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Assessment of hematobiochemical and stress responses in buffaloes (*Bubalus bubalis*) undergoing single and double stage diaphragmatic herniorrhaphy

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ABSTRACT

The present study was aimed to evaluate and compare the hematobiochemical alterations and stress responses in twelve female Murrah buffaloes diagnosed with diaphragmatic hernia and treated using two different surgical approaches. The animals were divided into Group I (n=6), subjected to single-stage diaphragmatic herniorrhaphy without laparo-rumenotomy, and Group II (n=6), subjected to double-stage repair involving laparo-rumenotomy followed by diaphragmatic herniorrhaphy. Haematological, biochemical, oxidative stress and inflammatory parameters were evaluated during the preoperative and postoperative periods. Haematological examination revealed changes in haemoglobin, packed cell volume, total leukocyte count, neutrophil count and lymphocyte count in both groups, with more pronounced leukocytosis, neutrophilia and lymphopenia in Group II. Among the biochemical parameters, blood urea nitrogen (BUN) decreased, whereas alanine aminotransferase (ALT), blood glucose, sodium, and chloride increased, while the remaining biochemical parameters did not show significant changes. Stress and inflammatory markers showed progressive changes, with increased serum cortisol, malondialdehyde and C-reactive protein, along with decreased total antioxidant capacity in both groups. Group II showed higher cortisol, malondialdehyde and C-reactive protein levels and lower total antioxidant capacity, suggesting greater inflammatory, oxidative and physiological stress. The study concluded that buffaloes undergoing single-stage diaphragmatic herniorrhaphy showed less pronounced hematobiochemical and stress alterations than those undergoing double-stage repair.

Keywords: Biochemical parameters, Buffaloes, Cortisol, C-reactive protein, Diaphragmatic herniorrhaphy, Haematology, Oxidative stress, Total antioxidant capacity

Diaphragmatic hernia is a major surgical affliction in bovines, with a comparatively higher occurrence reported in buffaloes than in cattle (Krishnamurthy *et al.* 1985). Clinically, diaphragmatic hernia, a form of internal herniation, is commonly manifested by persistent tympany unresponsive to medical treatment, reduced milk production, scanty faeces and progressive emaciation resulting from prolonged anorexia. In chronic cases, adhesions of the reticulum along with a fibrotic hernial ring are often observed (Sahu *et al.* 2003). In addition to recognized predisposing factors such as ingestion of foreign bodies, advanced pregnancy and recent parturition, certain anatomical peculiarities of buffaloes may further increase their susceptibility to this condition. Buffaloes affected with diaphragmatic hernia may also be susceptible to oxidative stress due to nutritional compromise and ischemia–reperfusion injury associated with recurrent tympany and increased intrathoracic pressure during surgical intervention (Trivedi *et al.* 2019).

Conventionally, treatment of diaphragmatic hernia in buffaloes involves a double-stage surgical approach, in which exploratory laparo-rumenotomy is performed first, followed by diaphragmatic herniorrhaphy through the post-xiphoid approach. Single-stage diaphragmatic herniorrhaphy has been attempted previously in buffaloes (Patel *et al.* 2011; Ghanshyam *et al.* 2020; Sutaria *et al.* 2021), which included rumenotomy followed by diaphragmatic herniorrhaphy through the same surgical incision. Since information on the haematobiochemical and stress responses associated with these two techniques is limited, the present study was undertaken to comparatively evaluate these parameters in buffaloes subjected to single- and double-stage diaphragmatic herniorrhaphy.

MATERIALS AND METHODS

The present clinical study was carried out on 12 buffaloes suffering from diaphragmatic hernia and presented to

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the Department of Veterinary Surgery and Radiology, College of Veterinary Sciences, LUVAS, Hisar. Following preliminary physical and clinical examination, all animals were subjected to radiographic evaluation in standing position and lateral recumbency. Based on radiographic findings, confirmed cases of diaphragmatic hernia were divided into two groups. Buffaloes without detectable potential foreign bodies were categorized as Group I (n=6) and subjected to single-stage diaphragmatic herniorrhaphy without laparo-rumenotomy. Buffaloes with potential foreign bodies in the reticulum were categorized as Group II (n=6) and subjected to double-stage diaphragmatic herniorrhaphy, comprising laparo-rumenotomy followed by diaphragmatic herniorrhaphy 24 hours later. Blood samples were collected at predetermined intervals in both groups during the preoperative and postoperative periods. Preoperative samples were collected prior to the first surgical intervention, that is, before diaphragmatic herniorrhaphy in Group I and before laparo-rumenotomy in Group II. Postoperative blood samples were collected at the completion of diaphragmatic herniorrhaphy (following repositioning of the animals from dorsal to lateral recumbency) and subsequently at 6 and 24 hours after diaphragmatic herniorrhaphy.

For haematological analysis, 3 mL of blood was collected aseptically from the jugular vein of each animal in sterile EDTA vials. Haematological parameters, namely haemoglobin (g/dL), total erythrocyte count (10^6/mm^3), total leukocyte count (10^3/mm^3), packed cell volume (%), platelet count (10^3/mm^3) and differential leukocyte count, including neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%) and basophils (%), were analysed using an MS4-S haematology analyser (JLS380 Melet Schloesing Laboratoires, 9 Chaussee Jules Cesar, 95520 Osny, France). For biochemical and stress parameter analysis, 5 mL of blood was collected from the jugular vein in plain vials without anticoagulant. Serum was separated and stored in aliquots at −20°C until analysis. Biochemical parameters including total protein (g/dL), albumin (g/dL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (mg/dL), alkaline phosphatase (AP), blood glucose (mg/dL), calcium (mg%), magnesium (mg%), phosphorus (mg%), sodium (mEq/L), potassium (mEq/L) and chloride (mEq/L) were estimated using Erba diagnostic kits with a TRANSASIA–ERBA EM 200 automatic serum analyser. Oxidative stress parameters evaluated in the present study included malondialdehyde (MDA) (µg/mL) estimated by the method of Ohkawa *et al.* (1979), and total antioxidant capacity (TAC) (µg/mL) estimated by the phosphomolybdenum method suggested by Prieto *et al.* (1999). Serum cortisol (ng/mL) and faecal cortisol (ng/g) concentrations were measured using a commercially available ELISA kit (Calbiotech Inc., USA). The inflammatory response was assessed by estimating C-reactive protein (ng/mL) using the Quanticon™ Bovine CRP ELISA Kit (Amplicon Biotech).

The statistical analysis was done by one-way-analysis of variance and Duncan's multiple range test.

RESULTS AND DISCUSSION

Haematological parameters: Significant perioperative alterations were observed in selected haematological parameters of buffaloes subjected to single-stage diaphragmatic herniorrhaphy without laparo-rumenotomy (Group I) and double-stage diaphragmatic herniorrhaphy (Group II) (Table 1). Haemoglobin declined significantly during the early postoperative period in both groups and approached preoperative values by 24 hr, with a more pronounced reduction in Group II. Total erythrocyte count and platelet count remained stable throughout the observation period. Packed cell volume decreased significantly at 6 hr in Group I before returning towards baseline at 24 hr, whereas no significant variation was observed in Group II. The transient reduction in haemoglobin and packed cell volume was likely due to perioperative haemodilution, fluid shifts, surgical stress and blood loss, while the greater haemoglobin decline in Group II plausibly reflected the additional tissue trauma associated with prior rumenotomy. Similar postoperative reductions in haemoglobin and packed cell volume have been reported by Khan (2009), Attia (2016), Wagner *et al.* (1991), Rosin (1981) and Millis *et al.* (1992).

Total leukocyte count increased significantly at 6 and 24 hr postoperatively in both groups, with consistently higher values in Group II, indicating a greater inflammatory response likely associated with pre-existing reticular foreign body involvement and additional surgical trauma. Similar findings have been reported by Khan (2009), Abdelaal *et al.* (2014) and Attia (2016). Neutrophil counts increased significantly in both groups, with an earlier and more pronounced neutrophilia in Group II, consistent with a stronger inflammatory response following surgery (Krishnamurthy *et al.* 1983; Turkar and Uppal, 2006; Khan, 2009; Ghanshyam *et al.* 2020; Hussain *et al.* 2021; Suryawanshi *et al.* 2023). Lymphocyte counts decreased significantly in both groups, particularly in Group II, indicating a corticosteroid-mediated stress leukogram to be associated with surgical stress, pain and inflammation. The lower preoperative lymphocyte values in Group II further suggested greater pre-existing stress (Krishnamurthy *et al.* 1983; Ghanshyam *et al.* 2020; Hussain *et al.* 2021; Suryawanshi *et al.* 2023; Sangwan *et al.* 2024). Monocyte count remained unchanged, while eosinophils and basophils were negligible throughout the study.

Biochemical parameters: The perioperative biochemical parameters are presented in Table 2. Total protein, albumin, AST, AP, creatinine, calcium, phosphorus and potassium did not vary significantly within or between groups, indicating that neither surgical technique caused marked disturbances in protein, hepatic, renal or mineral profiles during the observation period. ALT increased significantly at 6 and 24 hr in both groups without significant intergroup differences, suggesting transient hepatocellular or muscular

disturbance related to surgical and anaesthetic stress rather than hepatic dysfunction.

BUN decreased significantly at 6 hr in both groups before partial recovery at 24 hr, whereas creatinine remained unchanged, indicating preserved renal function. The transient reduction in BUN was probably to be associated with perioperative fluid therapy, haemodilution and altered protein metabolism. Blood glucose increased progressively and significantly in both groups, with consistently higher values in Group II. This hyperglycaemic response was likely mediated by activation of the hypothalamic-pituitary-adrenal axis, increased cortisol and catecholamine release, surgical stress, pain, anaesthesia and the effects of xylazine on insulin secretion and hepatic glucose output (Desborough, 2000; Ninu, 2009; Yadav *et al.* 2024). The higher glucose concentrations in Group II suggested greater metabolic stress associated with rumenotomy, prolonged disease and increased surgical trauma.

Among electrolytes, sodium increased mildly at 6 hr only in Group I, while chloride increased significantly at 6 and 24 hr in both groups, probably due to perioperative fluid therapy and redistribution of body fluids. Potassium remained stable throughout the study.

Oxidative stress and acute phase protein parameters: The oxidative stress and acute phase protein parameters are presented in Table 3. Total antioxidant capacity declined progressively in both groups, with consistently lower values in Group II, indicating depletion of antioxidant reserves due to increased oxidative stress. The greater decline in Group II probably reflected the combined effects of pre-existing inflammation and additional surgical trauma (Bisla *et al.* 2004; Khan *et al.* 2011; Lee and Kim, 2014; Tomsič and Nemec, 2022).

Malondialdehyde increased progressively in both groups and remained significantly higher in Group II, indicating enhanced lipid peroxidation and oxidative injury following surgery. The greater increase in Group II suggested increased oxidative stress associated with cumulative surgical trauma and reticular foreign body-related inflammation (Bisla *et al.* 2002; Khan *et al.* 2011; Patel, 2012; Trivedi *et al.* 2019).

Serum cortisol increased significantly throughout the perioperative period in both groups, with consistently higher concentrations in Group II, reflecting activation of the hypothalamic-pituitary-adrenal axis in response to surgical stress, pain and tissue injury. Higher preoperative cortisol values in Group II further indicated greater baseline

Table 1. Haematological parameters of both Group I and Group II (Mean±S.E.)

Parameter	Groups	Preoperative	Postoperative		
			At completion of diaphragmatic herniorrhaphy	At 6 hours of diaphragmatic herniorrhaphy	At 24 hours of diaphragmatic herniorrhaphy
Haemoglobin (Hb;-g/dL)	I	10.42^{bA}±0.22	9.30^{aA}±0.20	8.97^{aA}±0.17	10.10^{bA}±0.19
	II	10.03^{cB}±0.35	8.63^{abB}±0.19	8.30^{aB}±0.16	9.23^{bB}±0.22
Total erythrocyte count (TEC;-10^6/mm^3)	I	6.1± 0.07	5.95±0.09	5.91±0.08	5.91±0.06
	II	5.94±0.09	5.87±0.05	5.85±0.08	5.91±0.06
Total leukocyte count (TLC;-10^3/ mm^3)	I	7.89^{aA}±0.44	7.92^{aA}±0.31	10.71^{bA} ±0.35	10.82^{bA}±0.19
	II	10.58^{aB}±0.82	11.66^{aB}± 0.23	14.35^{bB}± 0.11	14.42^{bB}±0.30
Packed cell volume (PCV; - %)	I	32.73^{b}±1.27	30.80^{ab}±0.94	29.23^{a}±0.69	32.08^{b}±0.40
	II	32.83^{a}±1.22	30.17^{a}±0.95	30.33^{a}±0.76	^{a}±0.61
Platelets (10^3/mm^3)	I	279.83 ±16.01	243.33 ±15.91	265.00 ±20.47	288.17 ±7.56
	II	291.50 ±11.24	296.83 ±23.95	277.33 ±8.03	272.83 ±8.45
Neutrophils (%)	I	45.17^{aA}± 1.12	46.23^{aA}±1.09	58.33^{bA}±1.47	60.17^{bA}±0.60
	II	55.33^{aB}±1.73	70.12^{bB}±0.86	83.67^{cB}±1.28	84.67^{cB}±1.78
Lymphocytes (%)	I	53.01^{bA}±0.45	52.03^{bA}±1.15	40.16^{aA}±1.23	38.43^{aA}±2.11
	II	43.02^{cB}±1.19	28.55^{bB}±1.78	14.71^{aB}±0.45	14.00^{aB}±1.88
Monocytes (%)	I	1.60±0.22	1.57 ±0.22	1.51±0.22	1.40 ±0.22
	II	1.67 ±0.26	1.33 ±0.21	1.62 ±0.22	1.33 ±0.21
Eosinophils (%)	I	0.17 ±0.17	0.17 ±0.17	0	0
	II	0	0	0	0
Basophils (%)	I	0	0	0	0
	II	0	0	0	0

Means with different superscripts (A/B) in a column show significant difference between groups (P<0.05). Means with different superscripts (a/b/c) in a row show significant difference within group (P<0.05).

Table 2. Biochemical parameters of both Group I and Group II (Mean±S.E.)

Parameter	Groups	Preoperative	Post operative		
			At completion of diaphragmatic herniorrhaphy	At 6 hours of diaphragmatic herniorrhaphy	At 24 hours of diaphragmatic herniorrhaphy
Total protein (g/dL)	I	7.73 ±0.10	7.66 ±0.09	7.58 ±0.06	7.64 ±0.04
	II	7.65 ±0.12	7.57 ±0.11	7.42 ±0.14	7.53 ±0.12
Albumin (g/dL)	I	2.49±0.08	2.42±0.07	2.30±0.09	2.36±0.07
	II	2.42±0.07	2.37±0.07	2.34±0.08	2.35±0.07
Alanine aminotransferase (ALT;- IU/L)	I	77.00 ^{a}±2.06	81.02 ^{ab}±2.12	83.65 ^{b}±1.90	86.62 ^{b}±2.18
	II	76.45 ^{a}±1.89	81.65 ^{ab}±2.21	84.57 ^{bc}±1.85	87.93 ^{c}±2.01
Aspartate aminotransferase (AST; - IU/L)	I	68.15 ±4.19	71.67 ±4.19	74.92 ±3.96	79.53 ±3.28
	II	64.08 ±4.30	68.20 ±4.07	71.05 ±4.60	74.25 ±4.61
Alkaline phosphatase (ALP;- IU/L)	I	124.67±1.15	124.67 ±0.61	126.00 ±0.58	125.80 ±0.93
	II	122.33±1.23	123.67 ±1.17	125.00 ±1.03	124.33 ±1.12
Blood urea nitrogen (BUN;- mg/dL)	I	37.63^{b}±0.88	37.38^{b}±1.16	31.95^{a}±1.05	34.83^{ab}±1.02
	II	39.40^{b}±1.72	39.12^{b}±1.87	31.43^{a}±1.35	35.88^{ab}±1.51
Serum Creatinine (mg/dL)	I	0.78±0.02	0.80±0.02	0.80 ±0.02	0.79 ±0.02
	II	0.79 ±0.01	0.79 ±0.02	0.79 ±0.02	0.79 ±0.02
Blood Glucose (mg/dL)	I	69.55 ^{a}±2.25	80.48^{bA}±2.20	101.27^{cA}±3.39	122.45^{dA}± 2.59
	II	73.28 ^{a}±3.38	95.22^{bB}±4.19	116.85^{cB}±5.45	139.7^{dB}±1.16
Calcium (mg%)	I	10.02±0.10	10.10±0.10	10.16±0.09	10.12±0.09
	II	10.20±0.14	10.31±0.14	10.34±0.14	10.34±0.13
Phosphorus (mg%)	I	5.09±0.06	5.12±0.06	5.17±0.07	5.15±0.09
	II	5.04±0.14	5.09±0.13	5.12±0.14	5.14±0.14
Sodium (Na+) (mEq/L)	I	141.95 ^{a}±0.77	143.43 ^{ab}±0.88	144.67 ^{b}±0.89	143.97 ^{ab}±0.85
	II	140.95±0.80	144.00±1.54	145.22±1.46	144.25±1.47
Potassium (k+) (mEq/L)	I	4.29±0.03	4.3±0.03	4.3±0.03	4.30±0.03
	II	4.31±0.02	4.33±0.02	4.35±0.02	4.34±0.01
Chloride (Cl^{-}) (mEq/L)	I	101.35^{a}±0.76	102.85^{a}±0.76	107.00^{b}±0.91	111.77^{c}±0.59
	II	101.18^{a}±0.66	102.73^{a}±0.56	106.95^{b}±0.66	110.58^{c}±0.34

Means with different superscripts (A/B) in a column show significant difference between groups (P<0.05).Means with different superscripts (a/b/c/d) in a row show significant difference within group (P<0.05).

Table 3. Oxidative stress and acute phase protein parameters of both Group I and Group II (Mean±S.E.)

Parameter	Groups	Preoperative	Postoperative		
			At completion of diaphragmatic herniorrhaphy	At 6 hours of diaphragmatic herniorrhaphy	At 24 hours of diaphragmatic herniorrhaphy
Total Antioxidant Capacity (TAC;-µg/mL)	I	184.86^{dA}±4.75	167.25^{cA}±4.65	142.43^{bA}±5.01	111.55^{aA}±5.01
	II	166.66^{dB}±4.61	145.49^{cB}±6.36	115.93^{bB}±5.10	80.1^{aB}±5.60
Malondialdehyde (MDA; - µg/mL)	I	7.08^{aA}±0.04	8.64^{bA} ± 0.15	10.36^{cA}± 1.83	11.37^{dA}± 0.09
	II	7.85^{aB}± 0.09	9.62^{bB} ± 0.37	11.22^{cB}± 1.63	12.13^{dB} ± 1.48
Serum cortisol (ng/mL)	I	10.87^{aA} ± 1.53	13.72^{aA} ± 0.9	25.47^{bA}± 1.83	38.99 ^{cA}±0.88
	II	16.38^{aB}± 0.62	28.45^{bB} ± 1.25	41.12^{cB}± 0.75	55.77^{dB} ± 2.36
Faecal cortisol (ng/g)	I	149.95^{aA}±13.83	166.36^{aA}±13.16	186.1^{aA}±14.82	372.34^{bA}± 11.61
	II	245.29^{aB}±12.18	352.67^{bB}±18.88	371.58^{bB}±16.79	542.65^{cB}± 18.35
C- reactive protein (CRP;- ng/mL)	I	46.84^{aA}± 0.8	48.16^{aA}± 0.73	62.01^{bA}± 0.62	74.33^{cA}± 1.29
	II	53.12^{aB}±0.43	62.73^{bB}± 1.38	74.35^{cB}± 1.34	88.31^{dB}± 1.59

Means with different superscripts (A/B) in a column show significant difference between groups (P<0.05).Means with different superscripts (a/b/c/d) in a row show significant difference within group (P<0.05).

stress associated with chronic inflammation (Behl *et al.* 1995; Megahed *et al.* 2008; Patel, 2012). Faecal cortisol also increased in both groups, with the greatest rise at 24 hr, which was consistent with the delayed metabolism and excretion of cortisol into faeces (Möstl and Palme, 2002; Palme *et al.* 2005).

C-reactive protein increased progressively in both groups, and became significant at 6 and 24 hr in Group I and at each postoperative interval in Group II. The consistently higher values in Group II indicated a more pronounced acute phase inflammatory response and supported the presence of pre-existing inflammation associated with reticular foreign bodies (Eckersall, 2000; Petersen *et al.* 2004; El-Ashker *et al.* 2014; Mahour *et al.* 2025; Honale *et al.* 2026).

Overall, buffaloes undergoing single-stage diaphragmatic herniorrhaphy exhibited comparatively milder perioperative haematological, biochemical, oxidative stress and inflammatory alterations than those undergoing the double-stage procedure. The greater leukocytic, metabolic, oxidative and acute phase responses observed in Group II indicated increased systemic stress and inflammatory burden, likely resulting from pre-existing reticular pathology and the additional surgical trauma associated with laparo-rumenotomy.

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