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Histological and immunohistochemical study of an unusual type of gallbladder duplication

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Summary. Gallbladder duplication is a rare congenital anomaly, with an incidence of 1 in 3,800 autopsies. The correct diagnosis and treatment of this type of entity is important in clinical practice, because it may cause some clinical and surgical problems. In this report, we present the clinical case of a 28-year-old female with abdominal pain. Ultrasound of the upper abdomen showed a distended gallbladder with the presence of a septum that could suggest a congenital anomaly of the extrahepatic biliary system. During surgery, a distended and inflamed gallbladder with a lithiasis was found. In addition, a complete septum and double cystic duct were observed. The gross and histopathological evaluation of the surgical specimen allowed us to confirm the diagnosis of a Y- shaped type gallbladder duplication according to Boyden's classification. In conclusion, in presence of an atypical imaging of the gallbladder, diagnosis of this group of congenital anomalies should be considered in order to adequately plan surgical intervention if necessary.

Key words: Gallbladder duplication, Cholelithiasis, Cholecystectomy, Muscular differentiation, Immunohistochemistry

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Introduction

The gallbladder is one of the most common surgical specimens in pathology. The layers of the gallbladder include mucosa (surface epithelium and lamina propria), smooth muscle, perimuscular or subserosal connective tissue, and serosa. The muscularis mucosae and submucosa are not present (Mills, 2007). The structure and function of the gallbladder can be affected by several pathological conditions and congenital abnormalities. Duplication of the gallbladder is one of the most rare congenital anomalies, having an incidence of 1 in 3,800 autopsies (Boyden, 1926). The variable anatomy of this organ is well documented (Boyden, 1926; Harlaftis et al., 1977; Lamah et al., 2001; Singh et al., 2006; Causey et al., 2010). The duplication occurs because of outpouchings from the normal extrahepatic biliary system during the fifth and sixth week of gestation. These outpouchings typically regress; however, their persistence may result in the formation of an accessory gallbladder (Harlaftis et al., 1977; Causey et al., 2010).

Gallbladder duplication does not cause specific symptoms, and surgical treatment is indicated only when patients become symptomatic (Silvis et al., 1996; Khandelwal et al., 2010). Despite the advances in diagnostic techniques, a gallbladder duplication may be discovered during surgery or may even be missed intra-operatively, particularly when it has an intra-hepatic location (Singh et al., 2006). Preoperative diagnosis of this type of anomaly is especially important to prevent

possible surgical complications and second interventions (Singh et al., 2006; Hekimoglu et al., 2010). Several entities should be considered in the differential diagnosis, including folded gallbladder, choledochal cyst, Phrygian cap, pericholecystic fluid, gallbladder diverticulum, bilobed gallbladder and focal adenomyomatosis (Singh et al., 2006; Hekimoglu et al., 2010).

In this study, we report an incidental preoperative diagnosis, treatment and histopathological evaluation of one case of true Y-shaped gallbladder duplication and we discuss the diagnostic alternatives of this group of congenital anomalies from a morphological and histological standpoint.

Materials and methods

Case history

A 28-year-old woman with occasional abdominal pain for three days reported to the emergency department of the San Camilo Hospital (University of Valparaíso, San Felipe, Chile). The pain she experienced was associated with the intake of fatty food. The patient described right upper quadrant abdominal pain associated with bilious vomiting. She did not report to have had fever, diarrhea or any other symptoms.

Abdominal palpation was painful and Murphy's sign was negative. Sodium metamizole and meperidine were prescribed. However, the patient did not respond to this treatment. Extensive laboratory testing and abdominal ultrasound were performed. The results of the laboratory testing, including full blood cell count, coagulation test, C-reactive protein and biochemistry panel test were within normal limits. Ultrasound of the upper abdomen showed a distended gallbladder with the presence of a septum that could suggest congenital anomaly of the extrahepatic biliary system. In addition, a single calculus with a diameter of 1.8 cm was identified in one of the lumens (Fig. 1).

Due to the intensity of the abdominal pain and its resistance to treatment, an open cholecystectomy was performed. During the open surgery, a distended gallbladder with signs of inflammation, the presence of an external constriction along the organ and two cystic ducts with one unique cystic artery were identified. Both cystic ducts converged into the common hepatic duct forming the common bile duct. A calculus with a diameter of 1.8 cm was observed and removed from the distended and inflamed gallbladder. The surgical specimen was referred to the pathology unit for gross and both histological and immunohistochemical analysis.

Procedures

After surgery, the surgical specimen of the duplicated gallbladder was routinely fixed in 10% neutral buffered formalin. Subsequently, gross analysis was performed and the duplicated gallbladder was sectioned transversally from the fundus to the neck (cystic area) to realize a complete histological evaluation. All paraffin-embedded samples were cut in 5 μm thick sections for the histological and immunohistochemical analysis. The histopathological analysis was evaluated using haematoxylin-eosin and picrosirius stain at light microscopy.

The identification of the blood and nerve supply was determined by immunohistochemistry using the following antibodies: anti-laminin clone LAM-89 (Sigma-Aldrich, Steinheim, Germany), and prediluted anti-CD31 clone JC/ 70A (Master Diagnóstica, Granada, Spain).

The smooth muscle layer was evaluated by immunohistochemistry using the following muscular differentiation markers: prediluted anti-smooth muscle actin clone 1A4, prediluted anti-H caldesmon clone H-Cald, prediluted anti-desmin clone D33, prediluted anti-myosin clone SM-M10, and prediluted anti-smoothelin clone R4A (Master Diagnostica, Granada, Spain).

The immunohistochemical study of laminin was performed as previously described (Carriel et al., 2013). The antibodies CD 31 and all the muscular differentiation markers were performed using an automatic immunostainer (Autostainer 480, LabVision Fremont, CA) by an indirect polymer-peroxidase-based method followed by development with diaminobenzidine (Masvision, Master Diagnostica) as previously described (Aneiros-Fernandez et al., 2011).

Results

Gross and histopathological findings

Gross analysis of the surgical specimen revealed that

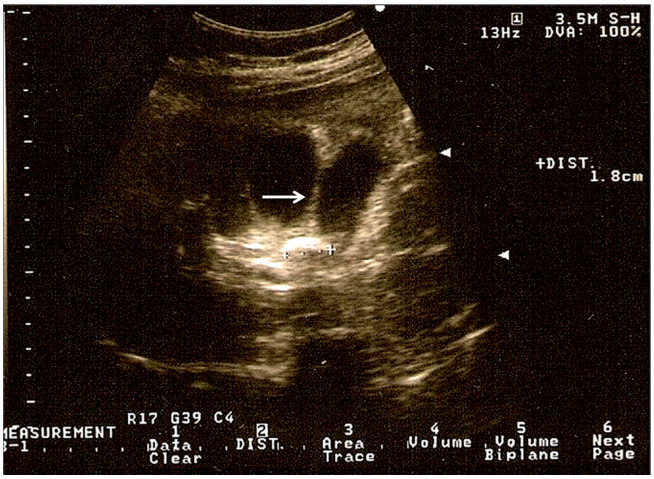

Fig. 1. Preoperative ultrasound image of the right upper abdominal quadrant. Note the septum (white arrow) that divides the gallbladder in two ovoid and anechoic structures, and a stone with a diameter of 1,8 cm.

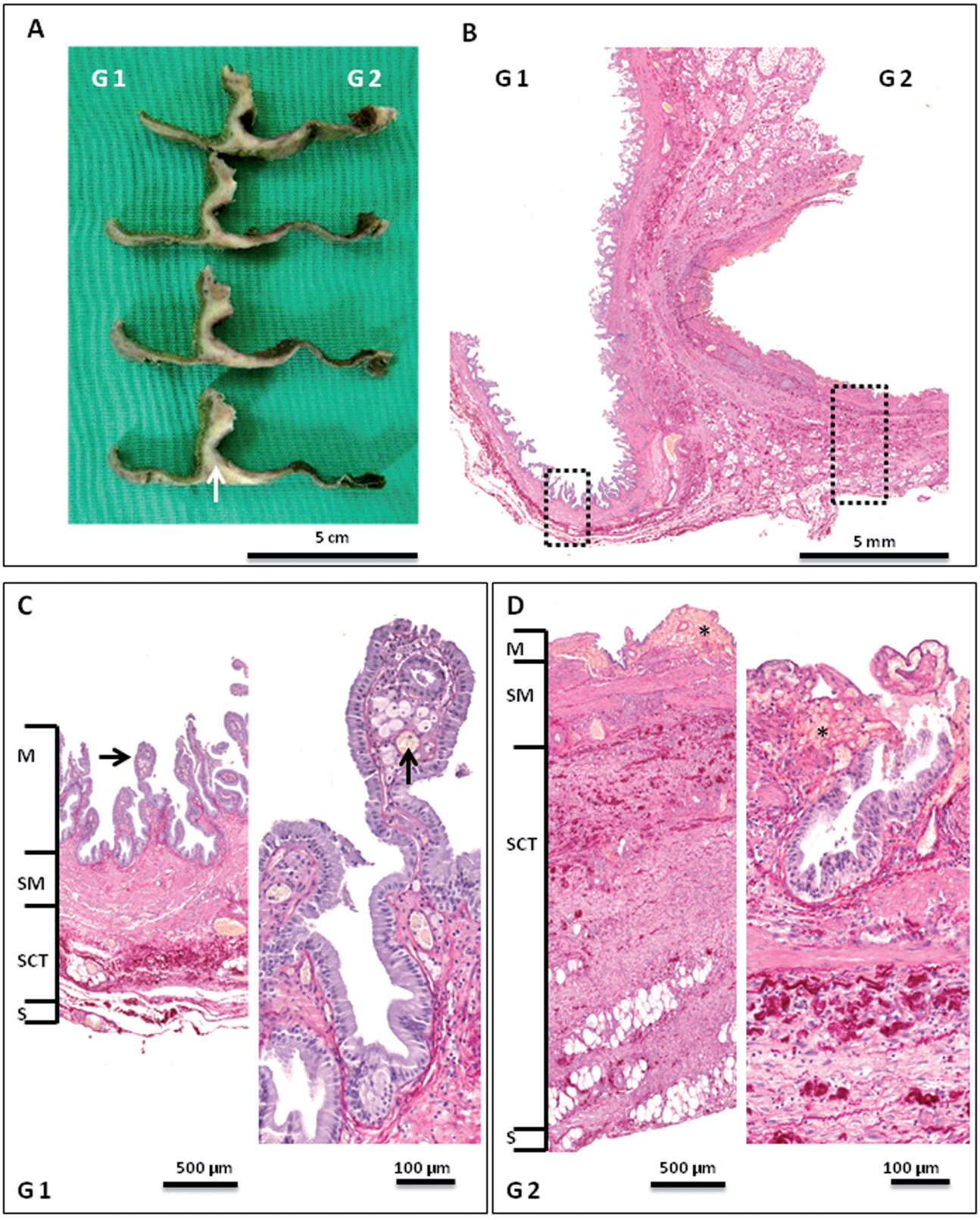

Fig. 2. A. Gross analysis of the surgical specimens, where it is possible to observe two partial gallbladders (G 1, G 2) separated by a complete septum (white arrow) coated at both surfaces by mucosa. **B.** Histological section of both gallbladders at low magnification. **C.** Histological analysis of the G 1, with evident cholesterolosis in the lamina propria (black arrow). **D.** Histological image of the G 2, where it is possible to observe the evident signs of inflammation and acute hemorrhage in the lamina propria (asterisk). M: mucosa; SM: smooth muscle; SCT: subserosal connective tissue; S: serosa.

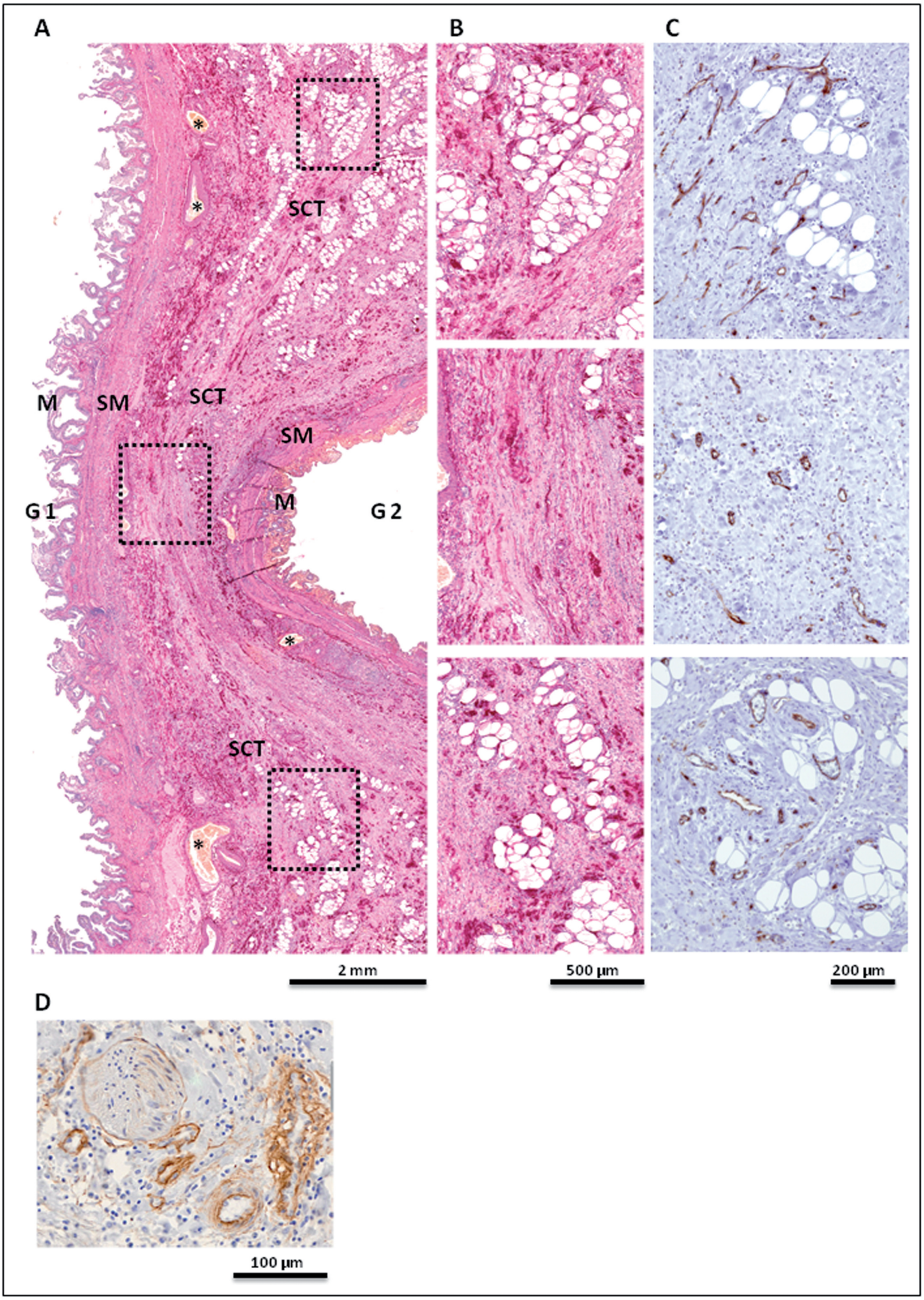

Fig. 3. Histological analysis of the longitudinal septum stained with picrosirius. **A.** Histological image at low magnification of the septum, note the independent histological layers of both gallbladders fused by the subserosal connective tissue (SCT). **B.** Magnification analysis of the SCT of the septum at different regions. **C.** Distribution of blood vessels in the SCT analyzed by CD31 immunohistochemistry. **D.** Laminin immunohistochemistry in the wall of blood vessels and peripheral nerves of the SCT. Asterisk: muscular arteries; M: mucosa; SM: smooth muscle; SCT: subserosal connective tissue; S: serosa.

the length of one gallbladder (G1) was 6 cm and that the width was 4 cm at the main perimeter. The second gallbladder (G2) had 7 cm of length and 4.5 cm of width at the main perimeter. Sectioned gallbladders showed the presence of a complete longitudinal septum coated by mucosa at both surfaces separating both gallbladders. In addition, two cystic ducts were identified. The G1 showed yellow spots on its mucosal surface (due to the accumulation of lipid in the lamina propria) without signs of inflammation. However, evident signs of inflammation in the mucosal surface were observed in the G2 (Fig. 2 A).

Histological analysis confirmed the gallbladder duplication with the presence of a septum coated by mucosa (Fig. 2B). The G1 showed a mucosa without signs of inflammation, and with the presence of macrophages with lipid content that confirms the diagnosis of cholesterolosis of the gallbladder (Fig. 2C). The histological analysis of G2 revealed the loss of epithelial surface, signs of a recent hemorrhage in the lamina propria and the presence of inflammatory infiltrate and edema. All of these findings confirm the histological diagnosis of chronic cholecystitis with acute hemorrhage of the G2 (Fig. 2D).

The histological study allows us to confirm that both gallbladders were completely independent, containing mucosa (surface epithelium and lamina propria), a discontinuous layer of smooth muscle, perimuscular or subserosal connective tissue, and serosa. The histological evaluation of the longitudinal septum showed that the two gallbladders were fused only by the subserosal connective tissue rich in collagen fibers and adipose tissue (Fig. 3). In relation with the blood and nerve supply, we observed the presence of muscular arteries and nerves in the lamina propria and perimuscular connective tissue of each gallbladder, and we identified by immunohistochemistry a few small blood vessels and nerve branches in the shared subserosal connective tissue (Fig. 3C,D). We observed that the muscular layer of the G2 was hypertrophic and thicker than the muscular layer of the G1. However, the immunohistochemical analysis revealed that smooth muscle layers of both gallbladders were positive for all the markers of muscular differentiation confirming that both gallbladders had fully developed muscle layers with contractile function (Fig. 4).

Finally, gross and histopathological evaluation of the surgical specimen confirmed the diagnosis of unusual Y-shaped gallbladder duplication. This duplicated gallbladder had two independent cystic ducts which became confluent close to their union with the common bile duct (Y-shaped type), and both gallbladder bodies were fused lengthwise by the subserosal connective tissue.

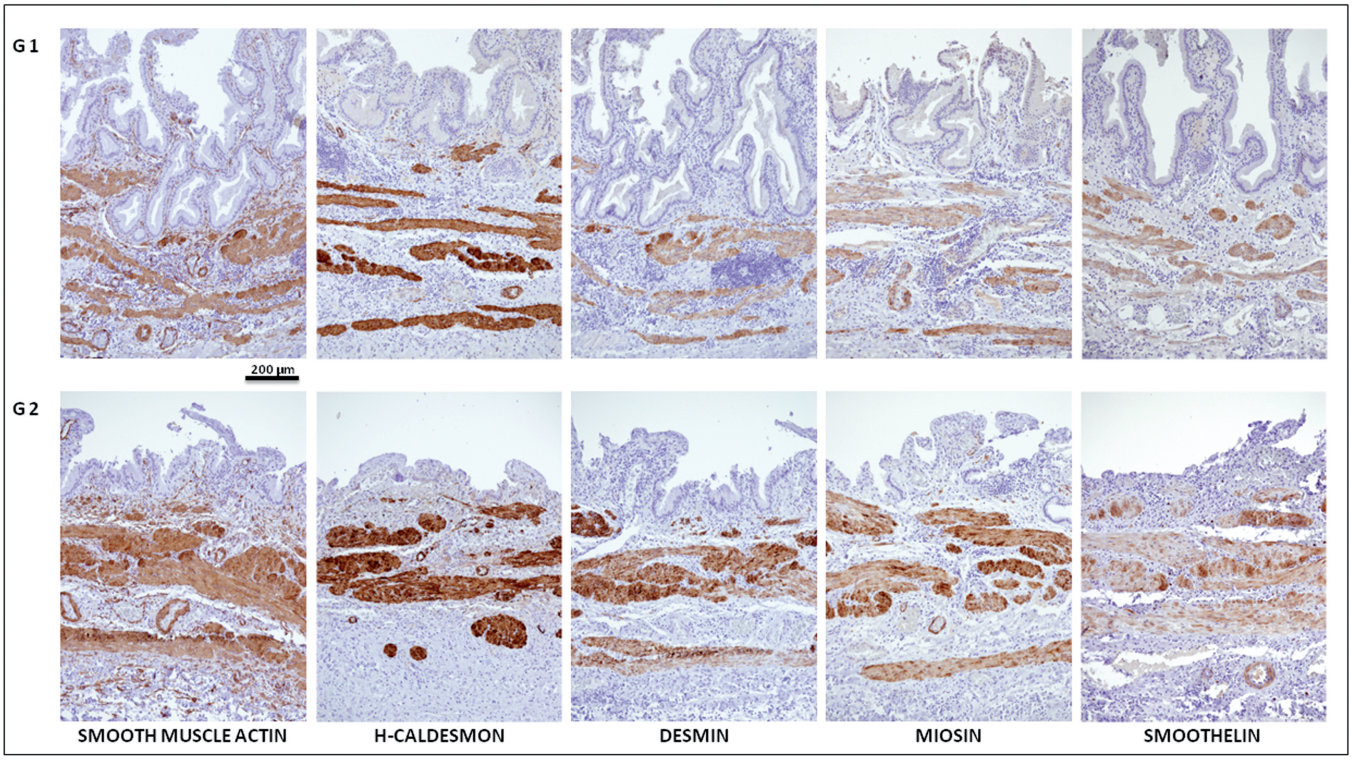

Fig. 4. Immunohistochemical analysis of the smooth muscle layers of the G1 and the G2.

Discussion

Gallbladder duplication is an extremely rare congenital anomaly of the extrahepatic biliary system, and the earliest review of this group of anomalies was published by Boyden in 1926 (Boyden, 1926). In multiple gallbladder anatomy, each gallbladder must have valves at the neck, a tunica muscularis, and the capacity to concentrate bile (Causey et al., 2010).

Several classifications have been proposed according to anatomic or embryological development of the gallbladder (Boyden, 1926; Harlaftis et al., 1977; Causey et al., 2010). The first classification was performed by Boyden in 1926, and according to this classification, congenital abnormalities of the gallbladder include "*vesica fellea divisa*" or bilobed gallbladder and "*vesica fellea duplex*" or true gallbladder duplication (Boyden, 1926; Rabinovitch et al., 1958). The true duplication is subdivided into the Y-shaped type (two cystic ducts united before entering into the common bile duct, unusually both gallbladders are adherent and occupy the same fossa) and the H-shaped type or ductular type (two separated gallbladders and cystic ducts entering separately into the common bile duct). The accessory gallbladder of the ductular type can be localized in the gallbladder fossa, the intrahepatic region, the subhepatic region or within the gastrohepatic ligament (Khandelwal et al., 2010). True gallbladder duplication is more common and occurs due to bifurcation of the gallbladder primordium during the fifth and the sixth week of embryonic life (Khandelwal et al., 2010). Harlaftis et al. (1977) classified gallbladder duplication in two main groups based upon embryogenesis. These were the type 1 or split primordial group which included the septate gallbladder, the V-shaped and the Y-shaped gallbladders. When the cystic primordium splits during embryogenesis, both gallbladders share a common cystic duct. Type 1 septated duplicated gallbladder occurs when there is a single cystic duct and both gallbladders are separated by a septum. Type 2 describes accessory gallbladders that are ductular or trabecular, meaning that they arise from a separate primordium from the biliary tree and have individual cystic ducts (Harlaftis et al., 1977; Causey et al., 2010). Recently, Causey et al. proposed a unified classification of multiple gallbladders based on Harlaftis's classification. In this unified classification, the authors incorporate a third group called combined gallbladders (Causey et al., 2010).

The gross and histopathological evaluations of the surgical specimen demonstrate that both gallbladders were fully developed and only fused lengthwise by the subserosal connective tissue. In accordance with previous works, the positive expression of the muscular differentiation markers, especially smoothelin suggests that both gallbladders were functional with a contractile capacity of the smooth muscle layer (Raparia et al., 2010; Aneiros-Fernandez et al., 2011). The differences

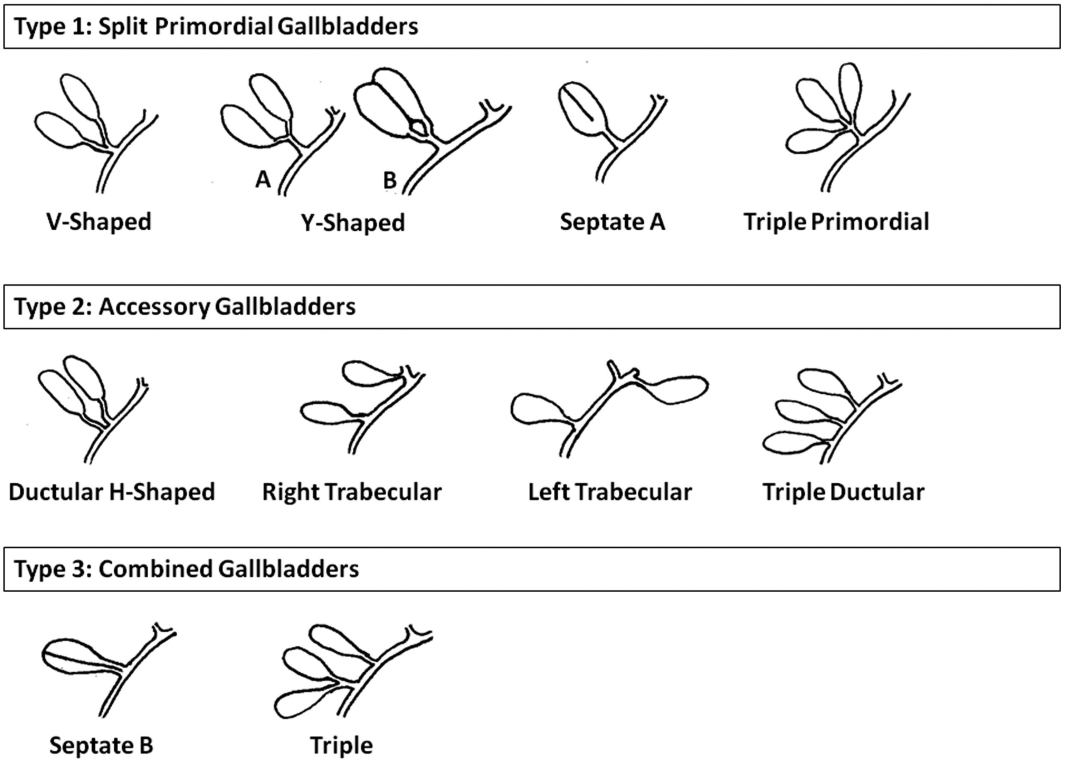

Fig. 5. Schematic representation of the different types of gallbladder duplication (modified from Causey et al., 2010).

on the intensity of the markers of muscular differentiation could be explained due to the inflammation and muscular hypertrophy of the G2 associated to the presence of a calculus. The histological analysis allowed us to confirm that our patient had true Y-shaped gallbladder duplication according to the classical Boyden's classification. However, our case differs with the classification published by Singh, 2006, where the author considers a Y-shaped gallbladder as two completely separate gallbladders, with two Y-shaped cystic ducts (Harlaftis et al., 1977; Singh et al., 2006; Causey et al., 2010). Due to the fact that our case is a true Y-shaped gallbladder duplication with gallbladder bodies fused lengthwise by subserosal connective tissue, we suggest the incorporation of our case in the most recent unified classification described by Causey, and we classify our gallbladder duplication as type 1 Y-shaped B (with fused bodies) (Fig. 5).

Patients with gallbladder duplication usually do not have any specific symptoms, and this group of anomalies is rarely diagnosed in the preoperative period (Ozmen et al., 2003). For surgeons and radiologists, it is very important to recognize these anomalies as a possible confusing issue, and to prevent iatrogenic injuries during surgery (Causey et al., 2010).

The diagnosis of gallbladder duplication is difficult, and successful preoperative diagnosis is noted in only half of the cases (Kim et al., 2009). In this sense, preoperative imaging is crucial in the study of this group of anomalies. However, these imaging methods are limited by the type of aberrant anatomy (Causey et al., 2010). Ultrasonography is definitively the initial imaging modality that can help in the diagnosis of these gallbladder anomalies (Senecail et al., 2000). Nevertheless, Magnetic Resonance Cholangiopancreatography (MRCP) has better diagnostic capability than ultrasound, and retrograde Cholangiopancreatography is considered the gold standard for diagnosis of these types of anomalies (Kim et al., 2009; Causey et al., 2010). Finally, the definitive diagnosis of true gallbladder duplication could be established during open surgery when there is an evident type 2 or 3 gallbladder anomaly or after surgery during gross and histopathological analysis when there is a type 1 with fused bodies.

In relation with the treatment of gallbladder duplication, surgery should be the treatment of choice only in symptomatic patients (Silvis et al., 1996; Ozmen et al., 2003; Causey et al., 2010; Khandelwal et al., 2010; Walbolt and Lalezarzadeh, 2011). Overall, patients with aberrant anatomy are more likely to undergo open surgery or laparoscopic surgery (Desolneux et al., 2009; Causey et al., 2010). Some authors recommended open cholecystectomy for these patients, because an additional anatomical anomaly can exist (Silvis et al., 1996). Open surgery gives the opportunity to the surgeon to palpate and explore the entire gallbladder fossa and the adjacent area. This enables the surgeon to diagnose cases of congenital anomalies of the gallbladder (Singh et al., 2006). Laparoscopic resection is a reasonable alternative and it is a very well described procedure in the literature (Ozmen et al., 2003; Kim et al., 2009; Causey et al., 2010; Khandelwal et al., 2010; Walbolt and Lalezarzadeh, 2011).

In general, the majority of authors suggest removing both gallbladders to avoid cholecystitis and symptomatic lithiasis in the remaining organ (Hekimoglu et al., 2010). The complete removal of gallbladders prevents a second surgical intervention in these patients (Silvis et al., 1996; Ozmen et al., 2003; Causey et al., 2010; Khandelwal et al., 2010; Walbolt and Lalezarzadeh, 2011). However, if its presence is not known before surgery, the second gallbladder could be missed during surgery, particularly when it has an intra-hepatic location (Gigot et al., 1997; Horattas, 1998; Hekimoglu et al., 2010).

In conclusion, gallbladder duplication is a rare and uncommon congenital anomaly of the gallbladder and extrahepatic biliary system, and knowledge about its existence is important in medical and surgical practice. With the presence of atypical imaging, preoperative diagnosis of this congenital anomaly should be considered in order to plan appropriate surgical intervention if necessary.

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