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Research Article

HAEMATO-BIOCHEMICAL AND INFLAMMATORY MARKERS OF FETOTOMY OPERATED BUFFALOES

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ABSTRACT: Hemato-biochemical and inflammatory markers (TNF, Interleukin and Fibrinogen) were compared between fetotomy operated and normally calved buffaloes. 50 buffaloes were used in the present study and were randomly divided into two groups. Group I comprised of 30 healthy buffaloes subjected to fetotomy operation, while as Group II comprised of 20 normally calved healthy buffaloes. The average values of serum Ca, P and protein were significantly ($p<0.05$) lower whereas serum glucose was significantly ($p<0.05$) higher in buffaloes subjected to fetotomy operation than the normally calved buffaloes. The average count (%) of neutrophils and eosinophils were significantly higher ($p<0.05$), in buffaloes subjected to fetotomy operation than the normally calved buffaloes. The monocyte counts did not showed any characteristic trend. The average values of fibrinogen was non-significantly ($p<0.05$) higher, whereas the values of TNF and IL-10 were significantly ($p<0.05$) higher in buffaloes subjected to fetotomy than the normally calved buffaloes. Dystocia is a stressful event which results in haemato-biochemical alterations and results in alteration of inflammatory molecules. The current study can assist in management of obstetrical cases in field conditions.

Key words: Fetotomy, buffalo, inflammatory markers, dystocia.

INTRODUCTION

The parturition that requires assistance of farmer or veterinarian for the extraction of fetus is known as dystocia. The frequency of dystocia varies from 2-23 % in buffalo (Mee 2008). Dystocia has been a long-standing problem in both the beef and dairy industry. It is one of the most serious complications of pregnancy in cattle, which is believed to be influenced by numerous factors such as pelvic area, calf's birth weight, age of dam, twin pregnancy, presentation, disposition, hormonal control, and nutrition of dam (Oakes *et al.* 2001). Haematological analysis is one of the useful indicators about prognosis and efficacy of treatment in various ailments (Vegad 2000). Acute Phase Proteins (APP) is a group of blood proteins that change in concentration in animals subjected to infection, inflammation, surgical trauma or stress (Murata *et al.* 2004). These APP's are considered to be non-specific innate immune components involved in the restoration of homeostasis and restrain microbial growth before animals develop acquired immunity to a disorder. The circulating concentrations of the APP are related to the severity of the disorder and the extent of tissue damage in the affected animal; quantification of their concentration can therefore provide diagnostic and prognostic information. Although parturition is a physiological process, dystocia is of great stress for both the dam and foetus (Sathya *et al.* 2010). Due to this stress there is generation of free radicals or reactive oxygen species (ROS) during normal birth as well as dystocia which consequently affects the hemodynamic of the body and leads to alteration in the biochemical parameters (Sathya *et al.* 2010). TNF and IL-10 have been observed to increase in buffaloes subjected to fetotomy by Galvao *et al.* (2012) and Mouihate *et al.* (2008). TNF and IL-10 are inflammatory markers and are suggested to be better indicators for severity and mortality.

Keeping these points under consideration the present study was designed to assess the changes in inflammatory markers and various haemato-biochemical parameters in dystocia affected buffaloes compared with the normally calved buffaloes.

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MATERIALS AND METHODS

Selection of animals

This study was conducted on 50 Murrah buffaloes, presented to Teaching Veterinary Clinics of Guru Angad Dev Veterinary & Animal Science University (GADVASU) Ludhiana. Complete history regarding gestation, duration of labour and previous handling or medication of the buffaloes, was recorded. In 30 the buffaloes, dystocia was corrected by fetotomy once mutations failed to deliver the foetus. For comparison 20 Murrah buffaloes maintained at GADVASU Dairy Farm, Ludhiana, Punjab, served as the control group. These buffaloes were having normal parturition.

Sampling

Blood was collected just after fetotomy/ parturition. 5 ml blood samples were collected aseptically in EDTA coated vials and anti-coagulant free vials for haematological parameters and biochemical parameters respectively. In addition 1 ml blood was collected in sodium fluoride vial and was used for blood glucose estimation. Immediately after collection, all the blood samples were brought to the laboratory in an ice box. Thereafter, the samples were centrifuged for 15 minutes at 3000 rpm; upper meniscus was marked, blood plasma was separated and stored immediately at -20 ^{o}C till further analysis

Complete blood count

Parameters like Haemoglobin (Hb), Packed Cell Volume (PCV), Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC) were estimated by using Automatic Analyzer (SIEMENS- ADVIA 2120 HEMATOLOGY SYSTEM) in Clinical Diagnostic Laboratory of Teaching Veterinary Clinical Complex of Guru Angad Dev Veterinary and Animal Science University, Ludhiana. Differential leukocyte counts were performed on Wright's-Leishman stained blood films (Schalm 2000).

Blood Biochemical analysis

VITROS DT- II Chemistry system (Ortho-Clinical Diagnostics, Johnson & Johnson Company) was used for estimation of following parameters: Calcium, Phosphorus, Total Protein and Glucose. The VITROS DT Slide method was performed using the VITROS DT Slides on VITROS DT60II/DTSCII Module. The VITROS DT Slide is a multilayer, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers (Syed *et al.* 2013).

Fibrinogen was estimated by hand held refractometer (Schalm 2000). TNF and Interleukin-10 were estimated by using a drop of serum in ELISA coated cups in ACS 180- Automated chemi-luminescence system for hormone and drug assay (Johnson and Johnson), in Clinical Diagnostic Laboratory of Teaching Veterinary Clinical Complex of Guru Angad Dev Veterinary and Animal Science University, Ludhiana.

Statistical analysis

All data was expressed as mean values with standard error (±SE). Comparison between the two groups was evaluated by student's t-test. P value of 0.05 was selected as a criterion for statistically significant differences.

RESULTS AND DISCUSSION

Haematology

Haemoglobin (Hb) and Packed Cell Volume (PCV)

In the present study the average value of Hb, PCV and TEC were significantly ($p<0.05$) lower in buffaloes subjected to fetotomy (Group I), than the normally calved buffaloes (Group II). The average Hb concentration in control group was 14.12±0.97 g/dl, where as in buffaloes subjected to fetotomy it was 11.81±0.95 g/dl on the day of calving. These results are in consonance with that of (Ahmed *et al.* 2009), who found the values as 14.90±0.34 g/dl and 11.51±0.28 g/dl in normally calved and dystocia affected buffaloes respectively. The PCV percentage was 29.38±2.47 % and 36.86±0.18 % in group I and II respectively on the day of parturition. These results are in consonance with that of (Ahmed *et al.* 2009), who found the values as 36.62±0.18 % and 31.80±0.37 % respectively in normally calved and dystocia affected buffaloes on the day of parturition.

Hb and PCV were lower in fetotomy operated buffaloes on the day of parturition. These findings are in consonance with that of (Ahmed *et al.* 2009). The lower levels of Hb and PCV in dystocia affected animals could be due to the higher levels of ADH due to stress causing increased retention of fluid (Moran *et al.* 2011).

Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC)

In the present study the average TEC was significantly lower in fetotomy operated buffaloes, whereas the average TEC was significantly higher in fetotomy operated buffaloes than the normally calved. The average TEC of group I and group II were 6.06±0.41 $X10^6$ (cells/µl) and 7.21±0.2 $X10^6$ (cells/µl) respectively on day of parturition. The TLC on the day of parturition was 12.95±0.24 $x10^3$ cells/µl and 10.02±0.13$x10^3$ cells/µl in group I and group II buffaloes respectively. The average TLC on the day of parturition was significantly ($p<0.05$)

lower in buffaloes subjected to fetotomy than the normally calved buffaloes (group II). This was in agreement with the findings of (Sathya *et al.* 2010) and (Hamit *et al.* 2011).

The reduction in TLC after parturition, even in normally calving animals, has been attributed to drainage of neutrophils into the inflamed and traumatised reproductive tract (Guidry *et al.* 1976). The increased TLC in group I as compared to group II has been attributed to higher blood histamine concentration or uterine bacterial contamination (Matharu *et al.* 1999, Singh *et al.* 1997).

Differential Leukocyte Count (DLC)

In the present study, the mean neutrophil count and eosinophil count (%) was significantly ($p<0.05$) higher in buffaloes subjected to fetotomy (Group I) than the normally calved buffaloes (Group II) on the day of parturition. The mean neutrophil count (%) was 64.00±2.92 and 54.60±3.60 in group I and II respectively. The mean lymphocyte count (%) was 32.80±2.82% and 46.00±4.13% in group I and II respectively on the day of parturition. The trend in Lymphocyte count was opposite to that of neutrophil count. In the present study there was no characteristic trend in the monocytes counts between the two groups. Such a non-significant ($p<0.05$) variation in monocytes counts in dystocia affected buffaloes during the course of observation was found in our study. In the present study the mean eosinophil count was 1.00±0.28 % and 0.20±0.20 % in group I and II respectively on the day of parturition. This finding is in consonance with the findings of (Singh *et al.* 1997) and (Sathya *et al.* 2010). The lower mean neutrophil counts in fetotomy operated buffaloes is due to the stress of dystocia which increases secretion of glucocorticoids from adrenal gland which decrease the adhesion of neutrophils on the endothelium and thus cause a shift of neutrophils from the marginal pool to the circulating pool, resulting in neutrophilia (Burton *et al.* 1995, Andreasen *et al.* 2000). There was no characteristic trend in the average monocyte counts between the two groups. The counts in the present study however remained within the normal physiological range as has been observed (Sathya *et al.* 2010, Hamit *et al.* 2011).

Biochemical parameters

The concentration of serum Ca, P and protein were significantly ($p<0.05$) lower in buffaloes subjected to fetotomy (Group I), than the normally parturiated buffaloes (Group II). In the present study the average values of serum calcium were 7.26±0.69 mg/dl and 9.52±0.14 mg/dl in group I and II respectively on the day of parturition. The concentration of phosphorus was 3.07±0.24 mg/dl and 4.84±0.10 mg/dl in group I and II respectively on the day of parturition. In the present study the average values of blood glucose were significantly ($p<0.05$) higher in buffaloes subjected to fetotomy (Group I) than the normally calved buffaloes (Group II) on the day of parturition. The concentration of blood glucose was 152.30±40.73 mg/dl and 47.20±3.15 mg/dl in group I and II respectively. The average concentration of blood protein was 7.56±0.14 g/dl and 8.98±0.21 g/dl in group I and II respectively.

These findings are in consonance with the findings of (Sathya *et al.* 2010) and (Hamit *et al.* 2011). The average value of serum calcium was significantly lower in buffaloes suffering from dystocia as compared to normally calving buffaloes. These findings are in consonance with the findings of Husnain *et al.* (2001) and Nayyar *et al.* (2006) who also reported lower serum levels of Ca and P in dystocia affected animals as compared to normally calving animals. There is controversial finding regarding level of Ca as predisposition or sequel of dystocia. Since Ca plays an important role in neuromuscular excitability, cell membrane permeability, muscle contraction and nerve

Table 1. Haemato-biochemical profile and inflammatory markers of fetotomy operated buffaloes (Mean±SE) in comparison with normally calved buffaloes.

Parameter	Fetotomy operated buffaloes (Group I)	Normally calved buffaloes (Group II)
Hb (g/dl)	11.81±0.95[a]	14.12±0.97[b]
PCV (%)	29.38±2.47[a]	36.86±0.18[b]
TLC $X10^3$ (cells/µl)	12.95±0.24[b]	10.02±0.13[a]
TEC $X10^6$ (cells/µl)	6.06±0.41[a]	7.21±0.20[b]
Neutrophil (%)	64.00±2.92[b]	54.60±3.60[a]
Lymphocytes (%)	32.80±2.82[a]	46.00±4.13[b]
Monocytes (%)	0.20±0.20[a]	0.10±0.10[a]
Eosinophils (%)	1.00±0.28[b]	0.20±0.20[a]
Calcium (mg/dl)	7.26±0.69[a]	9.52±0.14[b]
Phosphorus (mg/dl)	3.07±0.24[a]	4.84±0.10[b]
Glucose (mg/dl)	152.30±40.73[b]	47.20±3.15[a]
Total protein (g/dl)	7.56±0.14[a]	8.98±0.21[b]
Fibrinogen (g/dl)	0.56±0.16[a]	0.44±0.19[a]
TNF (pg/ml)	37.12±1.90[b]	28.26±2.70[a]
IL-10 (pg/ml)	1.16±0.42[b]	0.60±0.10[a]

*The values with different superscripts differ significantly ($p\leq0.05$) within a row.

impulse transmission, its deficiency may lead to reduced vaginal and uterine muscle tone which predisposes the animals to dystocia as proposed by Roberts (1980). Similarly the average serum phosphorus concentration was significantly lower in buffaloes subjected to fetotomy than the normally calved buffaloes leading to altered Ca: P ratio which is a predisposing cause of the dystocia. The elevated blood glucose level has been attributed to elevated cortisol and catecholamines, which occurs following various obstetrical procedures in response to stress (Breazile 1987, Nakao *et al.* 1997). The lower total protein concentration in fetotomy operated animals could be because of manipulation or haemorrhage during obstetrical manoeuvre, with subsequent extraction of intestinal fluid into the circulation as concluded by Sathya *et al.* (2010) and Hamit *et al.* (2011).

Inflammatory markers

TNF and IL-10 were used inflammatory markers as they are better indicators for severity and mortality. In the present study the average values of fibrinogen was non-significantly ($p<0.05$) higher, whereas the values of TNF and IL-10 were significantly ($p<0.05$) higher in buffaloes subjected to fetotomy than the normally calved buffaloes. The slightly higher average values of fibrinogen in dystocia affected buffaloes indicates inflammation (Kaneko *et al*. 1997). In the present study the fibrinogen concentration in group I and II were 0.56±0.16 g/dl and 0.44±0.19 g/dl respectively on the day of parturition, which were within normal range.

In the present study the average value of TNF was 37.12±1.90 pg/ml and 28.26±2.70 pg/ml in buffaloes subjected to fetotomy and normally calved buffaloes. The average values of IL-10 were significantly higher in buffaloes subjected to fetotomy than the normally calved buffaloes on the day of parturition. The average value of TNF was 1.16±0.42 g/dl and 0.60±0.10 g/dl and the average value of IL-10 was 106.70±4.06 and 65.04±6.08 pg/ml respectively in fetotomy operated and normally calved buffaloes.

The findings regarding the average values of TNF and IL-10 are totally in agreement with the observations of (Galvao *et al.* 2012, Mouihate *et al*. 2008), who also found the similar trend of TNF and IL-10 in postpartum dairy cows. Local inflammation is the first response of the immune system to noxious stimuli. When infections and tissue injuries overwhelm local defences, the organism responds by activating a wide ranging systemic response (Murata *et al.* 2004). Considering the higher values of TNF in dystocia affected buffaloes it can be assumed that this is a response to oxidative damage, or a response to tissue damage, for example that sustained during the trauma of fetotomy (Galvao *et al.* 2012). Similarly higher levels of IL-10 are an indication of inflammation. In the present study the higher levels of TNF and IL-10 in buffaloes subjected to fetotomy clearly indicates more uterine inflammation as compared to normally calving buffaloes. Fibrinogen is an acute phase reactant and its concentration in plasma may be greatly increased in chronic inflammatory disease (Kaneko *et al*. 1997). In most cases high level of plasma fibrinogen represents some ill defined response to tissue destruction or inflammation (Searacy 1969). Fibrinogen level increases within 24 hours of tissue injury. In chronic reactions it remains high as long as the disease is present and active (Mc-Cherry *et al.*1970). In the present study higher levels of fibrinogen in fetotomy operated buffaloes are an indication of uterine inflammation. Inflammatory cytokines are increased by manipulation in addition to the changes associated with uterine infection (Sheldon *et al.* 2001). Therefore, inflammatory marker expression can be evaluated in the female reproductive system in cases of dystocia and other uterine diseases.

CONCLUSION

Dystocia is a stressful event which results in haemato-biochemical alterations and results in alteration of inflammatory molecules. The current study can act as a guide in management of obstetrical cases in field conditions.

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