

## EVALUATION OF OXIDATIVE STRESS IN BUFFALOES UNDERGOING DIAPHRAGMATIC HERNIORRHAPHY WITH AND WITHOUT POSITIVE PRESSURE VENTILATION

**K.R. Trivedi<sup>1</sup>, Prajwalita T. Sutaria<sup>1\*</sup>, B.N. Suthar<sup>2</sup>, T.V. Sutaria<sup>2</sup>, S.T. Desai<sup>1</sup> and S.S. Chaudhary<sup>1</sup>**

### ABSTRACT

Oxidative stress is present in buffaloes with diaphragmatic hernia not only because of endogenous antioxidant deficiency but also because of reperfusion injury owing to intraoperative animal posture. This study was planned in 12 buffaloes with diaphragmatic hernia which was corrected surgically either with or without positive pressure ventilation. The extent of oxidative stress was estimated by studying MDA and SOD levels in blood. Both MDA and SOD increased at different stages of operation and did not differ significantly in animals with or without positive pressure ventilation.

**Keywords:** *Bubalus bubalis*, buffaloes, diaphragmatic herniorrhaphy, oxidative stress, malondehyde, superoxide dismutase

### INTRODUCTION

Diaphragmatic hernia is a chronic wasting disease, comprising of multi-organ dysfunctions.

It involves herniation of abdominal organs mainly reticulum through a rupture in the diaphragm at the musculotendinous junction (Bisla *et al.*, 2002). The buffaloes with diaphragmatic hernia are predisposed to deficiency of energy, proteins, vitamins and trace minerals, essentially required for biochemical protection of cells from over produced oxygen free radicals (OFRs). Further, the increased intra-thoracic pressure due to recurrent tympany and postural changes during diaphragmatic herniorrhaphy develop low flow state leading to ischaemia/ reperfusion in internal organs. Ischaemia/ reperfusion in the internal organs lead to over production of oxygen free radicals causing oxidative stress. The over-produced oxygen free radicals in the deficiency of endogenous anti-oxidant damages cell membranes through oxidative stress (Bisla *et al.*, 2003b). Hence, in the present study the oxidative stress of diaphragmatic herniorrhaphy under thiopentone anaesthesia with and without intermittent positive pressure ventilation in buffaloes was evaluated.

---

<sup>1</sup>Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar-Gujarat, India,

\*E-mail: drprajwalita@gmail.com

<sup>2</sup>Department of Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar-Gujarat, India

## MATERIALS AND METHODS

The present study was conducted on 12 animals diagnosed positive for diaphragmatic hernia through auscultation and transabdominal ultrasonography at 4 to 5<sup>th</sup> intercostals space. Rumenotomy was performed in all buffaloes through standard procedure followed by diaphragmatic herniorrhaphy 24 h later under general anesthesia. The buffaloes were sedated with xylazine 0.1 mg/kg body weight. General anesthesia was induced and maintained with Thiopentone sodium 5% intravenously with total dose not exceeding 20 mg/kg body weight. The buffaloes were intubated with 18 no cuffed endotracheal tube and oxygen was supplied with a flow rate of 6 lit/minute. The buffaloes were grouped as under:

Group 1 : Herniorrhaphy performed without intermittent positive pressure, ventilation (N=6)

Group 2 : Herniorrhaphy performed with intermittent positive pressure, ventilation (n=6)

In Group 2 intermittent positive pressure ventilation using manual compression of reservoir bag was carried out to manually ventilate the animal (10/minutes), while in group I animals were allowed to breath spontaneously.

Rectal temperature (°F), heart rate (beats/minute), respiration rate (breaths/minute) and ruminal motility (palpated rumen movement/3 minutes) were recorded.

Five milliliter blood was collected in sterilized K<sub>3</sub>EDTA vial (CML Biotech Pvt. Ltd., Kerala) from jugular vein to analyze the oxidative stress. Oxidative stress parameters namely lipid peroxidation (LPO) in terms of malondialdehyde (MDA) and superoxide dismutase (SOD) were estimated in erythrocyte.

Physiological parameters, LPO and SOD were estimated on the day of blood collection prior to herniorrhaphy before and after induction of thiopentone sodium anaesthesia, immediately on completion of herniorrhaphy, 24, 48, 72 h and 10<sup>th</sup> day after herniorrhaphy in Group I and II. While blood was collected only once in six healthy non pregnant non lactating buffaloes without D.H to estimate levels of MDA and SOD.

The oxidative damage to erythrocyte membrane was determined in terms of malondialdehyde (MDA) production by the method of Shafiq-U-Rahman (1984) as under. The SOD was estimated in accordance with the method described by Madesh and Balasubramanian (1998).

### Statistical methods

The data was analysed statistically and significance of difference was tested by one way ANOVA with Duncan test.

## RESULTS AND DISCUSSION

Oxidative stress is the manifestation of imbalance between the production of oxygen free radicals and bodys antioxidant defense, while oxidative damage is the sum of bimolecular injury due to by attack of reactive oxygen species (ROS) on living cells of body (Halliwell, 2007). The oxidative damage is not solely dependant on level of oxidative stress, but also from failure of repair or replacement systems, Hence an arbitrary increase in levels of 'biomarkers' of oxidative damage need not always imply a greater level of oxidative stress.

The present study could be included among the above mentioned stress situations because the buffaloes suffering from diaphragmatic hernia had a history of chronic starvation,

pregnancy or lactation stress (Krishnamurthy *et al.*, 1985). Bisla *et al.* (2003a) opined that buffaloes with diaphragmatic hernia show stagnant ischemia/ reperfusion phase of circulation due to recurrent tympany and pressure of herniated reticulum on the heart and lungs. This condition gets further aggravated when the affected animals were subjected to herniorrhaphy in supine position. Ischemia/ reperfusion condition lead to over production of oxygen free radicals (OFRs) that had deleterious effects on cellular membranes.

At all stages of rectal temperature in buffaloes of both groups were within the normal specified range (Radostits *et al.*, 2007) at all stages of observation. The heart rate between Group I and II did not differ significantly at any stages of observation but showed a increasing trend, similar increasing trend in heart rate was observed by Khan (2009) in buffaloes operated for D.H without positive pressure ventilation (PPV) and by Bisla *et al.* (2003a) in buffaloes operated for D.H with intermittent positive pressure ventilation (IPPV)

The mean respiration rate value in Group 1 and 2 was non significantly increased immediately on completion of herniorrhaphy. Increased respiration rate following herniorrhaphy in buffaloes were reported by Nassimi *et al.* (1986); Bisla *et al.* (2003a); Khan (2009). Narale *et al.* (2006) reported respiration rate was decreased significantly and on 8<sup>th</sup> post-operative day all physical parameter reverted to normal. In between Group 1 and 2, non significant difference was observed in respiratory rate at different stages of observation hence concluding that intermittent positive pressure ventilation during herniorrhaphy has non significant effect on respiratory rate.

In Group 1 and 2, the MDA value prior to rumenotomy was observed to be significantly higher ( $P<0.05$ ) as compared to Group 3 (Healthy

buffaloes without D.H). Bisla *et al.* (2002); Sahu *et al.* (2002); Bisla and Singh (2006) found a significantly higher level of MDA in buffaloes suffering with diaphragmatic hernia as compared to healthy buffaloes. Bisla *et al.* (2004) have opined that buffaloes suffering from D.H were deficient in endogenous antioxidants which lead to poor detoxification of over produce OFRs.

The mean value of malondialdehyde (MDA) was significant increased ( $P<0.05$ ) in both groups at prior to herniorrhaphy before induction, which corresponded to 24 h after rumenotomy. Koksai and Kurban (2010) have elucidated on the multiple etiologic factors of oxidative stress with surgical trauma of both laparotomy and gastrotomy as one of the major causal factors as observed in our study. Furthermore, in both Group 1 and 2 significant increase in MDA value was observed after induction with thiopentone anaesthesia before herniorrhaphy. This may be so as observation was made with the buffaloes in supine position and myocardial sensitization effect of thiopentone (Bisla *et al.*, 2004), the increase in MDA value was observed at 24, 48 and 72 h after herniorrhaphy as also observed by Bisla *et al.* (2003a).

While mean value of superoxidedismutase (SOD) was significant increased ( $P<0.05$ ) in both groups at prior to herniorrhaphy before and after induction of thiopentone sodium anaesthesia, immediately on completion of herniorrhaphy, 24, 48 and 72 h after herniorrhaphy. Khan (2009) found similar results in buffaloes operated for D.H without IPPV and attributed it to increased oxidative stress. Contrary to our result Joshi (2012) reported non significant decrease SOD value at 48, 72 and 96 h after rumenotomy in animals subjected to D.H and given different dose of  $MnCl_2$ . Bernabucci *et al.* (2005) explained that the SOD constitutes a major intracellular antioxidant

defense mechanism. They considered the SOD as the first line of defense toward pro-oxidants which form hydrogen peroxide from superoxide.

Hence, we conclude that an increase in oxidative damage causes the body to increase endogenous antioxidant protection measures. Furthermore, oxidative stress is present in buffaloes operated for diaphragmatic hernia with or without positive pressure ventilation.

### ACKNOWLEDGEMENT

We are thankful to our dean for providing the necessary facilities helping to make this work successful.

### REFERENCES

- Bernabucci, U., B. Ronchi, N. Lacetera and A. Nardone. 2005. Influence of body condition score on relationship between metabolic status and oxidative stress in periparturient dairy cow. *J. Dairy Sci.*, **88**: 2017-2026
- Bisla, R.S, J. Singh and D. Krishnamurthy. 2002. Assessment of oxidative stress in buffaloes suffering from diaphragmatic hernia. *Indian Journal of Veterinary Surgery*, **23**: 77-80.
- Bisla, R.S, J. Singh, K. Singh and D. Krishnamurthy. 2003a. Assessment of vitamin E as an anti-oxidant in pre and post operative treatment schedule of buffaloes subjected to trans-abdominal diaphragmatic herniorrhaphy. *Indian Journal of Veterinary Surgery*, **24**: 11-15.
- Bisla, R.S, J. Singh, R. Tayal, S. Singh and D. Krishnamurthy. 2003b. Assessment of oxidative stress in diaphragmatic hernia affected buffaloes subjected to trans-abdominal diaphragmatic herniorrhaphy. *Indian Journal of Veterinary Surgery*, **24**: 92-94.
- Bisla, R.S, J. Singh, S.K. Chawla and D. Krishnamurthy. 2004. Assessment of ascorbic acid as anti-oxidant in buffaloes during diaphragmatic herniorrhaphy. *Indian Journal of Veterinary Surgery*, **25**: 94-97.
- Bisla, R.S. and J. Singh. 2006. Complication of manganese chloride used in buffaloes subjected to diaphragmatic herniorrhaphy. *Indian Journal of Veterinary Surgery*, **27**: 24-26.
- Halliwel. 2007. Biochemistry of oxidative stress. *Biochemical Society Transactions*, **25**: 104-105.
- Joshi, K.N. 2012. *Therapeutic evaluation of vitamin-C and manganese chloride as antioxidants in diaphragmatic herniorrhaphy in buffaloes*. M.V.Sc. Thesis, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India.
- Khan, M.J.Z. 2009. *Studies on repair and assessment of oxidative stress in diaphragmatic hernia without positive pressure ventilation in buffaloes*. M.V.Sc. Thesis, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India.
- Krishnamurthy, D., J.M. Nigam, P.K. Peshin, D.N. Sharma and R.P.S. Tyagi. 1985. *Mongraph on Diaphragmatic Hernia in Bovines*. Directorate of Publication, Haryana Agricultural University, Hisar, India.
- Koksal, H. and S. Kurban. 2010. Total oxidant status, Total antioxidant status and Paraoxonase and arylesterase activities

- during laparoscopic cholecystectomy. *Clinics*, **74**: 663-665.
- Madesh, M. and K.A. Balasubramanian. 1984. Microtitre plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry and Biophysics*, **35**: 184-188.
- Narale, N.L., A.P. Bhokre and R.R. Shelar. 2006. Diagnostic parameters of Diaphragmatic hernia in buffaloes. *Royal Veterinary Journal of India*, **2**: 24-28.
- Nassimi, M.N., D. Krishnamurthy and P.K. Peshin. 1986. Physiological reactions associated with diaphragmatic hernia in bovines. *Indian Journal of Veterinary Surgery*, **7**: 14-19.
- Radostitis, O.M, C.C. Gay, K.W. Hinchcliff and P.D. Constable. 2007. *Veterinary Medicine: A Textbook of the Disease of Cattle, Horse, Sheep, Pigs and Goats*, 10<sup>th</sup> ed., Philadelphia, PA: Saunders, Elsevier, USA.
- Sahu, A., S.K. Chawla, D. Krishnamurthy, A.R. Tayal and S.M. Behl. 2002. Role of certain antioxidant combination in the management of diaphragmatic hernia in buffaloes. *Indian Journal of Veterinary Surgery*, **23**: 81-82.
- Shafiq-U-Rahman. 1984. Lead induced regional lipid peroxidation in brain. *Toxicol. Let.*, **21**: 359-364.