<u>Original Research</u>

Analyzing the Correlation Between Serum Biomarkers and Child-Pugh Score in Liver Cirrhosis

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ABSTRACT

Objective • This study aimed to analyze the diagnostic efficacy of serum biomarkers in liver cirrhosis patients categorized by Child-Pugh scores.

Methods • An observational cross-sectional study design was employed. A total of 110 liver cirrhosis patients, classified according to Child-Pugh scores and 60 healthy individuals were included in this study. Serum levels of adenosine deaminase (ADA), adiponectin (APN), matrix metalloproteinase-2 (MMP-2), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured.

Results • The levels of ADA, APN, MMP-2, ALP, ALT, and

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INTRODUCTION

Liver cirrhosis is a prevalent and potentially lifethreatening condition in clinical gastroenterology.¹ The complex pathogenesis of this disorder involves a multifaceted relationship of various factors, with chronic liver injury emerging as an important cause. This injury results from diverse causes such as viral hepatitis (notably hepatitis B and C), excessive alcohol consumption, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, and other etiologies, initiating a cascade of intricate events. These events, in turn, set the stage for the development of fibrosis, a precursor to the more advanced and severe state of cirrhosis.^{2,3}

Persistent inflammation, hepatocyte damage, activation of hepatic stellate cells, and abnormal wound healing processes collectively contribute to the deposition of excessive extracellular matrix, fostering the formation of fibrotic tissue within the liver.³ The progressive impairment of liver function in liver cirrhosis emphasizes its critical nature. This condition AST were significantly higher in the study group compared to the control group (P < .05). Furthermore, these levels increased with the severity of liver cirrhosis, with higher levels observed in patients with Child-Pugh class C. The positive diagnostic rates for joint detection in Child-Pugh class A, B, and C were 93.75% (30/32), 100% (34/34), and 100% (44/44), respectively.

Conclusions • Combined detection of serum biomarkers improves the diagnostic efficacy of liver cirrhosis. The diagnostic rates were higher when considering Child-Pugh scores, with the highest rates observed in class C. (*Altern Ther Health Med.* [E-pub ahead of print.])

may progress to malignant transformation if it remains untreated, significantly risking patient safety. Therefore, early diagnosis and proactive treatment become imperative in its clinical management.⁴

According to recent statistics, the global prevalence of liver cirrhosis is estimated at around 1-2% of the population.⁵ In regions struggling with a significant burden of chronic liver diseases, such as Asia and Africa, this prevalence may surge even higher.⁵⁻⁶ Liver cirrhosis has a high mortality rate, accounting for over 1 million deaths annually worldwide ^[6]. Mortality is primarily attributed to complications arising from liver cirrhosis, including liver failure, portal hypertension, and hepatocellular carcinoma. These complications collectively contribute to the severity and fatality associated with this condition.

Despite its high prevalence, the current diagnostic practices for liver cirrhosis have limitations.⁷ Common diagnostic approaches involve the detection of serum indicators and biochemical projections, offering valuable insights into various diseases. Although these methods have been employed for diagnosing liver cirrhosis, the combination of serum indicators and biochemical projections is not widely adopted in clinical practice. Biomarkers such as deaminase (ADA), serum adiponectin (APN), and matrix metalloproteinase-2 (MMP-2) have been extensively studied in the context of liver cirrhosis diagnosis.⁸

The biochemical indicators alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are commonly employed in liver function tests.⁷⁻⁸ ADA, an enzyme involved in purine metabolism, exhibits elevated levels in liver cirrhosis patients. The activity of ADA can serve as a marker for assessing liver damage and inflammation.⁸ However, it is important to acknowledge that ADA alone lacks specificity for liver cirrhosis and may be influenced by other factors such as viral infections or autoimmune diseases.

APN, an adipokine, contributes to regulating glucose and lipid metabolism. In liver cirrhosis, a decrease in APN levels has been observed.⁸ Low APN levels are linked to insulin resistance, inflammation, and the advancement of liver fibrosis. APN serves as a marker to assess the severity of liver cirrhosis and its related complications. MMP-2, an enzyme involved in tissue remodeling and extracellular matrix degradation, may exhibit increased levels of liver cirrhosis due to ongoing liver inflammation and fibrosis.⁹ MMP-2 can function as a marker to evaluate the extent of liver fibrosis and predict disease progression.⁷⁻⁹ However, further research is necessary to establish its clinical utility in liver cirrhosis diagnosis.

This study aims to address this gap by investigating the diagnostic efficacy of combining serum and biochemical indicators in patients with liver cirrhosis. The purpose of this study was to provide a more comprehensive understanding of the patient's condition and the extent of liver damage. The findings contribute to the positive diagnostic rate of the joint detection method compared to single detection, thereby enhancing the accuracy of liver cirrhosis diagnosis. This study offers a novel methodology that addresses the current limitations of diagnosis. The outcomes may significantly improve early detection, management strategies, and overall patient outcomes in the realm of liver health.

MATERIALS AND METHODS

Study Design

An observational cohort study was conducted, and a total of 110 adult patients with liver cirrhosis admitted to Cangzhou Central Hospital between April 2020 and June 2022 were selected as the study group. The control group consisted of 60 healthy individuals undergoing physical examination during the same period. The inclusion criteria were strictly adhered to for all study subjects, and there were no gender restrictions during enrollment. The study protocol received ethical approval from the Ethics Committee of Cangzhou Central Hospital (Ethics Committee approval number 202-09-22).

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) Patients in the study group were diagnosed with liver cirrhosis based on clinical testing results, specifically ultrasonography revealing features such as reduced liver size, uneven surface, enhanced or reduced intrahepatic echogenicity, and high portal blood flow; abnormal elevation of ALT, AST, ALP, and bilirubin was observed. (2) All clinical data for the study subjects were complete. (3) All study subjects demonstrated the ability to think independently and expressed willingness to cooperate with the study. (4) Both study subjects and their families were informed about the study's content and willingly participated.

Exclusion criteria were as follows: (1) Patients with tumor diseases were excluded. (2) Patients with severe extrahepatic organ diseases were excluded. (3) Patients with systemic diseases such as hypertension and type 2 diabetes were excluded. (4) Patients with mental illness or cognitive disorders were excluded. (5) Patients and their families who were unable to cooperate with the study due to various reasons were also excluded.

Detection of Serum and Biochemical Indicators

Serum-Related Indicators. All study subjects underwent detection of serum-related indicators, including ADA, APN, and MMP-2.

Biochemical Indicators. The study also involved the assessment of biochemical indicators, which included ALP, ALT, and AST.

Detection Methods. The specific detection methods were as follows: 3 ml of fasting venous blood was collected from the subjects, and the collected serum was saved for testing after routine centrifugation. Fully automated biochemical analyzers (NOVA Biochemical, Waltham, MA, USA) were used to detect the levels of ADA, APN, MMP-2, ALT, AST, and ALP in the patients. The ELISA method was used for ADA, APN, and MMP-2, and the rate method was used for ALT, AST, and ALP.

Pre-Testing Instructions. Patients were required to avoid taking antihypertensive, lipid-lowering, and hormonal drugs before testing and were instructed to fast for 24 hours before testing.

Criteria for Judgment

Child-Pugh Grading Criteria. In accordance with the Child-Pugh score,¹⁰ the 110 cirrhosis patients in the study group were categorized into grades as follows: 32 in grade A, 34 in grade B, and 44 in grade C. The score for patients in grade A was <6 points, indicative of good liver function; for those in grade B, the score ranged from 7 to 9 points, signifying moderate liver function impairment; and for patients in grade C, the score exceeded 10 points, denoting severe liver function impairment.

Reference Range for Serum and Biochemical Indicators. (1) Serum Indicators: ADA, APN, and MMP-2. The normal range for ADA in the human body is 1-25 U/L. The normal range for APN is 5-30 μ g/mL. The normal range for MMP-2 is 0-0.5 μ g/L. (2) Biochemical Indicators: ALP, ALT, and AST. The normal range for ALP in the human body is 45-135 U/L. The normal range for ALT is 0-35 U/L. The normal range for AST is 0-40 U/L.

Statistical Analysis

Data analysis was performed using SPSS 25.0 (IBM, Armonk, NY, USA). Quantitative data were expressed as

mean \pm standard deviation ($\overline{x} \pm s$), and comparisons were made using the *t* test. Qualitative data were presented as [n (%)], and the chi-square test (χ^2) was employed for comparisons. A significance level of *P* < .05 was considered statistically significant. The graphic presentation was carried out using GraphPad Prism 8.

RESULTS

Comparison of Baseline Data

The control group comprised 40 males and 20 females, with a mean age of (52.05 ± 8.41) years, ranging from 33 to 73 years old. In the study group, there were 74 males and 36 females, with a mean age of (52.63 ± 8.47) years, ranging from 37 to 76 years old. The disease course in the study group ranged from 3 to 14 months, with a mean of (8.42 ± 2.63) months. The Child-Pugh classification included 32 cases in class A, 34 cases in class B, and 44 cases in class C. There were no significant differences in gender, age, or other general information between the two groups (P > .05); refer to Table 1.

Comparison of Serum Biomarkers

The levels of ADA, APN, MMP-2, ALP, ALT, and AST in the study group were markedly higher than those in the control group (all P < .05). Furthermore, the levels of ADA, APN, MMP-2, ALP, ALT, and AST exhibited an increasing trend with the severity of liver cirrhosis. Specifically, the levels in class C were significantly higher than those in the control group, class A, and class B (all P < .05). Class B demonstrated significantly elevated levels compared to the control group and class A (all P < .05). Additionally, class A exhibited significantly higher levels than those in the control group (P < .05). Refer to Table 2 for detailed results.

Positive Rates of Individual Diagnosis and Combined Diagnosis in Different Child-Pugh Stages

As presented in Table 3, in Child-Pugh A patients, the positive diagnosis rates for ADA, APN, MMP-2, ALP, ALT, and AST were 71.88% (23/32), 71.88% (23/32), 53.13% (17/32), 71.88% (23/32), 68.75% (22/32), and 71.88% (23/32), respectively. The positive diagnosis rate for combined diagnosis reached 93.75% (30/32). For Child-Pugh B patients, the positive diagnosis rates for ADA, APN, MMP-2, ALP, ALT, and AST were 79.41% (27/34), 70.59% (24/34), 85.29% (29/34), 64.71% (22/34), 85.29% (29/34), and 70.59% (24/34), respectively.

The positive diagnosis rate of combined diagnosis was 100% (34/34). In Child-Pugh C patients, the positive diagnosis rates of ADA, APN, MMP-2, ALP, ALT, and AST were 81.82% (36/44), 45.45% (20/44), 50.00% (22/44), 45.45% (20/44), 50.00% (22/44), and 54.55% (24/44), respectively. Additionally, the positive diagnosis rate of combined diagnosis was 100% (44/44). The positive rates of combined diagnosis in Child-Pugh A, B, and C patients were significantly higher than those of ADA, APN, MMP-2, ALP, ALT, and AST individual diagnoses (all, P < .05).

Table 1. Comparison of General Information

| | Control Group | Study Group | | |
|------------------------------------|---------------|-------------|------------|---------|
| Variables | (n = 60) | (n = 110) | t/χ^2 | P value |
| Gender | | | 0.007 | .936 |
| Male | 40 | 74 | | |
| Female | 20 | 36 | | |
| Age | 3 3 -73 | 32-73 | | |
| Average Age (Years) | 52.0 5 ±8.41 | 52.63±8.47 | -0.42 6 | .67 1 |
| Disease Course (Month) | - | 3 -1 4 | - | - |
| Average Course of Disease (Months) | | 8.42±2.63 | | |
| Child-Pugh Classification | | | | |
| Grade A | | 32 | | |
| Class B | | 34 | | |
| Grade C | | 44 | | |

Note: Grade A representing good liver function, Grade B indicating moderate liver function impairment, and Grade C signifying severe liver function impairment.

| | Number | Serum Indicators | | |
|-----------------|----------|---------------------------------|-------------------------------|--------------------------------|
| Group | of Cases | ADA(U/L) | APN (µg/mL) | MMP-2 (µg/L) |
| Research Group | 110 | 40.23±5.05 ^{a,b} | 2 2.6 6 ± 2.17 ^{a,b} | 7 75.54±5.89 ^{a,c} |
| Grade A | 32 | 34.25 ± 2.55 ^{a,c} | $20.13 \pm 1.16^{a,c}$ | $768.13 \pm 2.84^{a,c}$ |
| Class B | 34 | $39.44 \pm 1.62^{a,b}$ | $22.33 \pm 0.48^{a,b}$ | $775.04 \pm 1.60^{a,b}$ |
| Grade C | 44 | 45.18 ± 2.43 ^{a,c} | $24.74 \pm 1.24^{a,b,c}$ | 781.31 ± 2.22 ^{a,c} |
| Control group | 60 | 14.62±4.07 ^{b,c} | 6.26 ±1.07 ^{b,c} | 15 5.21±4.04 ^{b,c} |
| | | | | |
| | Number | Biochemical Indicators | | |
| Group | of Cases | ALP(U/L) | ALT(U/L) | AST(U/L) |
| Research Group | 110 | 174.38±12.36 ^{a,b,c} | 60.57±5.14 ^{a,b,c} | 97.32±8.54 ^{a,b,c} |
| Grade A | 32 | $1.60.56 \pm 8.23^{a,c}$ | 54.52 ±3.08 ^{a,c} | 8 7. 16 ± 5.21 ^{a,c} |
| Class B | 34 | 17 3 . 15 ± 1.60 ^{a,b} | $60.16 \pm 0.95^{a,b}$ | 96.00 ± 2.50 ^{a,b} |
| Grade C | 44 | $18.5.37 \pm 8.35^{a,b,c}$ | $6.5.30 \pm 3.06^{a,b,c}$ | 105.71 ± 3.16 ^{a,b,c} |
| Cantural Canaum | 60 | 20 12±7 52bc | 15 27+4 8 7b.c | 21 23+5 18bc |

Table 2. Comparison of Serum Biomarkers

 ${}^{a}P < .05$ compared with the control group ${}^{b}P < .05$ compared with Class A

 $^{\circ}P < .05$ compared with Class B

Note: This table compares serum biomarkers (ADA, APN, MMP-2) and biochemical indicators (ALP, ALT, AST) between the research group and the control group, as well as within different Child-Pugh classes (Grade A, Class B, Grade C) in the research group.

Abbreviations: ADA, Adenosine Deaminase; APN, Adiponectin; MMP-2,Matrix Metalloproteinase-2; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase.

Table 3. Positive Rates of Individual Diagnosis and CombinedDiagnosis in Different Child-Pugh Stages

| | Child-Paugh Class A | Child-Paugh Grade B | Child-Paugh Grade C |
|-----------|-----------------------------|-----------------------------|-----------------------------|
| Variables | (n = 32) | (n = 34) | (n = 44) |
| ADA | 71.88% (23/32) ^a | 79.41% (27/34) ^a | 81.82%(36/44) ^a |
| APNs | 71.88% (23/32) ^a | 70.59% (24/34) ^a | 45.45% (20/44) ^a |
| MMP-2 | 53.13% (17/32) ^a | 85.29% (29/34) ^a | 50.00% (22/44) ^a |
| ALP | 71.88% (23/32) ^a | 64.71% (22/34) ^a | 45.45% (20/44) ^a |
| ALT | 68.75% (22/32) ^a | 85.29% (29/34) ^a | 50.00% (22/44) ^a |
| AST | 71.88% (23/32) ^a | 70.59% (24/34) ^a | 54.55% (24/44) ^a |
| Combined | 93.75% (30/32) | 100% (34/34) | 100% (44/44) |
| Diagnosis | 1 | | |

 $^{a}P < .05$ compared to combined diagnosis.

Abbreviations: ADA, Adenosine Deaminase; APN, Adiponectin; MMP-2, Matrix Metalloproteinase-2; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase.

DISCUSSION

This study investigated the diagnostic efficacy of combined serum and biochemical indicators, exploring their correlation with Child-Pugh scores. The study's findings provide valuable insight into the diagnosis of liver cirrhosis. Serum biomarkers, including ADA, APN, and MMP-2, have potential roles in evaluating liver damage, inflammation, and fibrosis. These biomarkers present potential implications for clinical practice and advancing research in the field.

Integrating biomarkers into the diagnostic landscape of liver cirrhosis represents an important step toward enhancing accuracy and promoting proactive clinical management. These biomarkers, carefully identified and analyzed, offer a nuanced understanding of the disease, enabling more precise and timely diagnoses. Their inclusion in routine diagnostic protocols can facilitate early detection and provide a continuous monitoring mechanism for liver cirrhosis.¹¹⁻¹²

Past studies¹¹⁻¹⁴ have demonstrated that the constant deterioration of cirrhosis poses a distinct risk of cancer transformation. Consequently, clinical diagnosis and treatment for cirrhosis patients should be initiated as early as possible to prevent the progression of their condition. Serum and biochemical indicator tests are routinely employed in the clinical diagnosis of various diseases, and their efficacy has gained widespread recognition^{15,16} Both serum and biochemical indicator tests assess the levels of metabolic products in patients' serum, providing valuable references for disease diagnosis, efficacy evaluation, and prognosis assessment.

It has been reported that the continuous impairment of liver function in cirrhosis patients results in significant changes in their serum biomarkers, making the detection of these indicators a crucial method for diagnosing early-stage cirrhosis.¹⁷ The human liver houses a variety of enzymes, and when the liver is damaged, some of these enzymes are released into the bloodstream.¹⁸ ADA, APN, and MMP-2 are relatively common serum indicators, and the alterations in ADA, APN, and MMP-2 levels are closely associated with the degree of liver damage in patients with cirrhosis.¹⁹

In biochemical indicator tests, the most frequently used markers for diagnosing cirrhosis are ALT, AST, and ALP. Pathological studies²⁰ have indicated that liver cell damage and necrosis in cirrhosis patients can elevate liver vascular permeability. During this phase, the levels of ALT, AST, and ALP in the patient's serum notably increase, and these changes can effectively indicate the severity of the patient's cirrhosis.

In this study, the levels of ADA, APN, MMP-2, ALP, ALT, and AST in the study group were significantly higher than those in the control group (all, P < .05), confirming that the serum biomarkers in patients with liver cirrhosis were markedly elevated compared to those in normal individuals. Furthermore, this study found that the levels of serum biomarkers in patients with liver cirrhosis increased with the severity of the disease, with levels in Class C patients significantly higher than those in Class A and B patients (all, P < .05). These findings are consistent with previous studies.²¹ Our findings also confirm that the detection of serum biomarkers in patients with liver cirrhosis can effectively reflect the degree of liver cell damage.

While both tests have proven valuable for the diagnosis of liver cirrhosis, studies on the combined application of these tests remain relatively rare.²² Our results showed that

the positivity rate for combined testing was significantly higher than that for single indicator testing (P < .05). Consistent with the results of Wan et al. and other studies, this study found that the positivity rate for the combined diagnosis of Child-Pugh A, B, and C patients was significantly higher than that for the single diagnosis of ADA, APN, MMP-2, ALP, ALT, and AST (all, P < .05).

These findings affirm that the diagnostic precision achieved by combining serum indicators with biochemical testing surpasses that of using a single indicator alone. This integrated diagnostic approach markedly enhances the efficiency of diagnosing patients with liver cirrhosis.

Study's Significance

The study's results have significant implications for advancing personalized medicine in liver cirrhosis. The identification of specific serum biomarkers enables the stratification of patients based on disease severity, progression risk, and treatment response, facilitating tailored therapeutic approaches for enhanced outcomes with minimized adverse effects. From a research perspective, the results open avenues for understanding the underlying mechanisms, guiding the development of novel therapeutic targets, and intervening to prevent or halt the progression of liver cirrhosis. Furthermore, the study encourages further exploration into the prognostic value of these biomarkers, shedding light on long-term outcomes and aiding in the identification of high-risk patient populations.

Study Limitations

Several limitations should be acknowledged in the interpretation of the study findings. Firstly, the relatively small sample size employed in the current study may impact the generalizability of the results, and employing a larger sample size would enhance the robustness and representativeness of the study population. Additionally, the study may have selection bias as participants were recruited from specific demographics or clinical settings, potentially limiting the applicability of the findings to broader populations or diverse clinical contexts.

The study design, possibly a cross-sectional approach, offers a snapshot of biomarker levels at a specific moment. In contrast, a longitudinal study design would yield more comprehensive insights into the dynamic changes of biomarkers and their association with disease progression over time. Furthermore, potential confounding factors, such as comorbidities, medications, or lifestyle factors, may not have been fully considered, impacting the accuracy of biomarkers in liver cirrhosis diagnosis. Lastly, the absence of assessed clinical outcomes related to biomarker levels, such as disease progression, liver-related complications, or patient survival, hinders a comprehensive understanding of the prognostic value of the identified biomarkers.

Future Directions

Several promising avenues for future research emerge from this study. Firstly, conducting validation studies with

larger cohorts and diverse populations would strengthen the evidence base and enhance the generalizability of the identified biomarkers' diagnostic utility in liver cirrhosis. Implementing longitudinal study designs is crucial for understanding the dynamic changes and stability of these biomarkers over time, enabling a more accurate assessment of their predictive value in disease progression. Additionally, conducting mechanistic studies is essential to unravel the underlying molecular pathways and mechanisms associated with the identified biomarkers. This deeper understanding could better explain the pathogenesis of liver cirrhosis and potentially reveal novel therapeutic targets for intervention.

CONCLUSION

In conclusion, our study highlights the significant improvement in diagnostic efficiency achieved through the combined detection of serum indicators and biochemical testing in patients with liver cirrhosis. This innovative approach enhances the accuracy of diagnosis and provides clinicians with a more comprehensive understanding of the patient's condition and the extent of liver damage. The notably higher positivity rate observed with the combined diagnostic method compared to single testing underscores its potential as a more robust and reliable diagnostic tool. These findings advocate for the integration of this combined testing method into routine clinical practice, offering a valuable means to optimize patient care, guide tailored interventions, and ultimately improve outcomes in individuals with liver cirrhosis.

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None

CONFLICT OF INTERESTS The authors report no conflict of interest

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available from the corresponding author upon request, subject to reasonable conditions.

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