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Clinical and Laboratory Studies on Dogs Suffering from Pyoderma in Correlation to some Microelements and Some Oxidative Stress Markers

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Abstract
The present study was carried out in the clinic of Faculties of Veterinary Medicine Suez Canal University, Ismailia and Cairo University, Cairo, Egypt on household dogs and cats admitted to both clinics and suffer from skin problems. The study was continued for one year. The clinical study was conducted on two groups of dogs. The first one was the diseased included ninety (90) dogs of different ages. The second group was clinically healthy fourteen (14) dogs used as a control group. Animals were examined clinically and special dermatological examination. Skin scraping and ear swapping samples were examined under microscope. Blood samples were taken for complete blood pictures and separation of plasma. Plasma used for analysis of zinc, Cu and oxidative stress markers including catalase, SOD and GPx. The history of diseased dogs suffered from pyoderma indicated that they were suffered from a previous mite, fungal infection, external parasitic infestation or a previous allergy signs. Clinical examination revealed that 17 out of ninety skin diseased dogs were diagnosed as Pyoderma with a ratio of 18.9 %. The most common lesions were erythema, papules, pustules, erosions, alopecia and Pruritus. The hematological analysis of pyoderma affected dogs showed anemia due to the highly significant decrease in hemoglobin value and MCHC value. A significant increase in total leukocytic count and lymphocyte % and a highly significant increase in granulocyte and monocytes % were prevalent. A significant increase in both catalase activity and SOD values in diseased dogs. A high positive correlation between plasma Zn and plasma Cu levels, with high negative correlation between plasma Zn level and plasma catalase activity. Very high negative correlation between plasma catalase activity and plasma MDA level was recorded with Moderate negative correlation between plasma SOD activity and plasma MDA level and a moderate negative correlation between plasma MDA level and plasma GPX level. The previous correlations of minerals levels, enzymes activities and the acute phase proteins could be used as biomarkers for pyoderma
Keywords: Dog pyoderma, correlation, catalase, clinical signs

Introduction:

The skin is the largest organ of the body with many different functions as immune protection, thermoregulation, sensation, vitamin D production. It acts as a barrier between the animal and the environment (Monteiro-Riviere, 2010). It protects the body against external physical, chemical, and biologic insults (Kanitakis, 2002). In small animal clinics, dermatological disorders constitute a majority of cases (Scott and Paradis, 1990). Grant and Thoday, 1994) recorded that skin diseases are common in small animal practice and they constituent approximately 25 % morbidity rate. Hill et al. (2006) stated that skin diseases are one of the primary reason's owners take a pet to the veterinarian. Skin diseases in pets don't represent problems to animals only but some of them are highly zoonotic to human such as ring worm and mange. Moriello (2003) and Verde (2005) mentioned that skin diseases of dogs have complex etiological agents which may be infectious and noninfectious and sometimes allergic.

Pyoderma is among the most common skin diseases in dogs caused by a bacterial skin infection as defined by Ihrke (1987). Mason (1991) and Meher, et al. (2018) observed that superficial pyoderma is the most common disease of cutaneous bacterial infection in dogs, The author added that deep pyoderma does not

occur spontaneously and often starting as a superficial pyoderma**.** Ihrke,(1990), Scott, et al. (1995) stated that canine pyoderma represents the second most frequently reported disease. Moriello, (2011) reported that pyoderma is among the most common causes of skin diseases in dogs. McKeever et al. (2009) reported that superficial pyoderma due to bacterial infection is confined to the stratum corneum of the inter follicular skin and hair follicles. Devriese et al. (2005) reported that *Staphylococcus pseudo intermedius* is the most commonly isolated bacteria from dog's skin. Other organisms such as Proteus spp., Pseudomonas spp. and *E. coli* may be involved (Scott et al., 2001). McKeever et al. (2009) added that *S. aureus, S. hyicus*, and *S. schleiferi* have also been isolated particularly in North America. The authors added that Methicillin-resistant species including *S. intermedius, S. aureus*, and *S. schleiferi* recently isolated from diseased dogs . Moreover, Meher, et al. (2018) reported that Coagulase positive *staphylococcus aureus* species was isolated from pyoderma affected dogs.

Lesions of pyoderma may be superficial and involve only the epidermis or may involve deeper structures in the dermis or subcutaneous tissue, therefore, it is divided into surface, superficial and deep pyoderma (Ihrke, 1987). The author added that the most common lesions of superficial pyoderma are crusted papules due to the transient nature of canine pustules, pruritus, epidermal collarettes, hyper pigmentation and alopecia. Mason (1991) described the clinical signs of surface pyoderma by superficial erosions of the stratum corneum, alopecia, erythema and pruritus. The author added that papules, pustules associated with hair follicles, epidermal collarettes, alopecia and hyper pigmentation. Cobb et al. (2005) stated that superficial pyoderma affects the superficial portion of hair follicles (bacterial folliculitis) or the epidermis (impetigo), causing pustules and pruritus. McKeever et al. (2009) and Meher, et al. (2018) stated that superficial pyoderma is associated with erythema, papules, pustules, epidermal collarettes and multifocal alopecia were obvious in short coated breeds.

Diagnosis of pyoderma is obtained through the evaluation of clinical signs, presence of characteristic skin lesions, and elimination of other possible causes of folliculitis and by cytological evaluation of the intact pustules content, exudative lesions and skin debris as reported by Coyner (2012), and Hillier et al. (2014). Chaudhary, et al. (2019) stated that in practice, the diagnosis of most cases of superficial pyoderma is based upon clinical signs and the presence of characteristic lesions with complementary aids to confirm the clinical diagnosis of pyoderma such as cytology, skin scraping examination for parasites, and isolation and culture of bacteria and fungus. Nair and Nauriyal (2007) and Shyma and Vijayakumar (2011) observed a significant lower hemoglobin concentration and total erythrocytic count and a significant increase in total leukocytic count with neutrophilia in blood of dogs affected with pyoderma as a hematological differences in blood of diseased dogs.

Oxidative Stress Markers and Skin Diseases:

Cross et al. (1987) reported that reactive oxygen species (ROS) are involved in many inflammatory skin disorders, skin cancer formation, cutaneous autoimmune diseases, phototoxicity, photosensitivity and skin ageing. Okayama, (2005) stated that the skin is exposed to endogenous and environmental pro-oxidant agents, leading to production of harmful generations ROS. The author added that the resulting oxidative stress damages proteins, lipids, and Deoxyribonucleic acid (DNA). Bickers and Athar (2006) mentioned that free radicals induce or contribute in the pathogenesis of skin diseases expressed as erythema, edema, wrinkling, hypersensitivity, keratinization abnormalities and skin cancer. Prado et al. (2008) added that free radicals, are involved in the pathogenesis of several diseases including the dermatologic affections. Moreover, Singh et al. (2011) mentioned that oxidative stress has been implicated to play an important role in the etiopathogenesis of various infectious, inflammatory and degenerative diseases including dermatitis.

Bickers and Athar (2006) reported that in case of skin diseases, the body possesses an array of potent antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione hydrogenase (GSH), GSH-peroxidase. Portugal et al. (2007) reported that in skin diseases, the body 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 SOD and CAT are appropriate indirect way to assess the status of antioxidant defense and estimation of Malondialdehyde (MDA) a byproduct of lipid peroxidation 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. Trouba et al. (2002) and Jaheen (2015) reported that there was a significant decrease in GPx and CAT values in blood of dogs suffering from pyoderma. Basha and Rani (2003) explained the decreased activity of SOD and CAT in skin diseases due to over production of free radicals by the inflammatory cells recruited to combat the infection consequently, the exhaustion of antioxidant system leading to decreased blood CAT activity. Tomsič et al. (2016) reported that total antioxidant capacity (TAC) and antioxidant enzymes, GPx and SOD, are commonly used as markers for antioxidant status and oxidative stress.

Meher, et al. (2018) recorded a significantly ($p<0.05$) higher lipid peroxidase (LPO) value 1.53 ± 0.06 and significantly lower SOD 0.69 ± 0.02 and CAT 0.54 ± 0.03 values in pyoderma affected dogs.

The Relation of Oxidative Stress Markers and Trace Elements in pyoderma

Micronutrients such as zinc, copper, manganese and selenium are essential components of certain endogenous antioxidants. Copper and zinc are required for activities of SOD (Aruoma, 1998 and Chow, 1998). Hefnawy & El-Khaiat (2015) stated that Copper is essential for the antioxidant activity of superoxide dismutase and ceruloplasmin. The authors added that copper, is an atypical antioxidant because it works indirectly as Cu /Zinc SOD catalyzes dismutation of the superoxide anion producing molecular oxygen and hydrogen peroxide the product is usually metabolized by glutathione peroxidase and catalase. Genther and Hansen (2014) reported that the activity of Cu/Zn superoxide dismutase, CAT and GPx is decreased in animals with Cu deficiency and increased in animal with Cu supplementation. Additionally, Ighodaro & Akinloye (2018) mentioned that SOD is Metalloenzyme requires a metal cofactor for its activity which are iron, Zn, Cu and Manganese. While, Hefnawy & El-Khaiat (2015) mentioned that several enzymes with antioxidant activity which do not require Cu as a cofactor, such as CAT and glutathione peroxidase, are known to be negatively influenced by Cu deficiency, increasing free radicals generated in the cells. Beigh et al. (2014) recorded significant lower levels of zinc (Zn) and copper (Cu) in dogs. Nath et al. (1984) and Kubesy et al. (2017) attributed the lower concentration of Cu and Zn to their enhanced utilization in the synthesis of antioxidant enzymes to counter oxidative stress. It is clear that pyoderma is a wide spread disease among house hold dogs. Mostly affected dogs were exposed to a previous infestation with external blood sucking parasites such as fleas or mites. The aim of study is to investigate the percentage of pyoderma affection in relation to other skin affections among household dogs and to find the correlation between levels of blood parameters and acute phase proteins in pyoderma affected dogs aiming to help in early diagnosis of pyoderma.

Methods

The present study was carried out in the clinic of faculties of Veterinary Medicine Suez Canal University, Ismailia and Cairo University, Cairo, Egypt on household dogs suffer from skin problems. The study was continued for one year**.**

1-Animals

The clinical study was conducted on skin diseased dogs came to the veterinary clinic of both faculties of veterinary medicine, Suez Canal and Cairo Universities. The first group was the diseased included ninety (90) dogs of different ages. The second group was clinically healthy included fourteen (14) dogs reared in the clinic along the period of the study and used as a control group. All animals were examined clinically according to Radostits et al (2000**)** and samples were taken after primitive clinical diagnosis. Treatment of all diseased animals was carried out according to both clinic rules with the owners.

2- Samples

Whole blood samples of both groups were collected from jugular vein in some cases and from cephalic vein (fore limb) in other cases using a clean sterile plastic syringe and transferred immediately to tubes on EDTA, then transferred in ice box to the laboratory to carry out complete blood picture using automatic cell counter. Whole anticoagulated blood samples were collected using heparinized tubes then centrifuged at 4000 rpm for 15 minutes to obtain plasma. The obtained plasma samples were freeze at -80℃ according to Schalm (1986). Skin Scrapings were taken from affected areas according to Houston et al. (2000). The samples were transferred to the laboratory for examination.

3- Methods:

1- Clinical Examination of the Animals:

1.1 The history of the disease was taken from the owners directly and recorded according to (Birchard & Sherding, 2005). A history sheet was designed to cover most case history especially of dermatological importance. Animal examination included measurement of body temperature, pulse and respiratory rate, examination of superficial lymph nodes and inspection of both skin and skin coat according to Radostits et al (2000) and Birchard & Sherding (2005).

1.2 Skin Scrapings were taken according to **(**Houston et al., 2000).

2.1 Hematological Examination:

Hematological procedures were carried out using full automatic blood cell counter (Model: PCE-210N). Total and differential leukocytic cell count, hemoglobin, erythrocyte and blood indices were recorded according to Schalm (1986).

2.2 Biochemical Examination;

Plasma levels of copper and zinc were estimated calorimetrically by using test kits supplied by Biodiagnostic company- Egypt according to Ventura and king (1951) and Hayakawa (1961).

3.1 Determination of Oxidative Stress Indices

Plasma level of SOD, Plasma catalase activity, Plasma glutathione peroxidase activity and Plasma Malondialdehyde levels were estimated calorimetrically according to Nishikimi et al. (1972); Aebi (1984); Paglia and Valentine (1967) and Ohkawa et al (1979) respectively by using test kits supplied by Biodiagnostic Company- Egypt.

4- Statistical Analysis

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 ± standard 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. Correlation between variables was evaluated using Pearson Correlation Coefficient (Mukaka, 2012).

Results

Clinical Investigation:

The clinical investigation revealed that the most prevalent problems in dogs was tick infestation in 25 dogs (27.77%) followed by pyoderma in 17 dogs (18.8%). Dermatophytosis came in the 3rd level as diagnosed in 16 dogs (17.77%). Flea allergic dermatitis was diagnosed in 15 dogs (16.66%) and atopic dermatitis was diagnosed in 10 dogs (11.11%), while demodicosis was the least problem as it recorded in only 7 dogs (7.77%). (table, 1)

Table (1) the distribution of dermatologic problems among examined diseased animals

Animal	Total	Dermatophytosis		Pyoderma		Mange		AD		FAD		Ticks infestation	
		No.	%	No.	%	No.	%	No.	%	No.	%	No	%
Dogs	90	16	17.77	17	18.8	7	7.77	10	11.11	15	16.66	25	27.77

Clinical findings:

Pyoderma was diagnosed clinically. Dogs affected by pyoderma showed congested mucous membranes, slight elevated temperature, normal pulse rate and slightly enlargement of superficial lymph nodes (table, 2) and over keratinization all over the body and presence of different pustules specially on the back of the diseased dogs (fig 1 & 2). Skin scrapings were negative for skin parasites and dermatophytes.

Table (2) The Clinical findings of dermatological affection

Animal	Affected Group	Mucus Membrane	Temperature range	Respiratory (Rate/ Minute)	Pulse Rate/m	Lymph Nodes
Dogs	Pyoderma	Congested	39-39.8	30-40	75-110	Enlargement

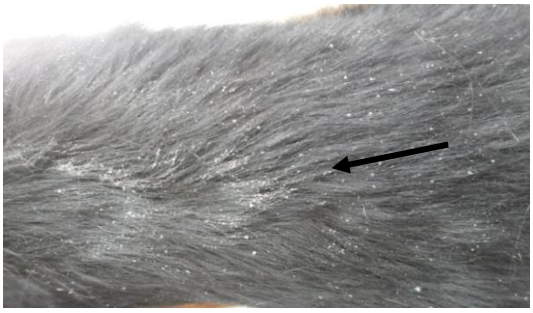

Figure (1) Pyoderma in Dog, Showed Excessive Kertinization

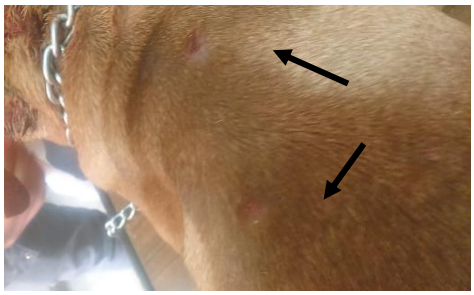

Figure (2) Pyoderma in Dog, Note Pustules on the Back

Hematological and biochemical parameters:

Table (3) showing that a highly significant decrease ($p \leq 0.01$) in hemoglobin blood level followed by a significant decrease in mean corpuscular hemoglobin concentration (MCHC) at ($P \leq 0.05$). Dogs were suffered from hemoglobin deficiency anemia. Leucocytes recorded a significant increase ($p \leq 0.01$) with a highly significant increase in granulocytes and monocytes, while lymphocytes recorded a significant increase in their numbers. Biochemical analysis of blood of Pyoderma affected dogs table (4), figure (4) showed no significant variations in the plasma Zinc and copper levels between the diseased dogs and control group. A significant increase ($p \leq 0.05$) in catalase activity and SOD values in pyoderma affected dogs in comparison with control group.

Correlation analysis

Pearson Correlation Coefficient in pyoderma diseased dogs:

Table (5) revealed a high positive correlation between plasma Zn levels and plasma Cu level, however, there was a high negative correlation between plasma Zn level and plasma catalase activity, additionally, the correlation analysis revealed a very high negative correlation between plasma catalase activity and plasma MDA level. Moreover, there was a moderate negative correlation between plasma SOD activity and plasma MDA level and a moderate negative correlation between plasma MDA level and plasma GPX level.

Table (3) Hematological Parameters of Pyoderma Dogs Affected Compared with Clinically Healthy.

Parameter	Measure	Control (n=14)	Pyoderma (n=17)
Hb (g/dl)	Mean	14.86	10.74**
	SE	±0.53	±0.74
N= Number of animals	SE slandered error	*= p ≤ 0.05	**=P ≤ 0.01
		±1.34	±2.82
RBCs (10^6/µL)	Mean	5.98	5.58
	SE	±0.25	±0.30
MCV (fL)	Mean	67.28	60.38
	SE	±2.25	±2.32
MCHC (%)	Mean	38.15	31.82*
	SE	±2.31	±0.76
WBCs (10^3/µL)	Mean	9.72	13.56*
	SE	±0.86	±1.65
Granulocytes /cubic mm	Mean	1.10	4.09**
	SE	±0.01	±0.07
Lymphocytes /cubic mm	Mean	7.01	7.03*
	SE	±0.05	±0.1
Monocytes /cubic mm	Mean	0.65	2.4**
	SE	±0.01	±0.03

N= Number of animals SE slandered error *= p ≤ 0.05 **=P ≤ 0.01

Figure (31) Blood Parameters of Pyoderma Affected Dogs Compared with Clinically Healthy.

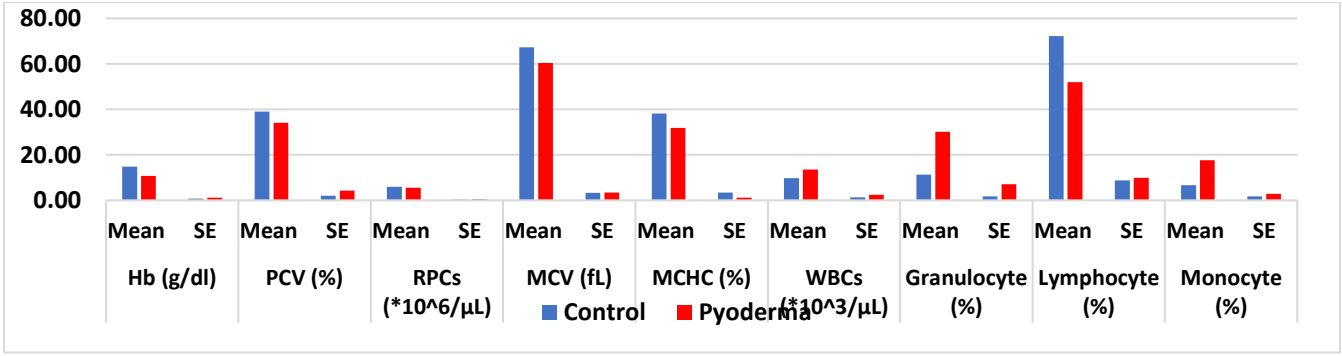

Table (4); Biochemical Findings of Pyoderma Affected Dogs Compared with Clinically Healthy Dogs.

Parameter	Measure	Control (n=14)	Pyoderma(n=17)
Zn (µ/ml)	Mean	0.822	0.914
	SE	±0.040	±0.811
Cu (µmole/ml)	Mean	0.087	0.092
	SE	±0.012	±0.018
Catalase (U/ml)	Mean	0.289	0.397*
	SE	±0.020	±0.035
SOD (U/ml)	Mean	2.280	3.450*
	SE	±0.170	±0.350
GPx (u/ml)	Mean	0.212	0.258
	SE	±0.014	±0.024
MDA (nmole/L)	Mean	0.212	0.017
	SE	±0.014	±0.004

N= Number of animals SE slandered error * = $p \leq 0.05$ ** =$P \leq 0.01$

Figure (4) Biochemical Findings of Pyoderma Affected Dogs Compared with Clinically Healthy Dogs.

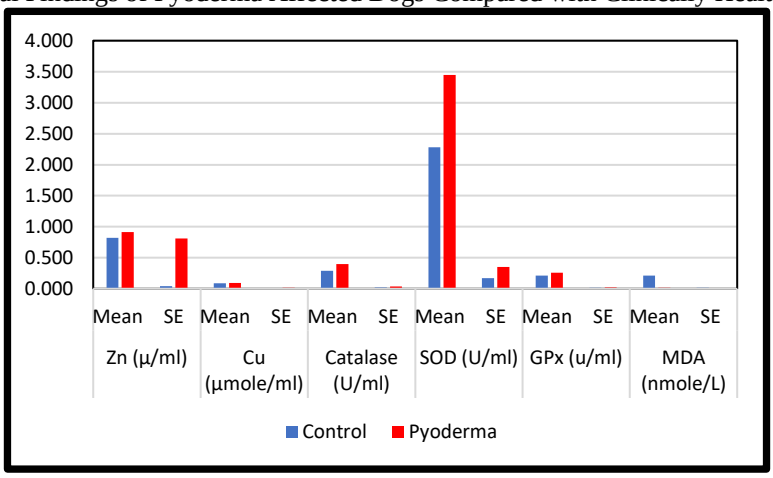

Table (5): Correlation between Trace Elements and Antioxidants of Dogs Affected with Pyoderma

	Zn	Cu	CAT	SOD	GPX	MDA
Zn	1	0.867[b]	-0.708[b]	0.253	0.394	0.331
Cu	0.867[b]	1	-0.370	0.438	-0.050	0.460
CAT	-0.708[b]	-0.370	1	0.017	0.172	-1.00[a]
SOD	0.253	0.438	0.017	1	0.420	-0.517[c]
GPX	0.097	-0.050	0.172	0.420	1	-0.667[c]
MDA	0.331	0.460	-0.100[a]	-0.517[c]	-0.657[c]	1

a- Very high positive/negative correlation (0.9 to 1.0)/(-1.0to-0.9)
b- High positive/negative correlation (0.7to0.9)/(-0.9to-0.7)
c- Moderate positive/negative correlation (0.5 to 0.7) / (-0.7 to -0.5)

Discussion

Prevalence of pyoderma in Dogs

The study revealed that pyoderma affection was diagnosed in 17 out of 90 diseased dogs with a ratio of 18.8%.The prevalence of other skin affections were discussed previously in Nafie, et al. (2021a) and. Nafie, et al. (2021b). Hill et al. (2006) mentioned that the most common dermatological problem in dogs was pyoderma. Khinchi (2019) recorded that 32 out of 205 dogs (15.61%) were positive for canine superficial pyoderma.

The clinical signs of pyoderma in diseased dogs include a slightly elevated temperature of some animals according to the temperature curve with congested mucous membranes and a pale m. m. in normal temperature dogs. Normal pulse rate and a slight enlargement of superficial lymph nodes were observed. Such results agreed that recorded by Yatoo *et al.* (2014) who observed a slightly elevated temperature in pyoderma diseased dogs. The observed lesions of pyoderma were erythema, papules, pustules, erosions, alopecia and pruritus. Similar signs were reported by Ihrke, (1987), Mason, (1991), Cobb et al. (2005) and Mckeever et al. (2009) who reported that pyoderma characterized by superficial erosions of the stratum corneum, alopecia, erythema and pruritus. The authors added that papules, pustules associated by hair follicles, epidermal collarettes, alopecia and hyper pigmentation. Meher et al (2018) reported that canine pyoderma was characterized by pustules, papules, pruritus, and alopecia.

The most common affected area observed was the hind quarter which associated mainly with anal saculities induces irritation and the animal mainly licks its hind quarter. Similar signs were observation by Allaker et al. (1992) and Harvey and Lloyd (1994) who suggested the possible transmission of bacteria from anal sac to the general hairy skin. Hematological picture of pyoderma table (3) and Fig (3) showed a highly significant decrease in hemoglobin values (Hb mg /dl) and a significant decrease in the mean corpuscular hemoglobin concentration (MCHC) values, however, no significant differences in hematocrit values were observed. The interpretation of decreased hemoglobin and MCHC values may be due to the previous infestation by fleas or mites in pyoderma affected dogs A similar result were obtained by Shyma and Vijayakumar (2011) who observed that diseased group of dogs showed a significant lower mean values of hemoglobin content and total erythrocyte counts when compared to the mean values of control animals. The values of total leukocytic count, lymphocyte %, granulocyte % and monocyte % were significantly higher in diseased dogs when compared to healthy ones. Similar results were recorded by Nair and Nauriyal (2007) who noticed a significant increase in total leukocytic counts with neutrophilia in blood of pyoderma affected dogs. Moreover, Rebar (1998) mentioned that leukocytosis, monocytosis and neutrophilia are indicator to skin inflammation.

The biochemical findings of blood of dogs suffering from pyoderma (table 4 & fig. 4) showed a significant increase in both plasma catalase and SOD mean values when compared with control ones. Similar findings were observed by Basha and Rani (2003) who reported that when the risk of oxidative damage increases, endogenous antioxidant production also increases, while, Trouba et al. (2002) recorded that the SOD value showed no statistical differences when compared to control in case of pyoderma infected dogs. Plasma zinc levels showed no significant differences in dogs affected with pyoderma when compared with healthy group (table 4 & fig. 4). Similar results were obtained by Trouba et al. (2002) and Kubesy, et al (2017) who recorded insignificant changes in the plasma zinc levels.

Correlation Analysis

Table (5) revealed a high positive correlation between plasma Zn levels and plasma Cu level indicated that both microelements are in direct correlation and both of them changed in the same direction , as well there was a high negative correlation between plasma Zn level and plasma catalase activity which indicate that both of the changed in inverse direction. Additionally, the correlation analysis revealed very high negative correlation between plasma catalase activity and plasma MDA level. Moreover, there was a moderate negative correlation between plasma SOD activity and plasma MDA level and a moderate negative correlation between plasma MDA level and plasma GPX level. Similar results were obtained by Beigh et al. (2014) who reported that MDA levels were negatively correlated with SOD activity and catalase activity in dermatophytosis affected dogs. Mladenov et al. (2015) reported that the antioxidant enzymes SOD and GPx negatively correlated with the MDA, while CAT displayed no correlation. Gurer et al. (1998) mentioned that the measurement of activities of antioxidant enzymes like SOD and catalase are indirect ways to assess the status of antioxidant defense and 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. Rice-Evans and Burdon (1994) mentioned that antioxidants function is 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 confirmed the negative correlation between antioxidant enzymes such as (SOD, Catalase and GPx) and MDA as a byproduct of lipid peroxidation.

Conclusion:

From this study it could be concluded that skin problems are widely prevalent among dogs' even household ones. One of the most common problems is pyoderma. The laboratory examination revealed several changes in hematological, biochemical parameters and Oxidative stress markers levels in plasma. The obtained results indicated the importance of analysis and measurement of these parameters in dogs suffered from pyoderma and in different skin diseases to overview the degree of skin affection and the level of

defense mechanisms of diseased animals. Additionally, antioxidants such as catalase, Superoxide dismutase and glutathione peroxidase may be possibly beneficial in the early diagnosis of skin diseases as markers. More researches are needed to establish a line of early diagnosis depending on acute phase proteins variations in skin diseases.

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