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Research Article

INVESTIGATION ON HAEMATO-BIOCHEMICAL PROFILING AND TOTAL IgE SEROLOGY IN DOGS WITH CANINE ATOPIC DERMATITIS

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ABSTRACT

Canine atopic dermatitis (CAD) is a prevalent inflammatory and pruritic skin disease of dogs, associated with IgE antibodies to environmental allergens with clinical disease apparent once a threshold of inflammatory response is reached. This activation of inflammatory response induces the production of free radicals which contribute to cellular damage and result in alteration in haemato-biochemical parameters in affected dogs. Therefore, the present investigation was planned to evaluate the haemato-biochemical profile and serum total IgE concentration in dogs affected with CAD. The study was conducted on 21 dogs with pruritic dermatological conditions diagnosed for CAD on the basis of Favrot's criteria. Blood samples were collected and processed to estimate haemato-biochemical parameters and serum total IgE concentration. The haematological profile of dogs with CAD revealed hypochromasia, normal to elevated leucocyte count with absolute lymphocytosis. Serum biochemical profile revealed hypoalbuminemia, normal to elevated globulin level, decreased albumin to globulin ratio, normal to elevated alkaline phosphatase, gamma glutamyl transferase and creatinine levels, indicating biliary and renal dysfunction in atopic dogs. Mean serum total IgE level in atopic dogs was non-significantly higher than that of healthy dogs. These altered biomarkers should be taken into consideration while initiation of specific targeted and ancillary therapy of CAD in dogs and could also be utilized for further assessment in response to therapy.

Keywords: Canine atopic dermatitis, Dogs, Haemato-biochemical profile, IgE concentration

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Canine atopic dermatitis (CAD) is a genetically predisposed inflammatory and pruritic allergic skin disease, associated mostly with IgE antibodies to environmental allergens (Halliwell, 2006; Hensel *et al.*, 2015). The prevalence of CAD is 3-15% in the general dog population and 3-58% in dermatopathic dogs presented to veterinarians (Saridomichelakis and Olivry, 2016). The age of onset of CAD typically ranges from 6 months to 6 years; however, over 70% of the dogs exhibit clinical signs between 1 to 3 years (Santoro, 2019). The most commonly encountered allergens of CAD include mold, followed by house dust and house dust mites with less frequency of outdoor allergens (Kim *et al.*, 2011). The clinical disease of CAD becomes apparent once a threshold of allergic inflammatory response is reached (Marsella, 2021).

The clinico-diagnosis of CAD in dogs is based on Favrot's criteria which include onset of signs under 3 years of age, dog living mostly indoors, glucocorticoid-responsive pruritus, affected front feet and ear pinnae with non-affected ear margins and dorso-lumbar area (Favrot *et al.*, 2010). The clinical presentation is sufficient for its diagnosis without essentially employing specific diagnostic procedures such as intradermal testing or IgE serology (Saridomichelakis and Olivry, 2016). Further, the activation of skin and systemic inflammatory response induces the production of free radicals resulting in cellular damage and ultimately cause alteration in haemato-biochemical parameters in dogs with CAD (Ferreira *et al.*, 2021). Till date, no systematic investigation has been conducted to study haemato-biochemical profile and total IgE serology in dog population affected with CAD in Hisar, Haryana. Therefore, the present investigation was planned to appraise the haemato-biochemical profile and total IgE concentration in dogs affected with CAD in our scenario.

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MATERIALS AND METHODS

The present investigation was conducted on dogs referred to Small Animal Medicine Section of Veterinary Clinical Complex (VCC), LUVAS with the history of pruritus, erythema, self-induced alopecia, excoriations, dry lustreless hair, hyperpigmentation, scaling and lichenification. Both superficial and deep skin scrapping examination was done to rule out any ectoparasitic infestation in affected dogs as per Miller *et al.* (2013). A total of 21 dogs with pruritic dermatological conditions negative for any ectoparasites were diagnosed for CAD on the basis of Favrot's criteria for its clinico-diagnosis (Favrot *et al.*, 2010). Apparently healthy dogs (n=6) above 1 year of age brought to VCC for routine health check-up and/or vaccination served as healthy control for comparison of serum total IgE level in affected dogs.

Collection, processing and preservation of blood samples: Approximately 5 ml of blood was collected aseptically from cephalic or saphenous vein. Of this, 1 ml

was poured into tube coated with K_3 ethylenediamine-tetra acetic acid (K_3 EDTA) for haematology and remaining 4 ml was poured in tube with clot activator for serum harvesting. Two aliquots of serum were prepared; one for serum biochemistry and other for total IgE ELISA and both were stored at -20° C till analysis.

Haematology: K_3 EDTA blood samples were analysed immediately for complete blood count using automated Haematology cell counter (MS4s, Melet Schlosing Lab, USA). The erythrocytic indices included haemoglobin (Hb) in g/dl, total erythrocyte count (TEC) in $10^6/mm^3$, packed cell volume (PCV) in %, mean corpuscular volume (MCV) in fl, mean corpuscular haemoglobin (MCH) in pg and mean corpuscular haemoglobin concentration (MCHC) in g/dl. The leucocytic and thrombocytic indices included total leucocyte count (TLC), lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocyte/platelet count in $10^3/mm^3$, respectively.

Serum biochemistry: The serum samples were analysed to estimate biochemical profile using automated random access clinical chemistry analyser (EM Destiny 200, Erba Diagnostics Mannheim GmbH, Germany). The protein profile included estimation of total proteins, albumin, globulin in g/dl and albumin to globulin ratio (A/G). Serum biochemical parameters of liver function measured were alanine aminotransferase (ALT), aspartate amino-transferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) in U/L, respectively; bilirubin (total), bilirubin (direct) and bilirubin (indirect) in mg/dl, respectively. Serum biochemical parameters of kidney function included urea and creatinine in mg/dl, respectively.

Serum total IgE measurement: The quantitative measurement of serum total IgE level was done using in-vitro Canine Immunoglobulin E ELISA Kit (Bioassay Technology Laboratory, China) following the manufacturer's protocol.

A standard curve was constructed by plotting the average OD for each standard on vertical (Y) axis against the concentration (mg/ml) on horizontal (X) axis. OD of sample (y) was measured at 450 nm and total IgE values (x) were calculated as per the formula, $y = 0.094 + 0.017$ or $x = (y - 0.094)/0.017$ and expressed in mg/ml of serum.

Statistical analysis: The data generated was analysed using statistical software package SPSS version 20.0. The results were presented as Mean ± Standard Error (S.E.). The values of haemato-biochemical parameters were compared with their normal reference values in dogs as laid in standard literature while the independent t-test was used to compare serum total IgE level between affected dogs and healthy control at a significance level, $p<0.05$.

RESULTS AND DISCUSSION

Haematological profile of dogs with CAD: Erythrocytic indices of dogs with CAD are depicted in Table 1. Values of Hb, TEC, PCV and MCV were found within normal limit (WNL) in maximum cases, while the values of MCHC and MCH were observed below the normal reference limit in most of the cases, indicating the hypochromasia without anaemia in atopic dogs. Significant decrease in MCH along with significant decrease in Hb, TEC and PCV has been reported by Walaa *et al.* (2008) in dogs with atopic dermatitis. In contrast, Ambily *et al.* (2022) revealed non-significant changes in values of MCH and MCHC with significant decrease in Hb and TEC in atopic dogs. Hypochromasia in atopic dogs in present study might be due to iron deficiency reported in atopic diseases, priming the mast cells for degranulation (Roth-Walter, 2022).

Leucocytic indices and platelet count of dogs with CAD are presented in Table 2. TLC was found within normal limit in maximum cases (n=12), followed by leucocytosis (n=7) and leucopenia (n=2). Absolute lymphocytosis was found in maximum cases (n=19) which might be attributed to the stimulation of immune system by chronicity of atopic dermatitis in affected dogs. The development of CAD is linked to alteration in both cutaneous and circulating lymphocyte populations, producing variety of cytokines involved in its pathogenesis (Pucheu-Haston *et al.*, 2015). This inflammation is primarily driven by imbalance between T helper cells, Th2 and Th1. Initially, only Th2 type response is typically observed in affected dogs which shifts to mixed Th1-Th2 response later on. Additionally, T cytotoxic (Tc) cells also contribute in atopic dermatitis with their recruitment in atopic skin (Majewska *et al.*, 2022). These facts support the finding of absolute lymphocytosis in the present investigation. Neutrophils count was observed within normal reference limit (n=12), followed by absolute neutropenia (n=5) and absolute neutrophilia (n=4). The monocytes, eosinophils and basophils count were within normal limit in most cases. In contrast, Walaa *et al.* (2008) reported significantly elevated TLC with elevated eosinophils count in atopic dogs and the eosinophils count remained significantly elevated even after treatment. Studies by Indian workers also reported leucocytosis, neutrophilia and eosinophilia in atopic dogs (Brar *et al.*, 2017; Singh *et al.*, 2022; Ambily *et al.*, 2022). Ferreira *et al.* (2021) analysed systemic and cutaneous inflammatory immune response in CAD. Dogs with canine atopic dermatitis extent and severity index (CADESI) between 10 to 34 tended to have higher

Table 1. Erythrocytic indices of dogs with canine atopic dermatitis (n=21) (Mean±S.E.)

Haematological Parameters	Mean ± S.E. (Range)	Normal Reference Values (Fielder, 2022. In: The MSD Veterinary Manual)
Hb (g/dl)	14.07±0.61 (8.8-19.7)	11.9-18.9
Within Normal Limit (WNL) (n=17)	13.92±0.47 (11.9-18.5)	
Below lower limit (n=2)	10.00±1.20 (8.8-11.2)	
Above upper limit (n=2)	19.40±0.30 (19.1-19.7)	
TEC (10^6/mm^3)	7.05±0.29 (5.36-9.66)	4.95-7.87
WNL (n=16)	6.42±0.17 (5.36-7.73)	
Above upper limit (n=5)	9.05±0.30 (7.97-9.66)	
PCV (%)	50.8±2.43 (31.7-72)	35-57
WNL (n=16)	47.11±1.25 (40.1-56.3)	
Below lower limit (n=1)	31.70	
Above upper limit (n=4)	70.30±0.62 (69-72)	
MCV (fl)	71.80±0.99 (59.1-81.4)	66-77
WNL (n=18)	71.66±0.65 (67-76.9)	
Below lower limit (n=1)	59.1	
Above upper limit (n=2)	79.35±2.05 (77.3-81.4)	
MCHC (g/dl)	27.84±0.35 (25.5-33.7)	32-36.3
WNL (n=1)	33.7	
Below lower limit (n=20)	27.55±0.19 (25.5-29.4)	
MCH (pg)	19.98±0.35 (16.4-23.9)	21-26.2
WNL (n=4)	22.35±0.55 (21.4-23.9)	
Below lower limit (n=17)	19.42±0.27 (16.4-20.7)	

Table 2. Leucocytic indices and platelet count of dogs with canine atopic dermatitis (n=21) (Mean ± S.E.)

Leucocytic Indices	Mean±S.E. (Range)	Normal Reference Values (The Merck Veterinary Manual, 2019)
TLC (10^3/mm^3)	13.55±1.67 (3.88-31.89)	5-14.1
WNL (n=12)	9.99±0.67 (5.81-12.93)	
Below lower limit (n=2)	3.89±0.01 (3.88-3.89)	
Above upper limit (n=7)	22.4±2.29 (15.35-31.89)	
Lymphocytes (10^3/mm^3)	4.83±0.39 (2.16-9.00)	0.4-2.9
WNL (n=2)	2.18±0.02 (2.16-2.20)	
Above upper limit (n=19)	5.1±0.38 (3-9)	
Monocytes (10^3/mm^3)	0.65±0.08 (0.15-1.46)	0.1-1.4
WNL (n=20)	0.61±0.07 (0.15-1.39)	
Above upper limit (n=1)	1.46	
Neutrophils (10^3/mm^3)	7.48±1.42 (1.11-25)	2.9-12
WNL (n=12)	6.03±0.66 (2.96-10.56)	
Below lower limit (n=5)	1.74±0.30 (1.11-2.82)	
Above upper limit (n=4)	18.99±2.33 (15.10-25)	
Eosinophils (10^3/mm^3)	0.28±0.04 (0.02-0.62)	0-1.3
WNL (n=21)	0.28±0.04 (0.02-0.62)	
Basophils (10^3/mm^3)	0.5±0.05 (0.01-0.22)	0-0.14
WNL (n=19)	0.06±0.01 (0.01-0.14)	
Above upper limit (n=2)	0.19±0.03 (0.16-0.22)	
Thrombocytes/Platelets (10^3/mm^3)	316.14±34.35 (81-612)	211-621
WNL (n=16)	373.69±33.29 (216-612)	
Below lower limit (n=5)	132±18.71 (81-168)	

Table 3. Serum protein profile of dogs with canine atopic dermatitis (n=21) (Mean±S.E.)

Parameter	Mean ± S.E. (Range)	Normal Reference Values (Kaneko *et al.*, 2008)
Total Proteins (g/dl)	6.34±0.27 (3.67-8.56)	5.4-7.1
WNL (n=11)	6.28±0.14 (5.65-7.06)	
Below lower limit (n=4)	4.44±0.35 (3.67-5.32)	
Above upper limit (n=6)	7.71±0.22 (7.18-8.56)	
Albumin (g/dl)	1.84±0.09 (0.92-2.46)	2.6-3.3
Below lower limit (n=21)	1.84±0.09 (0.92-2.46)	
Globulin (g/dl)	4.50±0.23 (2.75-6.76)	2.7-4.4
WNL (n=10)	3.57±0.14 (2.75-4.2)	
Above upper limit (n=11)	5.34±0.20 (4.69-6.76)	
A:G	0.42±0.03 (0.27-0.63)	0.7-2
Below lower limit (n=21)	0.42±0.03 (0.27-0.63)	

Table 4. Liver function profile of dogs with canine atopic dermatitis (n=21) (Mean±S.E.)

Parameter	Mean ± S.E. (Range)	Normal Reference Values (Kaneko *et al.*, 2008)
ALT (U/L)	29.09±5.10 (8.8-120.1)	21-102
Below to WNL (n=20)	24.54±2.41 (8.8-53.1)	
Above upper limit (n=1)	120.1	
AST (U/L)	14.32±1.73 (5.2-36.3)	23-66
Below to WNL (n=21)	14.32±1.73 (5.2-36.3)	
ALP (U/L)	107.14±24.11 (16-376)	20-156
Below to WNL (n=16)	52.63±7.37 (16-124)	
Above upper limit (n=5)	55.07±7.43 (163-376)	
GGT (U/L)	6.00±0.98 (1.3-19.2)	1.2-6.4
WNL (n=14)	3.82±0.43 (1.3-5.8)	
Above upper limit (n=7)	10.36±2.02 (6.5-19.2)	
Total Bilirubin (mg/dl)	0.09±0.01 (0.02-0.16)	0.1-0.5
Below to WNL (n=21)	0.09±0.01 (0.02-0.16)	
Direct Bilirubin (mg/dl)	0.01±0.00 (0-0.03)	0.06-0.12
Below lower limit (n=21)	0.01±0.00 (0-0.03)	
Indirect Bilirubin (mg/dl)	0.08±0.01 (0.02-0.16)	0-0.44
WNL (21)	0.08±0.01 (0.02-0.16)	

Table 5. Kidney function parameters of dogs with canine atopic dermatitis (n=21) (Mean±S.E.)

Parameter	Mean±S.E. (Range)	Normal Reference Values (Kaneko *et al.*, 2008)
Serum Urea (mg/dl)	46.91±2.53 (10.7-60.9)	21.4-59.9
Below to WNL (n=20)	46.21±2.55 (10.7-59.5)	
Above upper limit (n=1)	60.9	
Serum Creatinine (mg/dl)	1.48±0.06 (1.0-2.08)	0.5-1.5
WNL (n=11)	1.26±0.05 (1.0-1.42)	
Above upper limit (n=10)	1.73±0.06 (1.54-2.08)	

Table 6. Serum total IgE levels in dogs with canine atopic dermatitis (Mean±S.E.)

Parameter	Healthy (n=6)	Atopic (n=21)	Level of significance
Total IgE (g/ml)	3.28±0.42 (1.44-4.11)	3.60±0.12 (2.69-5.43)	p>0.05

neutrophils and eosinophils count, as well as neutrophil/lymphocyte ratio (NLR) in comparison to dogs with CADESI between 0-10. Histopathological analysis also revealed numerous neutrophils, macrophages and mast cells in atopic dogs. Ambily *et al.* (2022) found absolute eosinophils count to be positively correlated with NLR in atopic dogs.

Platelet count was found within normal limit in maximum number of atopic dogs (n=16), followed by

thrombocytopenia (n=5). Ambily *et al.* (2022) also reported non-significant alteration in platelets indices in atopic dogs. Overall, the haematological profile in atopic dogs in present investigation revealed hypochromasia, normal to elevated leucocyte count with absolute lymphocytosis.

Serum biochemical profile of dogs with CAD: Table 3 presents the serum protein profile of dogs with CAD. Total serum proteins were found within normal limit in more than half of cases (n=11), followed by hyperproteinaemia (n=6) and hypoproteinaemia (n=4). Serum albumin level revealed hypoalbuminemia in all the affected cases. Serum globulin level revealed hyperglobulinemia (n=11), followed by level within normal limit (n=10) in affected cases. Likewise, AG ratio was found below the normal reference limit in all the cases. Hyperproteinaemia in present investigation might be due to hyperglobulinemia observed in atopic dogs. In contrast, Walaa *et al.* (2008) reported lower values of total proteins and globulin in atopic dogs. Brar *et al.* (2017) also reported hypoproteinaemia in atopic dogs in Ludhiana (Punjab). Ferreira *et al.* (2021) analysed systemic inflammatory immune response in CAD which revealed hypoalbuminemia in affected dogs with a negative correlation of albumin level with CADESI. Ambily *et al.* (2022) also reported hypoalbuminemia with reduced AG ratio in atopic dogs. Conversely, Singh *et al.* (2022) revealed no significant changes in total proteins and albumin levels in atopic dogs in Mizoram. Overall, serum protein profile in the present investigation revealed hypoalbuminemia, normal to elevated globulin level and decreased AG ratio in atopic dogs.

Table 4 presents the liver function profile of dogs with CAD. The activities of serum ALT and AST were observed below to within normal limit in most cases. Serum ALP level was found within normal limit in maximum cases (n=15), followed by its elevated level in 5 cases. Serum GGT level was found within normal reference limit in 2/3rd of affected dogs (n=14), followed by its elevated level in 1/3th of the atopic dogs (n=7). Elevated ALP and GGT levels in few cases of CAD indicate biliary dysfunction in atopic dogs. In contrast, Singh *et al.* (2022) reported no significant changes in activities of serum ALT and ALP in atopic dogs. The levels of total bilirubin, direct and indirect bilirubin was found either below or within normal reference limit in all cases (n=21), indicating the normal hepatic function in atopic dogs. Likewise, Ferreira *et al.* (2021) also reported no significant alteration in total bilirubin level in atopic dogs.

Kidney function parameters of dogs with CAD are depicted in Table 5. Serum urea level was found within normal limit in most of the cases (n=20). Serum creatinine level was found either within normal limit (n=11) or above the upper limit of normal reference range (n=10). This elevated serum creatinine level indicates compromised kidney function in atopic dogs. Conversely, Singh *et al.* (2022) reported non-significant changes in blood urea nitrogen and creatinine levels in atopic dogs.

Serum total IgE level of dogs with CAD: Serum total IgE level in dogs with CAD is depicted in Table 6. Mean serum total IgE level in atopic dogs was observed non-significantly higher than that of healthy dogs. DeBoer and Hill (1999) advocated no correlation between total serum IgE level in young dogs and consequent development of signs of atopic dermatitis and claimed its limited diagnostic value in prediction of CAD. Likewise, Ledin *et al.* (2006) reported plasma IgE level low in young dogs and very high in adult dogs, irrespective of CAD status. Conversely, marked elevation in serum total IgE has been reported in atopic dogs by Indian workers (Brar *et al.*, 2017; Chaudhary *et al.*, 2019). Recently, Singh *et al.* (2022) evaluated serum total IgE using canine IgE rapid test kit in atopic dogs in Mizoram and revealed significantly elevated total IgE level in atopic dogs. Instead of measurement of total IgE only, allergen-specific IgE serum testing (ASIS) should be conducted which includes assays to detect circulating allergen specific IgE antibodies against a panel of allergens in atopic dogs (Hensel *et al.*, 2015). Chermprapai and Thengchaisri (2020) conducted ASIS testing for identifying allergens in dogs with atopic dermatitis in Thailand and revealed highest positivity for dust mites (69.57%). Tommaso *et al.* (2021) identified mites and pollens of Rumex acetosa and grasses to be the most prevalent environmental allergens in atopic dogs in Northern Italy using ASIS testing.

CONCLUSION

The haematological profile of dogs with CAD revealed hypochromasia, normal to elevated leucocyte count with absolute lymphocytosis. Serum biochemical profile revealed hypoalbuminemia, normal to elevated globulin level, decreased albumin to globulin ratio, normal to elevated ALP, GGT and creatinine levels, suggestive of biliary and renal dysfunction. Mean serum total IgE level in atopic dogs was non-significantly higher than that of healthy dogs. These altered biomarkers should be taken into consideration while initiation of specific targeted and ancillary therapy of CAD in dogs and could also be utilized for further assessment in response to therapy.

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