

PATHO-BIOCHEMICAL CHANGES IN BUFFALOES (*BUBALUS BUBALIS*) SUFFERING FROM PARTURIENT HAEMOGLOBINURIA

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ABSTRACT

The objectives of this study were to assess the role of glucose, bilirubin, cholesterol and alkaline phosphatase as aetiological factors in parturient haemoglobinuria (PHU) in buffaloes and gross and histopathological changes taking place in this disease. For this purpose, 120 buffaloes, including 60 PHU affected and 60 apparently healthy buffaloes were used. These animals were subjected to collection of blood, urine and tissue samples. Blood glucose, serum total bilirubin and serum alkaline phosphatase concentrations in PHU affected buffaloes were significantly higher ($P < 0.05$) than in healthy buffaloes. The colour of urine in PHU affected buffaloes ranged from red, dark red to coffee coloured and its pH was strongly alkaline. Urine of affected buffaloes was positive for haemoglobin (100%) and albumin (95%) but negative for sugar and ketone bodies. Microscopic examination of urine of affected buffaloes revealed no intact erythrocytes. Grossly, carcasses of buffaloes died of PHU were anaemic and jaundiced. Kidneys, liver and spleen were pale, enlarged and congested. Liver was friable and swollen. Epicardium and endocardium showed ecchymotic haemorrhages. Lungs were emphysemated and oedematous. Histopathologically, kidneys showed necrosis of tubular epithelium and deposition of casts along with atrophy of glomeruli. Liver exhibited centrilobular necrosis, haemorrhages and congestion. There was haemosiderin deposition in kidneys, liver and spleen. Heart muscles showed degenerative changes, whereas lungs were haemorrhagic, congested and emphysemated. It was concluded that significantly high blood glucose, serum total bilirubin and alkaline phosphatase were found in PHU affected buffaloes. Histopathologically, necrosis of tubular epithelium, deposition of casts along with atrophy of glomeruli, centrilobular necrosis and haemosiderin deposition were the main findings.

Key words: Buffaloes, parturient haemoglobinuria, serum biochemistry, urinalysis, gross and histopathological lesions.

INTRODUCTION

Parturient haemoglobinuria (PHU) is an acute disease of high yielding buffaloes and cows characterized by hypophosphataemia, intravascular haemolysis, haemoglobinuria and anaemia (Akhtar *et al.*, 2007a). This disease is prevalent (0.02-4.44%) throughout the Punjab, Pakistan (Raz *et al.*, 1988). It mostly affects buffaloes either in advanced pregnancy or in early lactation with majority of animals are in their 3rd to 6th lactations (Chugh *et al.*, 1996; Akhtar *et al.*, 2006).

The exact aetiology and pathogenesis of PHU is not known (Akhtar *et al.*, 2007b) and variety of aetiological factors have been reported to be associated with the disease in different parts of the world. Though hypophosphataemia is documented consistently in the affected buffaloes (Chugh *et al.*, 1996), great stress of digestive disorders in PHU can not be ignored. As a result of stress, glucocorticoids are released that probably stimulates glycogenolysis and gluconeogenesis, resulting in hyperglycaemia (Benjamin, 1978; Latimer *et al.*, 2003). In PHU, red blood cells are lysed most probably due to their exposure to altered function

and structure, a loss of normal deformability, and an increase in fragility due to impaired glycolysis and ATP synthesis (Wang *et al.*, 1985). Severe stress due to intravascular haemolysis in PHU affects the structural and functional activities of different body organs mainly liver, heart, kidneys etc. Keeping in view the diagnostic and prognostic significance, glucose, serum total bilirubin, cholesterol and alkaline phosphatase were determined in clinical cases of PHU along with urinalysis and pathological changes in buffaloes died of PHU.

MATERIALS AND METHODS

Experimental animals

A total of 60 buffaloes (*Bubalus bubalis*) suffering from PHU were randomly selected from field cases occurring in Faisalabad, Toba Tek Singh and Jhang districts of Punjab province of Pakistan. Simultaneously, 60 clinically healthy buffaloes of similar description from the same localities were included as controls. Affected buffaloes were from 1-8 parities having age 5-11 years and had history of normal parturition. The disease was diagnosed

clinically on the basis of specific signs such as haemoglobinuria and characteristic straining while defecating in early lactation or in advanced pregnancy (Digraskar *et al.*, 1991). The possibility of other diseases causing a reddish colouration of urine like babesiosis, leptospirosis and bacillary haemoglobinuria was ruled out through laboratory tests.

Sample collection and analysis

Blood samples were collected from the jugular vein of each animal, serum was separated and stored in aliquots at -20°C until analysed for biochemical parameters. Blood glucose (Cat. # GL 2586, Randox Laboratories Ltd., UK), serum cholesterol (Cat # 4248, Biocon Diagnostik, Germany), serum total bilirubin (Cat # 1965, Biocon Diagnostik, Germany) and serum alkaline phosphatase (Cat # 1622, Biocon Diagnostik, Germany) were determined photometrically using the diagnostic kits following the instructions of the manufacturer.

Urine samples were collected from each animal using a sterilized catheter into clean, dry, sterilized brown coloured glass bottles, brought to laboratory immediately and processed for gross, microscopic and biochemical analysis. These urine samples were examined for colour, pH, proteins, sugar, blood (haemoglobin) and ketone bodies following the procedures described by Benjamin (1978). For microscopic examination, urine sediment was obtained by centrifuging the sample at 3000 rpm for 5 minutes. A drop of sediment was placed on a clean, grease free glass slide, covered with a cover slip and examined under the microscope to identify erythrocytes, leukocytes (pus cells), epithelial cells, casts, crystals and bacteria. Maximum, minimum and mean number of RBCs, WBCs (pus cells) and epithelial cells per high power field per case was calculated and scored accordingly.

Gross lesions in organs including liver, lungs, spleen, kidneys, and heart were recorded in buffaloes died of parturient haemoglobinuria. Samples of morbid tissues were collected and preserved in 10% neutral buffered formalin. Tissue sections of 4-6 μm thick were cut, stained with haematoxylin and eosin stain and examined for histopathological studies.

Statistical analysis

The data regarding glucose, total bilirubin, cholesterol and alkaline phosphatase were analysed by using t-test. Chi-square was applied to know the difference in colour and pH of urine in healthy and PHU affected buffaloes on personal computer by using Minitab computer program (Anonymus, 2003). The level of significance was $P \leq 0.05$.

RESULTS

Blood glucose, serum total bilirubin and serum alkaline phosphatase concentrations were significantly

($P < 0.05$) higher in PHU affected buffaloes than healthy buffaloes (Table 1). The difference in serum cholesterol level between healthy and haemoglobinuria affected buffaloes was statistically non-significant.

The colour of urine in haemoglobinuric buffaloes ranged from red (28%), dark red (20%) to coffee coloured (52%), depending upon the severity and duration of illness. Chi-square analysis revealed significant difference in frequency of buffaloes passing urine of different colours ($\chi^2 = 7.006$, $df = 2$, $P < 0.05$). In healthy buffaloes, the colour of urine was yellow. The pH of urine in haemoglobinuric buffaloes (8.39 ± 0.26) was significantly ($P < 0.05$) higher than in healthy buffaloes (7.91 ± 0.25).

Urine of haemoglobinuric buffaloes was positive for haemoglobin (100%) and albumin (95%) but negative for sugar and ketone bodies. Urine of healthy buffaloes was negative for haemoglobin, albumin, sugar and ketone bodies. Microscopic examination of urine of affected buffaloes revealed no intact erythrocytes but few epithelial cells and crystals (amorphous phosphate and triple phosphate) were present.

Grossly, carcasses of buffaloes died of PHU were found anaemic and jaundiced. The urinary bladder was distended with red to coffee coloured urine. Kidneys were pale and enlarged. Liver was pale, congested, friable and swollen. Gall bladder was distended with thick bile. Spleen was enlarged and congested. Epicardium and endocardium showed ecchymotic haemorrhages. Lungs were emphysemated and oedematous. Small and large intestines did not show any lesion.

Histopathologically, kidneys showed necrosis of tubular epithelium and deposition of hyaline casts in the lower nephrons (Fig. 1). Glomerular atrophy with distension of urinary spaces (Fig. 2) and haemosiderin deposition were observed. The liver showed necrosis (Fig. 3), congestion and haemosiderin deposition. Spleen showed haemosiderin deposition nearly in all cases. Heart muscles showed haemorrhages and degenerative changes in the form of vacuolation, condensation of nuclei, pyknosis and necrosis of the cells. Lungs showed haemorrhages, congestion and alveolar walls were thickened. Atelactasis and emphysema was a prominent feature. Necrosis and sloughing of bronchiolar epithelium alongwith mild cellular infiltration were observed.

DISCUSSION

Increased blood glucose levels in PHU affected buffaloes observed in the present study were nearly the same as reported by Pandey and Misra (1987) in PHU affected buffaloes (74.87 ± 2.48 mg/dL). In ruminants, volatile fatty acids (VFA) are the main source of energy. Anorexia in PHU leads to non-availability of volatile fatty acids in sufficient quantity, therefore, affected animal has to depend upon oxidative glucose

metabolism for its energy requirements, causing glycogenolysis. Moreover, glucocorticoids released due to stress of digestive disorders in PHU probably stimulate glycogenolysis and gluconeogenesis, resulting in hyperglycaemia (Benjamin, 1978; Singh *et al.*, 1989; Latimer *et al.*, 2003).

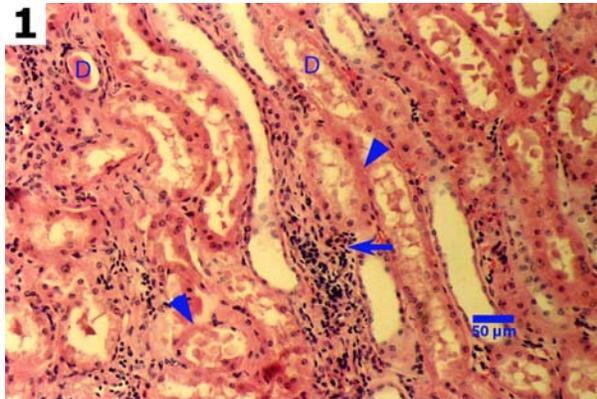


Fig. 1: Kidneys of parturient haemoglobinuria affected buffaloes showing necrosis of tubular epithelium (arrow heads), deposition of casts (D) and cellular infiltration (arrow) (200 X; H & E).

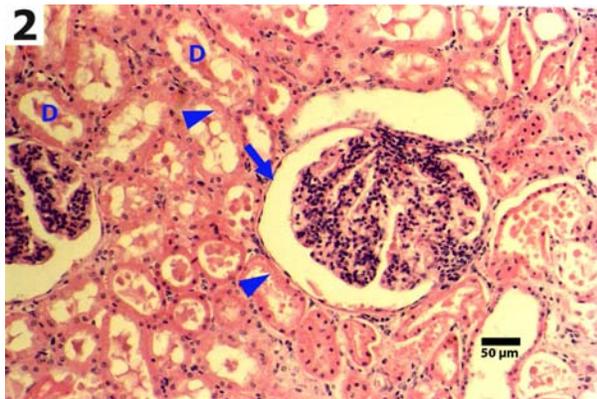


Fig. 2: Increased urinary spaces due to glomerulus atrophy (arrow) and lobulation, necrosis of tubular epithelium (arrow heads) and deposition of casts (D) in kidneys of parturient haemoglobinuria affected buffaloes (200 X; H & E).

A significant increase in the serum total bilirubin in PHU affected buffaloes (4.24 ± 2.49 mg/dL) compared to healthy buffaloes was observed in the present study. These findings are in agreement with the results (3.30 ± 0.51 mg/dL) reported by Digraskar *et al.* (1996), while the levels of 7.64 ± 1.19 and 7.9 mg/dL reported by Kurundkar *et al.* (1981) and Pirzada and Ali (1990), respectively, were higher than the present findings. The increase in the serum total bilirubin concentration in

PHU could be due to increased destruction of erythrocytes (haemolysis), hepatocellular damage, cholestasis and anorexia/dehydration (Benjamin, 1978; Kurundkar *et al.*, 1981; Pirzada and Ali, 1990). In severe haemolysis, hypoxia develops which affects the structure and functions of liver (Stogdale, 1981). Loss of hepatic functions results in decreased capacity for bilirubin uptake, conjugation and/or secretion (Latimer *et al.*, 2003).

The level of serum alkaline phosphatase (ALP) was significantly increased in PHU affected buffaloes (232.50 ± 58.68 U/L). The value of 378.00 ± 36.51 IU/L reported by Singh *et al.* (1992) was higher than the present findings. Increased serum ALP concentration could be attributed to the drastic fall in haemoglobin levels as a result of intravascular haemolysis which creates generalized hypoxia. The anoxic conditions developed in the liver, heart and kidneys cause damage to cell membranes, resulting in leakage of ALP (Cornelius, 1980). Histopathological findings observed in the present study and reported by Kurundkar *et al.* (1981) and Digraskar *et al.* (1991) in PHU affected buffaloes also support this view.

The colour of urine in PHU affected buffaloes ranged from red to coffee coloured, depending upon the severity and duration of illness. Urinalysis of PHU affected buffaloes in the present study revealed presence of haemoglobin and albumin, and absence of sugar and ketone bodies. On microscopic examination, no intact erythrocytes but few epithelial cells and crystals (phosphates) were observed. Similar observations were reported by Digraskar *et al.* (1991) and Jubb *et al.* (1993). Changes observed in urine could be attributed to damage to kidneys resulting from anaemic hypoxia caused by excessive haemolysis (Digraskar *et al.*, 1991). Kurundkar *et al.* (1981) reported presence of ketone bodies in PHU affected buffaloes which were contradictory with the results of the present study.

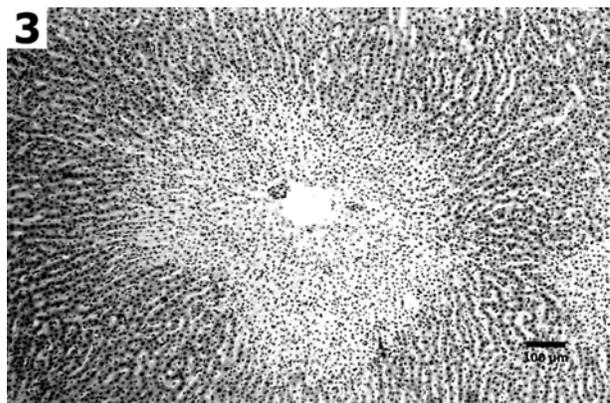


Fig. 3: Massive centrilobular necrosis in liver of parturient haemoglobinuria affected buffaloes (100 X; H & E).

Carcasses of buffaloes died of PHU were anaemic and jaundiced. Grossly, liver was pale, congested, friable and swollen along with distended gall bladder. Histopathologically, centrilobular necrosis, haemorrhages, congestion and haemosiderin deposition were observed. These changes in the liver could be attributed to excessive turnover of bile due to constant haemolysis (Digraskar *et al.*, 1991).

Lungs of PHU affected buffaloes were oedematous, haemorrhagic, congested, atelactic and emphysemated. These changes may be due to increased permeability and leakage of plasma resulting from prolonged anaemic hypoxia reported in this disease (Digraskar *et al.*, 1991). Prolonged hypoxia is thought to be the possible cause of pale, enlarged, necrosed, deposition of casts, atrophied glomeruli and haemosiderin deposition in the kidneys of PHU affected buffaloes (Kurundkar *et al.*, 1981; Digraskar *et al.*, 1991). Similarly, the degenerative changes observed in

heart muscles are also thought to be the effect of anaemic hypoxia. Haemosiderin deposition in the liver, spleen and kidneys could be attributed to haemolytic syndrome resulting from the release of haemoglobin (Sharma *et al.*, 1976; Digraskar *et al.*, 1991).

It was concluded from the study that colour of urine in PHU affected buffaloes ranges from red to coffee coloured with alkaline pH. Moreover, significantly high blood glucose, serum total bilirubin and alkaline phosphatase were observed in affected buffaloes. Haemoglobin and albumin were present in urine of PHU affected buffaloes. Grossly, carcasses of buffaloes died of PHU were anaemic and jaundiced along with enlarged and congested kidneys, liver and spleen. Necrosis of tubular epithelium, deposition of casts along with atrophy of glomeruli and centrilobular necrosis were the main findings in buffaloes died of parturient haemoglobinuria.

Table 1: Comparison of biochemical parameters (Mean \pm SD) in healthy and parturient haemoglobinuria affected buffaloes

Parameters	Healthy (n = 60)	Haemoglobinuric (n = 60)	P-Value
Glucose (mg/dL)	57.31 \pm 6.19a	76.05 \pm 7.77b	0.001
Total bilirubin (mg/dL)	0.57 \pm 0.13a	4.24 \pm 2.49b	0.001
Cholesterol (mg/dL)	202.73 \pm 27.85	207.64 \pm 29.08	0.347
Alkaline phosphatase (U/L)	80.35 \pm 22.89a	232.50 \pm 58.68b	0.001

Figures with different letters in a row differ significantly.

REFERENCES

- Akhtar, M. Z., A. Khan, M. Sarwar and A. Javaid, 2007a. Influence of soil and forage minerals on buffalo parturient haemoglobinuria. *Asian-Aust. J. Anim. Sci.*, 20: 393-398.
- Akhtar, M. Z., A. Khan, M. Z. Khan and G. Muhammad, 2007b. Haemato-biochemical aspects of parturient haemoglobinuria in buffalo. *Turkish J. Vet. Anim. Sci.*, 31(2): 119-123.
- Akhtar, M. Z., A. Khan, T. Zaman and N. Ahmad, 2006. Some clinico-epidemiological and biochemical observations of parturient haemoglobinuria in Nili-Ravi buffaloes (*Bubalus bubalis*). *Pakistan Vet. J.*, 26 (4): 151-156.
- Anonymous, 2003. Minitab Statistical Software, Minitab Release 13.20, Minitab Inc, USA.
- Benjamin, M. M., 1978. Outline of Veterinary Clinical Pathology. 3rd Ed., Iowa State Univ. Press, Ames, Iowa, USA.
- Chugh, S. K., M. M. Mata and K. S. Malik, 1996. Epidemiological observations on postparturient haemoglobinuria in buffaloes. *Indian J. Anim. Sci.*, 66: 1123-1125.
- Cornelius, C. E., 1980. Liver Functions. In: *Clinical Biochemistry of Domestic Animals*. (Kaneko, J. J., ed.). 3rd Ed., Academic Press, Inc. New York, USA.
- Digraskar, S., B. Singh and B. B. Deshpande, 1991. Epidemiology and clinico-pathology of haemoglobinuria in buffalo (*Bubalus bubalis*). *Livestock Advisor*, 16: 32-38.
- Digraskar, S., B. Singh and D. N. Rajguru, 1996. Blood chemistry and therapeutic management of metabolic haemoglobinuria in buffaloes. *J. Maharashtra Agri. Univ.*, 21: 100-102.
- Jubb, K. V. F., P. C. Kennedy and N. Palmer, 1993. *Pathology of Domestic Animals*. 4th Ed., Academic Press, Inc. New York, USA.
- Kurundkar, V. D., B. D. Deshpande, B. Singh and L. G. Anantwar, 1981. Biochemical and pathological changes in clinical cases of haemoglobinuria in buffaloes. *Indian J. Anim. Sci.*, 51: 35-38.
- Latimer, K. S., E. A. Mahaffey and K. W. Prasse, 2003. *Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology*. 4th Ed., Iowa State Univ. Press, Ames, Iowa, USA.
- Pandey, N. N. and S. K. Misra, 1987. Clinico-biochemical studies on nutritional haemoglobinuria in buffaloes and therapy. *Indian Vet. J.*, 64: 39-43.
- Pirzada, W. H. and S. Ali, 1990. Clinical case report of parturient haemoglobinuria in Nili-Ravi buffaloes (*Bubalus bubalis*). *Pakistan Vet. J.*, 10: 49-50.
- Raz, M. A., A. M. Rauf, M. A. Shah, I. Ahmad and M. A. Qureshi, 1988. Studies on the incidence and

- control of haemoglobinuria in buffaloes and cattle. Pakistan J. Vet. Res., 1: 22-31.
- Sharma, S. P., K. S. Malik and O. P. Gautam, 1976. Studies on biochemical and pathological aspects of haemoglobinuria in buffaloes. Indian J. Anim. Sci., 46: 336-339.
- Singh, N., R. Kumari and M. A. Akbar, 1989. Biochemical changes in blood metabolites in buffaloes with indigestion. Indian Vet. J., 66: 923-926.
- Singh, R., R. M. Bhardwaj and J. P. Rana, 1992. Serum enzymes profile in post-parturient haemoglobinuria in buffaloes. Indian Vet. J., 69: 591-593.
- Stogdale, L., 1981. Correlation of changes in blood chemistry with pathological changes in the animal's body: II. Electrolytes, kidney function tests, serum enzymes and liver function tests. J. South African Vet. Assoc., 52: 155-164.
- Wang, X. L., C. H. Gallagher, T. J. McClure, V. E. Reeve and P. J. Canfield, 1985. Bovine post-parturient haemoglobinuria: effect of inorganic phosphorus on red cell metabolism. Res. Vet. Sci., 39: 333-339.