Assessment of lipid profile and acute phase protein in Mycobacterium avium subspecies paratuberculosis infected and healthy goats

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ABSTRACT

Present study is based on 24 goats that were located in goat herds endemicly infected with Mycobacterium avium subspecies paratuberculosis (MAP) infection. Objective of the study was to access the variations in the lipid profile and acute phase proteins in the serum samples driven from non-infected (negative and physically healthy) and infected (positive and physically weak) goats with MAP infection, cause of incurable Johne’s disease (JD) in domestic livestock. Infected goats had significantly higher cholesterol and albumin levels and significantly ‘reduced level’ of high density lipoprotein (HDL) and ‘reduced level’ of the density of lipoproteins (LDL) in comparison to non-infected goats. Lipid profile and acute phase proteins could be further explored for their significance in pathogenesis and diagnosis of JD in domestic livestock including goats.

Key words: Acute phase proteins, Johne’s disease, Lipid profiling, Mycobacterium avium, Paratuberculosis

Johne’s disease (JD) is chronic granulomatous enteritis caused by Mycobacterium avium subspecies paratuberculosis (MAP) that can infect both animals and human population. Human population is acquiring infection through milk and milk products made from pasteurized milk as MAP is not in-activated during pasteurization and pose a serious public health problem (Grant et al. 2008, Singh et al. 2019).

Acute phase proteins are a class of proteins such as albumin, C-reactive protein, ceruloplasmin, transferrin, etc, whose plasma concentrations increase or decrease in response to various inflammatory and neoplastic conditions (Jain et al. 2011). Binding of C-reactive protein (CRP) to pathogen also interacts with specific receptors on phagocytes, induces anti-inflammatory cytokine production, and modulates neutrophil function. Ceruloplasmin in levels increase in the inflammatory, and neoplastic diseases and could have a prognostic role in the diagnosis of the infections that are persistent or recurrent (Natesha et al. 1992).

Cholesterol is known to regulate hormones and basic cellular metabolism in the body (Hu et al. 2010). LDL and HDL, transport, cholesterol back and forth between tissues and liver (Chien et al. 2005). Ability of macrophages to uptake Mycobacterium is compromised due to a low cholesterol content of their cell membrane and thus represents a key defect in the host defence system against tuberculosis (Kaul et al. 2004). Lipids and lipoproteins play a role in host defence (Khoovidhunkit et al. 2004). Cholesterol, HDL, and LDL levels are known to have potential for predictors of clinical outcome (Van Gorp et al. 2002). Despite ability of lipoprotein profile to be able to serve as predictor of clinical outcome of infections; its role has not yet been elucidated in goats afflicted with JD. We speculate that assessment of acute phase proteins and lipid profiling in host sera could aid in assigning clinical stage of JD.

Keeping in view all these facts, present study was conducted to compare the levels of serum lipid profile and acute phase proteins in goats infected with MAP to those of apparently healthy goats (Nielsen and Toft 2008).

MATERIALS AND METHODS

Compliance with ethical standards: All animal studies have been approved by the Institute Animal Ethics Committee (Registration number is 207 with CPCSEA) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (BMJ 1996). All the persons involved in the study gave their consent to their inclusion in the study.
Sample collection: Of the 24 goats sampled, 12 were apparently healthy and 12 were infected suffering from clinical JD and were also confirmed by ELISA, PCR and Microscopy tests. The age groups of these goats were 12 months and above (adults). Goats in our animal health livestock shed were received from different goat units (6) on regular basis, whenever goats were not responding to primary treatment against diarrhoea or weakness, since JD was endemic in these six goat units. Goat herds (24) sampled were located at Central Institute for Research on Goats (CIRG), Makhdoom (Uttar Pradesh) and Mhow, Madhya Pradesh (India) (Table 1). Adult goats of Barbari, Sirohi and Jakhrana, Non-Descript breeds were sampled and were clinical to advance stage of the disease (JD) with respect to physical condition. Goats located at CIRG were fed intensively by providing concentrate pellets, 6 h grazing, dry fodder (ad lib.), tree lopping and harvested green fodder. Despite intensive feeding of goats, some of the goats were getting weak and emaciated with or without diarrhoea on regular basis and were either culled or shifted to shed belonging to Health division. Serum were separated and stored at –20ºC till further analysis. ‘Indigenous ELISA kit’ was used to estimate sero-status (negative or JD positive). Blood and faeces were screened by PCR and microscopy for the confirmation of JD (Singh et al. 2013). Goats positive in the above three tests, twice at one month apart were considered positive for MAP infection. Serum samples from apparently healthy goats were found negative in 3 tests, were taken as negative controls.

Microscopic examination (Acid Fast Staining): Test was performed as per Singh et al. (2013). Representative MAP positive and negative samples are depicted in Fig. 1. 

ELISA: ELISA was performed as per method of Milner (1987) using plasma as samples. Analysis of OD values: S/P ratio value = [(Sample OD – Negative OD) / (Positive OD -Negative OD)]. Sample to positive ratios were derived to estimate corresponding status of JD in goats as per Collins (2002).

Isolation of DNA from faeces and blood and IS900 PCR: Isolation of DNA from faecal and blood samples was done as per the method of Singh et al. (2013) and Singh et al. (2010), respectively. IS900 PCR was performed as per Marsh et al. (1999) (using P-90B and P-91B primers (Singh et al. 2013). ‘MAP strain’ positive control DNA was provided by the Microbiology Laboratory, Central Institute for Research on Goats (CIRG, Makhdoom, India). Presence and yield of specific PCR product (413 bp) was analysed by 1.0% agarose gel electrophoresis.

Ceruloplasmin, Albumin, HDL, LDL, Cholesterol, C reactive protein estimations: Ceruloplasmin was estimated as per the method of Wolf et al. (2006). MAP Positive and negative serum samples were assayed photometrically by

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**Table 1.** Data represent the animal specification and health status of MAP positive and negative animal

| Animal no. | MAP positive goats | MAP negative goats |
|------------|--------------------|--------------------|
| Animal no. | Animal physical status and place of sampling | Breed | Animal no. | Animal physical status and place of sampling | Breed |
| S8669      | 2+, CIRG, Mathura   | Sirohi             | 117      | 4+, CIRG, Mathura   | Barbari |
| BE248      | 2+, CIRG, Mathura   | Barbari            | 435      | 3+, CIRG, Mathura   | Non-Descript |
| B1530      | 3+, CIRG, Mathura   | Barbari            | 153      | 4+, CIRG, Mathura   | Barbari |
| SE842      | 2+, CIRG, Mathura   | Sirohi             | 175      | 4+, CIRG, Mathura   | Barbari |
| BE250      | 3+, CIRG, Mathura   | Barbari            | 182      | 3+, CIRG, Mathura   | Barbari |
| 1593       | 3+, Mhow, MP        | Non-Descript       | 116      | 3+, Barbari         | Barbari |
| 1555       | 2+, Mhow, MP        | Non-Descript       | 123      | 4+, CIRG, Mathura   | Barbari |
| 1604       | 2+, Mhow, MP        | Non-Descript       | 181      | 4+, CIRG, Mathura   | Barbari |
| 1558       | 3+, Mhow, MP        | Non-Descript       | 180      | 3+, CIRG, Mathura   | Barbari |
| 1579       | 2+, Mhow, MP        | Non-Descript       | 166      | 4+, CIRG, Mathura   | Barbari |
| Goat–1 PC(GHB216) | 2+, CIRG, Mathura | Barbari            | Goat–1 NC | 4+, Kurkunda, Mathura | Non-Descript |
| Goat–2 PC(GHB126) | 2+, CIRG, Mathura | Barbari            | Goat–2 NC | 4+, Kurkunda, Mathura | Non-Descript |

4+ (Healthy); 3+ (Weak); 2+ (Weak and Suspected for JD); 1+ (Clinical cases of JD).
semi-automatic ‘Biochemical Analyzer’ (AGAPPE) using the commercial kits purchased from ERBA diagnostics Mannheim GmbH, Germany.

Statistical Analysis: Statistical analysis was done by Prism Pad software, USA

RESULTS AND DISCUSSION

Study was undertaken with respect to reports that lipids and lipoproteins play important role in host defences and immune responses against infections (Netea et al. 2009, Aora and Borah 2017). We attempted to assess differences in the concentration of lipid profiles (Cholesterol, LDL, and HDL) and acute phase proteins (Albumin, ceruloplasmin, C-reactive protein) between goats infected and un-infected with MAP.

Mean±SEM values of cholesterol (Fig. 2A), LDL (Fig. 2B), HDL (Fig. 2C), albumin (Fig. 2D), C-reactive protein (Fig. 2E) and ceruloplasmin (Fig. 2F) are shown. Significantly higher concentration of cholesterol (51.08±4.22 mg/dL) was recorded in MAP positive animals compared to MAP negative goat herd which was 27.93±3.55 mg/dL. In line with this, hypercholesterolemia impairs the initiation of adaptive immune response to tuberculosis and increased lipid levels aids to M. tuberculosis infections (Martens et al. 2008). In the battle between pathogen and the host, microbes utilize the lipids / lipo-proteins derived from the host to facilitate their survival and proliferation. For example, M. tuberculosis is known to catabolise cholesterol as energy source, which might facilitate the ability of this microbe to survive in macrophages (Feingold

Fig. 2. Lipid profile and acute phase proteins in MAP positive and negative animals.
Hypercholesterolemia led to increased mortality of mice infected with M. *tuberculosis* (Martens et al. 2008). Significantly high triglyceride levels in MAP positive goats have been reported by our group (Sharma et al. 2017). Thus by identifying these interactions, it may be possible to alter lipid/lipoprotein metabolism and generate favourable conditions for the host.

Interestingly levels of LDL (8.32±1.61 mg/dL) and HDL (14.90±1.66 mg/dL) were significantly lower in goats infected with MAP as compared to negative goats where the values were 13.97±1.77 mg/dL and 24.15±3.03 mg/dL respectively.

Several studies have reported that inflammation and infection are associated with decreased LDL (Khovidhunkit et al. 2004). In the present study also, we observed significant reduction in LDL levels in goats infected with MAP. Precise mechanism by which inflammation and infection decreases LDL and HDL levels is uncertain and is likely to involve multiple mechanisms (Khovidhunkit et al. 2004). Reduction in apolipoprotein A-I synthesis in the liver occurring during inflammation have been suggested to contribute to the decreased formation of HDL.

Inflammation results in decrease in Lecithin cholesterol acyl transferase (LCAT) which in turn leads to diminished cholesterol ester formation, thus hindering normal HDL formation. This ends up in decreased cholesterol contents of HDL. In addition, following infection, cytokines such as interleukin-1β, interleukin-6 also play major role in altering the stability and metabolism of lipoproteins. For instance, cytokine mediated enhanced enzymatic activity of secretary phospholipase A2 (sPLA2) and endothelial cell lipase, which metabolize key constituents of HDL, can modify HDL levels. Given the intricacy of lipoprotein metabolism, multiple pathways may be involved in subsiding HDL levels during infection and inflammation. Mice over-expressing sPLA2 have reduced HDL concentrations (De Beer et al. 2000) and HDL from these mice is catabolised more rapidly than HDL from normal mice. Thus it is possible that in our study, infection induced cytokine mediate higher lipase activity and thus reduced HDL levels that we have observed in MAP positive animals.

Infection can also mediate adjustments in lipid and lipoprotein metabolism in order to reshuffling of nutrient towards the immune cells involved in host defence and tissue repair (Khovidhunkit et al. 2004). For example, decreased reverse cholesterol transport has been reported following infection (both viral and bacterial) that helps in cholesterol conservation in immune cells such as macrophages (Sammalkorpi et al. 1988).

Upon infection, cytokines have been linked with the lipoprotein profile (Shaikh PZ). Tumour necrosis factor has been attributed to the cytokine responsible for coordination of both immune and inflammatory responses (Sharma and Thomas 2013).

Albumin levels were significantly higher (2.86±0.113 g/dL) in infected goats compared to non-infected MAP negative goat herd (2.325 g/dL), which is surprising because albumin concentration (negative acute phase protein) decreases following infections. True over-production of albumin is not known to occur in any animal. Therefore, any rise in albumin is only a relative hyper-albuminemia due to hemo-concentration as a result of water loss and dehydration. Higher albumin levels in JD positive cases might be due to the dehydration resulting from diarrhoea.

In cattle, weight loss despite adequate feeding accompanied by chronic diarrhoea are standard clinical signs of Johne’s disease (Singh et al. 2016). However, unlike cattle, diarrhoea is not a consistent or cardinal feature of goat paratuberculosis (Marnelln et al. 2005). Hyperprotenemia has been shown to occur in JD positive goats (Sharma et al. 2017). However, increased albumin levels in JD positive goats warrants further investigations.

In the current study, no differences were observed in C-reactive protein and ceruloplasmin levels among the MAP positive and negative goats. We speculate that since paratuberculosis is a chronic disease, it is conceivable that T cell exhaustion might possibly occur as a result of which diminished cytokine production by exhausted T cells could be responsible for the unaltered levels of the acute phase proteins. It would be worth estimating the levels of pro-inflammatory cytokines in JD positive and negative animals. However, given the fact that at the time of sampling, JD was endemic in domestic goat population and goats were in different stages of disease incubation and moreover age, sex and physical status of the animals might also influence the parameters observed.

Thus it could be concluded that goats infected with MAP had significantly higher cholesterol and albumin levels and significantly lower HDL and LDL levels as compared to the un-infected goats. Further studies with large number of JD positive and negative goats for their physical status, lipid profile and acute phase proteins could be explored for their role in pathogenesis and diagnosis of Johne’s disease in domestic livestock.

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