



Electrosurgical Excision and Differential Pathological Diagnosis of External Genital Tumors in Bitch

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ABSTRACT

Forty-five specimens from Various dog breeds were examined at animal reproduction research institute, agriculture research center, Egypt over 18-month period. Dogs' age ranged from 2 to 5 years. The mean weight was 25.6 kg. The specimens were diagnosed as vaginal mass, and bleeding, accompanied with ultrasonography imaging to find out any metastatic mass in abdomen region. A local anesthesia was induced by lidocaine HCL 2% (1-2ml) to provide analgesia following by xylazine -ketamine (1 mg/kg BW+10 mg/kg BW, I.M) for induction of general anesthesia. Then the tumor was excised by the electrosurgical technique (high frequency radio waves) which eliminates nerve and muscle stimulation; the cutting wave forms vaporize the cellular fluid causing cellular explosions. The tumor mass was preserved in formalin solution and submitted for histopathological and immunohistochemical evaluation using multi-cytokeratin, vimentin, S-100, CD79, p53, Ki 67, PCNA, estrogen receptor and progesterone receptors as well as actin and desmine. Thirty cases of Transmissible venereal tumors (TVT), six cases of Fibroma and three cases of Fibrosarcoma as well as three cases defined as Lipoleiomyoma or atypical leiomyoma (leiomyoma with bizarre nuclei) (ALM), and finally three cases leiomyosarcoma (LMS) of the vagina were distinguished. Moreover, we find that, malignant tumors (TVT, Fibrosarcoma and Leiomyosarcoma) were more frequent in older age while the benign were more in younger (fibroma and Leiomyoma). As German Shepard and Golden Retriever are more susceptible to TVT at average age 3 -4 years and 2-3 years whereas Pitbull and Rottweiler more affected with fibroma and fibrosarcoma at age 2-3 years while Leiomyoma (LM) and Leiomyosarcoma (LMS) were more frequent in Golden Retriever at age 2-3 and, 3-4 years respectively on the other hand Fibrosarcoma more frequently at 3-4 years age followed by Leiomyoma (LM) at 2-3 years age and finally Leiomyosarcoma (LMS) 3-4 years age. Finally, all the cases were subjected to a checkup monthly for six months after surgery and take special procedures and precautions if need in cases of malignancy. We concluded that, electrosurgery provides atraumatic, scalpel-like cutting precision and hemostasis, that resulting in reduced bleeding and tissue injury. Adding to, the important role of histopathology and immunohistopathological as well as hormonal receptors picture for diagnosis and differential diagnosis of vaginal tumors for the follow up of cases after surgery and take special percussions if need in cases of malignancy.

Key words:

Canine tumors;
electrosurgery;
Histopathology;
Immunohistochemistry;
Transmissible venereal tumors;
Fibroma;
Fibrosarcoma;
Lipoleiomyoma;
Leiomyosarcoma.

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1- INTRODUCTION:

Neoplasms of the female genital system of dogs occur mainly in old animals and early detection is difficult as in most cases (Dorn and Priester, 1987). Surgical

excision is the first therapeutic choice when total ablation is possible (Salomon, et al., 2004). Radio surgical incision should be made by using a smooth continuous motion. Unlike a scalpel blade, a radio surgical electrode cuts without pressure. The character

of the cut made with radio surgery is determined by the type of electrode, contact time with the tissue, intensity of power, nature of the radio wave frequency (Miller et al., 2004)

Electrosurgery uses radio frequency (RF) alternating current to heat the tissue by RF induced intracellular oscillation of ionized molecules that result in an elevation of intracellular temperature as means to cut, coagulate, desiccate, or fulgurate tissue (Boughton and Spencer 1987; Hainer, 1991; Reidenbach, 1993; Hyfrecator, 2000 and Barry et al., 2002). The benefits of electrosurgery include the ability to make cuts with limited blood loss (McCauley, 2003). Four waveforms are used in radiosurgery. The fully filtered, or continuous, waveform is a continuous high frequency wave that produces a smooth cutting action. The continuous waveform generates the least amount of lateral heat. When the continuous waveform is delivered by a fine electrode, the incision is comparable to that made by a scalpel (Miller et al. 2004).

The violent exertions associated with coitus in the dog render both sexes prone to genital injury and susceptible to transplantation of the tumor cells. The tumor may be also transmitted when a susceptible dog first licks the genitals of an affected bitch and then its own or those of another susceptible dog (Das and Das, 2000; Baba and Cătoi, 2007; Baştan et al., 2008 and Gupta and Sood, 2012). The histopathological features of genital tumors are previously differentiated as leiomyoma which is the most frequently in the bitch lymphomas (MacLachlan and Kennedy, 2002), melanomas (Temitope et al., 2010) and fibrosarcoma (Al-Kenanny, et al., 2013). While leiomyosarcomas are considered uncommon lesions (Schlafer and Miller, 2007). Transmissible venereal tumors (TVT) are normally seen on the genitalia of both male and female dogs, and could be extra-genital such as mammary gland, liver, spleen, kidney, and lung (Gupta and Sood, 2012). Mascarenhas et al. (2014) have excluded immunohistochemically the previous reports about the histiocytic tumor origin of TVT cells as expressing macrophage markers.

Immunohistochemistry plays a critical role in identifying the cell lineage and probable site of origin. Vimentin is an intracellular fibrous protein characteristic of mesenchymal cells. It is almost found in sarcomas, melanomas, and variably in lymphomas. Markers of proliferation as Ki-67, Proliferating cell nuclear antigen (PCNA) and p53 which are used for assessing the proliferation index in human as well as animal neoplasms. They also associated with DNA

repair and persists after the end of mitosis. Additionally, p53 which is one of most important tumor suppressor genes that involved in the development of neoplasia (Sanchez et al., 2009; Moro et al., 2010 and Stella et al., 2018). A screening panel consisting of cytokeratin (CK), S100 protein, vimentin, and leukocyte common antigen (LCA) should be applied first as a general approach to a tumor of unknown origin. In general, mesenchymal tumors and hematopoietic neoplasms of lymphoid lineage, express vimentin and/or epithelial markers LCA (CD45), but negative staining for S100 and CK, which are highly specific for melanocytic or neurogenic tumors (Kandukuri et al., 2017).

Effective treatment methods for external vaginal tumors include surgical excision, cryosurgery, immunotherapy and chemotherapy. Surgical excision is the most successful in animals with few, small, circumscribed, accessible lesion with no local invasion or metastases, although the recurrence rate can be as high as 50 - 68% in cases of large invasive tumors (Weir et al., 1987). Contamination of the surgical site with TVT cells is also a source of recurrence. Chemotherapy has been shown to be the most effective and practical therapy, by Vincristine sulfate being the most frequently used drug. A cure rate approaching 100% is achieved in cases treated in the initial stages of progression, especially in cases of less than one-year duration, disregarding the presence or absence of metastases (Boscos and Ververidis, 2004). Electrosurgical excision offers an added benefit of improved homeostasis, decreasing postoperative bleeding and negating the need to tie off bleeding vessels (Soleimani et al., 2013).

So, this study aimed to evaluate the surgical incisions and excision created by electrosurgical technique (high frequency radio waves) on genital tumors, as a new electrosurgical technique on genital tumors of bitch. Also, histopathological diagnosis and immunohistochemical evaluation for the specimens differential diagnosis were done for the follow up of cases after surgery and take special precautions if need in cases of malignancy.

2. MATERIALS AND METHODS:

Forty-five specimens from various dog breeds were examined at animal reproduction research institute, agriculture research center, Egypt over 18-month period. Dogs' age ranged from 2 to 5 years. The mean weight was 25.6 kg.

2.1. Physical examination.

2.2. Sonographic evaluations.

2.3. Abdominal and pelvic ultrasound for metastases.

2.4. Surgical interference:

i. Before the surgical operation the animal was fasted at least 12 hrs.

ii. Electrosurgical removal of the tumor mass was elected during the animal was prepared for surgery. Antibiotic was administered prior to operation to prevent the infection. A local anesthesia was induced by lidocaine HCL 2% (1-2ml) to provide analgesia following by xylazine -ketamine (1 mg/kg BW+10 mg/kg BW, I.M) for induction of general anesthesia. The animal was placed in a ventral or lateral recumbency with the legs hanging over the padded edge of the surgical table and the tail raised away. A purse string suture was placed around the anus to limit contamination during operation. The area was prepared for aseptic operation; urinary catheter was inserted into the urethral orifice to save the urethra.

Exposing for the tumor through the vaginal opening then holding of the tumor by forceps then complete excision of the tumor using monopolar loop electrode in cutting with hemostasis mode (Hermann Medizintechnik GmbH electrosurgical system HHF300). Fragile masses were removed manually. The urethral orifice was reconstructed and other residual tumor like tissues resected by monopolar loop electrode and the mucosal edges were sutured if needed and the vagina and the vulva were reconstructed to a normal anatomic appearance. The tumor mass was preserved in formalin solution and submitted for histopathological evaluation. Follow up the cases were done after one week then monthly for 6 months (Soleimani et al., 2013).

2.5. Pathological examination:

2.5.1. Histopathological examination:

The vaginal tissue specimens were examined for any gross pathological abnormalities. Then, initially fixed in 10% formalin solution, routinely processed and then stained with hematoxylin and eosin (H&E) and evaluated under light microscopy (Suvarna *et al.*, 2013)

2.5.2. Histochemical analysis: Tissue sections were stained with Masson-trichrome stain for the detection of collagen and elastic fibers changes and Sudan III for fat cell confirmation (Suvarna *et al.*, 2013).

2.5.3. Immunohistochemistry: It was performed on paraffin embedded sections using a panel of antibodies and avidin-biotin-peroxidase complex staining protocol (Dako, Glostrup, Denmark) for immunolabeling of multi-cytokeratin, vimentin, S-100, CD79, p53, Ki 67 Proliferating cell nuclear antigen PCNA, estrogen receptor (ER), progesterone receptors (PR), actin and desmine were performed to evaluate the immunohistochemical evaluation (Gruchala *et al.*, 1997 ; Mozos *et al.*, 1996; Rollon *et al.*, 2008). Sections were counterstained with Mayer's hematoxylin . Positive immunohistochemical controls for each one of the antibodies were used. For each antibody, a negative control was run by replacing the primary antibody with PBS buffer. The sections were incubated with the following primary monoclonal antibodies: mouse desmin Ab-1 (Clone D33, Dako, Glostrup, Denmark; 1:100) and mouse antibodies alpha smooth muscle actin (Clone 1A4, Ventana BenchMark, Tucson, AZ, USA; 1:250).

3-RESULTS:

3.1. Surgical interference:

In this study, the monopolar electrode (high frequency radio wave) exhibited a pronounced reduction in bleeding compared with conventional scalpel tool. This result suggested that the high operating temperatures and deep thermal coagulum associated with electrosurgery are always essential for hemostasis.

3.2. Incidence of tumor types in different bitch species and age:

From Tables 1: we find that, malignant tumors (TVT, Fibrosarcoma and Leiomyosarcoma) were more frequent in older age while the benign were more in younger (fibroma and Leiomyoma). As German Shepard and Golden Retriever are more susceptible to TVT at average age 3 -4 years and 2-3 years whereas Pitbull and Rottweiler more affected with fibroma and fibrosarcoma at age 2-3 years. While Leiomyoma (LM) and Leiomyosarcoma (LMS) were more frequent in Golden Retriever at age 2-3 and, 3-4 years respectively on the other hand Fibrosarcoma more frequently at 3-4 years age followed by Leiomyoma (LM) at 2-3 years age and finally Leiomyosarcoma (LMS) 3-4 years age.

Table 1: Summary of the No .and percentage of external genital tumor and their breed and age distribution (n.=45)

| Tumor type | Various dog breeds | | | | dog Ages | | | | | | | | | |
|--------------|--------------------|------|----------------|----|------------|------|------------------|-----|----------|-----|----------|------|----------|----|
| | Pitbull | | German Shepard | | Rottweiler | | Golden Retriever | | 2-3 year | | 3-4 year | | 4-6 year | |
| | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % | NO. | % |
| TVT | 6 | 13.3 | 9 | 20 | 6 | 13.3 | 9 | 20 | 9 | 20 | 12 | 26.5 | 9 | 20 |
| Fibroma | 6 | 13.3 | | | | | | | 3 | 6.7 | 3 | 6.7 | | |
| Fibrosarcoma | 3 | 6.7 | | | | | | | | | 3 | 6.7 | | |
| LM | | | | | | | 3 | 6.7 | 3 | 6.7 | | | | |
| LMS | | | | | | | 3 | 6.7 | | | 3 | 6.7 | | |

Table (2): Different used immunohistochemically used markers and their reaction in different tumor types in bitch vagina:

| The marker | Specificity | Positive immunoreactivity | Negative or weak immunoreactivity |
|--|--|---|---|
| Vimentin | Characteristic of mesenchymal cells, all sarcomas and melanomas and variably in lymphomas, hematopoietic neoplasms of lymphoid lineage, | TVT, LMS and Fibrosarcoma | Fibroma and LMs |
| Proliferative marker (PCNA), p53 and Ki67 | Proliferative markers are used for assessing the proliferation index | TVT, LMS and Fibrosarcoma | Fibroma and LMs |
| Leukocyte common antigen (LCA) | Epithelial tumors markers | TVT, LMS and Fibrosarcoma | Fibroma, and LMs |
| S100 and CK, ER and PR | Highly specific for melanocytic or neurogenic tumors | Melanoma and neurogenic tumors LMs strong expression LMS | TVT, LMs, LMS, fibroma and Fibrosarcoma LMS reduced expression LMS |
| SMA | Both for myogenic neoplasms (Variation in immunohistochemical reactivity to desmin & SMA) in LMS is diagnostic while both positive in LMs. | LMS | LMS |
| Desmine | | LMS | LMS |

In the present study, after excision of vaginal masses by the electrosurgical technique, they were differentiated using histopathological and immunohistochemical evaluation in relation to ultrasonography imaging. analysis of the forty-five tumor specimens from various dog breeds basing on the clinical examination, ultrasonographical, histopathological and immunohistochemical findings, revealed thirty cases of Transmissible venereal tumors (TVT), six cases of Fibroma, three cases of Fibrosarcoma as well as three cases defined as Lipoleiomyoma or atypical leiomyoma (leiomyoma with bizarre nuclei) (ALM), and finally three cases leiomyosarcoma (LMS) of the vagina.

3.3.1. Transmissible venereal tumors (TVT):

*Physical examination of those cases showed a large vaginal mass protruding from the vulva that appeared as a solitary, red, friable, often “cauliflower-like mass” (Fig.1) with history of lethargic, depressed and had serosanguineous vaginal discharge,

* Ultrasonographical evaluations revealed the presence of a heterogenous mass in the vaginal canal, measuring (5. 3× 3 .5 × 3 cm), extending from the vaginal fornix to the vestibule without any metastatic mass in the abdominal cavity.

* Pathological findings: In our study, this type was the most common observed type, they thirty cases that represented 67% of necropsies.

Macroscopically, most specimens revealed a nodular, friable, firm, pale-white, but multi-lobulated cauliflower-like mass which containing ulcerating, necrotic foci.

Microscopically, biopsied nodules were formed of neoplastic cells mainly (immature lymphocytes) with nodular arrangement as solid sheets, that supported by thin fibrovascular trabeculae. Those trabecular vessels revealed congestion and perivascular cellular infiltrations, mainly mature lymphocytes, plasma cells, neutrophils and macrophages (Fig.2). This supporting fibrovascular trabeculae were detected with Masson trichrome stain as blue collagen fibers (Fig. 3). Neoplastic cells were large, round with abundant vacuolated finely granular cytoplasm and large round nucleus. The Nuclei showed marginally clumped chromatin and contained one or more prominent nucleoli with few or frequently observed Mitoses (Fig. 4 and 5). Immunohistochemistry, as illustrated in Table (2) all ten cases, were positive for the novel markers of TVT in dogs., p53, PCNA, LCA (CD45 and CD 79) and vimentin, that, indicate the myeloid reticuloendothelial origin of lymphoid nature as well as high proliferative index and lymphoid malignancies (Fig. 6 ,7and8).

3. 3.2. Mesenchymal neoplasia: In our investigation, this type represented 33 % of specimens (15 out of 45) and distinguished to two types:

A- Vaginal Fibroblast cell tumor of the vagina: it was observed in this study in 9 specimens (6 cases vaginal fibroma and 3 cases vaginal Fibrosarcoma).

* Physical examination: It appeared as pale white vaginal tumor mass oval, large, firm, necked, with hemorrhagic base without necrosis that attached to vaginal wall and in some cases protruding from the vulva according to the time of formation (Fig. 9) with history of loss of body condition, depression a serosanguineous vaginal discharge.

* Sonographical evaluations, showed as a heterogenous mass in the vaginal canal, measuring about (5.2 × 3.4 × 4 cm) fibroma while Fibrosarcoma (5.2 × 3.2 × 5 cm), extending from the vaginal fornix to the vestibule, did not reveal any metastatic in the abdominal cavity.

* Pathological findings:

Grossly, they revealed large, firm, irregular, hard circumscribed growth mass with well demarcated outlines, white homogeneously cut surface and hemorrhagic base without necrosis in fibroma while in fibrosarcoma showed necrosis.

The microscopic examination of them showed spindle-shaped tumor cells forming interlacing and intersecting collagen bundles with a variable number of lymphocytes, eosinophils and plasma cells (Fig. 10). Abundant collagen fibers, that identified by Masson's trichrome staining (Fig. 11). In cases of fibrosarcoma, the neoplastic fibroblast cells showed cellular pleomorphism and nuclear hyperchromasia, karyomegaly with losing of polarity as well as most nuclei were oval shape containing one or more nucleoli (Fig. 12). The nuclear to cytoplasm ratio (N/C) was increased than in normal fibroblast. Giant cell was relatively few (Fig. 13). While in cases of vaginal fibroma revealed intersecting collagen bundles and fibroblast cells (Fig. 14).

Immunoreactivity, as illustrated in Table (2), showed Positive staining for vimentin (Fig. 15), P53 (Fig. 16) and LCA (CD45 and CD 79) (Fig.17) but negative staining for S100 and CK, which indicated lymphoid lineage.

B- Smooth muscle cell tumor of vagina:

In our investigation, this type of tumor noticed in six cases which are defined as three specimens of Lipoleiomyoma or atypical leiomyoma (leiomyoma with bizarre nuclei) (ALM), and finally three specimens as leiomyosarcoma (LMS), of the vagina.

*Physical examination, revealed the presence of large firm brownish-red, noncapsulated mass with areas of cyanosis and necrosis attached to vaginal wall (Fig.17) with history of body condition loss, depression with continuous bleeding as well as gradually increased in size over time.

*Sonographical evaluations revealed the presence of a heterogenous mass in the vaginal canal, one case of them measured as 7.4x6.4 cm, and the other case, measured about 6. 3 × 4 .3 × 3 cm.

* Pathological findings:

I-Lip leiomyoma of vagina (LM): It was atypical leiomyoma (leiomyoma with bizarre nuclei) (ALM) noticed in three cases.

Macroscopically, appeared firm, another part of the tumors included multiple nodules of varying sizes. The cut surface of these nodules was greyish-white in color Microscopically, the neoplasm was non-capsulated and predominantly composed of cells that resemble smooth muscle bundles admixed with lobules of mature adipocytes with minimal fibrous stroma (Fig.18) in which, the neoplastic smooth muscle cells invaded the adipose tissue stroma. Neoplastic cells were spindle, had an oval elongated nuclei and vesicular chromatins

well as abundant pink cytoplasm. Mitoses not observed (Fig.19). with the presence of bizarre leiomyoma cells in multiple foci with bizarre nuclei (Fig. 20). that appeared as numerous large atypical mononucleated or multinucleated cells with eosinophilic cytoplasm, karyorrhectic nuclei, prominent nucleoli and coarse chromatin.

II-Leiomyosarcoma of vagina (LMS): It noticed in three cases

Macroscopically, appeared large firm encapsulated brownish-red colored with areas of ulcerations and necrosis.

Microscopically, revealed proliferation of vacuolar degenerated and pyknotic smooth muscle-like cells that showing cellular polymorphism, anisokaryosis, multiple nucleoli as well as the presence of bizarre leiomyoma cells in multiple foci with bizarre nuclei and few tumor giant cells, in addition, appearance of perivascular lymphocytic aggregations with necrosis in some regions of the tumor with varying degrees

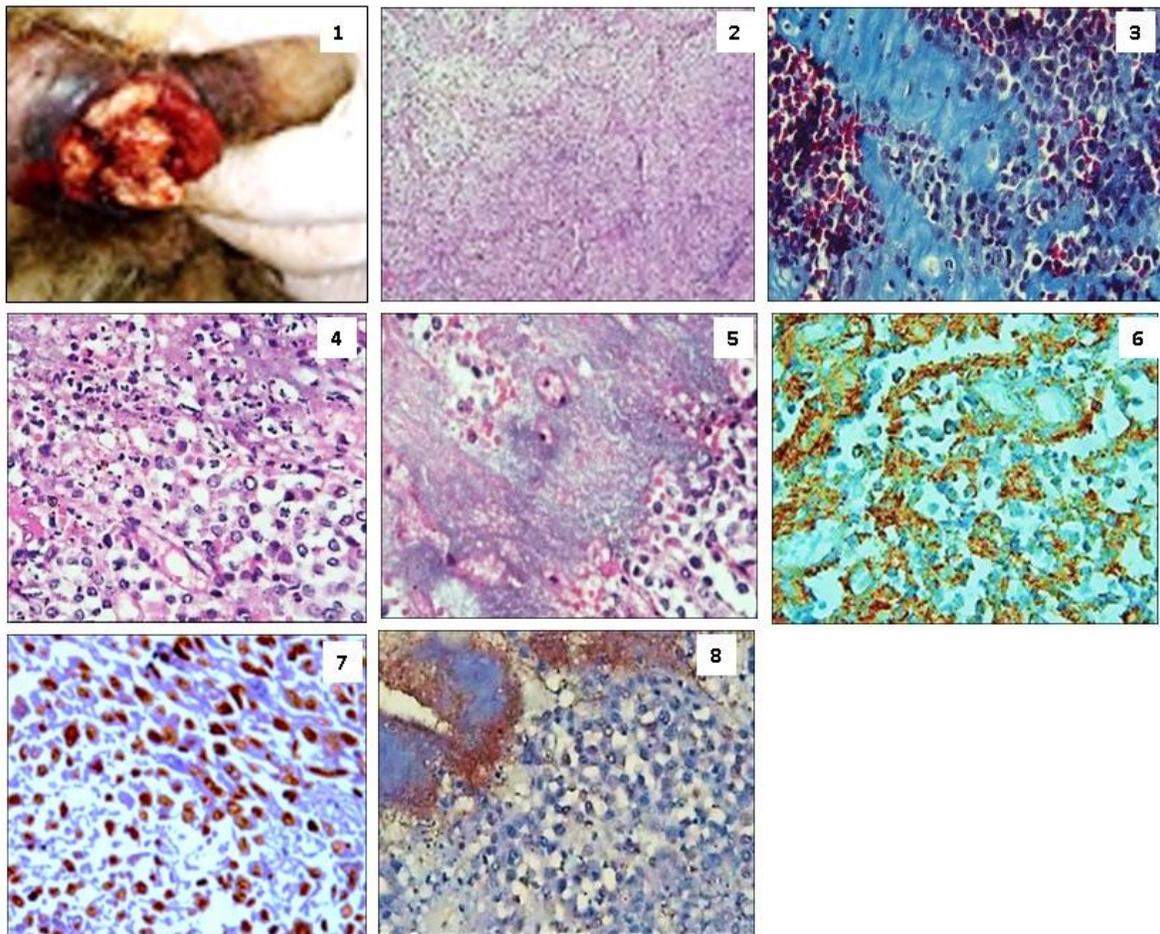


Fig. 1: A cauliflower like neoplastic growth of transmissible venereal tumor (TVT) that protruding from the vulva in bitch.

Fig. 2: Histopathology of biopsied nodule from the vagina. group of tumor cells arranged in sheets or rows within the stroma of thin fibrous connective tissue. H&E X 40.

Fig.3: Typical TVT revealed intersecting collagen bundles (Masson trichrome). X 40.

Fig. 4: Typical TVT cells, round to oval in shape, arranged in a cord like fashion along with a scattering of irregular connective tissue strands. H&E. x 400

Fig.5: The neoplastic cells infiltrated the dermis with hyperchromasia, indistinct cellular outline, round nuclei with numerous mitotic figures. H&E; x 400 .

Fig. 6: Moderate cytoplasmic immunoreactivity of vimentin in almost all neoplastic cells Mayer's hematoxylin counterstain x 400.

Fig. 7: Strong nuclear Proliferating cell nuclear antigen (PCNA) positivity in all neoplastic cells; Mayer's hematoxylin counterstain x 400.

Fig. 8: Strong P53 positive immunoreactivity in the neoplastic cells; Mayer's hematoxylin counterstain x 400.

. Moreover, a pronounced mainly hyalinized collagenous fibers admixed with the neoplastic cell (Fig.21) which confirmed by the Masson trichrome stain as greenish blue fibers (Fig. 22).

Immunohistochemistry, as described in Table (2), in the specimens of Lipoleiomyoma(LM) of dog vagina, revealed, either negative or weakly stained for p53, intensely stained for PR, strong positive diffuse uniform immunoreactivity to desmin and SMA and no immunoreactivity for cytokeratin or vimentin, Conversely. The cases of Leiomyosarcoma (LMS) showed strongly positive immunoreactive to p53, reduced PR expression. Variation in immunohistochemical reactivity to desmin and actin

SMA as a diagnostic feature of genital leiomyosarcomas strong diffuse cytoplasmic immunoreactivity for a-smooth muscle actin and weak or negative desmin (Fig. 23 and 24) and strong staining intensity for vimentin (Fig. 25) and p53 (Fig.26), as well as Ki67 (Fig.27), but pancytokeratin and S100 negative, also reduced progesterone receptors (PR) and estrogen receptors (ER) expression. While leiomyomas (LMs) either negative or weakly stained for P53 and Ki67, while intensely positive expression for estrogen ER in the epithelium (Fig. 28), and progesterone PR receptors in the stroma and smooth muscle (Fig. 29).

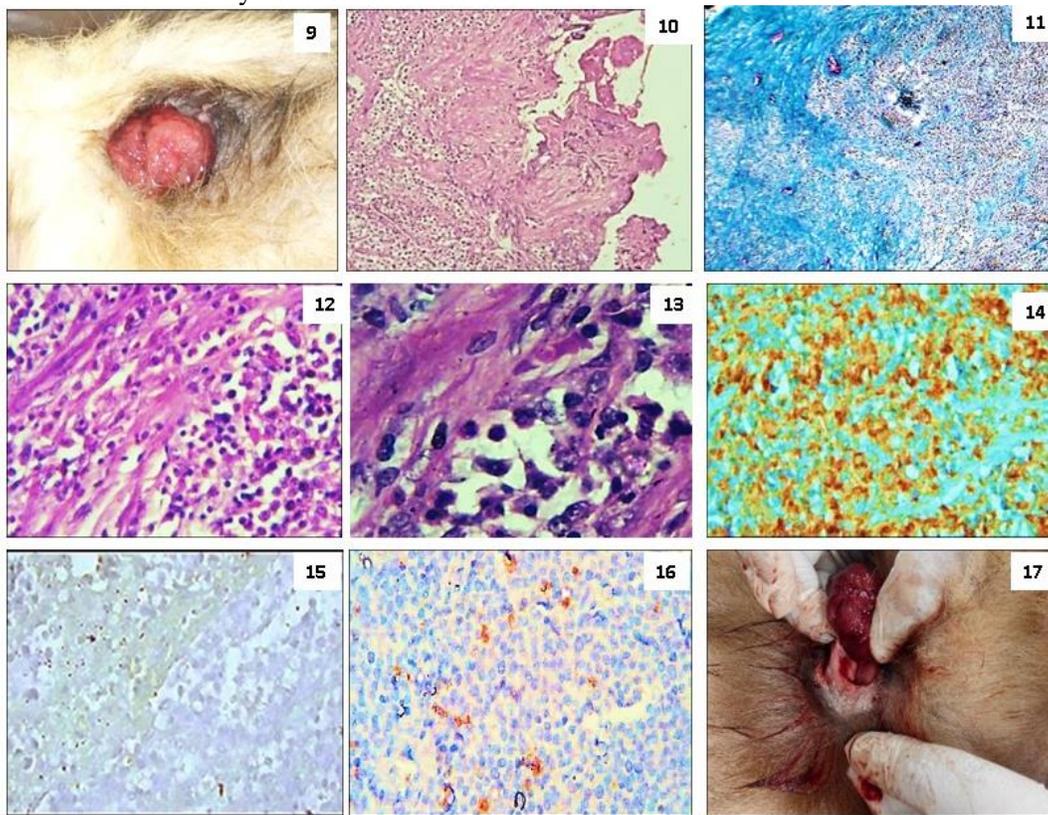


Fig. 9: Excised tumor mass measuring (5.2 × 3.2 × 5 cm). It is well demarcated and has a white cut surface. grow mass localized on vulvo-vaginal area of bitch and diagnosed as fibrosarcoma of vagina.

Fig. 10: vaginal fibrosarcoma revealed interlacing and intersecting collagen bundles H&E X 40.

Fig. 11: revealed interlacing and intersecting greenish blue collagen bundles (Masson trichrome) X 40.

Fig.12: vaginal fibrosarcoma in bitch showed fibroblast cells with cellular, nuclear pleomorphism H&E X 100.

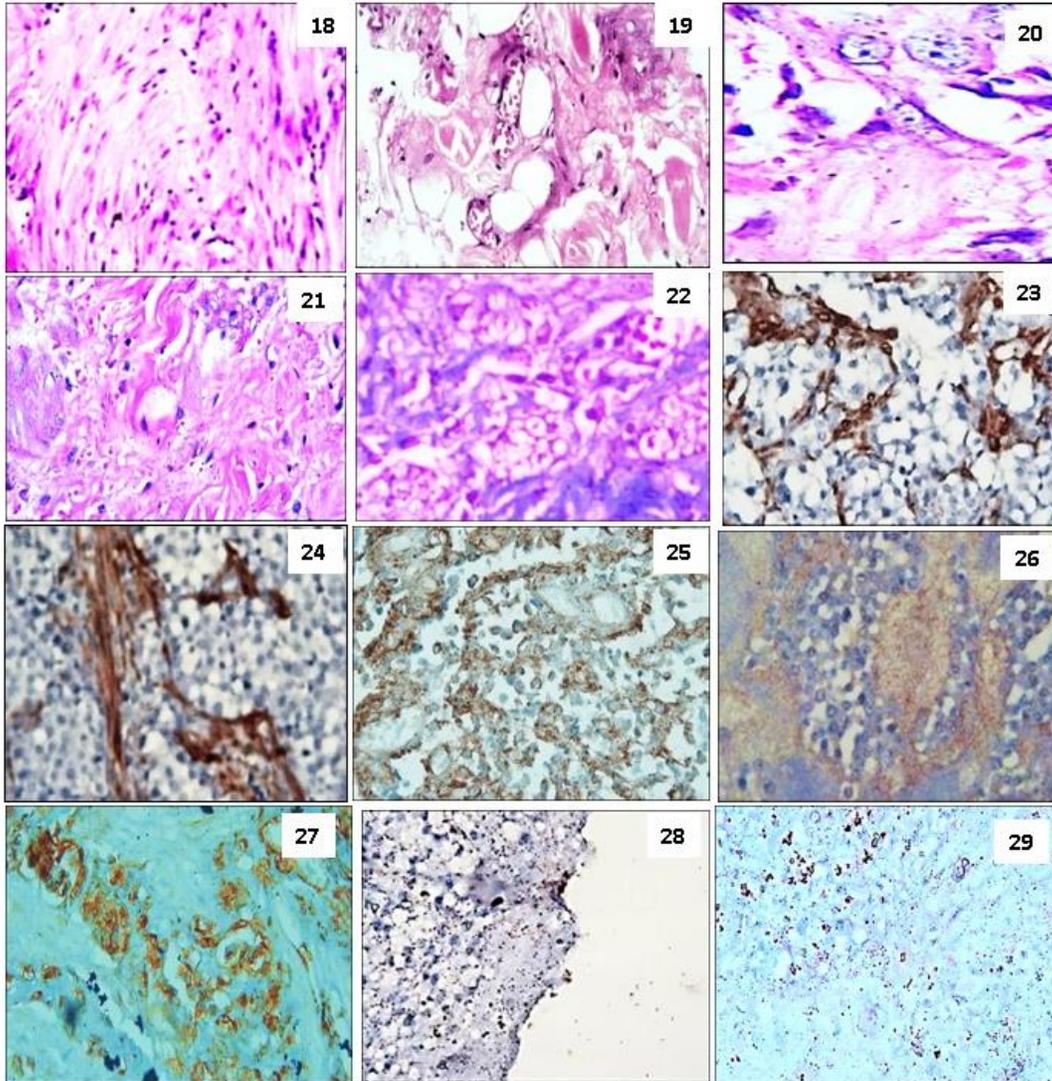
Fig.13: vaginal fibrosarcoma in bitch showed hyperchromatic nuclei (arrow) H&E. X 400.

Fig.14: vaginal fibrosarcoma in bitch showed Strong cytoplasmic immunoreactivity of vimentin in almost all neoplastic cells Mayer's hematoxylin counterstain X 100

Fig.15: vaginal fibrosarcoma in bitch showed Strong P53 positivity immunoreactivity in most of neoplastic cells Mayer's hematoxylin counterstain X 100.

Fig. 16: vaginal fibrosarcoma in bitch showed Strong nuclear CD79 positivity in almost all neoplastic cells Mayer's hematoxylin counterstain X 100.

Fig.17: revealed the presence of large firm brownish-red, noncapsulated mass with areas of cyanosis and necrosis attached to vaginal wall and diagnosed as Leiomyosarcoma of vagina



- Fig.18:** Leiomyoma of bitch vagina showing Haphazardly arranged, admixed with collagenous stroma. Smooth muscle bundles H&E X 40.
- Fig.19:** Lipoleiomyoma of vagina, neoplastic cells resemble smooth muscle bundles admixed with lobules of mature adipocytes with minimal fibrous stroma H&E. X 100.
- Fig.20:** bizarre leiomyoma cells in multiple foci with bizarre nuclei(arrow) H&E. X 400.
- Fig.21:** Leiomyosarcoma of vagina showing, proliferation of degenerated smooth muscle-like cells that densely interlacing with hyalinized collagenous matrix adding to noticed cellular polymorphism, anisokaryosis, , H&E. X 100.
- Fig. 22:** Leiomyosarcoma of vagina showing, proliferation of degenerated smooth muscle-like cells that densely interlacing with greenish blue collagenous fibers (Masson trichrome) X 100.
- Fig.23:** Leiomyosarcoma of vagina showing strong positive immunoreactivity of most neoplastic cells Intracytoplasmic to Smooth Muscle Actin- SMA Mayer's hematoxylin counterstain X 100.
- Fig. 24:** Leiomyosarcoma of vagina showing weak or slightly positive cytoplasmic immunoreactivity of the neoplastic cells to Demin Mayer's hematoxylin counterstain X 100.
- Fig.25:** Leiomyosarcoma of vagina showing strong intensity immunoreactivity of neoplastic cells tor vimentin, but stroma showed moderate for vimentin. Mayer's hematoxylin counterstain X 100.
- Fig. 26:** Leiomyosarcoma of vagina showing Strong nuclear P53 positivity in all neoplastic cells Mayer's hematoxylin counterstain X 100.
- Fig. 27:** Leiomyosarcoma of vagina showing Strong nuclear Ki67 positivity in all neoplastic cells Mayer's hematoxylin counterstain X 100.
- Fig.28** leiomyomas of vagina showing. Immunohistochemical positive expression for estrogen ER α in the epithelium Mayer's hematoxylin counterstain X 100.
- Fig.29.:** leiomyomas of vagina showing Immunohistochemical positive expression for progesterone PR receptors in the stroma and smooth muscle Mayer's hematoxylin counterstain X 100.

4-DISCUSSION:

Primary tumors not mean benign but mean first appearance of malignant tumor Vulva and vagina tumores are usually (71–82%) benign. Malignancy accounts for only a small percentage of vulvar and/or vaginal tumours, particularly if TVT is excluded. TVT alone accounts for between 37 % and 60 % of malignant tumors. Other common malignant tumors are leiomyosarcoma and squamous cell carcinoma (Brodeya and Roszel, 1967; Thacher and Bradley, 1983; Kydd and Burnie, 1986). Only single cases of mast cell tumor, epidermoid carcinoma, aemangiosarcoma, osteosarcoma and adenocarcinoma have been reported (Bilbrey *et al.*, 1989) . Another study reviewed 7 cases that presented with a vaginal and/or vestibular mass that resulted from a caudal extension of a bladder or urethral carcinoma (Magne *et al.*, 1985). The most common clinical signs of vulval/vaginal tumours are an abnormal vulvar discharge or the sudden appearance of a protruding mass. Many of the benign tumors are pedunculated, grow within the confines of the vagina and only at a later stage protrude through the vulvallis (Kydd and Burnie, 1986). Leiomyomas originate from the smooth muscle of the vagina or vestibulum and may be intramural or extraluminal (Withrow and Susaneck, 1986). Other presenting signs may be vulvar or perineal swelling, dysuria and tenesmus. Urinary incontinence does not occur (Thacher and Bradley, 1983).

Traditionally, the scalpel has been considered the surgical cutting tool of choice because of its precision, control, preservation of tissue integrity, and superior associated wound healing. However, its primary disadvantage is bleeding; consequently, numerous electro-surgical devices have been developed to provide hemostasis. Although hemostasis is improved, electro-surgical devices suffer from thermal damage to surrounding tissues, inferior wound healing (Loh *et al.*, 2009 and Pollinge *et al.*, 2003). In this study, the lack of hemorrhage, when using radio frequency surgery greatly reduced surgery time, this result suggested that the high operating temperatures and deep thermal coagulum associated with electro-surgery are always essential for hemostasis. So radiosurgery should consider an alternative to scalpels when performing surgery on easily deformable and highly vascular tissue (Miller *et al* 2004).

The average age on presentation depends on the type of tumor. In general, dogs with TVT present at younger age (average age of 4.4 years), whereas dogs with other tumors present at a much older age (10.8–11.2 years) (Brodey and Roszel, 1967; Thacher and Bradley, 1983; Withrow and Susaneck, 1986). Most malignant tumors are nonpedunculated. Vulvectomy has been described as a treatment for malignant tumors, but recurrence is common (Bilbrey *et al.*, 1989). In some cases, tumors have been deemed inoperable following exploratory episiotomy, and the animal subsequently euthanized (Thacher and Bradley, 1983). The average survival time is approximately 30 % shorter than those with benign tumors (Kydd and Burnie, 1986). A more extensive surgical resection technique combining vulvovaginectomy and perineal urethrostomy has been described for large and infiltrative tumors of this region (Bilbrey *et al.*, 1989).

From our results in Tables 1, the more incidence of malignant tumors (TVT, Fibrosarcoma and Leiomyosarcoma) in older age and conversely the benign tumors (fibroma and Leiomyoma) were more in younger. As German Shepard and Golden Retriever are more susceptible to TVT at average age 3 -4 years and 2-3 years, those findings nearly approach the previously reported by Behera *et al*, (2012) and Soleimani *et al.* (2013). Whereas Pitbull and Rottweiler more affected with fibroma and fibrosarcoma at age 2-3 years those results are parallel with Rollo *et al* (2008) and Al-Kenanny *et al* (2013). While Leiomyoma (LM) and Leiomyosarcoma (LMS) were more frequent in Golden Retriever at age 2-3 and, 3-4 years respectively on the other hand Fibrosarcoma more frequently at 3-4 years age followed by Leiomyoma (LM) at 2-3 years age and finally Leiomyosarcoma (LMS) 3-4 years age .Those findings coincide with Serin et al (2010) and Tsioli, et. al.(2011).

Extraluminal tumors present as slow growing perineal masses whereas intraluminal forms may present as polyps protruding through the vulvar lips especially when the animal strains or is in estrus. These masses may become traumatized and secondarily infected. Other signs may include vulvar bleeding or discharge, tenesmus, dysuria or even urinary obstruction. Most of vaginal and vulvar tumors are benign with good prognosis after surgical resection combined with ovariohysterectomy while malignant tumors carry a poor prognosis due to local recurrence and metastasis (James and McGavin, 2012).

Transmissible venereal tumors (TVT), also called contagious round-cell neoplasia of the dog that of reticuloendothelial origin, it was naturally occurring, located mainly on the external genital mucosae of both sexes. It clinically appears as a solitary, red, friable, often “cauliflower-like mass” with generalized metastases (Papazoglou et al, (2001).

Similar gross and microscopical findings were described in genital and extragenital TVT in dogs by Behera et al. (2012) and Soleimani et al. (2013) .As well as, immunohistochemistry, parallel with those reported previously by Vara et al. (2008) and Stockmann et al (2011). Moreover TVT believed to be consisted of immature leukocyte which indicated the lymphocytes of myeloid origin (Gross et al. (2005) and Marcos et al. (2006)).

Kandukuri et al. (2017) stated that Fibrosarcoma in bitch vagina are unusual mesenchymal tumors and can be found in any location of the body which indicates lymphoid lineage. In our investigation, our described vaginal fibroma and fibrosarcoma were similar to those observed by Neclu and Tiwari (2009) and Harriet (2010) in dog vaginal fibroma and fibrosarcoma. .

Kerliu et al. (2016) reported that leiomyoma or leiomyosarcoma of vagina is a spontaneously benign or malignant smooth muscle genital neoplasm, arise from smooth muscle of canine and the amount of collagenous stroma are variable. In our study, Lipoleiomyoma or atypical leiomyoma (leiomyoma with bizarre nuclei) (ALM), of the vagina. Histopathological and immunohistochemical as well as hormonal receptors expression noticed nearly consistent with the previously described cases of canine vulvovaginal Lipoleiomyoma that firstly recorded by Radi (2005) in dog and Takeda et al. (2011) in bovine but disagreed with observations of Kandukuri et al. (2017). Cooper and Valentine,(2002) reported that variation in immunohistochemical reactivity to desmin and actin SMA might be a diagnostic feature of genital leiomyosarcomas of domestic animals where there is diffuse and uniform immunoreactivity to desmin and SMA in leiomyomas, while bovine vaginal leiomyosarcoma had strong immunoreactivity to desmin and SMA with patchy reaction with vimentin in which Neoplastic cells showed strong staining intensity for vimentin, but stroma showed moderate staining intensity for vimentin (Avci et al. 2010). Additionally, neoplasms of smooth muscle and fibrous tumors in bitch vagina are mostly hormonally responsive as there is a

hormonal influence on the growth of vulvar/vaginal canine tumors (Vermeirsch et al., 2002).

Whereas the noticed Leiomyosarcoma of vagina (LMS) histopathological and immunohistochemical findings appeared similar to the previously observed by Firat et al. (2007) ; Millan et al. (2007); Ozmen et al. (2008) ;Hossein et al. (2009) ;Serin et al. (2010) and Tsioli et al. (2011) in canine vaginae and vulva as well as in genital malignant growths in cattle by Hewedi et al. (2012); Saut et al. (2013) and Tabrizi et al. (2015). Azimpouran (2016) recorded that immunoreactivity to PR, p53 and Ki67 is useful in distinguishing leiomyosarcomas from atypical leiomyomas as Leiomyosarcoma have strong, diffuse positive immunoreactivity to P53 and Ki67 while leiomyoma with bizarre nuclei Negative or weak immunoreactivity for P53 and Ki67 nuclear positivity. Also, reduced of ER and PR receptors in smooth muscle cell tumors was associated with increasing in malignancy.

4. CONCLUSIONS:

We concluded that: 1- Electrosurgical excision provides atraumatic, scalpel-like cutting precision and hemostasis, that resulting in reduced bleeding and tissue injury.

2- The important role of histopathology and immunohistopathological as well as hormonal receptors picture for diagnosis and differential diagnosis of vaginal tumors for the follow up of cases a after surgery and take special percussions if need in cases of malignancy.

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