Research Article

Substance P a potent prognostic indicator for canine mammary tumours- a comparative study[#]

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Abstract

Substance P, a neurotransmitter in the central and peripheral nervous system, is considered as a biomarker of pain due to its key role in nociception. But recent researches have proved the increased expression of Substance P and its receptors in human breast cancers and their role in its progression. Canine mammary glands and spontaneously occurring neoplasms in them are considered best models for human breast cancers. Hence as a preliminary study, the present research work was undertaken to assess the level of Substance P in dogs with mammary tumours in comparison to healthy dogs. From the dogs brought for ovariohysterectomy and mammary tumour resection, 20 female dogs were selected for the study. Signalment and detailed history regarding each dog were recorded. All the dogs were subjected to thorough preanaesthetic evaluation and were grouped into Group I (mammary tumour) and II (control group) with 10 dogs each. Blood was collected from all the dogs and serum was separated and stored at -20ºC for estimation of substance P. The same was performed using sandwich ELISA technique with canine specific Substance P ELISA kit. The values obtained for Group I and II were statistically analysed using independent t- test. The level of substance P was significantly higher in the dogs of Group I when compared to Group II.

Keywords: *Substance P, mammary tumour, dogs*

Substance P a neurotransmitter released in response to pain and inflammation in peripheral and central nervous system has been considered as a potential biomarker for pain (Yoon *et al*., 2019). Substance P, discovered by von Euler and Gaddum in 1931 was later identified as an 11 amino acid neuropeptide with the structure Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (O'Connor *et al.*, 2004). Research over the following years have proved the pronounced expression of Substance P and its receptors in tumours and it was found to promote angiogenesis, mitogenesis and tumour progression (Esteban *et al*., 2006; Javid *et al*., 2019). Ebrahimi *et al*. (2020) established strong evidence of the role of Substance P and its receptor (NK-1R) in the cell proliferation, migration, metastasis, angiogenesis and resistance of human breast cancer. Understanding Substance P, its receptors and actions have led to researches on application of drugs like Substance P receptor antagonists for breast cancer therapy (Robinson *et al*., 2016). Given the limited existing literature on the subject and the fact that canine mammary glands and spontaneously occurring

tumours associated with it are considered as best models for human breast cancers. The current study aimed to evaluate the concentration of substance P in dogs afflicted with mammary tumours in contrast to healthy counterparts undergoing ovariohysterectomy.

Materials and methods

The study was conducted in 20 female dogs brought for either mammary tumour resection or ovariohysterectomy procedures to the Kerala Veterinary and Animal Sciences University Hospitals at Mannuthy and Kokkalai. Prior to surgery, all dogs underwent comprehensive preanaesthetic assessments, encompassing clinical examinations and hematological and serum biochemical analyses. Based on these evaluations, dogs were categorized according to the American Society of Anesthesiologists classification and were allocated to two groups: Group I (undergoing mammary tumour resection) and Group II (undergoing ovariohysterectomy). Serum samples for Substance P evaluation were collected prior to surgical procedures and were stored at -20°C until analysis. Surgical interventions were conducted following standard protocols.

Substance P levels in the stored serum samples were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) technique employing a canine-specific Substance P ELISA Kit (Origin Diagnostics and Research, Karunagappalli, Kollam, Kerala). Statistical comparisons of the obtained values were performed using independent T-tests.

Results and discussion

The dogs in Group I were of mixed breeds including Labrador retriever, Dachshund, spitz, German shepherd dog, Dalmatian, Rottweiler and a non-descript
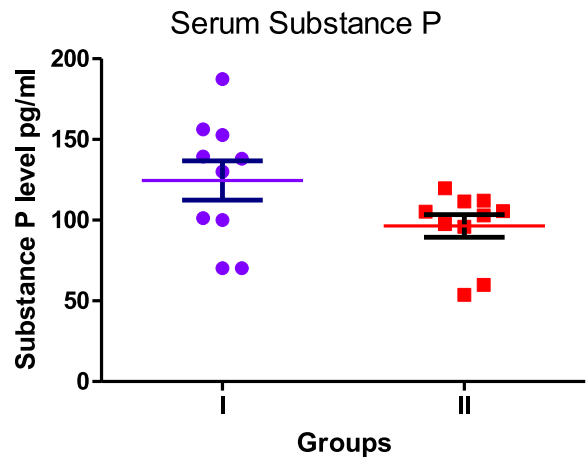

Fig. 1. Scatterplot depicting variation in Substance P values. Group I, mammary tumour has values varying from 70 to 187, whereas the values in control group are all within the normal expected range.

dog, aged five to 16 years weighing six to 36 kg. All had ASA status of either I or II. In contrast, dogs in Group II were all healthy non-descript breeds of dogs aged between one and four years weighing from six to 20 kg and all were assigned the ASA status I. Only those cases falling under class I or II were posted for elective surgery as those included in class ≥III were associated with increased risk of anaesthesia related death (Bille *et al*., 2012).

Substance P

Serum Substance P levels were found to be significantly higher (p <0.05) in dogs of group I with mean value 124.6 ± 12.13 pg/ml when compared to dogs of Group II with mean value 96.46 ± 6.98 pg/mL (Fig.1). The dogs in group I showed a higher level of Substance P even though they did not express any signs of pain during physical examination prior to surgical procedure and the values varied according to the histopathological type and stage of tumour (Table1 & Fig. 2). Ebrahimi *et*

Table.1 Substance P level in dogs with mammary tumour and control dogs

| SI. No. | Type of tumour | Grade | Substance P pg/ml | |
			Group I	Group II
1.	Intraductal papillary carcinoma	III	156.32	95.95
2.	Solid carcinoma	III	152.82	97.59
3.	Tubulopapillary carcinoma	III	139.49	119.82
4.	Carcinosarcoma	II	187.47	102.97
5.	Papillary carcinoma	II	100.16	53.82
6.	Ductal carcinoma	II	70.20	59.90
7.	Tubulopapillary carcinoma	II	101.33	105.54
8	Solid carcinoma	II	70.20	112.10
9.	Carcinosarcoma	III	138.08	105.31
10.	Carcinosarcoma	III	130.12	111.63

Fig. 2. Gross appearance of tumours included in the study

- A. Ductal carcinoma
- C. Intraductal papillary carcinoma
- E. Carcinosarcoma
- B. Solid carcinoma
- D. Tubulopapillary carcinoma
- F. Papillary carcinoma

al. (2020) opined that Substance P could be considered as a potent prognostic indicator of breast cancers as they could establish a strong correlation between cytoplasmic expression of Substance P and TNM staging of human breast cancers. Robinson *et al*. (2016) had suggested using Substance P receptor (NK-1R) antagonist drugs such as aprepitant and fosaprepitant to fight tumour progression which potentiates the sensitivity of triple negative human breast cancer cells to doxorubicin. Rodriguez *et al*. (2021) studied the effect of the same on potentiation of Cisplatin. Al-keilani *et al*. (2021) studied the immunohistochemical expression of Substance P in human breast cancer and identified the molecular subtypes of breast cancer in which Substance P values were higher. According to Ferreira *et al*. (2023), dogs and spontaneously occurring mammary tumours in dogs are the best models for research on human breast cancers. Researches to combat mammary neoplasms in dogs would definitely contribute a lot to the fight against breast cancer in human patients which is the most common cancer among women and is the second leading cancer, causing death (DeSantis *et al*., 2019). Drugs like gabapentin which are α2δ subunit ligands of the voltage gated calcium channels of central nervous system prevent intracellular influx of calcium and thereby hinder the release of substance P, might have a role in preventing tumour progression. Thus, substance P levels in dogs with mammary tumours, according to the type and grade, their response to substance P receptor antagonist drugs or drugs that prevent the release of substance P require further research in the future as it can contribute a lot to the fight against human breast cancer.

Conclusion

The concentration of Substance P was significantly higher in dogs with varying types and grades of mammary tumours than in normal healthy dogs. Further research on the correlation of level of Substance P in canine mammary tumours to the histopathological type and grade and its response to treatment with NK1-R antagonists are the need of the hour.

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