Significance of hepatic fibrosis markers in early diagnosis of type 2 diabetes mellitus complicated with nonalcoholic fatty liver disease

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Abstract

Objective: Hyaluronic acid (HA), laminin (LN), and collagen IV (C-IV) are major serum markers of liver fibrosis. This study evaluated the diagnostic value of various noninvasive indicators for hepatic fibrosis in patients with T2DM and NAFLD. Methods: Between January 2016 and September 2018, a total of 278 patients, which were grouped to normal, NAFLD and T2DM with NAFLD. Routine clinical and laboratory examinations were collected, including age, sex, blood routine, HbA1C, FBG, FCP, AST, ALB, PLT, TC, TG, HDL, LDL, serum fibrosis C-IV, HA, LN, NFS, APRI and FIB4 scores were calculated. Results: No statistical difference was found on age, ALT, AST, GGT, BMI, TG, CHOL, and Glu, APRI, FIB4, CIV, LN, and HA exhibited statistical significance. Further correlation analysis showed that HA, C-IV, LN, were positively correlated with NFS, APRI, and FIB4. Conclusions: Compared with healthy control group, ALT, AST, TC, TG, HDL and LDL in NAFLD group, T2DM patients with NAFLD group increased to different degrees, and the difference was statistically significant. The HA, C-IV, LN, NFS, APRI ratio index and FIB-4 index of T2DM patients with NAFLD were higher than those of NAFLD group and healthy control group, and the HA, C-IV and LN of NAFLD group were higher than those of healthy control group. Compared with non-fibrosis group, HA, LN, C-IV, ALT and AST in fibrosis group were significantly higher. Moreover, HA, LN and C-IV were positively correlated with NFS, APRI ratio index and FIB-4 index.

1. Introduction

In recent years, fatty liver has become an important public health problem in the world. The epidemiological survey of non-alcoholic fatty liver disease shows that the prevalence rate in western developed countries is 20%-33%, in China the prevalence rate is 12%-15%, the number of patients reaches 150-200 million, and the trend is increasing year by year. Non-alcoholic fatty liver disease (NAFLD) has surpassed chronic viral hepatitis and become the first in China. Large liver disease. At present, type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) co-occur, promoting the development of non-alcoholic fatty liver disease (NASH), liver fibrosis and progressive liver disease. NAFLD has become the primary cause of asymptomatic transaminase elevation, some of which will progress to end-stage liver disease, and some of which are closely related to liver tumors. At present, liver biopsy is still the gold standard for diagnosis of fatty liver and liver fibrosis, but it is an invasive examination, so it is not easily accepted by most patients. However, there is no typical clinical manifestation of NAFLD in its early stage. How to diagnose and monitor NAFLD in early stage has become a key issue to control the development of NAFLD to cirrhosis and even hepatocellular carcinoma. Hepatic fibrosis indicators collagen IV (C-IV), hyaluronic acid (HA) and laminin (LN) can reflect the early pathological changes of liver cirrhosis, but there are few studies on diabetes mellitus patients with non-fatty liver disease. The purpose of this study is to determine the levels of C-IV, HA, LN and other indicators of liver fibrosis in patients with type 2 diabetes mellitus and NAFLD, and to determine the correlation between C-IV, HA, LN and other non-traumatic liver
fibrosis indicators, such as NAFLD fibrosis score (NFS), AST/ALT ratio, FIB-4 index, APRI ratio index, and so on, in patients with type 2 diabetes mellitus and NAFLD. To explore the clinical significance of these changes in the process of transition from simple fatty liver to steatohepatitis and cirrhosis, so as to find a new way of early diagnosis and clinical treatment for patients with T2DM combined with NAFLD.

2. Materials and methods

2.1 Observers

This study selected 278 inpatients and health examinees from Luwan Branch Hospital of Ruijin Hospital from January 2016 to September 2018, aged 35-70. The patients were divided into three groups according to whether they had fatty liver: normal control group (78 cases), NAFLD group (80 cases), T2DM combined with NAFLD group (120 cases). There was no significant difference in age, BMI, glycated hemoglobin, fasting blood sugar, fasting C-peptide, albumin, platelet and HOMA-IR ($P > 0.05$). See Table 1. In addition, according to the high and low diagnostic thresholds of NAFLDFS scoring system, patients with T2DM combined with NAFLD were divided into three subgroups: NAFLDFS > 0.676 was subgroup of liver fibrosis, - 1.455 < NAFLDFS < 0.676 was subgroup of uncertainty, and NAFLDFS < -1.455 was subgroup of non-fibrosis.

2.2 standard of diagnosis

1) diagnostic standard of T2DM: Reference to WHO diagnostic criteria in 2006: fasting blood glucose (>$7.0$ mmol/L) and/or postprandial 2 h blood glucose (>$11.1$ mmol/L).

2) diagnostic standard of fatty liver: referring to the revised 2010 edition of the guidelines for the diagnosis and treatment of non-alcoholic fatty liver disease of the Fatty Liver and Alcoholic Liver Disease Group of the Chinese Medical Association; imaging diagnosis: two of the following abdominal ultrasonographic manifestations are defined as diffuse fatty liver: (1) diffuse enhancement of near-field echoes of the liver ("bright liver") with stronger echoes than that of the kidney; (2) display of intraparenchymal duct structure (3) The far-field echo of liver gradually attenuated.

2.3 Exclusion criteria

(1) Type 1 diabetes mellitus, gestational diabetes mellitus, secondary diabetes mellitus, and acute and chronic complications of diabetes mellitus require insulin therapy; (2) Severe damage of liver and kidney function; unstable or severe angina pectoris and cardiac insufficiency (NYHA grade III/IV); (3) be suffered from chronic consumptive diseases such as malignant tumors, hematological diseases, psychosis, autoimmune diseases and obvious digestive and absorption disorders. He suffered from acute cerebrovascular diseases in recent 6 months; (4) Have a long history of heavy drinking and drug abuse; exclude viral hepatitis; use drugs that affect glycometabolism, such as glucocorticoids.

2.4 Outcome measures

After admission, the patient’s height, weight, waist circumference and hip circumference were measured and BMI and WHR were calculated. All patients fasting for more than 10 hours after dinner. The next morning, venous blood was collected in the forearm for OGTT test to detect glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), fasting C-peptide (FCP), alanine aminotransferase (ALT), glutamic oxalase transaminase (AST), and white blood. Protein (ALB), platelet (PLT), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), collagen IV (C-IV), hyaluronic acid (HA), laminin (LN) and so on were measured, and HOMA-IR, liver fibrosis score (NFS), APRI ratio index and FIB-4 index were calculated.

2.5 Calculating formulas of different scoring indicators of liver fibrosis

(1)NAFLDFS = 1.675 + 0.037 × age (year) + 0.094 × BMI + 1.13 ×FBG regulation impaired or diabetes (yes = 1, not = 0) + 0.99 × AST / ALT - 0.013 × platelet (10$^9$/L) - 0.66 × albumin (g/dL). According to the high and low diagnostic thresholds of the scoring system, patients with T2DM and NAFLD were divided into three subgroups. NAFLDFS > 0.676 was the subgroup of hepatic fibrosis, - 1.455 < NAFLDFS < 0.676 was the uncertain subgroup, and NAFLDFS < -1.455 was the subgroup of non-fibrosis; (2) Patients whose APRI ratio index = AST (IU/L)/AST normal upper limit × platelet (10$^9$/L) < 100 APRI ratio index (<0.5) suggest no significant fibrosis, and those whose APRI ratio index > 1.5 indicate significant fibrosis; (3) FIB-4 index = age (year)×AST (IU/L)/[platelet (10$^9$/L)× ALT (IU/L)]/2 FIB-4 index < 1.45 excludes severe fibrosis, FIB-4 index > 3.25 indicates clear and significant fibrosis.

Table 1.

General information comparison (T-tests).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>age (mean±s)</th>
<th>BMI (kg/m$^2$)</th>
<th>HbA1c(%)</th>
<th>FBG (mmol/L)</th>
<th>FCP (ng/mL)</th>
<th>PLT (10$^9$/L)</th>
<th>HOMA-IR</th>
<th>ALB (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>78</td>
<td>51.9±10.64</td>
<td>23.3±2.68</td>
<td>5.5±0.31</td>
<td>4.9±0.52</td>
<td>2.0±0.84</td>
<td>203.38±54.31</td>
<td>1.50±0.01</td>
<td>42.4±6.32</td>
</tr>
<tr>
<td>B</td>
<td>80</td>
<td>54.40±10.92</td>
<td>27.7±5.08</td>
<td>5.7±0.41</td>
<td>5.0±0.49</td>
<td>3.3±2.20</td>
<td>218.69±51.79</td>
<td>1.50±0.01</td>
<td>43.1±6.06</td>
</tr>
<tr>
<td>C</td>
<td>120</td>
<td>59.10±11.01</td>
<td>26.49±3.77</td>
<td>9.0±1.87</td>
<td>7.59±2.55</td>
<td>2.58±1.80</td>
<td>202.10±54.31</td>
<td>1.50±0.01</td>
<td>40.15±6.32</td>
</tr>
</tbody>
</table>

Note: A: normal control group; B: pure NAFLD group; C: T2DM combined with NAFLD; BMI body mass index; HbA1c glycosylated hemoglobin; FBG fasting blood sugar; FCP fasting C-peptide; ALB albumin, PLT platelet; HOMA-IR.
Table 2.
Comparison of general data (±xs).

<table>
<thead>
<tr>
<th>Group</th>
<th>AST(IU/L)</th>
<th>ALT(IU/L)</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>C-IV (ng/mL)</th>
<th>HA (ng/mL)</th>
<th>LN (ng/mL)</th>
<th>NFS</th>
<th>APRI ratio index</th>
<th>FIB-4 index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21.92±10.29</td>
<td>21.38±24.31</td>
<td>4.72±1.01</td>
<td>1.2±0.62</td>
<td>1.35±0.28</td>
<td>3.09±0.77</td>
<td>54.08±13.63</td>
<td>57.76±47.95</td>
<td>94.45±22.29</td>
<td>1.455 &lt; NFS &lt; 0.676</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.50±9.28</td>
<td>2.97±16.22</td>
<td>5.39±1.03</td>
<td>1.79±0.95</td>
<td>1.18±0.21</td>
<td>3.69±0.82</td>
<td>52.03±11.24</td>
<td>77.91±67.15</td>
<td>95.16±28.67</td>
<td>NFS &lt; 1.455</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>21.67±11.29</td>
<td>27.55±21.27</td>
<td>5.05±1.24</td>
<td>2.33±1.24</td>
<td>1.09±0.26</td>
<td>3.51±0.95</td>
<td>64.74±39.30</td>
<td>88.18±62.91</td>
<td>94.92±27.96</td>
<td>NFS &lt; 0.676</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: TC total cholesterol; TG triglyceride; HDL high density lipoprotein; LDL low density lipoprotein; C-IV type IV collagen; HA hyaluronic acid; LN laminin; ALT alanine aminotransferase; AST glutathione aminotransferase; non-alcoholic fatty liver fibrosis score (NFS). Compared with the normal control group, P<0.05; compared with the simple NAFLD group, ¹P<0.05; compared with the nondeterministic group, ²P<0.05.

Table 3.
Comparison of three subgroups in patients with T2DM and NAFLD (±xs).

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>C-IV(ng/mL)</th>
<th>HA(ng/mL)</th>
<th>LN(ng/mL)</th>
<th>ALT(IU/L)</th>
<th>AST(IU/L)</th>
<th>ALB(g/dL)</th>
<th>PLT(10^12/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis group</td>
<td></td>
<td>66.94±35.21</td>
<td>98.16±61.57</td>
<td>95.83±28.01</td>
<td>28.77±23.22</td>
<td>23.60±12.31</td>
<td>40.02±6.08</td>
<td>203.14±54.37</td>
</tr>
<tr>
<td>Nondeterministic group</td>
<td></td>
<td>67.55±33.68</td>
<td>87.35±62.90</td>
<td>94.08±26.17</td>
<td>27.94±21.34</td>
<td>22.41±11.39</td>
<td>40.12±6.04</td>
<td>202.91±50.11</td>
</tr>
</tbody>
</table>

Note: Compared with the fibrosis group, ¹P<0.05. Compared with the unclear group, ²P<0.05.

2.6 Statistical methods

SPSS 19.0 software was used for statistical analysis. The measurement data were tested by T-test, and the relationship between them was tested by correlation test. The measurement data of skewed distribution are all taken natural logarithm, which is normalized and analyzed. Bilateral P<0.05 had statistical significance. Pearson correlation analysis was used for correlation analysis, P<0.05 was statistically significant.

3. Results

3.1 Comparison of general data

In this study, 278 inpatients and health examinees from Luwan Branch Hospital of Ruijin Hospital from January 2016 to September 2018 were selected, aged 35-70. The patients were divided into three groups: normal control group (78 cases), NAFLD group (80 cases), and T2DM combined with NAFLD group (120 cases). Compared with normal control group, ALT, AST, TC, TG, HDL and LDL of NAFLD, T2DM combined with NAFLD patients were all included. There were different degrees of increase, and the difference was statistically significant (P<0.05). The HA, C-IV, LN, NFS, APRI ratio index and FIB-4 index of T2DM patients with NAFLD were higher than those of NAFLD group and healthy control group (P<0.05), and the HA, C-IV and LN of NAFLD group were higher than those of healthy control group (P<0.05). Refer to Table 2.

3.2 Comparison of three subgroups in patients with T2DM and NAFLD

NFS > 0.676 in hepatic fibrosis subgroup, NFS < 0.676 in uncertain subgroup - 1.455 < NFS < 0.676 and NFS < 1.455 in non-fibrosis subgroup were divided into three subgroups. Compared with non-fibrosis group, HA, LN, C-IV, ALT and AST increased significantly among the three subgroups (P<0.05). Refer to Table 3.

3.3 Correlation analysis of fibrosis indexes with NFS, APRI ratio index and FIB-4 index

The correlation analysis of each fibrosis index (C-IV, HA, LN) with NFS, APRI ratio index and FIB-4 index showed that there was no significant correlation between the normal control group and NAFLD group, but there was a linear positive correlation between the two groups. Refer to Table 4, 5, 6.

Table 4.
Correlation analysis of hyaluronic acid with NFS, APRI ratio index and FIB-4 index.

<table>
<thead>
<tr>
<th>HA</th>
<th>NFS</th>
<th>APRI ratio index</th>
<th>FIB-4 index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.11</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td>B</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>0.28</td>
<td>0.31</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 5.
Correlation analysis of C-IV with NFS, APRI ratio index and FIB-4 index.

<table>
<thead>
<tr>
<th>C-IV</th>
<th>NFS</th>
<th>APRI ratio index</th>
<th>FIB-4 index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.33</td>
<td>0.24</td>
<td>0.65</td>
</tr>
<tr>
<td>B</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>0.24</td>
<td>0.38</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Table 6.
Correlation analysis of LN with NFS, APRI ratio index and FIB-4 index.

<table>
<thead>
<tr>
<th>LN</th>
<th>NFS</th>
<th>APRI ratio index</th>
<th>FIB-4 index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.51</td>
<td>0.55</td>
<td>0.41</td>
</tr>
<tr>
<td>B</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>0.21</td>
<td>0.42</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Note: Refer to Table 3.
4. Discussion

NAFLD is a predictor of pre-diabetes and a high-risk factor that was shown by many studies at home and abroad. In patients with NAFLD, the degree of fatty liver fibrosis may be aggravated by the presence of T2DM. Although many studies have reported the prevalence and risk factors of NAFLD in patients with T2DM, few studies have reported the degree of liver fibrosis and its risk factors in patients with NAFLD. Liver is an important organ to maintain the relative stability of blood sugar level. After liver fibrosis, the decrease of hepatic glycogen intake and the impairment of hepatic glycogen synthesis lead to the increase of HbA1c level. Foreign studies have shown that insulin resistance and glycolipid metabolic disorders in patients with NAFLD are more serious than those in patients with pure T2DM, which leads to higher cardiovascular mortality. In the past, it was considered that the prognosis of non-alcoholic fatty liver is good, and will not progress to liver fibrosis and cirrhosis. However, in recent years, some studies have found that some severe steatosis can be accompanied by steatohepatitis. Studies such as Chang Qiuxia and Gao Xin in Shanghai Zhongshan Hospital, they found that progressive hepatic fibrosis is up to 24.0% in patients with T2DM combined with NAFLD. With the progress of hepatic fibrosis, the symptoms of various metabolic disorders are further aggravated and will resulting in liver fibrosis and pseudolobular formation, also the liver fibrosis and cirrhosis appeared. T2DM is an independent risk factor for the development of non-alcoholic steatohepatitis to hepatic fibrosis. It can be seen that there is a close relationship between T2DM and hepatic fibrosis[3-5]. Patients with type 2 diabetes mellitus and NAFLD have more severe fatty liver and higher risk of developing hepatic cirrhosis and death. The predictive value of fibrosis markers such as hyaluronic acid, collagen type IV and laminin for diabetic steatohepatitis with or without hepatic fibrosis needs further study. In previous studies of viral hepatitis and cirrhosis, it was found that C-IV is the main component of reticular structure of basement membrane. Previous studies have suggested that C-IV can occur in early hepatic fibrosis. So, it was usually be used as an early indicator of liver fibrosis. Hyaluronic acid (HA) is a glycosaminoglycan synthesized by mesenchymal cells. The serum HA levels in patients with hepatitis and cirrhosis are increased. Some studies suggest that HA is one of the most valuable serological markers reflecting hepatic fibrosis. Laminin (LN) is a kind of non-collagen mucin, which can maintain the function of basement membrane. Normally, the content of LN in liver is very low. Only when chronic liver injury occurs, LN increases and forms structural changes of hepatic sinusoidal capillarization together with collagen type IV. In non-diabetic patients with NAFLD, the changes of serum HA, C-IV and LN levels can preliminarily reflect the severity of liver fibrosis. Therefore, the detection of liver fibrosis indicators provides a good objective indicator for the prognosis of NAFLD. However, the changes of serum C-IV, HA and LN levels in patients with T2DM combined with NAFLD whether can be used to predict the transition from fatty liver to steatohepatitis cirrhosis are still need more studies. In our study, we found that compared with the healthy control group, ALT, AST, TC, TG, HDL and LDL in NAFLD group and T2DM patients with NAFLD increased in different degrees, and the difference was statistically significant (P<0.05). The HA, C-IV, LN, NFS, APRI ratio index and FIB-4 index of T2DM patients with NAFLD were higher than those of NAFLD group and healthy control group (P<0.05), and the HA, C-IV and LN of NAFLD group were higher than those of healthy control group (P<0.05). It is further confirmed that patients with type 2 diabetes mellitus complicated with NAFLD have more severe fatty liver and higher risk of developing cirrhosis and death. In addition, we found that compared with non-fibrosis group, HA, LN, C-IV, ALT and AST in fibrosis group increased significantly, with statistical significance (P<0.05). Moreover, HA, LN and C-IV were positively correlated with NFS, APRI ratio index and FIB-4 index. Zhu C et al.[6] they detect the levels of HA, LN, PIIINP and C-IV in 228 patients with clinically diagnosed cirrhosis and without malignant tumors. They found that the levels of HA, LN and CVI may be positively correlated with the severity of liver dysfunction. However, considering that the correlation between them is relatively weak, it needs further verification. In recent years, Mollii et al.[7] further study selected 1346 healthy people for physical examination, and 361 patients with fatty liver were definitely diagnosed by ultrasound. The results showed that the liver function indexes ALT, AST of fatty liver patients were higher than those of non-fatty liver patients, and the difference was statistically significant (P<0.05). The four serum fibrosis indexes HA, LN, PCIII, C-IV of fatty liver group were higher than those of non-fatty liver group, and the difference was unified. The correlation analysis between liver function and serum hepatic fibrosis indicators showed that ALT, AST and fibrosis indicators HA, LN, PCIII were positively correlated, but not with C-IV. It was considered that liver function and serum hepatic fibrosis indicators could be used as an important basis for monitoring and diagnosing the progress of fatty liver disease. Deng WL et al.[8] also considered that serum hepatic fibrosis markers (HA, C-IV, PC-III) combined with liver function examination and ultrasound examination had important clinical value in the early diagnosis and assessment of the severity of IHS. There are also some scholars[9-11] in the detection of serum HA, PIII, C-IV and LN levels in chronic hepatitis and analysis. The results showed that the levels of serum HA, PCXIII, C-IV and LN in patients with moderate, high and cirrhosis of chronic hepatitis were significantly higher than those.
in the control group (P<0.05), and increased with the aggravation of liver inflammation and fibrosis. Some researchers\cite{12,13} believe that the detection of HA, PCIII, IV-C and LN can better reflect the degree of liver fibrosis and liver parenchymal damage, and provide a basis for clinical diagnosis and treatment. These results are basically consistent with the results of this study.

The incidence of liver fibrosis in patients with T2DM combined with NAFLD is significantly higher than that in patients with NAFLD. In the past, liver puncture is often used to determine whether there is liver fibrosis. Therefore, ultrasound examination and early combined detection of HA, C-IV and LN are helpful for early detection of liver fibrosis, early diagnosis and early intervention.

To sum up, by detecting hepatic fibrosis indicators C-IV, HA and LN, we explore the clinical significance of these indicators in the process of transition from simple fatty liver to steatohepatitis and cirrhosis, thus find a new way of early diagnosis and clinical treatment for patients with T2DM combined with NAFLD. However, whether this method can also be used to evaluate liver fibrosis in other types of diabetes mellitus patients with NAFLD remains to be further studied.

References
