

First of all, we express our sincere gratitude to the reviewers for acknowledging our research findings. A comprehensive response will be provided to address the inquiries raised by the reviewers pertaining to our research outcomes.

Reviewer #1:

In this paper, the authors performed a retrospective case-control study with the aim of classifying the morphology of the cystic duct to guide future clinical practice. In particular, they found that the conformation of the cystic duct was a risk factor for gallstone formation. I read the article with great interest, and my suggestions for improvement are as follows. - In the abstract, Methods section, I suggest changing the first sentence as follow: "A total of 300 patients were retrospectively enrolled from October 2021 to January 2022." - In the Introduction, abbreviations between brackets at the first mention of some terms are missing. Please, check and revise the manuscript accordingly. - In the Discussion, page 11, I suggest removing the sentence "This seems self-contradictory" at the beginning of the fourth paragraph and, moreover, I suggest changing the last sentence of the same paragraph as follow: "Therefore, the use of the near-infrared imaging with ICG technique for cholecystectomy should be taken into consideration, especially in patients with anatomical variants of the CD." - In the Discussion, fifth paragraph, page 12, I suggest removing the sentence "The RPD into the CD was not seen in 300 patients" considering that the same concept has been reported in the same paragraph. Please, try to be less repetitive and more precise in your affirmations regarding your study. - In the Discussion, page 14 - point n°3 "Mucous membrane repair", please add a reference supporting your statement. - The Legend of Figure 1 is scant and should be improved. - English language quality is quite good, but a native English speaker could probably help improve it further.

We thank the reviewer from the bottom of our hearts for recognizing the findings of our study and we are also very grateful for the valuable suggestions given to us. Based on the issues raised by reviewer 1, we made one-by-one revisions, while highlighting the revisions in yellow in the revised manuscript.

Reviewer #2:

This is a paper on anatomical morphology of cystic duct. The study is a great work that contributes to biliary operations, especially for laparoscopic cholecystectomy. It is understandable that a tortuous cystic duct may cause gallstones. I have one question. If bile stasis or viscosity has something to do with gallstone, how about a diameter of cystic duct?

We are grateful to the reviewer for recognizing our article.

There may be a correlation between the diameter of the cystic duct and gallbladder stones. According to Poiseuille's law, bile flux through the cystic duct is negatively correlated with bile viscosity and positively correlated with the diameter of the cystic duct. When the diameter of the cystic duct becomes small, the bile flux through the cystic duct decreases and bile viscosity increases, promoting the formation of gallstones. In addition, when the diameter of the cystic duct becomes thin, bile cannot easily flow through the cystic duct into the extrahepatic bile ducts, which easily leads to bile stasis and promotes the formation of gallstones. In conclusion, the thinner diameter of the cystic duct may be associated with the formation of gallstones.

Reviewer #3:

In this study, the univariate analysis showed differences between gallstone and non-gallstone groups in anatomical variations of the cystic duct (CD), and the multivariate analysis demonstrated morphology of the CD, which Zhu et.al. divided into four types, was significantly associated with gallstones. The authors concluded that a tortuous CD, which is classified as N-shaped, S-shaped, or W-shaped CD, is an important cause of gallstone formation, bile stasis, increased bile viscosity, and damage to the mucosa of the CD of mucus membrane, contributing to the stone formation. They also emphasized that when there are anatomical variations of the CD, the CD becomes longer, and the longer the CD is, the more likely it is to become tortuous, which not only leads to development of stones but also prone to bile duct injury during laparoscopic cholecystectomy. The authors also mentioned the usefulness of the near-infrared imaging with indocyanine green for cholecystectomy to minimize bile duct injury, that provides good visualization of the biliary tract anatomy.

This article is interesting in relating stone formation to the variation of the CD. My comments are as follows:

- 1. As the authors noted, there was no difference in the intrahepatic biliary anatomy in both groups. However, the variations in the intrahepatic anatomy are exhaustively explained. Thus, the Figure 2 might be deleted. Rather, I think it is better to explain intra- and extra-hepatic anatomical variations of the CD in more detail.**
- 2. Figure 2 is a little bit difficult to understand, and would be better be simplified.**
- 3. Abbreviations should be defined upon first mention in the text. e.g. EHBD, RPD.**

We are greatly appreciative of the reviewers' interest in our findings and their valuable suggestions for our article.

Question 1:

Anatomical variations of the intrahepatic and extrahepatic ducts are common. In laparoscopic cholecystectomy, we need to focus on the anatomical variations of both the intrahepatic and extrahepatic bile ducts. When the anatomical classification of intrahepatic bile ducts is Type D (Figure 2D), especially when the the right posterior duct joins the cystic duct, we tend to misidentify the the right posterior duct as the cystic duct, resulting in bile duct injury. Therefore, we also describe the anatomical variants of intrahepatic bile ducts in our article. Based on the anatomical morphology of the cystic duct, we propose a novel classification of the cystic duct and describe it in detail (Figure 3A-H). Based on our sample, we have documented the intrahepatic anatomical variants associated of the cystic duct. We found the following situations: the cystic duct converges into the right hepatic duct, the cystic duct converges into the left hepatic duct and the cystic duct converges into the confluence of the right and left hepatic ducts (Figure 4 A-C). However, unfortunately, due to the limited sample size, we did not conduct in-depth study and exploration.

Question 2:

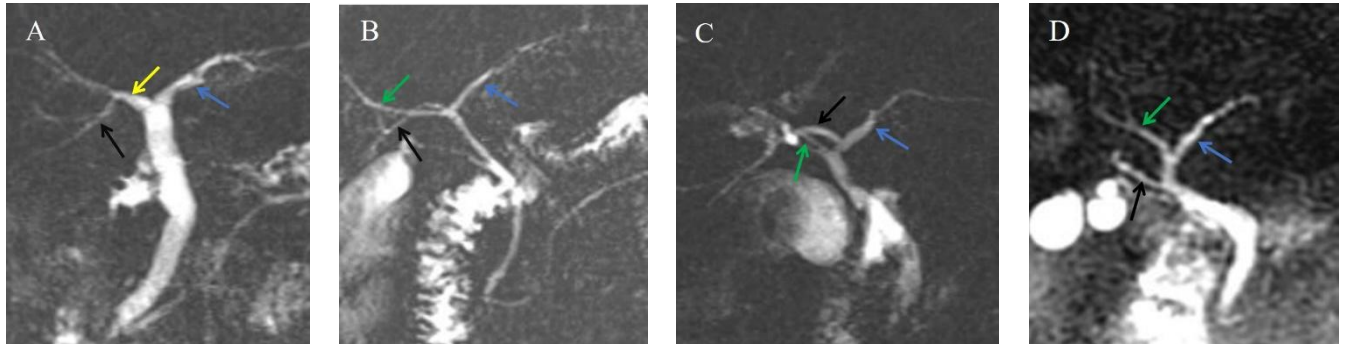


Figure 2 The classification of intrahepatic bile duct anatomy. A. Type A: the RPD (black arrow) converges into the RHD (yellow arrow); B. Type B: the RPD (black arrow) converges into the junction of the right and left hepatic ducts, and the three show a three-fork type; C. Type C: the RPD (black arrow) converges into the LHD (blue arrow); D. Type D: the RPD (black arrow) converges into the extrahepatic bile duct. RPD: right posterior duct; RHD: right hepatic duct; LHD: left hepatic duct.

Question 3:

Based on the suggestions made by reviewer , we have revised each of them, and the revisions are highlighted in yellow in the revised manuscript.