Clinical and Laboratory Studies in Correlation to Oxidative Stress Indices in Demodicosis Affected Dogs

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Abstract:
Skin diseases are the most common health problem among dogs. Demodicosis is the most important one. The study aimed to detect the clinical effect of demodicosis on dogs which come to veterinary clinic and the correlation between some blood parameters and some oxidative stress indices. Clinical and laboratory examination of the diseased dogs was carried out. The clinical study was conducted on two groups of dogs. The first one includes seven dogs (7.77%) out of 90 diseased dogs of different ages. The second group 14 dogs were clinically healthy used as a control group. Animals were subjected to physical examination followed by special dermatological examination. Skin scrapings were examined under microscope for mite’s detection. Blood samples were taken for hematological examination and biochemical analysis. Hematological parameters of demodicosis affected dogs showed a highly significant decrease in hemoglobin values. Regarding differential leukocytes, a significant increase in granulocytes absolute number was recorded. The biochemical findings of blood plasma obtained from dogs affected by demodicosis showed a significant increase in plasma SOD when compared with control ones and a highly significant increase in plasma MDA values. Correlation analysis revealed positive correlation between plasma zinc and copper levels and antioxidant enzymes as SOD, GPx and negative correlation between plasma SOD and plasma MDA levels. The study revealed the importance of oxidative stress...
indicators variations in diagnosis and pathophysiology of demodicosis.

**Keywords:** Dogs, Demodicosis, hematological changes, oxidative stress markers, correlation analysis

**Introduction:**
Demodicosis is a very common parasitic skin disease in dogs *(Mueller, 2004)*, in which a large number of *Demodex mites* are found in the skin *(Gortel, 2006)*. *Mueller et al. (2012)* and *Beigh et al. (2013)* described clinical signs of demodicosis that they are erythema, scaling, partial or complete alopecia, papules, follicular casts, pustules, and in severe cases, furunculosis, crusting, exudation and ulceration with focal draining tracts. Generally, the lesions begin on the face and limbs, but they may become generalized. Moreover, *Sivajothi et al (2015)* observed similar results with lichenification and cellulitis. The authors added that the clinical distribution of lesions was observed on face, around the eyes and ears, chin region, fore limbs, neck and lateral abdomen. The diagnosis of mange is typically based on clinical signs and is confirmed by the presence of mites in skin scrapings *(Carter, 2001)*. *Deb et al (2000)* and *Jyotsna et al. (2005)* reported that dogs affected with generalized demodicosis revealed significant reduction in total erythrocyte count, hemoglobin and packed cell volume resulting to anemia. Affected dogs also showed leukocytosis accompanied by neutrophilia, eosinophilia and lymphopenia with low absolute lymphocyte count.

*Nair & Nauriyal (2007)* recorded lower level of hemoglobin concentration and total erythrocytic count as well as normal level of total leukocytic count, neutrophil, lymphocyte and monocyte cells with presence of eosinophilia. *Dadhich (2008)* observed in a study on dogs with demodicosis a slight decrease in total erythrocytic count, hemoglobin and packed cell volume and an increase in total leukocytic count and eosinophilia. Similar results were obtained by *Sakina et al (2012)* who reported that the mean values of Hb, PCV and TEC were significantly lower, however MCV and MCH were significantly higher in dogs suffered from demodectic mange as compared to control group indicated macrocytic anemia in affected groups. The author reported that anemia might be due to the stress arising from the disease. The authors added that there was a significant increase in leukocytic count, neutrophil and eosinophil values.
Portugal et al. (2007) reported that in skin diseases, the body's antioxidant such as SOD, CAT, GSH, GSH-peroxidase (GPx) act synergistically to cause sequential degradation of peroxides and free radicals to combat oxidative damage. Gurer et al. (1998) reported that the measurement of antioxidant enzymes like SOD and CAT are appropriate indirect ways to assess the status of antioxidant defense and the estimation of Malondialdehyde (MDA)- a byproduct of lipid peroxidation- continues to be a reliable method to assess the degree of oxidative damage to cell membranes. Moreover, Fang et al. (2002) mentioned that estimation of antioxidant enzymes activities and levels of endogenous antioxidants in blood are indirect but reliable methods for assessment of free radicals’ activity and oxidative stress as well. Additionally, Tomsič et al. (2016) reported that total antioxidant capacity (TAC) and antioxidant enzymes, GPx and SOD, are commonly used markers of antioxidant status and oxidative stress.

Materials and methods:
the clinical study was conducted on two groups of dogs of different breeds and ages. the first group was fourteen apparently healthy dogs used as control group. the second group was the diseased included seven dogs out of ninety diseased dogs were affected with demodicosis. The study was carried out in the clinic of both faculties of veterinary medicine Suez Canal university, Ismailia, and Cairo university, Egypt. definitive diagnosis was carried by case history, physical examination, inspection of skin, skin coat, skin scraping, as well as body temperature, pulse, respiratory rate, examination of superficial lymph nodes. DIAGNOSIS was carried according to Birchard & Sherding (2005). Multiple skin scrapings were taken from the periphery of the lesions or alopecic areas until oozing of blood. After samples collections several drops of 10% sodium hydroxide (NaOH) solution were added then gently heated and examined under light microscope for the identification of mites (Houston, 2000). Anti-coagulated blood samples were drained then sent immediately for hematological analysis for complete blood pictures and separation of plasma. Plasma freeze at -80°c for biochemical analysis. The hematological procedures were carried out using fully automatic blood cell counter. Zinc, copper, SOD, Catalase, GPX and MDA were estimated using colorimetric test kits (Bio-Diagnostic Company Egypt)
according to manufacturer’s instructions (Hayakawa, 1961, Ventura and King 1951, Nishikimi et al. 1972, Aebi 1984, Paglia and Valentine 1967 and Ohkawa et al 1979) respectively.

Statistically, the obtained data was analyzed using statistical program of social science (SPSS) for windows, Version 24. Values of the measured parameters were expressed as mean value ± slandered error (S.E) and the difference between means of the two groups was determined by using one tail t-test and the significance was considered at P values <0.05 or 0.01. Correlations between variables were evaluated using Pearson Correlation Coefficient. The interpretation of Pearson correlation Coefficient according to (Mukaka, 2012).

Results and discussion:
The clinical investigation revealed that demodicosis was diagnosed in seven (7.77%) out of ninety dogs suffered from dermatological problems. These results coincided with Aujla (2000) who reported that the prevalence of canine demodicosis was 6.04% out of 281 examined dogs during the study. Similar factors were reported by Deepa et al. (2005) who stated that demodicosis is influenced by numerous factors such as alimentation, presence of stress factors, fitness and other diseases or pathogens. Shrestha et al (2015) found that prevalence rate of demodicosis was higher in stray dogs who suffer from poor management, poor body condition and improper nutrition. Dogs affected by demodicosis showed pale mucus membrane, slightly elevated pulse and respiratory rates. Demodicosis was manifested by diffuse alopecia, excessive scaling, erythema, characteristic offensive odor and severe itching. In most cases the lesions accompanied by secondary pyoderma. Similar signs were reported by Gortel (2006), Mueller et al. (2012), and Beigh et al. (2013) reported similar signs as erythema, scaling, complete alopecia, papules, follicular casts, pustules and in some cases furunculosis, crusting, exudation and ulceration with focal draining tracts. Microscopically examination of skin scrapings revealed presence of Demodex canis in samples taken from diseased dogs. These results were similar to those of Carter (2001) and Mueller et al. (2012) who reported that the diagnosis of demodicosis is made by deep skin scraping. Mueller and Bettenay (2010) stated that finding more than one mite is strongly suggestive of clinical demodicosis. Moreover,
Katariya et al (2018) screened all dogs in their study for the presence of mites. The authors recorded the presence of mites in eight dogs. Hematological parameters of demodicosis affected dogs showed a highly significant decrease in hemoglobin values. Similar results were recorded by Deb et al (2000), Nair and Nauriyal (2007), Dadhich, (2008) and Sudhakara et al (2014). Janus et al. (2014) interpreted the decrease in Hb level and PCV values to the deteriorated condition of affected dogs owing to reduced food intake, systemic illness, toxemia and septicemia caused by mites as well as secondary bacterial infection. Similar explanation reported by Pathak and Bhatia (1986). Regarding differential leukocytes, a significant increase in granulocytes absolute number was recorded this results agree with the results obtained by Dadhich (2008), Sakina et al (2012), Reddy et al (2015) and Katariya et al (2018). A highly significant increase in monocyte which coincided with the results obtained by Sudhakara et al (2014). Also, Rebar (1998) who mentioned that eosinophilia and monocytosis are the best indicators of dermatitis in case of demodicosis.

The biochemical findings of blood plasma obtained from dogs affected by demodicosis showed a significant increase in plasma SOD when compared with control ones. These findings agree with the results obtained by Dimri et al. (2008) Salem et al. (2020) and Kubesy et al (2020). They reported that the increase in plasma SOD activity could be attributed to upregulation in its synthesis to counteract free radicals. Moreover, Additionally, Singh et al. (2014) and Abdulaziz et al. (2019) mentioned that the reason of the observed elevation in plasma SOD levels in mange affected dogs may be attributed to its role as the first-line of defense antioxidant as it helps the body to remove the superoxide radicals by converting them to hydrogen peroxide (H2O2). However, some authors suggested that the increase in the antioxidant enzymes is not necessarily desirable, as the antioxidant enzymes are not always decreased in some disease conditions as reported by Russo and Bracarense (2016). The study revealed a highly significant increase in plasma MDA values. These findings were supported by Gurer et al. (1998) who reported that estimation of plasma malondialdehyde considered to be a reliable method to assess the degree of peroxidative damage of cell membrane, as it is the
most abundant aldehyde formed as a by-product during this process. Similarly, Cini et al. (1994) and Gurur et al. (1998) reported that estimation of plasma Malondialdehyde (MDA), a byproduct of lipid peroxidation, continues to be a reliable method to assess the degree of oxidative damage to cell membranes. The obtained findings were similar to that obtained by Beigh et al. (2013), Kubesy et al (2020) and Salem et al (2020) who observed increased plasma MDA levels in their study on demodicosis of dogs. The authors added that increased plasma MDA levels reflects increased membrane lipid peroxidation, providing evidence of enhanced free radicle generation in generalized demodicosis. Beigh et al. (2016) attributed the increase of plasma MDA to the oxidative stress interceded by pro-inflammatory cytokine liberation during external parasitic infestation. Demodicosis affected dogs (table 3) revealed moderate positive correlation between plasma zinc level and plasma Cu level and a moderate positive correlation between plasma Cu level and plasma SOD activity. Beigh et al (2013) recorded a strong positive correlation between SOD activity and Cu in both healthy and diseased dogs. The author added that a positive correlation between Zn and SOD in both healthy and diseased dogs. These results were explained by AL-Qudah et al. (2011) who reported that trace elements are required for the activity of a number of enzymes, including antioxidant enzymes. 

Ewans and Halliwell (2001) stated that copper along with zinc are essential components of the body’s antioxidant defense. Additionally, Ighodaro and Akinloye (2018) mentioned that SOD is a metalloenzyme and hence, requires a metal cofactor for its activity as iron, Zn and Cu. The correlation analysis in the present study revealed a high negative correlation between plasma MDA activity and plasma SOD activity in dogs affected with demodicosis. Similar results were obtained by Beigh et al (2013) who recorded that MDA levels were strongly negatively correlated with SOD activity in diseased dogs. Rice-Evans and Burdon (1994) mentioned that antioxidants function to delay or prevent ROS-induced cellular damage, and work by reducing local oxygen concentrations, impairing chain initiation reactions. However, Cini et al. (1994) reported that malondialdehyde (MDA) is a byproduct of lipid peroxidation that resulting in oxidative damage to cell membranes. The previous authors as well as the recorded results of table 3
confirmed the negative correlation between antioxidant enzymes such as (SOD, Catalase) and MDA as a byproduct of lipid peroxidation.

Table (1): Blood Parameters of Demodicosis Affected Dogs Compared with Clinically Healthy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=14) (Mean ±SE)</th>
<th>Demodicosis (n=7) (Mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>14.86 ±0.53</td>
<td>11.73** ±0.91</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>39.00±1.34</td>
<td>34.73±2.27</td>
</tr>
<tr>
<td>RBCs (10^6/μL)</td>
<td>5.98±0.25</td>
<td>5.28±0.23</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>67.28±2.25</td>
<td>64.20±2.02</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>38.15±2.31</td>
<td>33.50±1.03</td>
</tr>
<tr>
<td>WBCs (10^3/μL)</td>
<td>9.72±0.86</td>
<td>13.61±1.94</td>
</tr>
<tr>
<td>Granulocyte /cubic mm</td>
<td>1.10±0.01</td>
<td>2.6*±0.07</td>
</tr>
<tr>
<td>Lymphocyte /cubic mm</td>
<td>7.01±0.05</td>
<td>9.06±0.11</td>
</tr>
<tr>
<td>Monocyte /cubic mm</td>
<td>0.65±0.01</td>
<td>13.60**±1.85</td>
</tr>
</tbody>
</table>

*Significance p (0.05) ** Highly significance p (0.01)

Table (2): Biochemical Findings of Demodicosis Affected Dogs Compared with Clinically Healthy Dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=14) (Mean ±SE)</th>
<th>Demodicosis (n=7) (Mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (μ/ml)</td>
<td>0.822±0.040</td>
<td>0.786±0.052</td>
</tr>
<tr>
<td>Cu (μmole/ml)</td>
<td>0.087±0.012</td>
<td>0.079±0.015</td>
</tr>
<tr>
<td>Catalase (U/ml)</td>
<td>0.289±0.020</td>
<td>0.364±0.039</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>2.280±0.170</td>
<td>2.770*±0.124</td>
</tr>
<tr>
<td>GPx (u/ml)</td>
<td>0.212±0.014</td>
<td>0.225±0.022</td>
</tr>
<tr>
<td>MDA (nmole/L)</td>
<td>0.212±0.014</td>
<td>0.225**±0.022</td>
</tr>
</tbody>
</table>

*Significance p (0.05) ** Highly significance p (0.01)
Table (3) Correlation Between Trace Elements and Antioxidants of Dogs Affected with Demodicosis.

<table>
<thead>
<tr>
<th></th>
<th>Zn</th>
<th>Cu</th>
<th>CAT</th>
<th>SOD</th>
<th>GPx</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>1</td>
<td>0.670&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.112</td>
<td>0.382</td>
<td>-0.012</td>
<td>0.120</td>
</tr>
<tr>
<td>Cu</td>
<td>0.670&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>-0.406</td>
<td>0.568&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.040</td>
<td>0.408</td>
</tr>
<tr>
<td>CAT</td>
<td>-0.112</td>
<td>-0.406</td>
<td>1</td>
<td>0.277</td>
<td>-0.772&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.002</td>
</tr>
<tr>
<td>SOD</td>
<td>0.382</td>
<td>0.568&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.277</td>
<td>1</td>
<td>-0.266</td>
<td>-0.809&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx</td>
<td>-0.012</td>
<td>-0.040</td>
<td>-0.772&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.266</td>
<td>1</td>
<td>-0.009</td>
</tr>
<tr>
<td>MDA</td>
<td>0.120</td>
<td>-0.408</td>
<td>-0.002</td>
<td>-0.809&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.009</td>
<td>1</td>
</tr>
</tbody>
</table>

1. Very high positive/negative correlation (0.9 to 1.0)/(-1.0 to -0.9)
2. High positive/negative correlation (0.7 to 0.9)/(-0.9 to -0.7)
3. Moderate positive/negative correlation (0.5 to 0.7)/(-0.7 to -0.5)

References:


Carter G.R. (2001). External parasitic diseases of dogs and...


Tomšič, K., Seliškar, A., Lukanc, B., & Svete, A. N.