



## Comparison between Brush Cytology and Forceps Biopsy under Fluoroscopic Guidance for the Diagnosis of Proximal Cholangiocarcinoma

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### ABSTRACT

#### BACKGROUND

Cholangiocarcinoma is the second most common malignant liver cancer. Its early diagnosis plays an important role in the success of treatment. The aim of this study was to compare the use of cold forceps biopsy without cholangioscopy with brush cytology in the diagnosis of cholangiocarcinoma.

#### METHODS

In this prospective study, we enrolled 19 patients. Endoscopic retrograde cholangiopancreatography (ERCP) was performed for all individuals. Sampling was performed from the narrowing site using the brush method. Then, a cold forceps biopsy was performed under fluoroscopy.

#### RESULTS

The mean age of the patients was  $63.31 \pm 11.12$  years and most of them were men (63.16 %). The brush cytology and the cold forceps biopsy diagnosed 31.85% and 68.42% of the samples as cholangiocarcinoma, respectively. According to the McNemar test, there was a statistically significant difference between the diagnostic results of the brush cytology and cold forceps biopsy. So that more cholangiocarcinoma cases were diagnosed using forceps biopsy ( $p = 0.016$ ). No complications such as perforation, bleeding, cholangitis, and leakage were reported during the cold forceps procedure.

#### CONCLUSION

Cold forceps biopsy under fluoroscopy is better than cytology brush in the diagnosis of proximal cholangiocarcinoma. It is recommended to be used as a low-cost alternative in cases where cholangioscopy is not available.

#### KEYWORDS:

Cholangiocarcinoma, Cytology, Biopsy, Diagnosis

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## INTRODUCTION

Cholangiocarcinoma is the most common malignancy of biliary epithelial cell origin<sup>1</sup> and is the second most common malignant liver cancer in recent decades.<sup>2</sup> Early diagnosis plays an important role in the success of treatment and prognosis.<sup>3,4</sup> There are a variety of diagnostic methods, such as tumor marker measurement, imaging modalities, and histopathological examination by sampling.<sup>5</sup>

There are several methods for sampling, such as brush cytology, forceps biopsy, and endoscopic ultrasound fine needle aspiration (EUS-FNA),<sup>6</sup> which require a guided procedure. The most common guidelines include cholangioscopy, endoscopic ultrasound (EUS), and endoscopic retrograde cholangiopancreatography (ERCP) plus fluoroscopy.

Cholangioscopy is known as the preferred detection method for cholangiocarcinoma.<sup>7</sup> EUS-FNA is a suitable method for examining proximal bile duct lesions, which is similar to forceps biopsy.<sup>6</sup> Over the past three decades, biopsy and brush cytology under ERCP guidance have been the main methods of tissue examination.<sup>8,9</sup>

Brush cytology is a common technique due to its simplicity and short duration of performing, but its diagnostic sensitivity is low (about 30-70%).<sup>6</sup> Only a few cellular samples are taken in this procedure for histopathological examination, and it provides low study possibly.<sup>10</sup> Forceps biopsy is another method whose diagnostic sensitivity is reported to be about 36-81%.<sup>11</sup> In this method, forceps can easily enter the stenotic duct and give a complete tissue sample from the occlusion,<sup>10</sup> which also makes it possible to study the subepithelial level to assess the extent of cancer.<sup>6</sup>

To make a diagnostic comparison between the two methods of brush cytology and forceps biopsy, the study of Hasan Kulaksiz and colleagues<sup>10</sup> showed that the combined use of both methods was more sensitive than the use of each method alone (specificity of both methods is 100%, the sensitivity of brush and forceps alone are 49% and 69%, respectively, and 80% in combination use). A study by Hartman and others<sup>12</sup> also showed that fluoroscopy-guided sampling was better than cholangioscopy-guided.

Although cholangioscopy is a preferred method in the diagnosis of cholangiocarcinoma,<sup>7</sup> the lack of access to it in all medical centers and its high costs does not make it possible for all patients to use it, and physicians have

to choose the other alternative methods. In this regard, as mentioned earlier, the two methods of brush cytology and forceps biopsy are more popular in all medical centers due to their low cost and availability. Unfortunately, in the field of comparing the diagnostic power of these two methods, limited studies have been done, and enough resources are not available. Our aim in this study was to compare the use of cold-forceps biopsy without cholangioscopy with brush cytology in the detection of cholangiocarcinoma.

## MATERIALS AND METHODS

### Study design and participants

This was a prospective study. All patients with a diagnosis of proximal cholangiocarcinoma who had been referred to the gastroenterology clinic of Shahid Beheshti Hospital were entered in the study using census sampling.

In this study, cholangiocarcinoma was diagnosed based on a set of clinical and laboratory findings, and Magnetic resonance cholangiopancreatography (MRCP) results. Accordingly, the patients with no history of background disease including; surgery (iatrogenic bile duct injurie), trauma, bile duct injury, primary sclerosing cholangitis (PSC) such as underlying inflammatory bowel disease (IBD) included in the study. Cholangiocarcinoma considered when the patients presented high tumor markers and liver enzymes, clinical evidence of cholestasis such as icterus, pruritus, weight loss, and stenosis in MRCP.

### Data collecting

Study data were collected from the clinical records of eligible patients. These data were included in a researcher-made checklist that included two sections: demographic variables (sex and age) and laboratory variables (the result of liver enzymes, CA19\_9 tumor markers, and pathology report).

### Sampling protocol

For all 19 participants in the study, ERCP, with the use of Olympus cv-160 (Olympus Duodenoscope, Tokyo, Japan) was performed by a gastroenterologist, was done. First, the guidewire was passed through the narrowing site, and then the contrast agent was injected. This determined the length, intensity, and location of the stenosis. Then,

**Table 1:** Cross-sectional table of cold forceps biopsy and brush cytology

Variable	Brush cytology			
	Positive	Negative	Total	
Cold forceps biopsy	Positive	6	7	13
	Negative	0	6	6
	Total	6	13	19

from the inside of the stenosis, sampling was performed by the brush method (Cytology Brush SU- Diameter 2.6 mm, Length 180 cm ENDO FLEX, Germany). In the next step, cold forceps (Biopsy forceps–Diameter 1.8 mm, Length 160 cm, ENDO FLEX, Germany) sampling was performed directly using fluoroscopy-guided without cholangioscopy using small biopsy pans (Figure 1), and finally, the remaining of the contrast material in the ducts and branches was suctioned, and for establishing bile drainage, a plastic stent was placed in the involved branches and was crossed from the stenosis area. In order to obtain suitable tissue samples, the samples were taken from the three initial, middle, and terminal parts of the stenosis, which were more likely to have cholangiocarcinoma without mucosal necrosis.

#### Follow up

The follow-up program was designed to find the complications of the intervention: After ERCP, all patients, often with jaundice, were admitted to the hospital and monitored for at least 48 hours and were discharged if no complications occurred. Contact numbers were provided to patients that, in cases of abdominal pain, exacerbation of jaundice, fever, and chills, they could contact to receive the necessary advice. Besides, they were followed up after 1 week, 1 month, and 3 months after ERCP. During the evaluation, in the case of jaundice exacerbation, the plastic stents were replaced with another plastic stent, and in people with a definite diagnosis, metal stents were replaced.

#### Approval number of the institutional review board

This study was approved by our institutional review board [IRCT20161205031252N5].

#### Data analysis

Descriptive data were reported as frequency, frequency percentage, mean and standard deviation, and analytical

data were analyzed with McNemar, t test, and Mann-Whitney U tests. The significance level was considered to be 0.05. All analyzes were performed with Stata/SE 14.0 software.

#### RESULTS

19 people participated in this study. 63.16%<sup>12</sup> of the participants were men, and 36.84%<sup>7</sup> were women. The mean age of the patients was  $63.31 \pm 11.12$  years (male:  $59.58 \pm 8.50$ , female:  $69.71 \pm 12.78$  years).

The results of sampling showed that of the 19 examined samples, the brush cytology method diagnosed 31.58% (n = 6) of the samples as positive for cholangiocarcinoma (Figure 2-A) and 68.42% (n = 13) as negative for cholangiocarcinoma. In contrast, the cold forceps biopsy reported 68.42% (n = 13) of the samples as cholangiocarcinoma (Figure 2-B) and 31.58% (n = 6) as without cholangiocarcinoma (Table 1). According to McNemar's test, there was a statistically significant difference between the diagnostic results of the brush and the cold forceps method ( $p = 0.016$ ).

The mean laboratory parameters examined showed that in both diagnostic methods, the level of liver enzymes and CA19<sub>9</sub> tumor markers were higher in cases with the diagnosis of cholangiocarcinoma than in cases without the diagnosis, although this increase was significant only in CA19<sub>9</sub> tumor markers. The considered variables are shown by the diagnostic methods of brush and cold forceps in Table 2.

It should be noted that forceps biopsy reported 54% (seven cases) of the samples as positive while they were reported as negative with a brush. However, there were no cases in which the brush cytology sampling was positive, while forceps sampling was negative.

In the 3-months follow-up after ERCP, no complications such as perforation, bleeding, cholangitis, and leakage were reported for the cold forceps procedure.

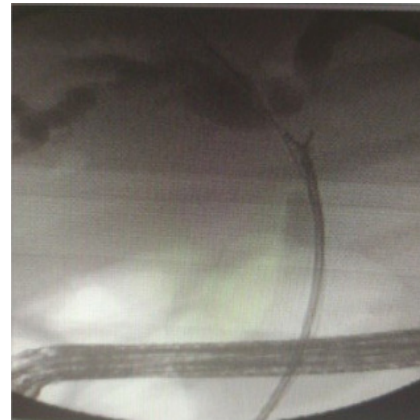
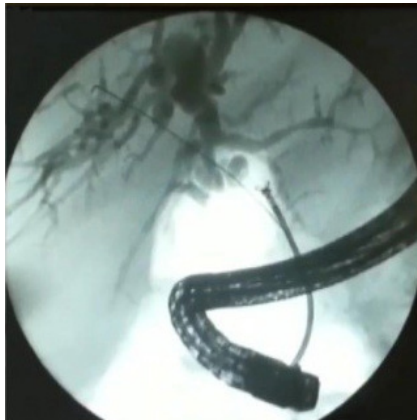
#### DISCUSSION

Based on the findings of our study, the brush cytology method and forceps biopsy method reported 31.58% (six cases) and 68.42% (13 cases) of the study samples as positive for cholangiocarcinoma, respectively. It should be noted that forceps biopsy reported 54% (seven cases) of the samples as positive while they were reported as negative with a brush. The reason for this finding is the difference in

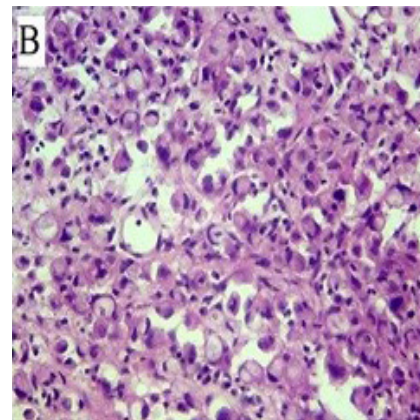
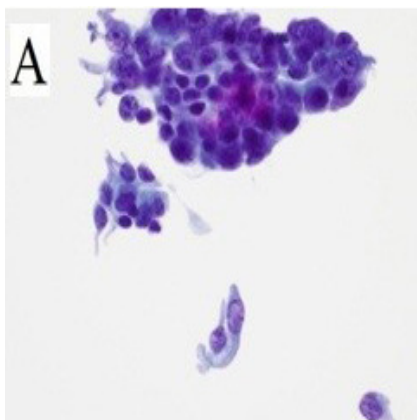
**Table 2:** The mean of laboratory parameters by sampling methods

Variables (SI unit)	Cold forceps biopsy			Brush cytology		
	Positive	Negative	<i>p</i> -value	Positive	Negative	<i>p</i> -value
CA19 <sub>9</sub> (U/mL)	432.08 ± 214.97	137.33 ± 44.56	0.001	515.50 ± 179.93	257.54 ± 201.26	0.011
Bill <sup>I</sup> (mg/dl)	15.75 ± 8.61	12.75 ± 6.18	0.356	18.47 ± 11.86	13.12 ± 4.97	0.379
ALP <sup>II</sup> (U/L)	1913.00 ± 823.89	1834.33 ± 594.58	0.837	2148.67 ± 1025.82	1767.92 ± 583.23	0.313
ALT <sup>III</sup> (U/L)	331.69 ± 179.64	370.67 ± 164.16	0.404	382.33 ± 194.55	326.31 ± 164.84	0.568
AST <sup>IV</sup> (U/L)	238.85 ± 141.76	279.50 ± 105.67	0.136	280.83 ± 202.60	238.23 ± 87.13	0.826

I Bilirubin  
 II Alkaline phosphatase  
 III Alanine aminotransferase  
 IV Aspartate aminotransferase



**Fig.1:** Forceps biopsy under fluoroscopic guidance



**Fig.2:** Microscopic findings of the stenotic site. A) Brush cytology smear. B) Forceps biopsy smear

the sampling method. The brushing method takes a few surface cell samples, which cannot be accurately examined by a pathologist, and the cases may be reported as negative,

but in the forceps method, a complete tissue sample with the appropriate depth is taken, and the diagnostic error will be less. A 2011 study by Kulaksiz and colleagues on 43

patients with proximal stenosis showed that the sensitivity of forceps sampling under the ERCP guide was higher than that of ERCP-guided brush cytology (69% vs. 49%).<sup>10</sup> The study by Inoue and co-workers on 110 patients with biliary stenosis (during 2009-2015) under controllable forceps biopsy (C-BF) and X-ray fluoroscopic guidance showed a high rate of technical success (99%), as well as sensitivity, specificity, and accuracy of 60%, 100%, and 72%, respectively.<sup>13</sup> In another study in 2012, Hartman and colleagues took 110 tissue samples from 89 patients with bile duct stenosis with unknown cause under fluoroscopic guidance (81 samples) and cholangioscopy (29 samples). Their findings showed the superiority of the diagnostic accuracy and sensitivity of forceps biopsy under fluoroscopic guidance compared with cholangioscopic guidance (accuracy 88% vs. 78%, sensitivity 76% vs. 57%, respectively), while the specificity in both methods was 100%.<sup>12</sup> Although we did not measure sensitivity, specificity, and accuracy in this study, our findings also showed that the forceps biopsy method significantly identified more cases of cholangiocarcinoma than brush cytology. Therefore, the results of these studies, in line with our study, show that the forceps biopsy method under fluoroscopic guidance can be useful in the diagnosis of cholangiocarcinoma.

Laboratory findings in both studied methods showed that the levels of liver enzymes and tumor markers CA19-9 were higher in positive cases than in negative ones. However, this increase was significant only in the 19-19-19 CA marker tumor. Perhaps the reason for this significant difference between these enzymes is the low sample size of the study. However, based on this finding, although the increased levels of liver enzymes are helpful to be suspicious of cholangiocarcinoma, increasing CA19-9 levels is more important. Weber's study findings support this claim.<sup>6</sup>

In a review study for forceps sampling, complications such as perforation, cholangitis, bleeding, and pancreatitis were reported.<sup>14</sup> In the study by Inoue and colleagues in 2017 on 110 patients, there were complications such as pancreatitis in 6.4% (seven cases), cholangitis in 2.7% (three cases), and periampullary perforation in 0.9% (one person) during sampling,<sup>13</sup> but in our study, no complication was seen throughout the follow-up period, which shows sampling must be done by an experienced and careful person.

One of the limitations of the present study was the small sample size compared with similar studies. The main reason was the low number of patients with the disease who were available to us, so researchers suggest that further studies be conducted with larger sample size. Another limitation was the lack of access to cholangioscopy as a preferred diagnostic method, which made it impossible to estimate the sensitivity and specificity of the two methods by examining all patients, so it is recommended that subsequent studies solve this limitation if possible.

## CONCLUSION

Standard cholangioscopy is usually expensive and is not available at most health centers. Therefore, cold forceps under fluoroscopy, which is less expensive than cholangioscopy and without any complications, if performed cautiously, has the potential to be used as an alternative in related cases when cholangioscopy is not available, in cases which reported negative with brush sampling, or in cases which EUS -FNA cannot be implemented.

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## ETHICAL APPROVAL

There is nothing to be declared.

## CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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