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N-cadherin expression in canine mammary tumours

A.K. Anseena[1], K. Krithiga[2], I.S. Sajitha[3], S.S. Devi[4], K.S. Prasanna[5], S. Anoop[6] and Nisna Niyas[1]
Department of Veterinary Pathology
College of Veterinary and Animal Sciences, Mannuthy, Thrissur – 680651
Kerala Veterinary and Animal Sciences University
Wayanad, India

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Abstract

Canine mammary tumours (CMT) are the second most common cancer in dogs after skin tumours and are frequently found in older, intact female dogs. Epithelial- Mesenchymal Transition (EMT) is identified as a major driving force in tumour progression and metastasis. The current study was intended to assess the expression of N- cadherin to analyse the invasive and metastatic nature of CMT. A total of 25 CMT cases presented to the University Veterinary Hospitals at Mannuthy and Kokkalai during the period from June 2022 to June 2023 were considered for the present study. By immunohistochemistry (IHC) 48 per cent of cases showed EMT by expressing the cadherin switch. Lymph node metastasis was observed in 4 out of 25 cases during the study period. The expression of N-cadherin could be appreciated in almost all cases which showed lymph node metastasis except for a single CMT sample. The present study confirms the relationship between the expression of N-cadherin in CMT and the presence of regional metastasis and tumour grade. Therefore, the presence of N-cadherin protein in mammary carcinomas could be considered for prognostication.

Keywords : *Canine mammary tumour, epithelial mesenchymal transition, N-cadherin, immunohistochemistry*

Mammary neoplasms are common in intact aged female dogs. The canine mammary tumour (CMT) is a useful model for studying human breast cancer (HBC) as they share similar clinical and molecular features. Epithelial Mesenchymal Transition (EMT) has been identified as

1. *MVSc Scholars, Department of Veterinary Pathology, CVAS, Mannuthy*
2. *Assistant Professor, BRTC, Thonnakkal, Thiruvananathapuram*
3. *Associate Professor, Department of Veterinary Pathology, CVAS, Mannuthy*
4. *Assistant Professor, Department of Veterinary Pathology, CVAS, Mannuthy*
5. *Assistant Professor and Head (i/c), Department of Veterinary Pathology, CVAS, Mannuthy*
6. *Professor, Department of Veterinary Surgery and Radiology, CVAS, Mannuthy*

a major player in tumour progression and metastasis. During EMT, cells lose epithelial polarity and acquire a spindle-shaped, highly motile mesenchymal like phenotype (Jechlinger *et al*., 2003). Cadherins are calcium-dependent transmembrane glycoproteins responsible for cell-to-cell adhesion and maintenance of normal structure of a tissue (Matos *et al*., 2006). E-cadherin is the major transmembrane component of adherent junction in most of epithelial cells. It is primarily localised at lateral cell borders (Wheelock *et al*., 2001). Neuronal and muscle cells in human embryo and adult tissues express N-cadherin (Hatta and Takeichi, 1986). "Cadherin switching" in EMT refers to the downregulation of E-cadherin and the upregulation of N- cadherin (Nakajima *et al*., 2004). Differential expression of these proteins is one of the key features in the process of EMT. It has also been proposed that N-cadherin bestows cancer cells with increased invasive and metastatic ability, independent of E-cadherin (Nieman *et al*., 1999 and Hazan *et al*., 2004). Hence, the acquisition of N-cadherin can be considered critical in tumour progression and thus the prognosis in cancer patients. The HBC studies have widely used these markers, but similar studies are sparse in animals. Hence, the present study aims to evaluate the expression of N-cadherin in CMT using IHC. Better diagnostic, prognostic and therapeutic methods for the management of CMT would be made possible by the knowledge gained from this study.

Materials and methods

A total of 25 CMT cases presented to the University Veterinary Hospitals were considered for the present study. Clinical history of animals such as breed, age, sex, reproductive status and gross appearances of tumour mass such as colour, size, consistency, attachment and status of draining lymph nodes were recorded. Tissue samples collected from surgically excised tumours were fixed in 10 per cent neutral buffered formalin for histopathology and immunohistochemistry. After 48-72 hours of fixation, tissues were trimmed and processed as per standard paraffin embedding technique (Wolfe, 2019). Tissue sections of 5 µm thickness were prepared and stained with Haematoxylin and Eosin (Bancroft and Layton, 2019). Four enlarged lymph nodes were also subjected to histopathological examination. Tissue sections were examined under the light microscope and the various histologic subtypes were determined according to the standard classification system put forwarded by Goldschmidt *et al*. (2011). Histological malignancy grading (HMG) was done as described by Clemente *et al*. (2010) based on three parameters such as tubule formation, nuclear pleomorphism and mitotic count. Expression pattern of the protein N-cadherin in the formalin fixed paraffin embedded (FFPE) tissues was studied by immunohistochemistry (IHC) as per technique elaborated by Ramos-Vara (2005). The antibody used to detect the protein was N-cadherin monoclonal antibody (Catalog No. PA6203, Clone YN01186m) procured from Elabscience, Texas, United States of America and secondary antibody from Quartett Germany. N-cadherin antibody was diluted to a concentration of 1:200 with primary antibody dilution buffer. Negative control staining was carried out by substituting the primary antibodies with antibody dilution buffer. Normal canine uterine tissue was used as positive control. IHC scoring was done according to Vakkala *et al*. (1999) based on per cent of cells stained and colour intensity and categorised into weak, moderate and strong.

The age of the affected animals ranged from four to sixteen years. The highest incidence of mammary tumour was noticed in patients aged more than eight years. A similar finding was also reported by Gill *et al*. (1997). Incidence was more in Labrador breeds (28%) followed by Dachshund (16%), Spitz (16%), German Shepherd (12%), Rottweiler (12%), Nondescript (8%), Dalmatians (4%), Lhasa Apso (4%) and this observation concurred with the findings of Devi *et al*. (2022). Among the total of 25 CMT cases, 24 were found in female, while only one was observed in male. Patel *et al*. (2019) also observed that mammary tumours were rare in male dogs and common in female dogs. Most of the female animals affected were nulliparous and obese. It was found that proliferation of normal mammary epithelium occurring in each oestrus cycle made the bitches highly prone to tumorigenesis (Rutteman, 1990). All the

tumours observed were malignant. Mathew *et al*. (2019) had reported that 10 per cent of the CMT were benign while 90 per cent were found to be malignant. This might be attributed to the limited sample size under study and the delayed presentation of affected animal for diagnostic confirmation. Caudal abdominal glands were found to be the most affected (32%) followed by the inguinal glands (24%), caudal thoracic glands (16%), cranial abdominal gland (16%) and cranial thoracic glands (12%) in the present study was also observed by Patel *et al*. (2019). The caudal glands are most involved, which can be attributed to higher amount of glandular tissue to maintain secretory activity longer than other pairs of glands (Fidler *et al*., 1967). Multiple glands were affected in seven cases (28%). This was in accordance with Hemanth *et al*. (2015) who reported multiple mammary tumours, involving more than single mammary gland in 15.68 per cent of cases. The smallest tumour was 4 cm in size, while the largest one was 17 cm with mean size of 10.24±4.16 cm.

Grossly, CMTs varied in size, colour and consistency. Draining lymph nodes were enlarged in four cases. Majority of the tumours were pedunculated (88%), while eight per cent were sessile and four per cent were non-pedunculated. The surface of tumours was ulcerated in 28 per cent and non-ulcerated in 72 per cent cases. Patel *et al*. (2019) reported out of 26 cases, ulcers were present on the surface of six (23%) tumours. The consistency of tumour was firm in most of the cases (56%), some were hard (28%) and others were soft (16%). Cut surface of firm masses were greyish white (50%), yellowish white (21%) and reddish white (29%), while in the hard masses it appeared reddish white (43%) and greyish white (57%). The cut-surface of soft masses was reddish white (50%) and greyish white (50%). Mathew *et al*. (2019) reported that most of the tumours were firm in consistency and cut surface appeared greyish white.

The histological subtypes of CMTs were also examined. The tumour cases included in the study were analysed using histopathology and categorised into various histological subtypes (Table1). Among the tumours categorised, the most frequently observed was tubulopapillary carcinoma followed by mixed mammary tumour and carcinosarcoma. As per Cassali *et al*. (2014) mixed tumours were the most frequent neoplasms in mammary gland of dogs.

Table 1. Histological classification of CMT (n=25)

Histopathology	Frequency	Percentage
Anaplastic Carcinoma	2	8
Carcinoma in-situ	1	4
Carcinosarcoma	3	12
Comedocarcinoma	1	4
Mixed Mammary tumour	5	20
Solid Carcinoma	2	8
Squamous cell carcinoma	1	4
Tubulopapillary	10	40
Total	25	100

The CMT graded were in grade III (67%) and II (36%). Mathew *et al*. (2019) and Devi *et al*. (2022) also reported an increased occurrence of grade II mammary tumours in the dogs of Thrissur district. Regional lymph node metastases were identified in four dogs, two of which had grade III mammary neoplasms (one each of comedocarcinoma and carcinosarcoma) and two were in grade II (one each of solid carcinoma and tubulopapillary). Raval *et al*. (2019) reported that two of the six examined lymph nodes showed metastases and were histologically mixed and solid carcinomas.

Immunostaining of N-cadherin with total score was depicted in Table 2. Membraneous expression of N-cadherin was observed in IHC. Buendia *et al*. (2014) reported that presence of more than five per cent of the mammary neoplastic epithelial cells exhibiting cytoplasmic and/ or membranous labelling of N-cadherin was considered to be positive for expression of the marker. When all CMT tissue samples (25 cases) were subjected to IHC, 48 per cent (12/ 25) of cases showed EMT by expressing the cadherin switch. Grade III carcinosarcomas and complex carcinomas typically showed strong immunopositivity for the protein, whereas grade II and III tumours showed either strong or moderate immunostaining. However, grade III tumours were found to

have strong expression of the protein when compared to grade II tumours. (Fig 1- 5). Similar findings were also reported by Buendia *et al*. (2014) that grade I carcinomas expressed N-cadherin at a lower level (8.1%) than grade II (30.3%) or grade III (25%) carcinomas. Lymph node metastasis was observed in 16 per cent cases within the study period. The expression of N-cadherin could be appreciated in almost all cases of lymph node metastasis except in a single CMT. Buendia *et al*. (2014) reported that at the time of diagnosis, metastases were present in the regional lymph nodes in 84 per cent of the N-cadherin positive tumours.

The results from this study showed a greater number of cases with N-cadherin expression than earlier report, which could be due to variations in the pathological presentation of the tumours studied by different researchers. In the present study, it was noticed that N-cadherin expression could be appreciated in three out of four cases having micrometastasis in regional lymph nodes. Similar findings by Di Domenico *et al*. (2011) who identified that N-cadherin has a key role in oral carcinogenesis, add strength to the findings of this study.

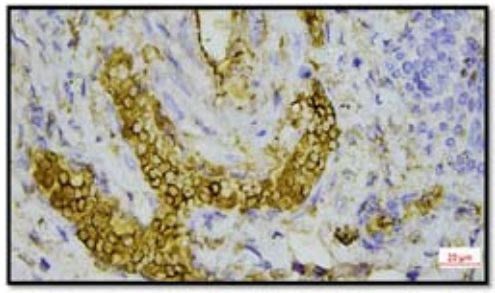

Fig.1. Uterus, dog – N-cadherin positive control - very strong immunostaining (IHC, x400)

Table 2. Immunohistochemistry of N cadherin in CMT (n=25)

Sl. No.	Tumour type	Immunohistochemistry N cadherin					Lymph node metastasis
		Grade	Intensity	% of cells	Total Score	Result	
1	Mixed Mammary tumour	2	-	0	-	Negative	Absent
2	Tubulopapillary	3	Weak	<25%	+ weak	Positive	Absent
3	Anaplastic Carcinoma	3	Moderate	25-50%	++ moderate	Positive	Absent
4	Solid Carcinoma	3	-	0	-	Negative	Absent
5	Carcinoma in situ	2	-	0	-	Negative	Absent
6	Tubulopapillary	2	Moderate	25-50%	++ moderate	Positive	Absent
7	Tubulopapillary	3	-	0	-	Negative	Absent
8	Carcinosarcoma	3	Strong	50-75%	+++ strong	Positive	Present
9	Mixed Mammary tumour	3	Strong	50-75%	+++ strong	Positive	Absent
10	Tubulopapillary	2	-	0	-	Negative	Absent
11	Carcinosarcoma	2	Moderate	25-50%	++ moderate	Positive	Absent
12	Mixed Mammary tumour	3	-	0	-	Negative	Absent
13	Tubulopapillary	2	-	0	-	Negative	Absent
14	Carcinosarcoma	3	-	0	-	Negative	Absent
15	Comedocarcinoma	3	-	0	-	Negative	Present
16	Tubulopapillary	3	Weak	<25%	+ weak	Positive	Absent
17	Tubulopapillary	2	Moderate	25-50%	++ moderate	Positive	Present
18	Tubulopapillary	3	-	0	-	Negative	Absent
19	Solid Carcinoma	2	Weak	<25%	+ weak	Positive	Present
20	Mixed Mammary tumour	3	Weak	25-50%	++ moderate	Positive	Absent
21	Tubulopapillary	3	-	0	-	Negative	Absent
22	Tubulopapillary	3	Moderate	25-50%	++ moderate	Positive	Absent
23	Squamous cell carcinoma	3	-	0	-	Negative	Absent
24	Anaplastic Carcinoma	2	-	0	-	Negative	Absent
25	Mixed Mammary tumour	3	Moderate	<25%	++ moderate	Positive	Absent

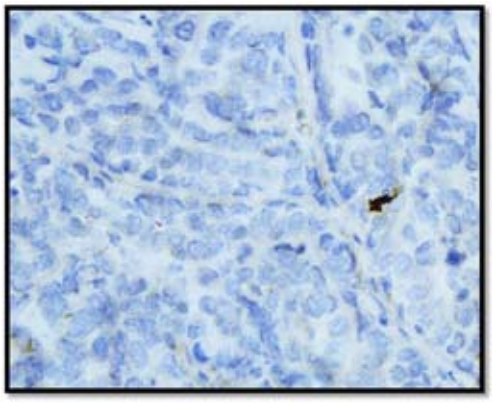

Fig.2. CMT - negative immunostaining of N-cadherin (IHC, x400)

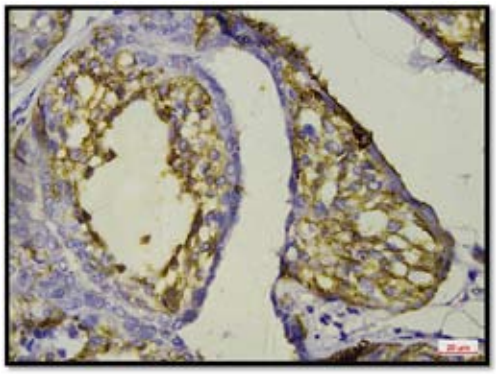

Fig.4. Mixed type carcinoma – strong immunostaining of N-cadherin (IHC, x 400)

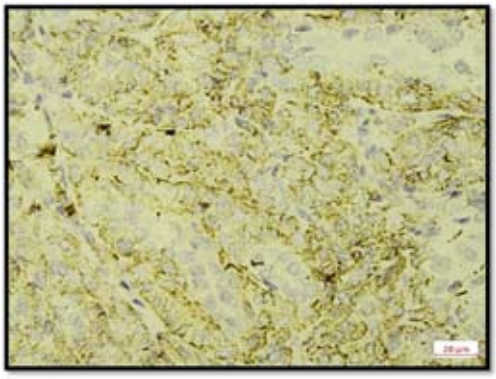

Fig.3. Tubulopapillary carcinoma – moderate immunostaining of N-cadherin (IHC, x400)

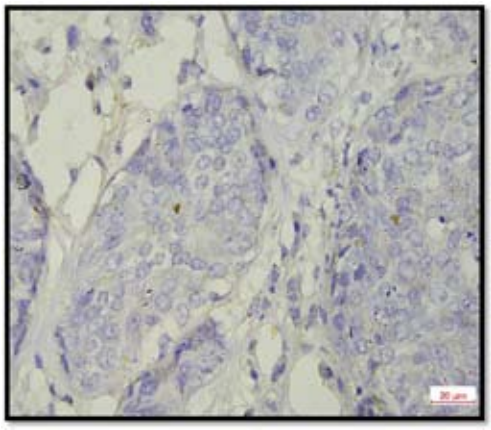

Fig.5. Mixed type carcinoma – weak immunostaining of N-cadherin (IHC, x 400)

Summary

The present study confirmed the relationship between the immunohistochemical expression of N-cadherin and prognostic factors such as the presence of regional metastasis and advanced malignancy in CMT. A local recurrence or distant metastasis could not be recorded in this study owing to the limited sample size and patients dying of advanced age or other health complications. However, the significance of this marker could not be overlooked considering its presence in most of the cases with lymph node metastasis, thereby announcing a possible poor prognosis. Further studies should focus on employing larger cohorts, genetic factors, development of therapeutic targets and personalized medicine.

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Conflict of interest

The authors declare that they have no conflict of interest

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