Received: 01 April 2023, Accepted: 18 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 5, 699-706

Unusual Multiple Primary Hepatic Tumors in Dromedary Camels: Pathological and Immunohistochemical Studies

Ibrahim Elmaghraby*, Abdel-Baset I. El-Mashad, Shawky A. Moustafa, Aziza A. Amin

Department of Pathology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.

***Correspondence**
Corresponding author: Ibrahim Elmaghraby
E-mail address: Ibrahim.elmaghraby@fvtm.bu.edu.eg

Abstract

Tumors have been rarely documented in the Arabian dromedary (*Camelus dromedarius*). Importantly, the current study investigated multiple primary tumors in the liver of dromedary camels, slaughtered at different abattoirs in Egypt during the period from January 2019 to February 2022. The study focused on the existence of two or more separate primary neoplasms, or one involving multiple sites in the same liver. The study conducted a comprehensive and accurate gross and histopathological description of the neoplastic cases. The use of special stains and diverse types of immunohistochemical-specific antibodies contributed significantly to the confirmed diagnosis of neoplastic cells. Interestingly, our results diagnosed unusual multiple primary hepatic tumors (prevalence 7/988, 0.7%), including one case each of cholangiocarcinoma-leiomyosarcoma, hemangiosarcoma-cholangiocarcinoma-leiomyoma, myelolipoma-osseous metaplasia, lymphosarcoma and three cases of leiomyomas. Detecting multiple primary hepatic tumors for the first time in the veterinary research area is a major challenge in the diagnosis and treatment strategies of tumors. Additionally, liver cirrhosis, amyloidosis, parasitic infection, and mycotic granuloma may be predisposing factors associated with increased overgrowth of primary mesenchymal hepatic tumors in camels.

KEYWORDS
Dromedary Camel, Histopathology, Immunohistochemistry, Multiple primary hepatic tumors, Abattoirs, Egypt

INTRODUCTION

Tumors have been rarely documented in the Arabian dromedary (*Camelus dromedarius*). Generally, it is assumed that camelids are susceptible to all the various tumor types that affect domestic animals. Unfortunately, although considerable veterinary and abattoirs attention to camelids, there is a decreased reporting of neoplasia in these species. The prevalence was determined in dromedary camels as 0.006%; of the reported cases, neoplasms of the skin and subcutaneous tissues are the most common (Alsobayil *et al*., 2018). Despite the fact that neoplasms arise in a broad variety of tissues, mesenchymal tumors of the liver in animals are rare. It is difficult to distinguish between each type of neoplastic cell by the routine H&E stain. Therefore, the use of special and immunohistochemical stains is helpful and confirmatory. Previous investigators diagnosed lipoma, cavernous hemangioma, and leiomyoma as benign tumors (Rezaie *et al*., 2015; Mohammed and Osman, 2017). Multicentric T-cell lymphoma (Simmons *et al*., 2005) and cholangiocarcinoma (Birincioğlu *et al*., 2008) were also described for the first time in camels. Most of these neoplastic disorders were published as case reports; no underlying etiologies or predisposing factors have been confirmed in camels.

Today, the situation of organs with multiple primaries is of increasing relevance and importance. Apart from a few reports in veterinary literature on the frequency of multiple primaries, the practical implications of the investigation of organs with multiple primaries are rarely discussed. Because of recent developments in treatment, diagnostic modalities, and increasing numbers of survivors, the risk of multiple primary neoplasms is increasing in humans (Copur and Manapuram, 2019; Zhao *et al*., 2020). To the best of the authors' knowledge, no work focusing on multiple primary tumors in the same liver has previously existed in veterinary research. Therefore, the objective of the present study was to determine the prevalence of multiple primary hepatic neoplasms in camels slaughtered at different abattoirs in Egypt, with the accurate gross and histopathological description of the neoplastic cases, immunohistochemical diagnosis and discussion of potential predisposing factors.

MATERIALS AND METHODS

Samples

The study examined a total of 988 single-humped camels (808 male and 180 female) slaughtered at different abattoirs (Toukh, Kerdasa, Warraq, and El-basateen) in Egypt during the period from January 2019 to February 2022. All data regarding the age, sex, and location of the suspected lesions were obtained. Any suspected gross neoplastic alterations in the examined livers were collected for further examination. A diligent search is made for primary or metastatic foci elsewhere during the necropsy of

ISSN: 2090-6277/2090-6269/

the animal that showed a positive liver lesion.

Histopathological examination

After careful gross examination, specimens from the liver were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 4 µm thickness, and stained by hematoxylin and eosin (H&E) for histopathological studies. Moreover, a special Van-Gieson's stain was used for muscles and connective tissue (Banchroft *et al*., 2013). The liver section revealed two or more separate primary neoplasms, or one involving multiple sites, considered positive for multiple primary tumors.

Immunohistochemistry

Immunohistochemical staining was performed on paraffin-embedded liver sections using the avidin–biotin–peroxidase complex method. The slides were incubated with primary antibody against αSMA (Clone 1A4, M0851, Dako, Germany) for leiomyoma and leiomyosarcoma, cytokeratin 20 (mouse anti-human, Dako, Carpinteria, CA, USA) for cholangiocarcinoma, monoclonal mouse anti-human CD31 antibody (Dako Cytomation, Denmark) for hemangiosarcoma. Moreover, CD3 (T- cell marker, Dako Glostrup, Denmark), and CD20 (B- cell marker, Dako Glostrup, Denmark) were used for lymphosarcoma. DAB was used as a chromogen and hematoxylin as a counter stain.

RESULTS

Based on the histopathological and immunohistochemical examination, seven cases out of 988 camels (prevalence 0.7%) had multiple primary hepatic tumors including one case each of cholangiocarcinoma-leiomyosarcoma, hemangiosarcoma-cholangiocarcinoma-leiomyoma, myelolipoma-osseous metaplasia, lymphosarcoma and three cases of leiomyomas (Table 1).

Cholangiocarcinoma-Leiomyosarcoma

Grossly, the liver showed multifocal, randomly distributed, whitish firm nodules (Fig. 1A), occasionally umbilicate appearance with a central depressed area was characteristic (Fig. 1B). Microscopically, intrahepatic cholangiocarcinoma was detected in the liver with leiomyosarcoma (Fig. 1C). The variant areas of leiomyosarcoma expressed positive immunohistochemical reaction by alpha-smooth muscle actin (Fig. 1D). Cholangiocarcinoma was an unencapsulated, poorly circumscribed, infiltrative neoplasm, effacing large areas of the hepatic parenchyma, separating, and surrounding remaining islands of degenerated hepatocytes. The tumor composed of cuboidal or columnar epithelial cells forms ductules (Fig. 2A) and acini (Fig. 2B), separated by abundant fibrous connective-tissue stroma. Occasionally, neoplastic ducts

Table 1. Multiple primary tumors in the liver of dromedary camels.

Pathological affection	Age (years)	Sex	Number of cases	Prevalence from the examined cases
Cholangiocarcinoma-Leiomyosarcoma	15	Male	1	0.10%
Hemangiosarcoma-Cholangiocarcinoma-Leiomyoma	9	Male	1	0.10%
Myelolipoma-osseous metaplasia	10	Male	1	0.10%
Lymphosarcoma	6	Male	1	0.10%
Leiomyoma	9-11	Male	3	0.30%
Total			7	0.70%

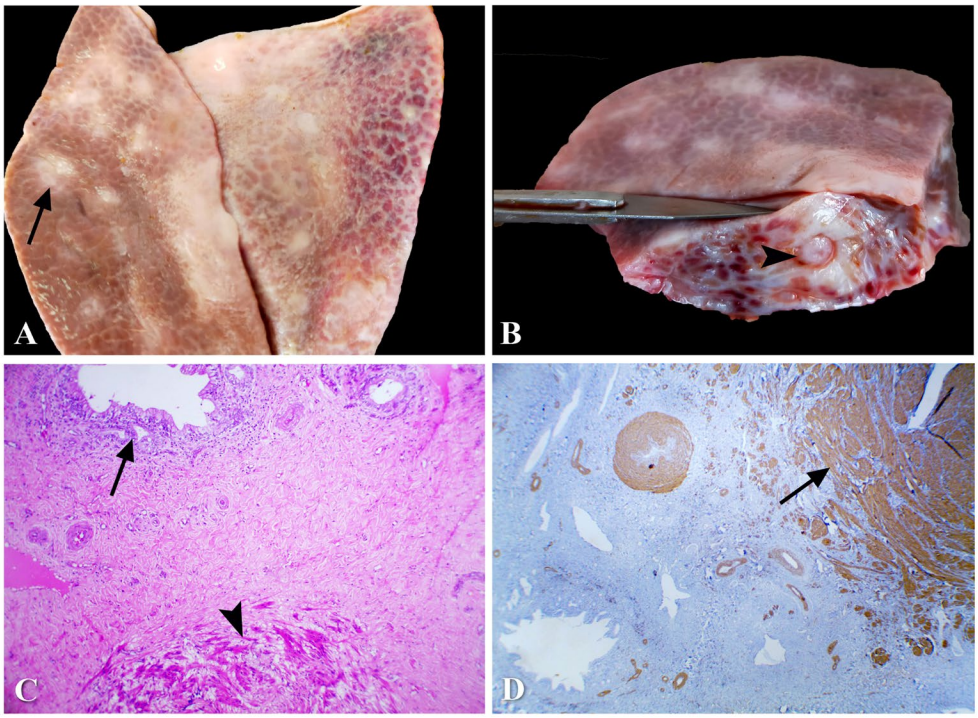

Fig. 1. Cholangiocarcinoma-Leiomyosarcoma, liver, camel. (A): Multifocal whitish firm nodules (arrow). (B): Umbilicate appearance (arrowhead) with the central depressed area. (C): Cholangiocarcinoma (arrow) with leiomyosarcoma (arrowhead) in a liver section, H&E, x 100. (D): Leiomyosarcoma (arrow) expressed a positive reaction for αSMA, x 100.

were distinctly lined by 1 to 2 cells thick and exhibited papillary formations within the lumen. The covering epithelium of well-differentiated ducts contained mucins. Neoplastic cells had variably distinct cell borders, moderate to abundant amounts of granular cytoplasm, and round to oval uniform nuclei with finely stippled chromatin and indistinct nucleoli. Mitotic figures were variable, with averages of 2-4 per high power field. Multifocally, poorly differentiated areas, rarely arranged in a certain pattern, characterized by pleomorphic neoplastic cells and hyperchromic nuclei (Fig. 2C). Immunohistochemically, the neoplastic cells expressed cytoplasmic positive reaction for Cytokeratin 20 antibodies (Fig. 2D). There were large areas of cirrhosis with numerous hemorrhages and small aggregates of lymphocytes and macrophages scattered throughout the tumor.

Multifocally, leiomyosarcoma was well-circumscribed, unencapsulated, moderately cellular neoplasm replacing the hepatic parenchyma. The tumor was composed of interlacing bundles and streams of smooth muscle cells, separated by interconnecting fibrovascular stroma (Fig. 3A). Separating and surrounding bundles of the smooth muscles, there were aggregates of the neo-

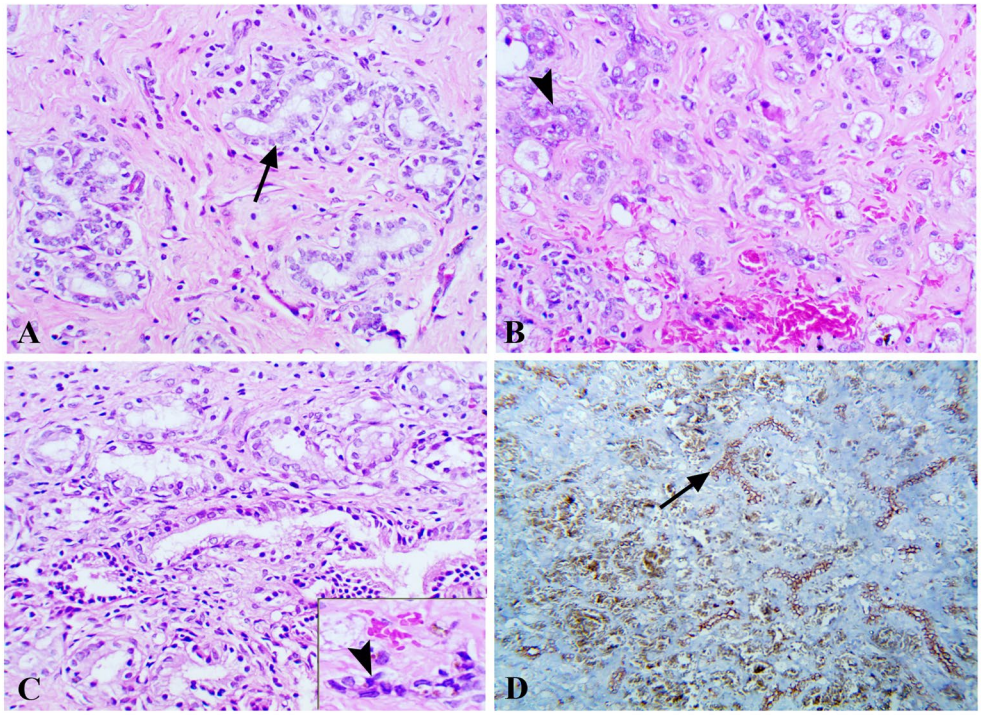

Fig. 2. Cholangiocarcinoma, liver, camel. (A): Neoplastic cells form ductules (arrow) separated by fibrous connective-tissue stroma, H&E, x 400. (B): Neoplastic cells form acini (arrowhead) on a dense fibrous stroma with hemorrhages and sparse numbers of inflammatory cells, H&E, x 400. (C): Poorly differentiated cholangiocarcinoma, H&E, x 200. Inset, pleomorphic neoplastic cells with hyperchromic nuclei (arrowhead), H&E, x 400. (D): Neoplastic cells (arrow) expressed cytoplasmic positive reaction for CK 20, x 200.

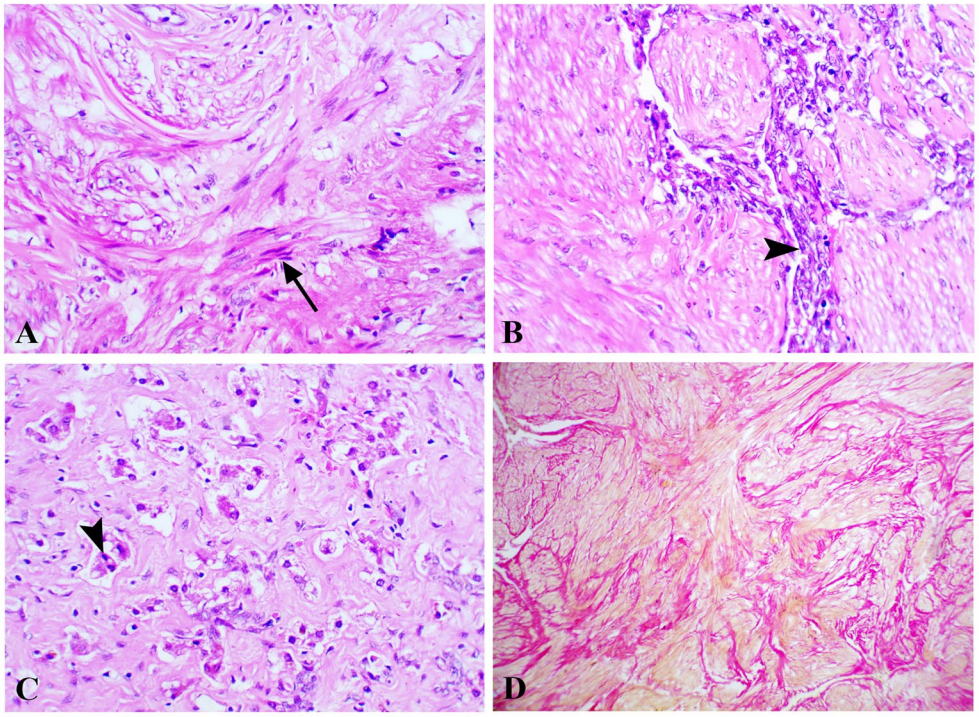

Fig. 3. Leiomyosarcoma, liver, camel, H&E. (A): Interlacing bundles and streams of smooth muscle cells (arrow), x 400. (B): Aggregates of the neoplastic cells (arrowhead) arranged in a trabecular pattern, x 400. (C): Neoplastic cells showing anisokaryosis, multiple nucleoli, scattered bizarre nuclei (arrowhead), and obvious mitotic activity, x 400. (D): The tumor cells stained positive yellow separated by interconnecting red fibrous stroma, (Van Gieson's stain), x 200.

plastic cells arranged in a trabecular pattern (Fig. 3B). Neoplastic cells were uniform fusiform cells, tightly packed with indistinct cell borders and abundant eosinophilic cytoplasm. Nuclei were elongated, with blunt ends, finely stippled to vesiculate chromatin and indistinct nucleoli. Occasionally, there were some degrees of anisokaryosis, multiple nucleoli, scattered bizarre nuclei, and obvious mitotic activity (Fig. 3C). The tumor mass stained positive yellow by Van Gieson's stain (Fig. 3D). Furthermore, there were multifocal areas of granulomatous inflammation in the center and periphery or adjacent to tumor mass, composed of necrotic cellular debris admixed with high numbers of viable and degenerate neutrophils, scattered septate fungal hyphae or spores, surrounded by large numbers of macrophages and lymphocytes.

Hemangiosarcoma-Cholangiocarcinoma-Leiomyoma

Grossly, the liver showed well-circumscribed, small-elevated dark red areas and multifocal, randomly distributed, whitish firm nodules (Fig. 4A). Microscopically, three different tumors were observed in the liver: hemangiosarcoma, cholangiocarcinoma,

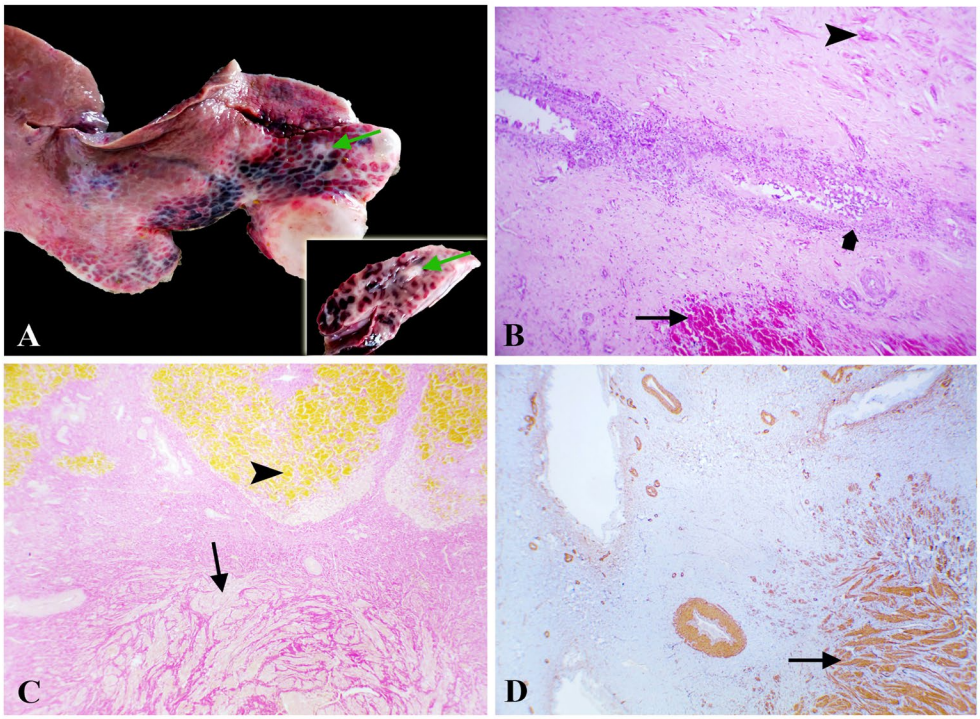

Fig. 4. Hemangiosarcoma-Cholangiocarcinoma-Leiomyoma, liver, camel. (A): Well-circumscribed, small-elevated dark red areas and multifocal, whitish firm nodules (green arrow). Inset, cut surface. (B): Hemangiosarcoma (arrow) with cholangiocarcinoma (thick arrow) and leiomyoma (arrowhead) in a liver section, H&E, x 100. (C): Leiomyoma stained positive yellow (arrow) and vascular channels of hemangiosarcoma stained dark yellow (arrowhead), (Van Gieson's stain), x 100. (D): Leiomyoma (arrow) expressed a positive reaction for αSMA, x100.

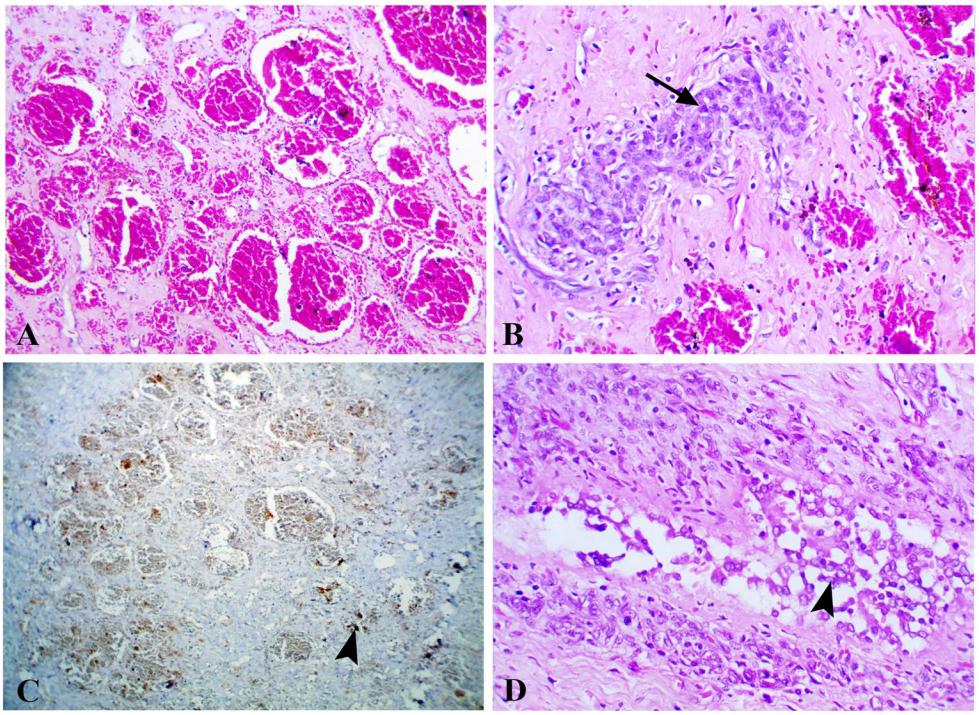

Fig. 5. Hemangiosarcoma (A, B, C) and Cholangiocarcinoma (D), liver, camel. (A): Formation of irregularly sized blood-filled vascular channels, H&E, x 200. (B): Solid cellular areas of pleomorphic neoplastic cells (arrow), H&E, x 400. (C): Neoplastic cells of hemangiosarcoma expressed a positive reaction for CD 31 antibodies (arrowhead), x 100. (D): Cholangiocarcinoma, neoplastic cells infiltrated the duct wall and formed solid cellular areas and trabeculae (arrowhead) within the duct lumen, H&E, x 400.

and leiomyoma (Fig. 4B). Neoplastic muscles stained positive yellow against red connective tissue stroma by Van Gieson's stain, while the vascular channels of hemangiosarcoma stained dark yellow (Fig. 4C). Immunohistochemically, leiomyoma was the only one expressed positive reaction for alpha-smooth muscle actin stain (Fig. 4D).

Hemangiosarcoma appeared as an unencapsulated, moderately cellular, infiltrative neoplasm effacing the hepatic parenchyma. The tumor is composed of pleomorphic spindle cells forming irregularly sized blood-filled vascular channels (Fig. 5A) and rarely arranged in solid cellular areas (Fig. 5B). Occasionally, neoplastic cells bulged into the vascular channel lumens and often wrapped collagen bundles. Neoplastic cells had variably distinct cell borders and a moderate amount of eosinophilic fibrillar cytoplasm. Nuclei were irregularly oval, with finely stippled chromatin, and occasional one to two variably distinct nucleoli. The mitotic rate averaged 1-3 per high power field. There was moderate anisokaryosis and anisocytosis. Immunohistochemically, the tumor cells expressed positive reaction for CD 31 antibodies (Fig. 5C). Multifocally, there were marked amyloidosis and atrophy of hepatocytes, varisized areas of hemorrhage, and fibrin thrombi with sparse numbers of scattered lymphocytes, plasma cells, and hemosiderin-laden macrophages. The remaining hepatocytes were swollen with vacuolated cytoplasm (fatty degeneration).

Cholangiocarcinoma was an unencapsulated, poorly circumscribed, infiltrative neoplasm, often involving intrahepatic large-size bile ducts. The tumor had similar characteristics to that observed in case I. The neoplastic cells infiltrated the duct wall and formed ductules and acini lined by cuboidal cells and separated by fibrous connective-tissue stroma or formed solid cellular areas and trabeculae within the lumen of neoplastic ducts (Fig. 5D). Neoplastic cells had variably distinct cell borders, moderate to abundant amounts of eosinophilic or vacuolated cytoplasm, and round to oval uniform nuclei with finely stippled chromatin. Multifocally, in poorly differentiated areas, there was moderate anisokaryosis and anisocytosis with average mitotic figures of 2-3 per high power field.

Leiomyoma was multiple, well-circumscribed, unencapsulated, moderately cellular neoplasm replacing the hepatic parenchyma. The tumor is composed of interlacing bundles of smooth muscle cells, separated by a fibrovascular stroma. Neoplastic cells were arranged in tightly packed spindle cells, with indistinct cell borders and eosinophilic cytoplasm; Nuclei were elongated cigar-shaped with blunt ends, finely stippled to vesiculate chromatin and indistinct nucleoli. Immunohistochemically, neoplastic smooth muscle expressed a positive reaction for alpha-smooth muscle actin antigen.

Myelolipoma-osseous metaplasia

Myelolipoma with osseous metaplasia was detected in one case. Grossly, the liver showed an irregular grayish-white circumscribed hard nodule (1 cm × 1.5 cm) (Fig. 6A). Microscopically, the hepatic parenchyma was effaced by a well-demarcated, moderately cellular, and unencapsulated neoplastic mass compressing adjacent hepatic tissue. The tumor is composed of a mixture of mature adipocytes and variable numbers of hematopoietic cells arranged in sheets on a moderate fibrovascular stroma, with the focal formation of immature woven bone (osseous metaplasia) within the neoplasm (Fig. 6B). The infiltrating hematopoietic cells of the myeloid and erythroid series were in various stages of maturation, admixed with mature red blood cells and variable amounts of eosinophilic proteinaceous fluid (Fig. 6C). Anisocytosis and mitotic figures were rarely observed. Multifocally, the hepatic interstitium was expanded by aggregates of hematopoietic cells admixed with lymphocytes and macrophages, and there was fatty degeneration or necrosis of the adjacent hepatocytes (Fig. 6D). The remaining hepatic parenchyma showed marked venous congestion and amyloidosis that stained positive by Congo red stain.

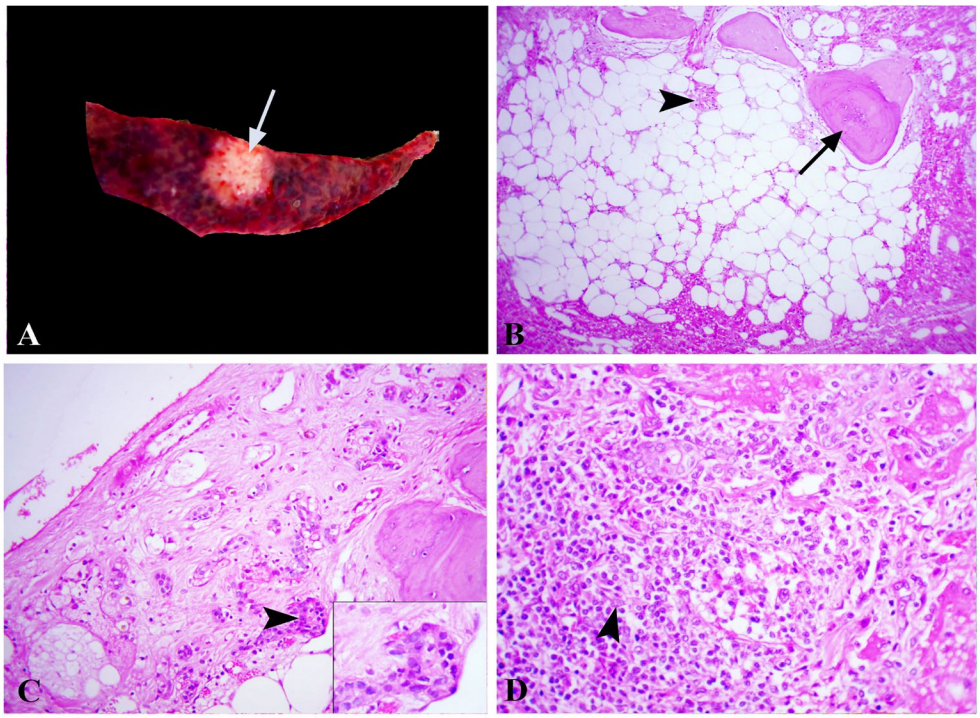

Fig. 6. Myelolipoma-osseous metaplasia, liver, camel. (A): Grayish white circumscribed hard nodule (arrow). (B): Myelolipoma is composed of a mixture of mature adipocytes and variable numbers of hematopoietic cells (arrowhead), with the focal formation of immature woven bone (arrow), H&E, x 100. (C): Hematopoietic cells (arrowhead) admixed with eosinophilic proteinaceous material, H&E, x 200. Inset, higher magnification, x 400. (D): Interstitial aggregation of hematopoietic cells (arrowhead) admixed with lymphocytes and macrophages, with necrosis of the adjacent hepatocytes, H&E, x 400.

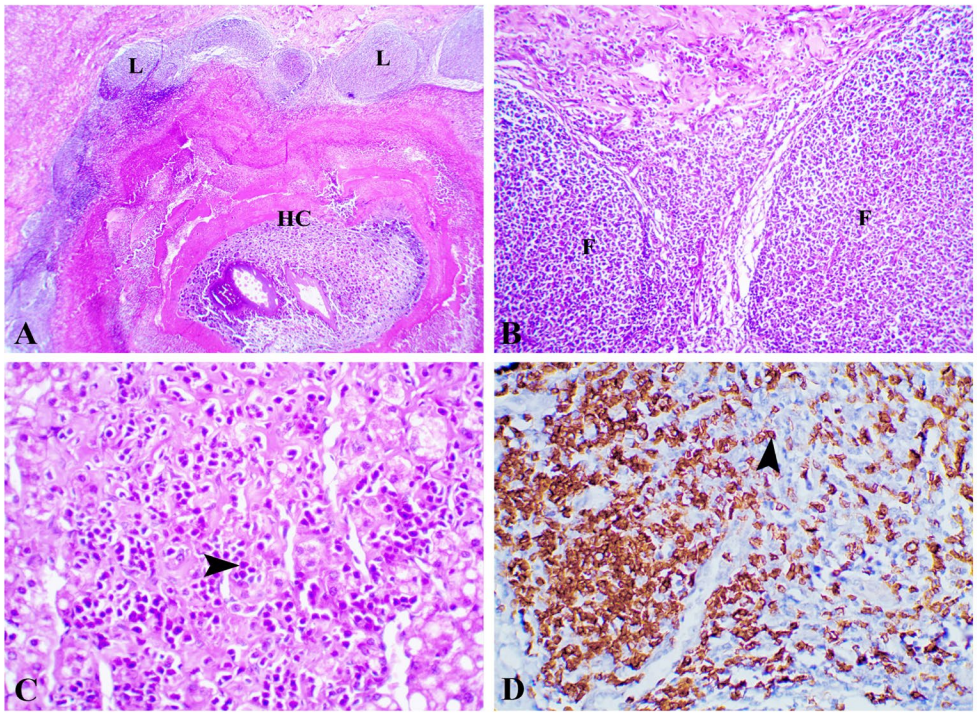

Fig. 7. Lymphosarcoma, liver, camel. (A): Neoplastic lymphocytes (L) replacing hepatocytes and surrounding immature hydatid cyst (HC), H&E, x 100. (B): The tumor is composed of round to pleomorphic lymphocytes arranged in follicles (F) with a fibrovascular stroma, H&E, x 200. (C): Lymphosarcoma (arrowhead) infiltrates the hepatic stroma, H&E, x 400. (D): Neoplastic lymphocytes (arrowhead) expressed a positive reaction for CD3, x 400.

Lymphosarcoma

Lymphosarcoma was observed in one case. Grossly, there were grayish-white nodules embedded in hepatic tissue. Multifocally infiltrating hepatic tissue; separating, surrounding, and replacing hepatocytes; and surrounding immature hydatid cyst was an unencapsulated, infiltrative, moderately cellular neoplasm (Fig. 7A). The tumor was composed of round to pleomorphic lymphocytes arranged primarily in follicles and less frequently in sheets with a fibrovascular stroma (Fig. 7B). Neoplastic lymphocytes infiltrated the hepatic stroma, had distinct cell borders, moderate amounts of eosinophilic granular cytoplasm, with a round to polygonal medium to large, hyperchromatic nucleus (Fig. 7C). There was moderate anisokaryosis, and anisocytosis with average mitotic figures 3-5 per high power field. Immuno-histochemically, neoplastic lymphocytes expressed a positive reaction for CD3 (Fig. 7D) but were negative for CD20. Therefore, the neoplasm was diagnosed as T-cell lymphoma. Multifocally, hepatocytes were distended with one or more round clear vacuoles.

Leiomyoma

Leiomyoma was observed in three liver cases. Grossly, the liver showed randomly distributed, multifocal whitish firm nodules. Microscopically, the tumors have similar findings to the leiomyoma previously described in this study (Hemangiosarcoma-Cholangiocarcinoma-Leiomyoma).

DISCUSSION

Importantly, the current study reported multiple primary tumors in the liver of Dromedary camels. Based on the present results, out of 988 one humped slaughtered camels, seven animals revealed multiple primary hepatic tumors (0.7%). Epidemiologically, multiple primaries are defined as more than one synchronous or metachronous tumor in the same individual (Vogt *et al.*, 2017). Regardless of the controversy about the definition, which differs from one study to another (Zhao *et al.*, 2020); the present study focused on the existence of two or more separate primary neoplasms, or a single one involving multiple sites in the same liver. The fact that animals may have multiple primary tumors is not new and already in 1977, a report found 2361 tumors in 1062 dogs and 250 in 120 other species categories (Priester, 1977). However, an overall decline in the incidence rates of multiple tumors in the same organ was observed. The process of multiple primary tumors is sequential and selective and contains stochastic elements. Primary tumors consist of multiple subpopulations of progenitor cells with heterogeneous properties, and the outcome of multiple tumors depends on the interplay of tumor cells with various host factors. Even within the same organ, however, heterogeneity of biological characteristics can develop rapidly (Williams *et al.*, 2018). For epidemiological studies, causes for multiple primary cancers may include various environmental, immune, and genetic factors (Vogt *et al.*, 2017). Camelids have unique features that differentiate them from ruminants, for example, dromedary camels have higher leukocyte numbers in the blood, extensive genetic defects, are resistant to hyperthermia, and withstand social stress and dehydration (Agnew, 2018). The interaction among host and different lifestyle-related factors induced single-nucleotide polymorphism (SNP) of tumor suppressor genes, oncogenes, and carcinogen metabolism-related genes, together with DNA methylation, which may play a significant role in the risk of multiple primary tumors (Zhang *et al.*, 2019).

Interestingly, our results diagnosed seven cases of multiple primary hepatic tumors (prevalence 7/988, 0.7%); including one case each of cholangiocarcinoma-leiomyosarcoma, hemangiosarcoma-cholangiocarcinoma-leiomyoma, myelolipoma with osseous metaplasia, lymphosarcoma and three cases of leiomyomas. Although our prevalence is lower than previously reviewed (2-17%) in humans (Vogt *et al.*, 2017), it is considered relatively high as it is only recorded in the liver. However, the prevalence rate in this study is extremely higher than that previously reported (0.006%) in camels (Alsobayil *et al.*, 2018). Veterinary oncologists should be aware that might be a second or third primary tumor in the affected organ. Cholangiocarcinomas were detected in the present work in two cases: one with leiomyosarcoma, and the other with hemangiosarcoma and leiomyoma. Cholangio-

carcinomas are uncommon malignant neoplasms of the biliary epithelium; only one case has been reported with seminoma in an 18-year-old male camel (Birincioğlu *et al*., 2008), but not described, in a retrospective study (Alsobayil *et al*., 2018). Cholangiocellular carcinomas have been recorded in dogs, cats, cattle, sheep, and horses but less frequently than hepatocellular tumors. Microscopically, cholangiocarcinoma can be distinguished from hepatocellular carcinomas by the typical acinar and ductular pattern; and the demonstration of mucins in the epithelium of well-differentiated ducts (Cullen, 2016). From an epidemiological standpoint, the presence of large areas of cirrhosis in the liver could be a potential risk factor for cholangiocarcinoma (Khan *et al*., 2019).

Primary hemangiosarcoma was diagnosed in one case with cholangiocarcinoma and leiomyoma in the same liver. The tumor has the same gross and microscopic pathological characteristics of hepatic hemangiosarcoma that are reported in dogs, cats, sheep, and cattle (Cullen, 2016). Primary hepatic hemangiosarcomas are most frequent in cats (Breithaupt, 2016). Distinguishing primary hepatic hemangiosarcoma in this study from metastatic lesions in the liver based on a diligent search for other foci elsewhere during necropsy (Cullen and Stalker, 2016). However, multiple organ involvement could be explained by the possibility of the multicentric origin of hemangiosarcomas.

Myelolipomas are benign neoplasms that are composed of a variable mixture of well-differentiated adipocytes and mature and immature hemopoietic cells, with the focal formation of immature woven bone (osseous metaplasia) within the neoplasm. Despite this well-differentiated histological pattern, myelolipomas must be differentiated from extramedullary hematopoiesis. Myelolipomas had been reported at a high rate in Goeldi's monkeys (Callimico goeldii) (Kleinschmidt *et al*., 2015), and rarely in the veterinary literature, particularly in the liver of cats and wild Felidae; and in the spleen, adrenal gland, and spinal cord of dogs (Cullen and Stalker, 2016; Kevin Keel *et al*., 2018). Whereas similar cases in camels were misdiagnosed as osteolipomatous metaplasia (Stroud *et al*., 1982; Rezaie *et al*., 2015). Metastasis had not been reported (Valli *et al*., 2017). The origin of myelolipomas is uncertain; there is some debate about the pathogenesis of this lesion. The speculative pathogenesis of Myelolipomas is a metaplastic transformation of a resident cell population triggered by various stimuli. The presence of immature woven bone within the neoplasm may be explained by the same pathogenesis or by the differentiation of adult, pluripotent mesenchymal cells into osteoblasts (osseous metaplasia) (Myers *et al*., 2012). Chronic ischemia associated with marked hepatic amyloidosis and venous congestion in the present study may be a contributing factor for myelolipomas observed in the liver of camels.

Lymphosarcoma was observed in one case, in the liver of 6 years old age camelus dromedaries, in the present study. Immunohistochemistry using antibodies against CD3, the neoplasm was diagnosed as T-cell lymphoma. Generally, lymphosarcoma was the most common of the reported cancers in the Camelidae family (Agnew, 2018), however, few reports were published in camelus dromedaries (Simmons *et al*., 2005; Raval *et al*., 2015). An unusual finding in our results is a primary arrangement of neoplastic lymphocytes in follicles or sheets surrounding the immature hydatid cyst, which may postulate the presence of hydatidosis as a predisposing factor for lymphosarcoma in camels.

The current study documented leiomyoma in three cases, while in another case, it was detected with hemangiosarcoma and cholangiocarcinoma in the same liver. Microscopically, the tumor is composed of multiple masses of interlacing bundles of typical smooth muscle cells, separated by a fibrovascular stroma. Primary leiomyoma of the liver is a rare benign tumor in animals and humans with unclear pathogenesis. Leiomyomas were previously reported in camels' uteri (Moustafa *et al*., 2004), during an abattoir survey in Egypt, but they had not been recorded in camels' livers. The suggestive possible origin of these tumors is the proliferation of smooth muscle of the hepatic vessels as reported by Esmaeilzadeh *et al*. (2007) in cattle's liver and by Wareth and Moustafa (2013) in camel's lung. The presence of multiple sites within the liver is regarded as the multicentric origin of leiomyomas.

Leiomyosarcoma was detected in one case with cholangiocarcinoma in the same liver. Despite the tumor being largely well differentiated, malignancy features were clear, based on the infiltrative nature of the neoplastic cells, cellular atypia, anisokaryosis, and obvious mitotic activity. Leiomyosarcomas were exceedingly rare in all domestic animals except those originating from the gastrointestinal tract wall of dogs. Recently, two cases of leiomyosarcoma were published in canines (Ochoa-Amaya *et al*., 2019; Kim *et al*., 2022); but no tumors were recorded in the liver. Leiomyomas and leiomyosarcomas in the present work were stained positively yellow by Van Gieson's stain. Immunohistochemistry, these tumors confirmed positivity for alpha-smooth muscle actin (Rubisz *et al*., 2019). Multifocally, the presence of mycotic granulomas within or adjacent to tumor mass in the liver may contribute to the pathogenesis of leiomyosarcomas which appeared etiologically linked to immunodeficiency (Bhatia *et al*., 2012). Histologically, it is insufficient to determine if a leiomyosarcoma is primary or metastatic. Generally, the presence of more than one focus within the same liver suggests a metastatic origin (Cullen, 2016); but no primary lesion was detected in other organs during careful necropsy. Therefore, the discovery of multiple masses of leiomyomas in the liver of camels in the present study is likely to be primary hepatic leiomyosarcoma.

CONCLUSION

Multiple primary hepatic tumors have been diagnosed in camels for the first time in the veterinary research area. The significant prevalence of multiple primary tumors in camels highlights the need to investigate the impact of genetic susceptibility - environmental exposures interaction and their role in cancer initiation, promotion, and progression. Detecting multiple primary cancers in animals is a major challenge in the diagnosis and treatment strategies of tumors. Primary mesenchymal hepatic tumors such as hemangiosarcoma, leiomyosarcoma, and myelolipoma were not previously recorded in camels. Additionally, liver cirrhosis, amyloidosis, parasitic infection, and mycotic granuloma may be predisposing factors associated with increased overgrowth of primary hepatic tumors in camels.

ACKNOWLEDGMENTS

The authors would like to thank all staff members of Pathology Department, Faculty of Veterinary Medicine, Benha University.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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