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ORIGINAL RESEARCH ARTICLE

Correlation of Red Blood Cell Acetylcholinesterase Enzyme Activity with Various RBC Indices

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Abstract Cholinesterases belongs to class hydrolases. There are two types acetylcholinesterase and butyryl cholinesterase. Acetylcholinesterase present in nerve endings and also in the RBC membrane. It helps to maintain the shape and size of RBCs. Any change in shape and size of RBCs may affect the activity of Acetylcholinesterase. Thus this study aimed to estimate RBCs Acetylcholinesterase enzyme activity in various types of anemias and correlate the RBCs Acetylcholinesterase enzyme activity with various hematological indices such as Erythrocyte Sedimentation Rate (ESR), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red cell Distribution Width (RDW) etc. After obtaining ethical approval from Institutional ethics committee total of 100 samples were collected from Clinical Biochemistry laboratory, Kasturba Medical College, Manipal, Manipal University. 25 were having normal RBC indices, 12 with hemolytic anemia, 26 with microcytic anemia and 26 with macrocytic anemia based on peripheral smear report and RBC indices. Acetylcholinesterase were measured using Ellman's method. RBC acetylcholinesterase activity was significantly increased in microcytic anemia (58.13 ± 5.4) and macrocytic anemia (76.87 ± 6.7) than normal group (37.62 ± 2.71). Also increased RBC acetylcholinesterase was seen in hemolytic anemia (48.11 ± 5.18) but the increase is not statistically significant. RBC acetylcholinesterase correlated negatively with hemoglobin ($r = -0.356$, $p = 0.001$) and positively with RDW ($r = 0.31$, $p = 0.003$). To conclude RBC acetylcholinesterase activity can be used as one of the potential marker for various types of anemia.

Keywords RBC acetylcholinesterase · Microcytic anemia · Macrocytic anemia · Hemolytic anemia · RBC indices

Introduction

Cholinesterase belongs to hydrolase class of enzymes, hydrolyses the acetylcholine to choline and acetic acid. It is exclusively present in animal tissues and important for nerve tissue functioning. Two main types of cholinesterase are Acetylcholinesterase and Butyrylcholinesterase. Role of acetylcholine as a neurotransmitter and its hydrolysis by cholinesterase is well understood. The main type for that purpose is acetylcholinesterase (also called choline esterase I or erythrocyte cholinesterase); it is found mainly in chemical synapses and red blood cell membranes [1].

More than a dozen enzymes have been recognized in the membrane of the human erythrocyte, but changes in activity associated with pathologic conditions are found regularly only with acetylcholinesterase. Although the physiologic functions of erythrocyte acetylcholinesterase remain obscure, the location of this enzyme at or near the cell surface gives it special significance in studies of cellular membranes and the activity alterations seen in several disorders.

The mature erythrocyte is a nonnucleated, biconcave, disc-shaped cell containing a high concentration of

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hemoglobin.The normal life span of RBC is 120 days. Changes in structural integrity of RBC membrane and functioning of RBC reduces the life span of RBC and leads to anemia [1].

Wintrobe in 1929, first introduced RBC indices like Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. RBC indices values were highly useful in elucidating the type of anemias. Red cell distribution width is the quantification of anisocytosis (Variation in the size of red cells). ESRis the rate at which red blood cells sediment in a period of one hour. It is a non-specific measure of inflammation [2].

Major classification of anemias are based on the size and shape of RBCs. Macrocytic anemia is large oval shaped RBCs due to the defects in nuclear maturation, seen in folate or B_{12} deficiency. In this condition MCV and MCH are increased, while the MCHC remains normal. There is anisocytosis, and RDW is often increased [2].

In microcytic anemia the size of RBCs are small due to defective hemoglobin synthesis, characterized by low MCV, MCH and MCHC. RDW is also increased in microcytic anemia [2].

If the abnormalities involve nuclear maturation, there is a lag in cell division, ultimately leading to a larger size of RBC than normal. In contrast, when there is a defective and delayed synthesis of hemoglobin, leads to to microcytosis [2, 3].

Acetyl cholinesterase is also found in hematopoietic, osteogenic, immune cells. But its role in these cells is not well understood [4]. Red blood cell membrane has a very important role maintaining normal shape of RBC. Any defect or damage to this membrane can adversely affect lifespan of circulating RBC and hence oxygen transport. Studies have shown the changes in the levels of RBC acetylcholinesterase in various types hemolytic anemias [5, 6]. However, possibilities of membrane abnormalities leading to change in enzymatic activities in different types of anemias has not received much attention. This study hypothesizes that, acetylcholinesterase may have an important role in maintaining the shape and integrity of RBC membrane and any change in size shape and RBC indices can influence the levels of this enzyme.

Methodology

After obtaining the ethical clearance from institutional ethics committee, Kasturba Hospital, Manipal, India this prospective study was undertaken. Totally 89 subjects of both the gender in the age group of 20–80 years were included based on their peripheral smear and hematological indices. There were 25 subjects with normal RBC indices, 26 subjects with microcytic anemia, 26 subjects with macrocytic anemia, and 12 subjects with hemolytic anemias based on peripheral smear reports.

4 ml of venous blood collected in EDTA vacutainer. Plasma and buffy layer was separated following centrifugation and the RBC's were washed, then the RBC acetylcholinesterase activity was measured. Plasma needs to be separated so as to prevent the false results due to activity of plasma cholinesterase/butyrylcholinesterase.

Method of Estimation of acetylcholinesterase enzyme activity

Acetylcholinesterase catalyzes the hydrolysis of acetylthiocholine to thiocholine and acetate. The catalytic activity of acetylcholinesterase is measured by following the increase of the yellow anion 5- thio-2-nitrobenzoate, produced from thiocholine when it reacts with 5, 5′-dithio-bis-2-nitrobenzoic acid (DTNB). The rate of formation of yellow anion was measured at 410 nm at 25 °C in a thermo stated cuvette.

$$\text{Acetylthiocholine} + \text{water} \rightarrow \text{acetic acid} + \text{thiocholine}$$

$$\text{Thiocholine} + \text{DTNB} \rightarrow \text{yellow anion}$$

Statistical Analysis

Data were compiled and ANOVA, Pearson's correlation were used wherever appropriate.

Results

Table 1, 2 and 3 implies, Hemoglobin levels are significantly decreased in microcytic ($p = < 0.0001$) and macrocytic ($p = 0.025$) anemia compared to normal study subject. The decrease level of hemoglobin in hemolytic anemia ($p = 0.453$) is not statistically significant. MCH and MCV levels are significantly decreased in microcytic anemia ($p = 0.005$, $p = < 0.0001$) and significantly increased in macrocytic anemia ($p = <0.0001$, $p = <0.0001$) compared to normal group (Figs. 1, 2 and Tables 4, 5 and 6).

Discussion

Large amounts of acetylcholinesterase (AChE) enzyme has been known to exist in the human erythrocyte anchored to the external surface of the membrane. It functions has been

Table 1 Demographic profile of the study subjects

Parameters	Normal (n = 25) Mean ± SEM	Microcytic (n = 26) Mean ± SEM	Macrocytic (n = 26) Mean ± SEM	Hemolytic (n = 12) Mean ± SEM
Age (years)	49.52 ± 3.82	46.38 ± 3.17	47.23 ± 3.79	40.66 ± 5.44
Male:Female	17:8	10:16	23:3	12:0

Table 2 Showing RBC Acetylcholinesterase activities in different types of anemia

Enzyme activity	Normal (n = 25) Mean ± SEM	Microcytic (n = 26) Mean ± SEM	Macrocytic (n = 26) Mean ± SEM	Hemolytic (n = 12) Mean ± SEM
Acetylcholinesterase	37.62 ± 2.71	58.13 ± 5.4*	76.87 ± 6.7*	48.11 ± 5.18

As per this table, there is significant increase in RBC acetylcholinesterase activities in microcytic (p = 0.029) and macrocytic (p = <0.0001) anemia compared to normal group. There is no significant increase in hemolytic anemia (p = 0.655) compared normal group

* ANOVA

Table 3 RBC indices in different types of anemia

RBC indices	Normal (n = 25) Mean ± SEM	Microcytic (n = 26) Mean ± SEM	Macrocytic (n = 26) Mean ± SEM	Hemolytic (n = 12) Mean ± SEM
Hemoglobin	14.39 ± 1.05	9.81 ± 0.51**	11.33 ± 0.69**	12.42 ± 0.7
ESR	31.4 ± 5.79	44.38 ± 6.28	39.37 ± 7.36	24.58 ± 6.7
MCH	28.76 ± 0.60	25.23 ± 0.73**	34.05 ± 0.65**	30.32 ± 1.45
MCHC	33.24 ± 0.38	32.34 ± 0.19	33. 16 ± 0.24	33.56 ± 0.62
MCV	87.75 ± 1.66	76.85 ± 1.31**	103.93 ± 1.36**	89.37 ± 3.35
RDW	15.5 ± 0.75	15.68 ± 0.39	16.65 ± 0.66	14.07 ± 0.29

** ANOVA

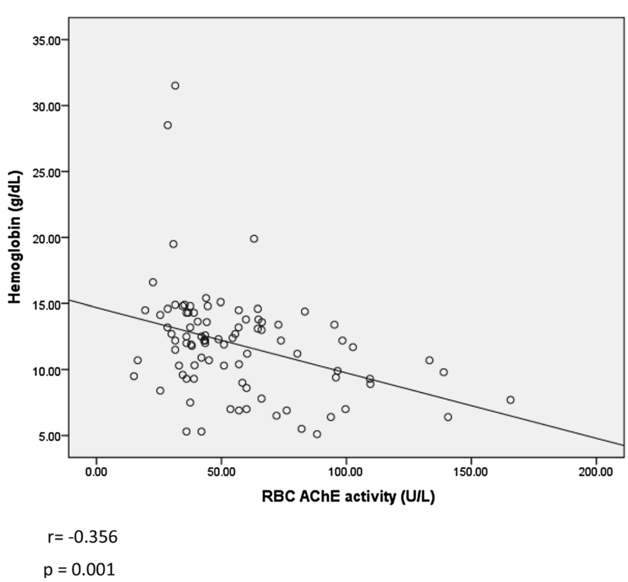

Fig. 1 Scattered plot showing correlation of AChE activities with Hemoglobin

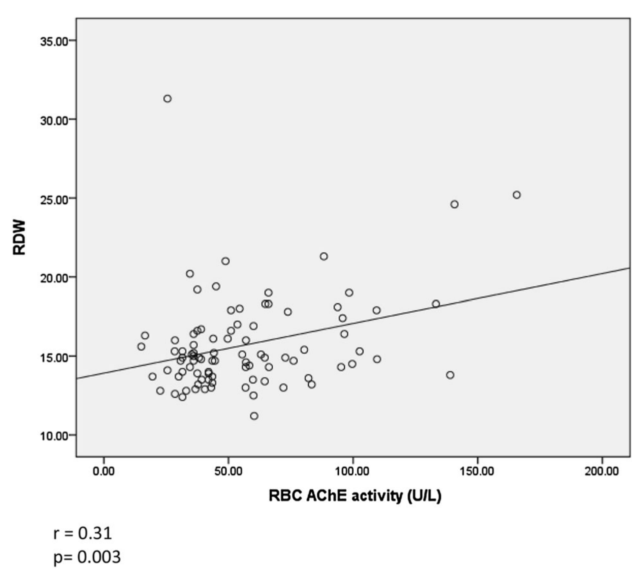

Fig. 2 Scattered plot showing correlation of AChE activities with RDW

Table 4 showing correlation of AChE activities with RBC indices in microcytic anemia

	r	P
Hemoglobin	−0.603	0.001**
ESR	0.426	0.038**
MCH	−0.23	0.256 NS
MCHC	−0.241	0.23 NS
MCV	−0.133	0.516 NS
RDW	0.443	0.023**

Pearson's correlation

NS not significant

** Significant

Table 5 showing correlation of AChE activities with RBC indices in macrocytic anemia

	r	P
Hemoglobin	−0.065	0.753 NS
ESR	−0.302	0.134 NS
MCH	−0.46	0.018**
MCHC	−0.661	<0.0001**
MCV	−0.689	<0.0001**
RDW	0.36	0.07 NS

Pearson's correlation

NS not significant

** Significant

Table 6 Showing correlation of AChE activities with RBC indices in hemolytic anemia

	r	P
Hemoglobin	−0.697	0.024**
ESR	−0.149	0.661 NS
MCH	0.198	0.559 NS
MCHC	0.144	0.673 NS
MCV	0.377	0.253 NS
RDW	0.356	0.283 NS

Pearson's correlation

NS not significant

** Significant

still been obscure [7]. The enzyme on RBC unlike the serum form has a half-life of over 3 months implying its role in the entire lifespan of RBC. Activity of this enzyme has shown to decrease with RBC aging indicating increase availability of acetyl choline in such RBC which may influence RBC metabolism and oxygen transport. This has been further supported by the study which showed in the presence of increasing concentrations of acetylcholine in vitro, RBC showed decrease in pH, increase pCO2 and increase of p50 (decrease of hemoglobin affinity to oxygen) [8].

This study showed a negative correlation of AChE with hemoglobin, MCH, MCHC and MCV both in macrocytic and microcytic patients. There was negative correlation of AChE with hemoglobin in hemolytic anemia. RBC acetylcholinesterase activity was found to be significantly increased in microcytic anemia (58.13 ± 5.4) and macrocytic anemia (76.87 ± 6.7) than normal group (37.62 ± 2.71). RBC AChE activity was increased in hemolytic compared to normal control but statistically not significant. All these observations support the previous theory that acetylcholine and decrease affinity of RBC to oxygen.

Studies have also observed an inverse relationship between acetylcholinesterase and RBC G6PD activity implying its role in changing intracellular metabolism [9]. Many studies have also supported the above findings who observed a consistent reduction in acetylcholinesterase activity in newborn infants affected with ABO hemolytic disease and in some individuals with autoimmune hemolytic anemia [10, 11]. Studies have indicated that, RBC Acetylcholinesterase activity is more in sickle cells where it may protect against sickling by increasing its oxygen affinity [12]. This study showed a positive correlation with RDW in both macrocytic and microcytic anemia. Indicating a role for this enzyme in maintaining the shape and integrity of RBC membrane.

Conclusion

1. This study showed AChE levels were significantly higher in both macrocytic and microcytic anemia indicating it may have a role in maintaining the shape and integrity of the membrane of RBC.

2.This study showed significant inverse correlationship between Hb, MCH, MCHC and MCV and AChE levels supporting the previous theory of correlation between acetylcholine and decreased affinity of RBC to oxygen.

3.Previous studies have shown the changes in the levels of RBC acetylcholinesterase in various types of hemolytic anemias but in this study the levels did not change significantly.

More studies required to be done to understand the complete role of this enzyme in RBC metabolism.

Limitation of the Study

This study did not take into account the causes and duration of anemia and treatment history in study subjects. This might have influenced the results generated.

Actual sample size was 47 in each group. Due to time constraint and unavailability of cases during the specified period we could able to do the study in small numbers.

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