Kinetic Alteration in Michaelis Menten Parameters of Human Erythrocyte Acetylcholinesterase in Diabetes Mellitus

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Summary: Kinetic characteristics of human erythrocytes acetylcholinesterase (AChE : EC 3.1.1.7) were determined in patients suffering from Diabetes Mellitus up to seven years. 85 samples of blood were assayed from the hospitalized patients along with parallel to the normal blood samples. The patients were divided in different groups depending upon the duration of the disease. A significant change in Michaelis Menten Parameters (akm, aVm) were observed in the patients divided in different groups. The kinetic approach will proceed these studies to provide a precise and easy method for accurate diagnosis of Diabetic Mellitus disease.

Introduction

The erythrocyte AChE is an externally oriented membrane-bound enzyme whose kinetic properties alter under clinically abnormal conditions, [1,8,9]. It seems to be a correlation between membrane changes and behaviour of the enzyme. It is of interest that for the erythrocyte membrane which contain more than a dozen enzyme, abnormality has been reported only in AChE, which suggests that the biochemical status of AChE is more closely linked to that of the erythrocyte membrane than the others [1]. The cyclic change within or on either sides of the membrane environments may define the nature of the changes encountered in kinetics of the enzyme. A huge diurnal fluctuations were also reported in different individuals in human menstrual cycle [2] and in pregnancy [3]. Such a huge oscillations focused our interest to characterise the enzyme as a marker of memberane changes in disease states. A systematic attempt has been made to evaluate the correlation between the enzyme activity of AChE with time course of the disease.

Methods

Selection of Patients

The values of aKm and aVm were determined in patients suffering from Diabetes Mellitus in all the nine groups. All groups consisted of male as well as female patients with the ages ranging between seventeen and fifty five years. The patients were admitted at the Medical C ward of

Khyber Hospital Peshawar and were diagnosed and referred for investigation by the physician. The first three groups consisted of patients in whom the duration of the disease was 3 months, 6 months and one year respectively. The remaining six groups belonged to the patients, who had been suffering from the disease for a duration of 2,3,4,5,6, and 7 years respectively.

Blood Samples

The donors gave blood at their own consent. Blood samples were drawn from the patients prior to the drugs given to them. In general, blood samples were collected by sterile venipuncture and added to freshly prepared acid-citrate-dextrose anticoagulant solution in the ratio of 1:4.

Preparation of enzyme

Immediately, the blood samples, 2 to 6 ml obtained at a time, were mixed and then centrifuged (2000xg, 5 min) 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 were washed 3 times with two volumes of ice-cold 0.9% (w/v) NaCl.

The enzyme (haemolysate) was prepared by adding 0.4 ml of the cells to 1000 ml 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 ml/1 pH 7.4).

Enzyme assay

The enzyme activity was assayed in replicate at 30°C and pH 7.4, using acetylthiocholine Iodide (ATChI) as substrate and 5,5-dithiobis (2-nitro benzoic acid) (DTNB) as colour reagent. To 6 ml of the haemolysate was added 100 µl of 10 mmol/1 DTNB (final concentration 160µmol/1) and then after a 10 min pre-incubation period, 50 µl of ATChI was added as 200 µmol/1 concentration. The change in absorbance (Δ E) at 412 nm due to the formation of 5-thio-2-nitrobenzoate yellow coloured anion was recorded per min by the method of Ellman [4].

Absolute activity

The absolute activity was expressed as Δ Ea/min per Δ Eb, where Δ Eb represents the absorbance due to haemoglobin content of the haemolysate measured at 540 nm [5].

Enzyme parameters

All the assays were run by the same observer at two concentrations of substrate, one was much lower (S_1 = 10 µmol/1) and the other much higher (S_2 = 160 µmol/1) than a provisional estimate of Km. The enzyme parameters aKm and aVm were calculated by fitting the corresponding given linear regression equations, which were derived from S/V versus S Plot [6] to the data.

$$aKm = (s_1/vs_1)\ (s_2-s_1)(s_2/vs_2) - (s_1/vs_1)-s_1$$

and

$$aVm = 1/\ [(s_2/vs_2) - (s_1/vs_1)/(s_2-s_1)]$$

where vs_1 and vs_2 represent absolute activities at s_1 and s_2, respectively.

Results

Haemotological values of the Michaelis Menten Parameters aK_m aV_m of the human erythrocyte AChE were estimated in randomly distributed hospitalized patients suffering from Diabetes

Table-1:Estimation of apparent Michaelis Menten parameters of human erythrocyte AChE in adults ill with Diabetes Mellitus.

Group No.	Diabetes Mellitus aK_m	aV_m
1.	38 ± 1.47 (9)	94 ± 0.70 (9)
2.	58 ± 1.67 (10)	98 ± 0.80 (10)
3.	60 ± 1.25 (8)	128 ± 0.80 (8)
4.	58 ± 1.76 (9)	100 ± 1.31 (9)
5.	46 ± 1.73 (10)	80 ± 1.43 (10)
6.	42 ± 2.05 (10)	120 ± 1.51 (10)
7.	40 ± 2.31 (11)	138 ± 2.34 (11)
8.	40 ± 1.37 (9)	62 ± 0.59 (9)
9.	41 ± 1.87 (9)	68 ± 0.39 (9)

Figures in parenthesis indicate the number of patients in a group.

Mellitus for nine different groups (table-1). Individual values in each group were pooled together and the mean values ± S.E. were calculated, for each group. The values were tabulated for general comparison, special graph (Fig.1) was prepared to depict the oscillation of these values with the duration of the disease. A significant increase in both the parameters were observed with the exception that the value of aVm was found below the normal on the 6th and 7th year of the onset of disease. The maximum peak of AChE for aK_m was observed

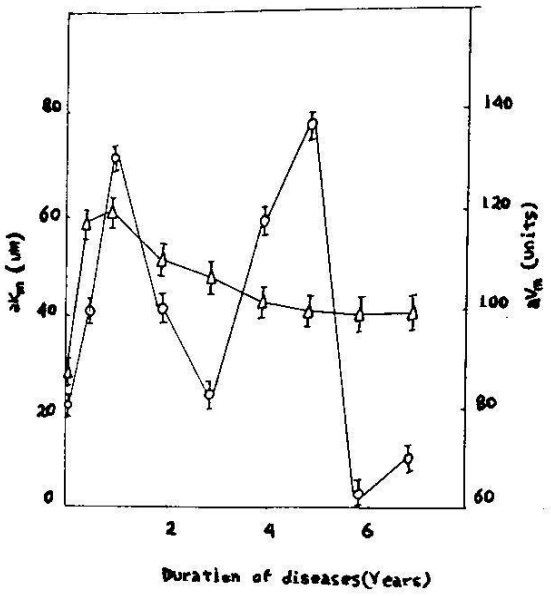

Fig.1 : Time course of AChE activities in Diabetes Mellitis, based on 85 patients.

when the duration of the disease was one year. While a declined was observed upto seven year. However two maximum peaks of AChE for aV_m were observed on Ist and 5th year of the onset of disease.

Discussion

The Michaelis-Menten Parameters aK_m demonstrated peak rhythm while the aV_m exhibited two peaks rhythm. The aK_m values were observed to that of the peak on the onset of the disease and then declines by forming a steady state level while the aV_m values fluctuates throughout the observation. The aKm declines when the acute course change to a more chronic stage. Fluctuations in enzyme activity may be due to various factors, activation or inhibition by soluble cellular compounds [6] or protein synthesis and degradation. Since there is no protein-synthesis in the human red blood cell, the fluctuation in enzyme activity must

be due, therefore, to other factors [7]. Similarly when cells are lysed, they loss some amount of activity, the aVm falls (11%) and aKm rises (45%). It was reported that on lysis the membrane lose (or altered) the components essential to protect the enzyme from the heat or inhibitor inactivation [8]. We therefore, also attempted to estimate, in addition to the parameters of the enzyme, the blood sugar. For further conformation some tests experiments were also done in vitro for applying various concentrations of sugar to the same sample and observed the effect that with increasing concentration the aKm rose and the aVm declined [9]. The overall observation suggest that changes in kinetics of the enzyme may be indicative of changes either the duration of the disease as well as with the increasing value of sugar in blood.

In summary the enzyme is characteristically very sensitive to changes in the organization, composition and lipid fluidity of the erythrocyte membrane, and these changes in the parameters of the enzyme can be employed as indicators of the particular disease provided the accurate mean values of the parameters in that disease are known.

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