1. Introduction

Biliary stones are a common disease of hepatobiliary surgery, among which gallstone is the most common biliary stone disease. Laparoscopic cholecystectomy is the main treatment for gallstones and has achieved good results, but about 10% to 26% of treated patients with gallstones will have secondary common bile duct stones [1]. Common bile duct stones can cause biliary obstruction, if the patient has fever, abdominal pain, jaundice and other biliary obstruction symptoms, the diagnosis is relatively easy. However, most patients with secondary choledocholithiasis do not have clinical symptoms of biliary obstruction in the early stage, which is easy to cause missed diagnosis and misdiagnosis. For such patients, if choledochal stones cannot be detected before surgery, residual choledochduct stones appear after surgery, which is easy to occur bile leakage, cholangitis, pancreatitis and other serious complications, increase the risk of secondary operation, seriously affect the prognosis of patients, and even endanger the life of patients [2,3]. How improve the preoperative detection rate of choledochal stones so as to adjust the surgical plan is the key to the treatment of patients with secondary gallbladder stones. Recent studies have shown that patients with secondary choledocholithiasis often show abnormally elevated part of the liver function index [4]. Index factors such as glutamyltransferase (GGT), alkaline phosphatase (ALP), and glutamate transaminase (AST) can be used not only to evaluate liver injury but also as a predictor of choledocholithiasis [5]. Small RNA (miRNAs) is a class of non-coding single-stranded RNA molecules encoded by endogenous genes that are about 22 nucleotides in length. Its role in ontogeny, cell differentiation, apoptosis and other physiopathological processes has been widely confirmed by [6]. In addition to the coding genes, the role of miRNA in gallstone formation has also received increasing attention. In large families, miR-210 has become one of the research hotspots because of its functional diversity and stability in low-oxygen environments. Most studies focus on the role that miR-210 plays in the development of various kinds of cancer, while there are few studies on other aspects. It has been shown that miR-210 is aberrantly expressed in gallbladder cancer cells and may play a role in gallbladder disease [7]. Therefore, the present study investigated the expression level of miR-210 in patients with choledocholithiasis and analyzed its correlation with serum GGT, ALP and AST levels.
2. Materials and Methods

2.1. General data
Clinical data of 82 patients with biliary stones admitted to the hospital from May 2020 to May 2022 were collected. They were grouped according to whether asymptomatic secondary choledolithiasis, including 40 cases in the observation group (with asymptomatic secondary choledolithiasis) and 42 patients in the control group. There were no significant differences between the general data including gender, age, BMI, number of stones, and combined chronic diseases between the two groups, as shown in Table 1.

2.2 Case selection criteria
Inclusion criteria: (1) age > 18 years, diagnosed with biliary calculi, and diagnosed with common bile duct calculi; (2) no intestinal obstruction or biliary tract infection; (3) no liver occupying lesions or obvious liver damage or infectious liver diseases; (4) liver function indicators such as GGT, ALP, and AST before surgery.
Exclusion criteria: (1) primary choledochal stones; (2) malignant biliary tumor, Mirrizi syndrome, ischemic cardiomyopathy; (3) acute and chronic hepatitis or alcoholic liver disease; (4) sclerosing cholangitis, congenital bile duct ectasia, pancreatitis, congenital jaundice and other diseases; (5) pregnant women; (6) acute and critical patients.

2.3. Detection indicators
2.3.1. Serum GGT, ALP and AST detection
5 mL of fasting venous blood was collected in the morning, and the serum was measured by centrifugation at 3500 r/min for 15 min. The instrument is AU640 automatic biochemical analyzer (Olympus, Tokyo, Japan), using rate method to detect serum GGT, ALP, and AST levels, and perform standardized operation strictly according to the operation steps of the kit instructions.

2.3.2. Mir-210 detection
By TRizol method, the serum to be taken, the total RNA was extracted by homogenizing, separation, precipitation, washing, and dissolution and the concentration and purity were detected by UV-photometer; RT-PCR was performed after sample RNA OD260 / OD 280 in the range of 1.8 to 2.0. First, RT kit, primers and total RNA was prepared, gently shaken and centrifuged, incubated on ice for 5 min and placed in the PCR instrument, start the PCR reaction, and reverse transcription to cDNA. RT-PCR was performed with cDNA as template: mir-210 reagent, U6 snRNA probe and cDNA samples were thawed on ice, a 20 μl reaction system (containing 1.33 μl cDNA samples) was prepared, negative control wells were set, and the 1.33 μl cDNA sample in the reaction system was replaced with Nuclease-free water. The centrifuged 96-well plates were placed in a PCR instrument to detect the mir-210 and U6 expression levels. Reaction conditions: 50℃ pre-denaturation for 2 min, 95℃ denaturation for 5 min, 90℃ annealing for 15 s, 60℃ extension for 60 s, a total of 40 cycles. Primer sequence: mir-210 forward 5'-CGCGACCGGCTAGAGG-3' and reverse 5'-AGCGCCGGGTGAATCG-3'; U6 forward 5'-CTGCTGATCGTCGCTCGTG-3' and reverse 5'-AAGCACGGGTG-TGCCATCT-3'. Primer synthesis was completed by Beijing Qingke Biotechnology Co., Ltd. Relative expression values of mir-210 were calculated using the 2-△△Ct method.

2.4. Statistical analysis
Data were analyzed using the SPSS statistical software (IBM, Armonk, NY, USA). The measurement data were expressed as (mean ± standard deviation), and the t-test was opted for the variance analysis between clusters, One-way ANOVA was used for the comparison between multiple groups; Correlation analysis using Pearson correlation analysis; Subject operating characteristic curve (ROC) was used for diagnostic value. and P<0.05 was considered as remarkable.

3. Results
3.1. Comparison of the clinical data between the two patient groups
In total, 289 patients with biliary stones were included in the study, and 65 developed cholecholithiasis, with an incidence of 22.49% (Table 1).

3.2. Comparison of the relative expression of serum GGT, ALP and AST and miR-210 in the two patient groups
The relative expression of serum GGT, ALP and AST and miR-210 was significantly higher than that of the
3.3. Analysis of the predictive value of serum GGT, ALP, and AST and miR-210 relative expression for choledocholithiasis.

Using GGT, ALP, AST and miR-210 as test variables, combined common bile duct stones as the status variable, the ROC curve =1), the results showed that the area under the serum GGT curve (AUC) was 0.782, 95%CI: 0.710–0.854. When the cutoff value was 98.72 U/L, the sensitivity of serum GGT for the diagnosis of choledocholithiasis was 84.61%, and the specificity was 82.60%. The AUC of the serum ALP was 0.776, 95%CI: 0.700 to 0.853. At the cutoff value of 124.50 U/L, the sensitivity of serum ALP was 76.92% and 79.46%. the AUC of serum AST was 0.681, 95%CI: 0.595–0.768. When the cutoff value was 34.27 U/L, the sensitivity of serum AST was 63.08% and 65.18%. The AUC of the relative expression of miR-210 was 0.568, 95%CI: 0.476–0.660. At a cutoff of 1.56, the sensitivity of miR-210 relative expression was 61.54% and specificity was 66.52%. See Tables 3, 4, and Figure 1.

3.4. Correlation analysis of miR-210 with serum GGT, ALP and AST in patients with choledocholithiasis.

Relrelative miR-210, ALP and AST in cholecholithiarias (r=0.756, 0.832 and 0.326, all P <0.05). See Table 5.

4. Discussion

Biliary stones are common digestive diseases, widely distributed in the population, and are one of the main diseases affecting human health. Due to the change of living environment, eating habits and other factors, the incidence control group, and the difference was significant (P <0.05). See Table 2.

Table 2. Comparison of the relative serum expression of GGT, ALP and AST and miR-210 in the two patient groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Example number</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>AST (U/L)</th>
<th>miR210</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>40</td>
<td>142.35±32.54</td>
<td>182.20±41.53</td>
<td>35.26±3.82</td>
<td>1.72±0.45</td>
</tr>
<tr>
<td>Control group</td>
<td>42</td>
<td>40.58±11.42</td>
<td>128.55±31.38</td>
<td>33.12±3.67</td>
<td>1.38±0.43</td>
</tr>
<tr>
<td>t</td>
<td>19.077</td>
<td>6.620</td>
<td>2.587</td>
<td>3.499</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
<td>0.011</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Serum GGT, ALP and AST and miR-210 alone diagnosed choledocholithiasis.

<table>
<thead>
<tr>
<th>Inspection index</th>
<th>Exploration of common bile duct</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Choledocholithiasis</td>
<td>No choledocholithiasis</td>
</tr>
<tr>
<td>GGT</td>
<td>34</td>
<td>9</td>
</tr>
<tr>
<td>ALP</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>AST</td>
<td>26</td>
<td>14</td>
</tr>
<tr>
<td>miR-210</td>
<td>28</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 4. Analysis of the ROC curves.

<table>
<thead>
<tr>
<th>Inspection index</th>
<th>AUC</th>
<th>Standard error</th>
<th>95%CI</th>
<th>Truncation value</th>
<th>sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT</td>
<td>0.782</td>
<td>0.037</td>
<td>0.710–0.854</td>
<td>98.72</td>
<td>85.00</td>
<td>78.57</td>
</tr>
<tr>
<td>ALP</td>
<td>0.776</td>
<td>0.039</td>
<td>0.700–0.853</td>
<td>124.5</td>
<td>76.19</td>
<td>76.83</td>
</tr>
<tr>
<td>AST</td>
<td>0.681</td>
<td>0.044</td>
<td>0.595–0.768</td>
<td>34.27</td>
<td>65.00</td>
<td>66.67</td>
</tr>
<tr>
<td>miR-210</td>
<td>0.568</td>
<td>0.047</td>
<td>0.476–0.660</td>
<td>1.56</td>
<td>61.90</td>
<td>65.85</td>
</tr>
</tbody>
</table>
of biliary stones in China showed a significant increasing trend [8]. Influenced by obesity, diabetes, lack of exercise and other factors, the onset age of biliary stones in China also tends to be younger and [9]. According to the type, choledocholithiasis can be divided into primary and secondary. Secondary choledocholithiasis is often formed by bile excretion into the common bile duct and [10]. Common bile duct stones occur at the lower end of the common bile duct, and most stones are bile pigment stones or mixed stones, which can lead to incomplete biliary obstruction; stones will shift in the biliary system with changes in the body, and the clinical manifestations are intermittent. Without timely treatment, it may lead to systemic toxemia and even endanger life safety [11]. Some stones are floating in the common bile duct, so there are no clinical symptoms such as biliary obstruction and stone incarceration, etc. [12], which greatly improves the difficulty of early diagnosis.

Liver function indicators play a significant role in the diagnosis and prediction of choledocholithiasis. GGT is widely distributed on the intrahepatic bile duct, especially during biliary obstruction, GGT activity will be significantly increased. If patients are complicated with cholangitis, the activity is higher [13]; GGT has liver secretion, mainly excreted through the bile duct. In the period liver function is impaired, the obstruction of common bile duct stones will directly affect the excretion of GGT, and the serum GGT level greatly increases [14]. AST is widely distributed in tissues such as myocardium, liver and kidney. Under normal circumstances, serum AST is at a low level, the corresponding cells are damaged when the body is in a pathological state, and AST in the cytoplasm will be released into the blood through damaged cells, resulting in high expression of serum AST [15]. ALP, an enzyme capable of dephosphorylating the corresponding substrate, is derived mainly from the liver, has extensive application in various examinations of hepatitis diseases and shows significantly high expression of [16] in the early stage of biliary obstruction. If the imaging methods such as B ultrasound and CT do not talk about the common bile duct stones, and the liver function indicators such as GGT, AST and ALP are obviously abnormal, it indicates that there may be stones in the bile duct, and further investigation of ERCP and MRCP for [17] is needed. This study showed that the serum GGT, AST and ALP indexes of patients in cholecholithiasis group were significantly higher than those of the control group, indicating that the patients had abnormal increases in liver function indexes such as GGT, AST and ALP, which was consistent with the research results of other scholars. The ROC curve shows that Serum GGT diagnosed with AUC was 0.782, 95%CI: 0.710–0.854, When the cutoff value is 98.72 U/L, A sensitivity of 84.61%, Specific degree is 82.60%; Serum ALP diagnosed with AUC was 0.776, 95%CI: 0.700–0.853, When the cutoff value is 124.50 U/L, A sensitivity of 76.92%, Specific degree for 79.46%; Serum AST diagnosed with AUC was 0.681, 95%CI: 0.595–0.768, When the cutoff value is 34.27 U/L, With a sensitivity of 63.08%, The specificity ratio was 65.18%. It can be seen that serum GGT alone has a better diagnostic efficacy for choledocholithiasis than serum AST and ALP.

The role of genetic factors in the formation of biliary stones cannot be ignored. Several studies have confirmed that not only mRNA is important for the formation of biliary stones, but miRNA is one of the important molecular mechanisms of biliary stone formation [18]. The action of miRNA on target genes can be divided into two types. The most common mode is that miRNA suppresses the translation process of the targeted mRNA while inhibiting the complete complementarity of mRNA and the target gene; the targeted miRNA reduces the protein expression level. The miRNA plays an important role in the process of cholesterol metabolism, and the key factor in the formation of biliary stones is abnormal cholesterol metabolism [19]. Representative miRNA such as miR-33a promotes ABCA1, miR-185 suppresses SREBP-2 expression, miR-122a suppresses CYP7A1 expression, etc. Changes in these pathways will have effects on cholesterol synthesis, transformation, transport and other processes, thus promoting the formation of biliary stones.

As a newly discovered miRNA, miR-210 plays an important role in the occurrence and development of many diseases [20]. Studies have shown that miR-210 can play a role in cell response, angiogenesis, and nerve function, and regulate the process of ischemic stroke, and its level is correlated with the severity of stroke [21]. The relative expression of miR-210-3p in lung cancer tissues was higher than that of adjacent tissues, and the expression level was related to the clinical classification and lymph node metastasis of lung cancer, which had great significance for the diagnosis, treatment and prognosis prediction of lung cancer [22]. High expression of miR-210 in adipose tissue promotes adipocyte proliferation and angiogenesis and suppresses its apoptosis by targeted downregulation of YWHAQ [23]. Treatment of miR-210 was highly expressed in glioma tissues, and downregulation of miR-210 expression could inhibit cell proliferation, migration and invasion ability, and promote cell apoptosis for [24]. Serum miR-210 shows high expression in elderly patients with sepsis, which is related to the severity of AKI patients with sepsis, and is also closely related to the prognosis of the condition [25-28]. Meanwhile, serum miR-210 was positively correlated with the degree index of kidney injury index, which could also laterally reflect the degree of kidney injury. It has been found that miR-210 and its potential target gene, ATP11A, are differentially expressed in both miRNA and mRNA profiles. ATP11A is a direct target of miR-210, and miR-210 is predicted to regulate the ABC transporter protein pathway. The expression level of ATP11A in gallstones was negatively correlated with miR-210 expression, and the upregulation of miR-210 decreased ATP11A expression in HGBEC. Differential miRNA expression exists between patients with and without gallstones, and miR-210 can target regulating ATP11A, which may affect the

<table>
<thead>
<tr>
<th>Statistic</th>
<th>GGT</th>
<th>ALP</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.756</td>
<td>0.832</td>
<td>0.326</td>
</tr>
<tr>
<td>P</td>
<td>0.007</td>
<td>0.015</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Table 5. Correlation analysis of miR-210 with serum GGT, ALP and AST in patients with choledocholithiasis.
formation of biliary stones. The author considers whether miR-210 can be used as one of the factors for the diagnosis and prediction of choledocholithiasis. This study showed that the relative expression level of miR-210 in patients with choledocholithiasis was significantly higher than that of control patients with biliary calculi (P <0.05), and the significant improvement in serum relative expression of miR-210 may be related to its high expression in bile, suggesting that it may be used as a marker for early diagnosis of asymptomatic or less symptomatic choledocholithiasis. The quantitative expression levels of miR-210 in serum and bile can be used for early diagnosis and prediction of choledocholithiasis. This study showed that the significant positive relationship between the relative expression level of miR-210 and serum GGT, ALP and AST in choledocholithiasis (r=0.756, 0.832, 0.326, all P <0.05), which partly predicted the occurrence of choledocholithiasis by miR-210. The ROC curve showed that the AUC of miR-210 was 0.568, 95%CI: 0.476–0.660. When the cutoff value was 1.56, the sensitivity of miR-210 was 61.54% and the specificity was 66.52%, which may be related to the number of samples and the severity of individual disease.

The limitations of this study are as follows: (1) the combined diagnostic efficacy of miR-210 and serum GGT, AST and ALP was not studied; (2) the mechanism between miR-210 and common bile duct stones needs to be further explored; (3) the sample size is too small, and the sample size was expanded; and (4) some factors that may influence diseases, such as smoking and exercise, were not counted and corrected. In the future, more complete and in-depth research will be conducted on the above problems.

5. Conclusion
In conclusion, the serum miR-210 level was upregulated in patients with choledocholithiasis, and their expression was positively correlated with serum GGT, AST and ALP, which can be used for early adjuvant diagnosis of choledocholithiasis.

Conflict of Interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
This study was approved by the ethics committee of Suzhou Hospital of Anhui Medical University.

Informed Consent
Signed written informed consent were obtained from the patients and/or guardians.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors’ contributions
PL and KH designed the study and performed the experiments, DW collected the data, XH analyzed the data, PL prepared the manuscript. All authors read and approved the final manuscript.

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miR-210 correlation with GGT, ALP, AST in choledocholithiasis

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