INCREASED ERYTHROCYTE RESISTANCE TO OSMOTIC LYSIS IN THE ACUTE HEPATITIS CAUSED BY TRUE HEPATOTROPIC VIRUSES non-A, non-B (NANB)

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ABSTRACT

An unexpected increase in erythrocyte osmotic resistance during viral hepatitis in two patients prompted study of effects of hepatitis (in Karad) on osmotic resistance. The test was performed by placing erythrocytes in saline solutions of decreasing osmolarity and osmotic fragility defined in terms of the saline concentration at which hemolysis begins. Study included 44 infected subjects (acute viral hepatitis non-A non-B). All forty four infected subjects showed abnormal results when compared to normal subjects. Increased erythrocyte osmotic resistance i.e. hemolysis begins between 0.45% to 0.40% of NaCl and is completed between 0.25% to 0.20% NaCl, whereas in normal subjects hemolysis begins between 0.50% to 0.45% NaCl and is completed between 0.35% to 0.30% NaCl. Osmotic resistance was observed with increase in total bilirubin (mean $\pm$ SD) (4.6 ± 3.6), direct bilirubin (3.0 ± 2.5), SGOT (58.8 ± 55.5) SGPT (114.2 ± 150.3) activity. Physiologic shifts in erythrocyte osmotic resistance may be due to changes in membrane lipid ratio.

KEY WORDS : Viral hepatitis, Osmotic fragility, hemolysis.

INTRODUCTION

Most patients with inflammation and infection of hepatic cell have target red cells. The shape, deformability and surface features of erythrocytes are determined by three interacting components i.e. the membrane, the hemoglobin and nonhemoglobin components (salts, water, substrates, cofactors and enzymes mainly of glycolytic pathway). Membrane function is affected by the kind and amount of haemoglobin present, by cell water, cation and anion content, by the energy levels of the cells and by lipoprotein content of phospholipid bilayer.

The liver is the key organ in the metabolism of cholesterol in humans. It is the only organ by which substantial amounts of cholesterol are excreted from the body either directly as free cholesterol into the bile or after conversion to bile acids (1). The liver plays important role in serum lipoprotein synthesis and metabolism. Hence liver malfunction is expected to produce changes in serum lipid and lipoprotein levels (2).

Free cholesterol is in rapid equilibrium between serum lipoproteins and red cells. The level of red cell cholesterol is influenced by bile salts and bilirubin (3), which shifts the serum / cell

partition of free cholesterol to cell phase and which inhibit the cholesterol esterifying mechanism. Any process or disorder affecting cholesterol exchange *in vivo* is capable of critically modifying the shape and behaviour of red cells which result in increased resistance to osmotic lysis.

MATERIALS AND METHODS

Blood Specimens :

Blood specimens were obtained from 44 infected subjects (non-A, non-B hepatitis proved serologically) registered in Krishna Hospital and Medical Research Centre, Karad & Cottage Hospital, Karad. Control samples were obtained from healthy subjects (subjects with known hemolytic disorders or other medical conditions that could affect erythrocyte fragility were excluded from the study).

All blood specimens were obtained by venipuncture into EDTA, bulb (1.0 mg/ml).

Erythrocyte osmotic fragility test :

This test was performed according to Dacie's method (4). Percent haemolysis for each tube including control was calculated. The estimations of the two serum transaminases i.e. SGOT, SGPT (5) and bilirubin (6) were done on Erba Chem-5, Transasia instrument.

RESULTS

For this study Dacie's osmotic fragility test was used to measure osmotic fragility because the speed and simplicity of the method facilitates the testing of large number of subjects and also

because the result is expressed as a single numerical value that leads itself to comparison between individuals or groups. The results of this study demonstrate a significant decrease in fragility during infective jaundice.

In control group blood hemolysis ends between 0.35% to 0.30% sodium chloride and beginning of hemolysis occurred between 0.50% to 0.45% sodium chloride concentration. But in the case of hepatitis patients hemolysis begins at 0.45% to 0.40% sodium chloride and it is completed at 0.25% to 0.20% sodium chloride concentration. Osmotic fragility results are reported in graphic form as shown in Fig.1. A shift towards increased fragility resistance was evident within the entire group of hepatitis individuals.

Entire hepatitis group is having high SGPT, SGOT and bilirubin levels as shown in Table 1.

DISCUSSION

The results of this study demonstrate a significant decrease in fragility during infective hepatitis. The mechanism underlying decreased osmotic fragility may include changed environment around RBC's which include increased amount of bile pigments, bile salts and enzymes like SGPT, SGOT and decreased efficiency of hepatic parenchymal cells. The osmotic resistance of red cells in hepatitis may be attributed to a characteristic increase in the surface to volume ratio (SA/V). It has been shown that in severe forms of liver disease, the red cell membrane takes up more cholesterol than phospholipid. The relative increase in cholesterol may produce an expanded membrane with increased microviscosity and

folded edges due to the intercalation of cholesterol at the core of the lipid bilayer (7,8).

In viral hepatitis parenchymal cells are involved in disease process that effect impaired lipoprotein synthesis, LCAT and hepatic lipase deficiency leads to abnormalities in clearance of remnant lipoprotein particles (7). Therefore, circumstances that produce an increase in non esterified cholesterol will lead to an increase in membrane cholesterol on both sides of the bilayer. This produces an expansion of the red cell membrane surface or decrease in membrane

fluidity that increase resistance to osmotic lysis. It has been shown that gains and losses of red cell cholesterol do not involve metabolic injury.

LCAT enzyme is involved in metabolism of serum free cholesterol and phospholipid. Lack of this enzyme will result in target cell formation rich in cholesterol and lecithin (8), which in turn may help in formation of target cells which are resistant to haemolysis.

Table 1. Biochemical Investigation in study group.

Investigation	Mean $\pm$ SD	Range for patients value
Total Bilirubin (mg%)	4.6 $\pm$ 3.6	1.7 - 14.2
Direct Bilirubin (mg%)	3.0 $\pm$ 2.5	0.5 - 9.2
SGOT (IU/L)	58.8 $\pm$ 55.5	30 - 400
SGPT (IU/L)	114.2 $\pm$ 150.2	42 - 1050
50% Haemolysis at	0.35 $\pm$ 0.03	0.33% - 0.36%
100% Haemolysis at	0.22 $\pm$ 0.02	0.20% - 0.25%

Fig-1 Changes in the osmotic fragility of red cells from a patient with non-A non-B hepatitis compared with normal red cell osmotic fragility pattern

Fig. 1. Changes in the osmotic fragility of red cells from a patient with non-A non-B hepatitis compared with normal red cell osmotic fragility pattern.

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