



Estimation of Blood Biochemical Constituents and Cortisol as Stress Predictors and Prognostic Biomarkers in Buffaloes Affected with Uterine Torsion

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ABSTRACT

Full term pregnant buffaloes (n=24) affected with uterine torsion were equally divided into four experimental groups based on duration of uterine duration *viz* 6-12 hours (Group I); 12-24 hours (Group II); 24-48 hours (Group III) and more than 48 hours (Group IV). The control (Group V) consisted of six normal full term pregnant buffaloes. The experimental buffaloes were treated by modified Schaffer's method. Those buffaloes, in which detorsion failed following modified Schaffer's method, were treated by caesarean section. As the duration of uterine torsion increased, there was a corresponding increase in blood glucose levels from Group I to Group IV. There was a definite reduction in serum total protein and albumin concentrations in buffaloes affected with uterine torsion. As the duration of uterine torsion increased, a concomitant increase in blood urea nitrogen (BUN) and greater increase in serum creatinine levels were noticed. The mean creatine phosphokinase (CPK) and aspartate aminotransferase (AST) levels ranged from 152.71±39.09 to 445.30±44.57 and from 80.84±2.19 to 110.83±2.67 U/L, respectively. The successful detorsion in treatment groups caused drastic reduction in serum CPK and AST values at 24 hours after detorsion and 30 days after fetal delivery. The mean serum cortisol level ranged from 44.02±3.30 to 79.39±1.78 ng/ml in buffaloes affected with uterine torsion and it was 12.17±0.54 ng/ml at 24 hours before calving in normally calved buffaloes. Prior to detorsion in all the treatment groups, tremendous increase in mean serum cortisol levels was observed. It can be concluded that serum biochemical parameters and cortisol concentration may help to predict the prognosis of uterine torsion in buffaloes.

Key words: Uterine torsion, biochemical constituents, cortisol, buffaloes.

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INTRODUCTION

Torsion of uterus in buffaloes usually occurs in the pregnant uterine horn and is defined as the twisting of the uterus on its longitudinal axis. It is one of the complicated causes of maternal dystocia in buffaloes culminating in death of both the fetus and the dam if not treated early. Because of the rapidity of fetal death that ensues following torsion and the uterine adhesions with visceral organs that develop, uterine torsion must be considered as an emergency (Selvaraju *et al.*, 2020). High incidence of uterine torsion recorded during advanced pregnancy, immediately before parturition (Karthick *et al.*, 2015) and mostly during the late first stage or early second stage of labour (Arthur *et al.*, 1996), although uterine torsion occasionally diagnosed at 57 days of pregnancy (Prakash *et al.*, 2018). The incidence of uterine torsion represented about 29.5 to 30.6 per cent of the buffaloes with dystocia (Amer *et al.* 2008). Recent researches indicated that there are changes in the blood biochemical constituents and hormonal changes which can be correlated with various factors like degree, site, severity of uterine torsion, fetal viability, time needed for cervical dilatation as well as occurrence of uterine rupture to predict the prognosis of the uterine torsion (Amer *et al.*, 2008). However, detailed studies on blood biochemical and cortisol concentrations consequent to the stress caused by uterine torsion in buffaloes and their relationship with the prognosis of uterine torsion of different degrees have not been reported in detail. Hence, an investigation in buffaloes was undertaken with the objective to correlate the blood biochemical parameters and serum cortisol levels as stress biomarkers to predict the prognosis of the uterine torsion in buffaloes.

MATERIALS AND METHODS

Twenty-four full term pregnant buffaloes were brought with the history of anorexia, colic, restlessness, frequent lying down and getting up were diagnosed for uterine torsion by vaginal and rectal examinations and selected for the study. Further, six normal healthy full-term pregnant buffaloes served as control. All the selected buffaloes affected with uterine torsion were randomly and equally divided into four experimental groups based on the duration of the uterine torsion viz., buffaloes affected with uterine torsion for the duration of 6-12 hours (Group I); 12-24 hours (Group II); 24-48 hours (Group III) and more than 48 hours (Group IV). The control (Group V) consisted of 6 normal full term pregnant buffaloes. All the buffaloes of groups I, II and III responded very well to modified Schaffer's method. Only 3 buffaloes in group IV yielded detorsion by modified Schaffer's method. Three buffaloes

from group IV subjected for caesarean section as per the standard procedure described by Arthur *et al.* (1996) following inverted 'L' block.

Blood samples were collected in the vacutainer containing heparin for blood glucose estimation and clotting activator for biochemical and hormonal analysis. The blood samples were collected (i) before detorsion (ii) 24 hours after detorsion and (iii) 30 days after fetal delivery in experimental groups. In control buffaloes, the blood was collected at 24 hours before and 24 hours after calving and 30 days after postpartum. The blood glucose level was estimated immediately after blood collection in experimental and control buffaloes. Then, the serum samples were separated and stored at -20°C until analysis of blood biochemical parameters. Serum samples were analysed in duplicate for cortisol by Radioimmunoassay (RIA) technique.

The completely randomized design (CRD) method was followed for the experiment and the data collected was analysed using SPSS® 20.0 software package. Post hoc analysis was done by Tukey's Honestly Significance Difference.

RESULTS AND DISCUSSION

Factors such as duration of the condition and severity of the uterine torsion have been suggested as determinants of the outcome in buffaloes (Amer *et al.*, 2008). When physical examination failed to yield a diagnosis or prognosis in difficult cases of uterine torsion (Ali *et al.*, 2011), blood biochemical profiles might aid in establishing a prognosis and developing a therapeutic plan (Amin *et al.*, 2011).

Blood glucose

In this study, the blood glucose level ranged from 143.33±10.32 to 172.50±6.69 mg/dl in buffaloes affected with uterine torsion (Table 1).

Table 1: Mean±SEM blood glucose levels (mg/dl) before and after detorsion in buffaloes.

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	143.33±10.52 ^{br}	88.5±4.48 ^{bq}	66.00±1.92 ^p
Group II	148.00±10.56 ^{br}	89.83±7.58 ^{bq}	62.00±3.78 ^p
Group III	168.5±23.66 ^{bq}	91.67±8.30 ^{bp}	57.67±3.23 ^p
Group IV	172.50±6.69 ^{br}	95.00±8.95 ^{bq}	62.67±3.88 ^p
Control	24 hours before calving	24 hours after calving	59.17±2.48 ^p
	100.83±1.41 ^{aq}	60.83±3.52 ^{ap}	

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control – Normal calving.

This was far beyond the physiological limit (35.45 to 92.47 mg/dl) in pregnant buffaloes (Sabasthin *et al.*, 2012). The increased blood glucose concentration in uterine torsion affected buffaloes might be due to the fact that buffaloes became anoxic which would have increased liver glycogenolysis or might be due to the stress condition and increase secretion of ACTH (Kaneko *et al.*, 1997). Moreover, the drastic increase in glucose was related to the increased level of cortisol found in this study which might have increased the gluconeogenesis as described by Payne (1987).

In control group, 24 hours before calving the blood glucose level was 100.83 ± 1.41 mg/dl which was in concurrence with the findings of Amer *et al.* (2008). This was higher than the normal (63.48 ± 1.60 mg/dl) blood glucose in buffaloes as described by Sabasthin *et al.* (2012). The secretion of fetal cortisol from fetal adrenal might have raised markedly and competed with action of P_4 in regulation of corticotrophin releasing hormone gene (Karalis *et al.*, 1996). At the time of approaching parturition, glucose utilization in the peripheral tissue decreased while the hepatic blood glucose level increased due to gluconeogenesis (Yang, 1997). As the duration of torsion increased, the mean blood glucose concentration also increased in this study. Due to inflammation and tissue damage caused by uterine torsion, there was inactivation of intracellular enzyme 11- β hydroxysteroid dehydrogenase localized in the syncytiotrophoblast. It might have prevented the rapid decline of cortisol in animals under stress due to uterine torsion and might have induced aerobic energy production associated with approaching parturition/ torsion resulting in increased blood glucose profiles. Hence, hyperglycemia could be an indicator of duration and a predictor of prognosis of uterine torsion in buffaloes.

Total protein and albumin

In this investigation, the serum total protein and albumin levels (Table 2 and 3) in uterine torsion affected buffaloes ranged from 5.44 ± 0.60 to 6.29 ± 0.21 and 2.71 ± 0.19 to 3.19 ± 0.13 g/dl, respectively. The physiological range of serum total protein and albumin was 5.63 to 8.10 g/dl and 2.48 to 4.70 g/dl, respectively in buffaloes (AbdEllah *et al.*, 2014). The decrease in serum total protein and albumin found in the study was in accordance with the report of Amer and Hashem (2008).

Table 2: Mean \pm SEM serum total protein levels (g/dl) before and after detorsion in buffaloes.

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	6.29 ± 0.21^a	6.76 ± 0.30	6.53 ± 0.86
Group II	6.28 ± 0.45^a	6.50 ± 0.27	6.86 ± 0.53
Group III	5.95 ± 0.23^a	6.02 ± 0.28	6.59 ± 0.51
Group IV	5.44 ± 0.60^a	5.92 ± 0.51	6.05 ± 0.49
Control	24 hours before calving	24 hours after calving	6.91 ± 0.23
	6.83 ± 0.31^b	6.97 ± 0.31	

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control – Normal calving.

Table 3: Mean \pm SEM serum albumin levels (g/dl) before and after detorsion in buffaloes

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	3.19 ± 0.13^a	3.30 ± 0.18	3.00 ± 0.07
Group II	2.98 ± 0.30^a	3.21 ± 0.06	3.52 ± 0.24
Group III	2.81 ± 0.20^a	2.93 ± 0.21	3.06 ± 0.28
Group IV	2.71 ± 0.19^a	2.86 ± 0.20	2.99 ± 0.21
Control	24 hours before calving	24 hours after calving	3.09 ± 0.17
	3.60 ± 0.14^b	3.21 ± 0.30	

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control – Normal calving.

The decrease in serum total protein and albumin could be attributed to the stress of uterine torsion leading to disturbed liver function, increased catabolism of proteins due to anorexia and increased inflammation causing movement of fluid along with protein into tissues. Injuries, edema and peritonitis in uterine torsion cases might also be a cause of decreased plasma protein (Dhindsa *et al.*, 2005). Therefore, the result of this study indicated that serum hypoproteinemia may occur in dystocia due to uterine torsion in buffaloes.

Blood urea nitrogen and creatinine

In the current study, the mean BUN levels (mg/dl) and serum creatinine levels (mg/dl) ranged from 28.35 ± 1.37 to 33.27 ± 1.87 and from 1.57 ± 0.08 to 2.24 ± 0.18 in buffaloes affected with uterine torsion, respectively (Table 4 and 5).

Table 4: Mean±SEM blood urea nitrogen (BUN) levels (mg/dl) before and after detorsion in buffaloes

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	28.35±1.37 ^{br}	19.67±0.50 ^{bq}	16.10±0.60 ^p
Group II	30.15±0.65 ^{br}	20.53±1.10 ^{bq}	16.57±0.73 ^p
Group III	32.47±0.78 ^{br}	21.75±0.73 ^{bq}	15.98±0.73 ^p
Group IV	33.27±1.87 ^{bq}	22.18±1.73 ^{bp}	18.05±0.50 ^p
Control	24 hours before calving 17.83±0.71 ^a	24 hours after calving 16.23±0.79 ^a	16.35±1.01

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control - Normal calving.

Table 5: Mean±SEM serum creatinine levels (mg/dl) before and after detorsion in buffaloes

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	1.57±0.08 ^{br}	1.23±0.06 ^{abq}	0.99±0.05 ^p
Group II	1.70±0.05 ^{bcr}	1.38±0.08 ^{bcq}	0.83±0.06 ^p
Group III	1.96±0.07 ^{cdr}	1.52±0.14 ^{bcq}	0.82±0.05 ^p
Group IV	2.24±0.18 ^{dr}	1.66±0.15 ^{cq}	0.98±0.11 ^p
Control	24 hours before calving 0.96±0.08 ^a	24 hours after calving 0.93±0.07 ^a	0.84±0.06

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control - Normal calving.

The physiological levels of BUN and serum creatinine was 26.00±0.67 mg/dl and 1.33±0.19 mg/dl, respectively (Sabasthin *et al.*, 2012). The high concentrations of serum creatinine and BUN in this study were in concurrence with the report of Amer and Hashem (2008). As the duration of uterine torsion increased, a concomitant increase in BUN and very high increase in serum creatinine levels were noticed in this study. In uterine torsion, ureters lying in the broad ligaments were constricted and thus the urine output is reduced and renal function might be altered (Schonfelder *et al.*, 2007). Moreover, the presence of stress induces a decrease in blood flow to kidneys, shock, dehydration and nephropathy resulting from toxic substances liberated by dead fetus might cause acute or chronic renal insufficiency, leading to decrease in the urea and creatinine elimination (Amer and Hashem, 2008 and Noakes *et al.*, 2009).

In control group, there was no much variation in the BUN and serum creatinine levels during different days of blood collection and the levels were within the physiological range. Hence, BUN and typically serum creatinine may be the indicators of renal damage in the uterine torsion which may indicate the duration of existence of condition in buffaloes.

Serum creatine phosphokinase (CPK) and aspartate aminotransferase (AST)

The mean serum CPK and AST levels ranged from 152.71±39.09 to 445.30±44.57 and from 80.84±2.19 to 110.83±2.67 U/L, respectively (Table 6 and 7).

Table 6: Mean±SEM serum creatine phosphokinase (cpk) levels (u/l) before and after detorsion in buffaloes

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	152.71±39.09 ^{bq}	106.20±16.74 ^{bp}	42.04±4.44 ^p
Group II	235.32±32.68 ^{cq}	131.81±23.03 ^{bp}	38.14±2.58 ^p
Group III	314.93±57.65 ^{cr}	207.49±27.83 ^{cq}	46.22±3.43 ^p
Group IV	445.30±44.57 ^{dr}	271.00±20.43 ^{dq}	40.20±1.68 ^p
Control	24 hours before calving 40.71±2.05 ^{qa}	24 hours after calving 30.56±1.37 ^{ap}	40.60±2.75 ^q

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control - Normal calving.

Table 7: Mean±SEM serum aspartate aminotransferase (AST) levels (u/l) before and after detorsion in buffaloes

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	80.84±2.19 ^{br}	71.72±0.84 ^{bq}	40.10±1.05 ^p
Group II	90.25±1.54 ^{cr}	79.72±0.79 ^{cq}	39.37±1.22 ^p
Group III	99.72±1.25 ^{dr}	87.24±1.56 ^{dq}	39.75±0.42 ^p
Group IV	110.83±2.67 ^{er}	90.37±1.42 ^{dq}	39.78±1.31 ^p
Control	24 hours before calving 58.76±1.39 ^{aq}	24 hours after calving 54.72±2.26 ^{aq}	38.30±1.03 ^p

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control - Normal calving.

The normal values reported for CPK and AST were 20.23±11.0 U/L (Ali *et al.*, 2011) and 22.29 to 68.71 U/L

(Farrag *et al.*, 1984), respectively in naturally calved buffaloes. The higher values of CPK and AST found in this study were in accordance with Amin *et al.* (2011). The increase in serum CPK and AST of this investigation might be attributed to the damage in uterine muscle cells affected with twisting of uterus as reported by Amin *et al.* (2011). In the present study, there was a drastic reduction in both CPK and AST after detorsion in all the experimental groups. It might be due to the healing of uterine muscle after detorsion as reported by Amin *et al.* (2011). As the duration of uterine torsion increased, both of these enzymes found to increase in this study. El-Din Zain *et al.* (1997) reported that the buffalo with a severe degree of uterine torsion or carrying a dead fetus had greater concentration of AST compared to those having a lesser degree of torsion or carrying a live fetus. Therefore both serum CPK and AST could be the good predictors of prognosis of uterine torsion in buffaloes.

Cortisol

In the study, the serum cortisol level ranged from 44.02±3.30 to 79.39±1.78 ng/ml in buffaloes affected with uterine torsion and it was 12.17±0.54 ng/ml at 24 hours before calving in normally calved buffaloes (Table 8).

Table 8: Mean±SEM serum cortisol levels (ng/ml) before and after detorsion in buffaloes

	Before detorsion	24 hours after detorsion	30 days after fetal delivery
Group I	44.02±3.30 ^{br}	24.87±1.94 ^{bq}	4.30±0.32 ^p
Group II	57.79±2.00 ^{cf}	28.87±1.13 ^{bq}	4.81±0.19 ^p
Group III	69.11±2.47 ^{dr}	33.48±0.76 ^{cq}	4.66±0.32 ^p
Group IV	79.39±1.78 ^{er}	41.78±2.15 ^{dq}	4.37±0.23 ^p
Control	24 hours before calving	24 hours after calving	4.46±0.20 ^p
	12.17±0.54 ^{af}	6.73±0.23 ^{aq}	

Values bearing different superscript (a, b, c, d, e) varied significantly among different groups on same day (with in same column)

Values bearing different superscript (p, q, r) varied significantly within each group on different days (with in same row)

Group I- torsion duration for 6-12 hours, Group II- 12-24 hours, Group III- 24-48 hours, Group IV- more than 48 hours, Control - Normal calving.

The level of cortisol reported in advanced pregnancy in cows was 6.00±0.54 ng/ml (Nessim, 2010). Prabhakar *et al.* (2002) reported the plasma cortisol concentration on the day of calving as 12.13±1.66 ng/ml. The increased levels of serum cortisol reported in uterine torsion affected buffaloes in this study was in accordance with the findings of Noakes *et al.* (2001) and Amer and Hashem (2008). Since the occurrence of uterine torsion was a stressful event, there were very high levels of serum cortisol in this study. Uterine torsion create inflammatory process in the uterus which might

involve the rise of cytokines and prostaglandins which inturn stimulates the stress axis which resulted in sharp rise in cortisol level in this study as opined by Rivest (2001). In the current investigation, as the duration of torsion increased there was a significant corresponding increase in serum cortisol levels. Rise in cortisol level was reported to be in proportion with the severity of tissue trauma (Schmidt and Brooker, 1982). Following detorsion, there was a drastic reduction in serum cortisol levels in all the groups in this experiment. It indicated the recovery from stress and also regenerative changes in the uterine musculatures (Amer *et al.*, 2008). Therefore, serum cortisol could be a confirmative indicator of uterine torsion and its prognosis in buffaloes.

CONCLUSIONS

From this study, it was observed that (i) the serum glucose, total protein, albumin, blood urea nitrogen and serum creatinine levels were influenced by uterine torsion in buffaloes (ii) there was drastic change in the serum CPK and AST levels in the buffaloes affected uterine torsion and (iii) very high elevation of serum cortisol levels was noticed in buffaloes with uterine torsion. Hence, it was concluded that serum biochemical parameters *viz.*, blood glucose, creatinine, CPK, AST and serum cortisol concentrations may help to predict the prognosis of uterine torsion in buffaloes under field conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest among themselves.

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