinergic nerves, keeping a view that during injury of myocardium in MI the AChE activity in serum is inhibited which is further confirmed because of increase of Km. The results are in conformity with the findings of the authors in this field, who also found decrease of AChE activity [Oka 1954; Basu *et al.* 1970]. None of these authors, however, reported Km and Vmax. The Km of this enzyme increased with increase in concentration of pesticides showing inhibition

(Hande & Pradhan 1990 a, b, Al-Jafari *et al.* 1995).

The present study concludes that there is increase of Km value of AChE in the serum of MI patients in comparison to control. It is suggested that these parameters are important in the diagnosis as well as for predicting prognosis in case of MI in addition to tissue specific CK- MB & SGOT. Increase of Km value of AChE in the serum of MI patients shall increase the sensitivity and specificity of biochemical diagnosis of myocardial necrosis and help in prognostication to reduce morbidity.

The present study confirms our previous observations on the heart of fish subjected artificial production of injury in the ventricular myocardium and inhibition of acetylcholinesterase by isoproterenol hydrochloride in *Rana tigrina* (Gaur & Kumar 1993, Gaur *et al.* 1999, Kumar *et al.* 2014)

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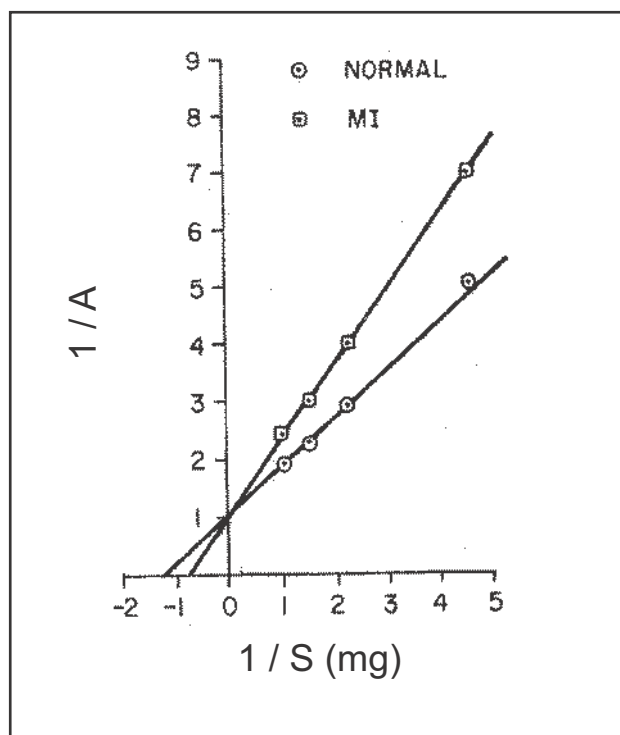


Fig. 2 Lineweaver-Burk plot of inhibition of AChE of human serum in myocardial infarction patients. (S) is the substrate concentration of acetylcholine iodide.

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