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Haemato-biochemical and trichographic studies on mucocutaneous lesions in dogs

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Abstract

The present study was carried out with the objective of assessment of haemato-biochemical and trichographic features of mucocutaneous lesions in dogs. Dogs presented with mucocutaneous lesions associated with various dermatological conditions were included in the study. Mange, atopic dermatitis, dermatophytosis, juvenile cellulitis, pyoderma and hypothyroidism with secondary Malassezia dermatitis were presented with mucocutaneous lesions in the perioral, periorbital and in the nostrils with least involvement of anogenital region. Haemato-biochemical analysis revealed anaemia, non-significant increase in total leucocyte count, neutrophilia, eosinophilia, elevated platelet count, hyperproteinaemia and hypoalbuminaemia. Elevated serum C-reactive protein was evident in all cases. Trichographic features revealed that highest number of hairs were included in the telogen phase of hair cycle with greater number of primary hairs. Hair fractures were evident in 20.8 per cent of hairs and trichoptilosis of hair tip was observed in 50.8 per cent of hairs studied. Demodex mites could be detected by hair pluck examination in all cases of demodicosis.

***Keywords**: Mucocutaneous lesions, trichography, telogen, trichoptilosis*

Skin is the largest and visible organ of the body which act as a physiologic and anatomic barrier between the animal and the external environment. Mucocutaneous regions in dogs mostly include the perioral or lip, periocular, nostril, anus and genital regions and are the most sensitive areas of the body with minimum hairs. Various dermatological conditions associated with lesions in

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the mucocutaneous region ranges from parasitic to more serious autoimmune diseases.

Proper diagnosis of dermatological conditions using the standard diagnostic techniques like microscopical examination of skin scraping and impression smear, culture and sensitivity, skin biopsy and histopathology are important for better response to treatment. Due to the wide range of underlying aetiologies, management of dermatological conditions in dogs require a multifaceted strategy with combination therapy. Trichography is a non-invasive and simple technique utilised in canine dermatology for the detection of fungal or parasitic elements, hair shaft defects, pigmentary disorders and hair cycle abnormalities (Negoita and Negoita, 2021). This non-painful diagnostic technique can be used for sample collection in dogs with lesions in the sensitive areas of the body with least trauma. Thus, the present study was carried out to assess the haemato-biochemical and trichographic features associated with mucocutaneous lesions in dogs.

Materials and methods

Dogs presented with dermatological conditions at Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai with mucocutaneous lesions were screened. Out of 92 dogs with lesions in periorbital, perioral, nostril, anus, vulva or prepuce, 16 dogs were selected for detailed study and six apparently healthy dogs brought for vaccination were selected as control. Whole blood samples were collected for the estimation of haemoglobin (g/dL), red blood cell count (10^6/ µL), volume of packed red cells (per cent), platelet count ($\times10^3$/µL), total leucocyte count (10^3/ µL), neutrophil (10^3/µL), eosinophil (10^3/ µL), monocyte (10^3/µL) and lymphocyte count (10^3/µL) using fully automated haematological analyser (ORPHEE Mythic 18 Vet). Serum samples were colorimetrically analysed for estimating total proteins and albumin using standard kits (Kits from SPINREACT S.A.U) as per the manufacturer instructions in semi-automated biochemical analyser (Hospitex – Screen Master T). Estimation of serum zinc and copper were done using atomic absorption spectrometry. Serum C - reactive protein (CRP) was estimated using commercially available ELISA Kit supplied by Origin diagnostics, Kerala. Estimation of serum thyroxine (T4) level of suspected canine cases of hypothyroidism based on clinical signs were done with Gamma counter using standard radioimmunoassay kit (BRIA MAG 4) as per manufacturer's instructions.

Trichography was performed by grasping the hairs with fingertips in the direction of hair growth with a firm even pressure, epilated them completely, laid them in the same direction on a microscopic slide with mineral oil, applied a coverslip and examined under low power objective of microscope. Hairs were plucked from the lesion site, left shoulder region and left flank region and five hairs from each site were selected randomly for detailed study. Trichographic variables such as relative abundance of primary and secondary hairs, hair cycle (anagen / telogen), hair shaft defects (hair fracture / trichoptilosis) and any parasites or adherent eggs were studied (Richman and Griffin, 2018).

The statistical analysis of data obtained was done using software SPSS, version 24.0.

Results and discussion

Out of 1317 dogs with skin diseases which were recorded in a duration of one year, a total of 92 dogs (6.98 per cent) had mucocutaneous lesions in association with various dermatological conditions. Dermatological conditions associated with mucocutaneous lesions in dogs in the present study included mange (37.5 per cent), atopic dermatitis (18.75 per cent), pyoderma (12.5 per cent), dermatophytosis (12.5 per cent), juvenile cellulitis (12.5 per cent) and hypothyroidism with secondary *Malassezia* dermatitis (6.25 per cent). Among these, mange was found to be the most frequent aetiology associated with mucocutaneous lesions in dogs. This upholds the findings of Ayodhya and Suryanarayana (2006) and Sharma *et al.* (2009). Alopecia and erythema were the predominant mucocutaneous lesions as suggested by Camkerten *et al.* (2009) and Bizikova *et al.* (2015). The lesions mostly localised to the perioral or lips (87.5 per cent),

around eyes (81.25 per cent), nostril (68.75 per cent), anal (18.75 per cent) and vulval (6.25 per cent) region of dogs.

Haemogram, platelet count and leucogram

The mean values of haemogram, leucogram, platelet count of dogs with mucocutaneous lesions are given in Table 1.

A significant decrease in the mean value of haemoglobin concentration, volume of packed red cells and red blood cell count was noticed in dogs with mucocutaneous lesions. Similar findings were recorded by Nair and Nauriyal (2007) in pyoderma, Kumar *et al.* (2013) in juvenile cellulitis, Beigh *et al*. (2016) in sarcoptic mange, Sharma *et al.* (2018) and Kamalu (2022) in demodicosis. This might be due to reduced food intake, stress, restlessness due to pruritus, loss of blood and skin protein associated with severe scratching, oxidative injury to the erythrocyte membrane due to the excessive production of free radicals by the mites along with toxaemia and septicaemia caused by mites as suggested by Prathiba *et al.* (2000) and Salem *et al.* (2020). Anaemia observed in hypothyroid dog might be due to the decreased erythropoietin levels in plasma due to decreased levels of thyroid hormones as opined by Roopali *et al.* (2020) and Kour *et al.* (2021). Normal haemoglobin concentration was noticed in dogs diagnosed with atopic dermatitis and dermatophytosis in present study. This upholds the findings of Bond *et al.* (2002) and Brar *et al.* (2017) in dermatophytosis and atopic dermatitis, respectively and this might be due to the small sample population when compared to other study. In contrary to these findings, Ambily *et al*, (2022) reported a significant decrease in haemoglobin concentration in atopic dogs. A significant increase was observed in the mean values of platelet count in dogs with mucocutaneous lesions on day of presentation. Similar findings were documented by Fouda *et al.* (2021) in canine dermatopathies which might be due to the nonregenerative anaemia or due to infection. Clinical relevance of such finding was not clear and not yet reported.

A non-significant increase in the total leucocyte count with significant increase in neutrophil and eosinophil count was observed in dogs with mucocutaneous lesions. Leucocytosis was noticed in dogs diagnosed with demodicosis, juvenile cellulitis and hypothyroidism associated with *Malassezia* dermatitis in the present study. Similar findings were also documented by Sharma *et al.* (2018) and Salem *et al.* (2020) in demodicosis which might be due to inflammatory reaction and secondary bacterial infection. Scott and Miller (2007) reported mild leucocytosis in dogs diagnosed with juvenile cellulitis and the leucocytosis observed in hypothyroid dog with secondary *Malassezia* dermatitis might be due to the decreased humoral immunity and T-cell activity which leads to secondary parasitic and bacterial infection as opined by Kour *et al.* (2021). Neutrophilia and eosinophilia were noticed in dogs diagnosed with demodicosis, scabies, hypothyroidism associated with *Malassezia* dermatitis and atopic dermatitis in the present study. Elevated neutrophil levels in such conditions might be due to leucotoxins released during the insult of cells and this might activate the release of neutrophils into the blood stream and eosinophilia might be due to release of histamine by mast cells associated with tissue irritation as opined by Kumar and Shekhar, (2020). A non-significant variation was noticed in the lymphocyte count and monocyte count. Similar findings were recorded by Thushara *et al.* (2016). Lymphopaenia was noticed in canine scabies which might be due to the activation of cell mediated immunity towards the mites (Sakina and Mandial, 2013). Besides, lymphopaenia was noticed in demodicosis by Solanki and Hasnani (2006) and Reddy *et al.* (2015).

Serum total protein, albumin, zinc and copper

The mean values of serum total protein, albumin, zinc and copper of diseased and control animals are presented in Table 2.

A significant increase in the mean value of serum total protein and decrease in serum albumin was noticed in dogs with mucocutaneous lesions. Dogs diagnosed with demodicosis and pyoderma had elevated serum total protein level and this might be due to the increased inflammatory response and elevated levels of immunoglobulins

against various pathogens associated with dermatological conditions as suggested by Shyma and Vijayakumar (2011) in pyoderma, Kumar and Shekhar (2020) and Salem *et al.* (2020) in demodicosis and Haritha *et al.* (2022) in dermatopathies. Conversely, Reddy *et al.* (2015) noticed normal levels of serum total protein in demodicosis and Brar *et al.* (2017) recorded a decreased serum total protein level in atopic dogs. Hypoalbuminemia was observed in animals diagnosed with demodicosis, juvenile cellulitis, atopic dermatitis, *Malassezia* dermatitis in the study. Similar observations were recorded by Rayulu *et al.* (2016) and Salem *et al.* (2020). This might be due to the leaching of protein through the damaged skin, parasitism and malnutrition associated with pruritus. Nutritional imbalance associated with atopic dermatitis leads to the over utilisation of dietary protein for the synthesis of globulin might be responsible for hypoalbuminemia as suggested by Brar *et al.* (2017) and Ambily (2022).

A non-significant decrease in the mean values of serum zinc and copper was observed in dogs with mucocutaneous lesions. Decreased levels of serum zinc and copper were reported in bdogs diagnosed with demodicosis, scabies and juvenile cellulitis. Similar findings were recorded by Beigh *et al.* (2016) and Nwufoh *et al.* (2019) suggested that decreased levels of serum zinc and copper observed in demodectic mange might be due to the increased utilization of antioxidant enzymes which required copper and zinc for its synthesis to prevent the oxidative stress associated with mange. Conversely, Arora *et al.* (2013) documented an increased serum copper level with normal zinc level in canine demodicosis. Decreased levels of serum zinc was observed in hypothyroid dog with secondary *Malassezia* dermatitis which might be due to the absence of zinc absorption ligands resulting in reduced absorption of zinc from gastrointestinal tract as explained by Logas *et al.* (1993).

Serum C-reactive protein

A statistically significant increase was noticed ($p<0.01$) in the mean value of serum CRP in diseased animals (34.94 ± 2.13 mg/L) when compared to control animals (7.83 ± 1.53mg/L). This was in accordance with the findings of Ulutas *et al.* (2011) and Salem *et al.* (2020). The reason for elevated levels of CRP in inflammatory conditions might be due to the release of cytokines into the blood stream. In parasitic dermatitis, it might be due to the release of inflammatory cytokines from the

Table 1. Haemogram, leucogram and platelet count of diseased and control animals

Variable	Diseased (n=16)	Control (n=6)	t-value	P-value
Hb (g/dL)	11.53 ± 0.66	15.62 ± 0.64	3.557**	0.002
VPRC%	32.66 ± 1.71	43.48 ± 2.13	3.489**	0.002
RBC (10^6/ µL)	5.06 ± 0.28	6.78 ± 0.34	3.378**	0.003
WBC (10^3/ µL)	14.54 ± 0.98	10.97 ± 1.05	2.057	0.053
Neutrophil count (10^3/µL)	10.79 ± 0.75	7.88 ± 0.76	2.208*	0.039
Eosinophil count (10^3/µL)	0.31 ± 0.04	0.11 ± 0.01	4.394**	< 0.001
Lymphocyte count (10^3/µL)	2.78 ± 0.22	2.20 ± 0.20	1.546	0.138
Monocyte count (10^3/µL)	0.67 ± 0.09	0.74 ± 0.09	0.407	0.689
Platelet count (*10^3/µL)	395.88 ± 40.53	250.33 ± 16.18	2.144*	0.044

*** Significant at 0.01 level; * significant at 0.05 level*

Table 2. Mean values of serum total protein, albumin, zinc and copper of diseased and control animals

Parameters	Diseased (n=16)	Control (n=6)	t-value	P-value
Total Protein (g/dL)	6.62 ± 0.23	5.72 ± 0.28	2.177*	0.042
Albumin (g/dL)	2.78 ± 0.11	3.65 ± 0.11	4.626**	< 0.001
Serum Zinc (mg/L)	0.996 ± 0.13	1.472 ± 0.16	2.012	0.058
Serum Copper (mg/L)	0.773 ± 0.11	1.009 ± 0.13	1.200	0.244

***Significant at 0.01 level; * significant at 0.05 level*

follicular epithelium due to the tissue damage and a systemic reaction induced by the parasite as explained by Martinez-Subiela *et al*. (2014). In canine scabies, increased levels of CRP might be due to the elevated pro-inflammatory cytokines in skin and blood (Beigh *et al.,* 2016) and in canine pyoderma this might be due to inflammatory reactions (Severo *et al.*, 2018).

Trichographic features associated with mucocutaneous lesions

The mean percentage of trichographic variable associated with various dermatological conditions are presented in Table 3.

In the present study, 76.25 per cent of hairs were primary and 23.75 per cent of hairs were secondary and the hair bulbs were more in telogen phase (57.9 per cent) than anagen phase of hair cycle (42 per cent). Similar findings were recorded by Diaz *et al.* (2004) who opined that the hairs in the anagen phase which came out from the follicle canal could not be easily plucked out and such hairs might be missed in the sampling process while hairs in the telogen phase could be easily plucked out. Labrador breed of dog diagnosed with hypothyroidism had more hairs in the telogen phase of hair cycle (73.33 per cent) (Fig.1). The predominant telogen phase of hair cycle observed in hypothyroid dog might be due to the fact that the hair follicles had prematurely entered and is retained in the telogen phase of hair cycle due to the deficiency of thyroid hormone as opined by Jaiswal *et al.* (2018) and Kour *et al.* (2021). Hair fractures were evident in 20.8 per cent of hairs examined (Fig. 2) and the hairs of dogs affected with scabies and hypothyroidism associated with *Malassezia* dermatitis had more occurrence of hair fracture (33.3 per cent) than other dermatological conditions. Normal hair tips were present at 49.2 per cent of hairs and trichoptilosis were present in 50.8 per cent of hairs (Fig. 3). Trichoptilosis was more evident in hairs of dogs affected with dermatophytosis (70 per cent) followed by scabies (66.67 per cent), hypothyroidism (66.67 per cent), atopic dermatitis (62.2 per cent), pyoderma (50 per cent), demodicosis (46.67 per cent) and juvenile cellulitis (10 per cent). The hair fracture and trichoptilosis observed in the present study might be due to any external injury associated with scratching, excessive grooming or licking and greater number of primary hairs might be due to the easy plucking when compared to secondary hairs as opined by Richman and Griffin (2018). In addition to these findings, cigar shaped *Demodex*mites were detected in trichographic examination of all the five cases of demodicosis included in the present study (Fig. 4). Mange infection especially demodicosis need deep skin scrapings for diagnosis which creates trauma and pain to animal. Hence trichography could be recommended as a non-invasive method for diagnosis of demodicosis in dogs. Similar findings were documented by Mueller *et al.* (2020).

Conclusion

Haemato-biochemical analysis associated with mucocutaneous lesions in dogs revealed anaemia, non-significant increase in total leucocyte count, neutrophilia, eosinophilia, hyperproteinaemia, hypoalbuminaemia and non-significant decrease in serum zinc and copper levels. Serum C-reactive protein level was found to be elevated in mucocutaneous lesions. Trichographic features included predominant

Table 3. Trichographic findings of hairs of dogs with mucocutaneous lesions

Variables (Per cent)	Anagen	Telogen	Fracture	Trichoptilosis	Primary hairs	Secondary hairs
Demodicosis	38.6	61	20	46.67	72	28
Scabies	40	60	33.3	66.67	46.67	53.33
Atopic dermatitis	44.4	55.5	22.2	62.2	84.4	15.6
Juvenile cellulitis	43.3	56.6	10	10	73.3	26.7
Hypothyroidism	26.6	73.3	33.3	66.67	60	40
Pyoderma	33.3	66.7	20	50	93.3	6.7
Dermatophytosis	40	60	20	70	83.3	16.7

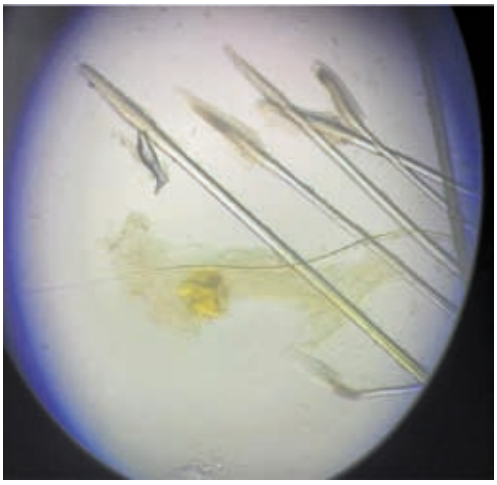

Fig. 1. Hair bulbs in telogen phase (10X)

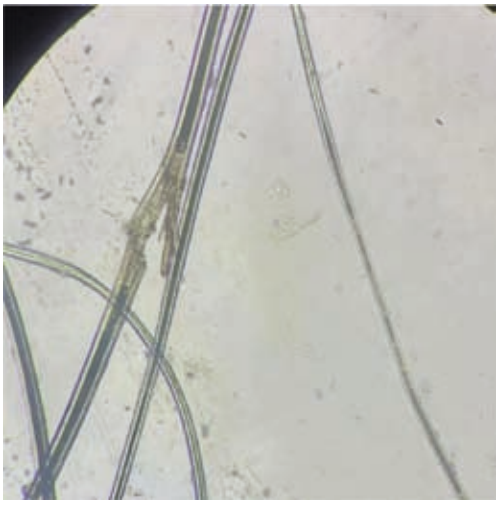

Fig. 2. Hair shaft fracture (10X)

Fig. 3. Trichoptilosis in hair tip (10X)

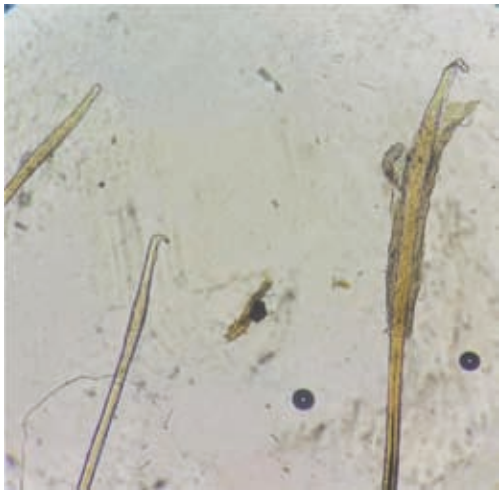

Fig.4. *Demodex* mites in hair pluck (10X)

telogen hair bulb with a greater number of primary hairs, hair fracture and trichoptilosis. Trichography could be recommended as a non-invasive technique for the diagnosis of mange especially demodicosis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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