

Osmotic Fragility of Erythrocytes in Duchenne Muscular Dystrophy

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Abstract

Osmotic fragility of erythrocytes was studied in ten Duchenne dystrophic patients and eight healthy controls. Lysis started in Duchenne erythrocytes at a concentration of 6.5 g/l NaCl and in the control erythrocytes at a concentration of 5.5 g/l NaCl. At all concentrations of sodium chloride (from 6.5 g/l to 4.5 g/l) significantly greater lysis was observed in the erythrocytes of dystrophic patients. The median corpuscular fragility (MCF) was also significantly greater in Duchenne patients as compared to their controls ($P < 0.001$). The increased osmotic fragility in Duchenne erythrocytes provides an evidence in support of the probable generalized membrane defect that manifest the disease (JPMA 34 : 127: 1984).

Introduction

The Duchenne form of muscular dystrophy (DMD) is the commonest of the muscular dystrophies and is inherited as a sex linked recessive trait. It produces progressive muscular weakness in boys and culminates in total disability, before the teens (Walton and Gardner Medwin, 1974). Although the pathogenesis of muscular dystrophies is not fully understood, much work on the muscular dystrophies in recent years lends support to the notion that the genetic defect may affect the surface membranes of muscle and other cells (Roland, 1976; Jones and Witkowski, 1983).

Many abnormalities have been observed in the erythrocytes of Duchenne dystrophic patients (Siddiqui, 1979), however a large proportion of erythrocyte research in Duchenne dystrophy deals with the osmotic fragility, surface properties and shape changes (Matheson and Howland, 1974; Fisher et al., 1976; Kim et al., 1980; Somer et al., 1979; Percy and Miller, 1975; Dellantonio et al., 1980; Matheson et al., 1976; Adornato et al., 1977; Roses and Appel, 1974; Mollica et al., 1980).

The report that the erythrocyte count in patients with Duchenne muscular dystrophy shows a larger proportion of echinocytes or stomatocytes as compared to the control erythrocytes (Roses and Appel, 1974), is doubtful as the morphology of erythrocytes is extremely susceptible to different types of cell treatment as well as the fact that such results are not readily reproducible. Same difficulty is faced in assessing the osmotic fragility as the reports for an increased osmotic fragility shown by the erythrocytes of Duchenne patients (Fisher et al., 1976) was contradicted (Adornato et al., 1977). However, more recently, Somer et al. (1979) and Kim et al. (1980) again indicated the presence of a less stable erythrocyte membrane in Duchenne patients and showed that the MCF was significantly higher in Duchenne patients than in normal cells.

Material and Methods

Heparinized samples of venous blood (3cc) were obtained from ten Duchenne dystrophic boys (ages 7 - 14 years) by venipuncture. Prior to this the patients had a complete diagnostic evaluation, which included serum CPK measurement and electromyographic studies. All these patients were attending the out patient clinic of Physical medicine department at Jinnah Postgraduate Medical Centre, Karachi. Control samples were obtained from eight young boys group, who were admitted to orthopaedic operations neuromuscular abnormalities. and the control boys were of the same age the centre for

unrelated to None of the patients suffering from any haematological abnormality. Along with each sample from patient, an appropriate age matched control sample was always processed, to minimize the effect of age on osmotic fragility. The amount of haemolysis at various concentrations of sodium chloride, buffered with sodium phosphate at pH 7.4 was determined by the method described by Dacie and Lewis (1975). All the samples were analyzed at 20 - 25°C within 3 hours after collection. Haemolysis was completed within 30 min. and after centrifugation the haemoglobin release was measured spectrophotometrically in the supernatant at 540 nm. For each sample percentage haemolysis was plotted against the different concentrations of sodium chloride in the phosphate buffer solution. The concentration of sodium chloride at which erythrocytes showed 50% lysis (MCF) were derived from the osmotic fragility curve.

The percentage of haemolysis was calculated as follows:

$$\% \text{ lysis} = \frac{100}{\text{O.D. at complete lysis}} \times \text{O.D. at the given concentration of NaCl.}$$

Results

The results of 10 different experiments (Table-I)

Table – I

Haemolysis (%) in Dystrophic and Control Erythrocytes at Different concentration of Sodium Chloride (Phosphate Buffer Saline pH 7.4).

Sodium Chloride concentration g/l	Normal controls (8)	DMD patients (10)	P-Value
1.0	100	100	
3.5	95.36 ± 0.68	96.92 ± 0.89	P = 0.8 - 0.7
4.0	86.88 ± 1.71	87.79 ± 2.07	P = 0.8 - 0.7
4.5	57.82 ± 3.64	72.94 ± 5.71	P < 0.01
5.0	20.39 ± 2.22	41.91 ± 5.71	P < 0.05
5.5	3.36 ± 0.43	13.02 ± 2.14	P < 0.001
6.0	0.00	2.43 ± 0.46	-
6.5	0.00	0.69 ± 0.32	-

Figures in parenthesis represent number of samples.

show that at all concentrations of NaCl from 6.5 to 4.5 g/l, significantly greater degree of haemolysis was shown by Duchenne erythrocytes as compared to their age matched controls. Where as such difference could not be observed at the 4.0 - 1.0 g/l NaCl concentrations in between the Duchenne and the control groups. A comparison of concentration for initial 50%, (MCF) and complete lysis was also made between controls and Duchenne erythrocytes (Table-II).

Table - II

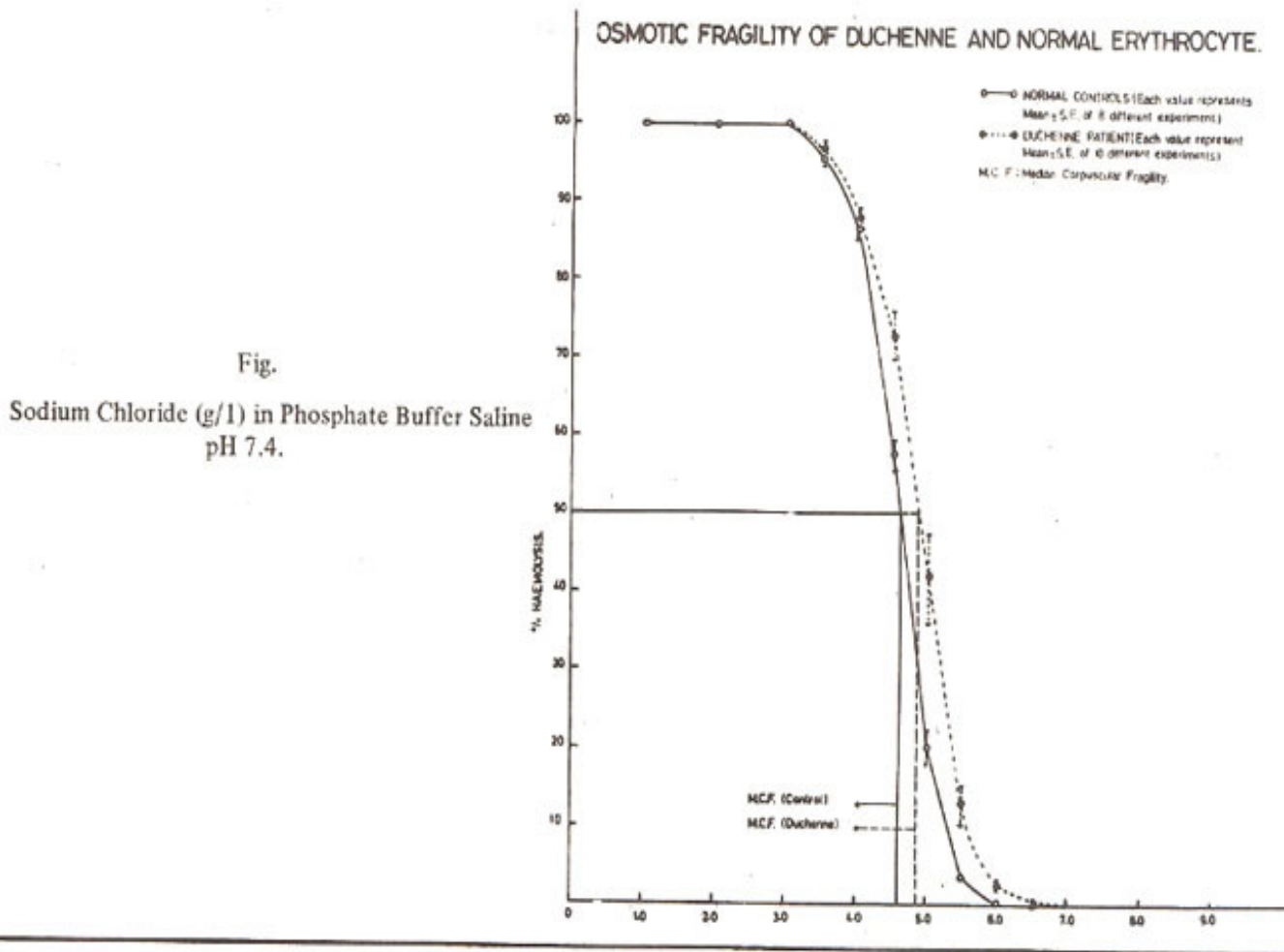
Comparison of initial Lysis, Median Corpuscular Fragility (M.C.F. 50% Lysis) and Complete Lysis in Dystrophic and Control Erythrocytes at Different Concentrations of Sodium Chloride (Phosphate Buffer Saline pH 7.4).

NaCl concentration for initial lysis (g/l)		NaCl concentration for 50% lysis (MCF) g/l		NaCl concentration for complete lysis (g/l)	
Control	DMD	Control	DMD	Control	DMD
5.5	6.0	4.7	4.6	3.0	3.5
5.5	6.0	4.6	5.0	3.0	3.0
5.5	6.5	4.3	4.9	2.0	3.5
5.5	6.5	4.3	5.1	3.0	3.0
5.5	6.0	4.6	4.9	3.0	3.0
5.5	6.5	4.6	4.7	3.0	3.0
5.5	6.5	4.6	5.0	3.0	3.0
5.5	6.5	4.7	4.7	3.0	3.0
	6.0		5.1		3.0
	6.5		5.1		3.0
5.5 ± 0.00*	6.3 ± 0.08*	4.55 ± 0.06*	4.91 ± 0.06*	3.0 ± 0.00	3.1 ± 0.07*
(8)	(10)	(8)	(10)	(8)	(10)
P < 0.001		P < 0.001		N.S.	

*Each figure represent Mean ± S.E.

Figures in parenthesis represent number of samples.

NaCl concentration for the initial lysis was 5.5 g/l, and for Duchenne erythrocytes at 6.30 ± 0.08 g/l. This difference was statistically significant ($P < 0.001$). Similarly, the NaCl concentration for 50% lysis (MCF) was 4.55 ± 0.06 g/l, for control and 4.9 ± 0.06 g/l for Duchenne erythrocytes. This again is statistically significant ($P < 0.001$). However, no significant difference was observed in NaCl concentration for complete lysis between controls and Duchenne erythrocytes. The fragility curve drawn for Duchenne and control erythrocytes was of characteristic sigmoid type (Fig.1).



It was noted that the curve representing Duchenne erythrocytes was slightly shifted towards right and also showed a slight tailing between 6.0 - 7.0 g/l NaCl concentrations which indicate an increased susceptibility of dystrophic erythrocytes to the hypotonic stress.

Discussion

Erythrocytes have the inherent property of deformation, which is an important rheological phenomena in blood circulation. Discocyte, echinocyte and discocyte stomatocyte are the fundamental shape changes which normal human erythrocytes may undergo in a reversible fashion (LaCell et al., 1976). Echinocytes occur in vivo, notably in uremia, peptic ulcer, heart disease and carcinoma of the stomach, as well as in certain anemias. Such shape transformations run through successive stages between biconcave discocyte and complete spherical form (Mohandas and Fea, 1975). It has been reported that erythrocyte swelling in a hypotonic medium hemolyzed immediately on reaching critical volume (Saari and Beck, 1975) and that these transformations become non reversible for erythrocytes after entering the spherical phase.

There is no direct evidence that the circulating Duchenne erythrocytes are deformed and the haemoglobin efflux studies under physiological conditions by Siddiqui and Pennington (1977) revealed no significant difference between Duchenne erythrocytes and the control cells. However, there are recent reports about the decreased deformation of Duchenne erythrocytes in vitro measured by different methods (Somer et al., 1979; Nash and Wyard, 1982). Tillmaun et al. (1979) observed that Duchenne erythrocytes exhibit reduced deformation only when they are pretreated with some echinogenic agent

or stressed upon by the saline environment.

The abnormally increased osmotic fragility in Duchenne erythrocytes observed in this study has previously been reported by many authors (Fisher et al., 1976; Godinet et al., 1978; Kim et al., 1979; Lloyd and Nunn, 1978; Ruitenbeck et al., 1979; Somer et al., 1979). In this series the Duchenne erythrocytes manifested a small but significant increase in osmotic fragility as compared to the controls. The median corpuscular fragility which is supposed to be a more reliable index of the change in osmotic fragility was also found significantly different in most of our patients. This study also supports indirectly the possible presence of a large number of deformed erythrocytes in Duchenne blood since the change in the initial lysis time shown by the Duchenne erythrocytes may indicate the presence of such susceptible or deformed cells.

Tillmann et al. (1979) observed that there seems to be a possibility that proportionally a large number of echinocytes in Duchenne blood may contribute towards the mild haemolysis observed in vitro at the concentrations of sodium chloride where normal cell does not show any haemolysis.

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