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RESEARCH ARTICLE

Studies on erythrocytic abnormalities with leukogram and cytological findings of dogs

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ABSTRACT

The study was conducted on blood samples of dogs to correlate the erythrocytic abnormalities with leukogram and cytological findings. The study was conducted on blood samples submitted to Centralized Clinical Diagnostic Laboratory in the Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana. The Leukogram analysis was carried out in all the 363 cases and detailed leukocytic abnormalities were recorded. The cases were categorized into those having normal total leukocyte count, leukopenia, leukocytosis and finally the cases having leukemoid response. On the basis of cytological examination, the cases were classified into neoplastic and non-neoplastic conditions. A good correlation was between presence of codocytes, schistocytes, echinocytes, polychromatophilic RBCs and leukocytosis and stomatocytes and polychromatophilic RBCs with leukopenia. In addition, acanthocytes, echinocytes and schistocytes were common in cases of lymphoma.

Keywords: Blood, correlation, cytology, dogs, erythrocytic abnormalities, leukogram

INTRODUCTION

Hematological examination is an important diagnostic tool and it is often used to correlate the physical examination and the medical history for diagnosis of diseases in both medical and veterinary practice[1]. Routine haematological examination includes complete blood cell count and blood smear examination for leukocytic and erythrocytic abnormalities[2,3]. Even in the age of molecular analysis, the blood smear remains an important diagnostic tool[4]. Erythrocyte morphological abnormalities have been reported to be associated with disease conditions. Morphology abnormalities like acanthocytes, schistocytes spherocytosis have been reported to be associated with different disease conditions in dogs[5,6]. However, anemia with erythropenia, without significant changes in erythrocyte indices, thrombocytopenia and leukocytosis and slight variation in differential white blood cell count was studied in dogs with mammary gland carcinoma[7]. In addition, canine lymphoma was the most common hematologic malignancy in dogs and in many aspects comparable to non-Hodgkin lymphoma in humans characterized by the involvement of multiple lymph nodes and the infiltration of various organs especially liver and spleen by neoplastic lymphocytes[8]. Therefore, the present study was undertaken to correlate the erythrocytic abnormalities with the leukocytic and cytological changes in dogs.

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MATERIAL AND METHODS

The present study was conducted on blood samples of dogs presented to Centralized Clinical Diagnostic Laboratory in the department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana, with effect from 1st July 2018 to 31st March 2019. A total of 363 cases of dogs were analyzed. Out of these, 259 were analyzed manually and 104 cases were analyzed using ADVIA 2120 Hematology System (Siemens, USA). Leukogram analysis was carried out in all 363 cases and detailed leukocytic abnormalities were recorded. The cases were categorized into those having normal total leukocyte count, leukopenia, leukocytosis and finally the cases having leukemoid response. Moreover, cytological examination of the smears submitted to the Centralized Clinical Diagnostic Laboratory was also performed. The smears were stained by Leishman stain. Based on cytological examination, the cases were classified into neoplastic and non-neoplastic conditions.

Statistical analysis

The data pertaining to different parameters was analyzed using Statistical Analysis Software (SAS-version 9.3, Institute, Cary, USA). The comparison between

Table 1. Correlation of morphological abnormalities of erythrocytes with leukogram findings.

Erythrocytic morphology	Normal TLC	Leukopenia	Leukocytosis	Leukemoid response
Acanthocytes	6.23^{a} ± 1.35	5.11^{a} ± 2.91	9.13^{a} ± 1.90	10.16^{a} ± 6.86
Blister cells	0.76^{a} ± 0.24	0.11^{a} ± 0.11	0.24^{a} ± 0.12	0.00^{a} ± 0.00
Codocytes	30.88^{a} ± 3.01	24.96^{a} ± 5.60	36.68^{a} ± 3.39	48.33^{a} ± 7.89
Dacrocytes	1.23^{a} ± 0.31	2.25^{a} ± 1.26	0.75^{a} ± 0.25	0.00^{a} ± 0.00
Echinocytes	45.09^{a} ± 5.29	38.18^{a} ± 9.89	52.58^{a} ± 5.73	18.5^{a} ± 12.35
Elliptocytes	0.11^{a} ± 0.11	0.00^{a} ± 0.00	0.86^{a} ± 0.85	0.00^{a} ± 0.00
Incomplete spherocytes	11.83^{a} ± 2.06	19.51^{a} ± 13.39	7.29^{a} ± 1.39	0.00^{a} ± 0.00
Keratocytes	0.23^{a} ± 0.08	0.37^{a} ± 0.17	0.24^{a} ± 0.12	0.00^{a} ± 0.00
Nucleated RBCs	0.15^{a} ± 0.08	0.07^{a} ± 0.07	0.20^{a} ± 0.07	0.00^{a} ± 0.00
Polychromatophils	1.65^{a} ± 0.44	0.40^{a} ± 0.19	4.01^{a} ± 0.72	4.5^{a} ± 4.11
Quatrefoil RBCs	0.87^{a} ± 0.17	0.40^{a} ± 0.31	0.90^{a} ± 0.18	1.5^{a} ± 0.84
Schistocytes	18.36^{a} ± 2.26	12.11^{a} ± 2.55	26.83^{a} ± 3.15	28^{a} ± 7.22
Spherocytes	50.84^{a} ± 3.39	41.40^{a} ± 11.53	39.13^{a} ± 3.63	25.16^{a} ± 6.78
Stomatocytes	0.75^{a} ± 0.21	1.81^{a} ± 0.74	1.11^{a} ± 0.30	0.00^{a} ± 0.00

Values with different superscript (a, b, c) within a parameter differ significantly ($p<0.05$)

the normally distributed quantitative variables was done using ANOVA along with Duncan correction. In addition, correlation and regression analysis was also carried out and the association between different erythrocytic abnormalities and leukogram findings was calculated.

RESULTS

In the present study, the erythrocytic abnormalities observed were correlated with the findings of the leukogram in all 363 cases (Table 1). The leukogram was classified into animals having normal TLC, leukopenia, leukocytosis and leukemoid response. The mean value of acanthocytes was more in dogs showing leukemoid response, followed by those having leukocytosis, normal TLC and leukopenia respectively. The mean values of blister cells were more in dogs showing normal TLC, followed by leukocytosis and no blister cells were observed in leukopenia respectively. The mean value of codocytes was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. The mean value of dacrocytes was more in dogs showing leukopenia, followed by normal TLC, leukocytosis. The mean value of echinocytes was more in dogs showing leukocytosis, followed by normal TLC and no echinocytes were observed in leukopenia and leukemoid response. The mean value of elliptocytes was more in dogs showing leukocytosis, followed by normal TLC, no elliptocytes were observed in leukemoid response. The mean value of incomplete spherocytes was more in dogs showing leukopenia, followed by normal TLC and leukocytosis, no incomplete spherocytes were observed in leukemoid response. The mean value of keratocytes was more in dogs showing leukopenia, followed by leukocytosis and normal TLC, no keratocytes were observed in cases showing leukemoid response. The mean value of nucleated RBCs was more in dogs showing leukocytosis, followed by normal TLC and leukopenia, no nucleated RBCs were observed in cases having leukemoid response. The mean value of polychromatophilic RBCs was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. The mean value of q RBC was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. The mean value of schistocytes was more in dogs showing leukemoid response, followed by leukocytosis, normal TLC and leukopenia. The mean value of spherocytes was more in dogs showing normal TLC, followed by leukopenia, leukocytosis and leukemoid response. The mean value of stomatocytes was more in dogs showing leukopenia, followed by leukocytosis, normal TLC respectively and no stomatocytes were observed in leukemoid response. The mean values of acanthocytes, blister cells, codocytes, dacrocytes, echinocytes, elliptocytes, incomplete spherocytes, keratocytes, neucleated RBCs, polychromatophilic RBCs, q RBCs, schistocytes, spherocytes and stomatocytes did not differ significantly in cases having normal TLC, leukopenia, leukocytosis and leukemoid response.

Table 2 depicts the correlation between serum chemistry findings with the findings of leukogram. The mean value of total protein was more in dogs showing leukopenia, followed by normal TLC, leukocytosis and leukemoid response. The mean value of albumin was more in dogs showing normal TLC, followed by leukocytosis, leukemoid response and leukopenia. The mean value of ALT was more in dogs showing leukocytosis, followed by normal TLC, leukopenia and leukemoid response. The mean value of AST was more

Table 2. Correlation of serum chemistry findings with the leukogram findings.

Parameter	Units	Normal TLC	Leukopenia	Leukocytosis	Leukemoid response
Total protein	(g/dL)	5.53^{a} ± 0.11	5.48^{a} ± 0.31	5.09^{a} ± 0.11	4.58^{a} ± 0.42
Albumin	(U/L)	2.21^{a} ± 0.05	1.89^{a} ± 0.08	2.07^{a} ± 0.05	1.98^{a} ± 0.45
Alanine aminotransferase (ALT)	(U/L)	93.69^{a} ± 10.07	88.42^{a} ± 33.56	129.62^{a} ± 15.68	66^{a} ± 18.30
Aspartate aminotransferase (AST)	(U/L)	75.85^{a} ± 7.63	102.13^{a} ± 58.03	105.51^{a} ± 12.36	103.5^{a} ± 43.95
Gamma-glutamyl transferase (GGT)	(U/L)	11.30^{a} ± 2.29	21.13^{a} ± 14.71	21.72^{a} ± 4.69	15.75^{a} ± 6.23
Total bilirubin	(mg/dL)	0.40^{b} ± 0.05	0.89^{b} ± 0.58	1.82^{b} ± 0.35	8.15^{a} ± 5.34
Alkaline phosphatase (ALKP)	(U/L)	242.17^{b} ± 24.98	270.29^{b} ± 73.94	348.61^{b} ± 36.25	2800.17^{a} ± 2409.03
Blood urea nitrogen (BUN)	(mg/dL)	66.47^{a} ± 10.62	65.02^{a} ± 10.62	50.60^{a} ± 4.10	54.33^{a} ± 21.31
Creatinine	(mg/dL)	5.95^{a} ± 0.49	4.70^{a} ± 1.11	3.63^{a} ± 0.34	3.68^{a} ± 1.47

Values with different superscripts (a, b, c) within parameter differ significantly ($p<0.05$)

in dogs showing leukocytosis, followed by leukemoid response, leukopenia and normal TLC. The mean value of GGT was more in dogs showing leukocytosis, followed by leukopenia, leukemoid response and normal TLC. The mean value of total bilirubin was more in dogs showing leukemoid response, followed by leukocytosis, leukopenia and normal TLC. The mean value of ALKP was more in dogs showing leukemoid response, followed by leukocytosis, leukopenia and normal TLC. The mean value of BUN was more in dogs showing normal TLC, followed by leukopenia, leukemoid response and leukocytosis. The mean value of creatinine was more in dogs showing normal TLC, followed by leukopenia, leukemoid response and leukocytosis. The mean values of total protein, albumin, ALT, AST, GGT, total bilirubin, ALKP, BUN and creatinine did not differ significantly in cases having normal TLC, leukopenia, leukocytosis and leukemoid response.

Table 3 depicts the correlation of hematological findings using hematological analyzer with findings of the leukogram. The mean value of anisocytosis was more in dogs showing leukocytosis, followed by normal TLC, and no anisocytosis were observed in leukopenia and leukemoid response respectively. The mean value of hypochromasia was more in dogs showing leukemoid response, followed by normal TLC and leukocytosis, and no hypochromasia were observed in leukopenia respectively. The mean value of polychromasia was more in dogs showing leukemoid response, followed by normal TLC and leukocytosis and no polychromasia were observed in leukopenia respectively. The mean value of

Table 3. Correlation of hematological findings using hematological analyzer with the findings of the leukogram (n=104).

Variables	Normal TLC	Leukopenia	Leukocytosis	Leukemoid response
Anisocytosis	0.14^{a} ± 0.07	0.00^{a} ± 0.00	0.16^{a} ± 0.09	0.00^{a} ± 0.00
Hypochromasia	0.06^{a} ± 0.03	0.00^{a} ± 0.00	0.06^{a} ± 0.05	0.25^{a} ± 0.25
Polychromasia	0.12^{a} ± 0.04	0.00^{a} ± 0.00	0.04^{a} ± 0.03	0.25^{a} ± 0.25
Normocytic	0.00^{a} ± 0.00	0.00^{a} ± 0.00	0.00^{a} ± 0.00	0.00^{a} ± 0.00
Microcytic	0.5^{a} ± 0.12	0.5^{a} ± 0.37	0.30^{a} ± 0.11	0.00^{a} ± 0.00
Macrocytic	0.04^{a} ± 0.02	0.00^{a} ± 0.00	0.09^{a} ± 0.07	0.00^{a} ± 0.00
CH (Cellular Hemoglobin)	20.20^{a} ± 0.37	19.37^{a} ± 0.37	19.94^{a} ± 0.40	19.5^{a} ± 0.62
CHCM (Corpuscular Hb concentration mean)	32.49^{a} ± 0.28	33.0^{a} ± 0.73	32.08^{a} ± 0.46	33.07^{a} ± 1.12
HCT	31.68^{a} ± 1.52	26.35^{a} ± 2.54	29.44^{a} ± 1.50	28.3^{a} ± 4.16
HDW (Hb concentration distribution width)	2.44^{a} ± 0.13	2.14^{a} ± 0.16	2.68^{a} ± 0.16	2.66^{a} ± 0.31
MCH	19.17^{a} ± 0.55	16.38^{a} ± 1.49	18.90^{a} ± 0.58	15.1^{a} ± 2.34
MCHC	31.04^{a} ± 0.89	27.9^{a} ± 2.40	29.98^{a} ± 1.03	25.57^{a} ± 4.04
MCV	62.23^{a} ± 1.04	58.75^{a} ± 1.38	63.22^{a} ± 1.26	45.85^{b} ± 13.38
MPV	13.47^{a} ± 0.87	13.3^{a} ± 2.04	14.07^{a} ± 0.95	14.4^{a} ± 2.54
RBC	5.18^{a} ± 0.26	4.53^{a} ± 0.45	4.70^{a} ± 0.23	4.82^{a} ± 0.80
RDW	15.01^{a} ± 0.38	14.72^{a} ± 0.51	16.35^{a} ± 0.58	17.3^{a} ± 2.12
WBC	12.74^{a} ± 1.45	4.37^{a} ± 0.47	29.07^{a} ± 1.94	72.22^{a} ± 30.95

Values with different superscripts (a, b, c) within a parameter differ significantly ($p<0.05$)

microcytosis was more in dogs showing normal TLC and leukopenia, followed by leukocytosis and no microcytosis were observed in leukemoid response. The mean value of macrocytosis was more in dogs showing leukocytosis followed by normal TLC and no macrocytosis were observed in leukopenia and leukemoid response. The mean value of CH was more in dogs showing normal TLC, followed by leukemoid response, leukocytois and leukopenia. The mean value of CHCM was more in dogs showing leukemoid response, followed by leukopenia, normal TLC and leukocytosis. The mean value of HCT was more in dogs showing normal TLC, followed by leukocytosis, leukemoid response and leukopenia. The mean value of HDW was more in dogs showing leukocytosis followed by leukemoid response, normal TLC and leukopenia. The mean value of MCH was more in dogs showing normal TLC followed by leukocytosis, leukopenia and leukemoid response. The mean value of MCHC was more in dogs showing normal TLC followed by leukocytosis, leukopenia and leukemoid response. The mean value of MCV was more in dogs showing leukocytosis followed by normal TLC, leukopenia and leukemoid response. The mean value of MPV was more in dogs showing leukocytosis followed by leukemoid response, normal TLC and leukopenia. The mean value of RBC was more in dogs showing normal TLC followed by leukemoid response, leukocytosis and leukopenia. The mean value of RDW was more in dogs showing leukemoid response followed by leukocytosis, normal TLC and leukopenia. The mean values of anisocytosis, hypochromasia, polychromasia, normocytic RBC, microcytic RBC, macrocytic RBC, CH, CHCM, HCT, HDW, MCH, MCHC, MCV, MPV, RBC, RDW and WBC did not differ significantly in cases having normal TLC, leukopenia, leukocytosis and leukemoid response.

In the present study, correlation between erythrocytic abnormality with leukogram finding was carried out ($p<0.05$) and there was significant correlation between normal TLC with the presence of spherocytes and polychromatophilic RBCs in the blood smears, between leukocytosis and the presence of codocytes, schistocytes, echinocytes and polychromatophilic RBCs in the blood smears. In addition, leukopenia correlated with the presence of stomatocytes and polychromatophilic RBCs in blood smears of dogs.

Correlation between spherocytes and q RBCs were found in TVT cases (Fig. 1 & 2) and echinocytes and schistocytes were found in lymphoma cases, acanthocytes were found in malignant melanoma whereas, acanthocytes and schistocytes were observed in malignant histiocytoma. The codocytes was observed in chronic active inflammation (Fig. 3 & 4).

In the present study, correlation between history and clinical signs with leukogram finding was also carried out ($p<0.05$) and there was significant correlation between

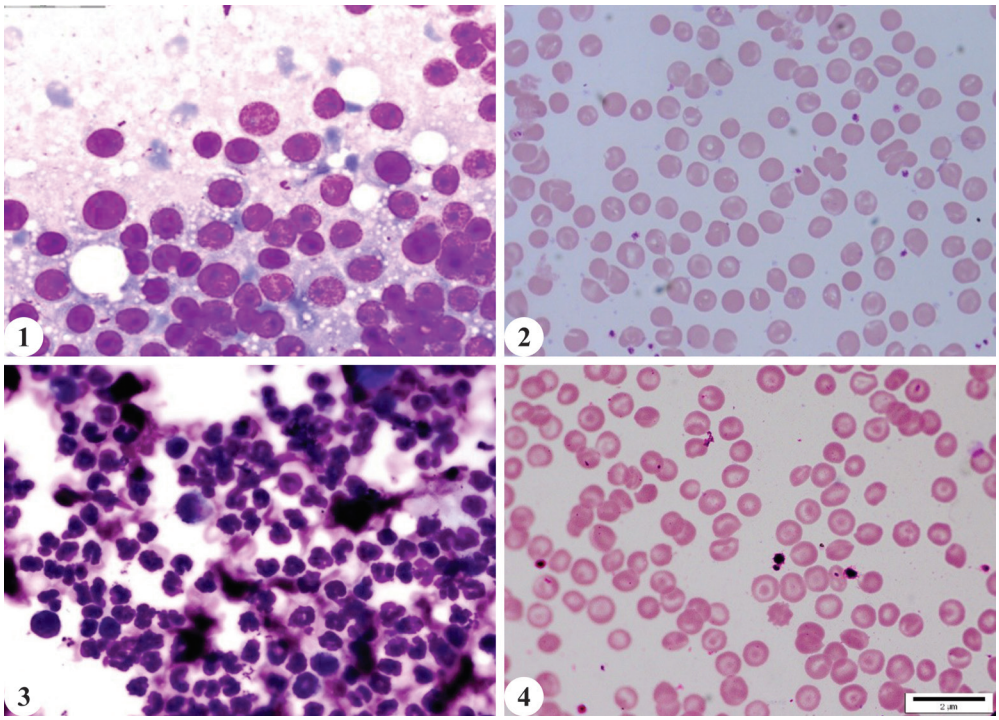

Fig. 1. Impression smear from tumor growth showing round, slightly pleomorphic cells having vacuolated cytoplasm, suggestive of TVT (Leishman stain x100); **Fig. 2.** Blood smear shows Quatrefoil RBCs in dog with TVT (Leishman stain x100); **Fig. 3.** FNAB showing large number of neutrophils and few macrophages, suggestive of chronic active inflammation (Leishman stain x100); **Fig. 4.** Blood smear shows codocytes in dog with chronic active inflammation (Leishman stain x100).

leukocytosis with the presence of clinical signs like blood in vomit, leukopenia with the history of presence of ticks, icteric mucus membrane and distented abdomen.

DISCUSSION

In accordance with our present study, earlier workers have also reported that codocytes and leukemoid response was observed in dogs having renal carcinoma[9]. Scientist observed that echinocytes and leukocytosis was reported in dogs having hemangiosarcoma[10]. Similarly, some researchers reported that hypoalbuminemia, elevated ALT, ALKP levels and leukocytosis was observed in dogs having IMHA[11]. Moreover, it is reported that hypochromasia, leukemoid response and codocytes were associated with chronic granulocytic leukemia in dogs[12], low RBC count, leucopenia, thrombocytopenia was associated with Ehrlichia and Anaplasma infection in dogs[13]. Researcher reported that high WBC counts and leukemoid response was associated with dogs having pyometra[14]. Anisocytosis, polychromasia, codocytes and leukocytosis were observed in dogs having renal carcinoma[9]. Reports of[15] revealed dog having renal failure showed clinical sign of blood in vomit along with leukocytosis. Leukopenia was observed in early cases of canine babesiosis having history of inappetence and ticks infestation[14]. Scientists reported that acanthocytes and schstocytes were observed in blood smear of dogs having lymphoma[16]. Earlier workers reported that dog with malignant melanoma showed acanthocytes and codocytes in blood smears[17]. Codocytes and acanthocytes were seen in blood smear of dogs suffering from chronic kidney diseases[18].

CONCLUSION

From the study it was concluded that erythrocytic abnormalities may provide some insight regarding changes in the leukogram and cytological diagnosis of dogs.

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