Research Article

Evaluation of Hemato-Biochemical Parameters, Histopathological Altration and Antioxidant Trace Elements in Demodicosis Infected Dogs

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Abstract | Canine demodicosis is a misery that causes panic to pet owners and caused by excessive proliferation of the normal skin commensal Demodex mites. Hemato-biochemical changes were previously reported in patient dogs with demodicosis due to the effect of stress caused by mites. This study was conducted on 38 dogs, 20 of them were used as control group and 18 were demodicosis infected dogs, for evaluation of hematological, biochemical and histopathological alterations. Beside detection of the antioxidant trace elements (Zn, Cu, Se) levels in dogs suffering from demodicosis. Results showed significant decrease in the value of (PCV) and (TEC). Affected dogs also showed leukocytosis associated with lymphocytosis. Hypothyroidism demonstrated by reduced levels of free T4 and significant decrease in antioxidant trace elements level (Zn, Cu, Se).

Keywords | Antioxidant trace elements, Biochemistry, Canine demodicosis, Hematology, Histopathology

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INTRODUCTION

Skin is the major and the most significant organ of the body which represents about 12-24% of the animal's bodyweight (Kutlay and Hoştürk, 2005). It performs many fundamental functions including protection against external physical, chemical, and biologic assailants, as well as preventing excess water loss from the body in addition to playing a vital role in thermoregulation (Kanitakis, 2002).

Dermatologic disorders still remain the most frequent and exasperating problems in canine medicine comprising about more than 20% of the cases received by veterinary clinics. The condition of dog's skin can be a significant mirror of the general health circumstances (Sue-Paterson, 2008; Nuttall et al., 2009), including the oxidative stress resulting from load of the free radicals due to excessive consuming of antioxidant enzymes and antioxidant trace minerals.

Skin is the major target of oxidative stress due to reactive oxygen species (ROS) and free radicals following exposure to environmental stimuli or from normal cellular metabolism. Oxidative stress is incriminated in the pathogenesis of various infectious and inflammatory diseases all over the body including dermatitis and is normally neutralized by antioxidants (Nachbar and Korting, 1995; Bickers and Athar, 2006; Singh et al., 2011).

Oxidative stress is associated with the decrease in the

effectiveness of antioxidant defenses. Antioxidants reduce the damaging effect of ROS and prevent ROS-induced cellular damage. However, increased or prolonged ROS action can devastate antioxidant defense mechanisms, contributing to the development of cutaneous disorders (Schafer and Buettner, 2001; Trouba et al., 2002; Paulsen and Carroll, 2013).

Antioxidant trace elements; zinc, copper and selenium are considered essential components of specific endogenous antioxidant and required for the activity of antioxidant enzymes, for example, Cu and Zn are required for activities of superoxide dismutase enzyme (Al-Qudah et al., 2011; Genther and Hansen, 2014; Ighotlaro and Akinloye, 2018).

Selenium acts as natural antioxidant. It is an integral part of the antioxidant enzyme Glutathione peroxidase, which inactivates hydrogen peroxide through its dissociation to water and oxygen, hence protects the body against oxidative damage (Fuchs, 1992).

Zinc metalloenzymes catalyze the conversion of O_2 radicals into H_2O_2 and antagonize the catalytic properties of iron and iron mediated xanthine oxidase (Gibbs et al., 1985; Sato and Bremner, 1993; Rossman and Goncharova, 1998; Saul, 2000; Zelko et al., 2002).

Animal's skin is usually exposed to attacks by different types of external parasites such as *Demodex* species; the most frequent cause of skin diseases in dogs (Muller et al., 1989; Verde, 2005). Canine demodicosis is a very common inflammatory noncontagious parasitic dermatosis caused by overpopulation of the host-specific follicular *demodex canis* mite (Singh et al., 2011a; Foley et al., 2021). Predisposing factors such as genetic factors, immunologic disorders, trace minerals deficiencies, oxidant and antioxidant levels, as well as immunosuppression exhibit a chief role that leads to mite proliferation in hair follicles resulting in development and progression of canine demodicosis (Beigh et al., 2013; Kumari et al., 2018; Gazi et al., 2019).

Canine Demodicosis is clinically manifested by 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 (localized) but they may become generalized. Multiple, deep skin scrapings from affected areas are the diagnostic test of choice. Microscopically, fusiform eggs, six-legged larvae, eight-legged nymphs or cigar-shaped adult mite may be seen (Horne, 2010; Mueller et al., 2011).

Demodectic dogs are in a state of significant oxidative stress, as indicated by decreased SOD activity due to overconsumption by ROS revealing exhaustion of antioxidant system (Beigh et al., 2013; 2014b).

Previous literatures (Dimri et al., 2008; Beigh et al., 2013) demonstrated significant decrease in Zn, Cu levels and SOD activity in dogs with demodicosis with lack of histopathological and biochemical alterations accompanied with such parasitic infestation. Therefore, the present work aimed to evaluate the hemato-biochemical parameters and histopathological alteration as well as antioxidant trace elements (Zn-Cu-Se) levels in demodectic dogs.

MATERIALS AND METHODS

Animals

All procedures of animals handling and samples collection were compatible with guidelines approved by the Ethics of Animal Experiments Committee, Faculty of Veterinary Medicine, Cairo University, Egypt.

Thirty-eight dogs with different breeds and sexes were enrolled in the present study. Their ages ranged from 3 months to 10 years. Out of 38 dogs there were 20 apparently healthy dogs served as control group and 18 were affected with demodicosis. Dogs were admitted to the small animal medicine teaching hospital, Faculty of veterinary medicine, Cairo University, Egypt during the period from October 2020 to December 2021. Thorough history, clinical signs were recorded and animals were exposed to a comprehensive clinical scrutinization, skin biopsy and skin scrapings were also undertaken.

Skin scraping

Multiple deep skin scrapings were performed from the periphery of the lesion in the direction of hair growth until capillary bleeding. The scraped sample was spread on a clear glass slide with several drops of tap water. The samples were examined microscopically under low and high power objectives according to (Hnilica and Patterson, 2016).

Blood Samples and hematological analyses

Whole blood samples: 3 ml of whole blood samples were collected on EDTA tube from each dog from the cephalic vein or saphenous vein and used for estimation of hematological parameters including packed cell volume (PCV), hemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC) and thrombin by using Diatron hematology analyzer, USA.

Serum samples: Blood samples were collected from diseased and apparently healthy dogs in plain tubes for separation of clear non hemolized serum. Sera were analyzed for estimation of alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, thyroid stimulating

hormone (TSH), free thyroxin (FT4), cortisol, copper (CU) and zinc (Zn) levels using specific kits according to manufactures instructions (spectrum diagnostic, Egypt) using Diatron chemistry analyzer (USA). Selenium (Se) measured in serum samples according to method described by (Lavu et al., 2012; Van Zelst et al., 2015) using Thermo scientific ICE 3300 Atomic Absorption spectrophotometer, Germany.

Skin biopsy and histopathological examination

Histopathological examination: Skin biopsy specimens were collected (using 3 mm circular punch at a depth of 2 mm) and fixed in 10% neutral buffer formalin, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 4-5 micron thickness and stained with hematoxylin and Eosin for light microscopic examination (Olympus BX50, Tokyo, Japan (Bancroft et al., 2012).

Statistical analysis

Statistical analysis was performed by SPSS version 20 (IPM Inc., Chicago). Descriptive statistics were presented as mean±SE (Standard Error). Diseased animals, blood parameters and trace elements were compared to control animals using student T-Test, P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

During careful scrutinization of demodicosis infected dogs, the most obvious clinical signs were erythema, scaling, alopecia, papules, and pustules of one body region as shown in Figure 1 or more severe as shown in Figure 2 that showing complete alopecia, furunculosis, crusting, exudation and ulceration with focal draining tracts with offensive odor of the skin.

Figure 1: Dog showing localized demodicosis.

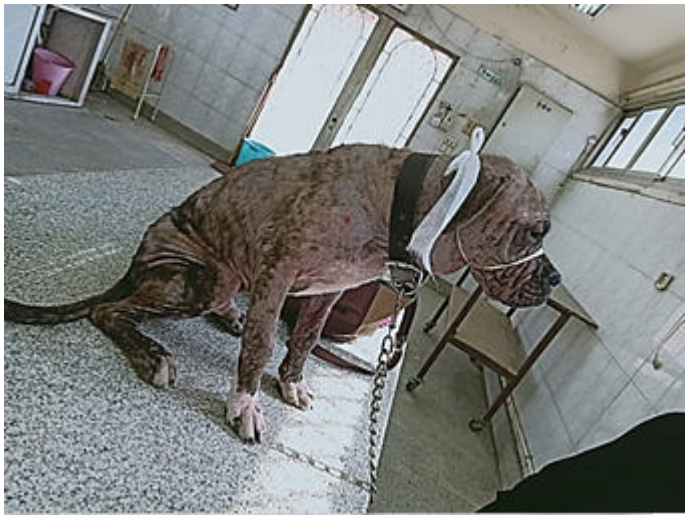

Figure 2: Dog showing generalized demodicosis.

Microscopic examination findings of multiple, deep skin scrapings from affected areas were shown in Figure 3. Skin biopsy specimens showed severe histopathological lesions of demodicosis. These lesions summarized as heavy mites infestation in different developmental stages in the stratum conium of the epidermis and in the follicles. Laminar orthokeratotic hyperkeratosis, vacuolated epidermal prickle cells, acanthosis and vaculation of keratinocytes of the infundibula. Furthermore, the dermis showed dermatitis characterized by moderate to marked inflammatory infiltration of the dermis by macrophages, lymphocytes and eosinophils. Necrosis of adnexa, folliculitis and per folliculitis were also recorded in examined cases as showed in Figure 4.

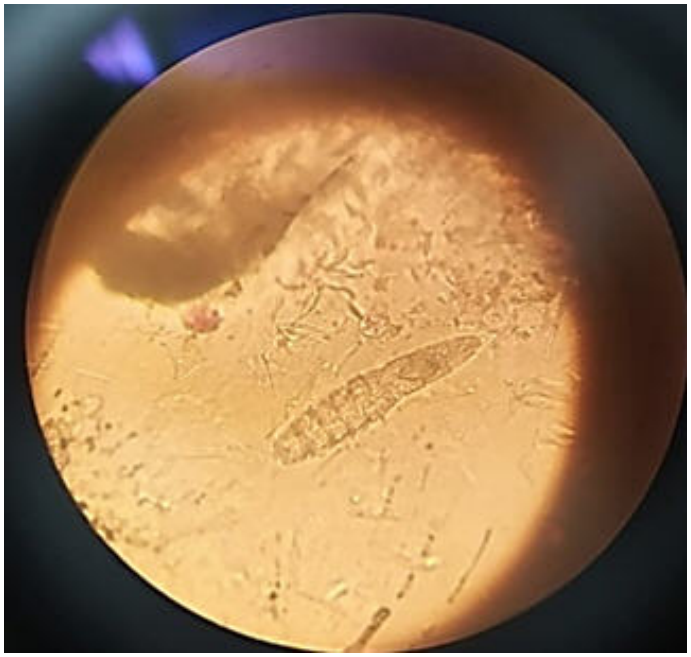

Figure 3: *Demodex Canis* adult mite under microscope.

The mean values of hematological, biochemical parameters and antioxidant trace elements (Zn-Cu and Se) were

illustrated in Table 1. Regarding biochemical analysis, as shown in Table 2, there was no significant difference in the level of (ALT) as well as significance decrease in (Free T4- creatinine-BUN) between affected dogs and apparently healthy dogs. There were significant decreases in (Zn, Cu, Se) levels in serum as shown in Table 3.

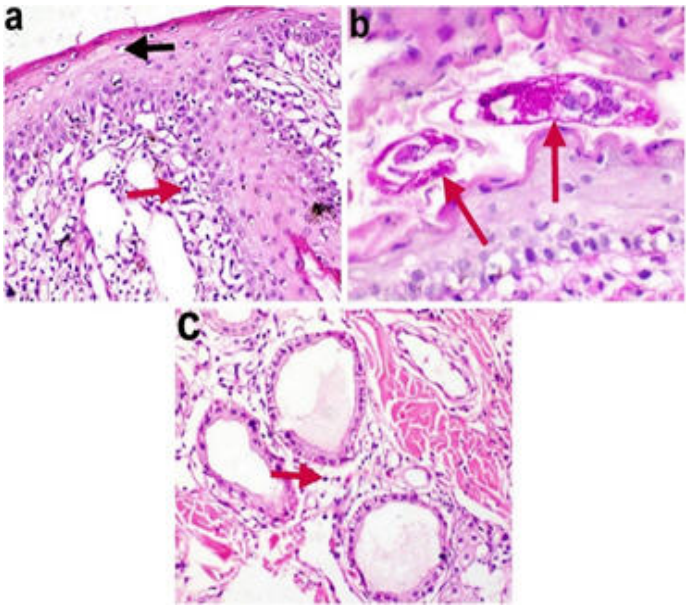

Figure 4: Photomicrographs of a skin biopsy of generalized demodicosis infected dog showing (a) vacuolar degeneration of the epidermal prickle cells (black arrow) and severe dermatitis (red arrow). (b) Demodex mite in the follicle (red arrows). (c) periglandular inflammatory cells infiltration (red arrow) (H & E, X 200 (a & c), X 400 (b).

Table 1: Hematological profiles in demodicosis infected dogs.

Parameters	Control (n=20) Mean±SE	Demodicosis (n = 18) Mean±SE	p Value
Hb (g/dl)	14.68±0.34	13.08±0.88	0.08
RBCs count (×10⁶U/l)	6.98±0.15	6.00±0.39*	0.02
PCV % (HCT)	45.84±1.06	38.28±2.67*	0.01
MCV (fl)	65.21±0.94	63.90±1.37	0.42
MCH (pg)	21.23±0.25	21.92±0.47	0.19
MCHC (g/dl)	33.55±0.30	34.08±0.33	0.25
RDWcv %	15.25±1.20	19.81±0.77*	0.00
Plts Count (×10³ U/L)	297.80±21.53	172.67±28.81*	0.00
WBCs Count (×10³U/l)	11.24±0.57	18.29±1.40*	0.00
Neutrophils%	66.89±1.30	40.16±5.75*	0.00
Staff %	2.70±0.20	3.00±0.00	0.17
Seg %	64.19±1.23	37.16±5.75*	0.00
Lymphocytes%	22.03±1.11	49.25±6.41*	0.00
Eosinophils %	7.20±0.5	6.55±0.60	0.42
Monocytes%	3.87± .28	4.03±0.36	0.73

S.E= Standard Error; *Means are significant at $p< 0.05$

Table 2: Serum biochemical findings in demodicosis infected dogs.

Parameters	Control (n=20) Mean±SE	Demodicosis (n = 18) Mean±SE	p value
ALT (U/L)	50.90±5.50	39.06±6.61	0.17
BUN (mg/dl)	19.90±1.18	14.12±1.05*	0.00
Creatinine(mg/dl)	1.01±0.059	0.78±0.034*	0.00
TSH (ng/ml)	33±0.04	27±0.05	0.41
Free T4 (pmol/l)	21.15±2.17	9.62±1.66*	0.00
Cortisol (nmol/l)	197.60±9.85	710.85±38.89*	0.00

S.E = Standard Error, *Means are significant at $p< 0.05$

Table 3: Serum antioxidant trace elements of demodicosis infected dog.

Parameters	Control (n = 20) Mean±SE	Demodicosis (n = 18) Mean±SE	p value
Selenium (mg/dl)	0.25±0.01	0.04±0.00*	0.000
Copper (mg/dl)	111.15±3.34	64.41±4.38*	0.000
Zinc (mg/dl)	102.7±2.38	64.25±3.83*	0.000

S.E = Standard Error *Means are significant at $p< 0.05$

Current study aimed to emphasize the antioxidant status, represented by antioxidant trace elements in dogs clinically suffered from *demodex* mite infestation confirmed by microscopical examination with recording the haemato-biochemical and histopathological alterations accompanied with such severely common dermatological problem.

The morphological appearance of *demodex* mite under the microscope is confirmative of the disease and this result agreed with that previously reported by (Houston, 2000).

Referring to histopathological findings, our results agreed with (Rojkoet al., 1978; Scott et al., 1996; Machado et al., 2012; Bond et al., 2020). Regarding erythrogram, our findings revealed significant decrease in the values of PCV and TEC which came in accordance with the findings previously stated by (Jyotsna et al., 2005; Patel et al., 2005; Nair and Nauriyal, 2007; Dadhich and Khanna, 2008; Singh et al., 2011b; Sakina et al., 2012; Beigh et al., 2014b; Reddy et al., 2015; Farag, 2016). The decrease in the values of (TEC) might be due to loss of skin protein that leads to anemia as reported by (Deb et al., 2000) or due to the stress arising from the disease as reported by (Sakina et al., 2012).

Regarding leucogram, our findings revealed a significant increase in TLC, accompanied with lymphocytosis. These findings agreed with (Jyotsna et al., 2005; Patel et al., 2005; Dadhich and Khanna, 2008; Singh et al., 2011b; Sakina et al., 2012; Beigh et al., 2014b; Farag, 2016). The

previous changes were explained by Sakina et al. (2012) who reported that leukocytosis may be due to generalized inflammation and response of leukocytes to prolonged antigenic stimulus in the form of chronic *demodex* mite infection, or due to a higher cutaneous inflammatory reaction as explained by (Reddy et al., 2015). There was no significant change in the level of eosinophils and this finding came in accordance with (Tsai et al., 2011; Mctaggart, 2013; Bate, 2013) who demonstrated a normal level of blood eosinophils in demodectic dogs.

Regarding biochemical analyses, our results settled with (Beigh et al., 2014b; Farag, 2016) who recorded non-significant change in the level of Alanine aminotransferase (ALT). Also, obtained results agreed with (Mederle et al., 2010; Bate, 2013; Mctaggart, 2013) who hypothesized presence of somewhat correlation between demodicosis and hypothyroidism, presumably because of suppression of the immune system allowing proliferation of mites. The increased number of mites and/or microbes due to immunosuppression can cause skin disease. However, disagreement with (Reddy et al., 2015) was noticed as he found that demodectic dogs showed no significant difference in free T4 levels when compared with clinically healthy dogs.

Considering the analyses of antioxidant trace elements, our results came in accordance with (Dimri et al., 2008; Beigh et al., 2013) who mentioned a significant decrease in Zn and Cu levels in dogs with demodicosis compared with clinically healthy control group.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the reduction in the levels of antioxidant trace element (Zn, Cu, Se) contributes to the development of demodicosis as a result of oxidative stress. The most consistent hematological and serum biochemical alterations associated with demodicosis infection in dogs were significant decrease in TEC and PCV. Presence of leukocytosis, associated with lymphocytosis. Presence of hypothyroidism as demonstrated by reduced levels of free T4. It is recommended to give a special attention and further studies regarding cases of hypothyroidism concurrently with dermatological affections because thyroid hormones are needed to initiate anagen phase of hair growth, stimulate erythropoiesis and regulate the function of immune system.

NOVELTY STATEMENT

This work highlighted the alterations in the levels of antioxidant trace elements; Zn, Se and Cu in demodectic dogs as well as hemato-biochemical and histopathological changes accompanied with such clinical condition.

AUTHOR'S CONTRIBUTION

Abdou OM put the idea and the protocol of work, El-Motaily NM collected the specimens and samples, Farag HS and El-Motaily NM applied the hemato-biochemical analysis, Ahmed KA performed the histopathological analyses, Saber M analyzed obtained results statistically, Farag HS and Saber M wrote the manuscript and Abdou OM revised the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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