**orignal reseach paper**

# Intracystic papillary carcinoma in the mammary gland of she-camel (*Camelus dromedaries*), Sudan

EDITOR COMMENT:

1. Clarifications sought: In the abstract section, the authors have commented that their survey over a one year time frame had diagnosed 45 tumours, of which 23 were malignant. Only one tumour had features suggestive of intra cystic palliary carcinoma. The main article has no mention about this fact. Better if authors summarize this information in the results section.
2. Line / word restructured: **Section Introduction**: ~~"The occurrence of intracystic papillary carcinoma in herbivores is meagre."~~ [to be changed to] "The occurrence of intracystic papillary carcinoma in herbivores is rare."
3. Line / word restructured: **Section Introduction**: ~~"Only there was a single report that on multicentric papillary cystadenoma in a cow (4)."~~ [to be changed to] "There is an only single reported case of multicentric papillary cystadenoma in a cow (4).
4. Clarification sought: Check whether you have tested for Cytokeratin 5/6 or cytokeratin 6/7.
5. Clarification sought: **2.2 Histopathological method:**dextrane polymer_hrp ?
6. Clarification sought: "~~grabe like structures"~~ [to be changed to] grape-like structures.
7. Clarification sought: [Priniting error likely] - [spelling mistake] "inrtacystic"
8. Formatting required: In the discussion section: (5; 6; 7; 8).
9. Rephrasing of sentences: Please go through reference articles about how these lines are written. In the **DISCUSSION** section:
    [1] This is in agreement with (13) and (2) who considered the cellular anaplasia and tumor invasion as a hallmark for malignancy.
    [2] (17) reported intracystic papillary carcinoma invasion in cyst wall, in vascular veins and lymphics.
    [3] (18) stated that the differentiation between them may be arbitrary.
    [4] (6) described IDPC *in situ* as non-invasive carcinoma extending into the small and medium sized ducts throughout the breast.
    [5] On the other hand, (12) is of the opinion that if the epithelium of the papillary carcinoma has features diagnostic for intra-ductal component the tumor should be classified as intra-ductal.
    [6] Furthermore, (19) suggested that papilliferous epithelium proliferations develop from the alveolar tissue within lobule, interlobular duct or teat sinus.
10. Clarification required: [Please check what you write – gross mistake in your paper.] CONSENT section: All authors declare that written informed consent was obtained from the animal for publication of this paper and accompanying images.

## ABSTRACT

**Aims of the Study**: Survey of udder disorders over one year to investigate the histopathological lesions of tumors in camel (Camelus dromedarius).

**Place of Study**: The study was undertaken in the Department of Pathology, Central Veterinary Research Laboratory, Ministry of Animal Resource and Fisheries, Khartoum, Sudan

**Study Design**: 45 tumors of camel udder tissues were obtained from Tumbool abattoir Al-Gazeera State; the gross and histopathological lesions were studied.

**Methodology:** The macroscopic pictures of samples were reviewed and described, then histopathological lesions were studied using H&E stain. Lastly immunohistochemical staining was done using estrogen, progesterone antibodies and CytoKeratin 5/6.

**Results**: Intracystic papillary carcinoma (IPC) was diagnosed in a 13-year-old non- pregnant, non- lactating local eco-type she-camel, Out of 23 malignant tumors. , the udder displayed multiple neoplastic masses entrapped in multioccular cystic spaces. The neoplastic cells were arranged in papilliferous fronds supported by fibro vascular stalk. These cells were pleomorphic cuboidal to low columnar epithelial cells with vesicular nuclei. Tumor invasion was encountered in the cystic wall and lymphatic vessels

**Conclusion:** To the best of our knowledge this may be the first intra cystic papillary carcinoma reported in the udder of a dromedary worldwide.



**Keywords**: Intracystic papillary carcinoma, immuno-stain mammary gland, the dromedary-she-camel, Sudan.


## 1. INTRODUCTION

Neoplasms of the mammary glands occur in all animal species. The prevalence of tumor in carnivores and herbivores and between captive and wild carnivores is quite different (1). This neoplasm is the most common in bitches; it ranks third after skin and lymphoma in queens (2). The available literature on papillary cystadenoma in food producing animal is rare (3). The occurrence of intracystic papillary carcinoma in herbivores is meagre. Only there was a single report that on multicentric papillary cystadenoma in a cow (4). To the best of our knowledge this may be the first of intra cystic papillary carcinoma report in the udder of dromedary worldwide.

## 2. MATERIALS AND METHODS:

### 2.1 Case history

During a survey of udder affections of slaughtered she-camels was conducted at Tumbool slaughterhouse, El-Gezira, State, Sudan. A thirteen-year-old, non-pregnant, non-lactating local eco-type she-camel with a history of chronic incurable udder affection was examined at slaughter. She originates from North Drafur State. The udder was slightly swollen, hard in consistency and the teat of the left hind quarter was distended approximately 2-3 times the normal size,

completely obstructed canal. On cut section the udder was lobulated, grayish-white with arborescent growth with irregular extensive branches hanging from the body of the udder filling and occluding the teat canal.

### 2.2 Histopathological method

Tissue specimens from udder were fixed in 10% formalin, processed routinely in paraffin wax and 5-6 µ sections were cut and stained with haematoxylin and eosin (H&E).

For tumor Immunohistochemistry using dextrane polymer_hrp for estrogen and progesterone receptor was done, and Ck 6&7.

## 3. RESULTS

At slaughter, the udder was slightly swollen, hard in consistency and the teat of the left hind quarter was distended approximately 2-3 times the normal size, and its canal was completely obstructed. On cut section the udder was lobulated, grayish-white with arborescent growth with irregular extensive branches hanging from the body of the udder filling and occluding the teat canal. Small multi-papillae forming bunch of grabe-like structures were projecting from these arborescent growths (fig 1).5

Histopathologically, the most prominent finding was complete obliteration of normal udder tissue which was replaced by multineoplastic masses entrapped in multioccular cyst or dilated ducts. The papillary fronds infiltrated into primary, secondary and tertiary branches (fig 2). Tumor invasion of the cyst wall and the lymphatics were detected. The lobules bordering the neoplastic masses presented areas of early tumor transformation with the papillary fronds appeared to be emerging from, or infiltrating into apparently normal secretory acini (fig 3). Each cyst was lined with one layer of flat to low cuboidal epithelial cells surrounded by fibrous tissue, stroma that varied in size from narrow to moderately wide in size. Each of the neoplastic masses composed of densely packed papilliferous fronds supported by fibrovascular core that appeared to be projected from the cyst wall (fig 4). Some papillary projections were covered with one or multi-layers of cuboidal to low columnar epithelial cells. On the other hand exophytic inrtacystic papillary fronds covered with pleomorphic poorly orientated undifferentiated epithelial cells were regularly encounterd together with stratification of the nuclei which formed irregular clusters. The nuclei were vesicular or clear with the ground-glass appearance. Dystrophic calcification was encountered in the epithelium and connective component of neoplastic masses. Moderate mononuclear cells reaction was observed in stromal connective tissue.

Immunostain for estrogen and progesterone were both negative while the ck 5/6 was highly positive (fig 5)

**Fig 1:** Macroscopic appearance of the tumor. A formalin fixed piece of the tumor showed a lobular pattern delineated by fibrous septa.

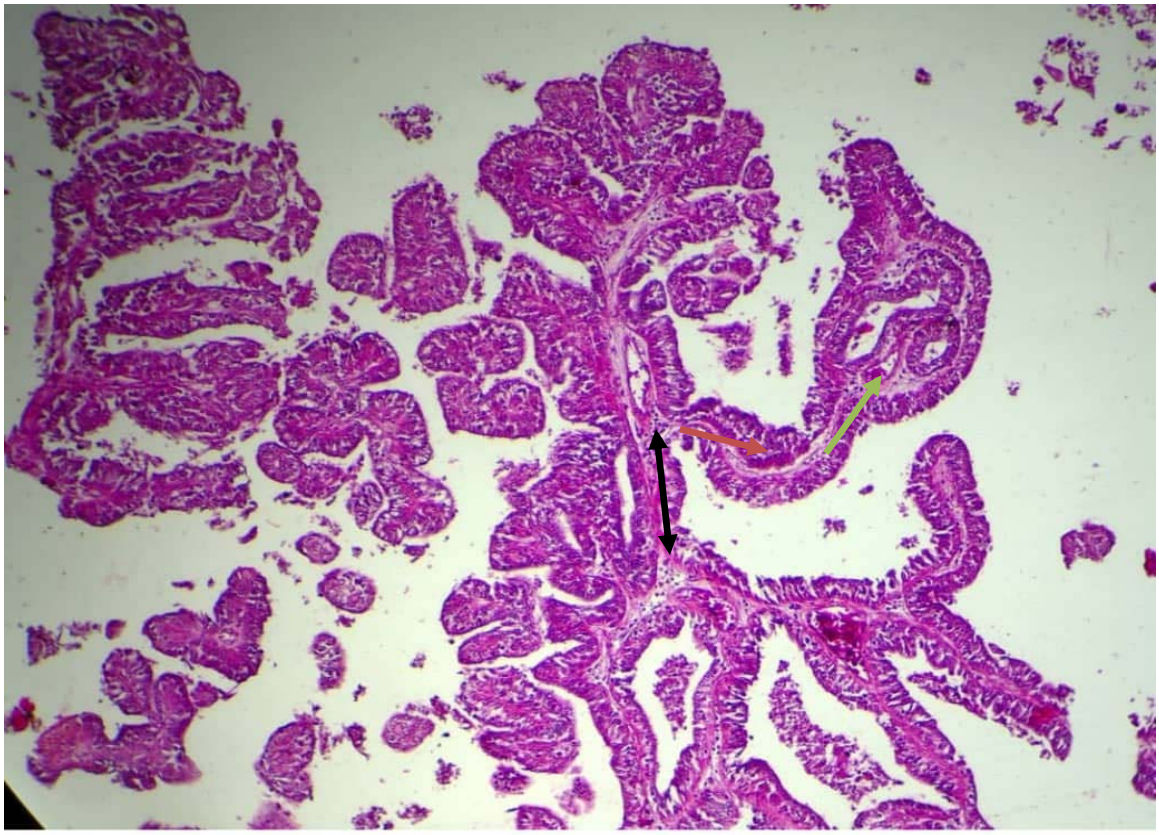

**Fig 2:** Intracystic papillary carcinoma, Note complex fibrovascular core with primary (black), secondary(red) and tertiary (green)branches H&E x10.

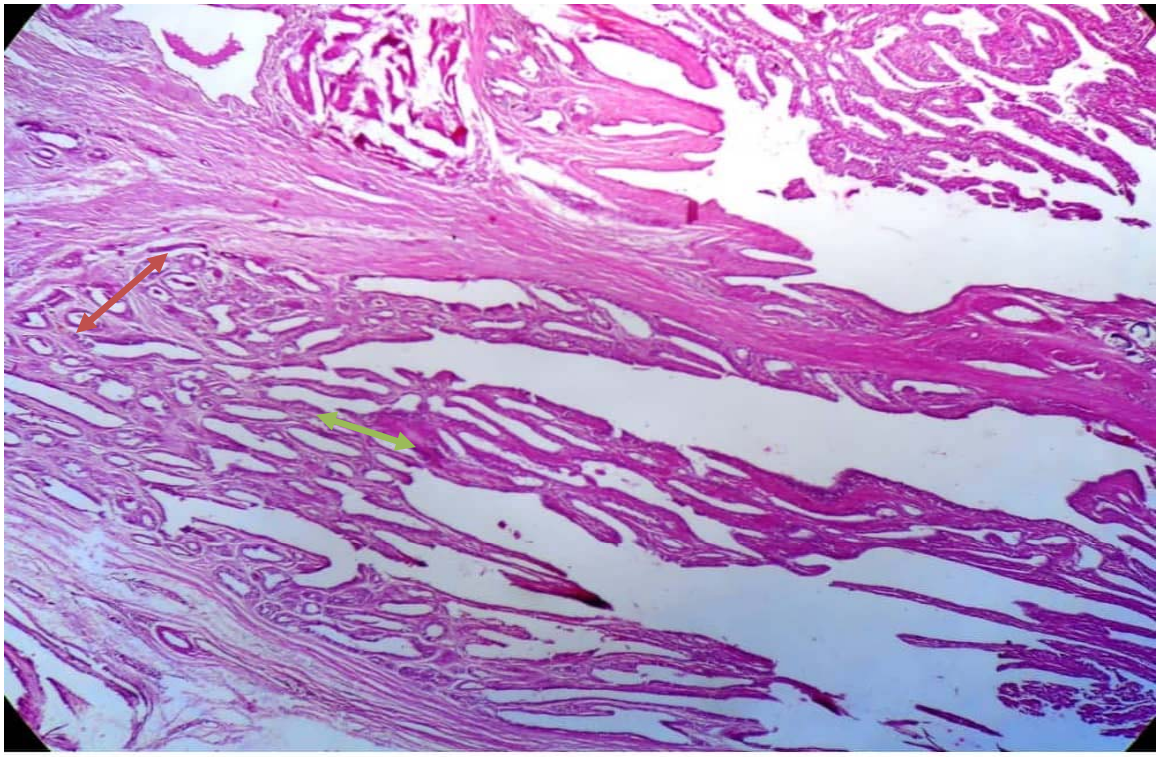

**Fig 3:** Intracystic papillary carcinoma Note, Early tumor transformation (green arrow) or infiltrated in apparently normal glandular tissues (red arrow) H&E x4.

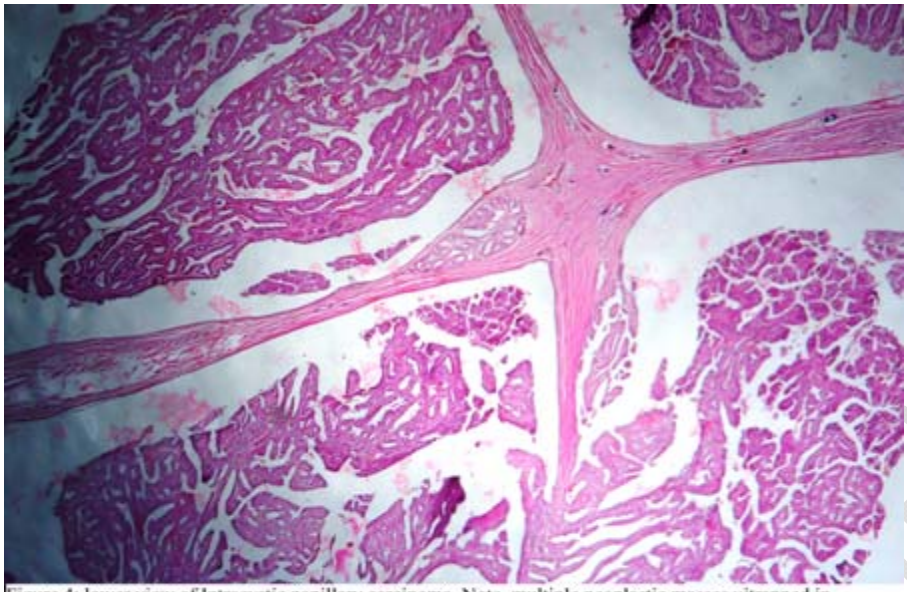

Figure 4: lower view of Intracystic papillary carcinoma. Note, multiple neoplastic masses uitrapped in multioccular cystic spaces, the fibrovascular stak projecting from the cyst wall, cyst wall invasion by tumor H&E.

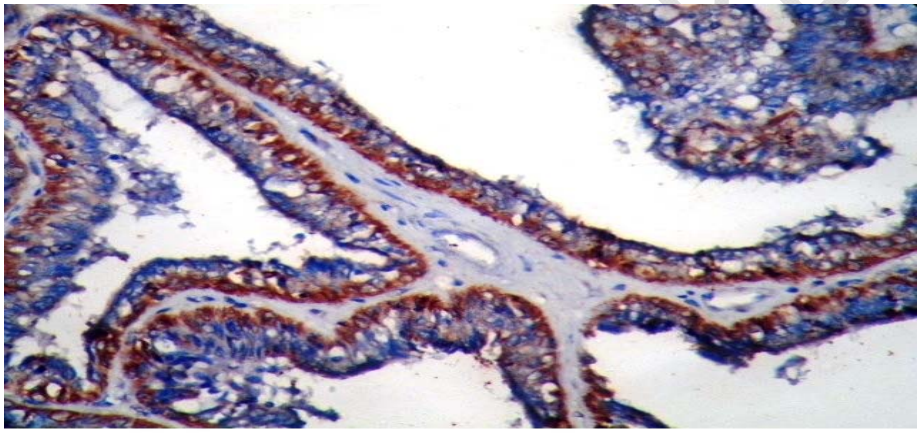

**Fig 5**: Udder of she camel with intracystic papillary carcinoma and positive for ck 5/6 Note, brown color, immunostain X40.

## 4. DISCUSSION

In the current study the masses were diagnosed as intracystic papillary carcinoma (IPC) based on the presence of papillary frond-forming pattern supported by fibrovascular stalk, which were considered as a diagnostic feature of papillary carcinoma (5; 6; 7; 8). These changes were consistent with those previously described for IPC in bitchs (9); a cow (4); zoo animals (10) and in the man (6; 11; 12). The neoplastic masses were projected in cystic cavity thus giving pathognomonic feature of intracystic neoplasm, this is in line with (6 ; 11) who stated that if intracystic component is present the tumor it should be classified as intracystic papillary carcinoma.

Based on the aforementioned morphological features and the relatively poorly differentiated plastic cells with cellular and nuclear pleomorphism and tumor invasion in cyst wall and lymphatics vessels, The tumor was described as carcinoma or malignant. This is in agreement with (13) and (2) who considered the cellular anaplasia and tumor invasion as a hallmark for malignancy. Furthermore, presences of empty or vesicular nuclei are considered as classic nuclear morphologic feature of papillary carcinoma (8; 14; 15). Other authors (16; 11), however, reported that invasion of papillary carcinoma occurs infrequently, as early small focus of stromal invasion and almost at the periphery of the lesion. (17) reported intracystic papillary carcinoma invasion in cyst wall, in vascular veins and lymphics.

Morphologically, IPC has the features of intraductal papillary carcinoma (IDPC) *in situ*. (18) stated that the differentiation between them may be arbitrary. However, (6) described IDPC *in situ* as non-invasive carcinoma extending into the small and medium sized ducts throughout the breast. On the other hand, (12) is of the opinion that if the epithelium of the papillary carcinoma has features diagnostic for intra-ductal component the tumor should be classified as intra-ductal.

Our study showed that certain lobules adjacent to the tumor revealed coexistence of both papillary fronds and apparently normal glandular tissue. It is difficult to draw any conclusion whether the papillary fronds emerging from, or infiltrating into, apparently normal udder tissue. Other investigators (19) (20) are of the opinion that adenocarcinomas (Tubular, papillary), arise from the alveolar epithelium . Furthermore, (19) suggested that papilliferous epithelium proliferations develop from the alveolar tissue within lobule, interlobular duct or teat sinus. Papillae were formed, when the ductules or alveoli become dilated and separated by thin stromal septa. When the septa break, the lobule is converted into single cyst with intraluminal papillae.

In lamb immune-histochemistry revealed the ductal epithelium to be positive for pan cytokeratin (AE1/AE3) and loose fibrovascular stroma was positive for vimentin and basal cells covering the ductal epithelium of alpha-smooth-muscle actin. Immuno-staining for the estrogen and progesterone receptors was negative may have resulted from a lack of cross reactivity to the ovine species of antibodies used. A diagnosis of mammary fibroadenoma was made based on the histological and immunohistochemical findings (21). In the mixed mammary tumor in camel, they found papillary section was moderate positive result for estrogen receptor and negative progesterone, while the fibroadenoma section was negative for both receptors (22)

## 5. CONCLUSIONS

To our knowledge this is the first time that the tumor cases reported in the field and found to be a problem in the udder of the camels

Intracystic papillary carcinoma IPC is described, the histopathology in one of best method for diagnoses tumor.

Further research should be tackled in order to determine the role of the neoplastic masses in the disease prevalence for better epidemiological consideration.

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## ETHICAL APPROVAL

All authors hereby declare that all animals experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards.

## CONSENT

All authors declare that written informed consent was obtained from the animal for publication of this paper and accompanying images.

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**To: International Research Journal of Oncology**

**To whom it may concern**

**Subject: Ethical Clearance**

This is to certify that the following authors: Abeer Abdella, Zakia Abass Khalid Mohamed and Muna Ahmed, were veterinary researchers, they did this work under title (Intracystic papillary carcinoma in the mammary gland of she-camel in Sudan) in Central Veterinary Research Laboratory, Soba Khartoum, Sudan. I can assure that they legally sampled the animal following all ethical procedures and they have run the requested laboratory test perfectly. The center administration and all authors agree to publish this article after submitting to this journal.

7/1/2019

With best regard

**Dr. Magdi Badawi**

**Director / Central Veterinary Research Laboratory**

**Date: 7/1/2019**