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Haematological, Biochemical and Therapeutic Aspects of Parturient Haemoglobinuria in Buffaloes

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Abstract.- A total of thirty buffaloes suffering from parturient haemoglobinuria were selected from district Chakwal during December 2010 – January 2011. Sixty apparently healthy buffaloes of similar description were also selected from the same areas as controls. Blood samples collected with and without anticoagulant (EDTA) were analyzed for various haematological and biochemical parameters. Significantly (P<0.05) increased mean corpuscular volume (60.72±8.49fL), mean corpuscular haemoglobin (21.46±2.52pg), red cell distribution width (16.80±1.81%), reticulocytes (1.25± 0.66%), platelets (208.13±85.26×10^3 /µl), plateletcrit (0.09±0.04%), mean platelet volume (4.99±1.85fL), serum alkaline phosphatase (164.20±88.95u/L), bilirubin conjugated (0.40±0.17mg/dl), bilirubin unconjugated (0.67±0.22mg/dl) and bilirubin total (1.07±0.39mg/dl) whereas; significantly (P<0.05) decreased serum calcium (2.12±0.11mmoles/L) and phosphorous (2.67±0.79mg/dL) were recorded in haemoglobinuric buffaloes compared to healthy controls. Non significant (P>0.05) differences were recorded in mean corpuscular haemoglobin concentration, platelet distribution width, serum glucose, cholesterol and copper concentrations between haemoglobinuric and healthy buffaloes. These thirty haemoglobinuric buffaloes were divided into three groups for clinical trial to compare and assess the recovery rates of three different treatment packages. Highest recovery rate (100%) was recorded for combined therapy of sodium acid phosphate and blood transfusion The recovery rates for combination of sodium acid phosphate with tranexamic acid and tranexamic acid with Novacoc forte injection were 70% and 50%, respectively.

Key words: Haemoglobinuria, hypophosphataemia, haemolytic anaemia, haemolytic syndrome, treatment of parturient haemoglobinuria, sodium acid phosphate therapy.

INTRODUCTION

Parturient haemoglobinuria is a non infectious haemolytic syndrome of milking buffaloes characterized by hypophosphataemia, intravascular haemolysis, haemoglobinaemia, haemoglobinuria and anaemia. A considerable number of buffaloes are affected every year in India and Pakistan during advanced pregnancy or early lactation (Dalir-Naghadeh *et al.*, 2006; Gahlawat *et al.*, 2007; Ghanem and El-Deeb 2010). The diversified etiological factors associated with this disease in different parts of the continent include dietary phosphorous deficiency, hypermolybdenosis, hypocuprosis and haemolytic agents from cruciferous/toxic plants such as saponin in sugar beets alfalfa and berseem whereas S-methyl cystein sulfoxide in brassica (Radostits *et al.*, 2007; Neto *et al.*, 2007; Brechbuhl *et al.*, 2008). Erythrocyte glycolytic activity is reduced due to decreased serum inorganic phosphorous resulting in less ATP production. ATP is required to maintain the normal deformability of erythrocytes. Due to its decreased level, erythrocytes loose their deformability, become rigid and ultimately leave the circulation due to decreased life span (Radostits *et al.*, 2007; Akhtar *et al.*, 2007b).

Increased molybdenum reduces phosphorous contents of the body by interfering with its absorption and increasing its elimination through urine (Dua, 2003; Kahn and Line, 2005). A copper containing enzyme superoxide dismutase is part of intraerythrocyte protection mechanism against oxidative damage. Activity of this enzyme is

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reduced due to copper deficiency rendering the erythrocytes vulnerable to Heinz body formation and ultimately haemolysis as a result of feeding on poisonous plants (Jain, 1993).

In Pakistan, information on multidimensional etiological aspects of this buffalo syndrome is quite scanty. Present study was therefore designed to explore etiological and therapeutic aspects important to prepare the control strategy of this disease.

MATERIALS AND METHODS

Buffaloes

Thirty haemoglobinuric buffaloes were selected from district Chakwal during December 2010 – January 2011. Parturient haemoglobinuria was diagnosed clinically on the basis of characteristic signs (red/coffee coloured urine and straining during defication) and epidemiological features (advanced pregnancy or recent parturition). Standard laboratory techniques (Cole *et al.,* 1973; Anwar *et al.,* 2005) were used to rule out other disease conditions causing red urine (haemoparasites and leptospirosis). Sixty apparently healthy buffaloes maintained under similar conditions were also selected as controls from the same areas.

Haematological studies

For haematological studies, blood samples were collected into 3ml sterile vacuum tubes containing EDTA (ethyl diamine tetra acetic acid) @ 1mg/ml from jugular vein of each animal. Blood samples were also collected from each animal into 4ml sterile vaccum tubes without anticoagulant; serum was separated and stored at -20 C until biochemical analysis.

Blood samples collected with anticoagulant were processed for determination of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) through automated haemotology analyzer Celltac a Mek 6420K (Nihon Kohden Japan) according to manufacturer's instructions. Reticulocyte percentage was determined after preparing staining solution and blood films according to recommendations of Dacie and Lewis (1991).

Biochemical analysis

Serum samples of haemoglobinuric and healthy buffaloes were analyzed through spectrophotometer using diagnostic kits as per manufacturer's recommendations for determination of glucose, cholesterol, alkaline phosphatase, bilirubin (total & direct), calcium and phosphorous.

Clinical trial

A randomized controlled trial was conducted to compare and assess the recovery rates of three different treatment packages for parturient haemoglobinuria. Thirty haemoglobinuric buffaloes were divided into three groups each comprising of ten animals for allocation of respective treatment packages as described below

Package I

Each animal of first group was treated by administering sodium acid phosphate (Merck) @ 80g as 20% solution in distilled water intraveinously as well as 80 g orally once daily till clinical recovery or death. Fresh blood was also administered @ 3 bags (500ml × 3) per animal on first day of treatment

Package II

Ten animals of the second group were treated with a combination of antifibrinolytic drug tranexamic acid (Transamine injection 250mg × 10) and sodium acid pohosphate. 80g sodium acid phosphate and 2.5g tranexamic acid (Transamine injection 250mg × 10) were mixed with 500 ml of 5% dextrose saline and intraveinously administered to each animal once daily till clinical recovery or death. A similar daily dose of sodium acid phosphate (80g) was also administered to each animal orally once daily till clinical recovery or death.

Package III

The remaining ten animals of third group were exposed to combined therapy of Tranexamic

acid and Novacoc Forte injection. 2.5g tranexamic acid (Transamine injection 250mg × 10) in 500 ml of 5% dextrose saline was intravenously administered to each animal once daily till clinical recovery or death. 500ml Novacoc Forte injection (Waseem Impex) was also intravenously administered to each animal only for one day *i.e.* first day of treatment.

In all three groups, the absence of haemoglobin in urine (after verification by the benzidine test) was taken as index of recovery.

Statistical analysis

Data of haemoglobinuric and healthy buffaloes with respect to haematological and biochemical values were analyzed by applying independent sample t-test whereas; treatment packages were compared with respect to their recovery rates and average number of doses utilized by applying Chi-square test for proportion and Kruskal Wallis H test respectively at 5% significance level using SPSS 17 software.

RESULTS

Haematological changes

Significantly (P<0.05) decreased MCH (21.46±2.52 g/dl), but significantly (P<0.05) increased MCV (60.72±8.49 fL), RDW (16.80±1.81%), reticulocytes (1.25±0.66%), PLT (208.13±85.26 × 10^3/µl), PCT (0.09±0.04%) and MPV (4.99±1.85 fL) were recorded in haemoglobinuric buffaloes. Non significant (P>0.05) difference was recorded between haemoglobinuric and healthy animals with respect to MCHC and PDW (Table I).

Biochemical changes

Significantly (P<0.05) decreased levels of serum calcium (2.12±0.11 mmoles/l) and phosphorous (2.67±0.79 mg/dl) and significantly (P<0.5) increased levels of serum alkaline phosphatase (164.20±88.95 u/l), billirubin conjugated (0.40±0.17 mg/dl), billirubin unconjugated (0.67±0.22 mg/dl) and billirubin total (1.07±0.39 mg /dl) were recorded in haemoglobinuric buffaloes. Non significant (P>0.05) difference was recorded between haemoglobinuric and healthy buffaloes with respect to serum glucose and cholesterol (Table I).

Table I.- Haematological and biochemical values (Mean±SD) of haemoglobinuric and healthy buffaloes,

Parameters	Haemoglobinuric buffaloes (n=30)	Healthy buffaloes (n=60)
Haematological parameters		
Mean corpuscular volume (fL)	60.72±8.49*	47.97±4.03
Mean corpuscular haemoglobin (pg)	21.46±2.52*	17.39±1.73
Mean corpuscular haemoglobin concentration (g/dl)	35.82±2.15	36.02±0.97
Red cell distribution width %	16.80±1.81*	16.08±0.70
Reticulocytes %	1.25±0.66*	0.21±0.16
Platelets (10^3 /µl)	208.13±85.26*	169.68±70.01
Plateletcrit %	0.09±0.04*	0.07±0.03
Mean platelet volume (fL)	4.99±1.85*	4.45±0.65
Platelet distribution width %	15.94±2.47	16.15±0.81
Biochemical components		
Serum glucose (mg/dl)	80.20±18.02	84.83±18.39
Cholesterol (mg/dl)	123.60±17.98	119.13±19.74
Alkaline phosphatase (u/l)	164.20±88.95*	120.20±76.99
Billirubin (conj) (mg/dl)	0.40±0.17 *	0.10±0.02
Billirubin (unconj) (mg/dl)	0.67±0.22*	0.28±0.06
Billirubin (total) (mg/dl)	1.07±0.39*	0.38±0.08
Calcium (mmole/L)	2.12±0.11*	2.20±0.08
Phosphorous (mg/dl)	2.67±0.79*	4.01±1.12

*Values at P< 0.05 are statistically significant

Clinical trials

Out of total 10 treated animals of group I; 7 and 3 animals recovered after 3 and 4 days respectively. The recovery rate of respective treatment package (package I) was 100% and average number of doses used was 3.3±0.48. Four and three animals of group II recovered after three and four days, respectively whereas; remaining three animals did not respond to treatment and died after four (one animal) and five (two animals) days. Recovery rate of respective treatment package (package II) was 70% and average number of doses used was 3.8±0.79. Two and three animals of group III recovered after three and four days respectively. The remaining five animals of this group did not respond to treatment and died after four (two animals) and five (three animals) days. The recovery

Table II.- Recovery rates of different treatment packages for parturient haemoglobinuria in buffaloes (n = 30).

Packages	Treated	Recovered	Died	*Average no. of doses used	**Recovery rate %
I (Sodium acid phosphate 80 g as 20% solution in distilled water i/v and 80 g orally once daily. Blood transfusion (3 bags × 500ml) for day I only)	10	10	0	3.3±0.48	100
II (Sodium acid phosphate 80 g and tranexamic acid 2.5 g (Transamine injection 250mg × 10) in 500 ml of 5% dextrose saline i/v with 80 g of sodium acid phosphate as 20% solution in distilled water orally once daily.)	10	7	3	3.8±0.79	70
III (Tranexamic acid 2.5 g (Transamine injection 250mg × 10) in 500 ml of 5% dextrose saline by slow i/v injection once daily. Novacoc injection 500ml i/v for day 1only)	10	5	5	4.1±0.74	50

*P = 0.048 (significant) Kruskal Wallis H test
**P = 0.000 (significant) Chi-square test for proportion

rate of respective treatment package (package III) was therefore 50% and average number of doses used was 4.1±0.74. These results are summarized in Table II.

DISCUSSION

The absolute values or red cell indices including MCV, MCH, MCHC and RDW are used for morphological classification of anaemia. Significantly (P<0.05) increased values of MCV and RDW; significantly decreased MCH whereas insignificantly changed MCHC indicate macrocytic anaemia (Anwar *et al.*, 2005; Dacie and Lewis, 1991). Increased MCV is also an indication of erythroid regenerative response resulting in release of immature cells of increased size (Feldman *et al.*, 2000). These cytomorphological changes of erythrocytes are attributed to decreased ATP production due to impaired glycolytic activity of erythrocytes in this disease (Radostits *et al.*, 2007; Akhtar *et al.*, 2007b).

No previous report is available on platelet parameters of haemoglobinuric buffaloes to compare the results of present study. This is first report which will advance the prevailing knowledge about this disease. Significantly (P<0.05) increased number of reticulocytes in haemoglobinuric animals indicate erythropoietic activity/regenerative response (Anwar *et al.*, 2005).

Significantly (P<0.05) increased level of serum alkaline phosphatase in haemoglobinuric buffaloes is attributed to generalized hypoxia due to decreaseed haemoglobin level as a result of intravascular haemolysis. The consequently developed anoxic conditions damage liver, heart and kidney cells resulting in leakage of alkaline phosphatase and thus increasing its concentration in general circulation (Akhtar *et al.*, 2008).

Significantly (P<0.05) increased concentration of unconjugated (indirect) bilirubin in haemoglobinuric buffaloes may be attributed to intravascular haemolysis, whereas; significantly increased conjugated (direct) bilirubin may be attributed to hepato-cellular damage, anorexia and dehydration. The hypoxic conditions developed as a result of severe intravascular haemolysis effect the structure and function of liver and decrease its capacity for bilirubin conjugation and secretion (Latimer *et al.*, 2003; Anwar *et al.*, 2005).

Significantly (P<0.05) decreased serum inorganic phosphorous concentration of haemoglobinuric buffaloes may be due to phosphorous deficient diets, ingestion of cruciferous/toxic plants, utilization of phosphorous for fetal bone development and its heavy drainage through milk in high producing animals maintained on low phosphorous rations (Akhtar, 2006). Low

phosphorous diets with high calcium contents induce hypophosphataemia by decreasing absorption of phosphorous from gastro-intestinal tract due to wider ratio of calcium and phosphorous. Deficiency of phosphorous is transferred from soil to plants and ultimately to animals due to prolonged feeding on phosphorous deficient plants particularly berseem (Akram *et al.,* 1990; Akhtar *et al.,* 2006; Dua, 2003, 2009).

Significantly ($P<0.05$) decreased serum calcium concentration of haemoglobinuric animals is in accordance with the findings of Radwan and Rateb (2007) whereas contrary to the previous reports of Akhtar *et al.* (2007b) and Ghanam and El-Deeb (2010).

Non significant ($P>0.05$) difference in serum glucose level of haemoglobinuric and healthy buffaloes is contrary to the previous reports of Akhtar (2006) and Akhtar *et al.* (2008). They reported significantly ($P \square 0.05$) increased serum glucose level in haemoglobinuric buffaloes as compared to healthy controls and attributed it to phenomenon of glycogenolysis which occurs as a result of anorexia. Ruminants depend on volatile fatty acids for main source of energy. Progressive loss of appetite in parturient haemoglobinuria leads to non availability of volatile fatty acids in sufficient quantity. Consequently the effected animals have to depend on glucose oxidation metabolism for their energy requirements leading to development of glycogenolysis and ultimately hyperglycaemia. Glucocorticoids released as a result of stress of digestive disorders in parturient haemoglobinuria may also stimulate phenomenon of glycogenolysis and gluconeogenesis resulting in development of hyperglycaemia (Latimer *et al.*, 2003).

The contradiction of these previous reports with the findings of present study with respect to serum glucose level of haemoglobinuric animals may be due to difference in time at which the effected animals were sampled. In parturient haemoglobinuria, anorexia develops progressively and the previous investigators probably sampled the majority of parturient haemoglobinuria affected animals few days after the onset of clinical signs when anorexia and resultant phenomenon of glycogenolysis and gluconeogenesis had been developed.

Non significant ($P>0.05$) difference in serum cholesterol level of haemoglobinuric and healthy buffaloes is in agreement with the previous report of Akhtar *et al.* (2008) whereas contrary to Ghanem and El-Deeb (2010).

Highest recovery rate was recorded for combined therapy of sodium acid phosphate and blood transfusion. Durrani *et al.* (2009, 2010) reported 18% efficacy rate for sodium acid phosphate. This difference is probably due to the reason that in present study sodium acid phosphate therapy is supported by blood transfusion and antifibrinolytic drug in group I and group II, respectively.

Hypophosphataemia decreases glucose utilization rate and ATP production in erythrocytes of haemoglobinuric buffaloes leading to decreased synthesis as well as reduction of glutathione. This process predisposes the erythrocytes to harmful effects of oxidants because reduced glutathione is required to protect erythrocyte membrane against lipid peroxidation and haemoglobin against oxidative denaturation. Its deficiency therefore leads to lipid peroxidation of red cell membrane and ultimately intravascular haemolysis. Sodium acid phosphate reduces lipid peroxidation by acting through antioxidant mechanism. Hence, sodium acid phosphate restores the plasma inorganic phosphorous level as well as the antioxidant potential of erythrocytes leading to increased red cell vitality (Gahlawat *et al.,* 2007). Whole blood transfusion helps in reducing anaemic hypoxia by improving haematological indices and ultimately the chances of recovery.

In conclusion, it is suggested that sodium acid phosphate along with whole blood transfusion is best treatment for parturient haemoglobinuria in buffaloes followed by combined therapy of sodium acid phosphate and antifibrinolytic drugs.

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