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STUDY AND MANAGEMENT OF RECURRED VAGINAL TUMORS IN BITCHES

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ABSTRACT
Bitches (n-14) of different breeds with recurred vaginal tumour were undergone both tumor resection and midventral complete bilateral ovariohysterectomy (OHE). This clinical research was conducted to find out proper treatment of recurred vaginal tumors as well as histopathogenesis of the same. Serum estradiol level estimated and analysed statistically in all animals, 138.71 ±20.26 pg/ml in pre-ovariohysterectomy as well as 63.71 ±16.88 pg/ml and 29 ±12.29 pg/ml at two and four months post-ovariohysterectomy period, respectively. Histopathological study by staining with Haematoxylin and Eosin stain of all vaginal tumors was confirmed the diagnosis of leiomyoma in six cases, fibroleiomyoma in four cases, leiomyosarcoma in two case and leiomyosarcoma alongwith adenocarcinoma in two cases. No evidence of local or distant metastasis was observed by exploratory laparotomy during OHE and by chest x-ray post-operatively in four cancer cases. Scanning electron microscopy (SEM) of the tumor tissue was done in four malignant cases. Anti-estrogenic aromatase inhibitor exemestane was tried post-operatively in all the cancer cases to control further growth. In all the cases no report of recurrence till one year post- operation was noticed. This result reports again the need of ovariohysterectomy in vaginal tumor and anti-estrogenic trials for the malignant birth canal tumor.

KEY WORDS: Vaginal tumors, Serum Estradiol, Panhysterectomy, Scanning Electron Microscopy, Exemestene.

INTRODUCTION

Vaginal and vulval tumors account for about 40% of reproductive tract tumor in canine (Johnston *et al.*, 2001) and are the 2nd most common canine reproductive system tumor after mammary tumor in bitches (Morris and Dobson, 2001) which. Out of 119 canine tumor cases presented in 2009 and 2010 in university clinics, Kolkata, India 14 was vulvovaginal tumors. All these were recurred case after primary excision elsewhere without ovariohysterectomy (OHE). The expression of oestrogen receptor-α (ERα) and progesterone receptor (PR) were found in genital tract tumor of canine and human (Milan *et al.*, 2007). Frequent metastasis of malignant tumor to illiac and abdominal lymphnode and infrequently to abdominal organ, lung and cervical spinal cord had been noticed (Meuten, 2002). Sometimes cancer becomes impossible to classify whether those are of epithelial or mesenchymal origin. That is why to get significant answer this study has been undertaken to evaluate the effect of ovariohysterectomy (OHE) on tumor recurrence probably mediated by the receptors. Another purpose of this study was to analyse serum estradiol level preoperatively & post-operatively for finding out hormonal influence on tumerogenesis. Study on metastasis of malignant Vulvovaginal tumors was followed to access their wickedness. Ultra structural study done in this work by Scanning Electron Microscopy (SEM) was imperative to investigate the cellular origin of the controversial histogenesis (Ferenczy, 1976).

MATERIALS & METHODS

All fourteen cases of vaginal tumours with symptom of "popped out" mass (Thacher and Bradley, 1983), vulval bleeding (Fig: 1A), tenesmus, dysuria and dyschezia presented in university clinics in the year 2009 and 2010 were recurred vaginal tumor cases after earlier primary tumor excision done elsewhere without OHE. Breed wise affected animals were 2 Spitzs, 6 Labrador Retriever, 2 German Shepherds, 2 mixed and 2 mongrel. Ages of affected bitches were between 1.5 years and 10 years.

After appropriate pre-operative evaluation all animal were undergone through midventral complete bi-lateral ovariohysterectomy along with tumor excision through episiotomy (Fig: 1B) on the same day. Atropine sulphate premedication, xylazine hydrochloride @1.5 mg/kg bwt i/m sedation were done outside the operation table and after a gap of 15minutes successively ketamine hydrochloride slow i/v @ 2mg/kg bwt and diazepam 1 mg/kg I/V in repeated doses were suitable for surgical operation with satisfactory muscle relaxation in these bitches. Sometimes second i/v admininistration of ketamine hydrochloride and diazepam required @ 1mg and 0.5 mg /kg bwt respectively until application of skin sutures (Lin, 1996). Surgical procedures were followed as per norms (Morrison, 2002).

FIGURE: 1A.Gross picture of vulvovaginal tumor

FIGURE: 1B. Resection of Vaginal tumor invading urinary meatus

Polycystic ovaries were found in few cases. Necessary post-operative medications were administered. Wound healings were uneventful. In four cases complete tumor removal was not possible to avoid the damage of urinary meatus. After histopathological confirmation of leiomyosarcoma in those four animals anti-estrogenic tablet Aromasin® (Exemestane) (Pfizer limited, Mumbai) 25 mg p.o. daily were given for 15 days starting after 2 months post-operation.

Periodical clinical check-up i.e. examination of vulva and vagina for any growth, checking for urinary incontinence of all operated animals were done on the day of postoperative blood collection and afterwards up to 1year at two month interval. No history or presence of any vulval discharge was observed during check up and telephone report. Histopathological analyses with Hematoxylin & Eosin stain (H&E) of all the excised tumors were done. Scanning Electron Microscopic (SEM) examinations (Bozzola and Russell, 1999) of vaginal sarcomas were made following histopathological examination. The tissue sample was preserved in 2.5% glutaraldehyde, processed, sputter coated (Bozzola and Russell,1999) and image was taken by Everhart-Thornley Detector (ETD) of FEI, Quanta 200 model, U.S.A. at 25 Kv. Visual Metastatic studies of malignant vaginal tumors were followed through Peri-operation exploration of abdomen as well as pre and post-operation thoracic radiography.

Analysis of serum estradiol preoperative as well as two and four months post-operation was done for hormonal influence on tumerogenesis. Serum esradiol was estimated by delayed competitive enzyme immunoassay method using the reagent of Monobind Inc., USA. This serum estradiol levels were analysed by statistical method using General Linear Model of SPSS software package developed as per the procedure of Snedecor and Cochran (1994).

RESULTS

TABLE 1. Mean±SE value of Serum Estradiol of all cases at different intervals (pg/ml)

Pre Ovariohyterectomy (E_2) (Pg/ml)	Post-ovariohysteectomy 2month (E_{2A}) (Pg/ml)	Post-ovariohysterectomy 4month (E_{2B}) (Pg/ml)
138.71±20.26	63.71±16.88	29±12.29

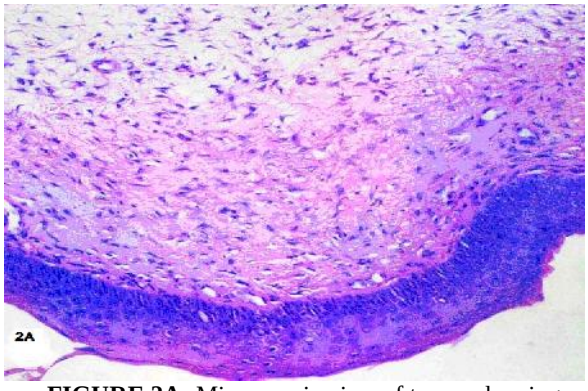

FIGURE 2A: Microscopic view of tumor showing Fibroleiomyoma H & E 40x.

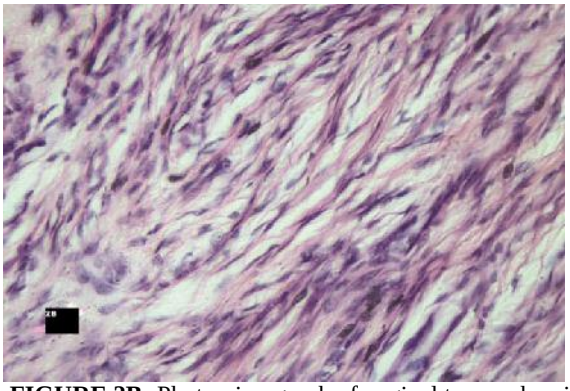

FIGURE 2B: Photomicrograph of vaginal tumor showing Leiomyoma.H& E 40x.

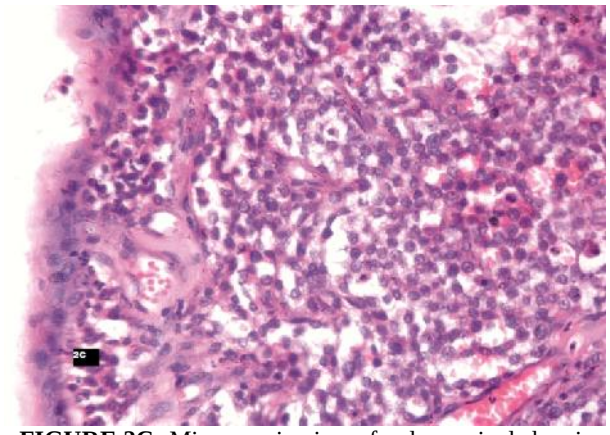

FIGURE 2C: Microscopic view of vulvovaginal showing leiomyosarcoma along with adenocarcinoma. H & E 20x

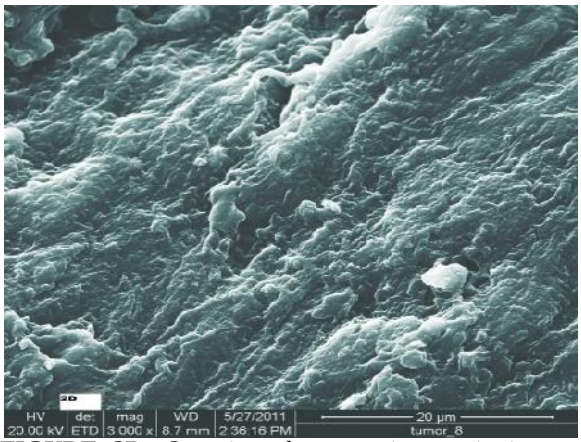

FIGURE 2D: Scanning electron microscopic image of vulvovaginal leiomyosarcoma .3000x Study on tumor recurrence

Above table show that the variation of pre and post-operative values of serum estradiol were highly significant and those between two post-operation periods also were significant. The hormone levels of intact bitches were slightly above the normal range.

Histopathological and ultra structural findings

The histopathological finding of six cases revealed typical case of leiomyoma (Fig:2B). Samples of tumors taken from other four bitches had microscopic picture of leiomyosarcoma. In two of the leiomyosarcoma cases carcinomatus elements were highly differentiated within the lining epithelium of vagina and it represented typical cases of adenocarcinoma alongwith Leiomyosarcoma (Fig: 2C). The slides obtained from vaginal tumor of last four bitches demonstrated a typical fibroleiomyoma (Fig: 2A).

SEM Study on the Leiomyosarcoma revealed that immature myofilaments were densely packed with irregular arrangement and often deposited in haphazard manner. Sometimes myofilaments were most abundant in well differentiated than that of the poor variety in which they appear as sparse and short bundles (Fig: 2D).

In all the cases no report of recurrence till one year post-ovariohysterectomy was noticed. No report of vaginal bleeding or urinary incontinence or dyschezia was noticed. Each animal was leading healthy life. In four cases where Aromasin® (exemestane) tablets were fed, spontaneous regression to very small sized tumor involving inaccessible area of urinary meatus were noticed within postoperative one year.

Study on tumor metastasis

Local and distant metastasis of leiomyosarcoma cases was not observed in abdomen and thorax as revealed by exploration of abdomen during ovariohysterectomy and radiography of thorax.

DISCUSSION

The normal Estradiol level in bitch is 2-10 pg/ml upto the peak level of 50-100 pg/ml at late proestrous. Higher level of pre-operative serum estradiol 138.71 ±20.26 pg/ml is perhaps because of presence of follicular cysts found in the ovaries during panhysterectomy in few of the cases and this probably has a key role in tumorogenesis through Estrogen receptors(ER) as shown also by Millán *et al*. (2007) in canine genital tract tumors either benign or malignant, pure or mixed. Significant positive correlations also were observed in receptor immunostaining in some vaginal and vulvar cell groups and the serum concentrations of estradiol-17β, but not with the serum progesterone concentration (Hilde *et al*., 2002). In the nucleus, estrogen mainly modulates gene transcription, and the resulting protein products determine the cell biological actions of the sex steroid (Levin, 2005). Removal of both ovaries were effective as evidenced by non-recurrence of the vaginal tumors though the higher post-hysterectomy estradiol (29 ±12.29 pg/ml) may be correlated with the post-menopausal estrogen produced by aromatase like enzyme from secondary source in human as observed by Noguchi *et al.* (1993). Primary tumour resection was not sufficient as recurrence rate of tumor was 100% in study group with intact bitches. Midventral incision was used for complete bilateral ovariohysterectomy because the flank approaches with limited exposure of the abdomen impending access to the ovaries or uterine body might cause post-operation remnant syndrome or perioperation complication (McGrath and Hardie, 2004).

In postmenopausal women conversion of adrenal androgens (androstenedione and testosterone) to estrogens (estrone and estradiol) is made by the aromatase enzyme through the cytochrome P450 enzyme complex in peripheral tissue like muscle and fat (Brueggemeier *et al*., 2005). Like estrogen deprivation through aromatase inhibition as effective and selective treatment for some postmenopausal patients with hormone-dependent breast cancer exemestane (Aromasin®, Pfizer Limited, Mumbai, India), a steroidal aromatase inactivator, the natural substrate androstenedione acting as a false substrate for the aromatase enzyme caused "suicide inhibition" of the later in four out of fourteen cases where complete tumor removal was not possible, histopathologically those cases were leiomyosarcoma and exemestane was given to check further growth of the tumor.

Tumor samples were preserved and histopathological study was done to access types of tumor prevalent in

kolkata. The histopathology of six vaginal tumor sections revealed leiomyoma, a positively correlative feature as also described by Robby *et al.* (2009), Meuten (2002), Nucci and Oliva (2009) and Jubb *et al.* (1993). Histopathological section of tumor taken from other four bitches revealed typical case of leiomyosarcoma. The histopathological features of leiomyosarcoma can be corroborated with the findings of Nucci and Oliva (2009), Meuten (2002), Weiss *et al*. (2003) and Robby *et al.* (2009). In two of the four leiomyosarcoma cases, carcinomatus element was highly differentiated within the lining epithelium of vagina and it represented a mixed and rare type of adenocarcinoma alongwith Leiomyosarcoma. The other four histopathological slides of vaginal tumor revealed a typical fibroleiomyoma. The microscopical features are similar with the descriptions of Meuten (2002) and Moulton (1978). Robbins (2002) suggested that 70% of vaginal tumors are benign which include mainly Leiomyoma (43%), Fibroleiomyoma, Fibroma, lipoma and malignants (30%) types mainly include Leiomyosarcoma. This observation is similar type as of the later worker but an inference about the above cannot be drawn as more number of clinical cases is needed.

Following the Ferenczy (1976).) and Morris *et al.* (1986**)** simple SEM was studied here and a few observations were noted. Corrosion casting SEM will be more useful for tumor angiogenesis study as shown by Walocha *et al*. (2003). However Transmission electron microscopy (TEM) will be much better for in-depth information for histogenesis. Meuten (2002) obsereved frequent metastasis of vaginal leiomyosarcoma to spleen and different abdominal lymph nodes whereas distant metastasis to lungs and other organs was infrequent. This study also demonstrated neither distant nor local and abdominal organ metastasis of malignant vaginal tumors in laparotomy exploration or radiographic record and physical examination and corroborates with the study by Robins (2002).

In brief it may be concluded that bi-lateral ovariohysterectomy as protocol can restrict tumor recurrence as experienced in these referred cases. Vulvo-vaginal tumor may be Leiomyoma, fibroleiomyoma or leiomyosarcoma and mixed rare type of leiomyosarcoma alongwith adenocarcinoma. Absence of both the ovaries cannot pull down the serum estrogen to minimum level all the times. Aromatase inhibitor can be tried for the treatment of malignant Vulvo-vaginal tumors as experienced in this pilot trial. SEM is not enough informative to understand the sub-cellular dynamics and pathogenetic development of tumor.

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REFERENCES

Bozzola, J. and L. Russell (1999) *Electron Microscopy*, 2nd ed. Canada, J.B. pub., pp :65-66.

Brueggemeier, R.W., J.C. Hackett and E.S. Diaz-Cruz (2005) Aromatase Inhibitors in the Treatment of Breast Cancer, *Endocrine Reviews*., 26: 331.

Cochran , P. (2010) Laboratory Manual for comperative veterinary anatomy and physiology, 2nd ed. USA.,D.C. Learning, pp: 31-33.

Ferenczy, A. (1976) The ultrastructural morphology of gynaecologic neoplasms. *Cancer.* , 38:463-486.

Hilde, V., Broeck WVD. and Paul, S. (2002) Immunolocalization of sex steroid hormone receptors in canine vaginal and vulvar tissue and their relation to sex steroid hormone levels. *Reproduction, Fertility and Development*, 14 (4): 251–258.

Johnston, S.D., Kustritz, M.V. and. Olsan, P.N.S. (2001) Canine and Feline Theriogenology. U.S.A., Elsiever, pp: 237-41.

Jubb, K.V.F., Kennedy, P.C. & Nigel, P. (1993) Pathology of domestic animals.3rd ed. London, Academic press.pp: 451.

Levin, E.R. (2005) Integration of the extranuclear and nuclear actions of estrogen. *Mol Endocrinol.*,19(8):1951-9.

Lin, H.C. (1996) Dissociative anesthetics. In Lumb and Jones' Veterinary Anesthesia, 3rd edition.Thurmon, JC, WJ. Tranquilli, GJ. Benson (eds.) Philadelphia, William and Wilkins.pp:241- 296.

McGrath, H. and RJ. Hardie (2004) Lateral Flank Approach for Ovariohysterectomy in Small Animals. Compendium, 26:197-201.

Meuten, D.J. (2002) Tumour in domestic animals. 4th edn. Iowa, U.S.A., Iowa state University press.pp:336-41.

Millán, Y., Gordon, A., Espinosa de los Monteros, A., Reymundo, C. and Martín de las Mulas, J. (2007**)** Steroid Receptors in Canine and Human Female Genital Tract Tumours with Smooth Muscle Differentiation. *Journal of Comparative Pathology* ,136(2-3)**:**197-201.

Morris, J. and Dobson, J. (2001) Small Animal Oncology. USA, Blackwell Science Ltd. pp: 171-174.

Morris, J.A., Oates K. and *S*taff, W.G. (1986) Scanning Electron Microscopy of Adenomatoid Tumours. *British Journal of Urology*, 58(2-4): 183–187.

Morrison, B.W. (2002) Cancer in dogs and cats: Medical and Surgical management 2nded. Teton new media.pp:296-98.

Moulton, J.E. (1978) Tumors in domestic animals, 2nd ed., London, University of California press, pp: 1-13.

Noguchi, T., Kitawaki , J., Tamura , T., Kim , T., Kanno, H., Yamamoto, T. and Okada, H. (1993) Relationship between aromatase activity and steroid receptor levels in

ovarian tumors from postmenopausal women. *J Steroid Biochem Mol Biol.,*44(4-6):657-60.

Nucci, M.R. and Oliva. E. (2009) Gynaecologic Pathology, UK Elsevier, pp:279-81.

Robbins, M. (2002) Reproductive Oncology. In:Slatter ed.Text book of Small Animal Surgery. 3rd edn., U.S.A., Elsiever, pp:2437-2443.

Robby, S.J., Mutter, G.L., Prat, J., Benley, R. and Russell, P. (2009) Robboy's pathology of female reproductive tract, U.K., Elsiever, pp: 470-75.

Snedecor, C.W. & Cochran, W.G. (1994) Statistical methods.8th edn., New delhi, Est-West press, pp: 254-268.

Thacher, C. & Bradley, R.L. (1983) Vulvar and vaginal tumors in the dog: a retrospective study. *J Am Vet Med Assoc.*183(6):690-2.

Walocha, J.A., Litwin, J.A. and Miodoński, A.J. (2003) Vascular system of intramural leiomyomata revealed by corrosion casting and scanning electron microscopy. *Hum Reprod.* , 18(5):1088-93.

Weiss, S.W., Goldblum, J.R. and Enzinger, F.M. (2001) *Enzinger and Weiss's soft tissue tumor.* St Louis, Mosby. pp: 727-731.