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Research Article

HISTOPATHOLOGICAL & IMMUNOHISTOCHEMICAL STUDIES OF CANINE EPITHELIAL TUMOURS

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ABSTRACT

A total of 40 samples of epithelial tumors in canine specie were collected in and around Hyderabad, Andhra Pradesh. Among these19 cases (47.5%) were benign and 21cases (52.50%) were malignant tumours based on histopathological and immunohistochemical studies. Benign epithelial cell tumours include papilloma 4 (21.05%), benign mammary tumours 4 (21.05%), sebaceous gland adenoma 3 (15.7%), perianal gland adenoma 3 (15.7%), melanoma 2 (10.52%), anal sac adenoma 2 (10.52%) and apocrine gland adenoma 1 (5.26%).Malignant tumours included mammary gland carcinoma 7 (33.33%), squamous cell carcinoma 4 (19.04%), sertoli cell tumour 3 (14.28%), ovarian adeno carcinoma 2 (9.52%), basal cell carcinoma 1 (4.76%), malignant melanoma 1 (4.76%), seminoma 1 (4.76%), prostatic carcinoma 1 (4.76%), transitional cell carcinoma 1 (4.76%). Proliferating cell nuclear antigen (PCNA) and argyrophilic nucleolar organizer regions (AgNOR) count were also used to determine the cell proliferation and prognosis of various tumours of epithelial origin.Tumors with high PCNA and AGNOR counts showed poor prognosis.

Key Words: *Epithelial Tumours, Papilloma, Sqcc, Seminoma, Adenoma*

INTRODUCTION

Cancer is the multiple diseases that hallmark self sufficiency in growth signal, insensitivity to antigrowth signal, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and metastasis. The progressive changes are reflected with pathology-normal epithelium, hyperplasia, carcinoma and invasive carcinoma. The prognosis depends on both clinical stage and molecular status (Vinay Kumar *et al.,* 2004). Dogs are the only species of animals, besides man in which there is a high incidence of spontaneous primary neoplasms. Pathological studies of canine neoplasm were carried out to know characters of various epithelial tumours. Assessment of the cell kinetics is a field of interest in modern oncology. The study of the parameters that reflect the cell cycle, phase of the neoplastic cell has been shown to be useful for the evaluation of biological behaviour of the tumours. In human neoplasms, immunohistochemical markers measure the kinetic parameters of the cells and have been used successfully as prognostic indicators. One of such marker is the proliferating cell nuclear antigen (PCNA). It is 36Kda nuclear polypeptide that functions as the cofactor for DNA polymerase delta which participates in the DNA synthesis and repair (Roels *et al.,* 1999). It is a reliable marker of cells undergoing proliferation.

Tumour with high proliferation rates have been associated with poorer prognosis (Suzuki *et al.,* 1992).A special staining technique, argyrophilic nucleolar organizer regions (AgNOR) count was also used to determine the cell proliferation and prognosis of various tumours (Lohr *et al.,* 1997).

MATERIALS AND METHODS

To study the pathology of canine neoplasms samples were collected from the various surgical wards in and around Hyderabad. The collected samples were preserved in 10% neutral buffered formalin for the histopathology and immunohistochemistry. Routine tissue processing has been carried out. Sections of 5

µ thickness were taken and stained with haematoxylin and eosin. Special staining techniques like argyrophilic nucleolar organizer regions (AgNOR) and proliferating cell nuclear antigen (PCNA) were performed to evaluate the prognosis of tumours.

The tissue sections were stained by modified silver colloid staining followed by Krishnamurthi *et al.,* (1998). The AgNOR dots in 100 non overlapping nuclei were counted under oil immersion objective (1000x magnification) and mean number of AgNOR dots per nucleus (AgNOR index) was calculated for each specimen.

Immunohistochemistry of proliferating cell nuclear antigen was done using the staining procedure followed by Pawaiya *et al.,* (2006). Formalin fixed paraffin embedded sections of 4 µ thickness were taken on to poly-l-lysine coated slides. Endogenous peroxidase was blocked by placing the sections in 3% hydrogen peroxide for 10 min and rinsed with 0.01M phosphate buffer saline (PBS) at pH 7.4 and incubated with 5% normal goat serum (Sigma, G9023), then with PCNA monoclonal antibodies. The immunoreactivity for PCNA was done by counting the positive cells in 10 randomly selected high power fields (x200) and the mean values were calculated (Karademir *et al.,* 1998; Yu *et al.,* 1992).

RESULTS AND DISCUSSION

Benign Tumors: Benign epithelial cell tumours include papilloma 4 (21.05%), benign mammary tumours 4 (21.05%), sebaceous gland adenoma 3 (15.7%), perianal gland adenoma 3 (15.7%), melanoma 2 (10.52%), anal sac adenoma 2 (10.52%) and apocrine gland adenoma 1 (5.26%). The mean number of AgNOR in benign tumours was significantly (P>0.05) lower than the malignant tumours.The AgNOR dots were large, round, less in number in benign tumours compared to malignant tumours.

Papilloma: Four cases revealed papilloma (21.05%). The growths were located in oral mucosa and urinary bladder, about 1-2 cm in diameter, finger like, pink coloured and hard in consistency. Histopathologically, tumour showed elongated dermal papillae covered with hyper keratotic epidermis and fibrovascular connective tissue stroma (Figure 1). Cells had eosinophilic cytoplasm with distinct vesicular nucleus and nucleoli. Histological features were similar to the findings of Singh *et al.,* (2004) and Krithiga *et al.,* (2005b)

Mammary Gland Adenoma: Four cases revealed Mammary gland adenoma (21.05%). Microscopically tumour revealed cuboidal to columinar shaped well differentiated luminal epithelial cells. Few tumours revealed proliferation of acinar cells along with fibrous tissue, cartilage, bone and /or fat (Figure 2) and that was in agreement with John Abrahon *et al.,* (2007).

Sebaceous Gland Adenoma: The sebaceous gland adenoma incidence was about 3(15.7%). Grossly tumours were 1-2 cm in diameter, irregular, lobulated, grey coloured with soft consistency. Two types of cells were found. At periphery, a rim of small basophilic reserve cells with hyperchromatic nuclei and little cytoplasm and at centre sebaceous epithelial cells were noticed .Cluster of neoplastic gland cells with vesicular nucleoli, cytoplasmic vacuoles and a rim of reserve cells were present at periphery and the findings were correlated with Gold Schmidt *et al.,* (2006).

Perianal Gland Adenoma: Perianal gland adenoma was found in 3 (15.7%) ,measuring about 1 – 4 cm diameter, with irregular, multilobular, white to brownish cut surface and soft in consistency(figure 3). Histologically, tumour revealed sheets of polyhedral cells (hepatiod cells), eosinophilic cytoplasm and vesicular nucleus with prominent nucleolus. At the periphery of lobules basaloid reserve cells of one layer thickness with small hyperchromatic nuclei were found (Figure 4) and these findings were similar to Goldschimdt *et al.,* (2002).

Melanoma: Out of 40 cases, 2 (10.52%) were melanomas found on the trunk region. Tumours were 0.8 cm in diameter, brown in colour with hard consistency. Tumour revealed islands of densely packed spindle shaped melanocytes which were separated by fibrovascular stromal tissue (figure 5). The readings were similar to Goldschmidt *et al.,* (2002) findings.

Anal sac Adenoma: Anal sac adenoma was observed in two (10.52%). Growth was irregular in shape, 1-2 cm in diameter and soft in consistency. Histologically, tumour revealed proliferation of glandular

epithelium and cells lining the individual glands were cuboidal to columnar in shape with normochromatic nuclei and the result was in confirmity with Goldschimdt *et al.* (2002) and also note brownred to brick colored PCNA positive nuclei (Figure 6).

Apocrine Adenoma: In the present study one case (5.26)% of apocrine adenoma was recorded. Histopathologically, intraluminal papillary invaginations were seen. Tumour cells werelarge, round to ovoid with prominent nuclei. Cells had varied amount of esinophilic cytoplasm, borders were deficient and separated by fibrous trabeculae. The observations are found in agreement with Shakir *et al.,* (1994) and Gold Schmidt *et al.,* (2002).

Malignant Tumours: Malignant tumours included mammary gland carcinoma 7 (33.33%), squamous cell carcinoma 4 (19.04%), sertoli cell tumour 3 (14.28%), ovarian adeno carcinoma 2 (9.52%), basal cell carcinoma 1 (4.76%), malignant melanoma 1 (4.76%), seminoma 1 (4.76%), prostatic carcinoma 1 (4.76%), transitional cell carcinoma 1 (4.76%). PCNA was associated with the cell proliferation in different tumours. The mean PCNA positive nuclei in epithelial tumour tissues varied from 14.23 ±1.22 to 321.38 ± 4.78. Malignant mammary adenocarcinomas showed highest PCNA counts. There exist a significant ($P< 0.01$) correlation between the AgNOR counts and PCNA index. Increased indices associated with malignancy and poor prognosis. The means of AgNOR dots per nucleus and PCNA index in different tumours were given in the table 1.

Mammary Adenocarcinoma: Incidence of mammary tumours was highest. Out of 40 cases7 (33.33%) were mammary tumours. Growths were located mostly in the abdominal and inguinal mammary glands and in two cases thoracic mammary glands were involved. Grossly tumours were usually lobulated. The tumour consisted of both epithelial and myoepithelial components. The amount of stroma varied considerably. In most of tumours necrotic areas were observed.

The tumours were characterized by formation of tubules with papillary projections. The stromal component also showed proliferation. Tubules were lined with cuboidal to columnar epithelium and some polyhedral neoplastic cells. The cytoplasm was eosinophilic, with hyperchromatic prominent nucleus. Few Tumours revealed cystic spaces of varying sizes, which were lined by flattened to cuboidal epithelium, eosinophilic cytoplasm with hyperchromatic nuclei. Also note the PCNA positive nuclei and AgNOR dots in nucleus (Figures 7& 8).

Squamous cell Carcinoma: Out of 40 cases 4 (19.04%) were squamous cell carcinomas. Grossly tumours were spherical to round in shape with various sizes, grayish white coloured with hard consistency. Histopathology of squamous cell carcinoma composed of irregular masses of neoplastic epithelial cells extending in to the dermis, showed a variable degree of squamous differentiation. Tumour revealed keratin pearls which were composed of concentric layers of squamous cells. Individual tumour cells had large, ovoid, vesicular nuclei with a prominent nucleolus and eosinophilic cytoplasm. Similar histopathological observations were reported by Gold Schmidt *et al.,* (2002).

Sertoli Cell Tumour: Sertoli cell tumour was observed in 3 (14.28%) dogs. The affected dogs had cryptorchidism and alopecia. Tumour was found on left side in all cases. Histopathological sections showed shrunken tubules with conspicuous lumen, containing multilayered cells arranged with their long axis perpendicular to the basement membrane. Cells were fusiform, eosinophilic cytoplasm with elongated nucleolus. The tubules were separated by collagenous stroma. These findings are in agreement with MacLanchlan *et al.,* (2002) and Thilagar *et al.,* (2002). Also note brown red to brick red coloured the PCNA positive nuclei (Figure 9).

Ovarian Adenocarcinoma: Ovarian epithelial adenocarcinoma was found in 2 (9.52%) cases the tumour masses were cystic and multilobulated. Cut surface showed thin yellow colored fluid in some lobules. Histopathology of tumour revealed arboriform papillae that projected in to lumen of cystic cavities. The papillae consisted of connective tissue strands that were lined by multiple layers of cuboidal epithelial cells AgNOR dots in nuclei (Figure 10). Some papillae showed mucinous material and areas of necrosis. Similar findings were noticed by MacLanchlan *et al.,* (2002)

Basal Cell Carcinoma: The incidence of BCC was 1 (4.76%) and grey in colour with hard consistency .Microscopically section revealed, attempted festoon formation of cells, consisting of 1 or 2 rows of small uniform size cells with eosinophilic cytoplasm and hyperchromatic nuclei. Nucleoli were inconspicuous and stroma was found between the cords of cells. Histological findings were in confirmity with Shakir *et al.*, (1994) and Gold Schmidt *et al.*, (2002).

Malignant Melanoma: Malignant melanoma was observed in 1 (4.76%) dog. Microscopically, tumour revealed pleiomorphic melanocytes of spindle to fusiform which contained little amount of intracytoplasmic melanin and also interwoven pattern of fusiform cells with an interstitial fibrovascular stroma. The results were in confirmity with Moulton (1961), Theilin *et al.*, (1987) and Goldschmidt *et al.*, (2002).

Seminoma: Seminoma incidence was about 1(4.76%). Oval shaped, pink coloured growth with soft in consistency was observed in right testis. Histologically, seminoma was diffusive type with sheets of polyhedral cells having sharp borders, central vesicular PCNA positive nuclei (Figure 11) and prominent nucleolus.Cytoplasm was scanty with eosinophilic nature, vacuoles were observed in some cells. Similar findings were observed by Dakshinkar *et al.*, (1991) and Krithiga *et al.*, (2005b).

Table1: AgNOR and PCNA counts in various epithelial tumours

S.No	Name of the tumour	AgNOR counts Mean ±S.E	PCNA counts Mean ±S.E
1	Papilloma	4.20±1.36	55.53±2.46
2	Benign mammary gland adenoma	2.81±1.02	14.23±1.22
3	Sebaceous gland adenoma	3.06±1.52	24.13±2.98
4	Perianal gland adenoma	4.21±1.23	42.36±2.69
5	Melanoma	---	32.14±4.96
6	Anal sac adenoma	4.32±1.05	48.32±5.32
7	Apocrine adenoma	3.12±1.02	45.23±5.24
8	Mammary Adenocarcinoma	6.21±0.41	321.38±4.78
9	Squamous cell carcinoma	6.23±1.42	224.67±6.45
10	Sertoli cell tumour	8.51±2.12	196.25±20.32
11	Ovarian adenocarcinoma	7.28±2.12	56.42±0.98
12	Basal cell carcinoma:	5.78±1.87	312.32±8.42
13	Malignant melanoma	--------	88.89±4.23
14	Seminoma:	8.51±2.12	102.32±2.45
15	Prostatic adenocarcinoma	5.26±1.54	88.42±9.54
16	Transitional cell carcinoma:	7.45±2.12	54.87±6.87

Note: *AgNOR and PCNA indices were positively correlelated.*
Correlation was significant (P<0.01)

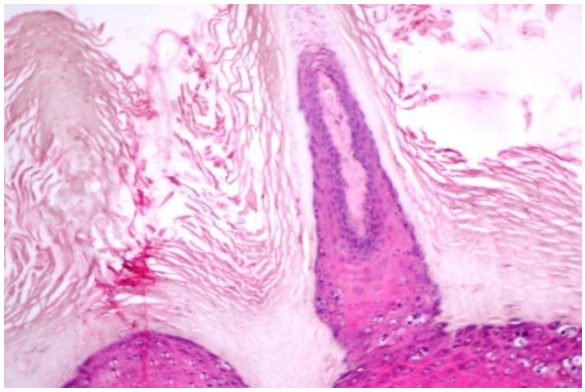

Figure 1: Skin papilloma showing elongated papillae with keratotic epidermis HEX100

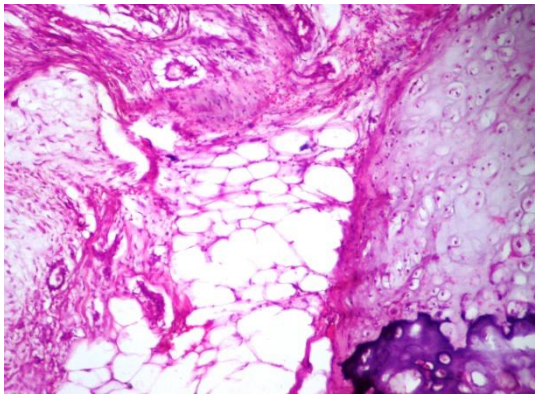

Figure 2: Benign mixed mammary tumor showing the areas of lipid, cartilage and fibrous tissue HEX200

Figure 3: Gross picture of Labrador showing perianal gland adenoma.

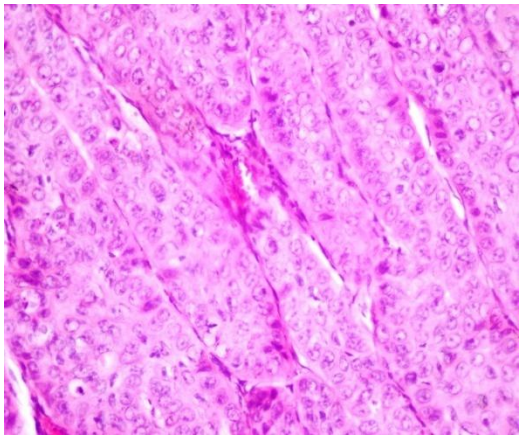

Figure 4: Perianal gland adenoma showing lobules of hepatoid cells surrounded by layer of reserve cells. HEX200

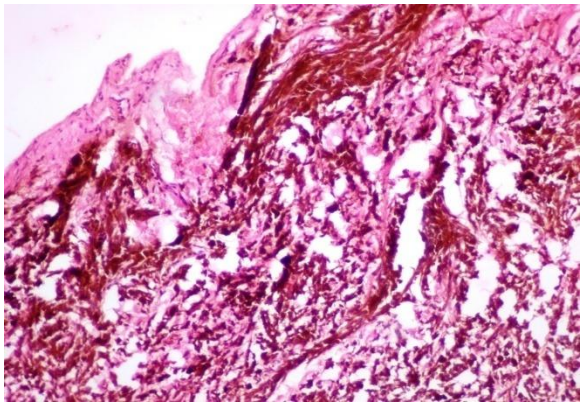

Figure 5: Melanomashowing the neoplastic cells in dermis containing brown pigment. HEX100.

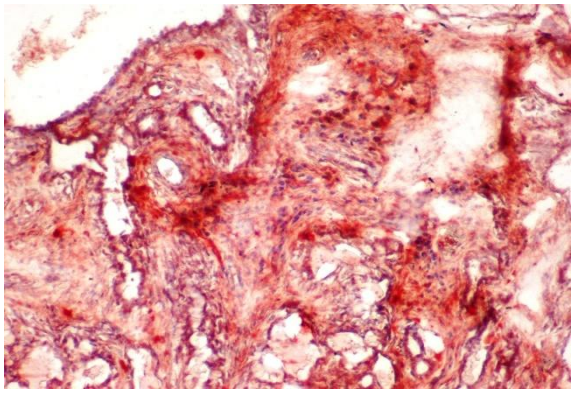

Figure 6: Anal sac adenoma showing PCNA positive nuclei. IP-AEC-MHX200.

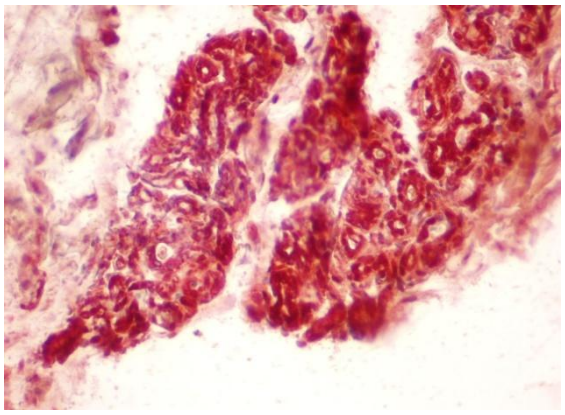

Figure 7: Papillary adenocarcinoma of mammary gland showing the PCNA positive nuclei.IP-AEC-MHX200.

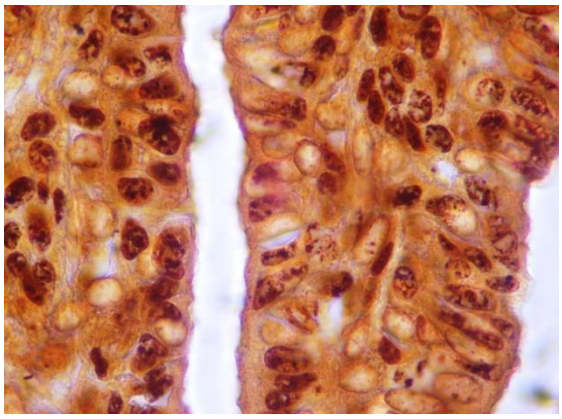

Figure 8: Papillary adenocarcinoma of mammary gland showing diffusivelyscattered dark stained AgNORdots. AgNORx200.

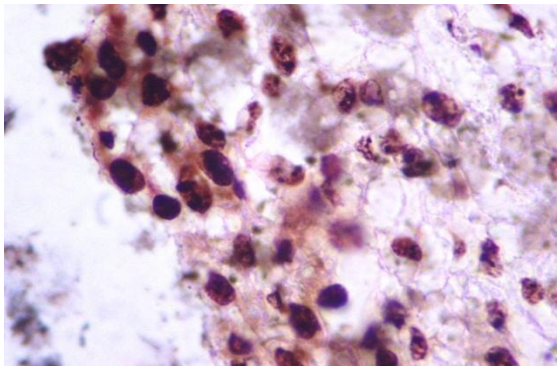

Figure 9: Sertoli cell tumour showing shrunken tubules with conspicuous lumen, cells with PCNA positive nuclei. IP-AEC-MHX200

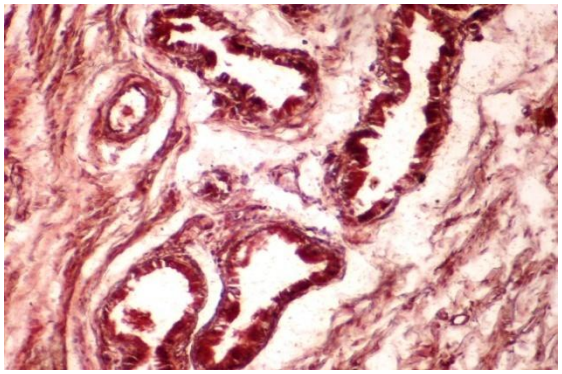

Figure 10: Ovarian adenocarcinoma showing the multiple AgNOR dots with in nuclei. AgNORx1000.

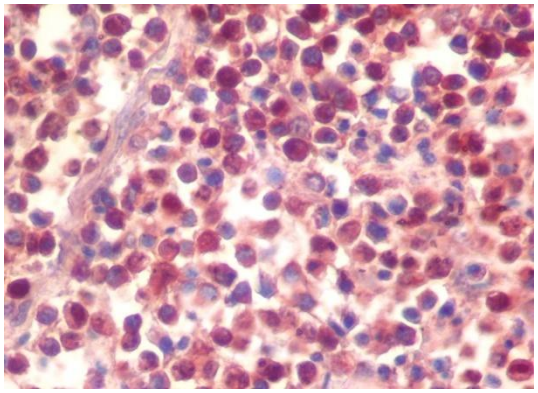

Figure 11: Seminoma showing the polyhedral cells with PCNA positive nuclei and moderate stroma. IP-AEC-MHX200.

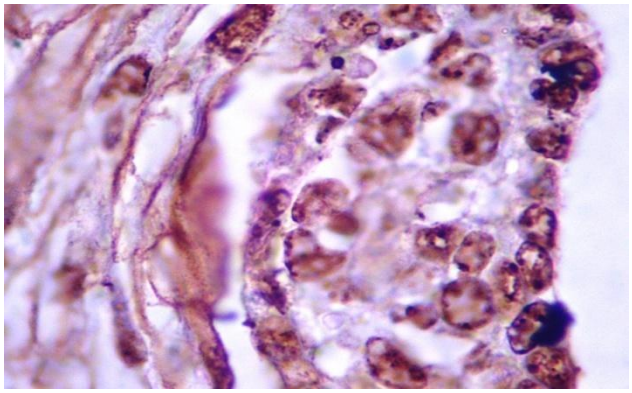

Figure 12: Prostatic adenocarcinoma showing the multiple AgNOR dots with in nuclei. AgNORx1000.

Prostatic Adenocarcinoma: Prostate adenocarcinoma was observed in 1 (4.76%) male Labrador dog of 12 year old. The tumour was 8 cm in diameter extending up to skin and was soft in consistency with grayish white surface. The dog showed symptoms of abdominal pain and urinary incontinence. Uptal Das *et al.,* (2000) recorded similar symptoms. Histologically, lobules of tumour tissue were separated by fibrous stroma and AgNOR dots in nuclei (Figure 12). The cells were intra alveolar in arrangement round to cuboidal in shape with moderately hyperchromatic nuclei. Ducts become distend into cystic structure (MacLachlan *et al.,* 2002).

Transitional Cell Carcinoma: Transitional cell carcinoma was found in 1 (4.76%) dog. Tumour was located at the trigone area of urinary bladder cream in colour and firm in consistency. Histologically, tumour revealed papillary growths that projected into lumen of urinary bladder Papillae were tall, covered by multiple layers of neoplastic urinary epithelium that had marked cellular atypica and forming signet ring cells microcysts. Tumour cells infiltrated in to the stalk and substantia propria. The results were in accordance with Moulton (1961)

Conclusion

Incidence of papilloma and mammary tumours was highest among beningn and malignant epithelial tumours respectively. The mean number of AgNOR in benign tumours was significantly (P>0.05) lower than the malignant tumours. Increased indices of AgNOR counts and PCNA associated with mailignancy and poor prognosis.

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