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Effect of Pre and Post-Surgical Correction of Left Displacement Abomasum on Oxidative Stress, Metabolic Status and Hematological Profile Changes in Serum of Dairy Cows of Pakistan

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HA, MAK, AAA and AZD conceptualized the hypothesis of this manuscript. MR conducted the research. MR, HA and AM performed the surgery. MR, NR, MAH and MTS statistically analyzed the data, wrote and edited the manuscript. HA and MAH reviewed the manuscript.

Key words
Biochemical, Hematological, Dairy cows, LDA, Oxidative stress

ABSTRACT

This study was aimed to evaluate the oxidative stress status (malondialdehyde, MDA; catalase, CAT), metabolic status (beta hydroxybutyric acid, BHBA; nonesterified fatty acid NEFA) and hematological changes (hemoglobin, total leucocytes, red blood cells, neutrophils, platelets, lymphocytes) in serum of dairy cows before and after the surgical correction of left displacement abomasum. Twenty-six cows were divided into two groups as control (Group-A) and treatment (Group-B), each of 13 cows. Left displacement abomasum was confirmed by clinical assessment and ultrasonography, and then was surgically treated through Dirksen technique. Blood samples of both groups were collected on days 0, 7, 14, 21, and 28. Levels of various oxidative, metabolic and hematological parameters were measured by authenticated standard methods. We observed that serum levels of MDA and BHBA were higher at day 0 to day 14 ($P<0.01$), while the level of nonesterified fatty acid (NEFA) was higher at day 0 to day 7 ($P<0.01$) which dropped down to normal at day 21 ($P>0.05$) post-surgery in Group-B than Group-A. Moreover, CAT level in serum decreased ($P<0.01$) at day 0 to day 7, then it gradually increased to normal on day 14 to day 21 ($P>0.05$) post-surgery in Group B compared to Group A. In addition, levels of hemoglobin, total leucocytes, neutrophils were higher at day 0 to day 7 ($P<0.01$), while red blood cells and lymphocytes were lower at day 0 to day 14 ($P<0.01$) which became normal at day 28 ($P>0.05$) in Group-B than Group-A. The level of platelets was higher at day 0 ($P<0.01$), then gradually decreased to normal at day 28 in Group-B than Group-A. In conclusion, left displacement abomasum is associated with metabolic, oxidative and hematological disturbances which can be rectified through surgical correction. However, it is recommended to provide antioxidant therapy in adjunct with surgical correction for early recovery.

INTRODUCTION

Abomasal displacement is a stem disorder affecting 0.9-6.3% of dairy cattle which mostly occurs within 4 weeks during the post-partum period. The anomaly is characterized by varying degrees of displacement and distention of the abomasum. The displaced abomasum harms the production of the animal, decreases fertility, and badly affects herd health (Qu *et al*., 2013). Auscultation and percussion are the conventional diagnostic approaches, in which a high-pitched ping sound can be heard. Some intensive techniques such as blowing air into the rumen by stomach tube, rectal examination, and abdominocentesis are used to differentiate between rumen tympany, rumen collapse, peritonitis, and left displacement abomasum (Mueller, 2011). The animal with displaced abomasum experiences febrile condition, elevated heart rate, and

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respiration rate (El-Attar *et al*., 2008).

Periparturient diseases like milk fever, fatty liver, ketosis, mastitis, retained placenta and metritis prone the cattle to abomasal displacement (Basiri *et al*., 2013). The cardinal signs of the LDA include anorexia, depression, milk drop, high concentration of ketone bodies, and non-esterified fatty acids (NEFA) in the blood (Melendez *et al*., 2017). During the transition period, liver function is compromised in dairy cows due to oxidative stress and inflammation. About 50% of cows have fatty liver after 4 weeks of parturition (Osorio *et al*., 2012).

For the diagnosis of abdominal disorders in cows, the use of transabdominal ultrasound has gained importance during the past 20 years (Toholj *et al*., 2014). Ultrasound is a helpful tool for the diagnosis of LDA (Li *et al*., 2018b). In some cows, ketone bodies are high in blood but they do not exhibit clinical signs known as subclinical ketosis. In high-producing dairy herds, subclinical ketosis is an important metabolic disorder characterized by decreased milk yield and reproductive efficiency with a higher culling rate (Shin *et al*., 2015). Ketosis can be diagnosed by measuring the ketone bodies i.e. beta-hydroxybutyric acid (BHBA), acetoacetate, and acetone levels in the blood, milk, and urine (Suthar *et al*., 2013).

A higher level of NEFA harms the inflammatory function and immune system of the animal due to metabolic stress and the production of reactive oxygen species (Corrêa *et al*., 2018). The elevated level of the NEFA enhances the inflammatory cytokines which negatively alter the gastrointestinal motility (Contreras and Sordillo 2011; Durgut *et al*., 2016b).

During negative energy balance, the concentration of BHBA and NEFA is increased in the blood (Ospina *et al*., 2010; Zurr and Leonhard, 2012). Elevated NEFA elevates the production of reactive oxygen species (Contreras and Sordillo, 2011; Durgut *et al*., 2016b) which consequently increases the acute phase protein and cytokines production in the body. This inflammatory response can change gastrointestinal motility with a resultant abomasal hypo-motility (Ghazy *et al*., 2016b).

Cells regulate reactive oxygen species (ROS) levels through antioxidative defense systems, whereas ROS stimulates antioxidant signaling between cells, leading to an enhanced antioxidative capacity, a process known as oxidative stress. Under normal conditions, catalase (CAT) is of no remarkable significance to most cell types, but in the existence of oxidative stress, it is the greatest adaptive antioxidant enzyme and shows a significant role in cell defense against oxidative damage (Hayat *et al*., 2020). Polyunsaturated fatty acids (PUFAs) in cell membranes are the main targets of ROS consequently; lipid peroxidation may harm the cell structure as well as function (Hayat *et al*., 2020). Furthermore, the decomposition of lipid hydro-peroxides yields a wide variety of end-products, like malondialdehyde (MDA), one of the most frequently used ROS biomarker, showing lipid peroxidation intensity (Gawal *et al*., 2004; Palmieri and Sblendorio 2007). Biochemical and hematological changes related to LDA may severely effects on the post-operational outcome (Dezfouli *et al*., 2013).

To the best of our knowledge, the changes in the levels of MDA, CAT, BHBA, NEFA, red blood cells (RBCs), hemoglobin (Hb), platelets (PLT), total leucocytes (TLC), neutrophils and lymphocyte have not been reported in LDA dairy cows in Pakistan. Hence, we hypothesized that LDA is associated with metabolic and oxidative disturbances which can be remedied through surgical correction. Therefore, the present study was planned to evaluate the level of oxidative stress, metabolic and hematological alterations in dairy cows before and after surgical correction of LDA.

MATERIALS AND METHODS

Ethical statement

This study was approved by the Ethical Review Committee at University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan (Approval no: DR/01; Dated: 01/01/2019).

Experimental animals

The study was carried out on Holstein Friesian cows (n= 26; age= 4-8 years) with left displaced abomasum (LDA) in different commercial Dairy farms around Lahore, Pakistan. The cows were divided into two equal groups viz A (control) and B (Treatment).

History and diagnosis

As per telephonic intimation various commercial dairy farms were visited and animals having a history of anorexia, sudden drop in milk yield, refusal of concentrates, feed intake less than 50%, mild dehydration, calving period, age of the animal, duration of calving, mastitis, metritis, retained placenta, ketosis, grain parts in scanty pasty feces or long time indigestion were selected for the current study. Clinically, LDA was diagnosed by auscultation and percussion. LDA was further confirmed by using a digital veterinary ultrasound scanner machine (Sonoscape Vet. China), with a linear array transducer (5-10MHZ). After confirmation, LDA was surgically corrected by Dirksen technique (Right flank Omentopexy).

Surgical correction

Right flank Omentopexy was performed by adapting

the Ghazy *et al*. (2016b) procedure. Briefly, 2% lignocaine solution was injected as a local anesthetic by practicing Farquharson technique and inverted L block technique. A 15 cm vertical laparotomy incision was made 10 cm caudal to the last rib. The displaced abomasum was confirmed between the rumen and the abdominal wall. A drip set was used to deflate the inflated abomasum. As the gas and fluid were removed, its size was reduced and it moved dorsoventrally. The abomasum was pulled underneath the rumen from the left side to the right ventral abdomen. The greater omentum was pulled out and pylorus was identified. The greater omentum was easily grasped towards the incision. Two stay sutures were prepared one from the cranial side and the other caudally to the incision. The greater omentum was tightly sutured by the absorbable 2-0 suture material adopting the continuous pattern. The skin incision was sutured by non-absorbable suture material No. 2 by ford interlocking suture pattern.

Blood sampling

Blood samples were collected from the coccygeal vein of each animal in plain vacutainer before surgery at 0 and 7th, 14th, 21st, and 28th day after surgery. Only blood samples used for CBC were collected in an EDTA vacutainer. The blood samples were transferred to the laboratory in the Department of Physiology, Faculty of bio-sciences, UVAS. The blood samples were centrifuged (HARRIER 18/80) at 3000 rpm for 15 min at 4˚C. The serum was separated and stored in Eppendorf tubes at -20˚C for further analyses.

Biochemical and oxidative stress analysis

Serum BHBA and NEFA were analyzed through commercially available kits (Randox; Ran But; Lot No. 487360; UK) and (Randox; NEFA; LOT No. 503 FA; UK) as per manufacturer's instructions. Serum catalase was analysed as described by Hadwan and Abed (2016). Serum Malondialdehyde was measured by following the protocol of Feldman (2004).

Hematological analysis

Hematological parameters including hemoglobin Hb (g/dL), TLCS (10^3/µL), RBCs (10^6/µL), PLTS (10^3 cells/µL), neutrophils (10^3 cell/µL), and lymphocytes (10^3 cells/µL) were analyzed by CBC Analyzer (Back Man Colter; China) at University Diagnostic Laboratories, Lahore, Pakistan.

Statistical analysis

The data were analyzed by using SPSS statistical software version 20. The data from the LDA cows and the control group before and after surgery were analyzed using the two-way repeated-measures ANOVA. All values were expressed as mean ± standard deviation (Mean± SD). The levels of significance were (P<0.01) and (P< 0.05).

RESULTS

Sonographic examination

Ultra-sonographic examination exposed the abomasal wall as a narrow echogenic line following the abdominal wall and the muscles. On the dorsal side of the abomasal, there was a gas cap with typical reverberation artifacts. While on the ventral side of the abomasum ingesta was appeared as echogenic indicated in Figure 1. These are the cardinal signs of LDA that can be explored by Ultrasonography.

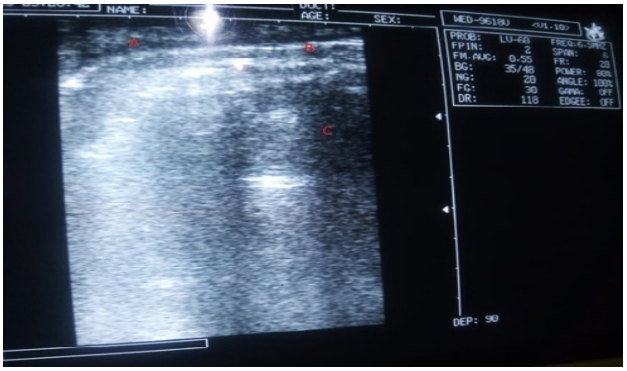

Fig. 1. Ultrasonography examination of Left Displaced Abomasum in a cow imaged from the left dorsal 12th intercostal space showed (A) abdominal wall, (B) abomasum wall, (C) Gas cap and Ingesta.

BHBA and NEFA serum concentrations and oxidative stress markers

Table I shows the effect of pre and post-surgical correction of left displacement abomasum on BHBA, NEFA concentration and oxidative stress markers of dairy cows**.** Level of BHBA in blood serum highly significantly (P<0.01) increased at day 0 to day 14, which dropped down (P>0.05) to normal at day 21 post-surgery in treatment (Group-B) as compared to the control (Group-A). Moreover, the level of NEFA highly significantly (P<0.01) increased at day 0 to day 7 which declined (P>0.05) to normal at day 21 post-surgery in group-B than group-A.

Serum level of MDA as extremely significantly higher (P<0.01) in treatment group-B at day 0 to day 14 which reduced (P>0.05) to normal at day 21 post-surgery as compared to the control Group-A. CAT level in serum highly significantly (P<0.01) decreased at day 0 to day 7 in treatment group, then it gradually increased (P>0.05) to normal on day 14 to day 21 post-surgery in group B compared to group A.

Table I. Effect of pre and post-surgical correction of left displacement abomasum on (BHBA and NEFA) and oxidative stress markers in dairy cows.

Parameters	Time of sampling (Days)	Control group	Treatment group	P value
BHBA (mmol/L)	0	0.809±0.11	1.80±0.48**	0.000
	7	0.81±0.08	1.51±0.43**	0.000
	14	0.83±0.07	1.25±0.39**	0.004
	21	0.84±0.07	1.00±0.26	0.219
	28	0.87±0.07	0.90±0.20	0.892
NEFA (mmol/L)	0	0.61±0.24	1.48±0.44**	0.000
	7	0.61±0.21	1.15±0.42**	0.001
	14	0.62±0.20	0.81±0.31	0.146
	21	0.59±0.19	0.69±0.24	0.439
MDA (nmol/L)	0	7.13±0.76	12.89±0.85**	0.000
	7	7.09±0.65	11.55±0.66**	0.000
	14	7.17±0.58	8.69±0.58**	0.000
	21	7.21±0.51	7.36±0.44	0.648
	28	7.44±0.35	7.33±0.44	0.756
Catalase (Ku)	0	1.39±0.95	0.39±0.30**	0.000
	7	1.41±0.92	0.63±0.23**	0.003
	14	1.42±0.88	0.91±0.19	0.44
	21	1.47±0.86	1.16±0.17	0.303
	28	1.46±0.78	1.49±0.21	0.987

Data are indicated as (M±SD) and ** shows the values are highly significant (P<0.01).

Hematological parameters

Levels of Hb, TLC, neutrophils were significantly (P<0.01) higher at day 0 to day 7 in treatment group as compared to control group which became normal (P>0.05) at day 28 red blood cells and lymphocytes were extremely significantly lower (P<0.01) at day 0 to day 14 in LDA affected animals as compared to control group which became normal (P>0.05) at day 28 post-surgery. The level of platelets highly significantly (P<0.01) increased at day 0 which gradually decreased to normal at day 28 in the treated group compared with the control group (Table II).

DISCUSSION

Left displacement abomasum is an important metabolic disorder in dairy cattle. The problem mostly happens after 3-4 weeks of parturition. It is reported that 44% of cases occurred in the first week while 52% of cases occurred after 3 to 4 weeks of parturition (Rohn *et al*., 2005). LDA is associated with metabolic disorder, hematological (Ghazy *et al*., 2016b), and oxidative stress (El-Deen and Aboulnasr, 2014).

Table II. Effect of pre and post-surgical correction of left displacement abomasum on hematological analysis in dairy cows.

Parameters	Time of sampling (Days)	Control group	Treatment group	P value
Hemoglobin (g/dL)	0	10.58±0.70	13.07±0.75**	0.000
	7	10.22±0.86	11.98±0.93**	0.000
	14	10.27±0.86	11.18±1.15	0.051
	21	9.98±1.19	10.37±0.83	0.514
	28	9.91±0.95	10.0±0.78	0.956
Total leucocytes count (10^3/µL)	0	6.46±0.88	8.69±0.73**	0.000
	7	6.68±1.01	8.08±0.57**	0.000
	14	6.91±0.98	7.65±0.64*	0.04
	21	7.06±0.85	7.21±0.51	0.816
	28	7.04±0.606	6.94±0.45	0.872
Red blood cells (10^6/µL)	0	6.54±0.24	5.55±0.25**	0.000
	7	6.55±0.36	5.67±0.20**	0.000
	14	6.54±0.23	5.96±0.23**	0.000
	21	6.55±0.32	6.31±0.38	0.152
	28	6.54±0.34	6.48±0.27	0.855
PLT (10^3cells/µL)	0	353.84±46.78	388.61±12.54**	0.001
	7	354.30±47.56	381.23±6.67	0.068
	14	353.46±46.69	378.84±6.51	0.080
	21	353.23±46.74	375.05±8.10	0.152
	28	353.53±46.85	374.30±9.46	0.184
Neutrophils (10^3 cell/µL)	0	2.76±0.42	3.77±0.27	0.000
	7	2.91±0.41	3.55±0.30	0.000
	14	2.91±0.40	3.14±0.14	0.127
	21	2.91±0.39	2.87±0.19	0.956
	28	2.90±0.38	2.82±0.17	0.759
Lymphocyte (10^3 cells/µL)	0	4.65±0.21	2.64±0.38**	0.000
	7	4.64±0.20	3.01±0.36**	0.000
	14	4.71±0.13	3.39±0.33**	0.000
	21	4.66±0.09	3.92±0.25**	0.000
	28	4.60±0.10	4.55±0.20	0.637

Data are indicated as (M±SD) and ** shows the values are highly significant (P<0.01), while * shows values are significant (P<0.05).

In the current study, the rumen was displaced dorsally by the abomasum and the abomasum was observed between the rumen and the abdominal wall (El-Attar *et al*., 2008). In all left displacement abomasum cow's heterogeneous appearance was imaged dorsally at 10th-12th intercostals spaces. This finding was similar to (Ok *et al*., 2002). Reverberations were produced due to gas

cap accumulation; it was observed dorsally. Ventrally hypo-echogenic ingesta were also visualized (El-Deen and Aboulnasr, 2014). At the left dorsal region gas cap with the reverberation, artifact appeared. It might be due to the reflection of ultrasound waves by abomasal gas and reverberation between the abomasal surface and the transducer. These reverberations appeared as running parallel lines to the abomasum surface. Its strength became weaker as the distance increased from the transducer (El-Deen and Aboulnasr, 2014; Ok *et al*., 2002).

The results of the current study revealed that serum BHBA level was significantly increased in LDA group at day 0 as compared to the control group. After surgical correction, serum BHBA level decreased and until day 21st, it returned to normal. Our findings are in accordance with previous findings (Cardoso *et al*., 2008; Antanaitis *et al*., 2014). Higher values of BHBA might be due to increased fat oxidation as a result of higher supply of NEFA to the liver with consequent production of ketone bodies for energy production. Ismael *et al*. (2018) reported a dramatic increase in serum BHBA in animal suffering from energy imbalance, hepatic lipidosis, and endotoxemia.

In the current stud the NEFA level was significantly increased in the LDA group on day 0 as compared to the control. It was decreased after corrective surgery and attained a normal level on day 14th post-operatively. Our findings are compatible with the previous literature indicating higher values of NEFA in LDA animals (Cardoso *et al*., 2008; Khalaphallah *et al*., 2016a; Van-winden and Kuiper, 2003). The most possible reason for higher values of NEFA might be the deposition of fatty acids in liver due to negative energy balance in the animals affected with LDA (Khalaphallah *et al*., 2016a).

Our findings in terms of expression of stress markers revealed that MDA level was increased in the LDA group which dropped to normal on day 21st post-surgery. These results are in agreement with the previously published data indicating higher MDA levels in LDA affected Animals (Maden *et al*., 2012; Hasanpour *et al*., 2011a; Mamak *et al*., 2013b). The possible reason for increased serum MDA level might be due to increase in stress in LDA affected cows. Furthermore, stress increased lipid peroxidation resulting in release of MDA as a major metabolite (El-Deen and Aboulnasr, 2014; Ismael *et al*., 2018). Additionally, cytokines, an acute-phase protein, and oxidative stress took part in inflammatory reactions and developed metabolic disorders (Devrim *et al*., 2012).

In the present study, serum CAT level was significantly decreased in the LDA group. Similarly, increase hydrogen peroxide and MDA is reported with decreased serum CAT level in LDA affected cows (Fiore *et al*., 2019). The possible reason for decreased CAT level might be due to insufficient antioxidant level and higher oxidation rate due to LDA (Durgut *et al*., 2016b; Sattler and Furll, 2001; Van-winden, 2002).

Various hematological parameters such as Hb, TLC, PLTS, and neutrophils were elevated in the LDA group which dropped down to normal post-surgery for LDA. Previous studies reported that increased Hb level might be, due to dehydration, lack of fluid intake, and absorption from the abomasum (Dezfouli *et al*., 2013; Al-Rawashdeh *et al*., 2017a; Ismael *et al*., 2018). The data regarding elevated TLC and PLTS levels in current study is in accordance with the previous studies (Dezfouli *et al*., 2013; El-Deen and Aboulnasr, 2014; Al-Rawashdeh *et al*., 2017a).

The elevated TLC and PLTS levels might be caused by immune response to mastitis, peritonitis and endotoxemia in respect to LDA (Ismael *et al*., 2018).

CONCLUSION

The present study concluded that ultrasonography is a valuable diagnostic tool for the diagnosis of LDA. Moreover, LDA is associated with metabolic, hematological and oxidative disturbances which can be rectified through surgical correction. However, it is recommended to provide antioxidant therapy in adjunct with surgical correction for early recovery. The 14 days post-surgery follow up period is sufficient to monitor the pattern of changes in metabolites of LDA affected cows

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Statement of conflict of interest

The authors have declared no conflict of interest.

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