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Trace minerals status and antioxidative enzyme activity in dogs with generalized demodecosis

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ABSTRACT

The present study was aimed to determine the levels of trace elements zinc, copper, iron, erythrocyte oxidant/anti-oxidant balance, vitamin C and β-carotene in dogs with generalized demodecosis. A total of 24 dogs with clinically established diagnosis of generalized demodecosis and 6 dogs as control were included in the study. In comparison to healthy control, zinc and copper levels were significantly ($P<0.01$) lower in dogs with generalized demodecosis, whereas iron levels were significantly ($P<0.01$) higher. Malondialdehyde (MDA) levels were significantly ($P<0.01$) higher in diseased dogs whereas activity of superoxide dismutase (SOD) and catalase were significantly ($P<0.01$) lower. β-carotene and vitamin C levels were significantly ($P<0.05$) lower in diseased dogs when compared to healthy control. SOD activity was positively correlated with zinc ($r_s=0.65$, $r_s=0.71$ and $P<0.05$) and copper ($r_s=0.51$, $r_s=0.63$ and $P<0.05$) in both healthy and diseased dogs. MDA levels were negatively correlated with iron ($r_s=-0.49$, $r_s=-0.78$ and $P<0.05$), β-carotene ($r_s=-0.26$, $P>0.05$; $r_s=-0.54$, $P<0.05$, respectively) in both healthy and diseased dogs and with SOD activity in diseased dogs only ($r_s=-0.68$, $P<0.05$). From the present study, it was concluded that generalized demodecosis in dogs is associated with significant alteration in trace elements and oxidant/anti-oxidant imbalance and this imbalance might be secondary to changes caused by demodectic mange.

1. Introduction

Canine demodecosis, caused by the proliferation of *Demodex canis* Leydig (1859) in hair follicle and sebaceous glands, is the most common and serious of all the canine dermatosis (Scott et al., 2001). Despite the high prevalence and severity of the disease, many aspects of canine demodecosis remain poorly understood. *Demodex canis* is considered to be a normal inhabitant of canine skin. The disease is thought to be the consequence of a genetically mediated specific immunodeficiency that allows the proliferation of the *Demodex* mites (Scott et al., 2001; It et al., 2010). Demodecosis can be categorized into localized and generalized forms (Scott et al., 2001). The prognosis in localized demodecosis is generally good with self-cure occurring in most cases. Generalized demodecosis is a complex disease whose exact pathogenesis remains unclear. The course of generalized demodecosis is usually unpredictable, being a potentially serious disease to an extent of

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euthanasia of the severely affected dogs (Scott et al., 2001). Although genetic and immunologic factors play important role in its development and progression some other factors such as the breed and breeding line, age, mineral/nutritional status, oxidant/antioxidant status, length of hair coat, stage of reproductive cycle and concurrent debilitating diseases like endoparasitism are also incriminated as predisposing factors (Ghubash, 2006; Dimri et al., 2008).

Trace elements are important for optimal metabolic function. They are essential components and cofactors of numerous enzymes and modulate the immune status of an animal. Zinc is an important component or activator of numerous metalloenzymes and hormones. It is also considered an essential element for the immune response, since it affects and cooperates in a specific way with various components of the immune system (Hirano et al., 2008). Copper is a natural component of many constitutional proteins of enzymatic properties like, ceruloplasmin, cytochrome oxidase, metallothionein, superoxide dismutase and also participates in numerous oxidation/reduction reactions (Gaetke and Chow, 2009). It demonstrates a haematopoetic effect, modulates immune processes and is necessary for the proper activity of many vitamins and hormones. Iron is considered the most dangerous transition element in body for its ability to readily accept and transmit electrons between the molecules. It participates in many metabolic processes required for normal functioning of the body. Its role in the process of cell death, consequent to oxidative damage is well documented (Emerit et al., 2001).

Because of unpaired electrons many trace elements (transition metals) participate in free radical generating reactions, like Fenton reaction. An increased content of reactive oxygen species (ROS) and exhausted defence mechanisms leads to cell dysfunction and incidence of various diseases (Lykkesfeldt and Svendsen, 2007). In skin diseases, the bodies antioxidant protection conferred by superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), GSH-peroxidase (GPx), and the antioxidant vitamins A, E and C (Portugal et al., 2007) act synergistically to cause sequential degradation of peroxides and free radicals (Bickers and Athar, 2006). The present study was aimed to determine the levels of selected transition metals, i.e. zinc, copper and iron, oxidant/antioxidant balance in the dogs affected with generalized demodecosis and the relationship between trace elements and antioxidant defence in dogs with generalized demodecosis.

2. Materials and methods

2.1. Selection of animals

Dogs of different sex and breed, in the age group between 6 and 24 months, presented to Teaching Veterinary Clinical Complex for dermatological problems were included in the study. A total of 30 dogs were studied, twenty four were diagnosed as generalized demodecosis by clinical and parasitological examination. Six healthy dogs were used as control group. The control dogs included dogs brought to the clinic for routine checkup, vaccination and/or for deworming. Dogs positive for *Demodex canis* mites with minimum of five affected areas (>10 cm^2 each), a single-affected body region (>100 cm^2) or at least one affected paw (pododemodecosis) were classified as generalized demodecosis. The infested dogs had a history of dermatological problem for at least 3–4 weeks before presentation and were not subjected to any medication for at least 30 days prior to collection of blood samples. The feaces of the diseased dogs were microscopically examined to rule out any gastrointestinal parasites/ova.

2.2. Dermatological examination

For parasitological examination the edge of active skin lesion was painted with glycerin and deeply scraped with a sterile scalpel until blood oozes. Scraps were collected in 10% potassium hydroxide solution and examined under a microscope after a gentle heating. The parasite was identified according to its morphological characteristics (Izdebska and Fryderyk, 2011) and the species identified after microscopic examination was found to be *Demodex canis*.

2.3. Blood sample collection

Ten ml of blood sample was collected in acid washed heparinized vials from each animal for the estimation of zinc, iron, copper, β-carotene and vitamin C. The blood samples were centrifuged at 3000 rpm for 10 min to harvest plasma and the remaining RBCs were used for the estimation of oxidative stress parameters.

2.4. Analysis of plasma zinc, copper and iron

Trace elements viz. zinc (Zn), copper (Cu) and iron (Fe) were estimated as per the method described by Kolmer et al. (1951), with little modification, using Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI). Two ml plasma sample was mixed with equal volume of concentrated nitric acid and kept on low heat (below 90 °C) for digestion on hotplate till volume reaches 1.5 ml. To this volume 2 ml of hydrogen peroxide was added and the sample was again digested till volume reaches 1.5 ml. The final volume of 10 ml was made by adding distilled water. The concentration of zinc, iron and copper in digested samples were then estimated by recording absorbance of digested samples.

2.5. β-Carotene estimation

The β-carotene was estimated as per the method described by Baker and Frank (1968). Two ml of 95 percent ethanol was added to 2 ml of plasma and vortexed vigorously for 1 min. To this 3.0 ml of petroleum ether was added and vortex mixed followed by centrifugation at 1500 rpm for 3 min. Finally 2.0 ml of supernatant was pipetted into a cuvette and the absorbance was read at 450 nm against petroleum ether as blank. The concentration of the carotene was calculated from the standard curve plotted within concentration range of 100–10 μg/dl of petroleum ether.