Oxidative stress, hematobiochemical parameters, trace elements and vitamins in dogs with zinc responsive dermatosis

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ABSTRACT

The aim of this study was to examine the hematobiochemical alterations and evaluate the oxidative stress indices in the blood of dogs with zinc responsive dermatosis. The study included 9 dogs with clinically established diagnosis of zinc responsive dermatosis and 6 dogs as a healthy control. The clinical disease was characterized by alopecia, erythema, hyperkeratotic foot pads and scaling around the eyes, chin, eyes, head and legs. The MDA levels were significantly (P<0.01) higher, whereas superoxide dismutase and catalase activity were significantly (P<0.01) lower when compared to healthy control dogs. Hematology revealed significant neutrophilia and lymphopenia, along with anemia. Biochemical examination revealed a significant (P<0.05) decrease in albumin, A:G ratio and glucose levels, and a significant increase (P<0.05) in globulin level. Plasma zinc, vitamin A and C were significantly decreased, however copper levels were increased. In conclusion, zinc responsive dermatosis is accompanied by oxidative stress and anemia, and affected animals are susceptible to infection.

Key words: biochemical, dermatitis, hematology, hyperkeratotic, oxidative stress, zinc

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Introduction

Zinc is an essential trace element found in all body tissues and body fluids. Eighty-five percent of body zinc is present in the muscles and bones, 11% in the skin and liver, and 2% to 3% in other organ tissues. Zinc concentrations tend to be higher in tissues with high epithelial proliferation rates, and normally parakeratotic sites, such as the nose, as well as keratinized pressure areas, such as the footpads, contain the largest zinc concentrations (COLOMBINI, 1999). Animals require a continuous supply of zinc as readily available body stores are limited and dietary requirements vary according to the species and physiological state of the animal. Young growing animals and breeding females require higher levels of zinc than healthy, non-reproducing adults (COLOMBINI and DUNSTAN, 1997). Moreover, absorption and utilization of zinc may be influenced by the presence of other dietary ingredients, such as phytate or excess calcium in the diet (WHITE et al., 2001).

In dogs, zinc-responsive dermatosis is a rare skin disease that is clinically classified into two distinct syndromes. Syndrome I is characterized by inherited impairment in the absorption or metabolism of zinc and is seen primarily in northern breed dogs (Siberian huskies, Alaskan malamutes and bull terriers) (WHITE et al., 2001). Syndrome II develops in rapidly growing, large-breed puppies on a zinc deficient diet, or with high phytates in the diet, or one containing an excess of vitamins, minerals (calcium and iron) that may inhibit zinc absorption. Cutaneous lesions are almost similar in both syndromes and include erythema at the mucocutaneous junction and pressure points, scaling, crusting, alopecia, and lichenification. However the severity is greater in syndrome II.

Oxidative stress has been implicated in playing an important role in the etiopathogenesis of various infectious, inflammatory and degenerative diseases (EWANS and HALLIWEL, 2001; IRSHAD and CHAUDURI, 2002) including dermatitis (ROMANUCCI et al., 2011; BEIGH et al., 2013a; BEIGH et al., 2014). Zinc is an important anti-oxidant and its deficiency has been reported in several infectious and inflammatory diseases, including dermatitis in dogs (BEIGH et al., 2013b). It plays an essential role in decreasing reactive oxygen species (ROS) production (FERNANDEZ et al., 2003) either by interacting with essential elements, such as Cu and Fe, and decreasing their tissue concentration (SANTON et al., 2003), by cell membrane stabilization, metallothionein (Mt) synthesis, or via superoxide dismutase (Cu/Zn SOD). The aim of this study was to evaluate the oxidative stress indices, hemato-biochemical, trace elements and vitamins in dogs with zinc responsive dermatosis.

Materials and methods

Out of 104 dogs with various dermatological problems, nine puppies of various breeds (German Shephard (n = 5), Labrador (n = 2), cross breed (n = 1) and Great Dane (n = 1) were diagnosed with zinc responsive dermatosis, based on history, clinical
symptoms, laboratory analysis and treatment response. The puppies presented with a history of inappetence, lethargy and mental dullness, pruritus, erythematous-crusted papules affecting nearly every region of the hairy part of the body and they had a history of calcium supplementation for more than 8 weeks. The infected dogs were reported to have been suffering from the clinical disease for at least 2-4 weeks before presentation for dermatological examination. None of the dogs had been treated with any kind of medicine, except routine calcium supplementations, for at least 30 days before taking blood samples. The control dogs include age matched dogs which came to the clinic for routine checkups. The feces of the animals were microscopically examined to rule out any gastrointestinal parasite/ova. Skin scrapings and skin cultures were taken from the dermatological lesions to rule out any ectoparasites, bacterial and fungal infections.

**Blood sample collection.** Ten mL of blood samples were collected from the dogs in acid digested heparinized vials for estimation of hemato-biochemical, trace minerals, vitamins and oxidative stress parameters. The un-clotted blood was used for the hematology estimation and the rest of the blood sample was centrifuged at 3000 rpm for 10 min to harvest plasma for the estimation of biochemical parameters, trace minerals and vitamins. The red blood cells (RBCs) were used for the preparation of 33 per cent packed erythrocyte for estimation of lipid peroxidation and 1 per cent erythrocyte lysate for the activity of antioxidant enzymes (catalase, superoxide-dismutase).

**Laboratory analysis.** The extent of lipid peroxidation was estimated in 33% of erythrocyte lysate as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by the method described by SHAFIQ (1984). The amount of lipid peroxidation was expressed as nmol MDA formed/mL of RBC’s, using a molar extinction coefficient of pure MDA of 1.5 ×10⁵. The activity of superoxide dismutase (SOD) in 1% erythrocyte lysate was determined by the method of MARKLUND and MARKLUND (1974). The assay is based on the ability of SOD to exhibit auto-oxidation of pyrogallol in the presence of EDTA. The activity was expressed as SOD units/mg Hb. The activity of catalase was determined by the method described by AEBI (1983). The activity was expressed as μmol of H₂O₂ decomposed/min/mg Hb using 36 as molar extinction coefficient of H₂O₂.

Un-coagulated blood was tested for estimation of hemoglobin (Hb), packed cell volume (PCV) (JAIN, 1986), total erythrocyte count (TEC) and total leukocyte counts (TLC). Stored plasma samples were analyzed for biochemical parameters using a commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman, India).

For mineral analysis, 2 mL of each plasma sample was analysed for trace elements viz. Zinc (Zn), Copper (Cu) and Iron (Fe), using a Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI) by the method described by KOLMER et al. (1951), with slight modifications.
Plasma vitamin A was estimated by the method based on the Carr-Price reaction and the plasma vitamin C was estimated by the 2, 4-dinitrophenylhydrazine method (BAKER and FRANK, 1968).

**Statistical analysis.** All statistical analyses were performed using the SPSS11.5 software package. Data were tested for normal distribution using the Kolmogorov-Smirnov test. Since the data were not normally distributed, the Mann-Whitney U-test was used to determine differences in the measured parameters between the two groups. All data were presented as mean ± standard deviation. A p-value less 0.05 was considered significant in all statistical analyses.

**Results**

Clinical examination of affected dogs revealed intense erythema on both the upper and lower lips, crusted lesions on the chin, and around the nose and eyes. Erythema and crusts were also observed in the interdigital areas. Hypotrichosis and crust formation were noted on the dorsal and lateral aspects of the metacarpal and metatarsal regions of all 4 limbs, along with the foot pads. The skin scarpings were negative for mites, and the fungal culture failed to show the growth of any pathogenic fungi. However, bacterial cultures in four dogs were positive for *Staphylococcus intermedius*.

Evaluation of oxidative stress indices revealed that the affected dogs had significantly (P<0.01) increased levels of MDA, whereas superoxide dismutase and catalase activities were significantly (P<0.01) decreased when compared to the control dogs (Table 1).

**Table 1. Oxidative/antioxidative stress indices in dogs with zinc responsive dermatosis**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 6)</th>
<th>Zinc deficient (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA(nmol MDA/mL of RBC’s)</td>
<td>2.30 ± 0.41</td>
<td>4.47 ± 0.32**</td>
</tr>
<tr>
<td>SOD (units/mg of Hb)</td>
<td>0.38 ± 0.25</td>
<td>0.27 ± 0.02**</td>
</tr>
<tr>
<td>CAT (μmol H$_2$O$_2$ decomposed/ min/mg Hb)</td>
<td>105.5 ± 3.25</td>
<td>87.20 ± 1.62**</td>
</tr>
</tbody>
</table>

**Significant at P<0.01**

The hematological examination revealed significantly (P<0.05) decreased Hb, PCV and TEC, and significantly (P<0.05) increased TLC, with neutrophilia and lymphopenia in affected dogs compared to the control (Table 2).
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Table 2. Hematological parameters in dogs with zinc responsive dermatosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 6)</th>
<th>Zinc deficient (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>11.26 ± 0.50</td>
<td>9.90 ± 0.34*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.78 ± 1.51</td>
<td>29.60 ± 0.15*</td>
</tr>
<tr>
<td>TEC (x10^6/mL)</td>
<td>5.80 ± 0.28</td>
<td>4.90 ± 0.23*</td>
</tr>
<tr>
<td>TLC (x10^3/mL)</td>
<td>10.34 ± 0.70</td>
<td>13.51 ± 0.30*</td>
</tr>
<tr>
<td>DLC (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>68.50 ± 2.04</td>
<td>77.08 ± 1.27*</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>25.83 ± 2.45</td>
<td>17.54 ± 0.81*</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.06 ± 0.25</td>
<td>2.4 ± 0.18</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2.33 ± 0.55</td>
<td>2.73 ± 0.17</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.28 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
</tbody>
</table>

*Significant at P<0.05

Table 3. Biochemical, trace elements and Vitamin profile in dogs with zinc responsive dermatosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 6)</th>
<th>Zinc deficient (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dL)</td>
<td>7.35 ± 0.10</td>
<td>7.47 ± 0.14</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.17 ± 0.18</td>
<td>2.68 ± 0.07</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>4.18 ± 0.09</td>
<td>4.79 ± 0.09</td>
</tr>
<tr>
<td>A:G Ratio</td>
<td>0.76 ± 0.01</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>90.62 ± 2.13</td>
<td>79.28 ± 2.86</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>28.75 ± 2.09</td>
<td>29.34 ± 1.53</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>22.26 ± 1.99</td>
<td>25.02 ± 1.16</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>256.34 ± 16.20</td>
<td>232.05 ± 14.29</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.1 ± 3.72</td>
<td>9.4 ± 2.89</td>
</tr>
<tr>
<td>Zinc (mg/mL)</td>
<td>1.46 ± 0.16</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>Iron (mg/mL)</td>
<td>1.66 ± 0.17</td>
<td>1.74 ± 0.04</td>
</tr>
<tr>
<td>Copper (mg/mL)</td>
<td>1.16 ± 0.07</td>
<td>1.40 ± 0.04</td>
</tr>
<tr>
<td>Vitamin A (mg/dL)</td>
<td>51.25 ± 3.14</td>
<td>42.97 ± 1.34</td>
</tr>
<tr>
<td>Vitamin C (mg/mL)</td>
<td>7.77 ± 0.72</td>
<td>6.23 ± 0.33</td>
</tr>
</tbody>
</table>

*Significant at P<0.05 and **Significant at P<0.01; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

The biochemical parameters revealed significantly (P<0.05) decreased albumin, glucose levels and significantly (P<0.01) increased globulin levels in affected dogs compared to the control, however no significant change was observed in protein levels. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol levels revealed no significant change between the two groups. The calcium levels were non-significantly higher in affected dogs. Trace elements revealed significantly (P<0.01)
lower levels of zinc and significantly (P<0.05) higher levels of copper in affected dogs, however no significant change was observed in iron levels. Vitamin A and vitamin C levels were significantly (P<0.05) decreased in affected dogs compared to the control group (Table 3).

**Discussion**

The diagnosis of zinc-responsive dermatosis must be based on a thorough history, physical examination, histopathological examination of skin biopsies and treatment response. In the present study, the animals were fed on generic adult dog food and had received calcium as a supplement for more than 8 weeks. Clinical signs were consistent with zinc responsive dermatitis. However, histopathological examination could not be performed because of the owner’s refusal. The cases were diagnosed as zinc responsive dermatosis on the basis of clinical symptoms, reduced plasma zinc concentrations and the recovery of puppies following oral administration of zinc and dietary corrections.

The present investigation revealed that affected dogs were in a state of significant oxidative stress, as indicated by increased MDA levels and decreased SOD and catalase activity when compared to healthy dogs. Zinc is an important anti-oxidant in the skin and its low concentrations in skin predispose the animals to oxidative damage (ROMANUCCI et al., 2011 and CUI and OKADA, 2000). Zinc acts as membrane stabilizer by preventing the formation of reactive oxygen species by means of a mechanism that involves the protection of sulfhydryl groups against their oxidation, and/or the inhibition of the production of ROS by transition metals (BRAY and BETTGER, 1990). Thus decreased zinc levels can cause an increase in ROS in the body, which might have been the reason for the increased MDA levels in the present study. Zinc has been found to inhibit endogenous as well as exogenous lipid peroxidation in both in vivo and in vitro conditions (CHVAPIL et al., 1973). SOD is a potent inhibitor of lipid peroxidation by catalyzing the dismutation of highly reactive superoxide radicals into $H_2O_2$ and $O_2$. Thus the decrease in SOD activity observed in the zinc deficient dogs could also be the reason for increased MDA levels. Increases in ROS levels and lipid peroxides cause overconsumption of SOD, resulting in its decreased activity in the affected animals. Also zinc is necessary for the synthesis and enzyme activity of Cu/Zn SOD (HALLIWELL and GUTTERIDGE, 2000) and thus decreased SOD activity could also be attributed to decreased zinc concentrations in the affected animals. The overconsumption of SOD leads to an increase in hydrogen peroxide level in the cells. This hydrogen peroxide is neutralized by a coordinated increase in catalase activity (CLEMEN and WALLER, 1987) thereby leading to increased utilization of catalase. Our results support the finding of ROMANUCCI et al. (2011) who suggested that oxidative stress is involved in the pathogenesis of canine zinc-responsive dermatosis.
The zinc deficient dogs were mildly to moderately anemic, as revealed by low Hb, PVC, TEC. Reduction in Hb content may be due to an increased rate of disruption or reduction in the rate of formation of erythrocyte (SHAKOORI et al., 1992) and the observed low TEC supports this hypothesis. Erythrocytes are highly susceptible to peroxidative damage due to the abundance of polyunsaturated fatty acids and the presence of a powerful transition-metal catalyst (RANJAN et al., 2006). The decreased blood Hb and TEC in the present study could be attributed to the increased damage to erythrocytes due to the increased lipid peroxidation caused by decreased serum zinc concentrations. The reason for decreased PCV could be attributed to the decreased cellular count in the blood. Zinc is essential for integrity of the immune system and its deficiency results in reduced immune-competence and decreased resistance to infections (PRASAD, 1996). The increase in leukocyte counts in the present study indicates the activation of the animal’s defense mechanism and immune system. The present study was in agreement with FRAKER et al. (1982) who observed zinc deficiency associated with impaired immunity.

Biochemical parameters revealed significantly higher levels of globulin and lower levels of albumin and glucose in affected dogs. The higher globulin levels observed could be due to increased production of gamma globulins in response to the infection. Albumin is an important extracellular anti-oxidant by virtue of its thiol groups, and it protects vital targets, such as erythrocytes, from copper ion-induced Fenton reaction, and inhibits ion-dependent free radical production (ROCHE et al., 2008). The decreased albumin levels in the present study could be due to its over utilization or sequestration to neutralize oxidative damage in affected animals. The hypoglycemia observed in the present study could be due to the increased need of the skin for glucose during inflammatory reaction (GOWDA et al., 1982). The non significant increase in plasma calcium observed in the present study was due to continuous calcium supplementation for more than 8 weeks. The trace mineral status revealed significantly lower levels of zinc in affected dogs, however copper and iron levels were increased. The decreased levels of zinc and occurrence of disease might have been precipitated by calcium supplementation since calcium is antagonistic to zinc absorption (SPENCER et al., 1984). There is a strong inter-relationship between zinc, copper and iron, and the increased levels of copper and iron levels in the present study could be due to the removal of the antagonistic effect of zinc on copper and iron absorption and utilization (SANDSTROEM, 2001). Transition elements, such as copper and iron, play an important role in catalyzing the formation of .OH, a powerful oxidant from H$_2$O$_2$ and O$_2$, through fenton reaction, and thus increased levels of these two minerals may also be one of the contributing causes of oxidative stress observed in the present study.

Vitamin A acts as an antioxidant by quenching singlet oxygen, neutralizing thiyl radicals, and acts in chain breaking by combining with peroxyl radicals (PALACE et al., 2008).
1999). Thus decreased levels of vitamin A can result in oxidative stress, as observed in the present study. The decreased vitamin A in the present investigation could be attributed to concurrent zinc imbalance, since zinc participates in the absorption, mobilization, transport and metabolism of vitamin A, mostly through its involvement in protein synthesis and cellular functions (CHRISTIAN and WEST, 1998). Vitamin C is a powerful antioxidant that reduces a wide range of oxidants including free radicals (BINDHUMOL et al., 2003). Thus, the decrease in the level of vitamin C in the plasma might be due to overutilization or sequestration of this antioxidant to neutralize the exaggerated production of ROS during inflammatory skin conditions.

**Conclusion**

The present investigation indicated that dogs with zinc responsive dermatosis are anemic and suffer from significant alteration in oxidative/antioxidant balance, trace elements and vitamins and are susceptible to various infections.

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**References**


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SAŽETAK
Istraživanje je provedeno radi određivanja hematološko-biokemijskih poremećaja i prosudbe pokazatelja oksidacijskog stresa u krvi pasa s cinkom izlječivom dermatozom. U istraživanje je bilo uključeno devet pasa s kliničkom dijagnozom dermatoze izlječive cinkom s šest z dravih kontrolnih pasa. Klinički se bolest očitovala alopecijom, eritemom, hiperkeratozom mekuši, ljuštenj em oko očiju, po bradi, glavi i nogama. Razine malondialdehida bile su značajno veće (P<0,01), dok je aktivnost superoksidne dismutaze i katalaze bila značajno manja (P<0,01) u usporedbi sa zdravom kontrolnom skupinom pasa. Hematološki nalazi pokazali su znatnu neutrofi liju, limfopeniju i anemiju. Biokemijskom pretragom ustanovljen je značajan pad koncentracije albumina (P<0,05), omjera A:G i razine glukoze te značajno povećanje razine globulina (P<0,05). Cink u plazmi, vitamin A i C bili su značajno smanjeni dok su razine bakra bile povećane. Zaključuje se da je dermatoza izlječiva cinkom također popraćena oksidacijskim stresom, anemijom, a zahvaćene životinje osjetljivije su na infekciju.

Ključne riječi: biokemijski pokazatelji, dermatoza, hematologija, hiperkeratoza, oksidacijski stres, cink