Diagnostic Significance of Agnor Counting in Bovine Tumors

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ABSTRACT

The present research was carried out to know the significance of AgNOR counting in diagnosis of bovine tumors. For this study tumor suspected tissue samples were collected from postmortems, college and city veterinary hospital, veterinary dispensaries in and around Tirupati and subjected for AgNOR staining and counting of AgNOR dots in different tumor cells. The AgNORs appeared as round to irregular, dark brown to black dots of varying sizes in the nuclei. The mean AgNOR count of the individual bovine tumors varied from 3.1 to 5.97. Highest and lowest mean AgNOR counts were observed in myxosarcoma and fibroma with the values of 5.97 and 3.1, respectively. The malignant tumors had numerous and smaller AgNORs when compared to benign or less malignant tumors.

Keywords: *Bovine tumors, AgNOR counting, Diagnosis, Benign and Malignant*

INTRODUCTION

Animal Husbandry is making a significant contribution to the national economy and socio-economic development in the country. Livestock farming is the main source of livelihood in rural India (Hegde, 2006) and it is an integral part of Indian agriculture and contributes to the well-being of its people. Cattle constitute a share of about 60% in India's total livestock population followed by buffaloes (32%), goats (4%) and sheep (2%). Livestock accounts for about 30% of entire output from agri sector (ASSOCHAM, 2013).Recently, the tumor incidence in bovines is relatively increasing. Cattle occupied the second place after dogs for incidence of all tumors (Marosfoi et al., 2009).

Many problems arise microscopically in differentiating the malignant aberrations from benign ones. Histopathology does not reveal all features which are of diagnostic and prognostic significance. So there is a need to employ procedures which can diagnose malignancy at the earliest and with accuracy. Studies have revealed the correlation between nucleolar function, size and the cell doubling time in human cancer cell lines, which has stimulated a revolution of the importance of the nucleus in tumor pathology. The Nucleolar Organizer Regions (AgNORs) correlates with the rate of proliferation, as can be estimated by Ki-67 and the percentages of the S phase cells and the mitotic cells. In quantitative terms, the number of AgNORs per nucleus suggests it to be a marker of the proliferative activity of the cell. Qualitatively, based on the shape, size and the pattern of distribution, AgNOR acts as a marker of pre-malignant or malignant change (Sandhya et al, 2011) . Hence the estimation of AgNOR parameters has been applied in tumor pathology both for diagnostic and prognostic purposes. Perusal of literature revealed that a little comprehensive work was conducted on diagnostic and prognostic evaluation of bovine neoplasms in India so far. Hence the present study has been undertaken to study the Importance of Nucleolar Organizer Regions (AgNORs) in diagnosis of different bovine tumors

MATERIALS AND METHODS

The materials for present study included the samples suspected for neoplasia of bovine origin submitted to the Department of Veterinary Pathology, College of Veterinary Science, Tirupati by the Department of Veterinary Surgery and Radiology and Veterinary Dispensaries in and around Tirupati. Samples were also collected from animals necropsied in the Department of Veterinary Pathology. Particulars like breed, sex, location, shape, colour and consistency of growths were recorded.

AgNOR Staining

AgNOR staining was performed as described by Krishnamurthi and Paliwal (1998). The sections were deparaffinized in xylene and hydrated through decreasing grades of ethanol to double distilled deionized water. The sections were then reacted with freshly prepared silver colloidal solution (1 part by volume of 2% gelatin in 1% formic acid and two parts by volume of 50% aqueous silver nitrate solution) in darkness for 35 min at room temperature. The silver colloidal solution was washed with double distilled ionized water. The sections were then treated with 5% sodium thiosulphate for 5 minutes and washed in double distilled deionized water, dehydrated through increasing grades of alcohol, cleared in xylene and mounted in DPX.
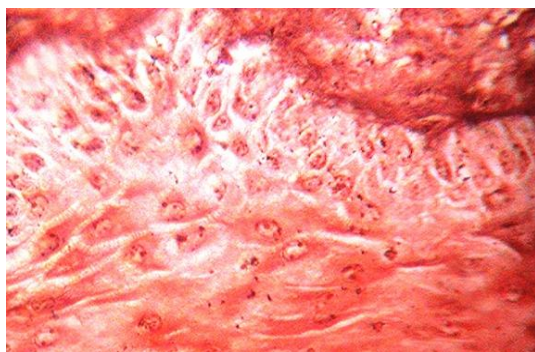
AgNOR Counting

The number of AgNORs present in each nucleus was counted in 100 non overlapping nuclei by using a 100x oil immersion lens. At this magnification, AgNORs are visible both within and outside the nuclei. The mean AgNOR value was calculated for each case and each type of tumor.

**Table1.** *Mean AgNOR counts in different bovine tumors*

S.no.	Type of tumor	Mean AgNOR count
1.	Papilloma	3.63
2.	Squamous cell carcinoma	5.06
3.	Adenoma	3.48
4.	Adenocarcinoma	3.5
5.	Basal cell carcinoma	4.85
6.	Fibroma	3.1
7.	Myxosarcoma	5.97
8.	Hemangioma	3.92
9.	Lymphosarcoma	4.25
10.	Leiomyoma	4.62
11.	Fibroleiomyoma	3.74
12.	Adamantinoma	3.44
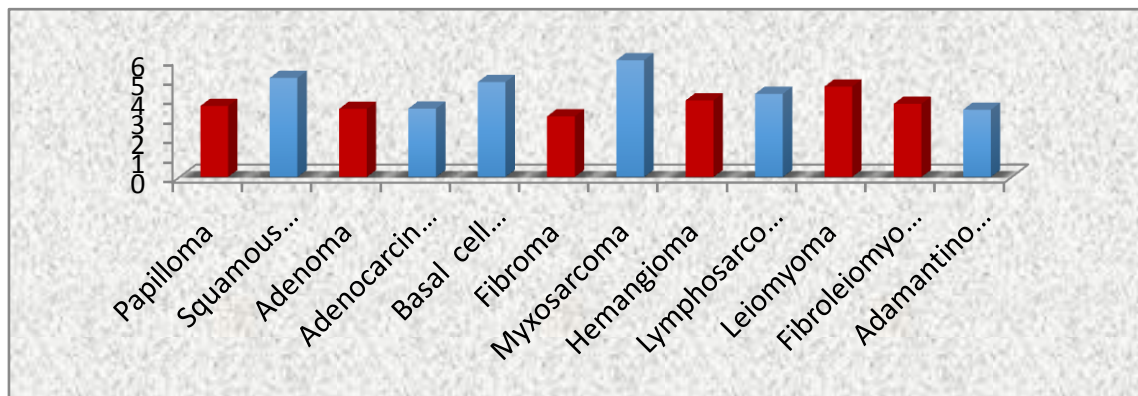
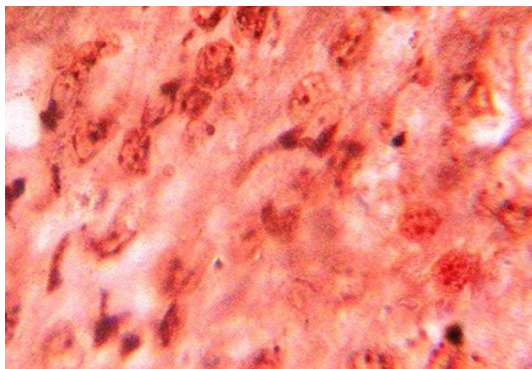**Fig1.** *Mean AgNOR counts of different bovine tumors*

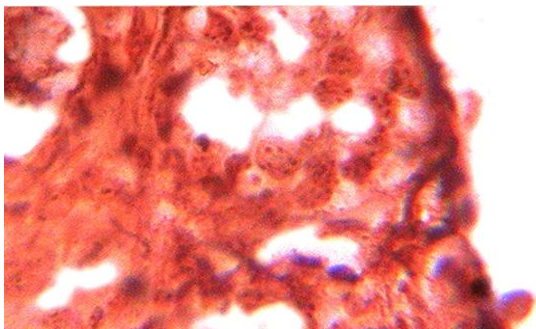**Fig2.** *Papilloma: Note 3-5 dark brown, large, round AgNOR dots in the nucleus of prickle cells. AgNOR: x 280.*
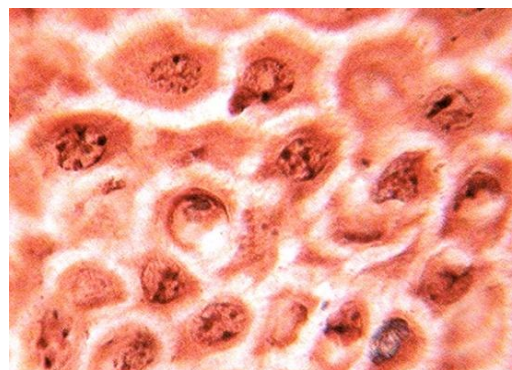
Fig3. *Papilloma: Note 3-5 dark brown, large, round AgNOR dots in the nucleus of pricklecells. AgNOR: x 700.*
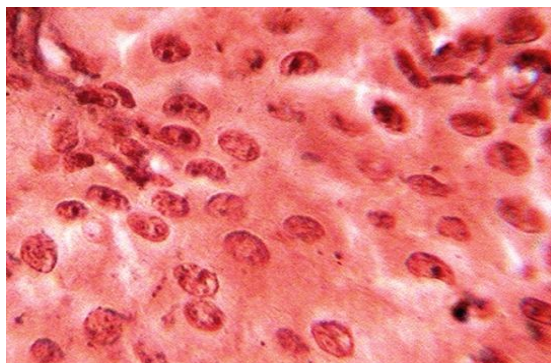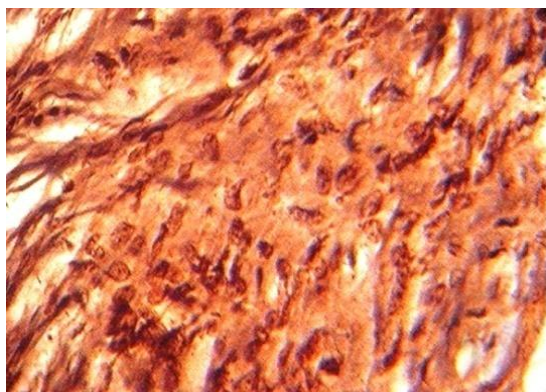
Fig4. *SqCC: Section showing 5 – 6 small, irregular AgNORs distributed throughout the nucleus. AgNOR: x 700.*
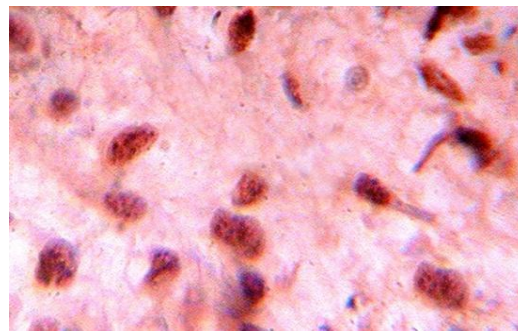

Fig5. *Papillary cystadenoma of ovary: Note 3 – 6 large, round AgNORs in the nuclei of cells of papillary projections into lumen. AgNOR: x 700.*


Fig6. *Basal cell carcinoma: Note 3 – 5 small, irregular AgNORs distributed throughout the nucleus in basaloid cells. AgNOR: x 700.*
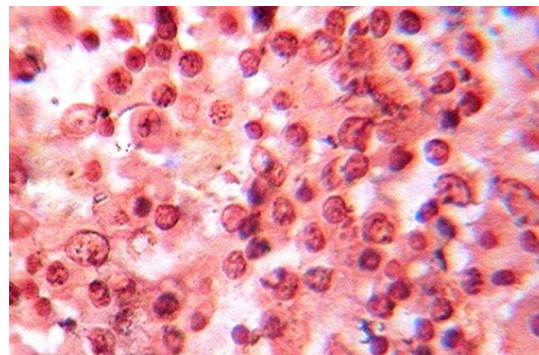

Fig7. *Soft fibroma: Note 2 – 6 large, round, regular black AgNOR dots in nuclei of fibroblasts. AgNOR: x 700.*

Fig8. *Myxosarcoma: Note numerous (4 – 7) small, irregular clusters of AgNORs distributed throughout the nucleus of myxomatous cells. AgNOR: x 700.*
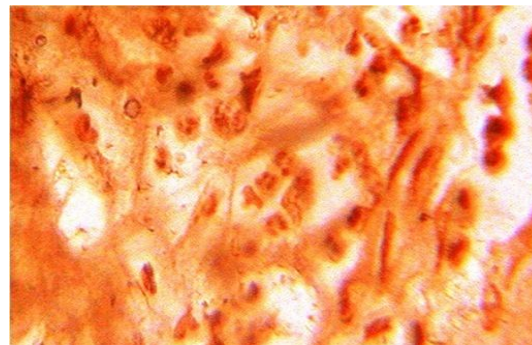
Fig9. *Hemangioma: Note 4 – 5 large round and uniform AgNORs in the neoplastic cells. AgNOR: x 700.*

Fig10. *Lymphosarcoma: Note numerous lymphocytes and lymphoblasts with 4-6 small, irregular AgNORs throughout the nucleus. AgNOR: x 700.*

RESULTS

AgNOR

AgNOR staining and counting was performed on different tumors. The AgNORs appeared as round to irregular, dark brown to black dots of varying sizes in the nuclei. The mean AgNOR count of the individual bovine tumors varied from 3.1 to 5.97 and a significant correlation was found between an increased AgNOR count and histological grade. The mean AgNOR counts of different tumors were shown in Table 1 (Fig. 1). The mean AgNOR count of benign tumors (3.75) was lower than that of malignant tumours (4.18). Highest and lowest mean

AgNOR counts were observed in myxosarcoma and fibroma with the values of 5.97 and 3.1, respectively. The malignant tumors had numerous smaller AgNORs in the nucleus whereas benign tumors had large, round, sharply defined and few AgNOR dots confined to the the nucleoli (Fig. 2 to 10).

DISCUSSION

In the present study, AgNORs appeared as round to irregular, dark brown to black dots of varying sizes in the nuclei which was in accordance with the observations of Chandrasekhar and Lalitha (1995). Malignant tumors had numerous, smaller and irregular AgNORs, dispersed throughout the nucleus whereas benign tumors had large, round, sharply defined and few AgNOR dots confined to the nucleoli. These findings were in accordance with the findings made by Crocker et al. (1989), Chandravathi et al. (2013) and Veena et al. (2014). In contrary, Sandhya et al. (2011) observed small, homogenously stained, regular AgNORs in benign tumors (papillomas) and large irregular dots or bizzare clusters in malignant tumors (SqCC). Variations in the size and number of the AgNOR dots might depend on the stage of the cell cycle, the transcriptional and metabolic activity of the cell or the number of NOR-bearing chromosomes in the karyotype (Sandhya et al., 2011).

The mean AgNOR count of different tumors in the present study varied from 3.1 to 5.97. Whereas Destexhe et al. (1995) and Palanivelu et al. (2013) observed it as 3.06 to 7.28 and 2.67 to 6.98, respectively. In the current study, malignant tumours (4.18) had higher mean AgNOR counts than the benign tumors (3.75). Correspondingly, Destexhe et al. (1995); Krishnamurthi and Paliwal (1998); Jelesijevic et al. (2003); Vajdovich et al. (2004); Pawan Kumar et al. (2010); Palanivelu et al. (2013); Chandravathi et al. (2013) observed significant difference between mean AgNOR counts of benign and malignant tumors. The increased mean AgNOR counts in bovine tumors might be due to increased cell ploidy, increased transcriptional activity and disorganization of chromosomal and AgNOR distribution resulting in formation of multiple, small and dispersed nucleoli in the active stages of cell proliferation (Sandhya et al., 2011).

In the present study, the highest and lowest mean AgNOR counts were observed in myxosarcoma and fibroma with the values of 5.97 and 3.1, respectively. The mean AgNOR count of papillomas in the current study was 3.63, whereas Krishnamurthi and Paliwal (1998), Sandhya et al. (2011) and Chandravathi et al. (2013) observed it as 3.24, 2.15 and 4.20, respectively. The mean count of SqCC in the present study was 5.06 whereas Dayananda et al. (2010), Palanivelu et al. (2013) and Chandravathi et al. (2013) found the values as 5.93, 5.57 and 6.23, respectively. The mean count in hemangioma was 3.92, in contrary, Dayananda et al. (2009) found it as 2.4 in dogs. In the current study, the mean AgNOR count of lymphosarcoma was 4.25, similarly, Thangapandiyan et al. (2012) observed it as 4.68 in canines.

The present research findings revealed that the mean AgNOR count was more in malignant tumors when compared to benign tumors. The quantitative (mean count per cell) and qualitative parameters (the shape, size and the pattern of distribution) of AgNOR can be used as supplementary factors for studying cell proliferation and to differentiate benign from malignant tumors.

ACKNOWLEDGEMENTS

REFERENCES

[1] Hegde N G 2006 Livestock Development for Sustainable Livelihood of Small Farmers. In Souvenir of the 39th Annual General Meeting and 48th National Symposium on "Energising Rural India – A Challenge to Livestock Industry. 26: 50-63.

[2] ASSOCHAM 2013 India's livestock population to reach 312 million by 2015: ASSOCHAM. Press Releases.

[3] Marosfoi L, Baba A I and Catoi C 2009 Morphological Study of Bovine Tumors. Bulletin of the University of Agricultural Sciences & Veterinary. 66(1): 147.

[4] Sandhya P G, Sitaramam E and Prasada Reddy K 2011 Role of Silver Staining Nucleolar Organiser Regions (AgNORs) in Lesions of the Oral Cavity. Journal of Clinical and Diagnostic Research. 5(5): 1011-1015.

[5] Krishnamurthi V and Paliwal O P 1998 Nucleolar organizer region count as a diagnostic marker for tumours and cell proliferation rate in certain neoplasms of animals. Indian Journal of Veterinary Pathology. 22(1): 6-10.

[6] Chandrasekhar V and Lalitha P S 1995 AgNOR count as a marker in Ethmoid Cacinoma in Bovines. Veterinary Record. 137: 24-25.

[7] Crocker J, Dravid A R, Boldy and Egan M J 1989 How should we count AgNOR. Proposal for the Standardized Approach. Journal of Pathology. 158: 185-188.

[8] Chandravathi T, Anjaneyulu Y, Anand Kumar A, Narasimha Reddy Y and Samatha V 2013 Histopathological & immunohistochemical studies of canine epithelial tumours. International Journal of Food, Agriculture and Veterinary Sciences. 3(3): 11-18.

[9] Veena P and Ravi Kumar A 2002 Homeopathic Treatment for Papillomatosis in a Calf. Indian Veterinary Journal. 79: 960.

[10] Destexhe E, Vanmanshoven P and Coignoul F 1995 Comparison of Argyrophilic Nucleolar Organizer Region by counting and image analysis in Canine Mammary Tumors. American Journal of Veterinary Research. 56(2): 185-187.

[11] Jelesijevic T, Jovanovic M, Milijana K and Sanja A K 2003 Quantitative and qualitative analysis of AgNOR in benign and malignant canine mammary gland tumors. Acta Veterinaria (Beograd). 53(5-6): 353-360.

[12] Vajdovich P, Psader R, Toth A Z and Perge 2004 Use of Argyrophilic Nucleolar Organizer Region method for Cytology and Histologic Examination of Lymph nodes in Dogs. Veterinary Pathology. 41: 338-345.

[13] Pawan Kumar, Ram K, Pawaiya R S and Puttaswamy M B 2010 Diagnostic significance of mitotic index and AgNOR count in canine mammary tumours. Brazilian Journal of Veterinary Pathology. 3(1): 41-45.

[14] Dayananda T S, Suguna Rao, Byregowda S M, Shilpa V T, Jayachandra K C and Satyanarayana M L 2010 Proliferation Index of Squamous Cell Carcinomas in Dogs. The Indian Veterinary Journal. 87: 9-12.

[15] Dayananda T S, Suguna Rao, Byregowda S M, Satyanarayana M L, Jayachandra K C and Shilpa V T 2009 Proliferation Index in Hemangioma and Hemangiosarcoma of Dogs. The Indian Veterinary Journal. 86: 454-456.

[16] Thangapandiyan M, Balachandran C and Murali Manohar 2012 Argyrophilic Nucleolar Organiser Regions (AgNOR) in Canine Lymphoma. The Indian Veterinary Journal. 89(2): 77-78.