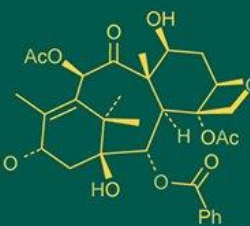
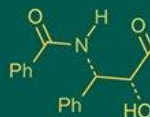
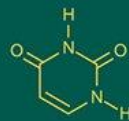
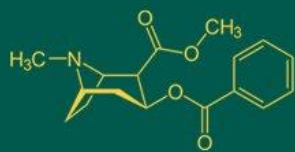


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Diagnostic significance of hematological parameters in benign and malignant lesions in dogs affected with mammary tumour

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Abstract

Canine mammary tumours are one of the most common malignancies diagnosed in female dogs. Despite advancements in diagnosis and treatment, tumours still result in significant morbidity and mortality. Distinguishing between benign and malignant mammary tumours in dogs is clinically challenging at early stages. A Complete Blood Count (CBC) analysis is typically performed as part of the preoperative work-up for mammary tumours. This study aimed to evaluate the utility of CBC parameters in diagnosing malignant canine mammary tumours from benign and analyse the diagnostic role of haematological parameters within benign and malignant mammary tumours. The period of study was from January 2024 to October 2024, a total of Forty-three cases suspected of canine mammary tumours were presented at Veterinary Clinical Complex, DUVASU, Mathura, out of which Thirty-Six dogs had neoplastic growth, while seven had inflammatory growth based on cytological examination. A total of Ninety-Seven cases of various dog cancers were documented throughout this time, indicating a 37.12% incidence of mammary tumours. Also, twenty-four samples were taken from the healthy dogs with the consent of the owner. Histopathological examination confirmed the diagnosis of these tumours. CBC parameters, including haemoglobin, Total White Blood Cell (TWBC) count, neutrophil count, lymphocyte count, RBC, Eosinophil, monocyte and platelet count, were collected from a total of 60 cases were calculated from the obtained CBC parameters for all cases. Malignant canine mammary tumours showed a significant increase in neutrophils ($81.32 \pm 1.53\%$) and a significant decrease in lymphocytes ($15.07 \pm 1.28\%$) compared to benign mammary cases. Among the CBC parameters evaluated, neutrophil count, lymphocyte count, RBC, WBC, Platelets, and Eosinophils were significantly altered in canine mammary malignancy compared to benign mammary masses. Measuring CBC parameters can be a fast, simple, inexpensive, and readily available method.

Keywords: Haematology, benign, malignant, mammary gland tumour

1. Introduction

Cancer poses a significant threat to both human and animal health. In the case of pets, cancer is a leading cause of mortality. Among human females, breast cancer is the most common tumour worldwide, with its incidence on the rise due to changes in environmental factors and lifestyle choices. Approximately one in ten new cancer cases diagnosed each year is breast cancer, making it the most prevalent malignancy in both developed and developing countries^[1]. In dogs, mammary tumours are the second most common neoplasm, with dogs being twice as likely as humans to develop cancer^[2]. Mammary tumours account for approximately 52% of all tumour cases in dogs^[3]. Dogs exhibit a wide range of tumours that share similarities in characteristics and incidence rates with human cancers, making them an excellent model for comparative oncology. Risk factors for carcinogenesis, such as obesity, environmental toxins, and ageing, are shared between humans and dogs. The similarities between canine mammary tumours and human breast cancer in terms of morphology, behaviour, and immunohistochemistry make dogs a valuable model for studying cancer^[4]. Research has

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shown that dogs with mammary carcinoma exhibit changes in their leukogram, with increases in total white blood cell count and neutrophils, decreases in lymphocytes, and reductions in mean haemoglobin levels [5]. These haematological parameters play a crucial role in the progression of cancer and can impact the prognosis of the disease. Studies have shown that haemoglobin and packed cell volume levels are indirectly linked to an increased risk of cardiac failure in cancer patients [6]. At the same time, elevated total leukocytic count is associated with a poorer prognosis [7]. Therefore, a comprehensive understanding of haematological parameters is essential for assessing and managing cancer in both humans and animals.

2. Material and Methods

From January 2024 to October 2024, a prospective study was conducted at the Veterinary Clinical Complex, DUVASU, Mathura, Uttar Pradesh, India. A total of 97 dogs with suspected canine mammary gland tumours were seen during this time; 36 of them had neoplastic development on their mammary glands, and 07 of them had inflammatory growth, according to the results of fine needle aspiration biopsy and ultrasonography tests. The study was preceded with the approval of the Institutional Animal Ethics Committee with Certification No. IAEC/22/2/4 and Letter No. 145/IAEC/24/1/27. Following the case's presentation, a thorough clinical examination was performed, and the following observations were recorded: the owner's contact information; animal's age, sex; breed and body weight; location of the lesion(s); number of glands involved; size of the affected gland; colour, texture, and consistency of the neoplasm; length of the illness; history of prior inflammation or injury; history of parity and spaying; and an ultrasonographic examination to determine whether the tumour had spread distantly to lymph nodes and visceral organs. Out of 36 neoplastic cases, 31 cytological samples, 36 blood samples and 21 tissue samples were obtained for further examination (Table 1).

2.1 Sample collection for blood examination

The dog's saphenous and cephalic veins were used to get the samples. The drawn blood was gently filled in an EDTA vial (2 ml) for the investigation of total blood parameters. 24 healthy animals served as the source of the control samples, with the consent of pet owners. To obtain complete blood parameters, blood taken in an EDTA vial was gently mixed and placed under an automated blood counting machine (NIHON KOHDEN MEK-6550 CELLTAC α). The one-way ANOVA was used to statistically analyse the blood sample data.

2.2 Fine needle aspiration fluid collection for cytology

A 22 G fine needle fitted to 5ml syringe, was carefully inserted into the affected mammary gland region, approximately up to the centre of the growth in 2-3 different directions, after the affected area had been thoroughly sterilised with a sterile swab soaked in 70% alcohol. The fluid was then aspirated by keeping the needle inside the growth. After removing the syringe, the aspirated fluid was gently spread out over a clean, grease-free glass slide to create a smear. The smear obtained from fine needle aspirates was stained with Giemsa stain for cytological analysis in order to determine the kind of neoplastic cells and analyse their properties in order to categorise the tumour into several

groups. Robinson's grading system was used for the cytological grading of canine mammary tumours [8].

2.3 Histopathology

This method is considered the gold standard for evaluating tissue alterations, classifying tumour types, and assessing the severity of malignancy. Tissue samples from mammary gland tumours were quickly preserved in 10% neutral buffered formalin (NBF) for at least 24 to 48 hours to guarantee the best preservation of cellular and tissue structure. After fixation, the tissues were subjected to standard histological processing, which involved dehydration using a graded series of ethanol concentrations (70%, 80%, 95%, and absolute), clearing with xylene, and embedding in paraffin wax. Tissue blocks embedded in paraffin were cut into sections of 4-5 μ m thickness with a rotary microtome. Sections were placed on clean glass slides and permitted to dry, after which they underwent deparaffinization in xylene and rehydration via a descending series of alcohol (absolute, 95%, 70%) to distilled water. The slides were stained with Harris's Hematoxylin for 5-10 minutes to reveal nuclear detail, then rinsed in running tap water. Differentiation was conducted with 1% acid alcohol. Afterward, slides were counterstained with Eosin Y for 1-2 minutes to colour the cytoplasm and extracellular matrix. Ultimately, the stained sections underwent dehydration through increasing concentrations of alcohol, were cleared with xylene, and were covered with a coverslip using a resinous mounting medium. The stained slides were subsequently analysed under a light microscope for histopathological assessment of tumour structure and cellular features. The histopathological samples were examined and categorized according to the Goldschmidt *et al.*, [9] along with the histopathological grading of CMTs utilizing the Elston and Ellis classification system [10].

3. Results

A total of 97 canine neoplastic cases were recorded at the Veterinary Clinical Complex, DUVASU, Mathura, Uttar Pradesh, India, during the study period. 36 cases of canine mammary gland tumours were found among the 97 neoplastic cases, accounting for 37.12% of the total. Based on cytological and ultrasonographic examination, the majority of canine mammary tumours were malignant. Of the 36 total neoplastic cases, 2 had distant metastases discovered in the lungs on ultrasonographic examination and had regional lymph node involvement based on histopathological examination.

3.1 Haematological parameters

Of the 36 cases of neoplasm in the current investigation, 9 Benign tumours, 27 Malignant tumours were found. Also, 24 healthy samples were compared with haematological parameters of benign and malignant cases. In our study, the Mean of haematological parameters of malignant condition was found significantly different compared to the benign and control groups. The one-way ANOVA was used to evaluate the mean, and standard error of the mean of benign, malignant and control as shown in Table 2.

The mean value of WBC in the malignant tumour was $18.98 \pm 1.85 \times 10^3 / \mu\text{l}$, compared to the mean of benign was $10.74 \pm 1.65 \times 10^3 / \mu\text{l}$. However, because of the large diversity in clinical values, these differences did not reach the statistically significant threshold.

The mean neutrophil count in the malignant condition was $81.32 \pm 1.53\%$, indicating a significant (P value < 0.05) increase in neutrophil count compared to the benign tumour and healthy condition which was $64.73 \pm 1.63\%$ and $55.43 \pm 0.92\%$, respectively.

The lymphocyte count in the malignant tumour condition was found $15.07 \pm 1.2\%$ which was significantly lower (P value < 0.05) than in the benign and control group with mean values of $29.85 \pm 2.88\%$ and $37.57 \pm 0.94\%$ respectively.

When comparing the malignant condition to the benign and control, there was a highly significant (P value < 0.05) drop in the mean monocyte count ($3.30\% \pm 0.69\%$) found in the malignant condition.

The eosinophil count mean value in the malignant condition was $0.60 \pm 0.16\%$, indicating an increase in eosinophil count compared to the benign and control which was $0.28 \pm 0.12\%$, and $0.11 \pm 0.03\%$, respectively.

The result shows that, when compared to the control and benign conditions the mean of total RBC count in the malignant condition was found to be decreased. The mean haemoglobin concentration in the malignant condition was $12.24 \pm 0.74\text{g/dl}$, indicating a decrease compared to the control.

The mean platelet count in the malignant condition was $341.3 \pm 17.57 \times 10^3/\mu\text{l}$, indicating a decrease when compared to the benign and control group. However, because of the large diversity in clinical values, these differences did not reach the statistically significant threshold (Fig.1).

3.2 Cytological examination of FNAC smears

Among the 36 neoplastic cases, 31 cytology samples were obtained by fine needle aspiration. The majority of samples (65%) belonged to cytological grade II. Tumours classified as grade II or III were considered malignant, whereas grade I tumours were considered benign (Fig. 2).

3.3 Histopathological Examination of Tissues

In the present study, 80% of tumours were classified as malignant, while 20% as benign on histopathology. The most common type of benign tumour was fibroadenoma and most common type of malignant mammary tumours was carcinoma mixed type. The other common tumors are also found shown in Fig. 3. On the basis of grading, 80% of tumors belonged to the grade II category.

4. Discussion

4.1 Haematological parameters

The significantly increased neutrophil count observed in malignant tumours may be associated with neutrophilic leucocytosis, which can be caused by acute or chronic inflammation or tissue necrosis as a result of malignancy. IL1 and TNF- α promote growth factor production, which can lead to neutrophilia. To fight against infection, leucocytes and phagocytic cells, especially neutrophils, create reactive oxygen and nitrogen species, which are typically produced in moderation [11]. However, under chronic inflammatory situations, there is a greater generation of these reactive species, which combine to generate peroxynitrite, a mutagenic agent. Thus, repeated tissue damage and regeneration in the presence of highly reactive oxygen and nitrogen species released by inflammatory cells interact with the DNA of proliferating epithelium, resulting in permanent genomic alterations such as point mutations, deletions, and rearrangements, among other effects. This eventually makes

the tissue more susceptible to tumour formation, which encourages the growth of neoplastic tissue in the mammary gland tissue [12].

The significant lymphopenia observed in malignant tumours may be due to reduced circulating T lymphocytes, which play a major role in tumour cell elimination. Disease development is the outcome of immune system alterations caused by ageing, particularly in circulating T-cells. These alterations reduce cellular immunity. Cancer cells generate immunosuppressive chemicals, including cytokines (IL-6, IL10), TGF β , and T-cell breakdown. These chemicals further weaken the immune system. As a result, these factors are likely to produce a decline in total lymphocyte count under tumour circumstances, accelerating cancer growth. This is also a potential explanation for why the majority of tumours appear in elderly people [13].

The reduction in monocyte count in malignant cases may reflect tumour-induced dysregulation of myeloid cells. As, myeloid cells are innate immune cells that comprise the mononuclear phagocytic system and derive from dendritic cells, monocytes, and macrophages. Given the critical role that myeloid cells play in stimulating and modulating immune responses, soluble factors released by tumour cells suppress canine myeloid cell activation and function, resulting in dysregulation of canine myeloid cells and, ultimately, cancer-related immunosuppression, which manifests itself in a variety of ways, including decreased monocyte counts and metastatic spread [14].

The elevated eosinophil count in malignant tumours may be caused by allergic reactions or parasite infections. According to several studies, mammary cancer cases have been associated with paraneoplastic eosinophilia, which is caused by the IL-5 cytokine and eosinophilic chemotactic factor [15]. The decreased RBC count and haemoglobin concentration observed in malignant tumours could be a result of Tumour cell-secreted cytokines including IL-1, which decreases erythrocyte half-life by sequestering iron, TNF- α , and IL-6, which decreases erythropoietin release, which lowers haemoglobin concentration and red blood cell count [16]. In addition to these findings, malignancy-associated erythrocytic alterations further highlight the role of angiogenesis as a critical pathological parameter. Tumour-induced angiogenesis is often structurally and functionally abnormal, leading to inefficient oxygen delivery despite increased vascular density. This results in tumour hypoxia, which promotes erythrocyte deformability, reduced lifespan, and impaired erythropoiesis. Hypoxia-inducible factors (HIFs) stimulate the release of pro-angiogenic mediators such as vascular endothelial growth factor (VEGF), perpetuating a cycle of defective neovascularisation and anaemia. Consequently, these erythrocytic changes not only reflect disease progression but also underscore angiogenesis as a key determinant influencing red blood cell dynamics in malignant conditions [17].

The decreased platelet count in malignant tumours could be the consequence of direct damage to the bone marrow, which would reduce its capacity to make thrombocytes [18]. Immunomediated thrombocytopenia is the other cause of thrombocytopenia. It arises when the body produces antiplatelet antibodies for no apparent reason. Thrombocytopenia encourages surface bleeding, which causes superficial blood loss and ultimately correlates with anaemia in tumour conditions [19].

4.2 Cytological examination

The predominance of grade II cytological smears (65%) and the high proportion of malignant tumours (80%) observed in this study are consistent with previous reports [20,21]. Cytological grading effectively differentiated benign (grade I) from malignant (grade II and III) mammary tumours [22].

4.3 Histopathological Examination

Histopathologically, fibroadenoma was the most common benign tumour, whereas carcinoma mixed type was the most common malignant tumour. The predominance of grade II tumours indicates that intermediate-grade malignancies constitute the major proportion of canine mammary neoplasms in the present study population.

Table 1: Clinical history of cases showing occurrence of canine mammary tumour

Breed wise occurrence of canine mammary tumour				
Breed		Number Of Cases		
German Shepherd		10		
Non-descript		7		
Rottweiler		5		
Labrador Retriever		4		
Pomeranian		4		
Indian Spitz		4		
Great Dane		1		
Beagle		1		
Total		36		
Occurrence of tumour on the basis of site of distribution				
Glands	Left	Right	Total	Percentage (%)
Cranial thoracic	1	2	3	5.89
Caudal thoracic	2	3	5	9.80
Cranial abdominal	4	2	6	11.76
Caudal abdominal	6	7	13	25.49
Inguinal	14	10	24	47.06
Spaying Status of animal				
Spayed Animal		11%		
Non-Spayed Animal		89%		

Table 2: Representation of haematological profiles of Malignant, Benign and healthy control of canine mammary gland tumour condition (n=36)

Blood parameters	Malignant		Benign		Control	
	Mean	Range	Mean	Range	Mean	Range
WBC ($\times 10^3/\mu\text{L}$)	18.98 ± 1.85	41.4 - 8.2	10.74 ± 1.65	15.7 - 6.36	10.29 ± 0.31	13.1 - 7.9
Neutrophil (%)	81.32 ± 1.53	89.8 - 64.4	64.73 ± 1.63	70.1 - 61.1	55.43 ± 0.92	62.3 - 48.5
Lymphocyte (%)	15.07 ± 1.28	25.4 - 7.7	29.85 ± 2.88	38.8 - 21.4	37.57 ± 0.94	42.7 - 30.9
Eosinophil (%)	0.60 ± 0.16	2.9 - 0.0	0.28 ± 0.12	1.7 - 0.0	0.11 ± 0.03	0.6 - 0.0
Monocyte (%)	3.01 ± 0.30	7.3 - 1.5	5.14 ± 0.27	6.1 - 4.2	6.89 ± 0.12	7.5 - 5.6
RBC ($\times 10^6/\mu\text{L}$)	5.52 ± 0.36	8.3 - 1.1	5.05 ± 0.44	6.5 - 3.3	7.12 ± 0.15	8.1 - 5.9
Hb (g/dl)	12.24 ± 0.74	18.7 - 2.7	11.83 ± 1.13	16.2 - 7.7	17.23 ± 0.31	19.3 - 14.1
Platelet ($\times 10^3/\mu\text{L}$)	341.3 ± 17.57	487 - 200	265.4 ± 61.59	426 - 1.3	302.3 ± 25.13	408 - 72

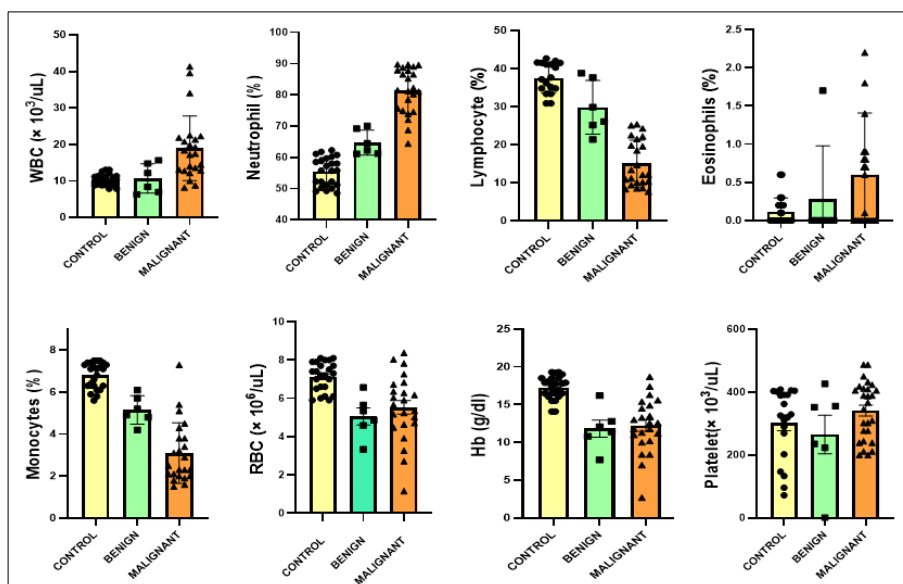


Fig 1: Graphical representation of different haematological parameters showing comparison between control, Malignant and Benign cases

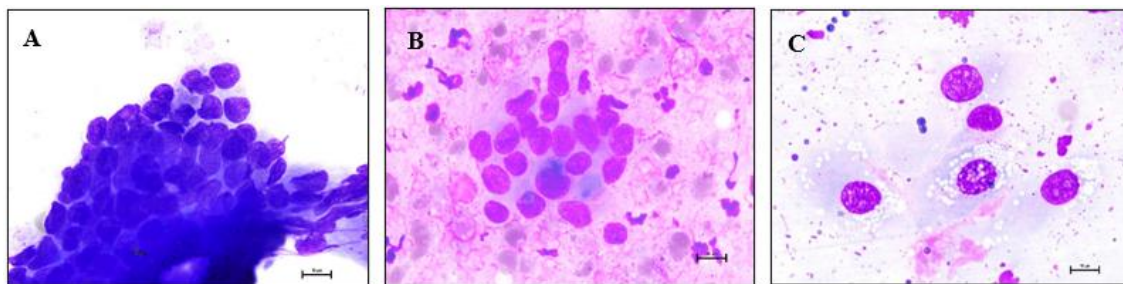


Fig 2: Cytological observation of different mammary gland tumours in dogs

- **Panel A:** Cytology showing uniform population of cells arranged in closely packed clusters. The nucleus has occupied the complete cell and cytoplasm is very scant. Nucleus is having smooth margins with clumped chromatin and indistinct nucleolus. Grade I category. Giemsa stain, 1000X;
- **Panel B:** Cytology showing cells mixed population of cells arranged in loose clusters as well as singly. Nucleus having irregular margins with noticeable nucleolus and granular chromatin. Grade II category. Giemsa stain, 1000X;
- **Panel C:** Cytology showing enlarged cell with vacuolated cytoplasm arranged singly. The size of cells is >5 times of RBC. The nucleus is hyperchromatic with slightly irregular margins and having clumped chromatin and noticeable nucleoli. Grade III category. Giemsa stain, 1000X)

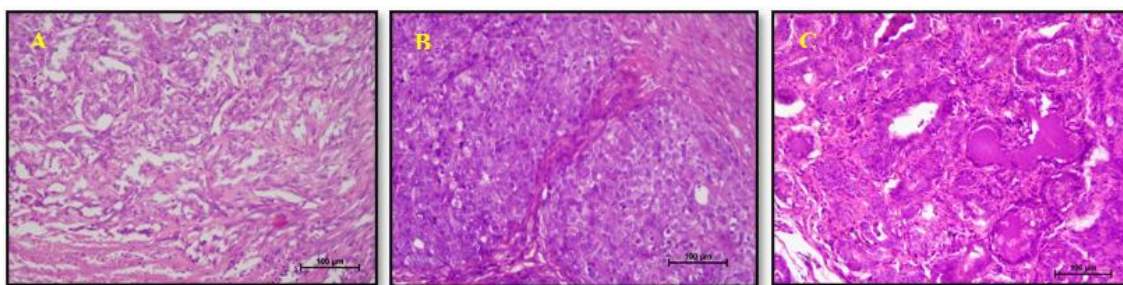


Fig 3: Histopathological observation of different mammary gland tumours in dogs

- **Panel A:** Fibrosarcoma, There is proliferation of fusiform cells with a distinctly interwoven pattern. Neoplastic cells have indistinct cell borders, a small amount of eosinophilic fibrillar cytoplasm, and oval to elongate nuclei that contain finely stippled chromatin and variably distinct nucleoli, 200X. HE.
- **Panel B:** Carcinoma In-Situ, Well demarcated nodules not extended up to the basement membrane that are densely cellular having closely packed cells arranged in irregular tubules, 200X. HE.
- **Panel C:** Intraductal Papillary Carcinoma, Presence of stratified population of epithelial cells. Fibrous connective tissue and myoepithelial cells are present as supporting stroma. papillary, arborescent growth pattern 200X. HE.

5. Conclusion

Early detection of tumours in dogs can greatly enhance survival chances. In this study, a strong correlation was found between the cytological and haematological parameters, as significant variation was noted in the haematological parameters in malignant cases according to cytological examination. This suggests that the haematological parameters alone may not be an accurate enough preliminary tool for identification of neoplastic condition, as research data regarding these tests is limited in canine. Therefore, cytological, histopathological, and molecular tests should be conducted to reach the appropriate diagnosis. The haematological analysis can be used as a routine examination as it is a non-invasive diagnostic tool and will help in the early

diagnosis of tumour conditions which can help in further treatment and prognosis of patients.

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