RESEARCH ARTICLE
JIVA 23 (3) December 2025
https://doi.org/10.55296/JIVA/23.3.2025.55-67

Received on 10.10.2025
Accepted on 12.12.2025

EPIDEMIOLOGICAL DETERMINANTS AND HISTOPATHOLOGICAL SPECTRUM OF CANINE MAMMARY TUMOURS: A STUDY FROM CENTRAL KERALA

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ABSTRACT

Canine mammary tumours (CMTs) are the most common neoplasms in intact female dogs and are of considerable interest in veterinary oncology due to their clinical significance and translational relevance to human breast cancer. This study investigated the epidemiologicaldeterminants and histopathological spectrum of CMTs in central Kerala, to identify risk factors and characterise tumour biology in a regional context. Fifty cases (48 biopsies and 2 necropsies) comprising 66 tumour masses were collected from the University Veterinary Hospitals at Mannuthy and Kokkalai and the Department of Veterinary Pathology, Mannuthy, between May 2024 and August 2025. Clinical variables including age, breed, sex, reproductive status, diet, body condition and glandular involvement were analysed. Histopathological classification and grading were performed on haematoxylin and eosin–stained sections following Goldschmidt *et al*. (2011) and Elston and Ellis (1991) criteria. The mean age of affected dogs was 8.94 ± 0.41 years, with peak incidence between 10 and 12 years. Labradors (36%), crossbreeds (22%) and German Shepherds (14%) were most affected. Females accounted for 96% of cases, with nulliparous animals forming the majority (56%). The inguinal glands were most frequently involved (58%), followed by caudal abdominal glands. All tumours were malignant, with carcinosarcoma (15.1%), carcinoma mixed type (10.6%) and solid carcinoma (10.6%) predominating. Histological grading revealed Grade II tumours as most common (56.1%), followed by Grade III (27.3%) and Grade I (16.7%). The predominance of aggressive histological subtypes and higher-grade tumours highlights the malignant profile of CMTs in this region and underscores their value as a comparative model for human breast cancer research.

Keywords: Canine mammary tumours, epidemiology, histopathology, carcinosarcoma, risk factors

INTRODUCTION

Canine mammary tumours (CMTs) are the most common neoplasms in female dogs, accounting for nearly half of all tumours, with about 50% being malignant and prone to recurrence and metastasis, particularly to the lungs (Brody *et al*.). Their global relevance is underscored by parallels with human breast cancer, with dogs serving as sentinel species in cancer epidemiology (Ferlay *et al*., 2024). The burden of CMTs has been widely documented, with Indian studies reporting variable incidence across regions, ranging from 13.3% in Himachal Pradesh to 39.9% in Gujarat (Dhami *et al*., 2010; Umeshwori *et al*., 2011; Sharma *et al*., 2018), while reports from Europe confirm their universal significance (Carvalho *et al*., 2023).

Epidemiological determinants include age, with risk rising after 5 years and peaking at 9–12 years (Cohen *et al*., 1974; Gunnes *et al*., 2017), and breed predisposition, notably in Poodles, Dachshunds, Spaniels, Boxers, Spitz, German Shepherds and Labradors (Zatloukal *et al*., 2005; Edmunds *et al*., 2023). Females are overwhelmingly predisposed, with risk strongly influenced by reproductive hormones; early spaying markedly reduces susceptibility, whereas delayed ovariohysterectomy, nulliparity and pseudopregnancy increase risk (Misdorp *et al*., 2002; Kwon *et al*., 2023). Lifestyle and environmental exposures, including obesity, high-fat diets and contact with pollutants, further contribute to tumour development (Owada *et al*., 2024).

Histopathologically, CMTs range from benign adenomas to aggressive carcinomas and carcinosarcomas, with malignant forms comprising 60–90% of cases (Rekha, 2007; Devi *et al*., 2022). Common subtypes include simple, complex, papillary, ductal, solid and mixed carcinomas, with carcinosarcomas reported more frequently in Asia (Tunc and Vural, 2024). Grading based on tubule formation, pleomorphism and mitotic index provides robust prognostic value, with higher grades correlating with poor outcomes (Elston and Ellis, 1991; Clemente *et al*., 2010; Sajeev *et al*., 2023). Given their multifactorial aetiology, biological aggressiveness and comparative oncology value, CMTs warrant focused investigation. However, consolidated data from southern India remain limited; hence, the present study evaluated epidemiological risk factors and histopathological diversity of CMTs with emphasis on their occurrence, biological behaviour and translational significance.

MATERIALS AND METHODS

Fifty cases of canine mammary tumours were analysed, comprising 48 excisional biopsy samples and two necropsy samples. Clinical data including age, sex, breed, colour, diet, reproductive status, affected gland, and prior history were recorded. Gross examination involved palpation of all mammary glands, measurement of tumour length and width using a digital vernier caliper, and calculation of tumour volume using the modified ellipsoidal formula $V = ½(W^2 \times L)$ (Faustino-Rocha *et al*., 2013). Each mass in multiple growth cases was measured separately. Tumours were further assessed for shape, consistency, cut surface colour, and gross alterations such as necrosis, ulceration, and cystic changes.

Tissues were fixed in 10% neutral buffered formalin, processed by paraffin

Fig. 1. Biometry using digital Vernier caliper

Fig. 2. Tumour mass with cystic space inside

Fig. 3. Tumour mass with necrosis of cut surface

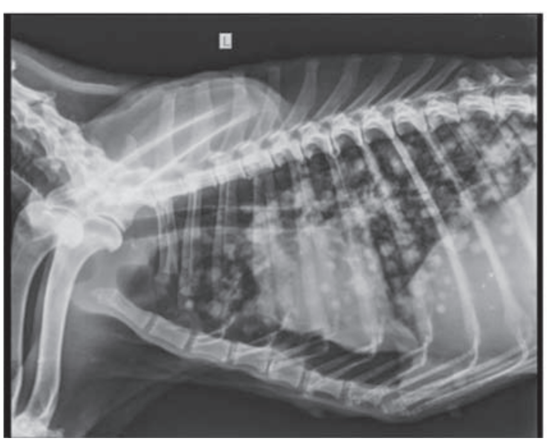

Fig. 4. Thoracic radiograph with numerous nodular metastatic foci

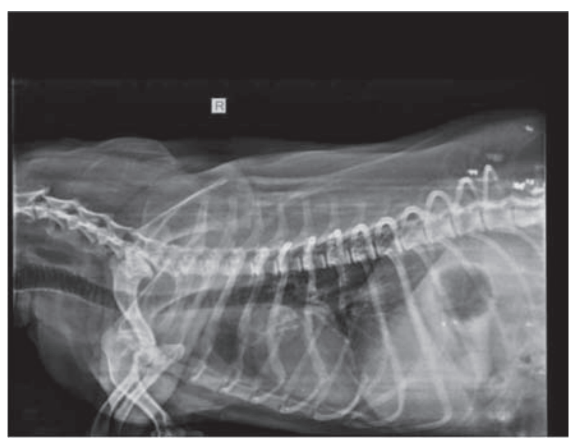

Fig.5. Thoracic radiograph with miliary metastatic foci

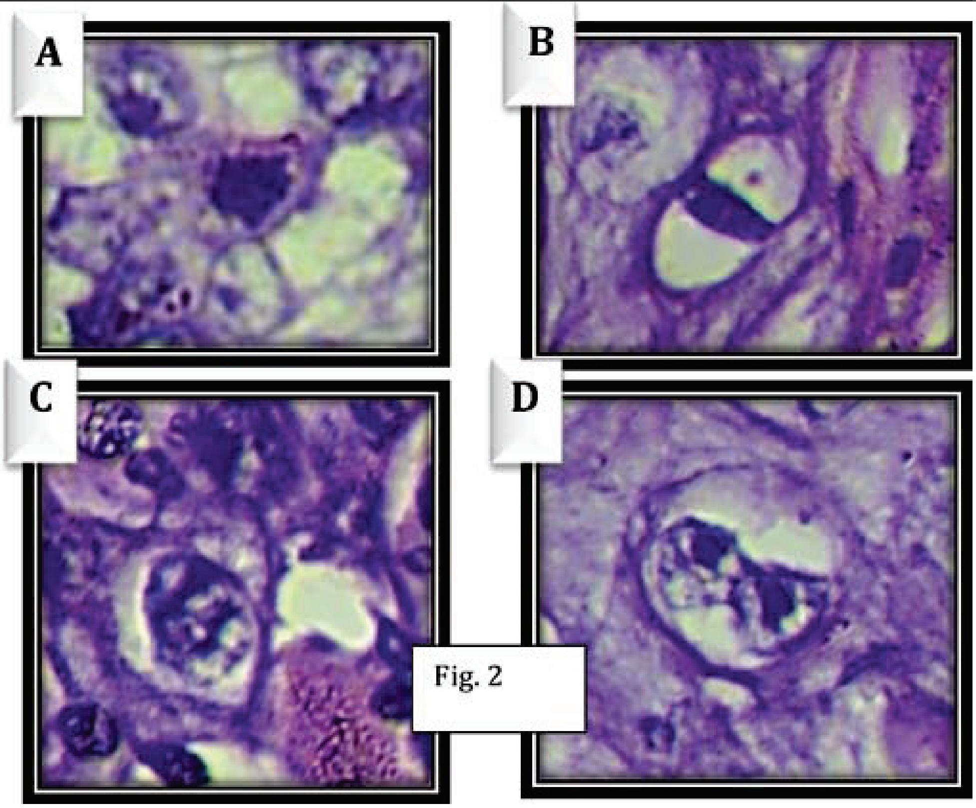

Fig. 6. Various stages of mitosis- prophase, metaphase, anaphase and telophase (H&E x 1000)

embedding, sectioned at 3–5 µm, and stained with haematoxylin and eosin (Suvarna *et al*., 2019). Microscopic evaluation was performed under a bright-field microscope (Olympus CX33). Only malignant tumours were classified, following the histological scheme of Goldschmidt *et al*. (2011). Histological malignancy grading (HMG) of carcinomas was done using the Nottingham system of Elston and Ellis as adapted for dogs by Clemente *et al*. (2010), based on tubule formation, nuclear pleomorphism, and mitotic count. Mammary sarcomas were graded using the French Federation of Cancer Centers Sarcoma Group (FNCLCC) system, as adapted by Augsburger *et al*. (2017), considering differentiation, mitotic index, and necrosis.

RESULTS AND DISCUSSION

Occurrence and Epidemiological Risk Factors

The mean age of occurrence of CMTs was 8.94 ± 0.41 years, ranging from 1 to 16.5 years. The highest incidence occurred in dogs aged 10–12 years (38%), followed by 7–9 years (28%), 4–6 years (18%), 0–3 years (6%), 13–15 years (6%) and ≥16 years (4%). These findings confirm the well-established age predisposition of CMTs, typically affecting

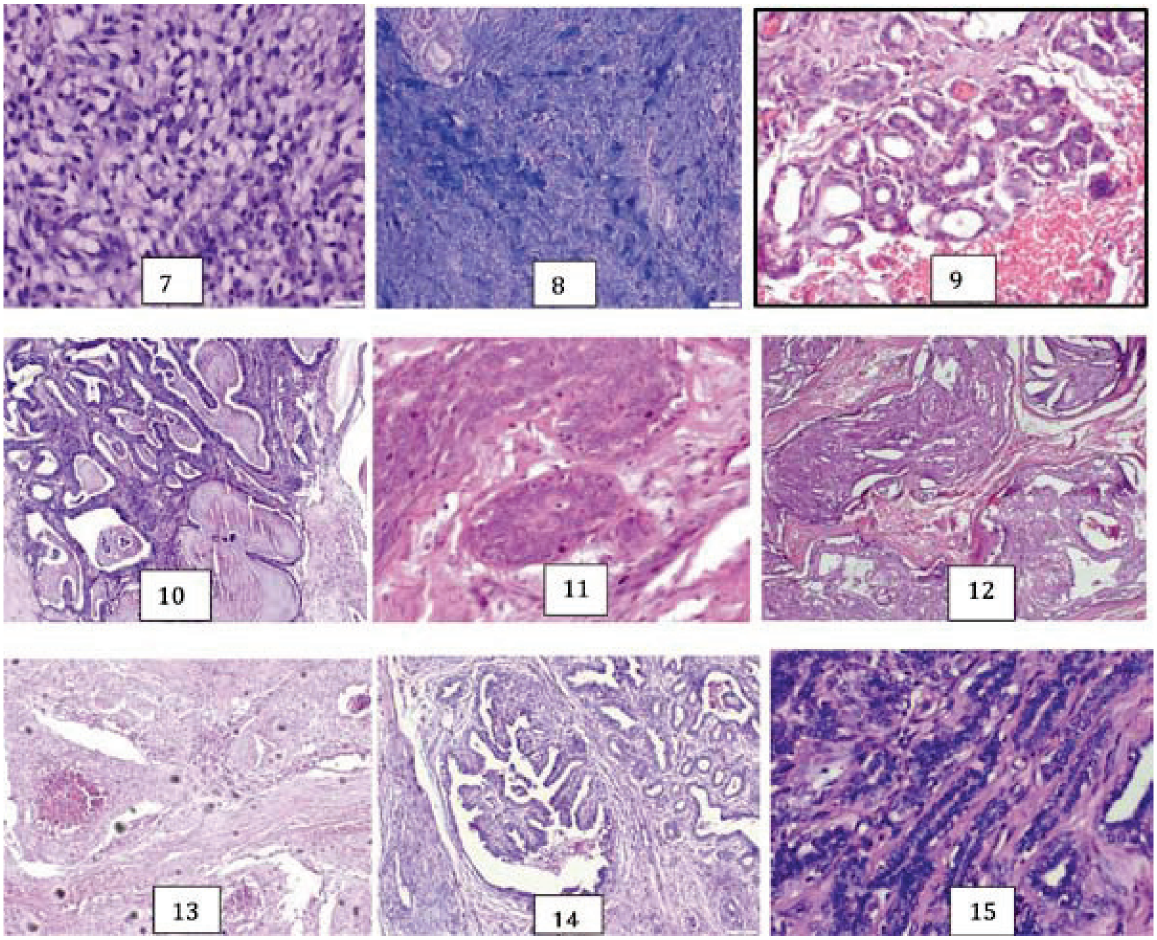

Fig.7 Fibrosarcoma - Malignant proliferation of fibrous connective tissue in interwoven pattern (H&E x 400)
Fig.8. Fibrosarcoma – fibrous tissue confirmed by Masson's trichrome staining (masson's trichrome x 400)
Fig.9. Ductal carcinoma - Bilayered cords of cuboidal cells with sparse cytoplasm and hyperchromatic nuclei (H&E x 400)
Fig.10. Cystic tubular carcinoma - Tubular structures within cystic spaces, lined by pleomorphic epithelial cells (H&E x 400)
Fig.11. Solid carcinoma- Pleomorphic solid epithelial sheets of cells arranged without lumen (H&E x 400);
Fig.12. Cribriform carcinoma carcinoma -Sieve-like arrangement of neoplastic epithelial cells forming multiple small lumina surrounded by cellular bridges (H&E x 400)
Fig.13. Comedocarcinoma - presence of necrotic areas within the center of neoplastic cell aggregates ((H&E x 100);
Fig.14. Intraductal papillary carcinoma- Papillae bordered with columnar cells having mild cytoplasm and open-faced nuclei (H&E x 400)
Fig.15. Tubular carcinoma - Irregular, infiltrative tubules lined by atypical cuboidal to columnar epithelial cells with vesicular nuclei (H&E x 400);

middle-aged to older dogs (Gunnes *et al*., 2017; Sharma *et al*., 2018), with increasing incidence attributable to cumulative genetic mutations, prolonged ovarian hormonal exposure during multiple estrus cycles, and declining DNA repair capacity. Breed distribution revealed that Labrador Retrievers were most frequently affected

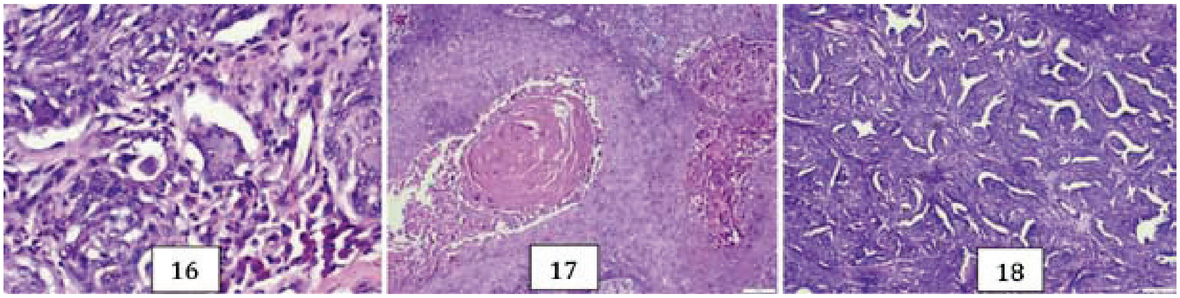

Fig.16. Anaplastic carcinoma- Pleomorphic and large or ballooned neoplastic cells (H&E x 400)
Fig.17. Adenosquamous carcinomaAreas of carcinoma admixed with neoplastic cells exhibiting squamous differentiation (H&E x 100)
Fig.18. Tubulopapillary carcinoma - neoplastic cells arranged in a sessile or pedunculated papillary fashion (H&E x 100)

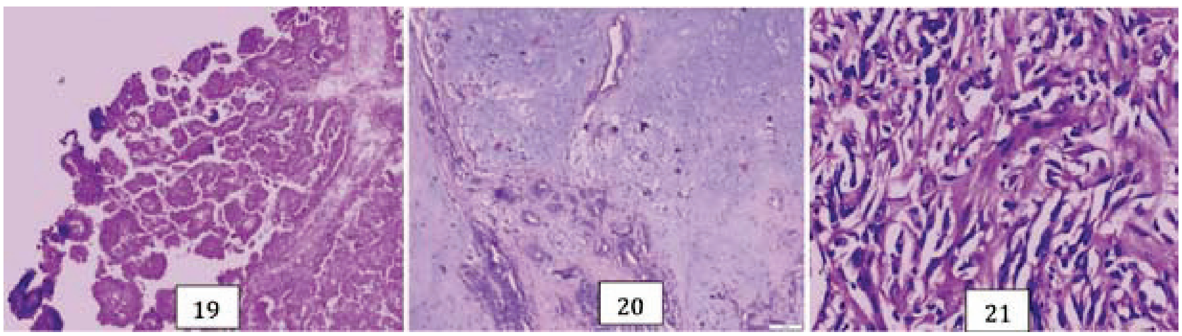

Fig.19. Micro papillary carcinoma - small intraluminal irregular aggregates & papillae without a supporting fibrovascular stalk & are surrounded by empty lacunar spaces (H&E x 400)
Fig.20. Carcinosarcoma - Malignant basophilic chondroid matrix and an uneven arrangement of neoplastic epithelial cells (H&E x 100)
Fig.21. Spindle cell carcinoma -Composed of elongated spindle-shaped neoplastic cells arranged in fascicles, resembling sarcomas (H&E x 400)

(36%), followed by crossbreeds (18%), German Shepherds (12%), Dachshunds (10%), non-descript dogs (8%), Rottweilers (6%), Pomeranians (4%), Siberian Huskies (2%), Boxers (2%), and Minipoms (2%). This pattern is consistent with previous reports highlighting high susceptibility in Labradors, German Shepherds, and Spaniel breeds (Zatloukal *et al*., 2005; Edmunds *et al*., 2023). Breed predisposition is likely shaped by hereditary oncogenic risk factors, polymorphisms affecting steroid receptors, and structural differences in mammary glands, in addition to breed popularity in the study area.

Sex distribution showed overwhelming female predominance (96%), with only 4% of cases in males. The rarity of male mammary tumours, consistent with earlier reports (Nair *et al*., 2021), is attributable to limited mammary tissue development and absence of cyclical hormonal stimulation. In intact bitches, repeated estrogen and progesterone stimulation during estrous cycles promotes epithelial proliferation and tumour initiation (John *et al*., 2022). Parity analysis indicated that nulliparous females constituted 56% of cases, while whelped dogs accounted for 30%, with 22% in parity group 1–3 and 8% in parity group 4–6. Five cases were spayed. These findings align with evidence that intact nulliparous bitches are at highest risk, whereas early spaying significantly reduces CMT incidence by eliminating cyclical hormonal stimulation (John *et al*., 2022; Kwon *et al*., 2023).

Nutritional and body condition assessment revealed that 86% of affected animals were maintained on home-prepared diets, 10% on mixed diets, and only 4% on commercial foods. Obesity was identified in 44% of affected dogs, while 30% were of normal weight and 26% were emaciated. High-fat homemade diets rich in red meat likely predispose to obesity and neoplasia, as obesity promotes tumourigenesis via increased aromatase activity, elevated estrogen levels, and chronic low-grade inflammation (Alenza, 1995; Lim *et al*., 2015; Nicchio *et al*., 2020).Environmental and management-related risk factors were also prominent. Nearly half of the affected dogs were reared outdoors (46%), followed by indoor (38%) and free-roaming (16%). The majority originated from urban environments (85%), with occasional exposure to pesticides, insecticides, plastic burning, or UV radiation. Environmental pollutants such as pyrethroids and persistent organic pollutants have been associated with mammary carcinogenesis (Andrade *et al*., 2010; Carvalho *et al*., 2023; Owada *et al*., 2024).

Clinical Characteristics

Gross examination of 66 affected glands revealed that inguinal glands were most frequently involved (43.9%), followed by caudal abdominal (18.2%), cranial abdominal (16.7%), caudal thoracic (12.1%) and cranial thoracic (9.1%) glands, with left-sided involvement being more common. Single gland involvement predominated (72%), while multiple glands were affected in 28% of cases. Single tumours likely represent independent neoplastic events, whereas multiple gland involvement suggests multicentric origin or intramammary metastasis, often indicating advanced disease requiring radical surgical management (Dileepkumar *et al*., 2014; Nadhiya *et al*., 2020). Tumour size varied widely, with an average volume of 349.23 ± 59.01 cm^3. The smallest lesion measured 2.89 cm^3, while the largest reached 2601.11 cm^3, suggesting late detection in most cases. Two distinct growth patterns were observed: invasive in 24% of cases and expansive in 76%, with invasive growth indicating aggressive biological potential.

All 50 dogs included in the study harboured malignant tumours, confirming the predominance of aggressive tumour types in the population. Recurrence was uncommon (6%), suggesting either adequate surgical margin resection or predominance of de novo tumour formation. Pulmonary metastases were detected radiographically in 26% of cases, appearing as multiple nodular opacities across lung fields, sometimes coalescing into larger masses (Fig. 4 &5). This highlights the aggressive nature of CMTs and the need for routine thoracic imaging, consistent with their recognised translational relevance to human breast cancer (Oliveira-Lopes *et al*., 2024).

Concurrent disease conditions were common, with haemoprotozoan infections being most prevalent (66%), followed by skin disorders (42%), respiratory difficulties (22%) and cardiac conditions (18%). These findings mirror earlier reports linking CMTs with systemic immune suppression characterised by increased Tregs, myeloid-derived suppressor cells, lymphocytopenia, neutrophilia, and altered CD4+/CD8+ ratios (Cimerman *et al*., 2024). Haemoprotozoan infections such as trypanosomosis, dirofilariasis, and babesiosis may exacerbate immunosuppression and predispose to secondary conditions, including opportunistic skin diseases.

Gross and Histopathological Features

Grossly, most tumours were ulcerated (71.2%), irregular (50%), and firmly attached (66.7%), with firm (48.5%) to hard (39.4%) consistency. On cut section, a reddish-white appearance was most common (48.5%), frequently accompanied by necrosis (54.5%), cystic changes (27.3%), and calcification (18.2%). These findings reflect rapid tumour growth, vascular insufficiency, and degenerative changes (Fig. 1 to 3).

Histopathological evaluation of 66 tumours confirmed all as malignant, exhibiting wide morphological heterogeneity. Carcinosarcoma was the most frequent subtype (15.1%), followed by carcinoma mixed type (10.6%) and solid carcinoma (10.6%). Intraductal papillary carcinoma, ductal carcinoma, and cystic papillary carcinoma each accounted for 7.6%, while adenosquamous carcinoma and intraductal tubulopapillary carcinoma were each 6.1%. Comedocarcinoma and malignant myoepithelioma (4.5% each) and less frequent subtypes including spindle cell, anaplastic, tubular carcinoma (3.0% each), and rare variants such as carcinoma in situ, cribriform, micropapillary, cystic tubular, complex carcinoma, fibrosarcoma, and cystic tubulopapillary carcinoma (1.5% each) were also documented (Fig. 7 to 21).

Grading revealed that most tumours were moderately differentiated (Grade II, 56.1%), followed by poorly differentiated (Grade III, 27.3%) and well-differentiated lesions (Grade I, 16.7%). Carcinosarcoma cases were predominantly Grade II, while solid carcinomas were mostly Grade III, reflecting their aggressive behaviour. Carcinoma mixed type and cystic papillary carcinoma were largely Grade II, while ductal carcinoma tended to be Grade I, indicating better differentiation. Adenosquamous carcinoma occurred in both Grade I and III forms, while papillary variants showed distribution between Grades I and II. Highly aggressive variants such as comedocarcinoma, anaplastic carcinoma, micropapillary carcinoma, and cribriform carcinoma were generally associated with higher grades (Canadas *et al*., 2019; Sajeev *et al*., 2023).

Association of Histological Grade with Clinical Variables

Correlation analysis revealed that tumour grade was not significantly associated with number of glands affected, tumour volume, age, or parity. However, mode of growth showed a significant correlation ($p < 0.05$), with invasive growth patterns more frequently associated with higher-grade tumours. This underscores the prognostic value of growth mode as an indicator of tumour aggressiveness.

SUMMARY

This study highlights the epidemiological, clinical, and pathological features of CMTs in central Kerala, demonstrating age, breed, parity, obesity, and environmental exposures as key risk factors. Tumours were predominantly malignant, frequently large, and often ulcerated, with inguinal glands most affected. Histopathology revealed marked heterogeneity, with carcinosarcoma being the most common subtype, and most tumours were of intermediate grade. The strong correlation between invasive growth and higher histological grade reinforces its importance in prognostication.

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