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Evaluation of clinical utility of serum enzymes of hepatic origin in clinically affected Marwari sheep of arid tract in India

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Abstract. The clinical utility of serum enzymes of hepatic origin was evaluated by determining their levels in the adult sheep of Marwari breed belonging to farmers' stock of arid tract of Rajasthan state, India. The animals were grouped into healthy and affected which comprised of fasciola infected, pneumonia affected, having enterotoxa122emia, and drought affected. The serum enzymes included were sorbitol dehydrogenase, malate dehydrogenase, glucose-6-phosphate-dehydrogenase, glutamate dehydrogenase, ornithine carbamoyl transferase, gamma-glutamayl transferase, 5'nucleotidase, glucose-6-phosphatase, arginase, and aldolase. In clinically affected animals the levels of hepatic serum enzymes were significantly ($p \leq 0.05$) higher as compared to respective healthy levels. In fasciola infected sheep the activities of all the enzymes were higher in comparison to healthy sheep. Degree of increase was least in pneumonia affected sheep. The G-6-Pase activity showed comparatively maximum change in fasciola infected animals as compared to other enzymes. As the activities of all the serum enzymes of hepatic origin were found increased in affected animals, it was concluded that they all had diagnostic significance exhibiting liver involvement. The increased activity was due to change of liver functions directly or indirectly.
Key words: drought, enterotoxaemia, enzymes, fasciola, hepatic, pneumonia.

Introduction. Certain tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage. The detection of alterations in hepatic functions can be done with the measurement of serum enzymes of hepatic origin (Varley 1988). Enzyme levels in serum rise due to increased membrane permeability, cell necrosis or cytosol leakage in the serum (Alemu et al 1977). Much attention has been paid to aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in veterinary clinical practice and several other hepatic serum enzymes i.e. gamma glutamyl transferase, 5'-nucleotidase, ornithine carbamoyl transferase, glutamate dehydrogenase, sorbitol dehydrogenase, arginase (Tennant 1999), aldolase, glucose-6-phosphatase etc (Wolf & Williams 1973) have been ignored. Moreover there is paucity of literature on the levels of these enzymes in sheep. To determine the diagnostic value of the blood serum level of the enzyme it is necessary to carry out investigations in animals when affected with various diseases (Goranov 1984) and then compare them with the normal range of values given for those animals, preferably of the same breed, and in similar environmental conditions. Marwari is a breed of sheep in arid tract playing an important role in the economy of marginal farmers. It is a melancholy that despite of immense quality characteristics of Marwari breed very little attention has been paid on the use of laboratory tools to ease out diagnosis. Studies on serum enzymes of hepatic functions have not been well

documented for sheep. Therefore, the present investigation was planned to determine levels of serum enzymes of hepatic origin at one platform and to find out their diagnostic significance in clinically affected Marwari sheep who are native to arid tract in India.

Materials and Methods. Serum enzyme levels were determined in 400 adult sheep of Marwari breed belonging to farmers' stock of arid tract of Rajasthan state, India. The animals were grouped into healthy (200) and affected (200). In healthy animals the blood samples were collected as a part of routine health checkup during moderate ambience (maximum temperature varied between 29 and 31 ^{0}C). The affected group comprised of fasciola infected (50), pneumonia affected (50), having enterotoxaemia (50) and drought affected (50) irrespective of sex.

All the samples were collected in sterile tubes without anticoagulants for serum separation. The serum enzymes included were sorbitol dehydrogenase (SDH, E.C. 1.1.1.14), malate dehydrogenase (MDH, E.C.1.1.1.37), glucose-6-phosphate-dehydrogenase(G6PD,E.C. 1.1.1.49), glutamate dehydrogenase (GD, E.C. 1.4.1.3), ornithine carbamoyl transferase (OCT, E.C. 2.1.3.3), gamma-glutamayl transferase (γGT, E.C. 2.3.2.2), 5'nucleotidase (5'NT, E.C. 3.1.3.5), glucose-6-phosphatase (G-6-Pase, E.C. 3.1.3.9), arginase (ARG, E.C. 3.5.3.1), and aldolase (ALD, E.C. 4.1.2.7). They were determined by the methods as described by King (1965) for SDH, MDH, G6PD, GD and G-6- Pase; by Brown & Grisolia (1959) for OCT; by Wolf & William (1973) for γGT; by Varley (1988) for 5'NT; by Manning and Grisolia (1957) for ARG and by Sibley and Lehninger (1949) for ALD.

Sorbitol dehydrogenase was determined by spectrophotometric method using a colour reagent with detection of fructose. Malate dehydrogenase assay used oxaloacetate substrate and reduced coenzyme and the procedure measured the rate of decrease in optical density at 340 mμ. Glucose-6-phosphate-dehydrogenase was determined by spectrophotometric method which used the rate of change of absorbance at 340 mμ. Glutamate dehydrogenase was determined by using adenosine-5'-diphosphate, which activated the enzyme present in serum and activity was recorded at 340 mμ using a spectrophotometer. For ornithine carbamoyl transferase determination, urea present in the serum was removed by incubating with urease. γ-Glutamyl-transferase hydrolysed the substrate γ-glutamyl-p-nitroanilide to yield p-nitroaniline and γ-glutamyl compounds. γ-Glutamyl-p-nitranillide is a chromogenic substrate and glycilglycine is a most commonly used acceptor molecule. Serum was incubated with substrate and the reaction was monitored at 405 mμ. As 5'-Nucleotidase is inactivated by nickel so that the hydrolysis was carried out with and without added nickel, and the difference gave the 5'-Nucleotidase activity. Glucose-6-phosphatase assay consisted of incubating the sample in the presence of a glucose-6-phosphate substrate buffered to pH 6.5 by means of a citrate buffer and precipitating the proteins to terminate the enzymic reaction and estimating the liberating inorganic phosphate. Arginase activity was assayed by determining the amount of arginine hydrolysed. A pink coloured complex was formed with urea and isonitrosopropiophenone. The colour reaction was depressed by the presence of arginine. The enzyme fructose-1,6-biphosphate aldolase, often simply called aldolase, was assayed by using an enzyme reaction, which was allowed to proceed for 30 minutes in the presence of the tris buffer. Following liberation of alkali-labile phosphate from triose esters at room temperature the reaction of free trioses with 2, 4 dinitrophenylhydrazine terminated. In this procedure the ratio of chromogenicity of triose phosphate to glyceraldehydes was 1.8. Serum enzyme activities were measured according to the reaction of each enzyme by using basic standard techniques. The required temperature maintenance was carried out according to the method for each enzyme and wherever required, necessary temperature corrections were done. All results of enzyme activities were expressed as per SI units in Units/litre written as U/l. Statistical significance for individual parameter between healthy and affected group was analysed (Snedecor & Cochran 1967). Abbreviations used in this paper: SDH=Sorbitol dehydrogenase, MDH=Malate dehydrogenase, G6PD=Glucose 6 phosphate dehydrogenase, GD=Glutamate dehydrogenase, OCT=Ornithine carbamoyl transferase,

OCT=Ornithine carbamoyl transferase, γGT=Gamma glutamyl transferase, 5'NT=5 nucleotidase, G-6-Pase=Glucose 6 phosphatase, ARG=Arginase, ALD=Aldolase.

Results. The mean values of serum enzymes are presented in Table 1. In affected group the mean values of all the enzymes increased significantly ($p \leq 0.05$) as compared to respective healthy mean value. All the enzymes showed highest values in the fasciola infected animals and least values in the animals having pneumonia. In fasciola infected animals maximum change was observed in G-6-Pase activity. It was 20 times of healthy mean value.

Table 1

Mean ± SEM values of serum enzymes in Marwari sheep

Serum enzymes UI^{-1}	Healthy group (200)	Affected group (200)			
		Fasciola infected (50)	Pneumonia affected (50)	Having enterotoxaemia (50)	Drought affected (50)
SDH	13.0±0.11	75.0±3.0[b]	25.0±0.6[b]	70.0±4.0[b]	60.2±4.0[b]
MDH	40.0±1.11	130.1±3.1[b]	50.1±2.5[b]	121.0±4.0[b]	109.3±3.2[b]
G6PD	4.0±0.02	25.0±1.0[b]	13.0±1.2[b]	20.0±1.5[b]	24.2±0.07[b]
GD	25.0±0.5	120.2±5.2[b]	40.0±4.2[b]	105.0±6.0[b]	119.2±4.3[b]
OCT	15.0±1.2	130.4±2.5[b]	22.0±2.3[b]	120.0±4.0[b]	125.6±5.4[b]
γGT	25.0±1.2	120.8±2.0[b]	30.0±1.4[b]	100.0±3.0[b]	115.5±4.1[b]
5'NT	15.0±1.4	80.5±2.0[b]	30.0±3.3[b]	65.5±5.0[b]	75.5±4.1[b]
G-6-Pase	3.0±0.06	60.0±5.0[b]	10.0±0.05[b]	57.7±4.0[b]	55.0±5.2[b]
ARG	9.0±0.07	65.0±3.10[b]	19.3±1.0[b]	52.61±3.2[b]	52.0±2.3[b]
ALD	9.0±0.07	60.0±2.0[b]	17.3±0.9[b]	42.3±2.3[b]	50.4±2.0[b]

Figures in the parenthesis indicate number of animals.
Superscript 'b' indicates a significant difference ($p \leq 0.05$) from repective healthy mean value.

Discussion. Higher activity of SDH could be due to hepatocellular injury (Tennant 1999). Serum SDH activity can be used as a reliable diagnostic test for hepatitis (Raghavendra & Rao 2000). Higher activity of MDH indicated the strategies of the animal to change the metabolic pathways for energy generation and glucose synthesis by gluconeogenesis (Abdel-Fattah et al 2002).

Increased levels of G6PD showed the increased metabolic activity of liver as the enzyme is important for glucose oxidation (oxidation of G-6-p) through the hexose mono phosphate (HMP) shunt which is essential for synthesis of fat. HMP pathway is the major source of NADPH, which maintains the reductive environment for all biosynthetic processes using NADPH as a cofactor (Kaneko et al 1999). Higher GD activity again showed the increased metabolism of the animal through liver as GD plays a central role in amino group metabolism. Whenever a hepatocyte needs fuel for citric acid cycle, GD activity increases, making alpha-ketoglutarate available and releasing NH_4^+ for excretion. GD is highly concentrated in liver (Keller 1971). Its higher level is suggestive of acute liver damage (El Samani et al 1985). The estimation of GD as a liver function test is being emphasized in animals (Tennant 1999).

Liver disease can produce increase in the OCT activity as OCT is an important enzyme of urea cycle. Determination of its serum level is important for detection of liver diseases and is used as an indicator of extent of hepatocellular damage. Serum levels of OCT are also elevated even in chronic diseases when there is an active liver necrosis (Blood et al 1979). Serum γGT is commonly used indicator of hepatobiliary disease in sheep (Saeed & Hussain 2006). Liver is the primary source of the enzyme 5'-nucleotidase (5'-NT). The determination has been most used in the cases where rise in serum ALP activity is there. Serum 5'NT increases in diseases of liver and biliary tract in a roughly parallel manner. Serum levels of 5'NT rises in obstructive jaundice (Kowlessar et al 1961); Inflammatory hepatic disease (Bardauill & Chang 1962) and damage in the liver tissues (El Samani et al 1985). Glucose-6-phosphatase can increase markedly in acute viral hepatitis or toxic necrosis of liver (Koide & Oda 1959). Glucose-6-phosphate is

dephosphorylated by G-6- Pase to yield free glucose which is exported to replenish blood glucose (Lehninger et al 1993). Measurement of G-6-Pase is also important in assessing gluconeogenic activity (Weber 1965).Serum arginase elevations have been demonstrated in progressive hepatic necrosis with unfavourable prognosis (Tennant 1999) and hepatocellular damage (Braun et al 1986) making it a useful tool for diagnosis of liver diseases. Elevated levels of serum ALD are useful predictors of liver damage (Alemu et al 1977); acute viral hepatitis or hepatic necrosis due to chemicals or drugs (Sibley and Fleisher, 1954); acute and chronic hepatic damage (Katz & Ducci 1958); and myocardial infarction in animals (Chazov & Savina 1958). It can be stated that the present investigation may be the first to provide values of certain serum enzymes at one platform *viz*. SDH, MDH, GD, G-6-PD, OCT, GGT, 5'NT, G-6-P, ARG, ALD etc in healthy and clinically affected Marwari breed of sheep which is indigenous to arid tract in India. The changes in the values in clinically affected animals could help in diagnosis of diseases and involvement of liver.

Conclusion. Higher levels of all the serum enzymes determined in the present study showed the changes of liver functions directly or indirectly as these enzymes are either formed by hepatocytes or participate in metabolic processes. It was concluded that any disease state of the body can affect the liver functions as a part of stress response. The present study provided the normal values of these enzymes in the sheep at one platform which could be helpful in the evaluation of hepatic functions in clinical cases.

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