

Pathological, histopathological, and immunohistochemical evaluation of vulvar fibropapilloma in a heifer and its therapeutic trial: A Case Report

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ABSTRACT

A 6-month-old heifer cow was admitted to a Veterinary clinic in Bani-Ghaleb, Assiut, Egypt, with a large, nodular, vulvar, broad base tumor that had an outer necrosed, rough core and smaller blackish nodules at the thigh region. The animal displayed a normal appetite, and the clinical parameters were within the normal physiological limits. The tumor was resected surgically, thermocautarization was applied and the animal received supportive treatment and antibiotic therapy to get rid of secondary infections. The tumor was photographed and fixed in neutral buffer formalin 10% for further histopathological assessments that showed hyperkeratosis, koilocytosis, and acanthosis of the stratified squamous epithelium. Occasional eosinophilic intracytoplasmic inclusions were observed. Immunohistochemical results revealed nuclear immunopositivity to cellular proliferation markers (PCNA and Ki-67) of the stratum basale as well as fibroblasts. Vimentin-positive labeling was remarkable in the dermal connective tissue. Myofibroblasts, smooth muscles of hair follicles, and dilated capillaries showed α -SMA positive reaction. Tumor recurrence didn't occur during a period of follow-up that extended to six months. We concluded that surgical excision with thermocautarization is the ideal treatment for genital fibropapillomas. Since the animal was sexually immature, genital papillomas are not restricted to venereal transmission. Further hormonal immunoassays are recommended.

Introduction

Bovine fibropapilloma is an infectious, highly contagious viral neoplastic skin disease. It is commonly observed in cattle, especially those younger than two years, and manifested as multiple benign tumors i.e. papillomas. The predilection sites for cutaneous warts are the shoulder, neck, eyelids, dewlap, legs, and brisket (Borzacchiello and Roperto, 2008). Papillomas of genital organs are reported in young cows and pigs following coitus particularly during their first breeding season (Oryan *et al.*, 2014). Approximately 14.5% and 9% of the diagnosed vulvar tumors are fibropapillomas (Yeruham *et al.*, 1999; Beytut, 2017). It is caused by oncogenic bovine papillomaviruses (BPV) that are small, circular, non-enveloped double-stranded DNA genomes whose diameter is about 50 nm and molecular weight is 8 kb (Batista *et al.*, 2013). Other varieties of genital tumors in cows include vaginal leiomyosarcoma, vaginal fibrosarcoma, and luteoma (Martz *et al.*, 2020). Fibroleiomyosarcoma and squamous cell carcinoma are listed as possible vulvar tumors (Timurkaan *et al.*, 2009; Avci *et al.*, 2010).

Until now, there are about 13 BPV types (Araldi *et al.*, 2017). Only six types of papillomaviruses have been well identified. Cutaneous papillomas are induced by BPV-1, BPV-2 and BPV-5. However, squamous papillomas of the upper gastrointestinal tract, skin, and teat papillomas are caused by BPV-3, BPV-4, and BPV-6 (Jelinek and Tachezy, 2005). With the exception of BPV-1 and BPV-2 which have the ability to infect family Equidae, papillomaviruses are strictly host-specific and are not capable of infecting hosts other than natural ones (Mathewos *et al.*, 2021).

The pathogenesis remains questionable. The virus can gain entrance to the body through microtrauma, scratches, or other skin defects after direct or indirect contact with the infected cattle. It initially infects basal cells, then it is transported to the nucleus where expression of oncoproteins (E5, E6, and E7) results in cell cycle disruption and production of numerous viral copies (Borzacchiello and Roperto, 2008). Viral tropism is

restricted to cutaneous and mucosal epithelial cells in which hyperplastic changes can result in either exophytic or endophytic lesions. In most cases, spontaneous regression of warts eventually occurs with the aid of a cell-mediated immune response. However, it may persist for many years, spread to other organs, or evolve into cancer of both epithelial and mesenchymal origins, especially in chronically immunocompromised animals or with the presence of carcinogenic environmental cofactors (Turk *et al.*, 2005).

Diagnosis of fibropapilloma relies on a clinical approach, and cytological and parasitological examination (Betiol *et al.*, 2012). Viral particles are visible by transmission electron microscope in affected tissue sections (Turk *et al.*, 2005). Polymerase chain reaction can be included as a sensitive technique for genotyping and identification of papillomaviruses (Nascimento *et al.*, 2012). Detection of viruses in blood can be achieved by using specific primers (Ogawa *et al.*, 2004). Mostly, fibropapillomas are not life-threatening conditions. However, the resultant organ dysfunction i.e. vulva and vagina, and the subsequent infection with opportunistic bacteria make choice of ideal treatment critical to restore the function and reduce economic losses (Mathewos *et al.*, 2021). The present report aimed to record a case of infectious fibropapilloma of bovine genitalia in a heifer with a pathological, histopathological, and immunohistochemical assessment of the detected tumor, as well as to explain how to deal with such cases to achieve the best results.

Case history, Clinical signs, and Management

A six-month-old cattle heifer, weighing approximately 150 kg, was presented to a Veterinary clinic in Bani-Ghaleb, Assiut, Egypt. The owner's complaint was the presence of a large circumscribed mass attached to the vulva. In addition, this mass was initially observed four weeks ago with a gradual increase in size. It was suspected to result in reproduction failure. The detected mass was firm in texture and attached to the ven-

tral and lateral borders causing somewhat shifting of the vulva to the left. In association, smaller blackish nodules were noticed at the region of the thigh (Fig. 1a). During the physical examination, the animal exhibited normal appetite, rumination, and defecation without any detectable abnormal symptoms. Clinical parameters were evaluated that were also within the normal physiological limits (Temp. 38.5C, HR. 65 beat/min. and RR. 26 breath/min.).

Sedation of the animal was induced using xylazine HCl (3 mg/kg, Xyla-get 20% ®: ADWIA Co., Egypt). The operation site was prepared routinely. Afterward, local infiltration anesthesia was carried out under the effect of lidocaine HCl 2% (Alexandria Co. for Pharmaceutical and Chemical Industries, Egypt). The mass was grasped using tumor forceps followed by a skin incision entirely around it. The mass was then removed, and control of bleeding was achieved using pressure hemostasis with a sterile tampon for 5 minutes. Then, thermocauterization of the cavity was performed. At last, the subcutaneous tissue was closed as usual. The other side of treatment, the medicinal one, included administration of 500 ml Dextrose 5% by intravenous infusion, Toldimphos sodium (1 mL/10kg; Tonotest, Drugest, Egypt) by intravenous injection and Procaine Penicillin & Dihydrostreptomycin Sulphate (1 mL/20kg; Pen & Strep, Norbook, Egypt) by intramuscular injection for five successive days to prevent infection.

One month after surgical excision and thermocauterization, the healing process was obvious. After two months, all lesions disappeared including those of the thigh, the skin returned to its normal condition and the vulva regained its normal pre-injurious appearance. The case was followed up for up to six months with no evidence of tumor recurrence neither at the primary site of infection nor at any other site of the body (Fig. 1b).

Histopathological examination

The excised tumor was photographed by a digital camera, cut into smaller pieces, and fixed in 10% neutral buffer formalin for preservation. It was referred to the Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Egypt. Fixed specimens were processed as usual. 3-5µm thick tissue sections were stained with Hematoxylin and Eosin for microscopic examination by light microscope, and were photographed using a Sc30 Olympus camera (Bancroft and Marilyn, 2002). Masson's trichrome staining was applied in some sections as a special stain to visualize collagen fibers (Foot, 1993).

Immunohistochemistry

Tissue sections were stained according to the steps explained by Petrosyan *et al.* (2002) for the expression of vimentin and alpha-smooth muscle actin (α SMA) as members of the type III intermediate filament proteins and PCNA, Ki-67 that represent cellular proliferation markers. Sections with 4 µm thickness were deparaffinized and rehydrated by graded alcohols. To reduce the nonspecific background staining due to endogenous peroxidase, the slide was incubated in UltraVision Hydrogen Peroxide Block for 10 minutes. Primary antibodies (anti-vimentin, anti-SMA, anti-PCNA, and anti-Ki-67, respectively) were applied and followed by the antibody enhancer. However, in the negative control section, the addition of primary antibodies was skipped. HRP polymer was added and color labeling was obtained after incubation with the peroxidase-compatible chromogen according to the manufacturer's recommendations. Sections were thoroughly washed with a buffer solution after each incubation step. Finally, sections were counterstained by Mayer's hematoxylin and cover-slip using an aqueous mounting media.

Results and Discussion

Definitive diagnosis of infectious fibropapillomatosis was achieved after pathological, histopathological, and immunohistochemical evalua-

tion of the extirpated mass. In line with our report, Agnew and MacLachlan, (2016) stated that the most common type of vulvar neoplasms was fibropapilloma, which was caused by bovine papillomaviruses. In contrary, Mathewos *et al.* (2021) stated that proliferative growths had not been observed in the vulva and the highest proportion of them was present in the neck and head with a percentage of 25.5 and 21.6%, respectively among the studied population. Grossly, the tumor exhibited elongation on the linea alba. Its internal surface was pedunculated, smooth, and fleshy, with minimal amounts of hemorrhage. The outer surface was lobulated with approximately 10 cm diameter and was covered with a thick rough blackish core possibly due to fecal contamination (Fig. 1c). The cut section revealed finger-like projections that were supported by fibrous stalks (Fig. 1d). Similar description of vulvar fibropapilloma was mentioned by Yeruham *et al.* (1999) who stated that it was a well-differentiated tumor protruding from the vulva, with a broad base and a mushroom-like shape. Otherwise, it was smaller in size with 3 cm diameter. Al-Salihi *et al.* (2020) observed a huge ulcerated papillomatous lesion in an adult cow's external genitalia that caused mating difficulties due to its tendency for bleeding. Another description was reported by Elzein *et al.* (1991) who explained that fibropapilloma of the vulva was deficient in magnitude. However, its diameter could reach 7 cm in some instances. It appeared as a verrucous multiple tumor, with a firm texture and grayish coloration.



Fig. 1. (a): Clinical picture of the vulvar fibropapilloma. (b): Six months after dealing with the case indicates no recurrence and full recovery. (c): Macroscopic postoperative view of the tumorous mass showing an irregular rough external surface with lobulation, a necrotic center, and a cyanosed periphery. (d): Greatly thickened epidermis and a cauliflower-shaped tumor on the cut section.

Histopathological examination revealed hyperkeratosis with extensive hyperplastic changes of the stratified squamous epithelium forming irregular processes towards the underlying dermis (Fig. 2a). Superficial cells showed hydropic degeneration of the cytoplasm that formed a halo around fragmented and pyknotic nuclei with keratohyalin granules accumulation (Fig. 2b). The rete ridges extended deeply towards a hypercellular-infiltrated connective tissue cells that showed, also, a myxomatous degeneration and acquired long and branching fibrils (Fig. 2c). Abundant mitotic figures and a pathognomic lesion in the form of eosinophilic intracytoplasmic inclusion bodies were evident in the epidermal cells (Fig. 2d). With Masson trichrome stain, the proliferated collagen fibers took the characteristic light blue or greenish coloration (Fig. 2e, 2f). This histological picture is compatible with those previously reported by Ataseven *et al.* (2016); Khalefa, (2016); Aydin *et al.* (2020) and Bertagnolli *et al.* (2020) who intimated them as the hallmarks of infectious fibropapilloma. A point of disagreement was mentioned by Özsoy *et al.*, (2011); Zhu *et al.* (2013), and Al-Salihi *et al.* (2020) who noticed intranuclear viral inclusions. However, in earlier research, Jones *et al.* (1997) stated that inclusion bod-

ies were scarcely observed in spontaneous papillomas.

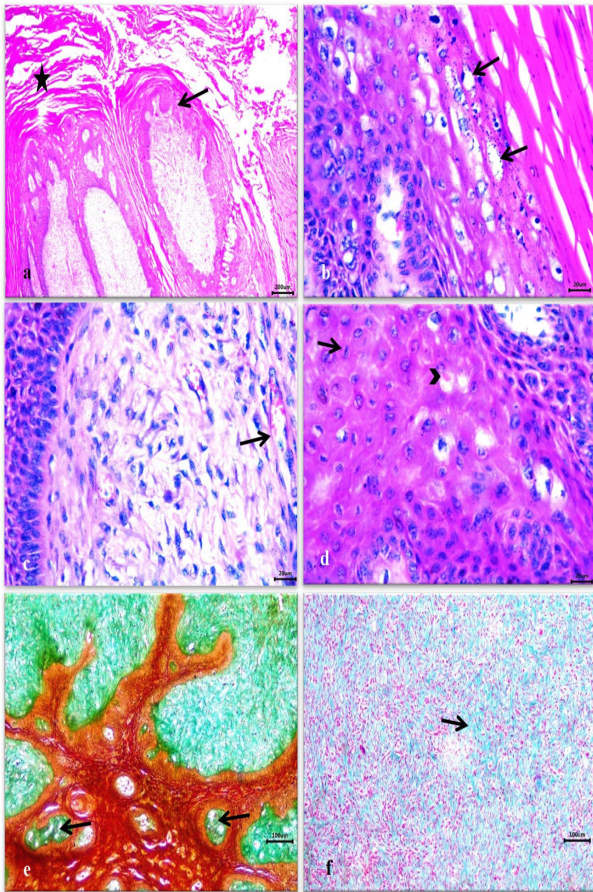


Fig. 2. (a): Showing hyperkeratosis (star), acanthosis, and papillae formation (arrow). (b): Koilocytosis with cytoplasmic vacuolation, nuclear karyorrhexis, and karyolysis of the squamous epithelium (arrows). (c): Degenerated and inflammatory infiltrated connective tissue cells associated with dilated vasculature (arrow). (d): Acidophilic intracytoplasmic viral inclusions (arrowhead) and numerous mitotic cells (arrow) (a-d: H&E). (e): A papular lesion with a fibrovascular core (arrows). (f): Proliferated light blue collagen fibers (arrow) (e, f: Masson trichrome stain).

Tumor tissue sections were studied for the immunorexpression of some of the type III intermediate filament proteins, vimentin, and alpha-smooth muscle actin (α -SMA). The results of immunohistochemical staining in the current study showed that vulvar fibropapilloma had a strong reactivity to vimentin. The dermal connective tissue revealed predominant immunopositivity that was stronger in islands of fibrous tissue within the rete ridges and in the superficial dermis. However, the epidermal cells reacted negatively to the marker (Fig. 3a). Although reports describing the vimentin expression are scant, similar results were obtained by Beytut (2017) who stated that vimentin-positive labeling is an important indicator for the fibrotic response to viral replication in cases of fibropapilloma. Dermal smooth muscle cells and those surrounding sebaceous glands and hair follicles showed an α -SMA positive labeling. However, negative immunoreactivity to the marker was obvious in the hyperplastic stratified squamous epithelium and those of skin appendages. An important observation is the α -SMA immunopositivity of the cytoplasmic stress fibers that are considered a major component of the irregular, star-shaped, or fusiform myofibroblasts which are distributed almost evenly throughout the dermal layers (Figs. 3b, c). We thought that these cells participated in the mechanism of tissue remodeling after the viral insult. Consistent with our observation, Zhao *et al.* (2018) stated that myofibroblasts are important effector cells involved in the process of fibrogenesis. Gabbiani (2003) explained that they are differentiated fibroblasts and cannot be distinguished from them without SMA immunostaining. According to Beytut (2017), a strong positive reaction to the marker was observed in the mature connective tissue, while myxomatous proliferations showed indisposed staining.

Tumor proliferation can be identified by various cellular markers. Mi

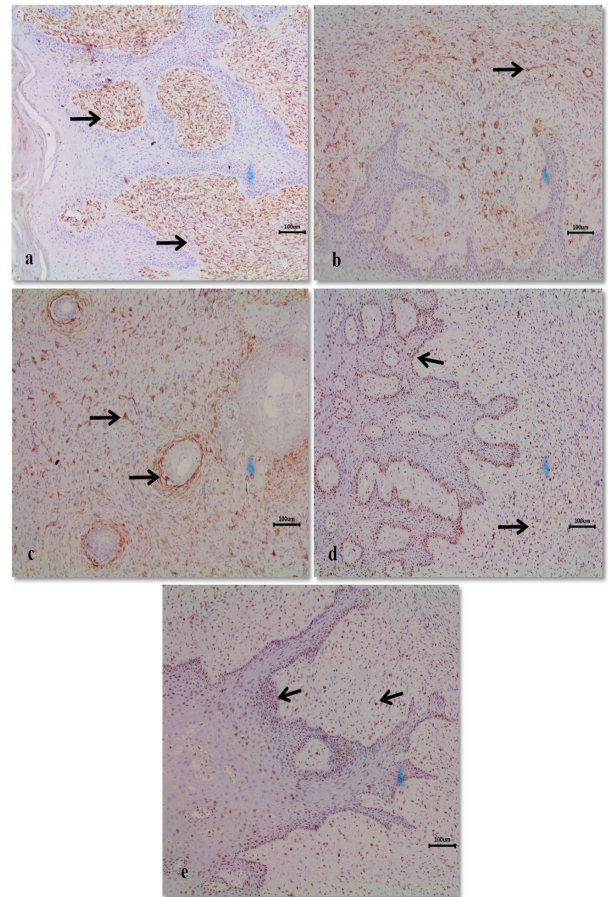


Fig. 3. Micrograph showing results of immunohistochemistry. (a): Islands of fibrous connective tissue revealed a strong vimentin-positive reaction (arrows). (b): Alpha smooth muscle actin expression in the fusiform-shaped dermal myofibroblasts (arrow). (c): α -SMA-positive labeling of hair follicle smooth muscles and myofibroblastic stress fibers (arrows) (d): Nuclear immunopositivity of stratum basale and dermal fibroblasts to PCNA and (e): Ki-67 (arrows) (a – e: IHC).

croscopic estimation of PCNA, Ki-67 that are involved in cellular growth and division is a useful tool in evaluating the proliferation rate of tumor cells by using immunohistochemical techniques. According to our observations, nuclear PCNA, and Ki-67 were prominently detected in the basal layer of the overlying epidermis, the cellular lining of the fibrovascular core, and some of the proliferating dermal fibroblasts (Fig. 3d, 3e). The number of PCNA/Ki-67- positively labeled cells was gradually diminished from the stratum basale superficially towards the stratum corneum and a few parabasal and stratum spinosum cells showed weak immunopositivity. Fibropapillomas are thought to have a high proliferative capacity that was proved by the increase in PCNA and Ki-67 expression. It had been shown that Ki-67 staining was closely related to PCNA immunoreactivity. In complete agreement with our results, Jelinek and Tachezy (2005); Özsoy *et al.* (2011) and Aydın *et al.* (2022) found an intranuclear positive reaction to the previously mentioned markers in the acanthotic epidermal and mesenchymal cells. Additionally, Beytut (2017) studied the tumor immunoreactivity to desmin and S-100 protein. Tunica media of the dermal blood vessels expressed positive reactivity, while the latter's strong immunolabeling was evident in the dermal and perivascular neural sheath, basal layer melanocytes, and vascular endothelial cytoplasm. Al-Salihi *et al.* (2020) stated that immunorexpression of P-53 can be used as a diagnostic marker for fibropapillomas in which strong cytoplasmic and perinuclear immunopositivity were evident in the corneal, basal and parabasal layers. In addition, a few cells of stratum granulosum and stratum spinosum showed a positive reaction. Aydın *et al.* (2022) observed an intranuclear positive reaction to BPV antigen in the stratum granulosum cell layer and an intracytoplasmic expression of HPRT in connective tissue cells.

Treatment of genital fibropapillomas still takes great attention due to their transmissible pattern, loss of reproductivity, and recurrence possibilities. In our report, we deduced that surgical intervention uprooted the

case. However, favorable results cannot be obtained unless the exposed subcutaneous tissues are thermocauterized. In agreement, Salib and Farghali (2011) stated that surgical excision is preferable for the treatment of warts over any other treatment options. Aydın *et al.* (2022) explained that cauterization had resulted in a great reduction in the incidence of hemorrhages and relapse of genital papillomas. In partial consensus with our results, Feyisa (2018) reported that ivermectin is the drug of choice for bovine papillomatosis and should be given at a dose of 0.2 mg/kg by subcutaneous route after manual excision of the largest lesion. Turk *et al.* (2005) and Aydın *et al.* (2020) found that autogenous vaccination succeeded in stimulating the immune response against papillomavirus by triggering the production of lymphocytes and interleukins. It was given subcutaneously at a dose of 10 ml 4 times at one-week intervals and prepared by a certain procedure involving about 30 gm from tumor tissue, 45 ml of tris buffer, 0.5% formalin, and crystal penicillin. According to Saied *et al.* (2020), the application of *Ocimum basilicum* ethanolic extract ointment has proved its efficacy in papillomas regression due to its direct antiproliferative, immunostimulant, and antioxidant effects. Recently, Geethanjali *et al.* (2024) described a multifaceted approach that could boost the therapeutic outcomes against the disease, starting with autohaemotherapy, followed by surgical removal of warts that were finally utilized to prepare an autogenous vaccine.

Conclusion

Fibropapilloma of bovine genitalia is not confined to venereal transmission. The case in our report did not come in heat and wasn't subjected to neither natural nor artificial insemination. The condition might be hereditary. Nevertheless, hormonal disturbances at a premature age could be incriminated in tumor development. Our report highlighted the demand for further hormonal immunoassays. Surgical resection is an indispensable treatment choice, particularly in animals intended for breeding. In such cases, thermocauterization is an excellent procedure to be applied after surgery; hence, it prevents the reoccurrence of the condition. Myofibroblastic activity in genital fibropapillomas can be estimated by the immunoeexpression of α -SMA that is utilized not only as an appropriate diagnostic marker but also to rule out other spindle cell-originated tumors such as malignant melanoma, leiomyoma, and leiomyosarcoma.

Conflict of interest

The authors declare that they have no conflict of interest.

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