HAEMATO-BIOCHEMICAL, SURFACE ULTRASCOPY AND THERAPEUTIC STUDIES ON PUERPERAL HAEMOGLOBINURIA IN DAIRY ANIMALS

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INTRODUCTION

Post-parturient haemoglobinuria (PPH) also referred as nutritional haemoglobinuria is an acute haemolytic disease of high producing dairy animals observed mostly in advanced pregnancy or within a month after parturition. The disease is of major economic significance due to loss of milk production and high mortality rate. In India, the disease has been reported in buffaloes and the line of treatment advocated by various workers comprised of parenteral administration of sodium acid phosphate, ascorbic acid, antifibrinolytic agents and oral medication of copper sulphate with variable results[1,2,3]. The present study reports the results of haemato-biochemical alterations and the comparative efficacy of parenteral infusion of copper glycinate in haemoglobinuric cows and buffaloes. Scanning electron microscopic studies of erythrocytes were also conducted to record morphological changes.

MATERIALS AND METHODS

Animals

Twenty-five buffaloes and twenty cows, 3-10 years old, tentatively diagnosed to be suffering from puerperal haemoglobinuria for varying periods of time, attended at dairy farms of the Punjab State, were included in the present investigation.

Sampling Procedure

Blood samples were collected once before institution of therapeutic measures and subsequently 48 hrs after clinical recovery in heparinized glass vials by jugular venepuncture from all the clinical cases of puerperal haemoglobinuria. Absence of haemoglobin in the urine was taken as the basis of clinical recovery. Blood samples from five of healthy postpartum and five prepartum cows and an equal number of post and prepartum healthy buffaloes were collected from the University herd for establishing control haemato-biochemical values. For SEM studies, 2-3 drops of free-flowing blood were collected in about 16 ml of 2% gluteraldehyde in 0.1M cold phosphate buffer, pH 7.4 from clinically healthy and haemoglobinuric cows and buffaloes by jugular venepuncture.

Analysis Procedure

Fresh heparinized blood samples were analysed for Hb, TEC, TLC, haematocrit and erythrocytic indices as per standard procedures. Blood samples were stained with new methylene blue stain for detection of Heinz bodies. Fresh blood smears fixed in methanol were stained with Giemsa's stain for detection of blood protozoan infection. For SEM studies, blood samples, fixed with 2% gluteraldehyde were stored for 2 hrs at 4°C. After fixation, the erythrocytes were washed with cold 0.1M phosphate buffer, pH 7.4. Subsequently, the erythrocytes were washed 3-4 times with triple distilled water and suspended in it. Thereafter, one drop of erythrocyte suspension was placed and allowed to dry on one surface of double-sided adhesive cellophane tape. Then a small piece of charged tape was stuck to the copper stubs. Sputtering was done with gold, and stubs were examined with JEOL electron microscope. Photographs were taken at 0 axis on Indu film at desired magnification. Plasma was separated from heparinized blood samples immediately after centrifugation and analysed for inorganic

phosphorus, copper and molybdenum content using the standard procedures.

Treatment Procedure

Therapeutic trials with copper glycinate were conducted in all the dairy animals. In the haemoglobinuric cows, single dose of copper glycinate @ 1.5 mg/kg body weight, to a maximum of 500 mg dissolved in 540 ml of normal saline was administered by slow intravenous drip. In the haemoglobinuric buffaloes, equivalent dose of copper glycinate was administered by intravenous drip followed by administration of another dose by intravenous drip after 36 hrs in half of the non-responsive cases (9) and a dose of 4 ml copper glycinate (100 mg/ml) by subcutaneous route in rest of the non-responsive cases (9).

RESULTS AND DISCUSSION

The incidence of puerperal haemoglobinuria in the present study was observed to be higher in postparturient buffaloes and cows with maximal observance recorded in 3rd and 4th lactation, respectively and in good milkers. These findings were similar to those recorded earlier[1,3,4]. The increased susceptibility in postpartum period could be ascribed to hypocupraemia and increased secretion of inorganic phosphorus in milk. The maximal milk yield recorded during 3rd and 4th lactation might have led to increased drainage of inorganic phosphorus from the circulation thus, further complicating hypophosphataemia as recorded in haemoglobinuric dairy animals in the present study. The observance of haemoglobinuric cases in prepartum buffaloes particularly in late gestation stage was similar to those recorded earlier[4]. However, there is no report in the literature on the occurrence of nutritional haemoglobinuria in cows in India as recorded in the present study. Even though acute haemolytic syndrome in cattle has been mostly associated with postpartum phase, frequently referred as PPH[5], but in the present study, the condition was recorded in prepartum haemoglobinuric cows also. The development of hypophosphataemia in prepartum haemoglobinuric dairy animals was ascribed to increased utilization of inorganic phosphorus for the foetal development.

In general, the clinical findings observed in the present study were similar to those described earlier[1,4,5]. The clinical signs in haemoglobinuric buffaloes and cows were not significantly variable except that their severity was more intense in buffaloes than cows. The severity of the clinical signs was also variable between pre and postpartum phases. The most consistent findings were sudden onset of the disease, loss of condition associated with passing of coffee coloured urine. As compared to cows, complete anorexia was observed in majority of the haemoglobinuric buffaloes which was associated with marked weakness, sharp reduction in milk yield in lactating animals and pallor conjunctiva. In majority of the animals, the temperature was normal in early stages. However, it was always observed to be subnormal in advanced stages of the disease where the history of onset of haemoglobinuria was of more than 48 hrs duration. Ruminal stasis, constipation and straining to pass scanty dry faeces were the marked symptoms in buffaloes, whereas, diarrhoeic faeces were also passed by some of the haemoglobinuric cows. Thumping heart sounds and jugular pulse was more prominent in cows than in buffaloes.

The significant decline in PCV and TEC values in haemoglobinuric animals was significantly correlated to the decreasing Hb levels and the decline in these haematological indices was indicative of severe form of anaemia resulting due to intravascular haemolysis. The severity of clinical findings was directly related to the severity of anaemia. Non-significant increase in MCV, MCH and MCHC values in haemoglobinuric cows and buffaloes as compared to healthy controls reflected development of macrocytic hypochromic anaemia. This was in accordance with the findings of Clegg and Evan[6]. Macrocytic hypochromic anaemia could be ascribed to hypocupraemia recorded in the present investigation. This form of anaemia could be related to increased erythropoietic activity associated with release of immature erythrocytes into the circulation in response to acute haemolytic crisis. Total leucocytic count

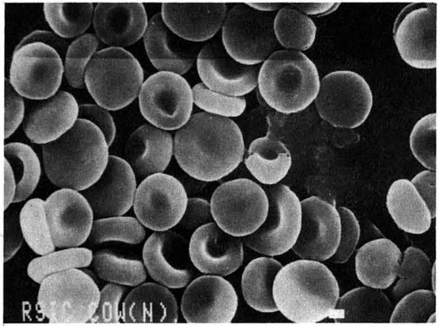

Fig.1. Biconcave erythrocytes in a healthy cow

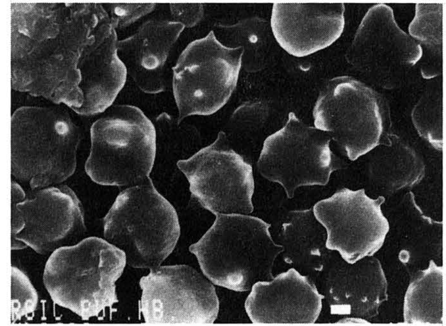

Fig. 2. Discocytes in a haemoglobinuric buffalo

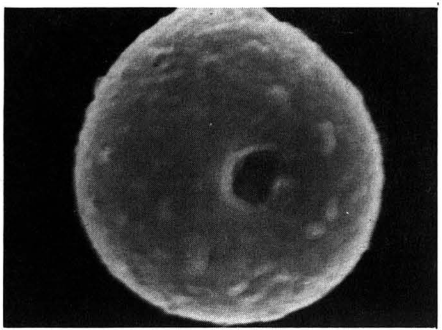

Fig. 3. A spherical erythrocyte of a haemoglobinuric buffalo with corrugated membrane

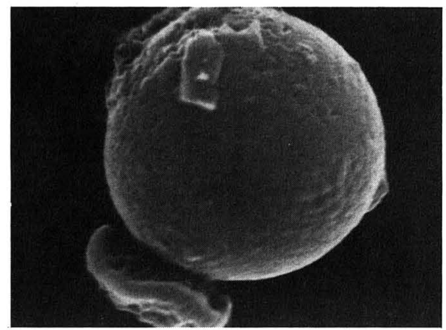

Fig. 4. A spherical erythrocyte of a haemoglobinuric cow with a Heinz body and exfoliated membrane

revealed leucocytosis, the increase being attributed to stress due to massive destruction of red blood cells.

Comparative analysis of results of haemato-biochemical studies revealed that severity of anaemia and hypophosphataemia was more marked in haemoglobinuric buffaloes than cows. Significant decline in plasma inorganic phosphorus content in haemoglobinuric cases had been reported by various workers[3,4,7]. The hypophosphataemia could be related to low levels of available phosphorus in the soil as well as in the plants in the affected areas as recorded in the present study.

Mean blood Mo content revealed a significant increase in haemoglobinuric animals which was inversely related to resultant hypocupraemia. Increase in Mo content of blood was supported by soil and plant Mo analysis which revealed significantly increased Mo content. Alkaline pH of soil recorded from the affected farms also increased the availability of Mo to the plant. Increased incidence of the disease recorded in the present study was related to prolonged and exclusive feeding of berseem (Trifolium alexandrinum L.), a leguminous forage. The present findings were supported by the results of Nayyar et al. who indicated that uptake of Mo was higher in leguminous crops[8]. The increased concentration of Mo in the berseem forage fed to animals might have interfered with the uptake of inorganic phosphorus and copper by the plants and also their absorption through the digestive tract, due to competitve inhibitory effect, thus setting in hypophosphataemia and hypocupraemia[9]. Mo is the most important factor, which in the presence of sufficient amount of S, exerts an antagonistic effect on Cu. Molybdenum and S form thiomolybdate complex in the rumen, which combine with Cu to form Cu-thiomolybdate, which interferes with the Cu absorption[10]. Increased Mo concentration in the system led to increased urinary excretion of phosphorus and Cu, thus, further precipitating hypophosphataemia and hypocupraemia. Postparturient haemoglobinuria has been recorded to be due to molybdenosis induced hypocupraemia in cattle in New Zealand[5,6].

Fresh blood smears stained with new methylene blue stain revealed Heinz bodies within the erythrocytes in all haemoglobinuric animals, their number being variable between 2-15 per cent of the erythrocytes. Usually, the Heinz bodies were peripheral but in some of the cases, their position was central. Usually the Heinz bodies were singular within the erythrocytes but in some of the erythrocytes, more than one were detected. Copper is associated with the activity of superoxide dismutase, which protects red cells from deleterious effects of superoxide anions which are normally generally generated in small amounts by auto-oxidation of Hb and in large amounts under oxidant stress. These superoxide radicals, if not destroyed, could result in inactivation of vital enzymes, denaturation of Hb, production of Heinz bodies and peroxidation of red cell membrane lipids with subsequent haemolysis[11]. The demonstration of Heinz bodies in the haemoglobinuric animals in the present study and hypocupraemia indicated that decreased activity of superoxide dismutase and hypophosphataemia might have precipitated the intravascular haemolysis.

Following SEM studies, the erythrocytes of healthy cows (Fig. 1) and Indian buffaloes generally appeared as biconcave discs but extensive poikilocytosis of erythrocytes of healthy buffaloes was also recorded. The degree of concavity of buffalo's RBC as compared to cow's RBC varied extensively giving the erythrocytes various shapes. Scanning electron microscopic studies revealed sphero-echinocytosis and sphero-acanthocytosis of erythrocytes in puerperal haemoglobinuric cows and buffaloes (Fig. 2) which reflected changes in membrane permeability and integrity. Most of the discocytes had irregular margins with appearance of variable forms of disco-echinocytes, sphero-acanthocytes or sphero-echinocytes (Fig.2). Fibrin-like deposits on the surface of erythrocytes of haemoglobinuric dairy animals were also detected. Some of the erythrocytes revealed corrugation of membrane (Fig. 3). SEM studies also revealed Heinz bodies like structure (Fig. 4). Reduced intraerythrocytic ATP levels along with low membrane phospholipid content had been recorded in haemoglobinuric dairy animals. These two factors might be responsible for the production of echinocytosis or sphero-echinocytosis thus, setting in intravascular haemolysis[12]. Cytomorphological changes recorded in the present study might also be ascribed to resultant anaemic anoxia.

Treatment of clinical cases of puerperal haemoglobinuria with parenteral infusion of copper glycinate, undertaken in the present study, is the first trial in India. The comparative evaluation of therapeutic response in cows and buffaloes revealed a significant variation. All the affected cows showed marked improvement in condition with disappearance of haemoglobinuria within 12-30 hrs following single intravenous administration of copper glycinate with majority of the cows showing clinical recovery by 12 hrs. However, in haemoglobinuric buffaloes the clinical response to the therapeutic measures was delayed. In seven of the buffaloes, marked improvement in condition was observed within 24 hrs following single intravenous infusion of copper glycinate. The clinical response following second administration of copper glycinate in delayed/non-responsive buffaloes was rapid and no marked variation in degree of response was observed with the route of administration of the second dose. Complete clinical recovery in these haemoglobinuric buffaloes was observed within 12-24 hrs of administration of second dose. The haemato-biochemical parameters showed significant improvement following therapy. The therapeutic trials with copper glycinate treatment resulted in absolute recovery of the haemoglobinuric animals which was comparable to the observations recorded in New Zealand, even though, the route of administration and the dosage of copper glycinate used varied.

It was cosidered that copper glycinate provided protection to red blood cells against oxidative stress through antioxidant mechanism. Thus, observance of significant level of hypocupraemia associated with significant increase in blood Mo content, detection of Heinz bodies anaemia and quick response to parenteral infusion of copper glycinate supports the view that this condition recorded in the Punjab State, India is somewhat comparable to that reported in New Zealand in haemoglobinuric cows.

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SUMMARY

Haemato-biochemical, surface ultrascopy and therapeutic studies on puerperal nutritional haemoglobinuria were conducted in 45 dairy animals. Maximal incidence was recorded in recently calved high yielding crossbred cows and buffaloes between

3rd to 6th calving. The increased incidence was correlated with exclusive and prolonged feeding of Trifolium alexandrinum L. a leguminous forage. Haematological studies revealed a significant decrease in TEC, Hb, PCV and increase in TLC values associated with macrocytic hypochromic and Heinz bodies anaemia in haemoglobinuric animals. Plasma biochemical alterations revealed hypophosphataemia, hypocupraemia and significant increase in molybdenum content. Scanning electron microscopic studies revealed sphero-echinocytosis and sphero-acanthocytosis in erythrocytes of haemoglobinuric animals. The haemoglobinuric animals showed complete clinical recovery following parenteral infusion of copper glycinate. The comparative evaluation of therapeutic response in haemoglobinuric cows and buffaloes in relation to soil-plant mineral status is discussed.

RESUMÉ

Haemato-biochimique, ultrascopie de surface et thérapeutique èetudes sur lédesordre haemoglobinurae a cause de mauvais alimentation ont étaients faites sur les 45 animaux laitière. L'incidence maximale a èté trouvez chez les animaux a grandes rendement, prevenants de croisement genetique, justment apres parturition et chez les buffles entre 3eme et 6eme déliverances. L'incidence augumenté a était lié a l'alimentation prolongé avee. T. alèxcomme le fourrage. Etudes haematologique a montrè une diminution significantive à C.E.T., Hb, V.C.P., et ume augumentation en C.L.T. associaé avee l'anémie de type macrocytique hypochromique et des corps d'Heinz les animaux haemoglobinurique. Alteration biochmique du plasma a motré hypophosphataemae, hypocupraemae et ume augumentation significantive en molybdene (Mo). Ultrascopie de surface a motré sphero-echincytose parmis les sphero-acanthocytose erythrocytes des animaux attaints d haemoglobinurae. Telle animaux ont-montraits ume guérison complete quant on domme traitment avèc glycinate de cuivre. Une evaluation comparative de reponse thérapeutique chez lés animaux haemoglobinurique en relation avec teneurs in minierales en des sol et des plants est discuté.