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Short Communication

OSMOTIC FRAGILITY OF BUFFALO RED BLOOD CELLS IN POST-PARTURIENT HAEMOGLOBINURIA

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SUMMARY

The osmotic fragility test was conducted on blood samples of 50 clinical cases of post-parturient haemoglobinuria in buffaloes along with 15 healthy animals each from villages and an organized farm to measure the resistance of erythrocytes to haemolysis by osmotic stress. The erythrocytes osmotic fragility was found to be higher in haemoglobinuric buffaloes as compared to healthy ones indicating increased membrane permeability and alteration of bilipid layer of red cells.

Key words: Buffaloes, post-parturient haemoglobinuria, osmotic fragility

Post-parturient haemoglobinuria (PPH) is an acute disease of cattle and buffaloes characterized by severe intravascular haemolysis, haemoglobinaemia, haemoglobinuria, anaemia and death due to anaemic anoxia. This disease entity is emerging as a potent threat to buffaloes not only in India but also in other buffalo rearing countries of the world. Though much work has been done and still in progress on various aspects of PPH including clinical manifestations, haematological, biochemical and histopathological changes, yet the specific etiological factor (s) and mechanism of erythrolysis are not fully understood. Deficiency of inorganic phosphorus in blood is a constant finding and cases respond to various degrees to phosphate therapy (Nagpal *et al*., 1968, Malik and Goutam, 1971, Gahlawat, 1998). Apart from phosphorus deficiency, oxidative stress is also responsible for red blood cell membrane abnormalities such as lipid per oxidation, increased rigidity of lipid bilayer, increased osmotic fragility and permeability and subsequently leading to haemolysis.

The present study was conducted on 50 clinical cases of post-parturient haemoglobinuria along with 15 healthy lactating, non-pregnant buffaloes each from villages (maintained under similar conditions) and organized farm. Blood samples were collected by jugular venepuncture in sterilized vials containing an anticoagulant. Samples from diseased buffaloes were collected after the completion of treatment. The blood samples immediately after collection were placed in ice bath and taken to laboratory for analysis.

The haemoglobinuric buffaloes included in the study were given two treatments. Twenty-five patient buffaloes (group-I) were given sodium acid phosphate 80 gm i/v as 20% solution and 80 gm orally once daily, and remaining 25 patient animals (group-II) received 50 gm sodium acid phosphate plus 7.5 gm ascorbic acid i/v as 20% solution and 50 gm sodium acid phosphate orally once daily for three days . The red cells were exposed to decreasing strength of hypotonic saline solutions and the degree of haemolysis was measured. The osmotic fragility test was done as per the standard method described by Schalm *et al.* (1975).

The osmotic fragility test is a measure of the resistance of erythrocytes to haemolysis by osmotic stress. The red blood cell is surrounded by a semipermeable membrane which is composed of a hydrophobic lipid bilayer with a protein skeletal meshwork attached to its inner surface by binding to integral proteins. The normal biconcave shape of most of mammalian RBCs represents the resting unstressed

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geometry of the cell. The biconcave shape results in a large surface area to volume ratio compared to that of sphere, allowing RBCs to undergo marked deformation while maintaining a constant surface area. This is important because an increase of 3-4 per cent of surface area results in cell lysis (Mohandas and Chasis, 1993). The lipid layer is impermeable to most molecules. Consequently, various membrane protein transport systems are utilized for movement of molecules into and out of RBCs. Mostly, the movement of anions, water, sodium, potassium, calcium, amino acids, glucose, adenine, adenosine and inocine, etc., take place through the membrane (Harvey, 1997).

If the red cell is placed in a hypotonic saline solution, osmotic equilibrium will be established by drawing water into the cells which then swell. In hypertonic saline solution, the cells will loose water and shrink. Pathologically increased permeability of red cell membrane leads to accumulation of water with cells, swelling of cells and finally escape of haemoglobin, through the widened pores of the cell membrane. *In-vitro*, this process can be produced by exposing red cells to hypotonic solutions. Normally, first sign of haemolysis appears at a salt concentration of 0.44 per cent and lysis is usually completes at 0.34 per cent salt concentration. The difference in saline concentration between beginning and complete haemolysis is called resistance (Schalm *et al.* 1975). It has been reported that red cells exposed to oxidative stress may be exposed to deleterious effects on both the membrane and cytosole. The membrane effects include an increase in osmotic fragility, an increase in membrane permeability, inactivation of membrane bound enzymes and cross linking of membrane constituents and membrane deformability may decrease due to alterations of polyunsaturated lipids (Vladimirov *et al.*, 1980).

In the present investigation, the mean osmotic fragility of haemoglobinuric buffalo erythrocytes in 0.5% salt solution, was found to be 96.75 ± 0.89 per cent, whereas corresponding values in farm healthy and village healthy were 81.17 ± 0.76 and 81.61 ± 1.49 per cent, respectively (Table). Similarly, these values in the treated buffaloes of group-1 and group-II were found to be 92.47 ± 0.41 and 91.65 ± 1.95 per cent, respectively. It is much clear from the above data that RBCs of patient buffaloes are very fragile whereas, that of recovered animals, it tends to come closer to healthy animals. In 0.60 per cent salt solution, osmotic fragility of healthy buffaloes of farm and village origin,was found to be 33.55 ± 0.92 and 32.62 ± 0.67 per cent whereas, in PPH patients of group-I and II osmotic fragility was 26.10 ± 0.79 and 19.47 ± 2.49 per cent, respectively. Here, it is again clear that red cells

Table
Mean osmotic fragility (%) of erythrocytes of healthy and haemoglobinuric buffaloes

Tube no.	Concentration of salt solution	Farm healthy	Village healthy	Haemo-globinuric	Post-treated buffaloes	
					group-I	group-II
1	0.00	100.00	100.00	100.00	100.00	100.00
2	0.10	100.00	100.00	100.00	100.00	100.00
3	0.20	99.72±0.49	99.92±0.15	99.75±0.34	99.57±0.54	99.35±0.48
4	0.25	99.27±0.37	99.74±0.34	99.20±0.36	99.09±0.60	99.23±0.37
5	0.30	98.97±o.46	99.09±0.48	98.73±0.45	98.92±0.43	98.35±0.46
6	0.35	98.65±0.46	99.44±0.58	98.24±0.57	98.59±0.73	97.01±0.76
7	0.40	98.13±0.47	97.79±0.74	97.84±0.68	98.07±0.62	96.01±1.27
8	0.45	97.18±0.68	97.10±0.66	97.35±0.83	95.09±1.03	94.21±1.89
9	0.50	81.17±0.76	81.61±1.49	96.75±0.89	92.47±0.41	91.65±1.95
10	0.55	51.83±1.09	52.48±0.98	95.97±0.95	49.74±1.16	51.74±2.21
11	0.60	33.55±0.92	32.62±0.76	94.96±0.72	26.10±0.79	19.47±2.49
12	0.65	14.99±0.84	15.28±0.56	76.28±1.25	11.28±1.00	11.54±2.23
13	0.70	6.49±1.11	7.62±0.99	42.33±1.27	5.96±1.65	4.64±0.95
14	0.75	3.05±1.05	3.68±0.90	12.49±1.64	3.00±0.78	2.28±0.72
15	0.80	0.99±0.87	0.43±0.61	6.02±0.60	2.17±1.10	1.90±0.64
16	0.85	0.14±0.25	0.00±0.00	4.02±1.05	1.18±0.80	0.93±0.74

of haemoglobinuric buffaloes are more fragile than healthy ones. The marked difference of osmotic fragility at 0.60 per cent between the animal groups I and II may be due to the difference in their therapy. The group-I animals were treated with sodium acid phosphate alone whereas group-II animals received vitamin C, a known antioxidant, may have a stabilizing effect on the red cell membrane leading to higher cellular resistance to hypotonic solution. Similar finding were reported by Ogawa *et al*. (1987) in haemoglobinuric cows.

The osmotic fragility curve of RBCs in haemoglobinuric, healthy and recovered buffaloes showed a sigmonoid curve. The osmotic fragility curve of PPH buffalo erythrocytes goes almost straight upto 0.60 per cent salt concentration, thereafter it declines steadily and slowly. Even in 8.5 per cent salt concentration there was 4.02 ± 1.05 per cent haemolysis. In healthy buffaloes, the curve goes almost straight up to 0 .45 per cent salt solution thereafter it followed the trend of PPH buffaloes and in 0.85 per cent salt concentration the haemolysis was negligible. In recovered patient of group-I and group-II, the curves traveled almost a straight path up to 0.50 per cent salt concentration and thereafter it declines regularly coming to nearly one per cent in 0.85 per cent salt solution.

So, the osmotic fragility of haemoglobinuric buffalo erythrocytes had a marked shift of the sigmonoid curve to the right as compared to the curve of healthy buffalo RBCs. The curve of recovered buffalo erythrocytes followed a strange path. At the start, it traveled almost a straight line and then started to decline regularly and intercepted the healthy buffalo erythrocytes curve at about 0.50 per cent salt solution concentration and thereafter it traveled below this curve i.e. there was shift to left. This may be due to the protective effect of sodium acid phosphate and vitamin C on red cell membrane and also due to more release of young erythrocytes into circulation which might be more active in generating ATP then the mature red cells. Probably, the stabilizing effect of the therapy might be due to its antioxidant activity. Consequently, antioxidant might help in avoiding inactivation of cell membrane enzymes and cross-linking of membrane cytoskeleton proteins and restoration of normal erythrocyte shape and metabolism. So it may be assumed that phosphorus deficiency, a regular finding in haemoglobinuric buffaloes, closely associated with RBCs metabolic disorders leading to suppressed glycolysis cycle, increased oxidative stress, increased osmotic fragility and haemolysis.

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