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Original article

The effect of hip dysplasia on some biochemical parameters, oxidative stress factors and hematocrit levels in dogs

E. Polat[1], M.C. Han[1], E. Kaya[2], S. Yilmaz[2], S.D. Kayapinar[1], S. Coskun[1], A. Yildirim[1], U.K. Can[1]

[1] Fırat University, Faculty of Veterinary Medicine, Department of Surgery, Elazig, Turkey
[2] Fırat University, Faculty of Veterinary Medicine, Department of Biochemistry, Elazig, Turkey

Abstract

In this study, it was aimed to investigate the effect of hip dysplasia on some biochemical parameters, oxidative stress factors and hematocrit values in dogs. Hematocrit values (HTC), serum calcium (Ca), phosphorus (P) levels, serum alkaline phosphatase (ALP), creatine kinase (CK) activities and oxidative stress factors were evaluated in a total of 27 dogs with healthy hip joints (n: 11) and hip dysplasia (n: 16). There was no statistically significant difference between the two groups in terms of HCT, Ca and P values ($p>0.05$). ALP and CK activities were found to be statistically significantly increased in the group with hip dysplasia compared to the control group with a healthy hip joint ($p<0.05$). While malondialdehyde (MDA) level, one of the oxidative stress factors, was increased in the group with hip dysplasia, decreased glutathione (GSH) levels, catalase (CAT) and glutathione peroxidase (GSH-Px) activities were significantly decreased. There was no significant difference between the two groups in terms of superoxide dismutase (SOD) level. As a result, it was determined that oxidative stress factors differ in dogs with hip dysplasia compared to dogs with the healthy hip joint.

Key words: alkaline phosphatase, hematocrit, hip dysplasia, creatine kinase, oxidative stress

Correspondence to: E. Polat, e-mail: erenpolat@firat.edu.tr, tel.: +905557466039

Introduction

Hip dysplasia is a biomechanical disease characterized by the incompatibility of the hip joint, which occurs under the influence of hereditary and environmental factors, and can be seen in all carnivores, especially in dogs. It was first described by Schnelle in 1935 as bilateral luxation of the coxofemoral joint (Gınja et al. 2006, Karabaglı et al. 2014, Schachner and Lopez 2015, Bostancı and Demirkan 2017, Polat 2021).

Hip dysplasia is a progressive disease seen mostly in large breed dogs such as Saint Bernard and German Shepherd. In the early stages of the disease in dogs, it is detected that there is laxity in the hip joint. In the later stages, a degenerative joint disease, characterized by osteoarthritis, develops due to the laxity in the joint. As the acetabulum loses its depth as a result of degenerative joint disease, the femoral head loses its rounded structure and becomes irregular. This mismatch in the hip joint eventually results in luxation of the femoral head (Dassler 2002, Captug and Bilgili 2007, Sarı and Bilgili 2011, Karabaglı et al. 2014, Polat 2021).

Dogs with hip dysplasia are known to show clinical signs such as lameness in their hind legs, muscular atrophy, exercise intolerance, and rabbit walking (Deny et al. 2000, Dassler 2002, Schachner and Lopez 2015). Deformations and inflammations in the musculoskeletal system can cause differences in hematocrit (HCT) and serum biochemical parameters. For example, while alkaline phosphatase (ALP) activitiy increases in damage to bone tissue, creatine kinase (CK) activity increases significantly in muscle problems (Teixeira and Borges 2012, Sharma et al. 2014).

Antioxidants neutralize free oxygen radicals in the body, thereby maintaining tissue integrity in living organisms. Glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) are some of these antioxidants. When antioxidants fail to neutralize oxidants, oxidative stress causes tissue damage due to the increase in toxic products such as malondialdehyde (MDA). Due to the imbalance between prooxidant and antioxidant defense, excessive production of reactive oxygen species (ROS - superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$)) pushes the cell towards oxidative stress. Subsequently, endogenous antioxidant defense mechanisms cannot protect the cell from oxidative damage and cell damage occurs (Mukherjee et al. 2013).

This study aimed to evaluate the relationship between HCT values, levels of some biochemical parameters (calcium (Ca), phosphorus (P), ALP and CK) and levels of oxidative stress factors (MDA, GSH, CAT, SOD, GSH-Px) in dogs with hip dysplasia and healthy dogs.

Materials and Methods

Ethical approval and creation of groups

This study was conducted in accordance with the principles of the Local Ethics Committee in the framework of the ethics confirmed by the Fırat University Animal Experiments Local Ethics Committee (13.01.2020, 2020/1, 371360). In this study, 27 dogs with healthy and dysplasia hip joints brought to Fırat University Animal Hospital were used. Healthy dogs constituted the control group (Group 1, n=11), dogs with hip dysplasia constituted the study group (Group 2, n=16) (Fig. 1).

Laboratory analysis

Blood samples were taken from the cephalic vein. Blood was taken into tubes with 10% EDTA for hematological examinations, or into 5 ml tubes with gel and clot activator for biochemical analysis.

Hematocrit values, levels of some serum biochemical parameters (Ca, P, ALP and CK) and levels of oxidative stress factors of dogs in both groups were determined and the relationship between them was evaluated. While determining the hematocrit values, *Mindray BC-5000VET* brand hemogram device was used. Ca, P, ALP and CK levels were determined using *GESAN CHEM 200 Automated Biochemistry Analyzer* brand serum biochemistry device.

To determine oxidative stress factors, blood samples in EDTA tubes were centrifuged at 3000 rpm for 15 minutes and plasma was obtained. Plasma was used to measure MDA level as a marker of lipid peroxidation. Whole blood was used for GSH and GSH-Px determination. Plasma separated EDTA blood samples were washed 3 times with saline (0.9% NaCl). After that, CAT and SOD activities, and hemoglobin (Hb) levels were determined in erythrocytes.

The MDA level was measured according to the method of Placer et al. (1966). This method was based on the reaction of thiobarbituric acid (TBA) and MDA, one of the aldehyde products of lipid peroxidation. The MDA level was expressed as nmol/ml. GSH level was determined by a kinetic assay using a dithionitrobenzoic acid (DTNB) recycling method of Ellman et al. (1961), GSH levels were expressed as μmol/ml. CAT activity was carried out by using the Aebi's method (1984). The principle of the assay is based on the determination of the rate constant, k (dimension: k) of H_2O_2 decomposition. The reaction contained 50 mM potassium phosphate buffer and 10 mM H_2O_2 (as substrate) reaction was started by the addition of the sample. CAT activities were expressed as kat/g Hb. The GSH-Px activity was measured by the Beutler method (1984).

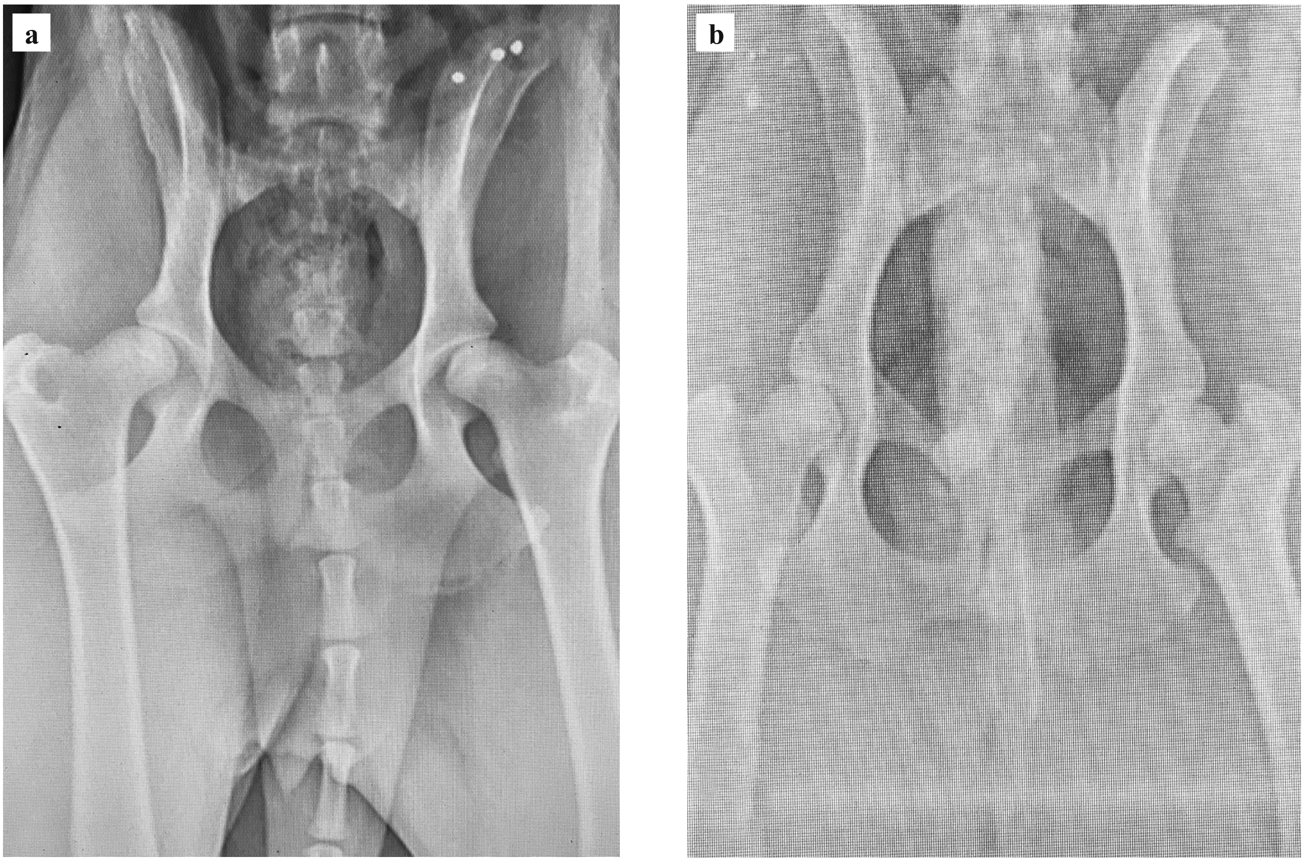

Fig. 1. Radiography of the dysplasic (a), and healthy (b) hip joint in dogs.

GSH-Px catalyzes the oxidation of GSH to oxide glutathione (GSSG), using H_2O_2. The rate of formation of GSSG was measured by the glutathione reductase reaction. The SOD activity was tested by quantifying superoxide anion (O_2•$^-$) generated by xanthine and xanthine oxidases reacting with nitroblue tetrazolium (Sun et al. 1988). To determine hemoglobin level, Frankel et al. (1970) 's method was used. According to this, ferricyanide oxidizes Fe^{+2} in hemoglobin and converts it from +2 to +3 valuable iron and it converts into methemoglobin. Thereafter, potassium cyanide and cyanomethemoglobin, a stable pigment, are formed. The absorbance of cyanomethemoglobin was read at 546 nm.

Statistical analysis

The results were expressed as mean ± standard error (S.E.). Shapiro-Wilk normality test was used to determine whether the raw values of all the measured parameters showed normal distribution. As a result of this test, it was found that the values in all parameters showed normal distribution. Independent samples test (t-test) was used to test whether significant differences existed among the groups. Statistical significance was accepted at a p-value <0.05. Statistical Package for Social Sciences (SPSS)/PC software program (Version 22.0; SPSS, Chicago, Illinois, USA) was used to perform the statistical analysis of the data.

Results

Relations between HCT, Ca, P, ALP and CK values of healthy and dysplasia dogs are presented in Fig. 2.

Table 1 presents the levels of MDA, GSH, and the activities of antioxidant enzymes tested in our study, which are CAT, GSH-Px, and SOD. The levels are presented in healthy (control) and with hip dysplasia dogs. MDA levels were increased in the control group; GSH levels and CAT and GSH-Px activities were reduced ($p<0.05$ and $p<0.001$, respectively). There were no statistically significant differences between the control and with hip dysplasia animals in SOD activities.

Discussion

Reactive oxygen species (ROS) are produced as a result of normal cellular metabolism in living organisms. These ROS are neutralized by antioxidant defense systems such as GSH, CAT, SOD, GSH-Px in the body. However, in some inflammatory conditions and diseases, the production rate of ROS increases. Accordingly,

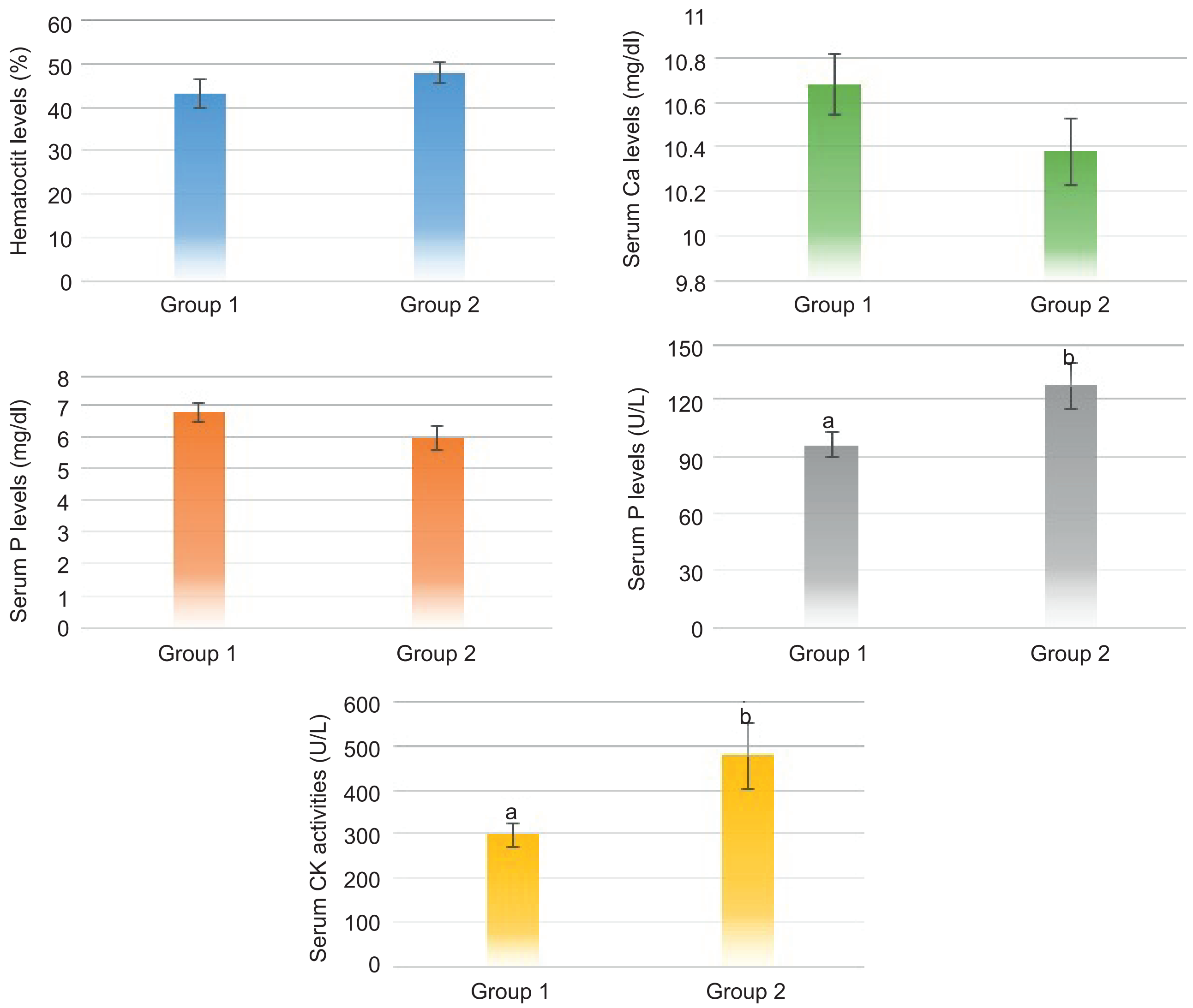

Fig. 2. The relationship of HCT, ALP, CK, Ca and P levels in healthy dogs with hip dysplasia.

Table 1. Statistical analysis of the relationship between levels of oxidative stress factors between healthy and hip dysplasia dogs.

	MDA (nmol/ml)	GSH (µmol/ml)	CAT (k/ g Hb)	SOD (U/g Hb)	GSH-Px (U/g Hb)
Control (Group 1)	7.94±0.12[b]	63.39±1.27[a]	151.79±6.12[a]	142.27±2.25[a]	83.04±2.38[a]
Hip Dysplasia (Group 2)	9.05±0.12[a]	57.45±1.45[b]	132.12±2.83[b]	137.15±2.41[a]	67.05±2.46[b]
p	0.001	0.05	0.05	-	0.001

oxidative stress occurs because antioxidant defense mechanisms are insufficient to neutralize ROS (Bırben et al. 2012, Ajadı et al. 2018, Altıner et al. 2018). This study aimed to investigate the effect of hip dysplasia disease causing osteoarthritis on oxidative stress. In conclusion, this study showed that the levels of antioxidant defense elements (GSH, GSH-Px, CAT and SOD) were lower in dogs with hip dysplasia due to the inflammatory reaction in the hip joint than in healthy dogs. When healthy dogs and dogs with hip dysplasia were compared, the differences in GSH, GSH-Px and CAT levels were found to be statistically significant, while the difference in SOD levels was not found to be statistically significant. It was determined that the MDA level, which is the final product of lipid peroxidation, was higher in dogs with hip dysplasia and this difference was statistically significant ($p<0.001$).

Ajandi et al. (2018) found that the MDA level was higher and the SOD level was lower in Boerboel dogs with hip dysplasia compared to healthy dogs, and the difference between the groups was statistically significant. However, they reported that the difference between GSH levels was not statistically significant ($p>0.05$). In this study, contrary to the another study, it was determined that the difference between SOD levels was not statistically significant ($p>0.05$), and the difference between GSH levels was statistically significant ($p<0.05$).

Diseases that cause deformities in the skeletal system, such as hip dysplasia, cause changes in the levels of some serum enzymes (ALP and CK) and elements (Ca, P) (Zılva and Pannal 1975, Prasad 1978, Or et al. 2001). The amount of ALP enzyme in the body is particularly closely related to the metabolic rate of bone tissue. Again, in diseases of these regions, the amount of ALP in blood may increase due to tissue destruction (Or et al. 2001). CK, which is indispensable for the physiology of muscle cells, can enter the bloodstream as a result of the disruption of the integrity of the muscle cells due to various conditions. In some studies (Szilagy and Sagi 1976, Candaş 1982, Lust et al. 1985, Wallace 1987, Bakır et al.1992) it was found that serum ALP level increased significantly in dogs with hip dysplasia. In this study, it was found that serum ALP levels in dogs with hip dysplasia increased significantly. Or et al. (2001) reported that, contrary to other studies, serum ALP levels did not show a statistically significant difference in dogs with hip dysplasia. In this study, the effect of hip dysplasia on serum CK enzyme level due to atrophy in hind leg muscles in the later periods was also compared. A statistically significant increase in serum CK enzyme level was found in dogs with hip dysplasia ($p<0.05$).

Some researchers investigated the effects of hip dysplasia on serum manganese (Mn), copper (Cu), cobalt (Co), magnesium (Mg), zinc (Zn), calcium (Ca) and phosphorus (P) levels in dogs (Or et al. 2001, Ajandi et al. 2018). Ajandi et al. (2018) found that hip dysplasia did not create a statistically significant difference in serum Mn, Cu, Co and Mg levels in Boerboel dogs ($p>0.05$). Again, Or et al. (2001) found that hip dysplasia did not create a statistically significant difference in serum Ca, P, Cu and Zn levels in Turkish Shepherd Dogs and German Shepherd Dogs ($p>0.05$). In this study, when serum Ca and P levels in dogs with hip dysplasia were compared with those in healthy dogs, no statistically significant difference was determined ($p>0.05$). It was found that this finding was similar to the results of other studies.

Conclusion

In conclusion, in this study, it was determined that in patients with hip dysplasia, which causes inflammatory reactions in the hip joint, serum ALP and serum CK levels are affected with oxidative stress factors. Therefore, especially in young dogs, information about the presence of hip dysplasia cases and the severity of the inflammation can be obtained by examining the levels of these oxidative stress factors and serum ALP and serum CK levels. In this study, it was determined that serum Ca and serum P levels were not statistically significantly affected by hip dysplasia. When dogs with hip dysplasia and healthy dogs were compared in terms of HCT levels, it was concluded that it would be beneficial to conduct studies that evaluate the ratio of blood cells to each other, although there was no statistically significant difference. Because the stress and necrotic tissues are caused by inflammatory reactions in the body they would cause an increase in some blood cells and a decrease in some blood cells.

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