

KINETIC STUDIES OF ERYTHROCYTES ACETYLCHOLINESTERASE WITH OTHER DIAGNOSTIC PARAMETERS IN CHRONIC RENAL FAILURE

Shereen Mohammad¹, Muhammad Irfan Shereen¹, Muhammad Haroon¹, Akhtar Ali²

¹Institute of Chemical Sciences, University of Peshawar - Pakistan

²Postgraduate Medical Institute, Lady Reading Hospital, Peshawar - Pakistan

ABSTRACT

Objective: To describe the relationship between an Enzyme acetyl cholinesterase (Ach.E, EC. 3.1.1.7) activity and other diagnostic parameters (urea, creatinine) used for diagnosing renal failure.

Material and Methods: The activity of the enzyme Ach.E of different ages (both males & females) was estimated in about 200 admitted renal failure patients along with other diagnostic parameters (urea and creatinine) in the nephrology unit of P.G.M.I. Lady Reading Hospital, Peshawar, by using Michaelis Menten kinetic parameters aKm (which shows the affinity of the enzyme towards the substrate) and aVm (which shows the rate of reaction) in pre dialyzed blood of patients suffering from chronic renal failure by the method of Ellman Gl. at 30C^o and pH 7.4, using acetylthiocholine iodide as a substrate. The activity of the enzyme was consolidated by a computerized program.

Results: The parameters (aKm & aVm) were found to be different from the normal in pre dialysed blood and activity of the enzyme was also low in all cases. A comparative set up was established among urea, creatinine, Hb% and activity of the enzyme Ach.E. The values of aKm, aVm, urea, creatinine, Hb and activity of the enzyme were 22,77,45,1.5 and 100% respectively in normal individuals, while these values were changed according to the severity of the diseases, resulting low activity of the enzyme.

Conclusion: This enzymatic technique has an advantage to show us, the activity of Acetylcholinesterase active site is externally oriented in the membrane of red blood cells, therefore any change occurring in the vicinity of the membrane will affect the activity of enzyme. This change can be used as a marker for the diagnosis and prognosis of disease.

Key Words: Enzyme, Acetylcholinesterase , Urea, Creatinine, Chronic, Renal failure.

INTRODUCTION

Acetyl cholinesterase is an externally oriented membrane bound glycolipoprotein¹, whose properties are not immutable and were shown to depend on the membrane micro environment². The kinetic properties of the enzyme change under many clinically abnormal conditions³. Long term exposure to aluminium affects the activity of Ach.E. Tissue specific effect of Ach.E in the canine heart showed that release of Ach.E from vagus nerve slow down heart rate and atriovascular condition⁴. Exposure to pesticides also slow down the activity of the enzyme and causes renal impairment⁵. Some organic drugs like methylmelonate decrease the activity of this enzyme. Carbamate and organophosphorus insecticides cause systemic toxicity due to inhibition of Ach.E activity⁶. Inhibition of the enzyme by plants phytomedicine is also studied by some authors⁷. Acetylcholinesterase

activities were studied in diseases like ABO type of the newly born hemolytic anemia, in pregnancy and in splenomegaly⁸. Such a huge oscillation focused our interest to characterize the enzyme as a probe of the membrane changes in chronic renal failure. The concentration of urea and creatinine increase ten times during 1-2 weeks of the renal shut down⁹.

Urea causes inhibition of the enzyme Ach. E in chronic uremia. Moreover the activity of the enzyme decreases due to high concentration of urea and creatinine and decreases 60% in heroin addiction¹⁰. Since the parameters aKm and aVm are calculated according to the duration of illness in chronic renal failure, it is of interest that for the erythrocyte membrane (which contains more than a dozen enzymes), abnormality may occur mostly in Ach. E enzyme. This tells that biochemical status of Ach.E is more closely related to erythrocyte membrane. Further, it may be of importance in understanding certain disease processes at the cellular level¹¹. The assay is based upon the hydrolysis of acetylthiocholine and subsequent detection of the evolved free thiol group by the formation of a yellow anion (5,5dithiobis, 2 nitrobenzoate) produced by the Ellman reaction determined spectrophotometrically.

Address for Correspondence:

Dr. Shereen Mohammad

Associate Professor Biochemistry

Al-Razi Medical College, Peshawar - Pakistan

Cell: 0300-5950396

E-mail: drshereenmohammad@gmail.com

MATERIAL AND METHODS

All reagents are obtained from E. Merck (West Germany). Blood samples were collected from nephrology unit P.G.M.I (LRH) at the time of admission for dialysis using ACD (acid citrate dextrose) as anticoagulant and transported to the Bio-Chemistry Laboratory LRH for analysis. Immediately, the blood samples (4 to 5 mls) were mixed with ACD and then centrifuged (at 5000 r/m) for 5 minutes at room temperature. The plasma, the top buffy coat and one third upper portion of the packed cells were sucked off and the remaining packed cells which were shown to be free of reticulocytes, leukocytes and thrombocytes were washed 3 times with 10 volumes of ice-cold 0.9% (w/v) NaCl. Haemolysate (enzyme) was prepared by adding 0.002 ml of washed cells to 50ml of ice-cold distilled water after about 15 min. This preparation was diluted with an equal volume of ice-cold potassium phosphate buffer (0.2 M, PH7.4). The enzyme was assayed by the method of Ellman GL in replicates at < 30°C and pH 7.4 using acetylthiocholine iodide as substrate, and 5, 5 dithiobis, 2nitrobenzoate as colour reagent. To 3mls of the enzyme preparation 50ul colour reagent was added and then, after 15 min. pre-incubation period, 25 µl of substrate was added. The change with time in the extinction at 412 nm was monitored continuously spectrophotometrically and reading was taken after one, two and three minutes in triplicates per min per gram hemoglobin

A total of 200 replicate assays were run by the same observer at each of two concentrations of the substrate, one was much lower ($s_1=10 \mu\text{m}$) and the other much higher ($s_2=200 \mu\text{m}$). Then a provisional estimate of Michaelis constant (aK_m or aV_m) of the enzyme were calculated. The two substrate concentrations method used here gives more accurate results as compared to multi substrate concentrations. The enzyme parameters were computed by fitting the corresponding linear regression equations, which were derived from S/V versus S plot to the data.

$$aK_m = \{(S_1/VS_1) (S_2-S_1)/(S_2/VS_2-S_1/VS_1)\}-S_1$$

$$aV_m = [(1/(S_2/VS_2 - S_1VS_1)/S_2-S_1)]$$

Where VS_1 and VS_2 represent absolute rates at S_1 and S_2 (substrates) respectively. The above equation is derived from a linear transform of the Michaelis Menten equation. The significance of mean difference were assessed by student's t-test (two tailed). The specific activity of the enzyme was calculated as.

$$S.A = \text{abs. (at 412 nm)} / \text{abs. (at 540 nm)} \times 66.667$$

Where 66.667 is a common factor.

Estimation of urea, creatinine and Hb were carried out by the routine kits methods by RF-500 spectrophotometer.

Table 1: Enzyme concentration and parameters

Group No.	Duration of Illness	aKm (μM)	aVm (Units)	Serum Urea (mg/dl)	Serum creatinine (md/dl)	Hb (gm/100)	S.A (%)
1	Normal	22.0+1.94 (20)	77.0+2.15 (20)	45.0+1.93 (20)	1.5+0.18 (20)	12.0+0.91 (20)	100
2	10 Days	33.0+1.4 (29)	70.0+0.9 (29)	142.0+2.07 (29)	6.0+1.1 (29)	9.0 +0..30 (29)	75
3	15 Days	36.0+0.50 (18)	60.0+0.33 (18)	144.0+2.29 (18)	7.2+0.56 (18)	8.2+0.16 (18)	70
4	20 Days	41.0+0.47 (32)	50.0+0.41 (32)	164.0+2.76 (32)	8.4+0.21 (32)	7.8u0.18 (32)	60
5	25 Days	46.0+.73 (27)	44.0+0.51 (27)	200.0+49 (27)	9.6+0.14 (27)	6.8+0.21 (27)	50
6	30 Days	52.0+0.73 (27)	39.0+0.25 (24)	208.0+0.78 (24)	11.2+0.88 (24)	5.8+0.82 (24)	45
7	Above 30 Days	60.0+0.27 (11)	29.0+0.22 (11)	240.0+2.45 (11)	12.4+1.04 (11)	5.4+0.26 (11)	40

Values are mean + S.D

Figures in parentheses indicate the number of samples analyzed.

RESULTS

The parameters aK_m and aV_m of the enzyme were assessed in duration of illness by using two substrate concentrations along with other diagnostic parameters (Urea, creatinine and Hb) as shown in Table 1. Since the data showed that the aK_m is directly proportional to the concentration of urea, creatinine and inversely proportional to Hb, while aV_m overall slightly decrease. In our results these values deviate from the normal and aK_m increases while aV_m decreases from the normal showing low enzyme activity. The hemoglobin values were also found to

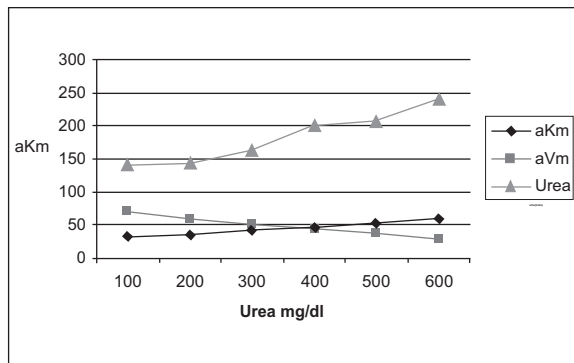


Fig. 1: Relationship of Urea with aK_m and aV_m

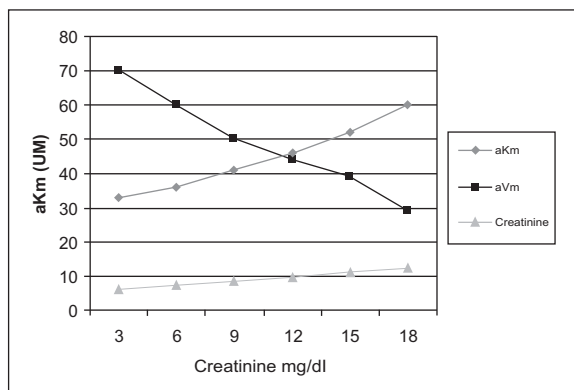


Fig. 2: Relationship of Creatinine with aK_m and aV_m

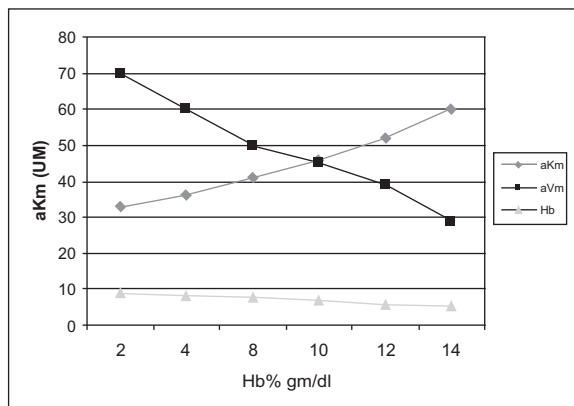


Fig. 3: Relationship of Hb% with aK_m and aV_m

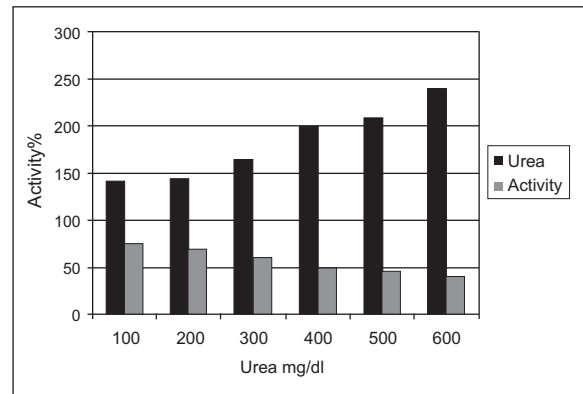


Fig. 4: Effect of Urea on the Activity of AchE

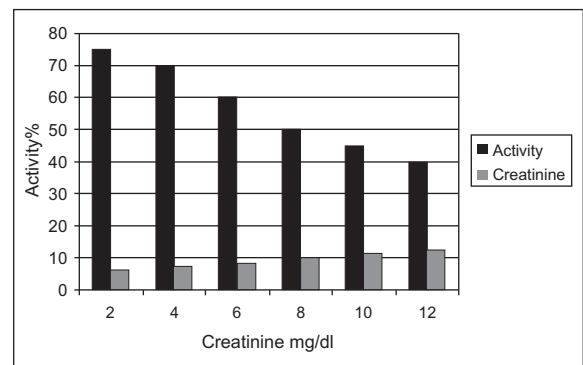


Fig. 5: Effect of Creatinine on the Activity of AchE

decreases from the normal due to severity of the illness and causing disturbed hemoglobin syntheses. Our results showed higher aK_m and lower aV_m in chronic renal failure, resulting from low enzyme activity. The relationship of aK_m and aV_m with urea, creatinine and Hb is given in Figs. 1, 2, 3, respectively while effect of urea and creatinine on the activity of the enzyme AchE is shown by bars in Figs. 4, 5, respectively. Data were analyzed statistically by two tailed method.

DISCUSSION

Renal failure is a burning problem of human health in Pakistan and increases day by day due to unhygienic conditions, imbalance diet, socioeconomic problems and stress. It has been well established that normal biochemical processes are the basis of health. In other words, all diseases are manifestations of abnormalities of biomolecules, biochemical reactions or processes. All of the agents that causes disease essentially affect one or more critical chemical reactions or molecules in the body. These molecules may be utilized in diagnosis of the diseases, and thus they may be referred to as biochemical diagnostic profiles if they had been originated from organs such as a liver, heart and kidney etc.¹² causing so many diseases including renal failure. It is suggested from time to time¹³⁻¹⁵ that the use of biochemical

investigations in relation to diseases can provide useful information. Kirstine Calloe et al¹⁶ recently reported that they can reveal their causes, can suggest rational and effective treatment, can assist in monitoring progress and can help in assessing response to therapy. It has been observed that renal failure is common throughout the world. Like some previous research workers¹⁷⁻¹⁹, the present work is to introduce an additional diagnostic aid to an ill kidney along with the previous diagnostic parameters (urea and creatinine). Michaelis Menten parameters (aKm & aVm) were estimated by two substrate designs, which gave more precise and accurate estimation as compared to multi design. Preliminary experiments indicate that in lysed cells aKm was 33, aVm was 70, urea was 142, creatinine was 6.0, Hb was 9 and activity was 75% from the onset of the diseases to ten days and these parameters changed due to high concentration and poisoning of urea and creatinine during the severity of diseases. Since, higher aKm and lower aVm indicated, the mixed type inhibition and denaturation of the enzyme, resulting in low activity of the enzyme. Reasons for the above facts are as reduction of number of nephrons leading to retention, especially of those waste products that depend on a high glomerular filtration rate for excretion. Shen T et al²⁰ also reported like our study, that the most prominent of these higher aKm and lower aVm as well as urea and creatinine, resulting in low Hb value. Due to high concentration of urea and creatinine, the activity of the enzyme changes, for instant in uremia. The activity of Ach. E. decreases by high concentration of urea while the poisoning of urea causes inhibition and loss of about 50 % of the activity of the enzyme. It is concluded in our results that high concentrations of urea and creatinine with high value of aKm and low value of aVm, show inhibition and denaturation of the enzyme Ach.E, resulting in low activity. As the enzyme is mainly present in the membrane of red blood cell and its active site is externally oriented, so any change occurring in the membrane vicinity will affect the activity of the enzyme and this activity can be used as a marker for the diagnoses and prognoses of diseases. The parameters aKm and aVm can be used as a tool for diagnosing of an illness. It can also be used in determining the efficacy of the artificial kidney used for dialysis. Estimation of parameters aKm, aVm and % activity in renal failure is a new enzymatic technique, and prediction of other diagnostic parameters from these can be done easily.

CONCLUSION

Changes in the kinetic parameters may be associated with integrity of the membrane and of complete characterization of aKm, aVm and enzymatic activity, while urea, creatinine and Hb will give prediction of each other i.e., one parameter can be

predicted from the other and the enzyme can be used as a tool for the diagnosis of diseases.

REFERENCES

1. Sussman JL, Harel M, Silman I "Three-dimensional structure of acetylcholinesterase and of its complexes with anticholinesterase drugs". (1993); *Chem. Biol. Interact.* 87 (1-3): 187-97.
2. Tougu V. "Acetylcholinesterase: Mechanism of Catalysis and Inhibition". *Current Medicinal Chemistry Central Nervous System Agents* (2001); 1(2): 155-70.
3. Drachman DB, Isselbacher KJ, Braunwald, E, Wilson, JD, Martin, JB, Fauci, AS Kasper, DL, eds (1998). *Harrison's Principles of Internal Medicine* (14 ed.). The McGraw-Hill Companies. pp. 2469-72.
4. Eubanks LM, Rogers CJ, Beuscher AE 4th, Koob GF, Olson AJ, Dickerson TJ, Janda KD "A molecular link between the active component of marijuana and Alzheimer's disease pathology". (2006). *Mol Pharm* 3 (6): 773-77.
5. R J Peiris- John. Exposure to Ach.E. inhibiting pesticides and chronic renal failure J. ceylon college of physicians. 2006; 51 (1): 42-43.
6. Whittaker V. "The Contribution of Drugs and Toxins to Understanding of Cholinergic Function". *Trends in Physiological Sciences*. (1990); 11 (1): 8-13.
7. Blumenthal D, Brunton L, Goodman LS, Parker K, Gilman A, Lazo JS, Buxton I (1996). "5: Autonomic Pharmacology: Cholinergic Drugs". *Goodman & Gilman's The pharmacological basis of therapeutics*. New York: McGraw-Hill. p. 1634.
8. Massoulié J, Perrier N, Noureddine H, Liang D, Bon S "Old and new questions about cholinesterases". *Chem Biol Interact.* (2008). 175 (1-3): 30-44.
9. Dori A, Ifergane G, Saar-Levy T, Bersudsky M, Mor I, Soreq H, Wirguin I "Read through acetylcholinesterase in inflammation-associated neuropathies". (2007).; *Life Sci* 80 (24-25): 2369-74.
10. Shaked I, Meerson A, Wolf Y, Avni R, Greenberg D, Gilboa-Geffen A, Soreq H. "MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase". (2009). *Immunity* 31 (6): 965-73.
11. Arie Ordentlich. The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions with Selected Organophosphate Inhibitors. *J Biol. Chem.* May (1996); 271: 11953-62.

12. Pohanka "Cholinesterases, a target of pharmacology and toxicology". *Biomedical Papers Olomouc*. (2011); 155 (3): 219-29.
13. Andre C. Affons, Daniele G. Increase susceptibility of brain Ach.E activity to methylmelonate in young rats with renal failure. *J. Metabolic brain deseases*. (2013); 20: P. 1.
14. Dacie and Lewis. A method of hemoglobin determination given in *Practical hematology 10th edn.* charchile London. 2006; page 271-3103.
15. Kaizer RR, Corria Mc, Gris LR, Da Rosa. Effect of long term exposure to aluminum on Ach. E activity in the CNS and erythrocytes. *Neurochemistry research*. (2008); 33 (11): 294-301.
16. Kirstine Calloe, Robert Goodrow, Soren peter. Tissue specific effect of Ach.E in the canine heart. *J. Am. Physio. Heart circ physio.* (2013); 305 (1): 66-75.
17. Pohanka M "Alpha 7 nicotinic acetylcholine receptor is a target in pharmacology and toxicology". (2012); *Int J Mol Sci* 13 (2): 2219-38.
18. Wang, BS, Wang, H, Wei, ZH, Song, YY, Zhang, L, Chen, HZ (2009). "Efficacy and safety of natural acetylcholinesterase inhibitor huperzine A in the treatment of Alzheimer's disease: an updated meta-analysis". *Journal of neural transmission (Vienna, Austria: (1996)* 116 (4): 457-65.
19. Massoulie J, Pezzementi L, Bon S, Krejci E, Vallette FM "Molecular and cellular biology of cholinesterases". *Progress in Neurobiology*. (1993). 41 (1): 31-91.
20. Shen T, Tai K, Henchman RH, McCammon JA "Molecular dynamics of acetylcholinesterase". *Acc. Chem. Res.* (2003). 35 (6): 332-40.

ONLINE SUBMISSION OF MANUSCRIPT

It is mandatory to submit the manuscripts at the following website of JMS. It is quick, convenient, cheap, requirement of HEC and paperless.

Website: **www.jmedsci.com**

The intending writers are expected to first register themselves and then attach/submit the manuscript. If processing fee is not submitted before should be deposited with Managing Editor in cash or can submit in the form of bank draft in the name of editor JMS. Also follow the format and check list of the Journal. Author agreement can be easily downloaded from our **website**. A duly signed author agreement must accompany initial submission of the manuscript.