

Pathomorphological and Immunohistochemical Findings of Cystic Mucinous Hyperplasia of the Gall Bladder

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ABSTRACT

A case of cystic mucinous hyperplasia is described in 5 years old, female, German Shepherd dog. Macroscopically the gall bladder was distended. There were multiple 0.1-0.3 cm size cystic papillary structures in many areas of gall bladder. In addition a 16 cm diameter paraovarian cyst containing serous fluid was detected in the cranial aspect of left ovary. Tissues were processed routinely and stained with haematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS) stains. The gall bladder cysts contained a mucinous secretion and were lined with cuboidal epithelium. Immunohistochemically, TGF- β 1, HER2 and Cx43 markers were mildly positive in cytoplasm of the gall bladder epithelial cells although there were no positive reactions with Ki67 and CEA markers.

Keywords: Dog, gall bladder, cystic mucinous hyperplasia, pathomorphology, immunohistochemistry.

INTRODUCTION

Cystic mucinous hyperplasia originating from the extrahepatic biliary system is rare and includes cystic mucinous hypertrophy, mucocoele of gall bladder and mucinous colecystitis (1,2). Until now these hyperplastic changes have been noted in the dog, sheep and cow (2-5). The condition generally has no clinical expression and is encountered incidentally at necropsy (6). To their best of the knowledge of the authors immunohistochemical studies have not been reported.

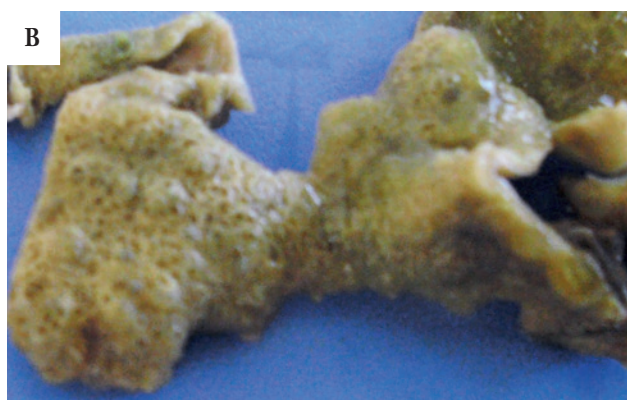
This case presents pathomorphological and immunohistochemical findings of cystic mucinous hyperplasia of the gall bladder of a 5 year old female dog.

MATERIALS AND METHODS

A 5 year old and female German Shepherd dog was referred to the clinic with complaints of anorexia and lethargia. At the owners request the bitch was euthanized. At necropsy, tissue samples were taken from the gall bladder were fixed in 10%

neutral formalin solution and processed routinely. Paraffin embedded tissues were cut at 5 μ thickness and stained with hematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS) stains.

Immunohistochemistry was carried out using indirect immunoperoxidase method (ABC-P). The tissue was examined using TGF- β 1, Connexin 43 (Cx43), carcinoembryogenic antigen (CEA), Her2 neu, and Ki67 markers. After deparaffinization and dehydration, peroxidase activity was blocked (Novocastra Peroxidase Detection Systems, Ready-to-Use, Germany). Trypsinization was performed by using 0.1% trypsin solution. Non-specific proteins were blocked with protein blocking sera (Novocastra Peroxidase Detection Systems (Ready-to-Use, Germany). The sections were incubated with primary antibodies [polyclonal rabbit anti TGF- β 1 (Santacruz Biotechnology Inc., USA), polyclonal rabbit anti Connexin 43 (clone GJA-1, Abcam, United Kingdom), monoclonal mouse CEA (clone COL-1, Neomarkers, USA), monoclonal mouse Her2neu (clone c-ErbB-2 oncoprotein,



Figures 1A and 1B: Macroscopic appearance of cystic exophytic changes in lumen of the gall bladder.

Novocastra, United Kingdom), monoclonal rabbit Ki67 (clone SP6, Labvision, USA) followed by biotinylated link antibody and Horse Radish Peroxidase (HRP) antibodies which were applied respectively (Novocastra Peroxidase Detection Systems, 250 tests, Ready-to-Use, Germany). AEC (Santacruz Biotechnology Inc., USA) was selected as chromogen. For counterstaining, Gill's haematoxylin was used. Sections were

mounted with glycerine. All sections were examined using a light microscope (Leica, Germany DM4000B).

RESULTS

Macroscopically the gall bladder was distended and filled with muddy-like gall. When cut open the surface of mucosa was thickened with a granular appearance. Many cystic structures with a green color were present varying between 0.1 to 0.3 cm in diameter (Figure 1A). On cut section these cysts were found to be filled with a greenish-brown colored material with a waxy consistency (Figure 1B).

A paraovarian cyst of 16 cm in diameter was present on the left ovary filled with clear serous fluid (Figure 2). Furthermore, multiple cystic structures were present on both ovaries varying between 1 to 3 cm with a similar appearance.

Microscopically it was noted that the cysts of the gall bladder were lined with one layer of columnar epithelium (Figure 3). These cystic structures and epithelial cells were mucin positive in many areas as judged by staining with PAS (Figures 4).

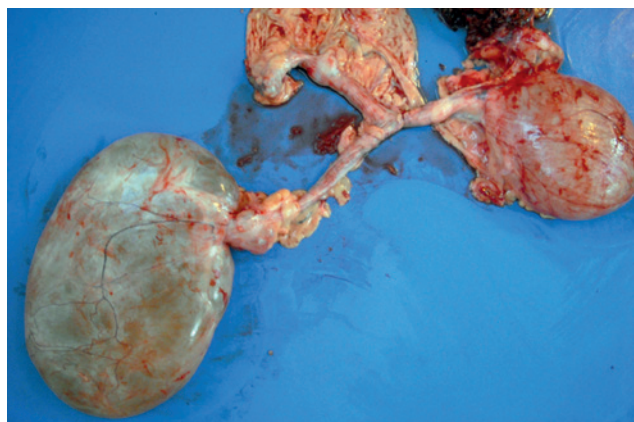


Figure 2: Paraovarian cyst filled with clear and serous fluid in left ovary.

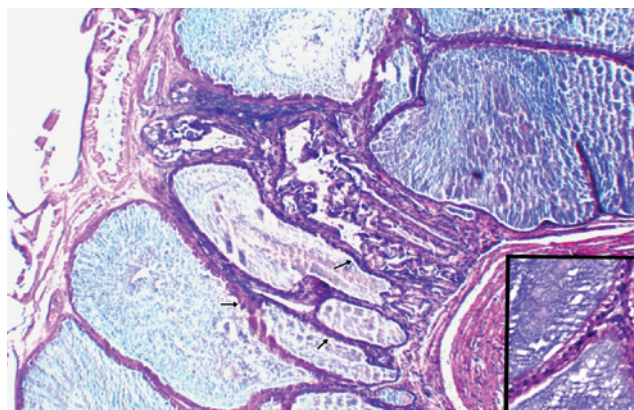


Figure 3: Hyperplastic and cystic mucinous glands (arrows), ×100 and ×400, H&E.

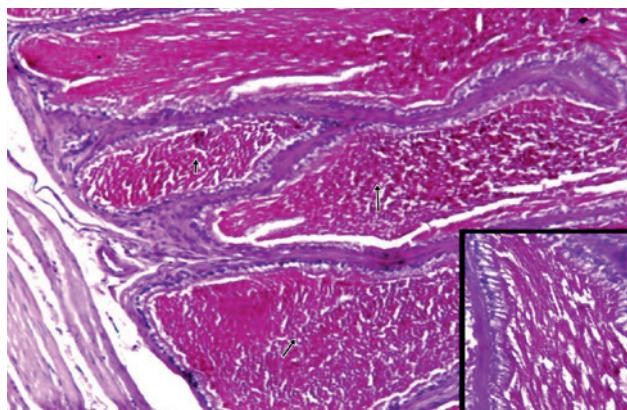


Figure 4: PAS positive gland cells (arrows) of gall bladder, ×100 and ×400, H&E.

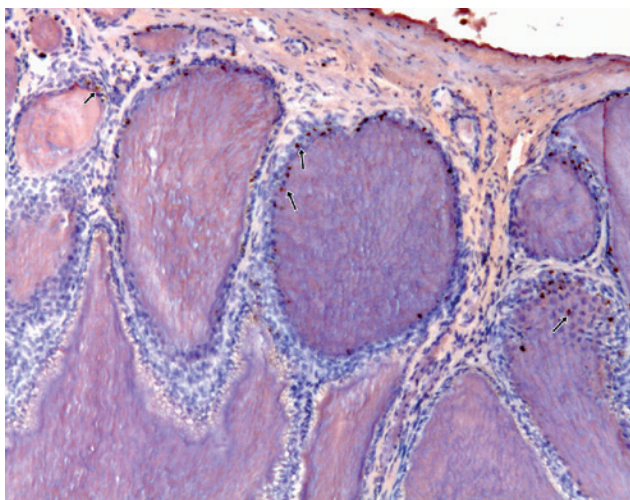


Figure 5: TGF-beta 1 mildly positivities (arrows) in gland cells, $\times 100$, ABC-P.

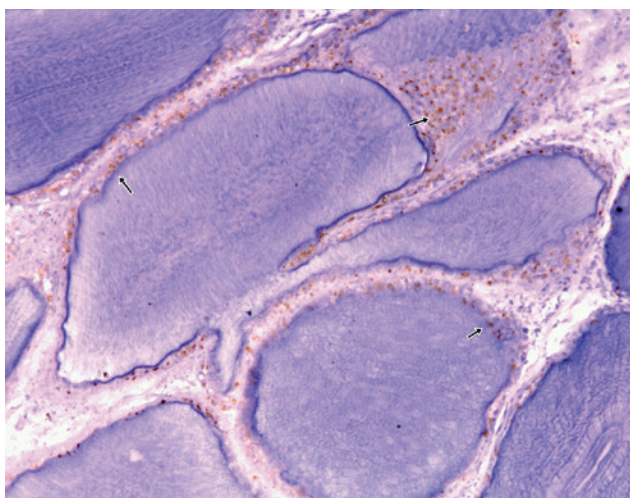


Figure 6: HER2 mildly positive (arrows) in gland cells, $\times 100$, ABC-P.

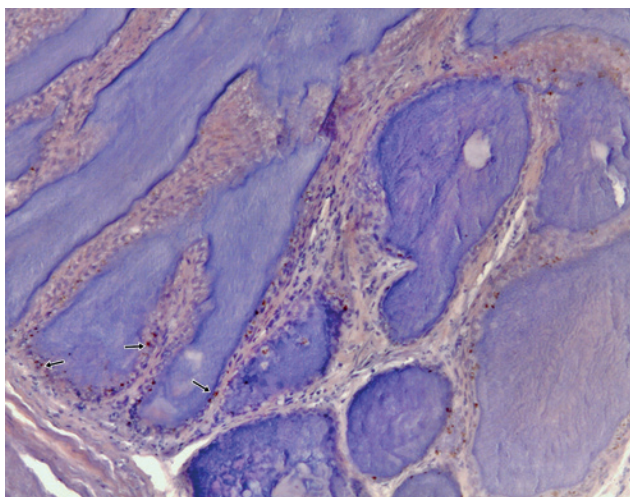


Figure 7: Connexin-43 mildly positive (arrows) in gland cells, $\times 100$, ABC-P.

The ovarian cysts varied size and were lined with flattened epithelium. In the left ovary fused cysts were lined with cuboidal epithelial cells. Furthermore, multilayer muscle bands enveloped these epithelial cells at their periphery.

Immunohistochemically, TGF- $\beta 1$, HER2 and Cx43 markers were mildly positive in cytoplasm of gall bladder epithelial cells although there were no positive reaction with Ki67 and CEA marker (Figures 5-7).

DISCUSSION

The etiology of cystic hyperplasia of gall bladder is unknown however there appears to be an association with age, progestagenic effects, chronic bacterial infections or parasitic infestations (3). Similar hyperplastic changes have been described in beagle dogs related to progestagen applications (3, 7). The effect of progesterone has been studied in different organs (7). Among the changes described are cystic mucinous hyperplasia of gallbladder, acinar proliferation of mammary gland, persistent ovarian corpora lutea, cystic uterine glandular hyperplasia, metritis and cervicitis. Often it is encountered related to pathological findings of the gallbladder resembling that of the current case (7). According to another study, factors which may result in hyperplastic changes of the gallbladder include release of growth hormones or excessive concentrations of hormones which may occur when dealing with conditions affecting the liver, exocrine pancreas or adrenal cortex (8). In sheep, gestation and prolonged progestagenic effects are considered to be a possible etiology (9).

In the case presented here the etiology of the mucinous hyperplasia of the gall bladder may possibly have been related to the progestagenic effect from the follicular and parovarian cysts. Previous cases have been documented in beagles, terriers, daschund, German shepherd dog, Labrador retriever and cocker spaniels. Although there have been no reports on breed predisposition (4), it is interesting to note that the present case was from a German shepherd. Age distribution for mucinous hyperplasia of the gall bladder has been estimated to vary from 8 to 12 years of age (4). The case presented here was younger.

Macroscopic findings of cystic mucinous hyperplasia are enlargement of the gall bladder, thickening of wall with papillary extensions and gall filled cystic structures with a diameter of a few millimeters and up to 8 cm (3, 6). Similar changes were also noted in the present case report.

Microscopically, according to the literature a monolayer

of cuboidal or columnar epithelium line the large cysts filled with mucinous secretions and are separated by distinctive stroma. In some epithelial cells, vacuolar degeneration may be seen. In some cases mononuclear cell infiltration in mucinous secretion or at the periphery may be present. The tunica muscularis is generally thin (3, 6). In the case presented here the large cysts and mucin positive areas were seen however there was no vacuolar degeneration or inflammatory cell infiltration. Furthermore there was no thinning of either the tunica muscularis or serosa.

On the other hand, adenomyomatous hyperplasia of the gall bladder as seen in humans is considered by some as a risk factor for *in situ* carcinoma of gallbladder epithelium. The microscopic appearance of adenomyomatous hyperplasia is similar to the present case (10). It is possible that these kinds of hyperplastic changes may have the potential for transformation to malignant gallbladders tumors.

To the best knowledge of the authors no immunohistochemical studies have been undertaken to study mucinous gall bladder hyperplasia. Immunohistochemical studies in this case detected epithelial proliferation using markers including TGF- β 1, Cx43 and HER2. A tendency for malignancy was observed through the use of Ki67 and CEA. HER2 was mildly positively reactive in hyperplastic gallbladder epithelium. HER2 is considered a crucial marker in determining proliferating cells especially in preneoplastic and neoplastic tissues (11). TGF- β 1 is another marker which detects proliferation in epithelial cells (12). It is considered to be more suitable for detection of mucosal hyperplasia originating from endoderm as it gives mild positive reactions in the cytoplasm of cells.

No immunochemistry reactions have been described regarding junction markers such as connexins. Connexins are composed of transmembrane proteins which give positive reactions during preneoplastic stages of cell proliferation (13). It has also been reported that reduced expressions of connexins are seen in the initial and progressive stages of neoplasia (14). Bearing this in mind, Cx43 was chosen to evaluate the connexin expression in the hyperplastic changes of the gall bladder, mild reactions were found to be present. For evaluation of the predisposition to malignancy Ki67 and CEA markers were investigated. Ki67 is expressed in cycling cells and is a useful marker for indicating cell proliferative activity (15, 16). Its expression rate is reduced in benign tumors or in the case of preneoplastic events like hyperplasia

although positivity is observed in malignant tumors of the gall bladder (15-17). No positive reactions were observed for Ki67 marker in the hyperplastic gallbladder cells indicating a lack of malignancy.

There is a dearth of information regarding CEA expression in the cells of the gall bladder both in human and veterinary pathology. Biochemical studies have reported that CEA macromolecules were found in normal bile. Another study has reported weak positive reactions in the apical surface of the cytoplasm of normal gallbladder epithelium as well as mild to strong positive reactions in different types of neoplasms of the gallbladder (18). In the present study no positive reactions for CEA were detected.

In conclusion, a description of the macroscopic, histopathologic and immunohistochemical findings is presented for a case of cystic mucinous hyperplasia of the gall bladder in a dog. The differences encountered with other studies may possibly be due to the younger age of this dog. Measurements of serum hormone levels such as estrogens and progestagens may have aided in further elucidating and gaining a better understanding of this condition.

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