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Original Article

Associations of severity of fatty liver with oxidative stress, SAA, CRP and degree of cerebral arteriosclerosis in cerebral arteriosclerosis patients who have fatty liver

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Abstract: Objective: To analyze the associations of severity of fatty liver with oxidative stress, serum amyloid protein A (SAA), C-reactive protein (CRP) and degree of cerebral arteriosclerosis (CAS) in CAS patients with the complication of fatty liver, and to explore the predictive values of risk factors for these patients. Methods: A total of 200 patients diagnosed with CAS in our hospital from October 2016 to November 2018 were selected, including 90 cases with fatty liver (observation group) and 110 cases without fatty liver (control group), and there were 123 males and 77 females. The general clinical data, liver function, oxidative stress status, inflammatory factor levels, and degree of CAS were compared between the two groups, and their correlations and influencing factors were explored. Results: 1) There were no significant differences in the age, gender, and high-density lipoprotein (HDL) level between the observation group and control group ($P>0.05$). The body mass index (BMI), total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) levels in the observation group were significantly higher than those in the control group. 2) The levels of liver function indexes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT), in the observation group were significantly higher than those in the control group, all ($P<0.05$). 3) In terms of the oxidative stress, the level of malondialdehyde (MDA) in the observation group was higher than that in the control group, while the levels of superoxide dismutase (SOD) and glutathione (GSH) in the observation group were obviously lower than in the control group, all ($P<0.05$). 4) The levels of inflammatory factors, including SAA, CRP and interleukin-6 (IL-6), in the observation group were much higher than those in the control group ($P<0.05$). 5) Observation group had higher whole blood low shear viscosity, whole blood high shear viscosity, and plasma viscosity than the control group ($P<0.05$). 6) ALT ($r = 0.422$, $P = 0.000$) and SAA ($r = 0.828$, $P = 0.000$) had positive correlations with the plasma viscosity, while GSH ($r = -0.719$, $P<0.001$) had a negative correlation with the plasma viscosity. Conclusion: The liver function index levels, oxidative stress status, and inflammatory factor levels in CAS patients may affect the severity of arteriosclerosis and fatty liver.

Keywords: Cerebral arteriosclerosis, fatty liver, oxidative stress, inflammatory factors

Introduction

Our population is aging, and the morbidity rates of cerebral arteriosclerosis (CAS) and fatty liver are increasing due to genetic factors, environment, and changing social lifestyle [1]. In CAS patients, the elasticity of arterial walls declines compared with the normal arterial wall, and there is luminal stenosis, so they are prone to cerebral arterial occlusion or vascular rupture [2, 3]. The severity of CAS and fatty liver is closely related to dyslipidemia. Both CAS and fatty liver are chronic diseases, and simple fatty liver can develop into cirrhosis or further into liver cancer under no effective control [4]. The number of patients with nonalcoholic fatty liver has increased year by year, and its morbidity rate is higher in CAS patients than that in normal people [5]. Accumulating studies indicate that inflammatory factors and oxidative stress are involved in the occurrence and development of fatty liver. Among oxidative stress factors, malondialdehyde (MDA) is a kind of lipid peroxide, often used to evaluate the oxidative stress status in patients. Moreover, superoxide dismutase (SOD) and reduced glutathione (GSH) are also oxidative stress factors [6]. Serum amyloid protein A (SAA), a polymorphic protein formed by the same gene cluster, can also be synthesized by liver cells, which can reflect the inflammatory level in the body and has a correlation with the degree of atherosclerosis [7]. In the present study, the associations of the liver function, oxidative stress, and inflammatory factor levels with fatty liver in CAS patients were explored, so as to provide a reference for the treatment of CAS and fatty liver.

General data and methods

General data

A total of 200 patients definitely diagnosed with CAS in our hospital from October 2016 to November 2018 were selected, including 90 cases with fatty liver (observation group) and 110 cases without fatty liver (control group), and there were 123 males and 77 females aged 35-78 years old, with an average age of (57.73 ± 10.31) years old. All patients enrolled met the diagnostic criteria for *Cerebrovascular Disease Classification (1995)* revised by the Fourth National Conference on Cerebrovascular Disease, and they were definitely diagnosed with CAS by brain CT examination. The fatty liver patients were definitely diagnosed by color Doppler ultrasonography. Exclusion criteria: patients with severe liver or kidney diseases, autoimmune diseases, a history of thrombosis and tumor, acute or chronic infectious diseases, or incomplete clinical data. The study was approved by the ethics committee of Linzi District People's Hospital and informed consents were signed by the patients.

Methods

The clinical data of all patients, including age, gender, weight and height, were retrospectively analyzed, and the body mass index (BMI) was calculated. After fasting for solids and liquids for 10 h overnight, 10 mL fasting peripheral blood was drawn from all patients, and the upper-layer serum was taken to detect the biochemical indexes, including total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT), using a full-automatic biochemical analyzer (Hitachi). SAA, C-reactive protein (CRP) and interleukin-6 (IL-6) were detected in the same way as above by immunoturbidimetry and using reagents and instruments provided by Shandong Bio-Instruments Co., Ltd.

Determination of oxidative stress indexes: 5 mL fasting venous blood was drawn in the morning, placed in a pro-coagulation tube for 15 min, and centrifuged at 3000 rpm for 15 min. Then the serum was extracted and cryopreserved at -20°C to detect the oxidative stress indexes, including MDA, SOD and GSH, by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) in strict accordance with the instructions of the kits (R&D, USA). All specimens and kits were placed at room temperature for 0.5 h for equivalence before application.

Statistical methods

SPSS 19.0 software was used for data processing, and the data collected were expressed as ($\overline{X}$ ± s). Chi-square test was used for the comparison of enumeration data, correlation analysis was performed between two factors, and logistic analysis was adopted for the relevant risk factors. $P<0.05$ was considered significant.

Results

Comparison of general data between observation group and control group

There were no significant differences in the age, gender and HDL level between observation group and control group ($P>0.05$). The BMI, TC, TG and LDL levels in observation group were significantly higher than those in control group, and the differences were statistically significant (**Table 1**).

Comparisons of the levels of liver function indexes between observation group and control group

The levels of liver function indexes, including ALT, AST and GGT, in the observation group were significantly higher than in the control group, all ($P<0.05$) (**Table 2**).

Comparison of oxidative stress between observation group and control group

In terms of oxidative stress, the average level of MDA in the observation group was higher than in the control group, while the levels of SOD and GSH in observation group were obviously lower than in the control group , all ($P<0.05$) (**Table 3**).

Table 1. Comparison of general data between the observation group and control group

General data	Observation group (n = 90)	Control group (n = 110)	P
Age (years old)	58.09 ± 9.73	57.82 ± 8.56	0.623
Gender (male/female)	62/28	83/27	0.095
BMI (kg/m²)	25.46 ± 3.24	20.75 ± 2.96	0.033
TC (mmol/L)	6.91 ± 1.05	4.87 ± 1.09	0.047
TG (mmol/L)	1.99 ± 1.07	1.23 ± 0.73	0.038
LDL (mmol/L)	3.46 ± 0.93	2.75 ± 0.52	0.025
HDL (mmol/L)	1.54 ± 0.55	1.40 ± 0.31	0.078

Table 2. Comparison of the levels of liver function indexes between the observation group and control group

Liver function	Observation group (n = 90)	Control group (n = 110)	P
ALT	94.23 ± 19.65	42.66 ± 17.93	0.001
AST	85.72 ± 14.93	38.78 ± 13.76	0.001
GGT	97.66 ± 9.75	40.29 ± 10.94	0.001

Table 3. Comparison of oxidative stress between the observation group and control group

Oxidative stress	Observation group (n = 90)	Control group (n = 110)	P
MDA (mmol/mL)	6.25 ± 1.76	1.39 ± 0.45	0.000
SOD (U/mL)	109.29 ± 20.72	532.51 ± 49.77	0.000
GSH (mg/L)	25.42 ± 5.45	63.93 ± 9.72	0.000

Table 4. Comparison of inflammatory factor levels between the observation group and control group

Inflammatory factor	Observation group (n = 90)	Control group (n = 110)	P
SAA (mg/L)	36.68 ± 15.43	15.85 ± 6.33	0.001
CRP (mg/L)	12.53 ± 8.56	2.95 ± 1.82	0.001
IL-6 (pg/mL)	202.45 ± 25.71	78.42 ± 6.95	0.003

Comparison of inflammatory factor levels between observation group and control group

The levels of inflammatory factors, including SAA, CRP, and IL-6, in the observation group were markedly higher than those in control group, all (P<0.05) (**Table 4**).

Comparison of degree of arteriosclerosis degree between the observation and control groups

The observation group had higher whole blood low shear viscosity, whole blood high shear viscosity, and plasma viscosity than control group, all (P<0.05) (**Table 5**).

Correlations of liver function, inflammatory factors, and oxidative stress with degree of arteriosclerosis

ALT (r = 0.422, P = 0.000) and SAA (r = 0.828, P = 0.000) had positive correlations with the plasma viscosity, while GSH (r = -0.719, P<0.001) had a negative correlation with the plasma viscosity (**Figures 1-3**).

Discussion

With the increase of population size and aging of society, the incidence of CAS is increasing. Cerebral hemorrhage in the elderly is often complicated by CAS [7]. The mortality rate of patients with cerebral hemorrhage is high, thus it has attracted much attention. However, there has been no better treatment method for cerebral hemorrhage in elderly patients, so it is of great importance to prevent CAS before cerebral hemorrhage [8]. CAS mainly occurs in large arteries, such as themcerebral arteries, aorta, and coronary arteries, and often involves other organs in the body [9]. The main cause of CAS is the abnormal level of blood lipids in the body and the attachment of cholesterol to the inner arterial wall, leading to arterial intimal thickening and plaque formation, and luminal stenosis [10]. At the same time, an abnormal level of blood lipids is closely related to the occurrence and development of fatty liver, and the morbidity rate of fatty liver is also gradually increasing due to changes in diet and decline in exercise [11]. A fatty liver can exist independently or interact with other diseases. The levels of blood lipid and cholesterol in fatty liver patients are significantly higher than those in people without fatty liver [12]. Studies have found that an increased level of SAA is a risk factor for fatty liver [13]. SAA is a polymorphic protein that can affect the metabolism of HDL in the body to a certain extent, and HDL possesses an anti-atherosclerotic effect, so the SAA level will be abnormally increased in patients with CAS and fatty liver [14]. Also, CRP and

Table 5. Comparison of degree of arteriosclerosis between the observation group and control group

Index	Observation group (n = 90)	Control group (n = 110)	*P*
Whole blood low shear viscosity (mPa.s)	10.35 ± 2.84	7.62 ± 1.83	0.001
Whole blood high shear viscosity (mPa.s)	6.65 ± 1.38	4.36 ± 1.27	0.001
Plasma viscosity (mPa.s)	2.13 ± 0.58	1.59 ± 0.38	0.001

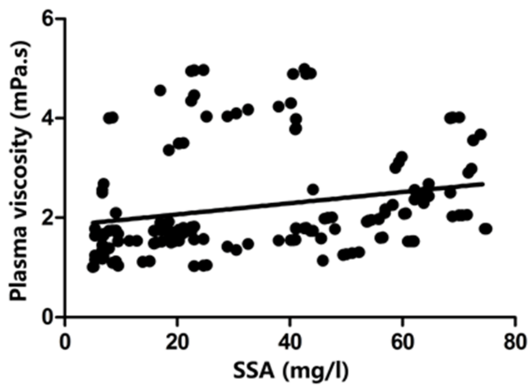

Figure 1. Correlation between SAA and plasma viscosity.

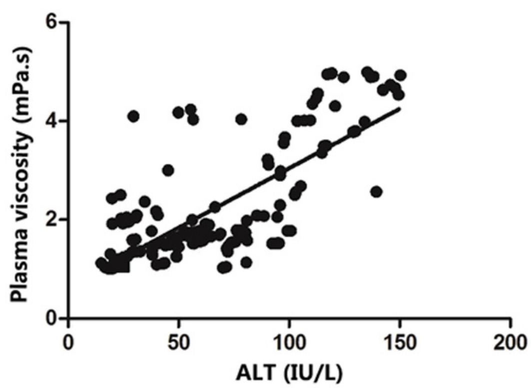

Figure 2. Correlation between ALT and plasma viscosity.

IL-6 are factors produced by inflammatory cells, that can aggravate the in vivo inflammatory response, leading to the accumulation of inflammatory cells in the body and promoting the release of inflammatory mediators [15]. The occurrence and development of fatty liver is also an inflammatory response process. In the present study, it was found that the levels of SAA, CRP, and IL-6 in CAS patients complicated by fatty liver were increased and significantly positively correlated with the plasma viscosity.

The final product of oxidative stress in the human body is MDA, which has cytotoxicity and

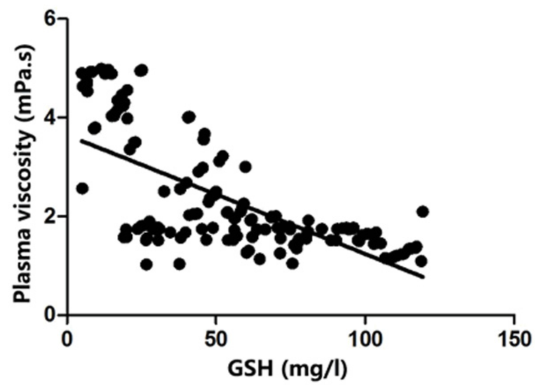

Figure 3. Correlation between GSH and plasma viscosity.

hidden carcinogenic properties, and an abnormal level of MDA can lead to abnormal apoptosis in vivo [16]. MDA is formed by excessive free radical oxidative stress in extracellular membrane lipids, which can result in mitochondrial enzyme metabolic disorders and damage liver cell function [17]. SOD is an active protease containing metal elements, which decreases superoxide radicals in the body to reduce harmful oxygen free radicals, thereby keeping a normal oxidative stress status [17]. Many studies have demonstrated that the level of SOD in fatty liver patients significantly declines, indicating that SOD can inhibit the oxidative stress and abnormal inflammatory response caused by excessive accumulation of fat in the body, and can also protect liver cells [18]. GSH, a polypeptide, has widespread effects and plays an important role in the tricarboxylic acid cycle and glucose metabolism in the body [19], which can not only provide enough energy, but also activates various peptides involved in the redox reaction. Moreover, GSH can bind to oxygen free radicals and protect the sulphydryl group in the cell membrane, thereby resisting the damage of other factors on organs [20].

Conclusion

It was found that the level of MDA in CAS patients complicated by fatty liver was significantly higher than that in patients without fatty liver, while the levels of SOD and GSH were lower than in those without fatty liver. This suggests that the oxidative stress status and inflammatory factor levels in CAS patients are closely associated with fatty liver.

Disclosure of conflict of interest

None.

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