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Biomarkers of Oxidative Stress in Canine Dermatitis

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ABSTRACT

Dermatitis in general represents the significant percentage of cases in small animal practice so the present study was conducted to record the changes in the oxidative stress parameters in allergic dermatitis in canine cases presented at the Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, R.S. Pura and Central Veterinary Hospital, Talab Tillo in Jammu region. Dogs were divided into four groups, Group A, Group B, and Group C representing bacterial, fungal and parasitic dermatitis and Control group containing normal healthy animals choosen randomly. The number of animals in each group was six. Blood samples were taken in heparinised vials and subjected to antioxidant analysis viz. SOD, Lipid peroxidise, catalase, Gpx and vitamin C. Significant increase in SOD, Lipid peroxidase and decrease in catalase, Gpx and vitamin C level was observed in dermatitis suffering dogs compared with the normal group. The activities of antioxidant enzymes catalase and superoxide dismutase, the first line of antioxidant defense against damaging effects of free radicals, were altered. The alterations in oxidative stress indices were more pronounced in cases with involvement of fungal dermatitis as compared to negative control group. The study shows that dermatitis induces marked changes in the antioxidants levels of dog that may have significance in diagnostic purposes.

Keywords: Allergic dermatitis, catalase, lipid peroxidise, super oxide dismutase, vitamin c

Canine Allergic Dermatitis (AD) has been defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is associated most commonly with IgE antibodies to environmental allergens (Halliwell, 2006). Although this definition encompasses many aspects of the pathogenesis and clinical aspects of the condition, it is important to remember that this disease has no pathognomonic clinical signs that permit a definitive diagnosis to be made upon initial owner interview and clinical examination (DeBoer and Hillier, 2006). Canine atopic dermatitis (AD) is a common, genetically predisposed, inflammatory and pruritic skin disease. The variation in clinical presentations, due to genetic factors, extent of the lesions, stage of the disease, secondary infections, as well as resemblance to other nonatopic related skin diseases complicate the diagnosis of the disease. Dermatitis in general represents the significant percentage of cases in small animal practice (Subramanian *et al*., 1989: Sharma *et al.,* 2008a). Dermatological disorders constitute a majority of these cases (Scott and Paradis, 1990). Dermatological disorders assumes great importance due to their effect on the animal such as distress, irritation and offensive smell besides being a potential source of a number of zoonotic diseases (Parish and Schwartzman, 1993). Canine allergic dermatitis (CAD) is most common form of non infectious dermatitis and constitutes a serious medical problem in veterinary medicine.

Oxidative stress in allergic dermatitis occur as a result of increased free radical production and have been implicated to play an important role in the pathogenesis of various allergic and inflammatory skin diseases both in human beings (Okayama, 2005) and in animals (Camkertan *et al.,* 2009; Dimri *et al.,* 2010).

MATERIALS AND METHODS

The present study was carried out to examine alterations in oxidative stress indices in dogs suffering from dermatitis and normal control dogs chosen randomly. Dogs suffering from dermatitis presented at Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology – Jammu as well as Central Veterinary Hospital, were divided into four groups, of six animals each. The four groups include Group A containing dogs with bacterial dermatitis, Group B containing dogs with fungal dermatitis, Group C containing dogs with parasitic dermatitis and Control group containing normal healthy dogs chosen randomly. In association with routine clinical sampling, the blood samples (approximately, 2 ml) were collected from each dog either from cephalic or recurrent tarsal vein, and using heparin (10 IU/ml of blood) as anticoagulant. Blood samples were centrifuged at 2000 rpm for 5 min in a refrigerated centrifuge to separate plasma. The plasma was collected in to a clean eppendorf tube. The packed RBC was re-suspended in PBS and was centrifuged for 5 min at 5000 rpm and the supernatant was discarded. The process was repeated for three times. Finally, 1:20 dilution of RBC hemolysate was prepared in distilled water for estimation of lipid peroxide (LPO), superoxide dismutase (SOD), and catalase (CAT), glutathione peroxidise (Gpx) and vitamin c spectrophotometrically.

Superoxide dismutase (SOD) activity was estimated as per the method of Marklund and Marklund (1974). Erythrocytes lipid peroxides (LPO) level was determined according to the method of Placer *et al*. (1966). GSHPx was determined by spectrophotometric determination of the nicotinamide adenine dinucleotide phosphate (NADPH) consumption rate in the presence of hydrogen peroxide at 340 nm as per methods described by Pleban *et al*. (1982) Vitamin C were colorimetrically determined using the phosphotungunstic acid method described by Kyaw (1978).

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS Ms package program (Windows Release 10.0). Results were expressed as means ± standard error and $p < 0.05$ was taken as the level of significance.

RESULTS AND DISCUSSION

Oxidative stress/anti-oxidant capacity

The oxidative stress/ anti-oxidant levels in different type of dermatitis are shown in table 1. The catalase activity revealed a significant decrease in all the three groups with values 85.83 ± 2.36 µmol H_2O_2 utilized/ min/ mg of Hb, 87.58 ± 0.70µmol H_2O_2 utilized/ min/ mg of Hb and 86.93 ± 0.92 µmol H_2O_2 utilized/ min/ mg of Hb respectively in group A, B and C when compared to healthy control 105.5 ± 3.36 µmol H_2O_2 utilized/ min/ mg of Hb. The catalase was significantly decreased in bacterial, fungal, parasitic dermatitis. Dimri *et al.* (2008a) found similar findings in the dogs suffering from demodectic mange. Decreased catalase activity was also noted in sheeps with Psoroptic mange (Dimri *et al.,* 2010). Catalase and SOD are primary antioxidant enzymes present in mammalian cells. SOD catalyzes the formation of O_2 from reactive oxygen species. A co-product of SOD activity is H_2O_2,

Table 1: Oxidative status in healthy and diseased dogs (Mean ± SE)

Parameters	Control (n=6)	Diseased dogs		
		Group A (n=6)	Group B (n=6)	Group C (n=6)
Catalase (µmol H_2O_2 utilized/ min/ mg of Hb)	105.5±3.36	85.83± 2.36*	87.58±0.70*	86.93±0.92*
SOD (U/mg of Hb)	0.38± 0.02	0.45± 0.01*	0.47±0.01*	0.45± 0.02*
Lipid peroxidise (nmol MDA/mg Hb)	0.23±0.08 .	10.10±0.18*	10.17± 0.02*	10.0± 0.02*
Gpx (U/mg Hb)	2.69±0.06	1.53±0.03*	1.54±0.03*	1.57±0.02*
Vit.C (mg/dl)	8.93±0.05	3.93±0.04*	3.95±0.04*	3.89±0.15*

*significant at 5% ($p<0.05$)

** significant at 1% ($p<0.01$)

which is converted to H_2O by catalase (Fang *et al.,* 2002). So the possible reason for decreased activity of catalase, in the present case could be because of increased activity of SOD, which might have resulted into increased production of H_2O_2 production and thereby increased utilization of catalase for converting H_2O_2 into H_2O. Decreased activity of catalase again indicates oxidative stress in dermatitis cases. Increased SOD activity and decreased catalase activity were also recorded in human vitiligo patients (Hanzneci *et al.,* 2005).

A significant increase was noticed in Lipid peroxidase in all the three groups with values 10.10 ± 0.18 nmol MDA/ml erythrocytes, 10.17 ± 0.01 nmol MDA/ml erythrocytes and 10.0 ± 0.02 nmol MDA/ml erythrocytes in group A, B and C , when compared to healthy control 7.32 ± 0.3 . Lipid peroxidase (LPO) activity was significantly increased in bacterial, fungal, demodectic and sarcoptic dermatitis which was in agreement with the findings of Dimri *et al*. (2008b) who found a significant increase in LPO activity in dogs with demodectic mange. Erythrocytes are highly susceptible to peroxidative damage due to abundance of polyunsaturated fatty acids and presence of powerful transition-metal catalyst (Ranjan *et al.,* 2005). Higher LPO levels in dogs with demodicosis in comparison to healthy control suggested enhanced oxidative damage to erythrocytes, either due to compromise in antioxidant defense or excess production of free radicals.

So enhanced LPO levels and reduced catalase activity, ascorbic acid concentration in blood indicates increased oxidative stress and compromised anti-oxidant defense in blood of dogs suffering from dermatitis. Similar finding were found in dogs with parasitic dermatitis (Dimri *et al.,* 2008a). Our finding were also supported by Camkertan *et al.* (2009) who suggested a possible relationship between oxidant/antioxidant imbalance and radical production due to inflammatory response

SOD revealed a significant increase in all the three groups with values 0.45 ± 0.01 U/mg of Hb, 0.47 ± 0.01 U/mg of Hb and 0.45 ± 0.02 U/mg of Hb resp. in group A, B and C when compared to healthy control 0.38 ± 0.02 U/mg of Hb. The SOD activity was increased in bacterial, fungal, parasitic dermatitis which was in agreement with the finding of Dimri *et al.* (2008a) who found increase in activity of SOD enzyme in dogs, suffering from demodectic mange. As SOD catalyze the formation of O_2 from reactive oxygen species, the possible reason of increase in SOD activity in the present study, could be then due to its up-regulation in its synthesis to counteract free radicals (Dimri *et al.,* 2008a). Significant decrease in the zinc and copper concentration also supported this hypothesis, since it may be, at least partially, due to enhanced utilization of these elements for synthesis of SOD. So increased SOD activity indicates increased oxidative stress in these cases.

The GPX level revealed a significant decrease in all the three groups with values 1.53 ± 0.03 U/mg Hb, 1.54 ± 0.03 U/mg Hb , 1.57 ± 0.02 U/mg Hb respectively, in group A, B and C when compared to healthy control 2.69 ± 0.06 U/mg Hb. Glutathione peroxidase is a selenium containing enzyme which reduces hydrogen peroxide and thereby serves as an alternative means of detoxifying activated oxygen. In the present study GPX level revealed a significant decrease in all the three groups when compared to healthy control indicating the oxidative stress resulting from and being caused by allergic inflammation. However Kapun *et al*. (2012) suggest that oxidative stress with increased lipid peroxidation could be involved in the pathogenesis of atopic dermatitis in dogs.

The plasma vitamin C level revealed a significant decrease in all the three groups with values 3.93 ± 0.04mg/dl, 3.95 ± 0.04 mg/dl and 3.89 ± 0.15 mg/dl respectively, in group A, B and C when compared to healthy control 8.93 ± 0.05 mg/dl. The vitamin C concentration was significantly decreased in allergic dermatitis, indicating the increased oxidative stress. Vitamin C is very important intracellular water soluble anti-oxidant, involved in recycling the alpha-tocopheryl radical back to alpha-tocopherol (Halliwel and Gutteridge, 1999). Reduced plasma ascorbic concentration has also been reported in stressful situations in cattle, owing to its enhanced rate of utilization without compensating increase in synthesis (Kolb, 1991). The decreased level of ascorbic acid in plasma in the present study might be due to overutilization or sequestration of this antioxidant to neutralize the over production of reactive oxygen species (ROS) during inflammatory conditions of skin.

The activities of antioxidant enzymes catalase and superoxide dismutase, the first line of antioxidant defence against damaging effects of free radicals, were also altered. The alterations in oxidative stress indices were more pronounced in cases with involvement of fungal dermatitis as compared to control group.

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