Investigations on Oxidative Stress in Post-parturient Haemoglobinuria in Buffaloes receiving Sodium Acid Phosphate Therapy

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ABSTRACT: The present investigation was carried out to assess the erythrocytic oxidative stress in post-parturient haemoglobinuria in buffaloes receiving sodium acid phosphate therapy daily till clinical recovery. Enhanced malondialdehyde levels indicating lipid peroxidation and very low reduced glutathione levels in erythrocytes of haemoglobinuric buffaloes suggested the involvement of oxidative stress in this disease. The decline of malondialdehyde levels to within normal limits and appreciable elevation of reduced glutathione levels following treatment with sodium acid phosphate revealed its antioxidant effects in haemoglobinuric buffaloes which seems to be the first report of its kind.

Key words: Oxidative stress, Haemoglobinuric buffaloes, Antioxidant therapy.

INTRODUCTION - Post-parturient haemoglobinuria (PPH) is emerging as a potent threat to buffalo industry in India and other buffalo rearing countries of the world. It is highly fatal owing to acute intravascular haemolysis and severe anemic anoxia. Phosphorus deficiency has been recorded as consistent finding in this disease and clinical cases do respond to sodium acid phosphate therapy (Pandey and Misra 1987). However, recent investigations emphasized the involvement of erythrocytic oxidative stress in this condition (Ogawa et al 1987; Singari et al 1989 and Chugh 1994). Therefore, it was considered appropriate to investigate the status of oxidative stress within the erythrocytes of haemoglobinuric buffaloes during therapy with sodium acid phosphate by measuring erythrocytic malondialdehyde and reduced glutathione levels.

MATERIAL AND METHODS - The present investigation was carried out on twelve clinical cases of PPH in buffaloes. Out of these, 10 clinical cases of PPH were treated by administering sodium acid phosphate @ 80g as 20% solution in double distilled water intravenously as well as 80g orally once daily till clinical recovery, whereas the remaining two clinical cases which did not receive any therapy served as untreated control. The blood samples were collected before and during therapy at different time intervals using heparin as anticoagulant. Blood samples from twelve clinically healthy buffaloes parturited within
45 days were also collected once to keep them as healthy control. The blood samples were analyzed for malondialdehyde (Ohkawa et al. 1979) and reduced glutathione (Beutler, 1971) levels in red blood cells. Plasma inorganic phosphorus was also estimated (Taussky and Shorr 1953).

**RESULTS AND DISCUSSION** - All haemoglobinuric buffaloes were within 60 days of parturition and during second to sixth lactation. Passage of coffee-coloured urine was the most prominent clinical sign. Anorexia and low milk yield became evident on third or fourth day of illness. There was straining at the time of defaecation. Duration of clinical illness before the onset of therapy varied from 0-2 days. All treated buffaloes recovered with sodium acid phosphate therapy, whereas the untreated ones died within 5-6 days of illness. On an average, 3-5 intra-venous as well as oral doses of sodium acid phosphate were required for clinical recovery. The biochemical alterations in PPH in buffaloes treated with sodium acid phosphate along with healthy and untreated controls are given in table 1.

| Parameters                              | Mean values |    |
|-----------------------------------------|-------------|----|
|                                         | Healthy (10)| Treated (10) | Haemoglobinuric (12) |
|                                         | Day 0 | Day 3 | Day 5 | Day 0 | Day 3 | Day 5 |
| Erythrocytic malondialdehyde (n moles/ml) | 237.03 ± 40.72 | 349.57 ± 34.45 | 285.32 ± 32.28 | 250.00 ± 21.95 | 351.48 | 392.93 | 466.65 |
| Erythrocytic reduced glutathione (mg %)  | 79.11 ± 6.44 | 31.57 ± 2.55 | 37.42 ± 3.02 | 56.26 ± 2.37 | 28.14 | 28.03 | 21.23 |
| Plasma inorganic phosphorus (mg %)      | 3.87 ± 0.21 | 2.10 ± 0.29 | 3.55 ± 0.48 | 5.26 ± 0.47 | 1.98 | 1.48 | 1.34 |

ab, bc & ac = P ≤ 0.01; Figures in parenthesis ( ) indicate number of animals.

The epidemiological observations, clinical signs and hypophosphataemia as recorded in present study were in agreement with earlier investigators (Singh 1999 and Singari et al. 1989). The findings revealed that sodium acid phosphate therapy restored the plasma inorganic phosphorus levels within normal limits at clinical recovery which was in agreement with Jubb et al. (1990) in haemoglobinuric cows. Mata and Bhardwaj (1985) proposed a hypothesis that in PPH of buffaloes, phosphorus deficiency decreases glucose utilization rate and ATP production by erythrocytes leading to decrease in synthesis as well as reduction of glutathione which predisposes the erythrocytes to adverse effects of oxidants. The resultant oxidative stress probably leads to lipid peroxidation of red cell membrane with eventual in-
travascular haemolysis. The present findings of almost 1.5 fold increase of malondialdehyde levels in erythrocytes of haemoglobinuric buffaloes indicated that there was enhanced lipid peroxidation in red cell membranes of PPH affected buffaloes. Stern (1985) reported that oxidative stress/damage in erythrocytes is usually manifested as lipid peroxidation. In present study, the administration of sodium acid phosphate effectively controlled the ongoing enhanced lipid peroxidation (oxidative stress) in erythrocytes of haemoglobinuric buffaloes as evident by its decline of malondialdehyde contents to within normal limits at clinical recovery. Here, it may be inferred that sodium acid phosphate acted through antioxidant mechanism in reducing lipid peroxidation.

In erythrocytes, the reduced glutathione protects haemoglobin against oxidative denaturation and its membrane against lipid peroxidation (Trotta et al 1982). In present study, the drastically lowered levels of reduced glutathione in erythrocytes of haemoglobinuric buffaloes indicated a very low antioxidant status in diseased buffaloes. There was an appreciable increase in reduced glutathione contents of erythrocytes on day 5 post-treatment indicating that sodium acid phosphate was effective in elevating the antioxidant status of erythrocytes in haemoglobinuric buffaloes. The rate of regeneration of reduced glutathione in red cells was reported to be slower in buffaloes as compared to other ruminants (Suzuki et al 1985) and this might be the reason for its slow elevation in erythrocytes following sodium acid phosphate therapy. Glucose-6-phosphate dehydrogenase enzyme maintains a continuous supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) which in turn is essential for the regeneration of reduced glutathione from oxidized glutathione. In PPH of buffaloes, the activity of glucose-6-phosphate dehydrogenase enzyme was reported to be low (Singari et al 1991) which might be a contributing factor for slow regeneration of reduced glutathione during clinical recovery from this disease. In two untreated haemoglobinuric buffaloes, the deterioration in values of erythrocytic malondialdehyde, erythrocytic reduced glutathione and plasma inorganic phosphorus continued leading to their death within 5-6 days of illness.

This seems to be the first report of status of erythrocytic malondialdehyde and reduced glutathione levels in PPH of buffaloes following treatment with sodium acid phosphate which revealed its anti-oxidant effects in haemoglobinuric buffaloes. Since phosphate ions have been reported to possess antioxidant action (Vladimirov et al 1980; Pokorny 1987), Sodium acid phosphate administered intravenously, in present study, might be able to restore red cell vitality due to direct antioxidant action of phosphate ions.

REFERENCES - Beutler, E. (1971). Red Cell Metabolism: A Manual of Biochemical Methods (Ed. Beutler E.). New York, Grune and Stratton, p.146. Chugh, S.K. (1994). Studies on certain aspects of oxidative stress in relation to pathogenesis and treatment of post-parturient haemoglobinuria in buffaloes. Ph.D. Dissertation, CCS Haryana Agricultural University, Hisar, Haryana, India. Jubb, T.F., Terrett, I.V., Browning, J.W. and Thomas, K.W. (1990). Haemoglobinuria and hypophosphatemia in pre-parturient dairy cows without dietary deficiency of phosphorus. Australian Veterinary Journal, 67: 86-89. Mata, M.M. and Bhardwaj, R.M. (1985). Possible alterations in erythrocyte metabolism and integrity in post-parturient haemoglobinuria. Indian Journal of Veterinary Medicine, 5: 67-72. Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358. Pandey, N.N. and Misra,
S.K. (1987). Clinico-biochemical studies of nutritional haemoglobinuria in buffaloes and its therapy. *Indian Veterinary Journal*, 64: 39-43. **Pokorny, J.** (1987). Major factors affecting the auto-oxidation of lipids. In ‘Auto-oxidation of Unsaturated Lipids’ (ed. H.W.S. Chan) London, Academic Press, Harcourt Brace Jovanovich Publishers, pp.141-206. **Singari, N.A., Bhardwaj, R.M., Mata, M.M. and Chugh, S.K.** (1989). The effect of hypophosphatemia on erythrocytic metabolism in post-parturient haemoglobinuria of buffaloes. *Indian Journal of Animal Science*, 59, 1235-1236. **Singari, N.A., Bhardwaj, R.M., Chugh, S.K. and Bhardwaj, S.** (1991). Status of erythrocytic G-6-PD in phosphorus deficiency haemoglobinuria of buffaloes. *Indian Veterinary Journal*, 68, 226-230. **Singh, R.** (1999). Studies on red cell shape and metabolism in relation to pathogenesis of post-parturient haemoglobinuria in buffaloes. Ph.D. dissertation, CCS Haryana Agricultural University, Hisar, Haryana India. **Stern, A.** (1985). Red cell oxidative damage. In: Oxidative Stress (ed. H. Sies.). London, Academic press, pp.331-349. **Suzuki, T., Agar, N.S. and Suzuki, M.** (1985). Red cell metabolism in experimental animal: pentose phosphate pathway, antioxidant enzymes and glutathione. *Experimental Animals (Tokyo)*, 34, 353-366. **Taussky, H.H. and Shorr, E.** (1953). A microcolorimetric method for the determination of inorganic phosphorus. *Journal of Biological Chemistry*, 202: 675-685. **Trotta, R.J., Sullivan, S.G. and Stern, A.** (1982). Lipid peroxidation and haemoglobin degradation in red blood cells exposed t-butylhydroperoxide: Effect of HMP shunt as mediated by glutathione and ascorbate. *Biochemistry Journal*, 204: 405-415. **Valdimirov, Yu.A., Olenev, V.I., Suslova, T.B. and Cheremisina, Z.P.** (1980). Lipid peroxidation in mitochondrial membranes. *Advances in Lipid Research*, 17: 173-249.