Editorial

Extracellular vesicles in bile: a game changer in the diagnosis of indeterminate biliary stenoses?

Seung Bae Yoon[1,2], Jae Hyuck Chang[1]

[1]Department of Internal Medicine, [2]Cancer Research Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea

Correspondence to: Jae Hyuck Chang, MD, PhD. Division of Gastroenterology, Department of Internal Medicine, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul, 137-701, Korea. Email: wwjjaang@catholic.ac.kr.

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Biliary stenoses are not infrequent clinical findings in various diseases or conditions. In some situations, differentiating the cause of biliary stenosis is difficult, and it often remains indeterminate. Indeterminate biliary stenoses can lead to repeated examinations, excess spending, and a delay in diagnosis and treatment. To date, the diagnosis of biliary stenosis has been based on histologic diagnosis through endoscopy. However, conventional brush cytology and intraductal biopsy via endoscopic retrograde cholangiopancreatography (ERCP) have low diagnostic sensitivity (1). Therefore, several methods have been attempted to improve this low sensitivity, including direct visualization of the bile duct or biopsy (direct peroral cholangioscopy, SpyGlass videocholangioscopy, intraductal ultrasonography, and confocal laser endomicroscopy) and molecular-biologic methods [fluorescent *in situ* hybridization of bile (2) and tumor marker gene mRNA analysis of bile (3)]. Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) is also a good method if the lesion is located in the distal bile duct or forms a mass; however, EUS-FNAB might not be appropriate for proximal biliary stenosis, because the test's sensitivity depends on the location of the biliary stenosis (4). Although these methods afford additional information to that of ERCP brush cytology and biopsy of bile duct in the diagnosis of biliary stenoses, there are associated technical difficulties and high costs that can prevent them from becoming routine tests. Importantly, these tests do not have much higher sensitivities than brush cytology and biopsy. Although an algorithmic approach to biliary stenosis has been suggested according to the location of the stenosis (5), the ultimate diagnosis using this method is imperfect and costly.

In *Gastroenterology*, Severino *et al.* reported the role of bile extracellular vesicles (EVs) in differentiating malignant from nonmalignant common bile duct stenosis (6). The group collected bile and blood samples from 50 patients undergoing therapeutic ERCP for biliary obstruction of malignant (n=25, pancreatic cancer or cholangiocarcinoma) or non-malignant origin (n=25, chronic pancreatitis or biliary stones). The median concentration of bile EVs from malignant patients was more than 10 times higher than that from nonmalignant patients. In addition, when the threshold value obtained from the preceding discovery cohort (n=20) was applied to the later verification cohort (n=30), it correctly classified all malignant and nonmalignant biliary stenoses (diagnostic accuracy, 100%). The diagnostic ability of bile EVs concentration was superior to those of serum carbohydrate antigen 19-9, as well as serum EVs concentration for malignant bile duct stenosis. If malignant biliary stenoses can be precisely diagnosed using a single bile EV concentration rather than an algorithmic approach, this might be a game changer in the diagnosis of indeterminate biliary stenosis.

EVs are a heterogeneous population of membrane-enclosed carriers that are released from a variety of cells into the extracellular space (7). They include exosomes

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(40–100 nm), microvesicles (50–1,000 nm), and apoptotic bodies (800–5,000 nm), which are grouped based on cellular biogenesis. While EVs were previously thought to be solely a mechanism for discarding nonfunctional cellular components, increasing evidences suggest that they are actually key players in intercellular communication (8). Several recent studies have implicated EVs and their cargo as having significant impact on the tumor microenvironment, and they also serve as promising biomarker candidates in the diagnosis and prognosis of cancers (9). Cancer cells produce relatively large amount of EVs, which are involved in tumor growth and differentiation (10). There is increasing research regarding EVs, and particularly concerning exosomes in the peripheral blood. One recent study showed that glypican-1, a membrane anchored protein, is expressed specifically in cancer-cell-derived exosomes of pancreatic cancer patients, and levels of glypican-1-positive cancer exosomes in the peripheral blood correlate with tumor burden and patient survival in pancreatic cancer (11). These were reliably detected in early pancreatic cancer, as well as in pancreatic cancer precursor lesions. Another recent study found that miRNA, as well as proteins in the serum exosome was helpful in the diagnosis of pancreatic cancer (12). Arbelaiz *et al.* recently reported that proteomic signatures of serum EVs have diagnostic utility in the differentiation of cholangiocarcinoma and hepatocellular carcinoma from primary sclerosing cholangitis; however there were no significant differences between malignant and benign biliary stenosis regarding serum EV concentration (13).

Interestingly, Severino *et al.* used bile rather than the serum to evaluate EVs for cancer diagnosis. Bile samples are easily obtained during therapeutic ERCP, which is essential to treat biliary stenosis. Therefore, no additional interventions are needed for bile EV collection. Because bile is a non-circulating fluid that is in direct contact with the tumor, it can be a valuable and unaffected source of EV evaluation. Accordingly, this study showed that the bile EV concentrations in the malignant stenoses were significantly higher than those in the benign stenoses. In contrast, there were no significant differences in serum EV concentrations between in patients with malignant and benign stenoses. Using bile, one study reported that the biliary EV microRNA-based panel differentiates cholangiocarcinoma from primary sclerosing cholangitis using multivariate organization of combinatorial alterations (14). Severino *et al.* used the bile EV concentration to discriminate biliary stenoses without further analysis of the proteins or microRNA extracted from the EV. Proteomic profiles and microRNA analysis are somewhat complicated and therefore difficult to be made routine diagnostic procedures.

This article suggests that measuring bile EV concentrations is superior to other methods in the differentiation of biliary stenoses. However, there are a few concerns to be discussed. First, although the diagnostic accuracy was 100% in the verification cohort, one patient with malignant biliary stenosis in the discovery cohort would have been diagnosed as having a benign stenosis when using the threshold (9.46×10^{14} nanoparticles/L). Therefore, using bile EVs is not 100% accurate in the diagnosis of biliary stenoses. This study enrolled only 25 cancer patients. Accordingly, its results are not generalized to the entire population of patients with malignant biliary stenoses. Future, larger studies are needed to verify the results of this study. In addition, many cases of indeterminate biliary stenosis are early pancreatobiliary cancers that are not fully visible in imaging studies. In contrast, most patients with malignant stenosis included in this study were at advanced stages. It could affect the bile EV concentrations and the results. Furthermore, the need for new technologies, such as nanoparticle tracking analysis, can hinder the usefulness of bile EV measurement. In order to become a routine diagnostic method, the measurement of EV concentration must be simplified.

The rapid measurement of bile EV concentration is a promising diagnostic tool in the differentiation of benign and malignant biliary stenoses. However, the analyses for detecting and measuring EV are not robust. Simple, standardized methods are needed. Future studies with a large number of patients and more developed EV detection technology are expected. Then, we will find out that EVs test is a real game changer in the diagnosis of indeterminate biliary stenoses.

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Footnote

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References

1. Glasbrenner B, Ardan M, Boeck W, et al. Prospective

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evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. Endoscopy 1999;31:712-7.
2. Smoczynski M, Jablonska A, Matyskiel A, et al. Routine brush cytology and fluorescence in situ hybridization for assessment of pancreatobiliary strictures. Gastrointest Endosc 2012;75:65-73.
3. Kim TH, Chang JH, Lee HJ, et al. mRNA expression of CDH3, IGF2BP3, and BIRC5 in biliary brush cytology specimens is a useful adjunctive tool of cytology for the diagnosis of malignant biliary stricture. Medicine (Baltimore) 2016;95:e4132.
4. Mohamadnejad M, DeWitt JM, Sherman S, et al. Role of EUS for preoperative evaluation of cholangiocarcinoma: a large single-center experience. Gastrointest Endosc 2011;73:71-8.
5. Singh A, Gelrud A, Agarwal B. Biliary strictures: diagnostic considerations and approach. Gastroenterol Rep (Oxf) 2015;3:22-31.
6. Severino V, Dumonceau JM, Delhaye M, et al. Extracellular Vesicles in Bile as Markers of Malignant Biliary Stenoses. Gastroenterology 2017;153:495-504.e8.
7. Hirsova P, Ibrahim SH, Verma VK, et al. Extracellular vesicles in liver pathobiology: Small particles with big impact. Hepatology 2016;64:2219-33.
8. Maas SL, Breakefield XO, Weaver AM. Extracellular Vesicles: Unique Intercellular Delivery Vehicles. Trends Cell Biol 2017;27:172-88.
9. Zomer A, van Rheenen J. Implications of Extracellular Vesicle Transfer on Cellular Heterogeneity in Cancer: What Are the Potential Clinical Ramifications? Cancer Res 2016;76:2071-5.
10. D'Souza-Schorey C, Clancy JW. Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers. Genes Dev 2012;26:1287-99.
11. Melo SA, Luecke LB, Kahlert C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 2015;523:177-82.
12. Madhavan B, Yue S, Galli U, et al. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int J Cancer 2015;136:2616-27.
13. Arbelaiz A, Azkargorta M, Krawczyk M, et al. Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. Hepatology 2017;66:1125-43.
14. Li L, Masica D, Ishida M, et al. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. Hepatology 2014;60:896-907.

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