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Original Article

Assessment of the Profile of Enzymatic Changes in Sick Dogs with Heart Disease in Iran

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Abstract

In a recurrence, the levels of superoxide dismutase enzymes (SOD), lactate dehydrogenase (LDH) glutamate pyruvate transaminase (GPT), the levels of hemoglobin (HB) and hematocrit (PCV) were measured) in 20 healthy dogs and 30 dogs with heart disease (control).

The mean SOD values between healthy and diseased dogs showed a significant difference. SOD concentration was significantly decreased in sick dogs as compared to healthy dogs. Whereas, LDH, GOT and GPT enzymes concentration were significantly increased in dogs with heart failure as compared with the control. However, the mean values of HB and PCV showed no significant differences between the groups of dogs. There was also a significant correlation between SOD, age and PCV value in diseased dogs.

In healthy dogs, there was no significant correlation between SOP values and other factors. The deficiency of the SOD enzyme over time generates free radicals such as free O2 molecules and reduces the neutralization of these free radicals resulting in the damage to the specific organ such as heart and causing heart disease.

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INTRODUCTION

Free radicals are not only naive in natural biochemical processes but also they play an important role in pathological cases. Hydroxyl radicals are the most active free radicals capable of reacting with a wide variety of cellular components [1,2]. As an example, it recovers amino acids and converts them into Schiff. It also alters the chemical characteristics of the purine and pyrimidine pools and attacks the membrane lipid. Toxicity of free radicals could be neutralized by many cell protective enzymes and antioxidants which limits the damage caused by these materials [3, 4]. Probably these protective mechanisms do not work independently but act as a cascade of reactions. One of the protective enzymes within the cells is superoxide dismutase (SOD) [5,6]. This enzyme is found either as a zinc/copper enzymes in the cytoplasm or in the form of protein containing manganese in the mitochondria of mammalian cells. This enzyme eliminates superoxide radicals by combining it with protons and leads to the formation of hydrogen peroxide and oxygen.

$$2O_2 + 2H^+ \quad H_2O_2 + O_2$$

When the superoxide anion approaches the enzyme, the anion is absorbed by the regions with the positive charge and directed to the active region of the enzyme and results in activation of the catalase that is located inside the peroxisome and the cytoplasm and hydrolyses the hydrogen peroxide into the water and oxygen molecules [7, 8].

$$2\,H_2O_2 \quad H_2O + O_2$$

The deficiency of this enzyme likely results in heart failure in sick dogs.

MATERIALS AND METHODS

A total of 30 dogs (17 females and 13 males) previously diagnosed with heart disease, aged from 60 to 120 months and body weight from 10 to 50.0 kg were recruited for this study.

All dogs were diagnosed with myocarditis and were symptomatic with clinical signs of heart disease that included exercise intolerance, cough or arrhythmia. Therefore, they underwent a clinical and electrocardiographic examination. Sick dogs showing rhythm disturbances underwent a 24-hour Holter ECG analysis. The ECG examination was performed using a BTL SD08® device (BTL, UK) with dogs in right lateral recumbency.

In the meantime, twenty healthy (10 females and 10 males) dogs were selected as control. Dogs evaluated as healthy based

Table 1: ±Mean concentration of the enzymes obtained from the blood samples withdrew from healthy and sick dogs. Data were considered significant where the P value was equal to or smaller than 0.05.

	SOD Relative *Fluorescence Unit* (*RFU*)	LDH (International Unit)	GOT (International Unit)	GPT (International Unit)	PCV (%)	HB (g/dl)
Healthy dogs	1372.7 ± 541.6	54.9 ± 26.1	28.8 ± 7.8	29.5 ± 10.2	47.3 ± 6.7	15.7 ± 2.8
Sick dogs with heart failure	732.7 ± 212.6	369 ± 66.6	71.6 ±11.5	65.7 ± 13.7	42.45 ± 6.7	15.7 ± 2.8

on their history including physical examination and results of hematological and serum biochemical analysis (data not shown). All dogs were housed separately in the kennels with access to free water and fed regularly with high-quality commercial Unauthenticated.

All dogs were bleed through the anterior femoral vein and the blood samples were examined for the concentration of following enzymes using commercial kits as follow: supper oxide dismutase (SOD) (Kyman, USA), lactate dehydrogenase (LDH) (DGKC method kit), glutamate pyruvate transaminase (GPT), and glutamate oxalostatic transaminase (GOT) using a kit based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method, as well as, hemoglobin (based on hemoglobin Cyanomet), and Hematocrit (based on microhematocrit method). Blood samples for the determination of TAC levels were immediately centrifuged at 1500 × g for 15 minutes at 4 °C. Plasma was separated and immediately frozen at -80 °C until use. Haemolysed red blood cells for the determination of SOD activity were prepared immediately after blood collection, following the manufacturer's instructions, and stored at -80 °C until analysis.

STATISTICAL ANALYSIS

All data were analyzed using t-test and Mann-Whitney U tests with the help of two statistical programs. Mean, standard deviation and correlation were calculated and significant relationship was obtained when the P value was smaller than 0.05.

DISCUSSION

This study provides information on the values of Sod, LDH, CPK, GPT, and creatinine enzymes in healthy individuals and sick dogs, using commercially available kits applied to an automated biochemical analyzer to reduced analytical variations, considerable reductions in time and use of smaller sample volumes. It has been reported the results of the full validation of an automated assay for the measurement of cupric reducing antioxidant capacity in the serum of dogs are highly reliable [9]. Therefore, we employed the assay kit for the assessment of SOD in plasma or serum in both healthy and sick dogs. It has been reported that there is a significant correlation between antioxidant capacity and oxidative damage based on the age of the animal and the sex-related differences humans [10,11] but not in dogs. There are several analytical methods for detection of the superoxide radical based on SOD activity and production of H2 O2 [12,13].

There is a significant difference between the measured values of SOD, LDH, CPK, GPT and creatinine enzymes in healthy individuals as compared with the sick dogs with heart disease. The level of SOD was significantly lower in sick dogs suffering from heart disease compared to healthy dogs. In the meantime, there was a significant correlation between values of SOD enzymes and the level of hemoglobin in healthy subjects and sick dogs. But, why is there no solid correlation between SOD and other enzymes? This is due to Enzyme timing because, in the first 24 hours after the infarction, the amount of both CPK and LDH enzymes reach the Peak and then decrease in the first 48 hours, and in the case of The GOT enzyme, it reaches the peak in the first 72 hours and then decreases. However, the SOD enzyme is not a function of time and depending on the life of red blood cells (120 days), decreases over a long period of time.

By reducing the content of SOD enzymes during the onset of heart, the production of free radicals, including superoxide (O_2) is increased which causes lesions and provide a context for heart failure.

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