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Lipid Variations as Prognosticators of Cardiovascular Risks in Dairy Cattle with Mastitis

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ABSTRACT: *Clinical mastitis a deadly problem for dairy farmers and economy. Current study was designed to check serum lipid profile alteration with respect to cardiovascular diseases in clinical mastitis cattle. A total of sixty five samples were collected and divided into two groups for this case control study on the basis of clinical examination. For analysis, 40 cases and 25 controls were included. Statistical analysis was done by applying student "t" test using GraphPad Prism software 6.0. There was non-significant reduction (P=0.3) of serum total cholesterol (TC) and pronounced elevation (P=0.01) of triglyceride (TG) in cases as compared to controls. Mastitis group presented mild decrease (P=0.07) in high density lipoprotein-cholesterol (HDL-C) and highly significant elevation (P=0.0003) of low density lipoprotein (LDL-C) as compared to healthy cattle. Moreover, diseased cattle also manifested marked elevation (P=0.01) of very low density lipoprotein-cholesterol (VLDL-C) as compared to controls. Conclusively, clinical mastitis is associated with painful, swollen udder and abnormalities in milk. It also contributes to dyslipidaemia that could be used as useful indicator for evaluation of cardiovascular risks in cattle with clinical mastitis.*

Keyword: *Dairy cattle, clinical mastitis, cardiovascular disorders, dyslipidaemia*

INTRODUCTION

Mastitis is an infection of udder tissue that induces physical and bacterial changes in milk as well as pathological alterations in the mammary gland (Radostits et al., 2006; Ashraf and Imran, 2020). It is a multi-factorial disease that affects dairy cows on a global scale and has a significant economic impact (Das et al., 2018). Due to reduced milk production, poor milk quality, higher treatment costs and early culling of infected animals, it is becoming more important disorder

(Qayyum et al., 2016). The disease is classified as clinical or subclinical on the basis of severity of udder inflammation. Depending on the causative pathogen it may be environmental or contagious in origin (Belay et al., 2022).

Clinical mastitis is the destructive malady of dairy animals, which results in huge economic losses to the dairy industry of Pakistan (Hameed et al., 2012). It can be detected by physical examination of the animal. It is characterised by the increased body temperature, discoloration, swelling, pain and discomfort in the mammary gland. Physical and chemical alterations in milk were also observed in clinical findings (Sarvesha et al., 2017).

The prevalence of mastitis is less during first lactation stage while its occurrence become higher with increasing number of lactations. Its occurrence is also linked with exotic breeds that are more susceptible to mastitis (Sadashiv et al., 2014). The ineffectiveness of treatment for mastitis is related to a number of factors, including inadequate veterinary care, antibiotic resistance, pathological changes in udder tissue and numerous causative pathogens (Adesola, 2012).

Lipid metabolism is challenged in dairy cows significantly during periparturient period to meet the energy requirements (Turk et al., 2013). Alteration in serum lipid profile is the crucial component of the energy needs and physiology of the transition dairy cows (Gross et al., 2013). Alterations in lipid profile in mastitic cattle have been reported (Kovačić et al., 2019; Abdel-Hamied and Mahmoud, 2020). However, available literature about the effect of clinical mastitis on lipid profile in cattle is still scarce.

According to our knowledge, there is no published data on cardiovascular disease (CVDs) assessment in mastitic cattle in Pakistan. In order to evaluate risks of CVDs in dairy cattle suffering from clinical mastitis, quantitative variations of lipid profile are investigated in present study.

MATERIALS AND METHODS

Sampling Facility and study design

The study was approved by the Ethical Review Committee of Institute of Zoology, University of the Punjab, Lahore. The research was carried on a total of 65 cattle, 3 to 7 years old, in their first to fifth parity belonging to the rural farms of Pattoki, District: Kasur and Bhera, District: Sargodha in Punjab province of Pakistan.

Study was performed from December 2021 to February 2022. Dairy cattle were kept in rural setups. All animals were fed with mixed ration and milked by hand, twice a day. Before sampling, a comprehensive proforma was prepared to record the etiological factors i.e., parity number, lactation period, age, milking method, milk yield, farm condition and common feed.

Visual examination of udder and teats was done on farm. California mastitis test (CMT) was performed to distinguish between healthy and infected cattle. On basis CMT screening test, cattle were categorized in two groups i.e., Control group and Mastitis group. The control group was comprised of healthy cattle. While, mastitis group included cattle with clinical symptoms like udder infection, abnormal milk production, reduced appetite, ruminant contraction, elevated respiratory and heart rate, water loss and hyperthermia.

Blood samples were collected from jugular vein in aseptic conditions. After phlebotomy, samples were left for thirty minutes at room temperature and centrifuged for fifteen minutes at 3000 rpm to collect serum. Then, the serum was stored at -80°C, until further biochemical usage.

Serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) of control and mastitis group were evaluated using commercially available kits of "Monlab", Spain through chemistry analyzer (Robert Riele Photometer 5010). Whereas, levels of very low density lipoprotein cholesterol (VLDL-C) were calculated

by the method of Friedewald et al. (1972).

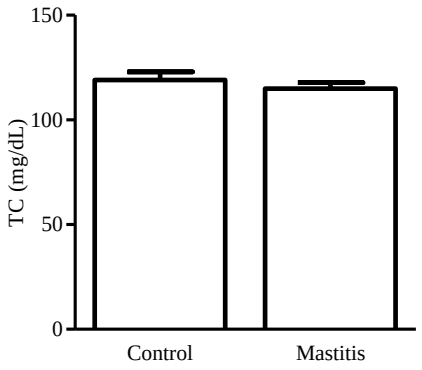
STATISTICAL ANALYSIS

Biochemical comparison between control and mastitis group was done by applying independent student "t" test using Graph Pad Prism version 6.0 software. Data of individual groups was expressed as Mean ± SEM. Mean values were significant at significance level $P \leq 0.05$.

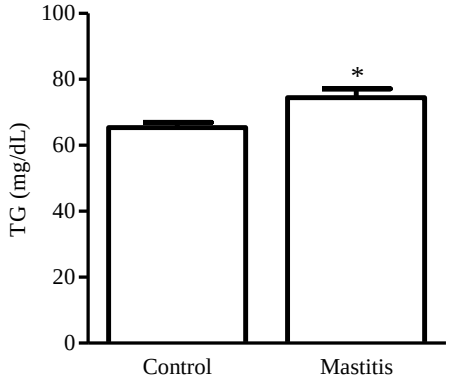
RESULTS

Table 1 indicated the overall comparison of lipid profile in control and mastitis groups. Control vs mastitis group comparison demonstrated non-significant difference (P=0.3) with 3% decrease in TC levels in mastitis group as compared to controls (Figure 1a). The significant increase (P=0.01) of 13% in TG levels was found in mastitis group as compared to controls. (Figure 1b). While, non-significant difference (P=0.07) with 12% reduction of HDL-C was noticed in mastitis group, when compared with Controls (Figure 1c). Moreover, control vs mastitis group comparison depicted prominent difference (P=0.0003) with 49% elevation of LDL-C levels in mastitis group (Figure 1d). Lastly, statistically marked difference (P=0.01) was observed in mastitis group with 13% increase of VLDL-C as compared to controls (Figure 1e).

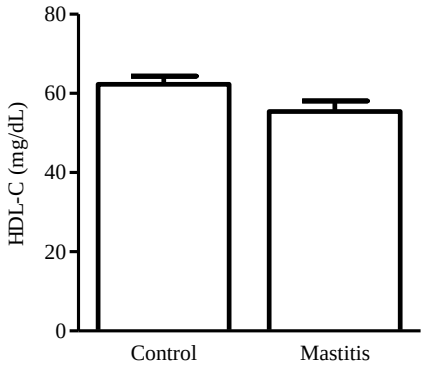
(a)



(b)



(c)



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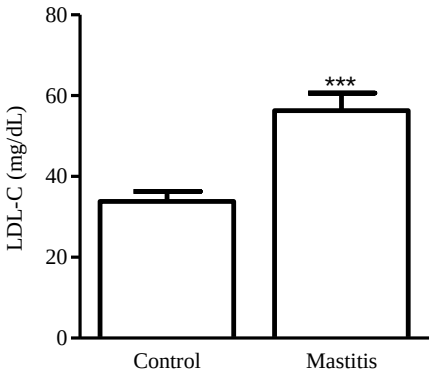

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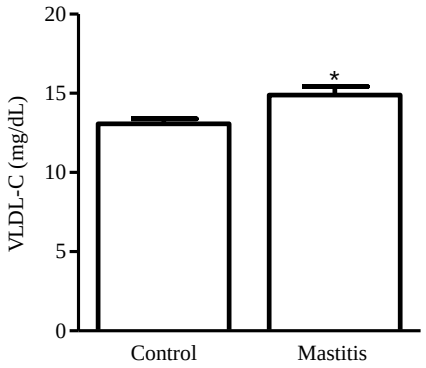

Figure 1: (a-e): An overall presentation of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C). Values

are Mean ± SEM in control and mastitis group. Significant at *P ≤ 0.05 and ***P ≤ 0.001

Table 1: A comprehensive presentation of lipid profile in control and mastitis cattle

Parameters	**Mean ± SEM**		**P-value**	**% Difference**
	Control (n=25)	**Mastitis** (n=40)		
TC (mg/dL)	119.00± 3.88	115±2.83	0.30	3↓
TG (mg/dL)	65.36±1.61	74.45±2.65	0.01	13↑*
HDL-C (mg/dL)	62.28±2.08	55.45±2.64	0.07	12↓
LDL-C (mg/dL)	33.81±2.58	56.29±4.37	0.0003	49↑***
VLDL-C(mg/dL)	13.07±0.32	14.89±0.53	0.01	13↑*

↓: Decrease, **↑:** Increase.

, *** indicate significance at P ≤ 0.05, and 0.001, respectively

TC; Total cholesterol, TG; Triglyceride, HDL-C; High density lipoprotein cholesterol, LDL-C; Low density lipoprotein-cholesterol and VLD-L; Very low-density lipoprotein-cholesterol.

DISCUSSION

Present study determines changes in lipid biochemical parameters of cattle suffering from clinical mastitis and their association with cardiovascular risks. Mastitis is an inflammatory response due to pathogen invasion in the mammary gland. Macrophages, leukocytes and other inflammatory cells create reactive oxygen species (ROS) during an inflammatory reaction, which helps to kill bacteria but also damages tissues around them (Pham, 2006). Oxidative stress may develop due to ROS. High amount of ROS and lipid hydroperoxides produced from oxidative stress may also contribute to the death of cells and tissues (Ryman et al., 2015). Moreover, organoleptic properties of milk are degraded by excessive ROS accumulation in milk and lowers milk quality (Novac et al., 2022).

In the current study, milk presented physical abnormalities like blood flakes, abnormal consistency, pus and discolouration. Poor quality milk was not recorded in healthy cattle. Similar trends were observed by Radostits et al. (2006).

Changes in lipid metabolism occur during acute phase response (APR) including alterations in the HDL particle. Antioxidant proteins are removed from HDL-C particle during inflammatory response, whereas, proinflammatory proteins enriched it (Feingold and Grunfeld, 2010). Alteration in serum lipid profile and lipid metabolism is important for the energy requirements and affects the physiology of peripartum or transition cows (Gross et al., 2013; Arfuso et al., 2016) but also during high lactation periods and later during some pathological diseases i.e., subclinical and clinical mastitis (Kovačić et al., 2019).

Cholesterol serves as a building block for all steroid hormones, bile acids and is essential for transmembrane signalling, membrane trafficking, and cell growth (Fernández et al., 2004). Inspite its significance, imbalance in cholesterol amounts may have detrimental effects on cells and can result in conditions like atherosclerosis (Maxfield and Tabas, 2005). Our findings demonstrate non-significant reduction in serum total cholesterol (TC) levels in cattle with clinical mastitis when compared to healthy controls. Ali et al. (2017) recorded similar results in plasma samples of cows affected with clinical mastitis. It was reported by Kovačić et al. (2019) that TC decreases during inflammation. Moreover, inflammatory mediators such as LPS, TNF and IL-2 lower blood cholesterol concentrations (Khovidhunkit et al., 2004).

Triglycerides (TG) are major source of energy, produced by fatty acids and glycerol combination (Walker et al., 1990). Formation of TG occur in liver and it is packaged in form of very low density lipoprotein (VLDL). Clearance of triglyceride rich lipoproteins (TGRLs) is catalysed by lipoprotein lipase (LPL) and ineffective

delipidation of TGRLs induce production of cholesterol enriched remnants. Small sized remnants pass through endothelial layer and taken up by arterial wall's macrophages (Borén and Williams, 2016). These remnants and cytotoxic free fatty acids can give rise to proatherogenic adhesion molecules and inflammatory mediators (Nordestgaard, 2016; Sandesara et al., 2019).

Present study has shown significantly higher concentrations of serum TG in cattle with clinical mastitis than in healthy cattle. These results are in accordance with (Kovačić et al., 2019). Elevated levels of triglycerides have been associated with increased production of VLDL due to insulin resistance, which results in formation of small dense LDL molecules, triglyceride rich atherogenic remnants and HDL particles (Adiels et al., 2008).

Metabolic dysregulation results in hypertriglyceridemia due to high production of VLDL, delayed clearance of remnants by liver and disturbed activity of lipoprotein lipase in peripheral tissues. As far as humans are concerned, higher levels of TG and TGRL remnants have been documented as risk factors for the development of CVDs (Hassing et al., 2012). Hence, it can be depicted that elevated TG concentrations can increase chances of CVDs in animals.

Normal levels of HDL-C not only play important role as antioxidative, anti-apoptotic and anti-inflammatory particle but also prevents the risks of atherosclerosis (Ali et al., 2012). In our study, HDL-C concentrations in mastitis group were slightly lower than in healthy controls. It is most likely as a result of lipoprotein particle remodelling and cholesterol translocation from HDL to other lipoprotein particles (Tabet and Rye, 2009). According to Feingold and Grunfeld (2010), cholesterol reverse transport is decreased during APR. Moreover, reduced serum HDL-C levels may be due to impaired liver secretion of apolipoprotein A, which plays primary role in formation of HDL-C (Esteve et al., 2005). Hence, it

increases chances of atherosclerotic events.

LDL-C particles are the important carriers of cholesterol in bloodstream (Trinick and Duly, 2005). LDL particles in circulation are taken up by endothelial lining of arterial wall and then trapped in arterial intima. They may undergo oxidation and ingested by macrophages to promote atherogenesis (Freeman, 2010). LDL-C is investigated as the most important atherogenic lipoprotein (Hirayama and Miida, 2012). During inflammatory response, different changes to low density lipoprotein cholesterol (LDL-C) increases atherosclerotic events. Oxidation of small dense particles of LDL-C is more likely to occur to initiate atherogenesis (Ivanova et al., 2017).

LDL-C levels displayed significant elevation in mastitic cattle compared to healthy cattle. Higher levels of LDL-C can be attributed to hypertriglyceridemia which results in increased amounts of small, dense LDL particles and remnant lipoproteins due to accumulation of VLDL and disturbances in delipidation of VLDL and LDL (Packard et al., 2020). Elevated levels of LDL-C are also due to genetic defects that affect structure of apolipoprotein B of LDL and function of LDL receptors or polygenic disorders disturbing lipid metabolism (Freeman, 2010).

Dyslipidaemia is a significant risk factor for the development of CVDs, primarily characterised by high levels of low-density lipoprotein cholesterol (LDL-C) and decreased HDL-C concentrations (Poss et al., 2011).

VLDL-C contributes to the transportation of hepatic triacylglycerol to the adipose tissue (Satyanarayana and Chakrapani, 2006). VLDL-C remnants are significantly atherogenic due to their smaller size, high cholesterol concentration and proinflammatory properties because of their triglyceride concentration (Nordestgaard, 2016).

The VLDL-C levels in mastitis group showed significant elevation in current study. It may be due to excessive triglycerides accumulation in the mastitic cattle. Moreover, it can be

attributed to large amount of fatty acids in liver which results in higher TG levels and their secretion as VLDL cholesterol (Khovidhunkit et al., 2004).

CONCLUSION

These alterations in lipid profile possess serious threats in developing cardiovascular diseases in cattle suffering from mastitis, which results in sudden economic loss to the farmers. Hence, the results of this investigation can be helpful to minimize the economic risks associated with the mastitis infection. Moreover, it is mandatory to have regular lipid biomarkers analysis to minimize the cardiovascular risks in cattle infected with clinical mastitis.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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