



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(5): 276-277

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Received: 09-07-2019

Accepted: 13-08-2019

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## Mutation in the BRCA2 gene and change of tissue architecture in canine mammary tumour

**D Rajkhowa, DJ Kalita, DP Saikia and T Upadhya**

**Abstract**

Blood samples were collected with anticoagulant from 20 female dogs brought for treatment of mammary tumour in College of Veterinary Science, AAU, Khanapara. DNA was isolated from blood and amplified BRCA2 (Exon 27) showing the mutation of adenine to cytosine was confirmed as cancer positive samples. Histopathological sections prepared from tumour positive mammary tissues revealed the cartilaginous structures around mammary ducts, ductules filled with fat globules, marked hypertrophy and presence of inflammatory cells in the endothelial lining of blood vessels, hypertrophy and hyperplasia of epithelial lining of mammary alveoli. From the present study it can be concluded that a massive changes of tissue architecture is occur in canine mammary tumour.

**Keywords:** Canine, Mammary Tumour, Histopathology, fat globules, hypertrophy, hyperplasia and mammary alveoli

**Introduction**

Molecular genetic techniques providing information about the specific and subtle genetic changes have been quite useful in the identification of tumours (Sidransky, 2002; Weissleder and Ntziachristos, 2003; Vogelstein and Kinzler, 2004) <sup>[6, 8, 7]</sup>. However, change of tissue architecture cannot be determined until histopathology of the concerned tissue is performed. The mammary tumour exhibit a complex histological pattern because they comprise elements from the epithelium and the mesenchyme and have the capacity to undergo malignant transformation (Geovanni., *et al.* 2012) <sup>[2]</sup>. Considering the facts, tumour positive mammary tissues was used in the present study for histopathological study to record the change of tissue architecture.

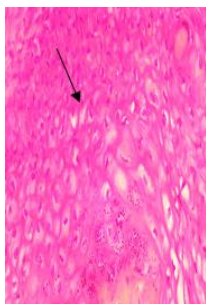
**Material and Methods**

Blood samples were collected in vacutainer tube with anticoagulant. DNA was isolated from blood following Sambrook and Russell (2001) <sup>[5]</sup>. A total of 20 female dogs (9 Labrador, 7 German spits and 4 German shepherd) above 5 years of age brought for treatment in the Directorate of clinics as well as in the Department of Veterinary Surgery, College of Veterinary Science, AAU, Khanapara were selected for the study. *BRCA2* (exon 27) genes were amplified from the extracted DNA using specific primers (Enginler *et al.* 2014) <sup>[1]</sup>, synthesized by GCC Biotech, India. One half of the purified PCR product was used for sequencing (Eurofins Genomics India Pvt. Ltd). Sequence was analysed using Mega 5 software. SsiI was used to digest the amplified PCR products of *BRCA2* gene for detection of mutation. The tumour masses for histopathological examination were collected from 10 tumour positive cases. After excision, the tissue samples were kept in 10% formalin for fixation and histopathology was performed following standard method.

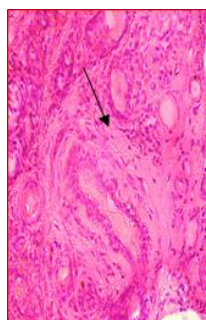
**Result and Discussion**

The extracted DNA was used as template for amplification of *BRCA2* gene at optimum annealing (50 °C). The size of the PCR products was 535 bp and nucleotide sequence result, confirmed a change of adenine to cytosine (a → c) in the cancer positive samples (Rajkhowa and Kalita, 2019) <sup>[4]</sup>. Histopathological changes in the tissues were studied from the positive cases, immediately upon their excision from the body. The portions representing the characteristic lesions were selected. Grossly, some of the tumours were single and some multiple, as mammary tumours vary greatly in size. Consistency of a few tumours were soft and edematous whereas some were firm and fibrotic. Histological sections showed some

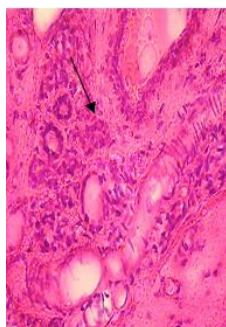
amount of glandular and connective tissue, that varied with the individual tumour. Presence of some cartilaginous structures around the mammary ducts depicted signs of early development of mixed mammary tumour (Fig.1). In another section, some yellowish fatty substances filled up the mammary ductules suggesting that the gland may have been in dry stage (Fig.2). Endothelial lining of the blood vessels were hypertrophied and there were presence of inflammatory cells in the stroma (Fig.3). The lining epithelial cells of the mammary alveoli showing hyperplasia and hypertrophy, projected into the lumen and gave it a glandular appearance, indicating papillary adenocarcinoma (Fig.4). Further, the smooth muscle cells contained deeply coloured cigar shaped nuclei in the interlobular septa of the mammary gland, depicting tumour (Fig.5). The findings of the present experiment correlates the findings reported by Klopffleisch and Gruber, (2009) [3] who also detected simple adenocarcinoma in canine mammary tumours with marked infiltration of the surrounding connective tissue. The tumour cells were pleomorphic arranged in glandular tubules on the contrary to non-neoplastic mammary gland epithelium characterized by well differentiated tubular arrangement of epithelial cells without cellular pleomorphism and infiltration of the adjacent tissue. From the present study it can be concluded that histopathology study of the canine mammary tumour revealed a massive changes of tissue architecture.



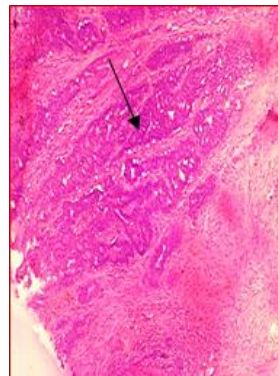
**Fig 1:** Cartilaginous structures around mammary ducts



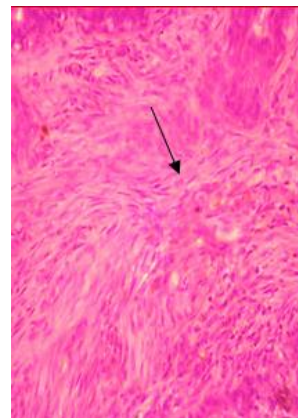
**Fig 2:** Mammary ductules filled with fat globules and yellowish fatty substances



**Fig 3:** Endothelial lining of blood vessels showing marked hypertrophy and presence of inflammatory cells



**Fig 4:** Hypertrophy and hyperplasia of epithelial lining of mammary alveoli projecting into lumen and giving it appearance of a gland



**Fig 5:** Smooth muscle cells with cigar shaped nuclei

#### Acknowledgements

The authors are highly thankful to the Department of Biotechnology, Govt. of India for financial assistance to carry out this work

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