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A prospective study on haemato-biochemical aspects of atopic dermatitis in dogs#

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Abstract

A prospective study on haemato-biochemical aspects of canine atopic dermatitis was conducted in the Department of Clinical Veterinary Medicine, College of Veterinary and Animal Sciences, Mannuthy during the period from 2019 to 2021. Sixteen dogs diagnosed with atopic dermatitis using characteristic clinical (Favrot's) criteria with exclusion of other pruritic skin diseases, together with elevated Ig E levels and ten healthy animals formed the subjects for the present study. Whole blood samples were collected from atopic and healthy controls and haemato-biochemical parameters were estimated. Haemato-biochemical studies of atopic dogs revealed anaemia with leukocytosis, neutrophilia and eosinophilia and hypoalbuminaemia with reduced AG ratio. Absolute eosinophil count that was found positively correlated with neutrophil to lymphocyte ratio (NLR) in atopic dogs, is of diagnostic significance in quantifying inflammatory response which helps in instituting customized treatment to atopic animals in clinical practice. Hypoalbuminaemia observed in atopic animals in the present study indicates the need for nutrient supplementation in the therapeutic protocol of the disease.

Keywords: *Atopic dermatitis, haemogram, neutrophil to lymphocyte ratio, dogs*

 # Part of PhD thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Canine atopic dermatitis (CAD) is defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens. Dogs with atopic dermatitis usually have pruritus and secondary skin lesions that have a particular distribution around the face, concave aspect of the ear pinnae, ventral abdomen, flexor aspects of elbow, carpal and tarsal joints, interdigital skin, and perineal area. Major clinical signs of CAD are those related to pruritus, such as frequent grooming, licking or scratching resulting in erythema, lichenification and excoriations of the affected skin. Clinical symptoms may be seasonal or, more frequently, nonseasonal depending on the allergens involved (Olivry, 2010).

Atopic dermatitis in dogs has been suggested as an animal model for human atopic dermatitis as its clinico-pathological features in dogs are similar to that of human beings. Unlike other types of allergic dermatitis in dogs, atopic dermatitis is a challenging disease for pets and pet owners (Gedon and Mueller, 2018) and, even for veterinary practitioners around the globe. The present paper deals with the haemato-biochemical aspects of atopic dermatitis in dogs and its correlation with systemic inflammatory markers such as neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR).

Materials and methods

Sixteen dogs diagnosed with atopic dermatitis using characteristic clinical (Favrot's) criteria (Favrot *et al*., 2010) with exclusion of other pruritic skin diseases, together with elevated Ig E levels (>91 IU/mL) and ten apparently healthy dogs formed the subjects of the present study.

Whole blood samples (2mL) were collected from sixteen diseased and ten apparently healthy dogs in EDTA coated vacutainer tubes on the day of presentation for estimating erythrogram, leucogram and platelet counts, using the standard technique as described by Feldman *et al*. (2000). The values of haemogram of atopic dogs were compared with healthy controls. Correlation of platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) with absolute eosinophil count was assessed in atopic animals and compared with healthy controls.

About 15 mL of blood was collected from sixteen diseased and ten apparently healthy dogs, in tubes with clot activator and allowed to clot and this was then centrifuged at 3000 rpm for 15 minutes. All the biochemical analyses were performed using standard kits (Kits from M/s Agappe diagnostics) as per the manufacturer's instructions in a semi-automated biochemical analyser. The values obtained for atopic dogs were compared with healthy controls.

Results and discussion

Haemogram

The mean total erythrocyte count, haemoglobin and volume of packed red cells in healthy controls were 6.65 ± 0.25 mill/mm^3, 13.49 ± 0.65 g/dL and 39.64 ± 1.55 per cent, respectively and their corresponding values in atopic animals on the day of presentation were 5.24 ± 0.28 mill/mm^3, 11.67 ± 0.56 g/dL and 32.68 ± 2.27 per cent, respectively. A statistically significant decrease in total erythrocyte count ($p \leq 0.01$), haemoglobin ($p \leq 0.05$), and volume of packed red cells ($p \leq 0.05$) were observed in diseased animals (Fig. 1-3), when compared to healthy controls and these results were similar to the observations made by Fouda *et al.* (2021) in their study on haemato-biochemical and histopathological aspects of various dermatopathies in dogs. The observations in this study were contrary to the findings of Sharma *et al.* (2015) and Brar *et al.* (2017), who observed no significant difference in these values in diseased animals when compared to the control group. The reason for anaemia in atopic dermatitis might be due to inflammation, which prevents the body from using stored iron for the synthesis of red blood cells. Drury *et al*. (2016) and Rhew and Oh (2019) reported association between atopic disease and iron deficiency anaemia of chronic inflammation in atopic children.

The mean corpuscular volume

Table 1. Haemogram in atopic animals and healthy controls

Variables	Atopic (n=16)	Healthy (n=10)	t-value	P-value
TEC (x 10^6/µl)	5.24 ± 0.28	6.65 ± 0.25	3.415**	0.002
Hb (g/dL)	11.67 ± 0.56	13.49 ± 0.65	2.091*	0.047
VPRC (%)	32.68 ± 2.27	39.64 ± 1.55	2.221*	0.036
MCV (fL)	64.96 ± 2.06	61.9 ± 2.00	1.000^{ns}	0.327
MCH (pg)	22.44 ± 0.66	20.81 ± 0.98	1.429^{ns}	0.166
MCHC (g/dL)	34.7 ± 0.51	33.53 ± 0.79	1.304^{ns}	0.204
RDW (fL)	17.81 ± 0.81	17.96 ± 0.95	0.121^{ns}	0.905
RPR	0.056 ± 0.01	0.06 ± 0.012	0.429^{ns}	0.672

Significant at 0.01 level; * significant at 0.05 level; ns non-significant

(MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean red cell distribution width (RDW) were 61.9 ± 2.00 fL, 20.81 ± 0.98 pg, 33.53 ± 0.79 g/dL and 17.96 ± 0.95fL respectively when compared to 64.96 ± 2.06 fL, 22.44 ± 0.66 pg, 34.7 ± 0.51 g/dL and 17.81 ± 0.81fL in healthy controls (Table 1). No statistically significant differences were noticed between the diseased animals and healthy controls in values of mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean red cell distribution width as documented by Sharma *et al.* (2015) and Brar *et al.* (2017) and Fouda *et al.* (2021).

Leucogram

Mean total leucocyte count (TLC) in healthy controls was 10.56 ± 0.63 (x 10^3/µl) and its corresponding values in diseased animals was 14.24 ± 0.94 (x 10^3/µl). Significant (P ≤ 0.01) increase was noticed in total leucocyte count in diseased animals when compared to healthy control. The mean values of neutrophils, lymphocytes, monocytes and eosinophils of healthy controls were 6.88 ± 0.54, 3.00 ± 0.25, 0.60 ± 0.06 and 0.08 ± 0.03 (x 10^3/µl), respectively and that of diseased animals were 10.03 ± 0.84, 2.87 ± 0.19, 0.55 ± 0.06 and 0.79 ± 0.11 (x 10^3/µl) respectively (Table 2). Statistically significant (P≤0.01) leukocytosis with neutrophilia and eosinophilia (Fig. 4) observed in diseased animals compared to healthy control was in agreement with the findings of Gupta and Prasad (2001), Sharma *et al.* (2015), Brar *et al.* (2017) and Fouda *et al.* (2021) and the reason for leukocytosis might be attributed to cellular and humoral immune response associated with the disease. Neutrophilia in atopic dermatitis was due to primary and secondary infections resulting from bacterial over load, with subsequent mobilization of granulocytic pool from bone marrow as documented by Schalm (1963)

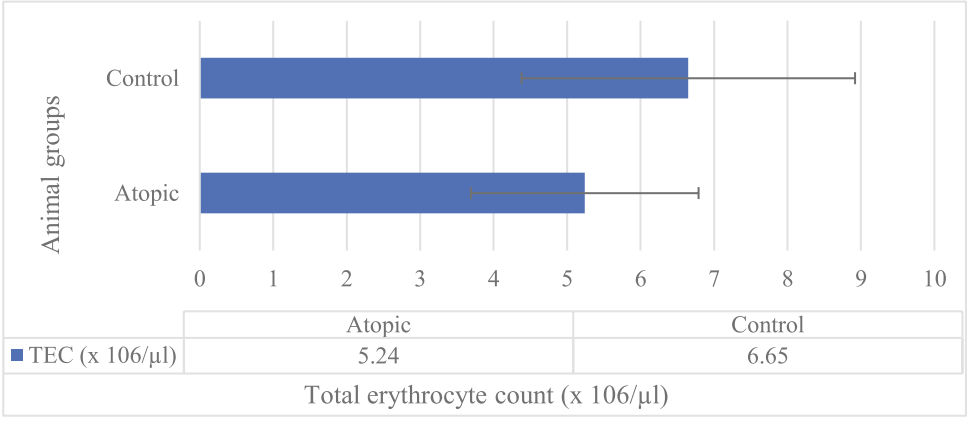

Fig. 1. Mean total erythrocyte count (x 10^3/µl) in atopic animals and healthy controls

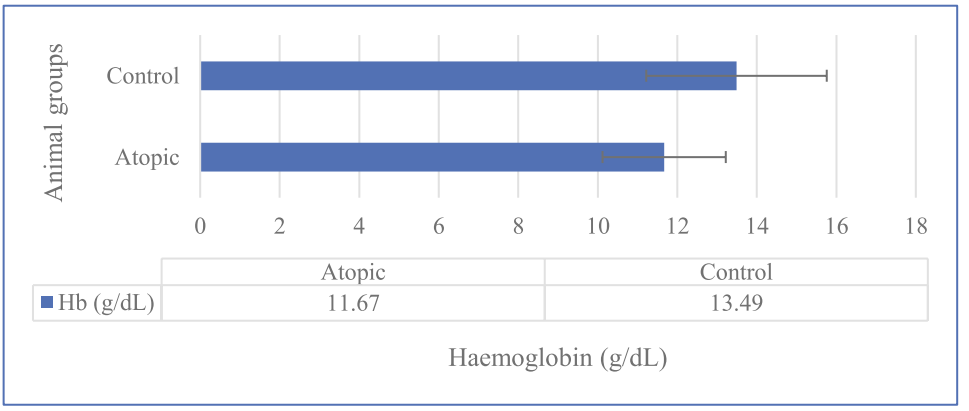

Fig. 2. Mean haemoglobin (g/dL) in atopic animals and healthy controls

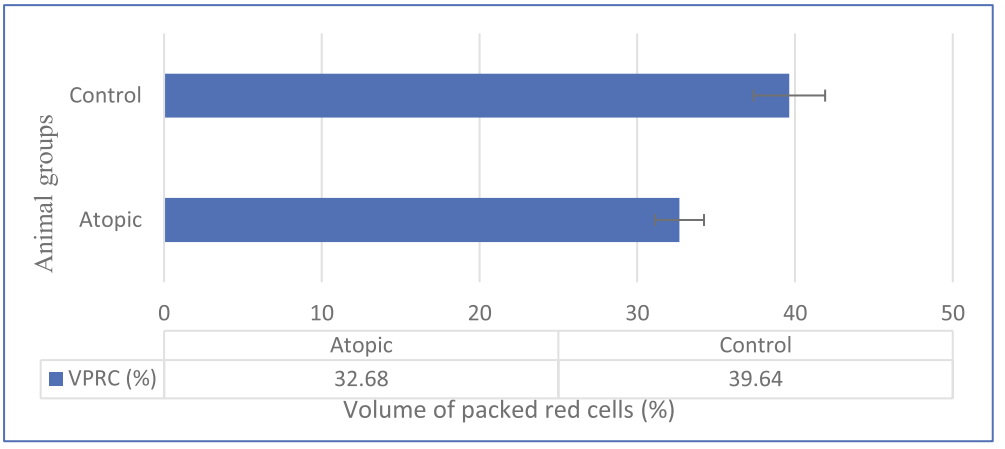

Fig. 3. Mean volume of packed red cells (%) in atopic animals and healthy controls

Table 2. Leucogram in atopic animals and healthy controls

Variables	Atopic (n=16)	Healthy (n=10)	t-value	P-value
Total leukocyte count (x 10^3/µl)	14.24 ± 0.94	10.56 ± 0.63	2.848**	0.009
Neutrophil (x 10^3/µl)	10.03 ± 0.84	6.88 ± 0.54	3.163**	0.004
Lymphocyte (x 10^3/µl)	2.87 ± 0.19	3.00 ± 0.25	0.410	0.686
Monocyte (x 10^3/µl)	0.55 ± 0.06	0.60 ± 0.06	0.497	0.624
Eosinophil (x 10^3/µl)	0.79 ± 0.11	0.08 ± 0.03	4.808**	0.001
NLR	3.77 ± 0.41	2.47 ± 0.33	2.244*	0.034

*** Significant at 0.01 level; * significant at 0.05 level; ns non-significant*

and Gupta and Prasad (2001). Eosinophilia observed in atopic dogs in this study might be due to increased Ig E receptor mediated mast cell stimulation as explained by Wuersch *et al.* (2006), who recorded eosinophilia in atopic dogs. In contrary to this, Wilkie *et al.* (1990) and Collie *et al.* (1997) opined that though eosinophilia was observed in atopic dermatitis, it may not be always associated with the disease.

No significant difference in lymphocyte count observed between diseased and healthy animals in this study was in accordance with the observations made by Sharma *et al.* (2015) and Brar *et al.* (2017). In contrary to this finding, Latimer (1995) reported the occurrence of lymphocytosis in allergic dermatitis. A statistically significant ($p \leq 0.05$) increase in neutrophil to lymphocyte ratio (NLR) found in atopic dogs when compared to control group,

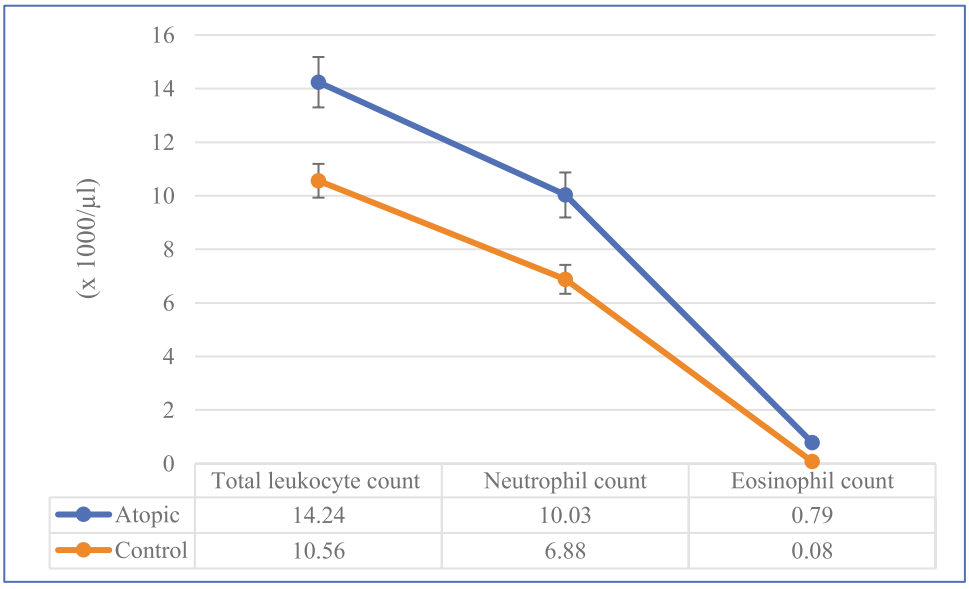

Fig.4. Total leukocyte, neutrophil and eosinophil count in atopic and healthy controls

Table 3. Platelet indices in atopic animals and healthy controls

Variables	Atopic (n=16)	Healthy (n=10)	t-value	P-value
Platelet count (x 10^3/µl)	416 ± 43.88	362.7 ± 50.73	0.778	0.444
MPV (fL)	7.88 ± 0.24	7.62 ± 0.52	0.502	0.620
PCT (%)	0.37 ± 0.04	0.30 ± 0.04	1.152	0.261
PDW (%)	16.21 ± 0.59	17.81 ± 1.28	1.279	0.213
PLR	160.5 ± 24.44	120.92 ± 16.12	1.178	0.250

was in agreement with the results of Jiang and Ma (2017) in human atopic patients. Sen *et al.* (2014) reported NLR as a measure of systemic inflammatory response in psoriasis. A significantly high NLR in atopic animals in this study reflected an inflammatory response as documented by Jiang and Ma (2017) in human patients.

Table 4. Correlation of PLR and NLR with Eosinophil count

Variable	Correlation with eosinophil count	P-value
NLR	0.523*	0.038
PLR	-0.250^{ns}	0.350

** Significant at 0.05 level; ns non-significant*

Platelet indices

The mean platelet count of healthy controls and diseased animals were 362.7 ± 50.73 (x 10^3/µL) and 416 ± 43.88 (x 10^3/µL) respectively (Table 3). Though not statistically significant, an increase in platelet count was noticed in diseased animals (Fig. 5). Nonsignificant increase in platelet count, platelet to lymphocyte ratio (PLR), mean platelet volume (MPV) and platelet crit (PCT) was observed in atopic dogs compared to healthy controls in the present study. In contrary to this, a significant increase in platelet count was observed by Fouda *et al.* (2021) in atopic dogs and Jiang and Ma (2017) in atopic human patients.

Correlation of PLR and NLR with eosinophil count

Systemic inflammatory responses in atopic dermatitis were documented by many researchers round the globe (Mu *et al.,* 2014, Werfel *et al.,* 2015 and Bao *et al.*, 2016). Significantly high levels of systemic inflammatory markers like NLR and PLR found associated with disease severity in inflammatory diseases like systemic lupus erythematosus and psoriasis as suggested

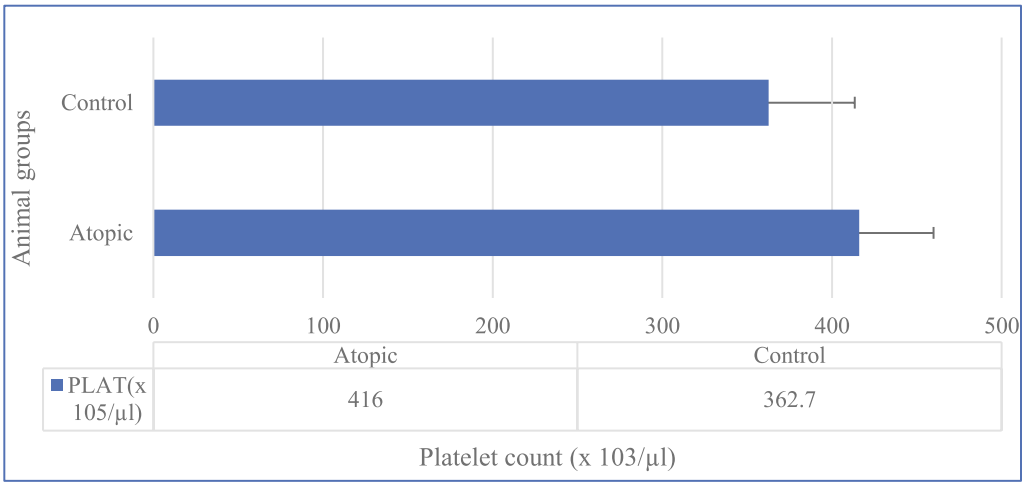

Fig. 5. Mean platelet count (x 10^3/µl) in atopic animals and healthy controls

Table 5. Serum biochemical values in atopic animals and healthy controls

Variables	Atopic (n=16)	Healthy (n=10)	t-value	P-value
Total protein(g/dL)	6.71 ± 0.19	6.98 ± 0.23	0.928^{ns}	0.363
Albumin(g/dL)	2.71 ± 0.1	3.14 ± 0.13	2.576*	0.017
Globulin(g/dL)	4 ± 0.14	3.85 ± 0.15	0.699^{ns}	0.491
A:G ratio	0.69 ± 0.03	0.82 ± 0.04	2.536*	0.018

** Significant at 0.05 level; ns non-significant*

by Kim *et al.* (2016), Qin *et al.* (2016) and Wu *et al.* (2016). Positive correlation between the NLR and inflammatory markers like eosinophil counts was documented by Sen *et al.* (2014) and Wu *et al.* (2016) and they recommended NLR as a cheap and easily available laboratory marker to measure systemic inflammation in allergic skin diseases. A statistically significant correlation of NLR with absolute eosinophil count was observed in the present study (Table 4 and Fig. 6). Similar correlation was noticed by Furuta *et al.* (2014) and Jiang and Ma (2017) in human atopic patients. However, no correlation was noticed between PLR and eosinophil count in atopic dogs in this study as against positive correlation reported Jiang and Ma (2017) in human atopic patients.

Serum biochemical analysis

The serum total protein, albumin, globulin and AG ratio in healthy controls were 6.98 ± 0.23 g/dL, 3.14 ± 0.13 g/dL, 3.85 ± 0.15 g/dL and 0.82 ± 0.04 respectively and its corresponding values for diseased animals were 6.71 ± 0.19 g/dL, 2.71 ± 0.1 g/dL, 4 ± 0.14 g/dL and 0.69 ± 0.03 (Table 5). Serum albumin level was significantly decreased in diseased animals, when compared to healthy animals in the present study (Fig.7). A non-significant decrease in values of serum total protein could be observed in diseased as compared to control group. Statistically significant (P≤0.05) hypoalbuminaemia and reduced AG ratio (Fig. 8) observed in the present study was in agreement with the findings of Sharma *et al.* (2015) and Brar *et al.* (2017). However, no significant difference was noticed in serum total protein and globulin levels of diseased animals by Sharma and Gupta (2005), whereas Sharma *et al.* (2015) and Brar *et al.* (2017) observed a significant reduction in both the values. The significantly decreased albumin values of atopic dogs in this study might be due to nutritional impairment resulting from general

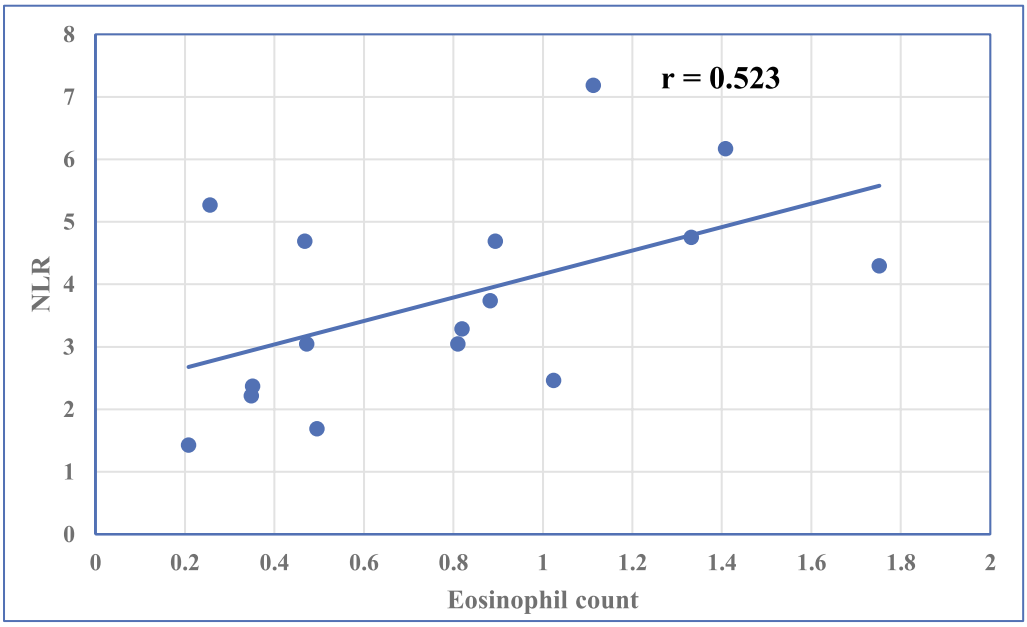

Fig. 6. Correlation of NLR with eosinophil count (n=16)

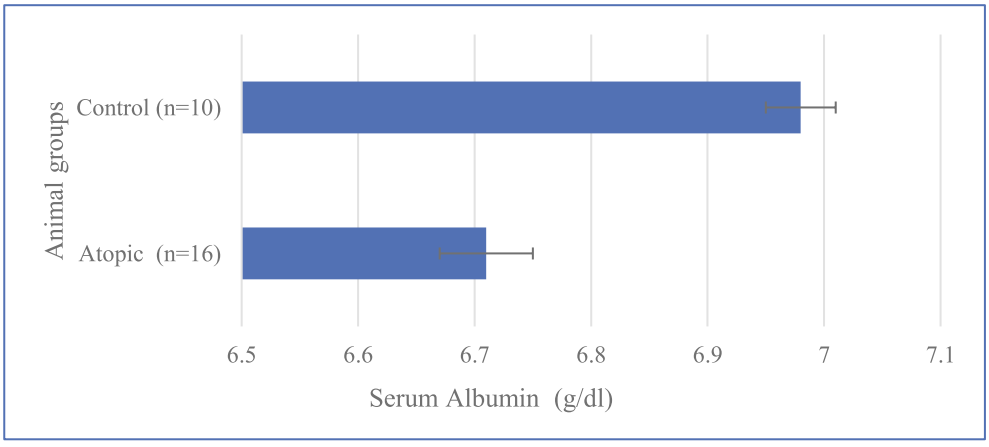

Fig. 7. Serum albumin in atopic and healthy controls

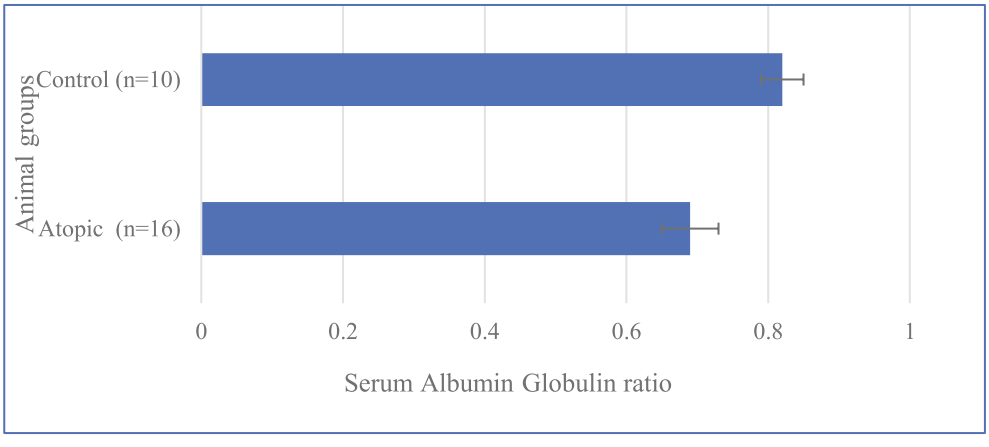

Fig.8. Serum albumin globulin ratio in atopic and healthy controls

weakness arising from severe dermatitis and excess utilization of dietary proteins for globulin synthesis as explained by Brar *et al.* (2017) in their study on haemato-biochemical aspects of atopic dermatitis in dogs.

Conclusion

In the present study the haemato-biochemical aspects of atopic dermatitis in dogs and its correlation with systemic inflammatory markers like neutrophil to lymphocyte ratio (NLR) and Platelet to lymphocyte ratio (PLR) was discussed. Haemato-biochemical studies of atopic dogs revealed anaemia with leukocytosis, neutrophilia and eosinophilia. A significantly high neutrophil to lymphocyte ratio (NLR) in atopic animals in this study reflected an inflammatory response, which seems well correlated with absolute eosinophil count in atopic dogs. This is having much diagnostic significance, and it can be made use of quantifying the systemic inflammation associated with the disease, which in turn helps in instituting customized treatment for atopic animals. Hypoalbuminaemia with reduced AG ratio was observed in the study and this might be due to nutritional impairment resulting from general weakness arising from severe dermatitis and excess utilization of dietary proteins for globulin synthesis which indicates the need for nutrient supplementation in the therapeutic protocol of the disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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