Association of TNF-α, IL-10, HSP70 and HSP90 gene with uterine torsion in Murrah buffaloes

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Abstract

Uterine torsion is a critical obstetrical emergency in buffaloes often results in dystocia and poses a fatal risk to both dam and fetus. Traditional diagnostic methods fall short in determination of the underlying cause and severity of the condition. This study aimed to explore the genetic component of uterine torsion with special emphasis on the expression of inflammatory mediators. To our knowledge, molecular insights into this disorder in buffaloes are scarce. The expression profiles of pro-inflammatory cytokine TNF-α, anti-inflammatory cytokine IL-10 and heat stress proteins HSP-70 and HSP-90 were evaluated in uterine torsion-affected buffaloes using peripheral blood mononuclear cells. Results revealed a significant upregulation in the expression of TNF-α (1.832±0.176), IL-10 (2.275±0.302), HSP-70 (2.033±0.301) and HSP-90 (2.126±0.112) when compared to normal calved buffaloes. These findings indicate increased inflammatory and stress responses in torsion cases. These biomarkers could serve as prognostic indicators which enables timely therapeutic intervention. Further exploration of gene regulation may aid in development of precision treatment strategies for this obstetrical challenge.

Key words: Murrah buffalo, uterine torsion, prognosis, pro-inflammatory cytokine, anti-inflammatory cytokine and heat stress proteins

Introduction

Uterine affections are common cause of reproductive problems in Buffaloes (Kapadiya et al., 2015). Uterine torsion is major problem in buffaloe which often result into mortality (Chandra Prasad et al., 2018). Uterine torsion refers to the twisting of the gravid uterus along its longitudinal axis, a condition first reported by Boutrolle in 1766 (Purohit et al., 2011). It is recognized as one of the most common causes of dystocia in bovines accounting for 56–80% of dystocia cases (Srinivas et al., 2007). In buffaloes, it contributes to approximately 53–83% of dystocia cases presented at referral hospitals (Purohit et al., 2012). This condition is extremely stressful for buffaloes at the time of parturition (Manju et al., 1985). Uterine torsion generally affects pluriparous animals in advanced pregnancy (Roberts, 1986). Studies suggest that the origin of torsion is more likely foetal than maternal, as animals usually do not experience torsion in subsequent pregnancies (Purohit et al., 2012). Factors like large and active foetuses during late gestation and early labour may trigger uterine rotation (Noakes et al., 2009). Inadequate amniotic fluid volume compared to foetal size may also predispose certain animals to torsion (Schönfelder and Sobiraj, 2005). If not diagnosed and managed promptly, the condition can become fatal for both the dam and the calf (Mekonnen and Moges, 2016). The uterine wall becomes necrotic and fragile due to compromised blood flow, increasing the risk of rupture (Ghuman, 2010).

Accurate and timely diagnosis plays a vital role in the management of uterine torsion cases. Traditional diagnostic methods like rectal and vaginal examinations may not be sufficient to determine the exact cause or severity of the torsion (Ghuman, 2010). Therefore, more advanced diagnostic approaches are needed to understand the pathological status and to apply the most effective therapeutic intervention (Mekonnen and Moges, 2016). With advancements in veterinary diagnostics, the focus has shifted towards developing prognostic criteria to better manage such reproductive emergencies. However, despite significant research on clinical aspects of uterine torsion, limited studies explore the molecular mechanisms associated with this condition. Inflammatory responses are known to play a critical role in the pathophysiology of uterine torsion. The necrotic uterine tissue releases various inflammatory mediators, which may influence the clinical outcome. Yet, data regarding the expression of such cytokines in affected animals remain scarce (Abrol et al., 2020). This gap in understanding highlights the need to explore inflammatory gene expression in uterine torsion cases. A molecular perspective can offer deeper insights into the systemic inflammatory response and guide future diagnostic strategies.

Genetic and molecular studies represent a promising area of investigation for understanding uterine torsion (Abrol et al., 2020). Exploring the gene expression profiles of cytokines in affected buffaloes may help determine whether the condition has an inflammatory or hereditary origin. Studying genes like tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) and heat shock proteins (HSP 70 AND HSP 90) may provide valuable information (Biradar et al., 2024) about the balance between pro- and anti-inflammatory responses during uterine torsion. These biomarkers could potentially serve as prognostic indicators for assessing the severity and outcome of the condition. A better understanding of cytokine expression may also help in developing targeted therapeutic approaches. Thus, the current study aims to examine the expression patterns of TNF-α, IL-10 and heat shock proteins (HSP 70 AND HSP 90) in peripheral blood mononuclear cells of uterine torsion-affected buffaloes.

Materials and methods

The present study was conducted in uterine torsion affected buffaloes, which were presented to the Obstetrical Unit, Department of VGO, N.T.R. CVSc, Andhra Pradesh and from local dairy farms were selected. These were referred from coastal districts in and around Gannavaram. The uterine torsion affected buffaloes were examined and their occurrence were recorded and analysed. Uterine torsion affected buffaloes will be assigned to Group-I (n=6), while normal calved buffaloes will be assigned to Group-II (n=6).

History and clinical examination

Complete history with regards to parity, age, gestational length, onset of clinical signs of parturition and previous calvings were recorded. Rectal temperature, heart rate, respiration rate and condition of conjunctiva were recorded in all the uterine torsion affected buffaloes during general clinical examination. Detailed obstetrical examination was performed to determine the degree (90° - 360°), the side (right or left) and site (pre- or post-cervical) in cases of torsion of uterus.

Sample collection and processing

Blood samples were collected aseptically from the jugular vein of buffaloes (within one hour of either uterine torsion presentation or normal parturition) into EDTA-coated and clot-activator vacutainers for haematological and biochemical analyses, respectively, with serum separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until use.

Gene expression analysis of inflammatory cytokines

Peripheral blood (4 mL) was collected aseptically in EDTA tubes from experimental animals and peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque density gradient centrifugation (Sigma-Aldrich, Cat. No. 10771) as per the manufacturer's protocol. The PBMC pellet was washed with PBS,

preserved in RNA later (Invitrogen) and stored at -20°C until RNA extraction. Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen, USA), following the manufacturer's instructions with minor modifications (Browne et al., 2020). RNA purity and concentration were measured using a Nano spectrophotometer and samples with acceptable 260/280 and 260/230 ratios were used for cDNA synthesis.

First-strand cDNA synthesis was carried out using the iScript™ Select cDNA Synthesis Kit (Bio-Rad, USA) according to the supplier's guidelines. Primers for target genes (TNF-α, IL-10, HSP70, HSP90) and the reference gene (GAPDH) were designed using Primer3Plus based on buffalo-specific sequences from NCBI and validated using BLAST. Real-time quantitative PCR (qPCR) was performed on a Bio-Rad CFX96™ system using SYBR Green. Assay optimization included conventional PCR, melt-curve analysis and determination of ideal primer-template ratios. Gene expression levels were normalized to GAPDH and analyzed using the 2^−ΔΔCt method. Amplicon specificity was confirmed by agarose gel electrophoresis using standard DNA markers.

Statistical analysis

The results obtained on various parameters were represented as Mean±SE. The data was analysed as per the method of Snedecor and Cochran (1994) using SPSS software ver. 25.0. Comparison between fresh and frozen samples for various parameters were performed using unpaired t-test. Relative gene expression (fold change) of the target genes was calculated using 2^-ΔΔCT method. Significance difference between groups with respect to fold change was analysed using analysis of variance (ANOVA) and multiple comparison was performed by using Duncan multiple range test. P values <0.05 were considered to be significant.

Results

Relative mRNA expression of certain inflammatory cytokine genes in normal calved and uterine torsion affected Murrah buffaloes

The mRNA expression levels of certain inflammatory cytokine genes were studied in blood samples collected from Murrah buffaloes. The samples were divided into two groups viz. normal calved buffaloes and uterine torsion affected buffaloes. The RNA was isolated from peripheral blood mononuclear cells, converted to cDNA and used for gene expression studies. The quantification was expressed as relative transcript levels using GAPDH as reference gene. Normally calved buffaloes group was used as control and comparisons were made with uterine torsion affected buffaloes group.

mRNA expression of TNF – α in normal calved and uterine torsion affected Murrah buffaloes

The Mean±SE values of ΔCq, ΔΔCq and fold change of TNF – α in normal calved buffaloes and uterine torsion affected Murrah buffaloes were presented in table 2. The mRNA expression of TNF-α was significantly (P<0.05) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The amplicon size of qPCR product of TNF-α visualized on 2% agarose gel was presented in figure 1.

mRNA expression of IL-10 in normal calved and uterine torsion affected Murrah buffaloes

The Mean±SE values of ΔCq, ΔΔCq and fold change of IL-10 in PMBC of normal calved buffaloes and uterine torsion affected Murrah buffaloes were presented in table 3. The mRNA expression of IL-10 was significantly (P<0.05) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The amplicon size of qPCR product of IL-10 visualized on 2% agarose gel was presented in figure 2.

mRNA expression of HSP-70 in normal calved and uterine torsion affected Murrah buffaloes

The Mean±SE values of ΔCq, ΔΔCq and fold change of HSP-70 in PMBC of normal calved buffaloes and uterine torsion affected Murrah buffaloes were presented in table 4. The mRNA expression of HSP-70 was significantly (P<0.05) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The amplicon size of qPCR product of HSP-70 visualized on 2% agarose gel was presented in figure 3.

mRNA expression of HSP-90 in normal calved and uterine torsion affected murrah buffaloes

The Mean±SE values of ΔCq, ΔΔCq and fold change of HSP-90in PMBC of normal calved buffaloes and uterine torsion affected Murrah buffaloes buffaloes were presented in table 5. The mRNA expression of HSP-90 was significantly (P<0.05) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The amplicon size of qPCR product of HSP-90 visualized on 2% agarose gel was presented in fig. 4.

mRNA expression of GAPDH in normal calved and uterine torsion affected Murrah buffaloes: GAPDH was used as housekeeping gene and the amplicon size of qPCR product of GAPDH was visualized on 2% agarose gel presented in figure 5.

Discussion

The present study documented that the changes in the TNF-α expression in uterine torsion affected and normally calved buffaloes as 1.832±0.17592 and 1.00±0.00, respectively. The present study also revealed that the mRNA expression of TNF-α was significantly (P<0.05) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The results of the present investigation were in accordance with the studies of Mahmoud et al. (2020) who reported that the level of TNF-α (pg/mL) was higher in uterine torsion affected

buffaloes (18.31 ± 0.24) when compared with normal pregnant buffaloes at full term (4.94 ± 0.11). Wani et al. (2018) reported that the values of TNF-α (pg/mL) was significantly ($p<0.05$) higher in buffaloes subjected to

Table 1: Sequence of primers and product size of the genes under study

Base pair (bp) size	143	185	205	91	116
Reverse primer (5-->3)	5'AACCAGAGGGC TGTTGATGG3'	5'AGTAAGCTGT CCAGTTGGTCC3'	5'ATCGGTGAA GGCCACATAGC3'	5'GGCAGCCAT GTAACCCATCG3'	5'CCGTTCTCT GCCTTGACTGT3'
Forward primer (5-->3)	5'GAAGTTGCTTG TGCCTCAGC3'	5'TCAGCACTACT CTGTTGCCTG3'	5'TCCCCATCCG AACCGTATCA3'	5'CACAAGCAC ATACGGCTGGAC3'	5'AAAGTGGAC ATCGTCGCCAT3'
Genes	TNF-α	IL-10	HSP - 70	HSP – 90	GAPDH

Table 2: Showing the Mean±SE of ΔCq, ΔΔCq and fold change of TNF-α in normal calved and uterine torsion affected Murrah buffaloes

Group	**ΔCq**	**ΔΔCq**	**Fold change**
Normal calved buffaloes (Group-I)	4.0163±0.13662	0.00±00	1.00±00[a]
Uterine torsion affected buffaloes (Group-II)	3.1725±0.20629	-0.8438±0.17592	1.8320±0.19692[b]

Means with different superscripts (a, b) in a column for fold change differed significantly ($p<0.05$).
ΔCq = Cq (Target) – Cq (Reference), ΔΔCq = ΔCq (Treatment) – Cq (control); Fold change = 2^- ΔΔCq

Table 3: Showing the Mean±SE of ΔCq, ΔΔCq and fold change of IL-10 in normal calved and Uterine torsion affected Murrah buffaloes

Group	**ΔCq**	**ΔΔCq**	**Fold change**
Normal calved buffaloes (Group-I)	4.9638±0.29839	0.00±00	1.00±00[a]
Uterine torsion affected buffaloes (Group-II)	3.8188±0.46662	-1.1450±0.20268	2.2753±0.30234[b]

Means with different superscripts (a, b) in a column for fold change differed significantly ($p<0.05$).
ΔCq = Cq (Target) – Cq (Reference); ΔΔCq = ΔCq (Treatment) – Cq (control); Fold change = 2^- ΔΔCq.

Table 4: Mean±SE of ΔCq, ΔΔCq and fold change of HSP-70 in uterine torsion affected and normal calved Murrah buffaloes

Group	**ΔCq**	**ΔΔCq**	**Fold change**
Normal calved buffaloes (Group-I)	3.9525±0.91914	0.00±00	1.00±00[a]
Uterine torsion affected buffaloes (Group-II)	2.9888±0.92103	-0.9638±0.25340	2.0328±0.30117[b]

Means with different superscripts (a, b) in a column for fold change differed significantly ($p<0.05$).
ΔCq = Cq (Target) – Cq (Reference); ΔΔCq = ΔCq (Treatment) – Cq (control); Fold change = 2^- ΔΔCq.

Table 5: Mean±SE of ΔCq, ΔΔCq and fold change of HSP-90 in normal calved and uterine torsion affected Murrah buffaloes

Group	**ΔCq**	**ΔΔCq**	**Fold change**
Normal calved buffaloes (Group-I)	0.6300±0.60886	0.00±00	1.00±00[a]
Uterine torsion affected buffaloes (Group-II)	-0.1175±0.11054	-1.0825±0.07554	2.1264±0.11159[b]

Means with different superscripts (a, b) in a column for fold change differed significantly ($p<0.05$).
ΔCq = Cq (Target) – Cq (Reference) ΔΔCq = ΔCq (Treatment) – Cq (control)

fetotomy (37.12±1.90) than the normally calved buffaloes (28.26±2.70). Further they opined that the buffaloes with uterine torsion had elevated levels of TNF-α, which was due to a response to tissue injury or oxidative damage.

The present study recorded that the fold change in the IL-10 expression in uterine torsion affected and normally calved buffaloes as 2.2753±0.30234 and 1.00±0.00, respectively. The present study also revealed that the mRNA expression of IL-10 was significantly ($P<0.05$) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The results of the present investigation were in line with the studies of Wani et al. (2018) who reported that the values of IL-10 (pg/mL) was significantly ($p<0.05$) higher in buffaloes

subjected to fetotomy (1.16±0.42) than the normally calved buffaloes (0.60±0.10). IL-10 was an anti-inflammatory cytokine, functions chiefly in suppressing the pro-inflammatory environment and played a crucial role in prevention of inflammation and autoimmune disorder (Sanjabi et al., 2009).

The present study documented the fold change in the HSP-70 expression in uterine torsion affected and normally calved buffaloes as 2.0328±0.30117 and 1.00±0.00, respectively. The present study also recorded the fold change in the HSP-90 expression in uterine torsion affected and normally calved buffaloes as 2.1264±0.11159 and 1.00±0.00, respectively. The mRNA expression of HSP-70 and HSP-90 were significantly ($P<0.05$) up-regulated in uterine torsion affected buffaloes compared to normal calved buffaloes. The elevated HSP-70 and HSP-90 levels in buffaloes affected with uterine torsion might be due to prolonged exposure to stress.

HSPs are thought to play important roles in environmental stress tolerance and temperature adaptation (Sorensen et al., 2003). HSPs act as molecular chaperones, maintaining cellular homeostasis and increasing cell survival (Collier et al., 2008). It protects cells from subsequent stress exposure (Byoung Hwa Roh et al, 2008). HSP70i (HSP70.1 and HSP70.2) is the most temperature sensitive member of the HSP family and it is activated by a variety of physiological, pathological and environmental stressors (Beckham et al., 2004).

Among the HSPs, HSP70 has a significant role in cell thermo tolerance (Beckham et al., 2004) and animal survival (King et al., 2002). Choe et al. (2010) carried out research to discover altered proteins by means of MALDI-TOF-MS mass spectrometry in the uterine washings of clinical endometritic cows and reported an upregulation of heat-shock protein (HSP) 27 and heat-shock protein (HSP) 70 in the endometrium of clinical endometritic cows and suggested their role in maintaining the cell stability and integrity. In spite of this, Muthukumar et al. (2014), discovered heat shock protein 70 (HSP 70) in the cervical fluid of buffaloes and this protein has since been postulated as a possible marker(s) for estrus detection in buffaloes. Nevertheless, results of the present study documented an elevated levels of the mRNA expression of both HSP-70 and HSP-90 in uterine torsion cases which could be attributed to the stress level malady connected during or at the time of uterine torsion, despite the fact that the expression is quite not obvious in the normal calved animals.

Conclusion

To sum up , it was clearly evident from the results of the present study that the mRNA expression levels of certain genes such as TNF-α, IL-10, HSP-70 and HSP-90 were upregulated in uterine torsion affected buffaloes compared to normal calved buffaloes which indicates tissue injury and stress and these could be used as a scale for evaluating the prognosis of the animal condition so as to initiate early treatment.

Conflicts of interest: The authors declare that they have no conflict of interest.

Ethical standards: The present experiment comply the ethical laws of the country in which they were performed.

References

1) Abrol A, Singh M, Sharma A and Kumar P. 2020. A review on diagnostic and prognostic techniques employed during bovine uterine torsion. Veterinary Sciences: Research and Reviews 6: 7-13.
2) Beckham J T, Mackanos M A, Crooke C, Takahashi T, O'Connell-Rodwell C, Contag C H and Jansen E D. 2004. Assessment of cellular response to thermal laser injury through bioluminescence imaging of heat shock protein 70. Photochemistry and Photobiology 79: 76–85.
3) Biradar SR, Komatwar SJ, Dongre VB, Jadhav PV, Bhosale AV, Vaidya MM 2024. Molecular characterization of heat shock protein 70 gene in Deoni cattle (*Bos indicus*). Journal of Livestock Science 15: 203-207 doi. 10.33259/JLivestSci.2024.203-207
4) Browne D J, Brady J L, Waardenberg A J, Loiseau C and Doolan D L. 2020. An analytically and diagnostically sensitive RNA extraction and RT-qPCR protocol for peripheral blood mononuclear cells. Frontiers in immunology 20:11:402.
5) Byoung Hwa Roh M D, Dae Hyun Kim M D, Moon Kyun Cho M D,Young Lip Park M D and Kyu Uang Whang M D. 2008. Expression of Heat Shock Protein 70 in Human Skin. Annals of dermatology 31:184.
6) Cells as a Photoprotective Function after UV Exposure. Annals of Dermatology 20: 184-189.
7) Chandra Prasad B, Ravi Kumar P, Tangamani A and Srinivas M, 2018. Cesarean section for management of delayed cases of uterine torsion in buffaloes (Bubalus bubalis). Journal of Livestock Science 9: 84-87
8) Choe C, Park J W, Kim E S, Lee S G, Park S Y, Lee J S and Kang D. 2010. Proteomic analysis of differentially expressed proteins in bovine endometrium with endometritis. The Korean Journal of Physiology & Pharmacology 14: 205-212.
9) Collier R J, Collier J L, Rhoads R P and Baumgard L H. 2008. Genes involved in the bovine heat stress response. Journal of Dairy Science 91: 445-54.
10) Ghuman S P S. 2010. Uterine torsion in bovines: a review. Indian Journal of Animal Sciences 80: 289.
11) King YT, Lin CS, Lin JH and Lee WC. 2002. Whole-body hyperthermia induced thermotolerance is associated with the induction of heat shock protein 70 in mice. Journal of Experimental Biology 205: 273-278.

12) Mahmoud E A, Essawi W M and Neamat-Allah A N. 2020. Influence of uterine torsion in water buffaloes (Bubalus bubalis) with insights into the hematological and biochemical prognostic values regarding to manual correction. Tropical Animal Health and Production 52: 3165-3171.
13) Manju T S, Verma S K, Gupta R C, Manadskhot V M and Krishnaswamy A. 1985. Profiles of some plasma biochemical constituents associated with uterine torsion and following its correction by laparohysterotomy in buffaloes. Indian Journal of Animal Reproduction 6: 57-61.
14) Mekonnen M and Moges N. 2016. A Review on Dystocia in Cows. European Journal of Biological Sciences 8: 91-100.
15) Muthukumar S, Rajkumar R, Karthikeyan K, Liao C C, Singh D, Akbarsha M A and Archunan G. 2014. Buffalo cervico-vaginal fluid proteomics with special reference to estrous cycle: heat shock protein (HSP)-70 appears to be an estrus indicator. Biology of Reproduction 90: 97-1.
16) Noakes DE, Parkinson DJ and England GCW. 2009. Maternal dystocia Arthur's Veterinary Reproduction and Obstetrics 9th edition, W B Saunders Company. pp 324-356.
17) Purohit GN, Barolia Y, Shekher C and Kumar P. 2011. Diagnosis and correction of uterine torsion in cattle and buffaloes. Raksha Technical Reviews 2: 11-17.
18) Purohit G N, Kumar P, Solanki K, Chandra Shekher and Yadav S P. 2012. Perspectives of Foetal Dystocia in Cattle and Buffalo. Veterinary Science Development 2: 1-12.
19) Roberts S J. 1986. Diseases and accidents during the gestation period. Diagnosis and treatment of the various types of dystocia. Injuries and diseases of the puerperal period. Veterinary obstetrics and genital diseases (Theriogenology). Woodstock, VT: SJ Roberts, 230-359.
20) Sanjabi S, Zenewicz LA, Kamanaka M and Flavell RA. 2009. Anti-inflammatory and pro-inflammatory roles of TGF-β, IL-10, and IL-22 in immunity and autoimmunity. Current Opinion in Pharmacology 9: 447-453.
21) Schonfelder A and Sobiraj A. 2005. Etiology of torsio uteri in cattle: a review. Schweizer Archiv fur Tierheilkunde 147: 397-402.
22) Sorensen J G, Kristensen T N and Loeschcke V. 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters 6: 1025–1037.
23) Srinivas M, Sreenu M, Lakshmi Rani N, Subramanyam Naidu K and Devi Prasad V. 2007. Studies on dystocia in graded Murrah buffaloes -a retrospective study. Buffalo Bulletin 26: 40-45.
24) Wani A A, Mavi P S, Rafee M A, Brar P S and Gandotra V K. 2018. Haemato-biochemical and inflammatory markers of fetotomy operated buffaloes. Exploratory Animal and Medical Research 8: 85-89.