A STUDY OF SPECTRIN AND LIPID PEROXIDATION OF RED BLOOD CELL MEMBRANE IN THALASSAEMIA CARRIER.

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ABSTRACT

The aim of the present work is to understand the lipid peroxidation of RBC membrane and the spectrin protein content of RBC membrane cytoskeleton of thalassaemic carrier state (trait) of β and hemoglobin E variant (HbE). We have measured the hemoglobin (Hb), malondialdehyde (MDA) and spectrin content of RBC membrane of thalassaemic carrier. The spectrin content (α and β band) of both β and HbE carrier was not changed than normal individuals. However, lipid peroxidation of RBC membrane was significantly increased in both β and HbE trait, and Hb level was also decreased in thalassaemic carrier. It may be assumed that oxidative damage by excess lipid peroxidation may have no role on irreversible membrane damage in β thalassaemia and HbE thalassaemia carrier.

KEY WORDS : Spectrin, malondialdehyde, HbE carrier, β carrier, Lipid Peroxidation.

INTRODUCTION

A thalassaemia syndrome is caused by any of several mutations including deletion of the β globin gene (1,2) and this leads to deficiency of hemoglobin / red blood cell and to an excess of insoluble-globin chain (3). In Eastern India, the most common inherited disorder is β and E β thalassaemia and this disease is a major public health problem in social life. β thalassaemia has been detected in large number in almost all parts of the country (4,5). A high incidence of β thalassaemia and that of HbE in Eastern India remains yet to be characterised (6). Heterozygotes (traits) of both β thalassaemia and HbE are prevalent in Eastern India (7). Spectrin, the

main component of skeleton protein of the erythrocyte membrane has an ability to maintain the normal shape (8) and is intimately related to the maintenance of phospholipid asymmetry across the erythrocyte membrane bilayer (9). Erythrocytes exposed to higher oxidative stress reflect increased accumulation of 'activated oxygen' or free radicals (10). This activated oxygen increases malondialdehyde (MDA) a secondary breakdown product of lipid peroxidation (11).

The diagnosis of thalassaemia patients and their carrier are being carried out regularly in our thalassaemia counselling centre. We have

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found that HbE and β thalassaemia carrier have lower hemoglobin level than that of control. Although studies of lipid peroxidation of red blood cell membrane of β and E β thalassaemia (7) and the studies of abnormal characterization of red cell membrane skeleton (9,11-15) have been performed, there is an apparent lack of knowledge of autooxidative process in hetero-zygotes like β and HbE thalassaemia carrier. In view of this, it is essential to study the oxidative attack in spectrin component of red blood cell membrane as well as to study the MDA, the index of lipid peroxidation of red cell membrane of β and HbE thalassaemia carrier.

MATERIALS AND METHODS

The study comprised of β thalassaemia carriers (n=20), carriers of HbE mutant (n=20) and normal individuals (n=20). Agarose gel electrophoresis was used for identification of Hb variant. The diagnosis of the carriers was established on the basis of complete blood count, hemoglobin electrophoresis with tris-EDTA buffer (pH 8.1) and estimation of fetal hemoglobin. After diagnosis of the β and HbE carrier, the venous peripheral blood of these carriers was collected in acid-citrate-dextrose solution for the study of spectrin, a cytoskeleton protein of red cell membrane.

The packed RBC were isolated by centrifugation at 3,000 rpm for 10 min. at 4°C. The plasma and buffy coat were removed by aspiration and the red cells were washed with 0.9% NaCl solution. Red blood cells were treated with proteolysis inhibitor (final concentration 2 mmol/L diisopropyl fluorophosphate) and 10 mg/ml each of leupeptin and pepstatin. The inhibitors

were added with gentle shaking and incubated at 37°C for 30 min. (16). Washed red cells were hemolysed in 5mM sodium phosphate buffer (pH 8.0) (17) containing 100 mg/L of phenyl-methylsulfonyl-fluoride and 0.1 mmol/L EDTA and centrifuged at 15,000 rpm for 20 min. at 0-4°C.

PAGE in the presence of SDS and 2-mercaptoethanol was performed as described by Laemmli (18). 50 mg protein was applied in each lane. The gels were stained with coomassie blue R-250. The gel was scanned by Beckman Appraise densitometer (USA) at 600 nm.

MDA with thiobarbutaric acid - trichloroacetic acid - hydrochloric acid (19) and protein of RBC membrane were assayed by Bradford Method (20). The results were analysed by Student's 't' test and by Anova (parametric) analysis.

RESULTS AND DISCUSSION

The α and β band of spectrin of red cell membrane of β and HbE thalassaemia trait were not changed than control (Table 1, Fig.1). MDA level was higher in β and HbE trait and Hb level was significantly decreased in both β thalassaemia and HbE trait (Table 1). Parametric anova analysis of the results revealed that there was an interaction of MDA level and Hb percentage of β and HbE thalassaemia carrier as shown in F values in Table 2. However increased level of MDA of both β and HbE thalassaemia carrier may not have some role on the reflection of damage of spectrin in this group (Tables 1 and 2).

Spectrin is intimately related with the maintenance of phospholipid asymmetry across

the erythrocyte membrane bilayer (21) and is attached to the plasma membrane by linkage of β -spectrin to ankyrin, which in turn, binds to the cytoplasmic domain of band 3, by linkage of protein 4.1 to glycophorin C (22) as well as by

direct interactions of spectrin and protein 4.1 with the phospholipid of the inner lipid bilayer. The membrane skeleton provides the stability against deformability which is essential for the survival of the red cell membrane cytoskeleton (23).

Table 1. Relative percentage of spectrin (α and β) band, the level of MDA of RBC membrane and Hb percentage of β and HbE carrier.

Group (n)	Mean Age (Years)	Hb. (g/dl)	MDA level (nmoles/mg protein)	Spectrin(%)	
				α	β
Control (20)	45 $\pm$ 1.49	13.4 $\pm$ 0.19	0.42 $\pm$ 0.012	16.6 $\pm$ 0.38	19.3 $\pm$ 0.78
β carrier (20)	41 $\pm$ 3.01	10.8 $\pm$ 0.38	0.57 $\pm$ 0.03	16.9 $\pm$ 0.53	18.4 $\pm$ 0.80
HbE carrier (20)	41 $\pm$ 2.91	10.5 $\pm$ 0.40	0.55 $\pm$ 0.04	16.0 $\pm$ 0.60	18.9 $\pm$ 0.09

* Mean $\pm$ SE., ** NS - Non significant vs Control., *** P< 0.005 vs Control.

Table 2. Anova table considering MDA, β spectrin and Hb in control β and HbE trait.

Source of variance	Degree of freedom	Sum of Squares (SS)	Mean of Squares (MSS)	F
Between Groups	2	48.64	24.32	8.41
Between parameter	2	28473.60	14236.80	4925.87
Group x parameter	4	45.67	11.42	3.95
Error	171	364.17	2.89	

There was a significant variation between Hb and MDA parameters and between all the groups at 0.005 level.

Fig. 1. SDS-PAGE analysis of red cell membrane of β and HbE carrier.

A :- α band of spectrin (2,40,000 dalton M.W.),
B :- β band of spectrin (2,20,000 dalton M.W.).

1. Normal, 2. β thalassaemia, 3. β carrier.
4. β thalassaemia, 5. HbE carrier.

Normally there is a strict physical separation between iron via its carrier or storage proteins (transferrin or ferritin). The same protective

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mechanisms apply to the covalent or high affinity association of haem iron with carrier problems (24). This separation is required in order to prevent the iron ions from catalysing lipid and protein peroxidation via their participation in the Fenton reaction (25). The increased generation of superoxide radicals (O_2^-) in thalassaemia due to autoxidation of α and β chain aggravate the situation as it may release iron from ferritin reductively which is then participate as a catalyst in Heber-Weiss reaction with subsequent release of free radicals (24) which damage the spectrin of RBC membrane by the action of lipid peroxidation or membrane thiol oxidation or both of these (26).

The auto-oxidative process may change the membrane lipid peroxidation in β and HbE thalassaemic carrier and this process may lead to the cleavage of polyunsaturated fatty acids forming excess MDA (Tables 1 and 2). It may be assumed that increased lipid peroxidation cannot influence the irreversible membrane damage in carrier state of β and HbE thalassaemia.

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