



RESEARCH ARTICLE

PATHOLOGY

HEMATOLOGICAL AND BIOCHEMICAL STUDIES IN TUBERCULIN TEST POSITIVE REACTORS.**MOHANKUMAR SHETTAR^{1*}, T.S.NALINI², K.R ANJAN KUMAR³,
P.RAVIKUMAR⁴ AND H.R. AZEEMULLA⁴**

Veterinary Pathology, Veterinary College, Hebbal, Bangalore-24

¹ *Ph D Scholar, Department of Pathology, Veterinary College, KVAFSU, Bangalore – 560024² Professor (Rtd) ,Department of Pathology, Veterinary College, KVAFSU, Bangalore – 560024³ Assistant Professor, Department of Pathology, Veterinary College, KVAFSU, Bangalore – 560024⁴ Veterinary Officers, Department of AH & VS, Govt of KarnatakaDr. **MOHANKUMAR SHETTAR**Ph D Scholar, Department of Pathology, Veterinary College, KVAFSU, Bangalore –
560024

*Corresponding author

ABSTRACT

The current study was conducted to correlate the interrelationship between the tuberculosis positive reactors with non-reactors with respect to the hematological and biochemical parameters. A total of 126 animals from an organized dairy farm were subjected to single intradermal tuberculin test following an outbreak and a total of 15 animals each from both the reactor and non reactor group were included for the study. There were significant alterations in Erythrocyte sedimentation rate, Hemoglobin content, Total erythrocyte count, Monocyte, Neutrophil and Eosinophil number & Mean corpuscular volume values in reactors when compared to non-reactors.



KEY WORDS

Tuberculosis, Hematology, Biochemistry

INTRODUCTION

Tuberculosis is a disease of high zoonotic potential and has been a scourge of man and animals from time immemorial. Active animal tuberculosis outbreaks represent possible sources of infection to both animal and human populations^{1, 28}. The Office International des Epizooties classifies BTB as a list B transmissible disease of public health importance and is of high significance to the international trade of animals and animal products¹⁹. Tuberculin testing has traditionally been used to determine the prevalence of infection in animals and human. Variation in the blood picture is a good aid in the diagnosis and prognosis of a disease and as such it has been a subject of study in several diseases of animals. The present study was taken up to understand the correlation between different hematological features in the typical cases of tuberculosis in dairy herds with that of non reactors.

MATERIALS AND METHODS

A total of 126 animals in an organized dairy farm at University of Agricultural Sciences, Bangalore, India were screened for tuberculosis using Single Intradermal (SID) technique, with Purified protein derivative (PPD) prepared from *M. bovis* strain AN-5 containing 1mg of PPD per ml (20,000 tuberculin units), obtained from Biologicals Division IVRI, Izatnagar, UP. A total 126 animals were included and 0.1 ml (2,000 units) of tuberculin was injected intra-dermally. The animals that showed reaction were called as positive reactors and with no reaction were called as non-reactors. A number of 15 animals each, irrespective of breed and age from both the positive reactors and non reactors were randomly selected for studying the hematological and biochemical changes.

The whole blood samples were collected in clean and dry tubes with and

without anticoagulant (EDTA at 1-2mg per ml of blood), for studying haemogram and biochemical parameters in post intra dermal injection of tuberculin. The following parameters were studied as per the standard techniques¹⁴. Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC): Total Leukocyte Count (TLC): Hemoglobin Concentration (Hb), Differential Leukocyte Count (DLC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC).

Serum samples were subjected to estimate total serum proteins, Albumin, Globulin Subtracting Albumin Concentration in total proteins, Total Cholesterol, Serum calcium Serum inorganic phosphorous, Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxalo-acetic transaminase (SGOT) and Alkaline phosphatase. Results of the studies were analyzed statistically adopting student "t" test for significance²⁵.

RESULTS AND DISCUSSION

Haemogram:

The results of hematological evaluation of tuberculin test positive reactors (n=15), and non-reactors (n=15) have been presented in Table. 1. In tuberculin test positive reactors there was an increase in erythrocyte sedimentation rate, mean corpuscular volume, eosinophil & monocyte number and decrease in total erythrocyte count & hemoglobin. There was also increase in mean hemoglobin concentration and decrease in Packed cell volume and Mean corpuscular hemoglobin concentration, which were not statistically significant (p>0.05). Erythrocyte sedimentation rate was not considered as a specific diagnostic value in tuberculous animals unlike in human beings, where it is

used frequently to evaluate the degree of the infection (Rao, 1992). In the present investigation significant increase in the ESR

values in the positive reactor ^{22, 23} was observed and could be used to evaluate probable degree of infection.

Table 1
Haemogram In Tuberculin Positive And Negative Reactors

PARAMETERS	REACTORS (15)	NON REACTORS (15)
ESR mm fall in 1 hr	3.67+0.79 *	1.38+0.57
PCV %	29.05+1.746	31.09+1.772
Hb gms/100ml	6.90+ .240 *	9.02+0.216
TLC per cmm	8039+631.2	8348.+258.51
TRC per cmm	4.52+0.587 *	7.38+0.648
LYMPHOCYTE%	62.7+2.08	66.9 + 1.26
NEUTROPHILS %	19.24+1.44 *	27.61+0.844
EOSINOPHILS %	11+1.807*	2 + 0.701
MONOCYTES %	4.05+0.846 *	0.047+0.047
BASOPHILS %	0.47+0.3	0.047+0.047
BAND CELLS %	2.33±.665	2.05+ .519
MCV Cubic Microns	87.41+13*	48.75+4.187
MCHC %	25.08+1.395	28.05+0.844
MHC Micro grams	18.30+2.231	13.83+0.7445

Figures in the parantheses indicates the number of animals used in the study *P<0.0

Decreased values of packed cell volume (PCV), Hemoglobin (Hb) & Total erythrocyte count (TEC) were observed in the tuberculin reactors as well as in animals with tubercular lesions ^{5, 8}. This decrease in PCV, Hb and TEC values could be attributed to poor health condition and chronicity of the disease and bone marrow atrophy. The leukocyte count was statistically insignificant (P 0.05) in the present study. However, slightly higher values of Total leukocyte count (TLC) and lymphocytes and lowered neutrophil count recorded in reactors when compared to non-reactors.

Further, it was observed that there was moderate degree of leukocytosis in six animals and leukopenia in seven animals. In the present study lymphocytosis in five cases and lymphopenia in seven cases were observed in the animals with tubercular lesions indicating a variation in lymphocyte numbers compared to other researcher's findings ^{16, 22}.

Neutropenia was observed in all the cases except in one where there was

moderate degree of neutrophilia ^{4, 16, 24, 27}. Haemogram of tubercular animals and reactors have shown considerable variation and this could be attributable to the advanced stage of the disease. The animals with discrete lesions may not indicate any change in the blood picture though it may elicit a positive tuberculin reaction and in chronic cases approaching fatal termination, a slight neutrophilic reaction along with anaemia may be observed ²².

Monocytosis was a common picture observed in blood samples of tubercular cattle ^{4, 10, 16}, whereas no significant difference was observed in monocyte percentage between reactors and non-reactors ²². However, in the present study mild to moderate degree of monocytosis was observed in six animals with tubercular lesion. As stated by Wintrobe (1974) and opined by Kaufmann (1990), monocytes have an important role in the cellular response to the tubercle bacillus and it is responsible for degradation of the phospholipids of the bacterial cell wall,

monocytes later transforms into epithelioid cell which is a characteristic feature in tubercular granulomas^{15, 30}. Hence monocytosis can be regarded as an evidence of active extension of tuberculous process.

In the present study increase in eosinophil percentage was highly significant in the reactor group which probably was due to the increase in the eosinophils²⁰, which was indicative of a benign period in the course of the disease; where as some of the researchers did not observe eosinophilia^{10, 12, 16, 23, 26}.

Biochemical studies:

The changes in the various biochemical constituents of serum in tuberculin test reactors and non-reactors have been summarized in Table 2. The results indicated that there was no significant difference in the total protein contents between the reactors and non-reactors but there was significant difference in the level of albumin, globulin and A/G ratio (P<0.05).

Reactors showed an increase in globulin content and decrease in albumin content and resulting in altered A/G ratio. In addition the total serum cholesterol in reactors were significantly higher when compared to the non-reactors (P<0.05) There was increase in the levels of SGPT, SGOT and serum calcium and decrease in inorganic phosphorous, which were statistically significant (P<0.05). Further there was increase in Alkaline phosphatase, though not statistically significant (P>0.05). Significant difference in total protein values were not seen between reactors and non-reactors in the present study. However, an increased level of serum total protein was observed in the animals, which had generalized tuberculosis which was observed in cases with large caseated tubercular lesions⁹. This variation in the protein spectrum was probably due to moderate and severe forms of pulmonary and generalized infection¹¹.

Table 2
Biochemical studies in tuberculin positive and negative reactors

PARAMETERS	REACTORS (15)	NON REACTORS (15)
SERUM CALCIUM mg %	9.8410.1427	6.7 0.338 ± 0.331
PHOSPHATE(in) mg %	4.88 ±0.611	7.38 ± 0.331
ALP iu per 100 ml	43.28 ±4.57	36.66 ± 3.037
SGOT IU per 100ml	80.66 ± 3.6	54.8 ± 2.889
SGPT IU per 100 ml	25.71 ±0.139	19.28 ±1.307
CHOLESTEROL mg %	156.47 ± 1.244	125.9 ±6.602
TOTAL PROTEINS gm %	6.948 ±0.189	6.75 ± 0.288
ALBUMIN gm %	2.69 ±0.146	2.807 ± 0.115 3.7
GLOBULIN gm %	4.29 ±0.143	0.901 ± 0.326
A/G RATIO	0.639 ±0.021 *	0.103

Figures in the parentheses indicate the number of animals used,*- P < 0.05

Albumin content in the serum of reactors was not variable when compared to non-reactors. However, altered albumin levels were reported by other researchers^{6, 9, 11, 13, 16, 17}. Significant increase in globulin content was observed in reactors when compared to non-reactors, which resulted in alteration in A/G

ratio in animals with tubercular lesions^{6, 16, 17}.

In the present study, total serum cholesterol was found to be significantly higher in the reactors when compared to non-reactors. Further, it was also observed that the animals with tubercular lesions had elevated serum cholesterol levels, which could be due to



extensive tubercular lesions⁹.

An increase in the levels of serum calcium and decreased serum inorganic phosphorus in the reactors were recorded in the present study.

However, marked decrease in the serum calcium level and increase in phosphorus content in tuberculous cattle²⁹, decrease in inorganic phosphorous levels with no change in serum calcium in tuberculin reactor^{2, 16, 18} was also observed by other researchers. Reactors with lesions showed

significant increase in the levels of transaminases which could be due to process of necrosis or degeneration taking place in the body^{3, 7}.

Bovine tuberculosis still continues to be a problem in developing countries, to understand the extent and prevalence of the disease, a routine tuberculin testing has to be carried out. Though the hematological and biochemical values are not much significant, alterations in the A/G ratio was significant indicator in tuberculosis.

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Abbreviations

%	Percent
P	
ALP	Alkaline Phosphatase
BTB	Bovine Tuberculosis
cmm	Cubic millimeter
DLC	Direct Leucocyte count
EDTA	Ethylene diamine tetra acetic acid
ESR	Erythrocyte sedimentation rate
gms	grams
Hb	Haemoglobin
hr	Hour
IU	International Unit
IVRI	Indian Veterinary Research Institute
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume



ml	Milliliter
mm	Millimeter
n	Number of samples
OIE	Office des epizootics
PCV	Packed cell volume
PPD	Purified protein derivative
SGOT	Serum glutamic oxalo-acetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SID	Single Intradermal
TEC	Total Erythrocyte Count
TLC	Total Leukocyte Count
UP	Uttar Pradesh