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LETTER TO THE EDITOR

OSMOTIC FRAGILITY OF NORMAL AND SICKLE HAEMOGLOBIN CONTAINING RED BLOOD CELLS

Sir,

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Abnormalities in osmotic fragility have been observed in hereditary spherocytosis, hereditary elliptocytosis, pyruvate kinase deficiency, thalassaemia and iron-deficient red cells (1). Harris et al have reported a decrease in osmotic fragility of the red cells of sickle cell anaemia patients from Africa (2). The Indian patients are genetically and clinically different from the African patients (3). Though the abnormalities in the glucose metabolism and a greater loss of K^+ ion from the red blood cells of our patients during incubation have been reported (4, 5), data pertaining to the osmotic fragility of these cells are not available. Therefore, we have estimated the osmotic fragility curve of both fresh and incubated red blood cells of the homozygous sickle cell disease patients.

Blood smaples from 8 adult normal healthy subjects, 11 healthy sickle cell trait cases and 10 homozygous sickle cell disease patients were collected. The patients were in steady state at the time of blood collection and no patient had received transfusion during the last three months prior to the study. The haemoglobin (mean ± SEM) for normal (AA), sickle cell trail (AS) and sickle cell patients (SS) subjects were 14 ± 0.34 g/dl, 13.2±1.4 g/dl and 10.9±1.0 g/dl respectively. The reticulocyte counts (mean±SEM) of AA, AS and SS sample were 0.64±0.26%, 1.2±0.57% and 6.9±3.5% respectively. The sickle cell disease patients had lower hamoglobin level ($P<0.001$) and higher reticulocyte count ($P<0.001$) than the normal and sickle cell trait cases. Immediately after the blood collection, the fresh red cell osmotic fragility test was performed in all the test samples. 1 ml of whole blood was incubated in sterile tubes at 37°C in a water bath for 24 hours. After incubation the red cell osmotic fragility test was again done for all three groups of samples.

Osmotic fragility test was performed (1) using haparinised venous blood. The O.D. was measured in Elico Digital UV spectrophotometer (Model CL – 54 D) at a wave length of 540 nm. Percentage of lysis of red blood cells was then calculated. Fragility curves were drawn taking percentage (%) of lysis against the (%) of NaCl solution. The data were analysed by Student's 't' test.

The concentration of NaCl solution in which 50% lysis occured or the median corpuscular fragility (MCF) point for fresh AA, AS, SS red cells were found to be at 0.41%, 0.38% and 0.33% respectively. After

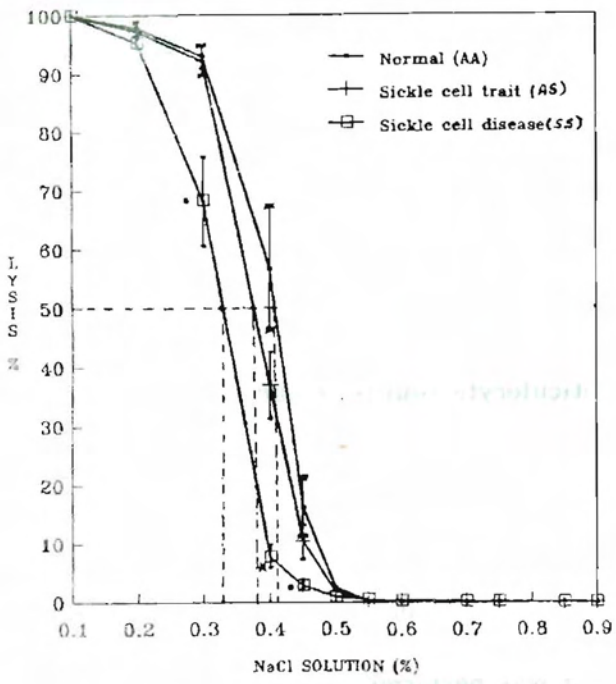

Fig. 1 : Fresh blood osmotie fragility curve, values are mean ± SEM, *P<0.05 Vs both AA & AS, *P<0.001 Vs both AA & AS.

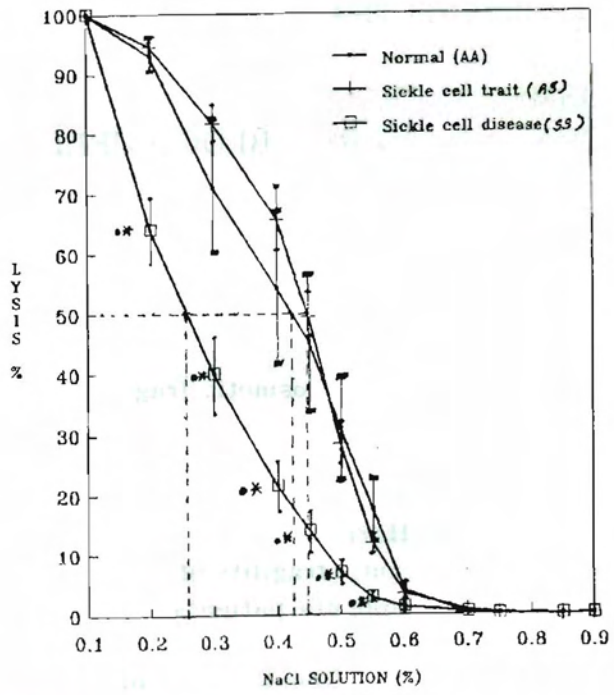

Fig. 2 : Post incubation osmotie fragility curve, values are mean ± SEM, *P<0.05 Vs AA and *P<0.001 Vs AS.

24 hours of incubation the MCF values for AA and AS red cells were 0.43%, 0.45% and for SS it was at 0.26% NaCl solution (Fig. 1 & 2).

It was observed that the osmotic fragility curve of both fresh and 24 hours incubated SS red blood cells showed a shift to the left as compared to AA and AS red cells, suggesting an increased resistance to hypotonic NaCl solution. The mean osmotic fragility values of fresh SS red cells from 0.3% to 0.5% NaCl solution were significantly less than the AA and AS red cells. The mean osmotic fragility values of incubated SS red cells were also significantly different than the AA and AS values. The mean osmotic fragility values of AS red cells were not different than the AA red cells both in fresh and incubated conditions.

A decrease was observed in the osmotic fragility of fresh and 24 hours incubated red cells of sickle cell disease patients as compared to that of normal (AA) and sickle cell trait (AS) red blood cells. The loss of phosphatidylcholine from the SS red cells may be a possible cause for the decreased osmotic fragility of red cells (6). Franck et al (1983) have reported such type of change in sickle erythrocytes (7). The fragility decrease in sickle red cells may be due to the greater loss of potassium from the red cells (5). A similar trend has been reported in red cells of thalassaemia patients (1).

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