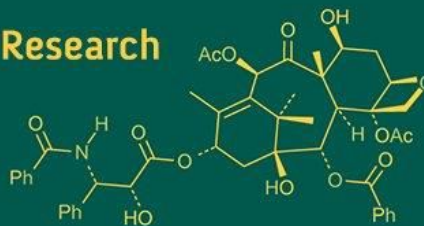
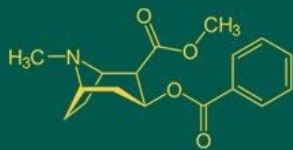


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Evaluation of Argyrophilic nucleolar organizer regions (AgNORs) and mitotic index in high and low-grade canine mast cell tumor

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Abstract

The present study was conducted to assess the significance of AgNOR count in different grades of canine mast cell tumor and to investigate its significance with mitotic index. A total of 53 samples of mast cell tumor received in the Department of Veterinary Pathology during a 41-month period (January 2022 to May 2025) were analysed. Grade wise they were divided into high (n=28) and low grade (n=25). Extremities especially legs were the most common site for mast cell tumor. The average AgNOR count and distribution of AgNOR dots were calculated for different grades of tumors. There was significantly higher AgNOR count in high grade mast cell tumor ($P=0.003$). Mitotic index was significantly more in high grade tumor than low grade ($p<0.0001$). AgNOR served as a quick, easy and accurate way to assess cell proliferative activity in mast cell tumors.

Keywords: AgNOR, canine, grade, mast cell tumor and mitotic index

Introduction

Mast cell tumor (mastocytoma) is one of the most commonly diagnosed cutaneous tumor of dogs, accounting for approximately 7-27% of skin tumors (Blackwood *et al.*, 2012; Prezoisi *et al.*, 2004; Kiupel *et al.*, 2011) [1, 2, 3]. The exact etiology remains unclear, although genetic mutations in c-KIT proto-oncogene alterations are frequently involved in mast cell tumor (London *et al.*, 1999; Cruz *et al.*, 2020; Webster *et al.*, 2007) [4, 5, 6]. Mast cell tumors in dogs can appear in various forms, ranging from small, distinct lumps to larger, aggressive lesions that may be ulcerated and inflamed, with some showing sudden changes in size due to the release of inflammatory substances. Mast cell tumors are also associated with paraneoplastic syndrome caused by the degranulation of mast cells, resulting in oedema and eosinophilia (Kiupel *et al.*, 2011; Strefezzi *et al.*, 2009) [3, 7].

A new 2-tier histologic grading system has been proposed by Kiupel *et al.*, 2011 [3] for canine cutaneous mast cell tumor. Low-grade tumors are linked with more favourable prognosis, whereas high-grade tumors leads to shorter survival time as well as higher metastasis risk (Marouda *et al.*, 2024; Sledge *et al.*, 2016; De Nardi *et al.*, 2022) [8, 9, 10].

AgNOR (Argyrophilic Nucleolar Organizer Region) staining is a specialized histochemical technique used in pathology and cytology to assess cellular proliferation by detecting nucleolar organizer regions (NORs) (Yadav *et al.*, 2023; Ettinger *et al.*, 2006) [11, 12]. These regions are loops of DNA that transcribe ribosomal RNA and are associated with specific argyrophilic (silver-binding) proteins. The rapidly dividing or malignant cells show an increased number and irregular distribution of AgNOR, which is valuable in grading tumors, evaluating prognosis and monitoring the effectiveness of therapy. Mitotic index is the basic parameter to assess the proliferative activity of neoplasms which is done on routine H&E stain. Higher mitotic count has been associated with high grade tumor (Strefezzi *et al.*, 2009; Romansik *et al.*, 2007) [7, 13].

Material and Methods

Total of 53 mast cell tumors received in the Department of Veterinary Pathology from January 2022 to May 2025 were included in this study. Tissues fixed in 10% formalin were processed for histopathology and stained with hematoxylin and eosin and toluidine blue as

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per standard protocol (Bancroft, 2010) [17]. AgNOR staining was done on the 4µm sections as per Cocker *et al.*, 1989 [15]. Histopathological grading was performed as per the newer grading system (Kiupel *et al.*, 2011) [3]. For AgNOR counting, total number of AgNOR dots were counted per 100 nuclei. The mitotic index was calculated by counting mitotic figures in a 2.37 mm² area. Hot spot areas of the tumor, characterized by high cellularity and increased mitotic figures were selected to calculate the mitotic figures (Donovan *et al.*, 2021) [16].

An unpaired t-test was used to assess the significant difference between low and high-grade tumors with respect to AgNOR count and mitotic index. P value of less than 0.05 level of confidence was considered significant. Statistical analysis was done using GraphPad Prism v 9.5.1 (GraphPad, Boston, USA).

Results and Discussion

Out of total 53 cases of mast cell tumor, 33 (62.26%) were in males and 20 (37.73%) in female dogs. The average age of dogs affected with MCT was 6.25±0.34 years. The mean incidence age for males and females was 6.34±0.63 and 6.19±0.39 years, respectively. Half of the cases were recorded in 6-9 year age group. Most common breeds suffering from MCT were Labrador retriever (13), non-descript (9), American bully (5), Pug (4) and Beagle (4). MCT was most commonly recorded in the extremities (30), trunk (15), head and neck (8). In the present study out of 53 cases, 28 (52.83%) were diagnosed as high-grade and 25 cases (47.17%) as low grade.

The low-grade tumor was composed of chain or rows of well differentiated mast cells having round nuclei and cytoplasm containing abundant granules (Fig.1). Mitotic count was generally low in low-grade tumor. High grade tumor was containing markedly pleomorphic neoplastic cells having pleomorphic vesicular nuclei and multiple prominent nucleoli. They showed karyomegaly, bizarre nuclei, binucleation, multinucleation and frequent mitosis. High-grade MCT were extending deep into the dermis and hypodermis with areas of edema, necrosis and eosinophils. Frequent mitotic figures were observed in high-grade tumor. Low grade tumor was containing infiltrating cords or chain of neoplastic cells with collagen hyalinization, whereas high-grade was composed of solid cluster of cells with thin delicate stroma (Fig.2). Frequent cutaneous ulceration were also noted in high-grade tumor (Fig.2). Mean AgNOR count of mast cell tumor was 2.48±0.14 and 1.90±0.12 for high and low grade respectively. The average mitotic index was 14.1±2.14 and 2.88±0.41 MF/2.37mm² for high and low grade MCT respectively.

The average age for the incidence of mast cell tumor was similar as those of previous workers (Kiupel *et al.*, 2011; Stefanello *et al.*, 2015) [3, 17]. Cruz *et al.* (2020), Pakhrin *et al.* (2007) and Kok *et al.* (2019) also found extremities and trunk as most commonly affected sites. Labrador retriever was the most commonly affected breed which was also reported earlier by other workers (Kiupel *et al.*, 2011; Webster *et al.*, 2007; Shoop *et al.*, 2015) [3, 6, 23]. In our study there were more cases of high-grade tumor, which was in contrary to the previous findings (Kiupel *et al.*, 2011; Cruz *et al.*, 2007; Stefanello *et al.*, 2015) [3, 5, 17]. Variation might have been due to different geographical factors, different breed distribution and different exposure factors. The mitotic index, as well as the mean AgNOR count, was

significantly higher in high-grade MCT compared to low-grade MCT. In the present study mean AgNOR count was significantly (**, P=0.003) higher in high-grade MCT than low-grade as reported by (Marouda *et al.*, 2024; Yadav *et al.*, 2023) [8, 11]. (Table 1). Also in high-grade tumor larger AgNOR dots were noted than low-grade tumor. Mitotic index was significantly (****, p<0.0001) higher in high-grade than the low-grade MCT (Table 1). The higher mitotic count in high-grade tumor is indicative of more number of proliferating cells in M-phase of cell division as compared to the low-grade MCT (Kumar *et al.*, 2010) [24].

Histopathologic grading of mast cell tumor still remains useful for assessing the prognosis alongside the mitotic index and AgNOR count. Despite the rise of more advanced molecular techniques, AgNOR analysis remains a practical tool in diagnostic pathology due to its cost effectiveness and simplicity in application, besides providing valuable insights into the proliferative behaviour of cells (Vijaykumar *et al.*, 2018) [25].

Table 1: Mean AgNOR score of different grades of mast cell tumor

Mast cell tumor	mAgNOR count	Mitotic index
High grade (n=28)	2.48±0.14	14.1±2.14
Low grade (n=25)	1.90±0.12	2.88±0.41

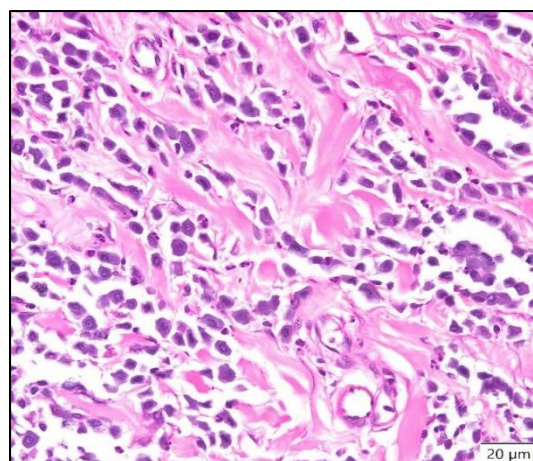


Fig 1: Low-grade mast cell tumor having low cellularity. The cells are arranged in rows and small groups with distinct cytoplasm full of granules. H&E x400

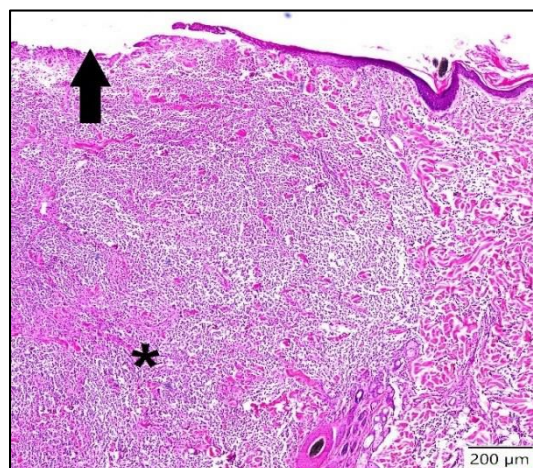


Fig 2: High-grade mast cell tumor extending deep into dermis (asterisk) with epidermal ulceration (arrow). H&E x40

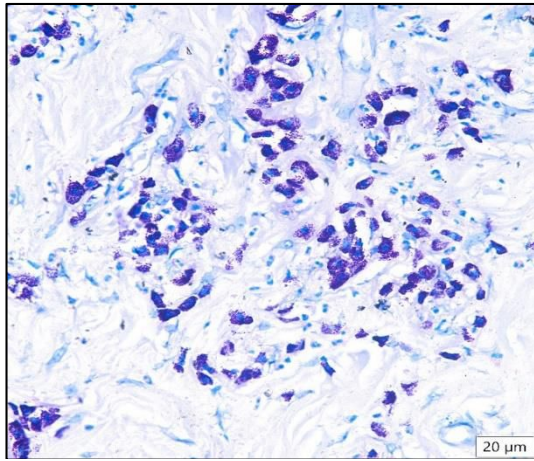


Fig 3: Mast cell tumor with purple colour metachromatic granules. Toluidine blue x400.

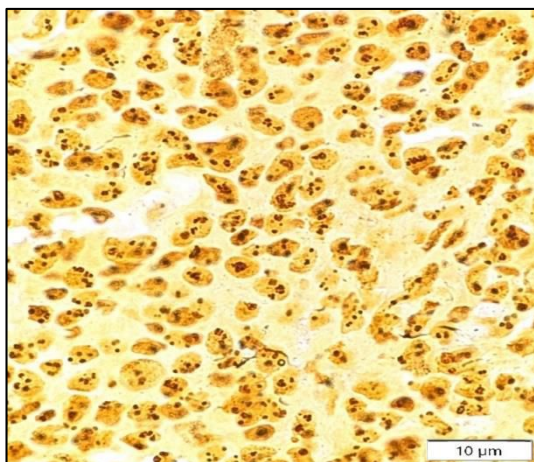


Fig 4: Numerous AgNOR black dots inside the nucleus of high-grade mast cell tumor. AgNOR x1000.

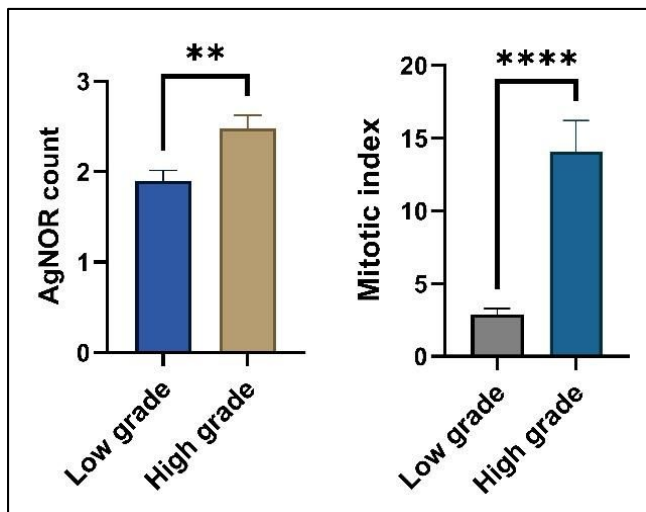


Fig 5: Average AgNOR and mitotic index of different grades of mast cell tumor. Results are presented as Mean ± SEM (**, $P=0.003$; ****, $p<0.0001$)

Conclusion

In the present study proliferative marker AgNOR and mitotic index of canine mast cell tumors were correlated with different grades. There was statistically higher AgNOR count and mitotic index in high grade than low grade mast cell tumors. Larger AgNOR dots were also observed in high-grade MCT. Therefore, it can be concluded from this

study that AgNOR count and mitotic index, when used in conjunction with histopathology, serve as effective markers of cellular proliferation and prognosis.

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