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Pathological and immunohistochemical studies on skin and subcutaneous neoplasia in Arabian Camels (Camelus dromedarius)

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Abstract

Background: Tumors have been rarely documented in the Arabian dromedary (Camelus dromedarius).

Methods: Post-slaughter inspections of different skin tumors in one-humped Dromedary camels slaughtered at different abattoirs in Egypt during the period from January 2019 and February 2022. The age of the examined animals ranged from 4 to 15 years. The pathological swelling was collected from affected camels and then subjected to a detailed study to record the different gross and microscopic findings.

Results: Our study revealed the presence of 13 cases out of 988 showed neoplasia with an incidence rate of 1.3% including papilloma (0.1%), fibropapilloma (0.1%), lipomas (0.2%), adenoma (0.1%), squamous cell carcinoma (0.1%), and myxosarcomas (0.7%).

Conclusions: Myxosarcomas is the most common tumor diagnosed in this study that was immunohistochemically confirmed by positive cytoplasmic immunoreactivity for vimentin. Meanwhile, benign cutaneous tumors are less frequent in camels such as papilloma, fibropapilloma, lipoma, and adenoma.

Keywords: Camelus dromedarius; Histopathology; Immunohistochemistry; Skin tumor; Egypt.

1. Introduction

Camels are regarded as one of the most important sources of milk and meat in several regions of Egypt, including the Nile Valley and Delta, as well as the desert regions, where this species also serves as a means of labor and transportation. Phenotypically, six camel breeds are reared in Egypt; Baladi, Maghrabi, Fallahi, Sudany, Somali, and Mowaled (Othman et al., 2017).

Neoplasms of the skin and subcutaneous tissues are the most frequently recognized neoplastic disorders in domestic animals because they are easily seen by the owner; they are brought to the attention of the veterinarian and usually are easy to be removed. Therefore, neoplastic skin specimens are frequently submitted for histopathologic evaluation (Ginn et al., 2007; Goldschmidt and Goldschmidt, 2016). Skin and subcutaneous tissue tumors had various etiological factors including the possibility of the intervention of intrinsic and extrinsic risk factors such as hormonal conditions, genetic and immunological factors, solar radiation, ionizing radiation, and viral and chemical factors. The appearance of skin tumors has a higher frequency in animals and humans that spend most of their time outdoors, exposed to ultraviolet rays, compared to those that stay indoors (Goldschmidt, 1998).

Cutaneous neoplasms have been reported as sporadic cases in camels. A case report of squamous cell carcinoma (Al-Sobayil and El-Amir, 2013; Siddiqui et al., 2013; Alsobayil et al., 2018) basal cell carcinoma (Al-Hizab et al., 2007), fibropapilloma (Oryan et al., 2011), fibroma, lipoma and fibromyxosarcoma (Al-Sobayil and Elamir, 2013), lipoma at ischiorectal fossa underneath the skin in the left side of the perianal area (Kaswan et al., 2013), squamous papillomas, fibropapillomas, sebaceous adenomas, melanocytomas and malignant melanoma (Khordadmehr et al., 2016) and cases of cutaneous papillomatosis in Saudi Arabia (Khalafalla et al., 2017), in Sudan (Ure et al., 2011; Khalafalla et al., 2018) has been described in dromedary camels. The classification of skin tumors is difficult due to the complex structure, and the ectodermal and mesodermal origin of skin components, in association with structural and physiological peculiarities of the skin in different animal species and breeds (Baba and Câtoi, 2007).

Vimentin, a 57-kDa protein, is one of the most widely expressed and highly conserved proteins of the type III IF protein family. Vimentin expression is elevated in a variety of tumor cell lines and tissues, including prostate cancer, breast cancer, endometrial cancer, central nervous system tumors, malignant melanoma, and gastrointestinal tract tumors such as pancreatic, colorectal, and hepatic cancers (Satelli and Li, 2011). The mesenchymal origin of some skin tumors was confirmed by using antibodies to vimentin (Van Zeeland et al., 2006). Therefore, the present study aimed to describe the gross morphology, histopathologic and immunohistochemical features of skin and subcutaneous tumors in dromedary camels at different abattoirs in Egypt during the period from January 2019 to February 2022.

2. Materials and methods

2.1. Samples collection

A total of 988 one-humped slaughtered camels were examined (808 male and 180 female) in Toukh, Kerdasa, Warraq, and El-Basateen abattoirs during the period from January 2019 to February 2022. The age of the examined animals ranged from 4 to 15 years. The specimens that had any suspected gross alterations were collected for further examination.

2.2. Histopathological examination

After careful gross examination, specimens from the skin and subcutaneous tissue were fixed in 10% neutral buffered formalin for 2 days, routinely processed, embedded in paraffin, sectioned at 4 μ m thickness, and stained by hematoxylin and eosin for histopathological studies (Bancroft et al., 2013). The histopathological examination of skin tumor samples was conducted in the Department of Pathology, Faculty of Veterinary Medicine, Benha University, Egypt using an Olympus BX43 microscope, Tokyo, Japan.

2.3. Immunohistochemistry

Immunohistochemical staining was performed on paraffinembedded skin sections using the avidin–biotin– peroxidase complex method following the protocol of Ramos-Vara and Beissenherz, (2000). The slides were incubated with primary antibody against vimentin (Mouse monoclonal antibody, M0725, EnVision DAB system, Dako Corp., Carpinteria, CA, diluted 1:2000). DAB (3,3 diaminobenzidine tetrahydrochloride; Sigma, St. Louis, MO) was used as a chromogen and hematoxylin as a counter stain.

3. Results

Based on histopathological and IHC examination, our results diagnosed skin tumors in 13 cases out of 988 camels (13/988, incidence 1.3%) including one case of each of the following Squamous cell papilloma, Fibropapilloma, Adenoma, and Squamous cell carcinoma; as well as 2 cases of Lipoma and 7 cases of Myxosarcoma (Table 1).

3.1. Squamous cell papilloma

Squamous cell papilloma was diagnosed in one case in the skin of the scrotum. Microscopically, observed mild to moderate acanthosis, and hyperkeratosis. The neoplasm was composed of multiple epithelial proliferations of variable width, the surface of which was covered with keratinized epithelium (Fig. 1A). In the spinous layer, cytoplasmic vacuolation was seen in the prickle cells (Fig. 1B). Mitotic figures were rarely observed.

3.2. Fibropapilloma

Fibropapilloma was noticed in one case in the skin of the forelimb and characterized by the presence of thickened rough ulcerated surface (Fig. 1C) that was yellowish-white and hard in the cut section (Fig. 1D). Microscopically, the surface covering epithelium showed acanthosis and hyperkeratosis associated with finger-like projections of epidermal pegs extending deep into the fibromatous connective tissue of the dermis (Fig. 1E). Moreover, extensive proliferation of connective tissue and fibroblasts were evident in the dermis and subcutaneous area where the neoplastic spindle cells arranged in long thick interlacing bundles with whorls formation and the oval to tapered nuclei in the neoplastic cells with finely fragmented chromatin (Fig. 1F).

3.3. Lipoma

Lipomas were observed in two cases in the skin; one of them in the scrotum and the other in the ventral abdominal wall. The swelling appeared as well-demarcated, encapsulated, and grayish-white to yellow gelatinous masses (Fig. 2A). Microscopically, the tumor formed from fat cells with variable sizes and shapes where the cytoplasm of neoplastic cells was replaced with large clear fat vacuoles with peripheralization and compression of nuclei. Strands of collagenous fibers are seen scattered among fat cells dividing neoplasm into lobules (Fig. 2B).

3.4. Adenoma

Adenoma was observed in 1 case in the skin of the neck near to head region and was grossly characterized by the presence of a firm, ball-like swelling that showed hemorrhagic content in the cut surface (Fig. 2C). Histopathologic findings of sweat gland revealed the presence of a demarcated mass that frequently replaced large areas of normal dermal and hypodermal tissue (Fig. 2D). The mass was subdivided into lobules of varying size composed of irregular packets and cords of cells. The entire mass was dissected by a dense fibrous stroma (Fig. 2E). The acini were lined by flattened neoplastic cells and their lumina were dilated with granular eosinophilic secretions (Fig. 2F).

3.5. Squamous cell carcinoma

Squamous cell carcinoma was detected grossly in the skin of the scrotum as a hemorrhagic ulcerated lesion. Microscopically, the tumor composed of nests of neoplastic epidermal cells invades and proliferates into the dermis surrounded by fibrovascular stroma. These nests formed from concentrically laminated masses of intensely eosinophilic keratin in the center surrounded by peripheral polyhedral neoplastic cells (Fig. 3A).

3.6. Myxosarcoma

Myxosarcoma was observed in seven investigated cases; 3 cases in the skin of the scrotum, 2 cases in the forelimb, one case in the check, and one in the back. Myxosarcoma grossly appeared as a firm, irregular, pale yellow ulcerated hemorrhagic multilobulated subcutaneous mass with an indistinct fibrous capsule. In the cut section, the mass was subdivided into distinct pale translucent lobules accompanied by gelatinous (mucinous) fluid oozed from the cut surfaces (Fig. 3B). Histopathologically, moderately cellular neoplasm consisted of numerous fibroblasts with long branching fibrils suspended in an abundance of extracellular matrix was detected (Fig. 3C). Occasionally, extensive areas of hemorrhage were observed in between the neoplastic cells (Fig. 3D). Individual neoplastic cells were pleomorphic, stellate or spindle-shaped, and contained hyperchromatic round to oval nuclei with fragmented chromatin, and multiple prominent nucleoli. Mitotic figures were infrequent (less than 3 per high power field) (Fig. 3E). The neoplastic cells expressed positive immunoreactivity staining for vimentin (Fig. 3F). Interestingly, other investigated cases showed myxosarcoma as circumscribed foci deep in the dermis of the skin of the scrotum (Fig. 3G). Immunohistochemically, vimentin expression was prominent in these neoplastic foci (Fig. 3H).

Table (1): Tumors in the skin of dromedary camels.

Pathological affection	Age (years)	Number of affected cases	Sex	Location	Incidence of the neoplastic lesions (n=988)
Squamous cell papilloma	10	1	Male	Skin of scrotum	0.1%
Fibropapilloma	5	1	Male	Skin of forelimb	0.1%
Lipoma	9 &10	2	Male Female	Skin of scrotum & Ventral abdominal wall	0.2%
Adenoma	6	1	Male	Skin of neck	0.1%
Squamous cell carcinoma	10	1	Male	Skin of scrotum	0.1%
Myxosarcoma	4 & 10	7	Male	Skin of scrotum, check, forelimb & back	0.7%
Total		13			1.3%



Fig. 1: H&E-stained sections of skin, camel showing, Papilloma (A, B), Fibropapilloma (C, D, E, F). (A) Basal cell hyperplasia, acanthosis (A), and hyperkeratosis (H) (x100), (B) Cytoplasmic vacuolation in the prickle cell layer (x400), (C) Gross picture of fibropapilloma showing thickened rough ulcerated skin surface (arrow), (D) Cut section of fibropapilloma that was appeared yellowish white in color and hard in consistency (arrowhead), (E) Thickened epithelium covering associated with finger-like projections of epidermal pegs (arrow) extending deep into the dermis (x40), (F) The neoplastic spindle cells arranged in long thick interlacing bundles with whorls formation (W, x100).



Fig. 2: Lipoma (A, B), Adenoma (C, D, E, F), skin, camel. (A) Gross picture of lipoma showing well-demarcated, grayish-white to yellow gelatinous masses, (B) Neoplastic fat cells (FC) with variable sizes and shapes with the presence of collagenous fibers strands (asterisk) scattered among fat cells dividing neoplasm into lobules (x100), Notes also peripheralizations of nuclei (arrowhead, inset, x400), (C) Firm, ball-like swelling (arrow) in the skin of the neck near to head region that showed hemorrhagic content in cut surface (arrowhead, inset). (D) Adenoma appeared as a demarcated mass that replaced large areas of normal dermal and hypodermal tissue (H&E, x100), (E) The neoplastic mass composed of irregular packets and cords of cells which is entirely enclosed by a dense fibrous stroma (H&E, x400), (F) The acini were lined by flattened neoplastic cells and their lumina were dilated with granular eosinophilic secretions (H&E, x400).



Fig. 3: Squamous cell carcinoma (A), Myxosarcoma, (B, C, D, E, F, G, H) skin, camel. (A) Nests of neoplastic epidermal cells (arrowhead) invade and proliferate into the dermis surrounded by fibrovascular stroma (asterisk) (x100), and the neoplastic nest formed from concentrically laminated masses of intensely eosinophilic keratin in the center surrounded by peripheral polyhedral neoplastic cells (inset, x400). (B) Pale yellow ulcerated hemorrhagic multilobulated (asterisk) subcutaneous mass with an indistinct fibrous capsule, in cut section, the mass appeared as distinct pale translucent lobules accompanied with gelatinous (mucinous) fluid oozed (inset). (C) Moderately cellular neoplasm consisted of numerous fibroblasts with long branching fibrils suspended in an abundance of diffusely pale pink mucinous stroma (x100), (D) Extensive areas of hemorrhage (H) in between the neoplastic cells (x400), (E) Individual neoplastic cells were pleomorphic, stellate or spindle-shaped, and contained hyperchromatic round to oval nuclei with stippled chromatin (arrowhead, x400). (F) The neoplastic cells showing positive brown staining for vimentin (arrowhead, x400), (G) Circumscribed foci (CF) of myxosarcoma deep in the dermis of the skin of scrotum (x40, H&E), inset, showing numerous fibroblasts with long branching fibrils suspended in an abundance of diffusely pale pink mucinous stroma (x100, H&E), (H) Prominent vimentin expression in these neoplastic foci (NF) (x40, inset, x400).

4. Discussion

In the present study, a total of 988 one-humped slaughtered camels were examined (808 male and 180 female) during the period from January 2019 to February 2022. Camel neoplasms (13/988) were categorized into 6 different types arranged according to their incidence: myxosarcomas, lipomas, squamous cell papilloma, carcinoma, fibropapilloma, and adenoma. Previous reports by Valentine and Martin, (2007) stated that cutaneous and mucocutaneous neoplasms were most common in llamas and alpacas (22 animals), particularly fibroma/fibropapilloma (12 tumors), squamous cell carcinoma (6 animals), and fibrosarcoma (4 animals).

The incidence rate of tumors in the skin of camels in this study was 1.3%. This rate is extremely higher than that previously reported (0.006%) in dromedary camels (Alsobayil et al., 2018). Meanwhile, our percentage is extremely lower than that reported in one-humped camel (15.24%) by Khordadmehr et al., (2016). However, in other animal species, the prevalence of skin or subcutaneous tissue tumors was as follows dogs (42.79%), cats (42.2%) (Ciaputa et al., 2017); cattle (78.95%), and buffalo (21.05%) (Shruthi et al., 2018).

In the current work, papilloma was diagnosed in one case in the skin of the scrotum and microscopically characterized by mild to moderate acanthosis, and hyperkeratosis. Our findings entirely agreed with Khordadmehr et al., (2016), meanwhile; Barakat et al., (2013) reported skin warts on the lips and lower jaw of the camel. In addition, our findings disagree with that mentioned by Khalafalla et al., (2018) who found wart-like lesions on the hands and legs of two herders attending the infected camel herd that showed similar lesions on the chest, legs, and lips where papillomavirus (PV) is the causative agent in these cases as confirmed by PCR and immunoperoxidase test. However, the sites where the papilloma may be found are greatly dependent on husbandry practices used in agriculture whereas in cattle it is mostly observed at sites of abrasion as the virus can enter the epidermis and produce the cutaneous lesions, including the ears following tattooing (Goldschmidt and Goldschmidt, 2016). We have generally suggested that papilloma in camels would be associated with chronic trauma by camel wrestling. Our suggestion is supported by Gross et al., (2008) who illustrated squamous cell papilloma as delicate, fimbriated masses that were smaller than viral warts where the lesions are frequently observed on the face, eyelids, feet, and conjunctiva with no known predisposition either to age or breed.

Fibropapilloma was noticed in one case in the skin of the forelimb and characterized by the presence of thickened covering epithelium showed acanthosis and hyperkeratosis associated with finger-like projections of epidermal pegs extending deep into the fibromatous connective tissue of the dermis. Our results were similar to those reported by Oryan et al., (2011); Khordadmehr et al., (2016). Schulman et al., (2003) found fibropapilloma on the nose, lip, and cheeks in llamas and alpacas and these tumors were positive for papillomavirus (PV) specific primers as detected by PCR.

Lipoma was detected in two animals in the skin of the scrotum and ventral abdominal wall. Similar results were reported by Kaswan et al., (2013) in camels and Orioles et al., (2021) in farmed striped sea bream. Additionally, Valentine and Martin, (2007) reported 2 cases of lipoma in mesentery and subcutis of llamas.

Interestingly, camelids' sweat glands were located deeper to the dermis, unlike other animal species, that suggests playing a role in evaporative cooling as thermoregulatory action, particularly in hot climates (Abdou et al., 2006; El-Shafey et al., 2017). In our study, microscopical examination of the skin section showed the presence of many lobules of varying size composed of irregular packets and cords of cells in deeper areas of the dermis where the affected acini were lined by flattened neoplastic cells and their lumina were dilated with granular eosinophilic secretions. These findings were in partial agreement with Khordadmehr et al., (2016) who reported sebaceous adenomas and sebaceous ductal adenomas in the skin of camels.

There were several predisposing factors for the development of squamous cell carcinoma (SCC) including Chronic sun exposure, lack of pigment, and thin haircoat in the exposed area. The action of sunlight or ultraviolet radiation possibly induced mutations of the p53 tumor suppressor gene leading to overexpression of this protein. The geographical region of the present study with prolonged exposure to intense sunlight per day may increase exposure to ultraviolet radiation (UV). In cats, predilection sites for UV-induced SCC are eyelids, pinnae, and nasal planum. In dogs, the ventral trunk, digits, limbs, scrotum, and lips are the most common sites. In llamas and alpacas, SCC has been reported in ocular tissue, perineum, and haired skin (Mauldin and Peters-Kennedy, 2016).

In the present research, squamous cell carcinoma was detected grossly in the skin of the scrotum as a hemorrhagic ulcerated lesion. Microscopically, the tumor composed of nests of neoplastic epidermal cells invades and proliferates into the dermis surrounded by a fibrovascular stroma. These cords formed from concentrically laminated masses of intensely eosinophilic keratin in the center surrounded by peripheral polyhedral neoplastic cells. These findings were in partial agreement with that reported by Siddiqui et al., (2013) who found squamous cell carcinomas were the most common tumors in the medial toenails of the forelimbs in dromedary camel. Interestingly, two different types of SCC were reported in camels; well-differentiated and poorly differentiated types were distinguished from each other by horn pearls in the center whereas, in the poorly differentiated type, no hom pearls could be observed (Al-sobayil and El-amir, 2013). Additionally, a barbed wire laceration in a 12-yearold llama is considered a predisposing cause for the development of squamous cell carcinoma (Rogers et al., 1997).

In the current work, myxosarcoma was characterized grossly by the presence of a firm, irregular, pale yellow ulcerated hemorrhagic multilobulated subcutaneous mass that showed pale translucent lobules accompanied with gelatinous (mucinous) fluid oozed from the cut surface. Microscopical examination revealed the presence of spindle-shaped cells with prominent criteria of atypia and suspended in an abundance of extracellular matrix. This result agreed with Singh et al., (2006) and Campos et al., (2015). Meanwhile, other studies confirmed the presence of bluish extracellular mucopolysaccharides by using an Alcian blue stain (Cagnini et al., 2011; Wada and Nagata, 2021). The mesenchymal nature of neoplastic spindle-shaped cells in this work was confirmed by their expression of vimentin. Interestingly, our results were in complete accordance with the finding of Cagnini et al., (2011); Headley et al., (2011); and Campos et al., (2015).

Myxosarcomas are rare soft tissue sarcomas that may occur at any anatomic location of the body, but the most common locations are the skin and subcutaneous tissue as a circumscribed mass in the dermis (Liptak and Forrest, 2013). Myxosarcomas are malignant neoplasms derived from fibroblasts or other primitive mesenchymal cells that produce a prominent extracellular matrix composed of mucinous-rich stroma that is considered an important feature to distinguish myxosarcomas from histologically similar sarcomas like fibrosarcoma (Goldschmidt and Hendrick, 2002).

Myxosarcoma has been reported in cattle and buffalo (Khalil et al., 2020), in dogs (Headley et al., 2011; Campos et al., 2015; Wada and Nagata, 2021), rabbits (Von Bomhard et al., 2007), Syrian hamster (Cagnini et al., 2011), tiger (Shilton et al., 2002), ferret (Van Zeeland et al., 2006), European hedgehog (Singh et al., 2006) and humans (Liu et al., 2002).

It seems that the diagnosis of skin tumor in dromedary camels need considerable veterinary and abattoirs attention to overcome a decreased reporting of neoplasia in these species.

Conclusion

Our investigation on the skin of camels clarified different neoplastic lesions where myxosarcoma was the most predominant type of skin neoplasms in one-humped camels in the current study. Meanwhile, benign cutaneous tumors are less frequent in camels such as papilloma, fibropapilloma, lipoma, and adenoma in comparison to the prevalence rate of malignant types.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contribution

Conceptualization, IE, AE, SM, AA; Sample collection, IE; Diagnosis, AE, SM, AA; Methodology, investigation, and data curation, IE, AE, SM, AA; IE collected literature then drafting the manuscript in consultation with AE, SM, AA; Review and editing, AE, SM, AA. All authors read and approved the final manuscript. **References**

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