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Aarif Ali
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India.

Bilal Ahmad Mir
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Rahil Razak Bhat
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Omer Khalil Baba
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Ishraq Hussain
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

S Mudasir Rashid
Faculty of Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Showkeen Muzamil
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Sheikh Bilal Ahmad
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Manzoor-ur Rahman Mir
Molecular Biology Lab, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Science & Technology (SKUAST-K), Srinagar, Jammu and Kashmir, India

Correspondence
Manzoor-ur Rehman Mir
Professor & Chief Scientist, Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e Kashmir University of Agriculture Science & Technology (SKUAST-K), Alusteng, Shuhama, Srinagar, J&K, India

Metabolic profiling of dairy cows affected with subclinical and clinical mastitis

Aarif Ali, Bilal Ahmad Mir, Rahil Razak Bhat, Omer Khalil Baba, Ishraq Hussain, S Mudasir Rashid, Showkeen Muzamil, Sheikh Bilal Ahmad and Manzoor-ur Rahman Mir

Abstract
The study was conducted to determine the effect of subclinical mastitis (SCM) and clinical mastitis (CM) on biochemical parameters of cows. A total of 200 samples of milk and blood, were collected from different areas of District Rajouri of J&K state. Before collection of blood and milk samples, the selection of animals from which samples had to be taken were put forward to screening by different cowside tests i.e. California Mastitis Test, electrical conductivity and pH. Subclinical mastitis were screened by California Mastitis Test (CMT), milk pH and electrical conductivity (EC) of milk. After screening Blood and milk samples were collected from 120 healthy, 50 SCM and 30 CM affected cows. Blood samples were analyzed for biochemical parameters. The present study was designed to study the possible biochemical profile (Glucose, Triglyceride, Albumin, Cholesterol, Globulin, HDL and TP) in the plasma of selected animals. Our study showed that total protein concentration was significantly ($p \leq 0.05$) higher in subclinical and clinical mastitic animals, compared to the control groups. Albumin level of the subclinical and clinical infected animals was significantly ($P < 0.05$) lower than the control group. Other parameters like glucose, cholesterol, triglyceride and HDL of subclinical and clinical mastitic animals showed non significant results as compared to the control group. Further, significant ($p \leq 0.05$) increase was observed in EC and pH of milk from SCM and CM infected animals compared to the control groups. Our study demonstrates that there is a significant increase in the levels of total protein, EC and pH in mastitic animals as compared to control groups and a significant reduction in the levels of albumin with the mastitic groups compared to control groups. Also glucose, cholesterol, triglyceride and HDL of subclinical and clinical mastitic animals showed non significant results as compared to the control group.

Keywords mastitis, subclinical, clinical, biochemical, CMT

Introduction
One of the most important food consumed by human beings is the milk. Due to the presence of essential components in the milk, it is universally regarded as a complete diet [1], to the surveys carried out in field, mastitis is considered as the number one disease affecting livestock [2]. Due to the tremendous rise in the population worldwide the demand for milk has increased. But due to various factors the production of milk is affected. All over the world, one of the serious problems faced by dairy farmers is Mastitis that decreases the production of milk and causes severe economic loses [3]. Mastitis is an inflammation of the mammary gland characterized by physical, bacteriological, pathological alterations in milk and tissue of mammary gland. Depending upon the severity of infection, it is classified as subclinical or clinical and on the basis of causative agent it is referred to as contagious and environmental based [4]. The typical signs of clinical mastitis are swollen, painful, discolored udder, sudden decrease in milk production and change in milk composition, whereas in subclinical mastitis there are no apparent or visible signs seen. However in both forms of mastitis there is an increase in somatic cell count and decrease in milk yield [5]. The occurrence of mastitis is lower in first lactation whereas its prevalence increases with higher number of lactations. The prevalence of mastitis is also associated to breed with crossbred and exotic breeds more prone for mastitis. The prevalence of changes in mastitis may be regional, breed wise, therapeutic, managemental and various causative microorganisms in the environment [6]. Lack of proper veterinary services, antimicrobial resistance, pathological alterations in the mammary gland and various causative organisms are all the factors that are attributed to the failure of treatment in Mastitis [7].

The main aim of the present study was to evaluate the biochemical changes in plasma of bovine animals affected with mastitis.

Materials and Methods

Study design and animals: The study was carried on 200 bovine animals of the hilly areas of district Rajouri. In this study the animals were grouped into three groups: Healthy (120), Subclinical (50) and Clinical (30).

Screening of animals

The screening of animals was done by performing following cowside tests on spot.

California Mastitis Test (CMT)

The milk samples collected were subjected for California mastitis test (CMT).

Electrical Conductivity (EC) test

Differential electric conductivity of milk samples (i.e. separate EC of each quarter) was measured by digital electric conductivity meter as described by the manufacturer (Eutech, Singapore).

Measurement of pH in Milk

Milk pH in all milk samples was measured by digital electric pH meter as described by Muhammad *et al*., (2013) [8]

Selection of Animals

The Blood samples were collected from jugular vein in 200 animals of different areas of district Rajouri (J&K). Out of these, 50 were diagnosed for subclinical mastitis, 30 for clinical mastitis and 120 were normal healthy on the basis of physical tests (CMT, pH, electrical conductivity).

Blood sampling

Blood samples were stored in (2-3 ml) EDTA vials and transported to the laboratory within one hour in a thermo flask with ice. Blood was centrifuged at an RPM of 3000 for 10 minutes to extract plasma and stored in separate vials at –20ºC for biochemical analysis.

Biochemical analysis

The Plasma separated from blood was analyzed for Glucose, Triglyceride, Albumin, Cholesterol, Globulin, HDL and TP by using semi-automated biochemical analyzer. All the biochemical parameters were estimated by using commercially available kits.

Statistical analysis

The data from individual groups are presented as the mean ±standard error of the mean (SEM). Difference between groups were analysed by using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test and minimum criteria for statistical significance was set at $p \leq 0.05$ for all groups.

Results and Discussion

Subclinical mastitis and clinical mastitis are of great economic importance as both forms are associated with reduction in milk yield which causes heavy economic losses. The present study was carried out to study the biochemical profile of control as well as diseased mastitic animals. In our study biochemical estimation revealed significantly ($p \leq 0.05$) higher values of total protein in mastitic animals as compared to the control groups. Significant increase in total protein (TP) level was observed in SCM cases as compared to healthy animals, however a non-significant rise was observed in CM infected animals. However, reduced TP values in mastitic cases were observed by Zaki *et al*. (2008) [9]. This may be attributed to the decreased albumin levels after the immune response to the udder infection [10]. Significantly ($P < 0.05$) lower average values of glucose, Cholesterol, triglyceride, HDL were observed in SCM infected animals, however no significant change were observed in values of CM infected than healthy animals. Albumin level of the subclinical and clinical infected animals was significantly ($P < 0.05$) lower than the control group as shown in table 1. The hypoalbuminaemia indicates stress condition that enhances protein catabolism as happens in various infections and trauma. Albumin has been widely considered as a negative acute phase protein [11]. According to the studies of Diwivedi *et al.*; 2004 [12] a non significant increase in average values of Albumin in mastitic cows was recorded. Significant increase in total protein levels of subclinical cases as compared with control group was observed, however non significant rise was observed in clinically infected animals as shown in table1. Globulin level of subclinically and clinically infected animals was 2.38 g/dl and 2.06g/dl respectively which was significantly lower than the control group animals (2.90 g/dl). Other biochemical parameters like glucose, cholesterol, triglyceride and HDL of subclinical and clinical mastitic animals showed non significant results as compared to the control group.

Further, significant increase in EC of milk from SCM and CM infected animals was observed as compared to the control groups. This is in accordance with the reports of sripad *et al.* 2013 [13]. The rise in EC of milk in mastitic animals could be due the elevated levels of ions such as sodium, potassium, calcium during the inflammation of the mammary gland. Also pH of subclinically and clinically infected animals showed significant results as compared to the control group. The increase in the pH of affected milk in the could be due to increased permeability of the gland tissue to blood components results in higher values in milk. Partially it might also be due to increased movement of bicarbonate ions into milk, since the lactose production decreased and the alkaline salts from the blood enters, the milk become more alkaline showing pH above 7.0 as indicated by Rao (1990) [14].

Table 1: Mean±SEM activities of Glucose, Total protein, Cholesterol, Triglyceride, Albumin, HDL and Globulin in plasma from Healthy, Subclinical and Clinical mastitis.

Parameters	Healthy	Subclinical	Clinical
Glucose	52.85± 1.02^a	52.20± 0.80^a	52.26± 0.99^a
Total protein	7.15 ± 0.18^a	7.90± 0.10^b	7.30± 0.13^a
Cholesterol	103.90±2.14^a	103.80±2.30^a	103.66±2.14^a
Triglyceride	75.11± 3.13^a	72.32± 4.26^a	73.52± 1.72^a
Albumin	3.95±0.11^a	3.54±0.14^a	3.10±0.08^b
HDL	50.82±1.45^a	47.57±2.09^a	46.42±0.75^a
Globulin	2.90±0.10^a	2.38±0.14^b	2.06±0.11^a

Values with superscripts a, b and c differ significantly ($P<0.05$) in a row.

Table 2: Mean ± SEM activities of E.C and pH in plasma from Healthy, Subclinical and Clinical mastitis.

Parameters	Healthy	Subclinical	Clinical
pH	6.20±0.05^a	7.10±0.06^b	7.40±0.08^b
E.C	3.23±0.04^a	4.35±0.09^b	5.23±0.09^c

Values with superscripts a, b and c differ significantly ($P<0.05$) in a row

Conclusion

In conclusion, the changes in the biochemical parameters could be used as important indicators of the physiological or pathological state (mastitis) of the animal. Our study demonstrates that there is a significant increase in the levels of total protein, EC, pH in subclinical and clinical mastitis as compared to the control healthy groups. Also levels of albumin and globulin of the subclinical and clinical infected animals were significantly lower than the control group. Other biochemical parameters like glucose, cholesterol, triglyceride and HDL of subclinical and clinical mastitic animals did not show statistically any significance as compared to the control group.

In addition, we conclude that the total protein, albumin, globulin, CMT, pH, EC are ideal for early detection of subclinical and clinical infected quarters. This will help in the selection of diseased dairy animals for either segregation or treatment.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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