

## A Preliminary Study on the Characteristics of Lactate Dehydrogenase (LDH) Enzyme Levels in Serum and Synovial Fluid of Dogs

R. Ibrahim and R. Zurina

Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
43400 UPM, Serdang, Selangor  
Malaysia

E-mail of Corresponding Author: [rashid@vet.upm.edu.my](mailto:rashid@vet.upm.edu.my)

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### Introduction

The enzyme lactate *dehydrogenase* (LDH) catalyses the interconversion of lactic and *pyruvic* acids. It is widely distributed in the body in relatively high concentration in the tissues, which makes increased plasma LDH a good indicator of tissue damage. The lack of tissue specificity of LDH has been partly circumvented by the discovery that it exists in a variety of molecular forms called *isoenzymes*. Five *isoenzymes* LDH1, LDH2, LDH3, LDH4 and LDH5 are present in different proportions in various tissues. The *synovial* fluid analysis is the most effective clinico-pathologic procedure available to determine the cause, type, duration and prognosis of the joint disorders caused by infectious agents. Increased LDH is a good indicator of tissue damage (Vessell *et al.*, 1962; Veys *et al.* and Wieme, 1968). However, little work has been done to date on enzyme levels in canine and feline *synovial* fluid. Many studies had been carried out in large animals such as in horses (Rejnö *et al.*, 1976, Lieberg *et al.*, 1977; Panndorf *et al.* and Grun *et al.*, 1978; Brock *et al.*, 1985; Yancik *et al.*, 1987) camels (Kataria *et al.* and Bharia, 1989), cattle and pigs (Bartel *et al.*, 1972; Grun and Weder, 1978). Enzyme levels have also been studied in human (Apple *et al.* and Rogers, 1986).

The objectives of this research were to determine characteristics of LDH *isoenzyme* level in serum and *synovial* fluid in clinically healthy dogs; to see the relationship between the LDH enzyme levels with age, body weight and sex in clinically healthy dogs; to find the correlation between the enzyme levels in the serum and the *synovial* fluid; and correlation between the enzyme level and pathologic changes found in the dogs.

### Materials and Methods

**Synovial Fluid:** *Synovial* fluid samples from the left and right stifle joints were collected from 40 clinically healthy dogs that were chosen randomly from different ages, sexes and body weights. Sedative drug (*acepromazine*, 0.1 mg/kg) was given if necessary. The dogs were placed on lateral recumbency for the *synovial* fluid collection. Sterile hypodermic needles (18G - 22G) ranging in length from 1 to 2 ½ inches was used to collect the *synovial* fluid. (the stifle joint was prepared aseptically prior to the *arthrocentesis*) using small-capacity syringes (2.5-3ml). The *synovial* fluid could also be collected after necropsy. In such cases the dogs were euthanised using Dolthelal (sodium pentobarbitone at dosage 5mg/5kg). *Synovia* samples were stabilised with 3.8% sodium citrate immediately after collection to prevent coagulation (1 part citrate + 9 part *synovia*). The samples were then treated for 15 minutes with 75 IU of *hyaluronidase* in 0.1 ml of saline solution per 1.0 ml of fluid to reduce viscosity. The samples were mixed on the cortex mixer and *hyaluronidase* was dissolved. Then it was left to stand for 20 minute before being centrifuged at 2000xg at 6°C for 30 minutes. The supernatants were collected for analysis. Lactate dehydrogenase (LDH) enzyme in the *synovial* fluid was quantified further to five known *isoenzymes*, using electrophoresis method (LD *Isoenzyme* Electrophoresis Procedure, Helena Laboratories, Texas).

**Serum Samples:** Venous blood samples were taken from the cephalic vein from each dog, from which the *synovial* fluid samples had been collected (blood samples were taken prior to euthanasia in the dogs). The blood samples from the cephalic vein which were collected in plain tubes were centrifuged a 3000 rpm for 20 minute before the serum were analysed using the

LDH diagnostic kit. The same laboratory method was applied as in the *synovias* on the collected blood serum to measure the level of the serum enzymes.

The comparison could be made from the results to show any significant correlation between the level of the enzymes in the serum and the enzymes in the *synovial* fluid from the same dogs.

### Results and Discussion

**Serum LDH:** There is no significant different between sex for the total LDH in serum of dogs. There is only significant difference between body weights. The range for total LDH in serum of dogs below 10 kg. were 149.5 ± 18 B-B unit/ml and the range for LDH *isoenzymes* were: LDH 1: 17.61 B-B unit/ml, LDH 2: 36.30 B-B unit/ml, LDH 3: 46.51 B-B unit/ml, LDH 4: 29.93 B-B unit/ml, and LDH 5: 29.08 B-B unit/ml. The range for LDH *isoenzyme* for dogs with body weight from 11 to 20 kg were: LDH 1: 25.33 B-B unit/ml, LDH 2: 37.82 B-B unit/ml, LDH 3: 66.89 B-B unit/ml, LDH 4: 43.04 B-B unit/ml, and LDH 5: 41.82 B-B unit/ml.

The composition of LDH *isoenzyme* in serum had been determined in the serum samples obtained from clinically healthy dogs. The mean value of the LDH *isoenzyme* in serum of clinically healthy dogs of different sex, age and body weight is tabulated. There is no significant different between sex, age and body weight of clinically healthy dogs.

The correlation between each LDH *isoenzyme* with the blood parameters showed that there are no strong correlations for ALT, BUN, creatinine and serum (protein plasma) in clinically healthy dogs. For RBC it gives slightly negative correlation between LDH4 ( $r=-0.33$ ). For WBC, LDH3 have positive correlation ( $r=0.474$ ) when

compared to LDH2 ( $r=0.329$ ). In PCV, LDH2 had positively correlated ( $r=0.49$ ) compared to LDH4 ( $r=-0.346$ ).

The comparison between each LDH isoenzyme showed that there was strong correlation between LDH3 and LDH2 ( $r=0.708$ ) where as there was strong negative correlation between LDH5 with LDH3 ( $r=-0.887$ ) followed by LDH2 ( $r=-0.838$ ) and LDH1 ( $r=-0.562$ ). The percentages of the LDH isoenzymes in clinically healthy serum dogs were 11.78% LDH1, 17.59% LDH2, 31.11% LDH3, 20.02% LDH4 and 19.45% LDH5.

**Synovial LDH:** Determination of the characteristics of lactic dehydrogenase enzyme level in *synovial* fluid in clinically healthy dogs encountered some problems such as small volume and high viscosity of *synovial* fluid was obtained from the left and right stifles. The technique had been improved and dilution factors had been introduced in the analytical methods. The determination of the characteristic of lactic dehydrogenase enzyme level in *synovial* fluid would be completed.

The normal trend of LDH in serum of clinically healthy dogs ( $n=40$ ) had been determined. The result of *synovial* fluid from the dogs' stifle joints had not been satisfactory and more work is needed. The *synovial* fluid analysis for LDH levels in normal and affected dogs would be completed.

### Conclusions

There was no difference between Serum from clinically healthy dogs of different sex, age and body weights. There is no strong correlation between LDH isoenzyme with blood parameter, however, there was a strong correlation between each LDH isoenzyme. Percentage of the LDH isoenzymes in clinically healthy dogs serum was 11.78% LDH1, 17.59% LDH2, 31.11% LDH3, 20.02% LDH4 and 19.45% LDH5.

Future research is required, for example, the research in total LDH serum and *synovial* fluid, to see the correlation of the LDH isoenzyme with haemogram and biochemistry, other problems in the body systems (liver, lungs, muscles, heart), in hemolytic anemia, blood parasites, and other pathologic changes. Other research is needed in relation to cytology and histopathological finding (*synovial* fluid and articular membrane) and samples of *synovial* fluid from induced arthritis (Wegner et al. and Mühlback, 1971; Nayak et al., 1990).

### Benefits from the study

The characteristics of LDH in serum had been successfully carried out in the laboratory. The characteristics of LDH in *synovial* fluid had encountered several problems one of that was the small volume of *synovial* samples obtained from the stifle joints. The technique is being improved and it is hoped that successful analysis can be carried out in the laboratory.

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### Graduate Research

None.