RELATIONSHIP BETWEEN SERUM PTX3, OPN, AND RESISTIN LEVELS AND THE SEVERITY OF STEATOSIS AND RELATED FIBROSIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Objective: *To analyze the relationship between serum pentameric protein 3 (PTX3), osteopontin (OPN), and resistin levels and the severity of steatosis and related fibrosis in patients with nonalcoholic fatty liver disease (NAFLD).*

Methods: *A total of 147 patients with NAFLD admitted to our hospital from January 2019 to January 2020 were enrolled in the observation group and divided into a mild group (n = 52), a moderate group (n = 49), and a severe group (n = 46) according to the number of fatty liver lesions found in histopathological examinations. Forty healthy individuals who received health checkups at our hospital during the same period were recruited in the control group. In the morning within 24 hours of being admitted to the study, 10-ml fasting blood samples were drawn from each participant. The levels of serum PTX3, OPN, and resistin of each participant were detected using enzyme-linked immunosorbent assay, and the levels of NAFLA fibrosis indicators such as procollagen III (PC III), hyaluronic acid (HA), type IV collagen (PC IV), and laminin (LN) were determined using enzyme-linked immunosorbent assay. The correlation of serum PTX3, OPN, and resistin levels with NAFLD-related fibrosis was analyzed using Spearman rank correlation, and the risk factors of steatosis in patients with NAFLD were investigated using logistic regression analysis.*

Results: *In the observation group, the levels of OPN and resistin were significantly higher than those in the control group, but the PTX3 levels were significantly lower; in the moderate and severe groups, the levels of OPN and resistin were significantly higher than those in the mild group, but the PTX3 levels were significantly lower; and in the severe group, the levels of OPN and resistin were significantly higher than those in the moderate group, but the PTX3 levels were significantly lower, and the difference was statistically significant ($P<0.05$). Regarding the PC III, HA, PC IV, and LN levels, in the observation group, they were significantly higher than those in the control group; in the moderate and severe groups, they were significantly higher than those in the mild group; and in the severe group, they were significantly higher than those in the moderate group, and the difference was statistically significant ($P < 0.05$). According to the Spearman analysis, the levels of PC III, HA, PC IV, and LN were negatively correlated with PTX3 ($r = -0.482$, -0.516, -0.471, and -0.519, respectively; $P<0.05$ or <0.01) and positively correlated with OPN ($r = 0.584$, 0.463, 0.491, and 0.516, respectively; $P<0.05$ or <0.01) and resistin ($r = 0.425$, 0.468, 0.512, and 0.472, respectively; $P<0.05$ or <0.01). The findings of the logistic regression analysis show that PTX3 is an independent protective factor affecting steatosis in patients with NAFLD ($P<0.05$) and that OPN and resistin are PTX3 independent risk factors affecting steatosis in patients with NAFLD ($P<0.05$).*

Conclusions: *The levels of PTX3, OPN, and resistin in the serum of patients with NAFLD are abnormally expressed and change according to the severity of steatosis. They have a certain correlation with the related fibrosis indicators in patients with NAFLD, which may support the diagnosis and evaluation of the disease.*

Keywords: *Serum, PTX3, OPN, resistin, nonalcoholic fatty liver disease, degree of steatosis, fibrosis.*

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Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to the clinical-pathological syndrome that is mainly characterized by excessive fat deposition in liver cells caused not by alcohol but by other confirmed risk factors of liver damage. It is an acquired metabolic stress-induced liver injury related to insulin resistance and genetic susceptibility[(1)]. Patients may experience fatigue, dyspepsia, dull pain in the liver area, hepatosplenomegaly, overweight, visceral obesity, increased fasting blood glucose, dyslipidemia, hypertension, etc[(2)]. In recent years, as people's living standards have improved and as life has become more fast paced, obesity, diabetes, and metabolic syndrome have become more prevalent

worldwide. The incidence of NAFLD, which is one of the important causes of chronic liver disease, is increasing year by year, and some patients develop life-threatening liver fibrosis as a result[3]. Pentameric protein 3 (PTX3), a long-chain pentamer protein, is a new type of reflecting factor of local inflammation and injury[4]. Recent studies have found that PTX3 is associated with insulin resistance and type 2 diabetes, and the former is one of the important factors that induce NAFLD[5].

Osteopontin (OPN), a secreted phosphorylated glycoprotein, can participate in many of the body's pathological processes[6]. In recent years, studies have found that OPN plays a role in the development of cirrhosis[7]. Moreover, resistin, a white fat cell-derived peptide hormone, has been found to be abnormally expressed in patients with NAFLD and thus may be related to the development of NAFLD[8]. In this study, we recruited patients with NAFLA admitted to our hospital from January 2019 to January 2020, aiming to analyze the relationship between PTX3, OPN, and resistin levels and the severity of steatosis and related fibrosis in patients with NAFLD.

Materials and methods

General materials

A total of 147 cases with NAFLD admitted to our hospital from January 2019 to January 2020 were enrolled in the observation group, and the inclusion criteria were as follows:

• All patients were in accordance with the diagnostic criteria for NAFLD presented in an article titled "Guidelines of prevention and treatment for nonalcoholic fatty liver disease: a 2018 update" by the National Workshop on Fatty Liver and Alcoholic Liver Disease of the Chinese Society of Hepatology[9];

• All patients' B-mode ultrasonography showed that the right hepatic capsule and diaphragm echo were unclear, that the near-field echo of the liver area was diffusely enhanced, that the liver echo >kidney and spleen echo, a mild or moderate enlargement of the liver, round stewed edges, and an obscure internal hepatic vein structure;

• All patients and their families signed an informed consent form.

Exclusion criteria:

• Female and male patients with a daily alcohol intake ≥40 g and ≥80 g, respectively, for five or more years;

• Patients with viral hepatitis, autoimmune hepatitis, and various cirrhosis;

• Patients with serious dysfunction of major organs such as the heart, liver, and kidney;

• Patients with fatty liver due to other reasons, such as drug use;

• Patients with genetic diseases such as hereditary hemochromatosis and hepatolenticular degeneration;

• Patients who declined to take part in or withdrew from the experiment.

There were 147 cases in the observation group (75 men and 72 women) with an average age of 45.15±5.10 years and a mean body mass index (BMI) of 21.12±1.45 kg/m^2. According to the severity of fatty liver lesions in the histopathological examination of each patient, the observation group was divided into a mild group (containing 5-10 fat or ⅓-⅔ of liver cell steatosis per unit area), a moderate group (containing 10-25 fat or more than ⅔ of liver cell steatosis), and a severe group (containing 25-50 fat or more than ½, or almost whole, liver cell steatosis). There were 52 cases in the mild group (27 men and 25 women) with an average age of 45.33±5.15 years and an average BMI of 21.15±1.48 kg/m^2. There were 49 cases in the moderate group (25 men and 24 women) with an average age of 45.10±5.15 years and an average BMI of 21.16±1.52 kg/m^2. There were 46 cases in the severe group (23 men and 23 women) with an average age of 45.02±5.19 years and an average BMI of 21.10±1.42 kg/m^2.

In the same period, the healthy individuals who received health checkups at our hospital were recruited in the control group.

Inclusion criteria:

• No serious dysfunction of major organs such as the heart, liver, and kidney;

• No long-term drinking history;

• Patients who signed an informed consent form.

There were 40 cases in the control group (20 men and 20 women) with an average age of 45.18±5.15 years and an average BMI of 21.21±1.43 kg/m^2. There was no statistically significant difference in the data related to age, gender, and BMI among the participants in each group (P>0.05).

Observation indicators

In the morning within 24 hours of admission, 10-ml of fasting blood was drawn from all patients, and 10-ml of fasting blood was drawn from the healthy participants during the health checkup. The blood samples were centrifuged at 3,000 r/min for

15 min, and the serum was carefully separated and stored in a refrigerator at -80°C. The levels of PTX3, OPN, and resistin in the serum of each participant were detected using enzyme-linked immunosorbent assay, and the levels of NAFLA fibrosis indicators such as procollagen III (PC III), hyaluronic acid (HA), type IV collagen (PC IV), and laminin (LN) were determined using enzyme-linked immunosorbent assay.

Statistical analysis

The data of this experiment were analyzed by software SPSS20.0. All measurement data were represented by ($\bar{x}\pm s$), and a t-test was used to compare the two groups; the enumeration data were expressed in percentages and analyzed using a χ^2 test. The correlation of PTX3, OPN, and resistin levels with NAFLD-related fibrosis was analyzed by Spearman rank correlation, and the risk factors of steatosis in patients with NAFLD were investigated using logistic regression analysis. The statistical results were statistically significant when P<0.05.

Results

Comparison of PTX3, OPN, and resistin levels of the participants in each group

In the observation group, the levels of OPN and resistin were significantly higher than those in the control group, but the PTX3 levels were significantly lower; in the moderate and severe groups, the levels of OPN and resistin were significantly higher than those in the mild group, but the PTX3 levels were significantly lower; and in the severe group, the levels of OPN and resistin were significantly higher than those in the moderate group, but the PTX3 levels were significantly lower, and the difference was statistically significant (P<0.05). See Table 1.

Group	n	PTX3 (ng/ml)	OPN (μg/L)	Resistin (mg/L)
Observation group	147	0.82±0.21[a]	63.18±5.16[a]	25.16±4.25[a]
Mild group	52	0.98±0.15	46.29±4.85	23.15±3.85
Moderate group	49	0.81±0.21[b]	61.38±4.12[b]	25.10±4.16[b]
Severe group	46	0.67±0.24[bc]	85.14±6.32[bc]	27.15±4.85[bc]
Control group	40	1.86±0.25	32.16±3.10	19.75±4.26

Table 1: Comparison of PTX3, OPN, and resistin levels of the participants in each group ($\bar{x}\pm s$).
Note: a was compared with the control group, [a]P<0.05; b was compared with the control group, [b]P<0.05; c was compared with the control group, [c]P<0.05.

Comparison of PC III, HA, PC IV, and LN levels of the participants in each group

Regarding the PC III, HA, PC IV, and LN levels, in the observation group, they were significantly higher than those in the control group; in the moderate and severe groups, they were significantly higher than those in the mild group; and in the severe group, they were was significantly higher than those in the moderate group, and the difference was statistically significant (P<0.05). See Table 2.

Group	n	PC III (μg/L)	HA (μg/L)	PC IV (μg/L)	LN (μg/L)
Observation group	147	138.76±28.43[a]	92.46±28.43[a]	73.25±26.59[a]	128.34±33.15[a]
Mild group	52	116.49±5.79	85.12±6.18	58.20±5.61	120.85±5.12
Moderate group	49	135.49±4.76[b]	93.46±5.19[b]	72.51±6.35[b]	130.45±6.83[b]
Severe group	46	152.16±5.75[bc]	108.34±7.21[bc]	82.43±7.49[bc]	145.87±7.52[bc]
Control group	40	95.76±23.15	75.29±21.05	57.34±18.42	98.26±21.41

Table 2: Comparison of PC III, HA, PC IV, and LN levels of the participants in each group ($\bar{x}\pm s$).
Note: a was compared with the control group, [a]P<0.05; b was compared with the control group, [b]P<0.05; c was compared with the control group, [c]P<0.05.

Correlation analysis of PTX3, OPN, and resistin levels and fibrosis indicators in patients with NAFLD

According to the Spearman analysis, the PC III, HA, PC IV, and LN levels were negatively correlated with PTX3 (r = -0.482, -0.516, -0.471, and -0.519, respectively; P<0.05 or <0.01) and positively correlated with OPN (r=0.584, 0.463, 0.491, and 0.516, respectively; P<0.05 or <0.01) and resistin (r=0.425, 0.468, 0.512, 0.472, P<0.05 or <0.01). See Table 3.

Indicators		PC III	HA	PC IV	LN
PTX3	*r*	-0.482	-0.516	-0.471	-0.519
	P	<0.001	0.021	0.025	0.023
OPN	*r*	0.584	0.463	0.491	0.516
	P	<0.001	0.024	<0.001	0.002
Resistin	*r*	0.425	0.468	0.512	0.472
	P	0.035	0.012	<0.001	0.031

Table 3: Correlation analysis of PTX3, OPN, and resistin levels and fibrosis indicators in patients with NAFLD.

Analysis of steatosis risk factors in patients with NAFLD

The findings of the logistic regression analysis show that PTX3 is an independent protective factor affecting steatosis in patients with NAFLD (P<0.05),

and OPN and resistin are PTX3 independent risk factors affecting steatosis in patients with NAFLD (P<0.05). See Table 4.

Indicators	β	*SE*	*P*-value	*95%CI*
PTX3	-0.328	0.253	0.016	0.242-0.648
OPN	0.622	0.409	0.002	1.032-1.799
Resistin	0.729	0.042	0.012	1.131-2.022

Table 4: Analysis of steatosis risk factors in patients with NAFLD.

Discussion

NAFLD is a disease commonly encountered in clinical practice. In recent years, studies have found that the disease seems to undergo a rejuvenation trend, which seriously threatens the health of patients[10]. Previous studies have shown that the incidence of NAFLD is related to a variety of factors, including inflammation, insulin resistance, and lipid metabolism. Adipose tissue, an important endocrine organ, can secrete various cytokines and thus plays a role in the pathogenesis and development of NAFLD[11]. PTX3 is a fat factor in the human body that is mainly expressed in adipose tissue and embryos[12]. Studies have shown that low PTX3 expression is closely associated with fat storage. Moreover, it is a crucial indicator of inflammation. PTX3 is also involved in immune defense and the development of atherosclerosis and has various biological effects, such as protection and inflammatory injury. Recent studies have found that OPN is closely related to the development of fibrosis[13]. Studies have shown that, in a liver fibrosis mouse model, high OPN expression in liver tissue can directly up-regulate the expression of transforming growth factor-β mRNA, induce the activation of hepatic stellate cells, and result in liver fibrosis. Moreover, OPN can promote the proliferation of hepatic stellate cells by up-regulating the downstream signaling molecule extracellular regulatory protein kinase 1 in the synaptic signaling pathway, thereby aggravating the degree of liver fibrosis. In recent years, studies have found that OPN originates in macrophages in adipose tissue and has a certain relationship with metabolic liver disease[14].

Resistin is a white fat cell-derived peptide hormone that was discovered in 2001. Animal experiments have shown that resistin is highly expressed in the fat tissue of obese mice and is increased in serum to varying degrees[15]. However, the relationship between resistin and liver fibrosis is not completely clear. In this study, we found that, in the observation group, the levels of OPN and resistin were significantly higher than those in the control group, but the PTX3 levels were significantly lower; in the moderate and severe groups, the levels of OPN and resistin were significantly higher than those in the mild group, but the PTX3 levels were significantly lower; and in the severe group, the levels of OPN and resistin were significantly higher than those in the moderate group, but the PTX3 levels were significantly lower, and the difference was statistically significant (P<0.05). These results indicate that PTX3, OPN, and resistin are abnormally expressed in the serum of patients with NAFLD, and they change based on the severity of steatosis, which may be related to the development of NAFLD.

To further analyze the relationship between PTX3, OPN, and resistin levels and the severity of steatosis and related fibrosis in patients with NAFLD, we conducted Spearman rank correlation in this study, the findings of which showed that PC III, HA, PCIV, and LN levels were negatively correlated with PTX3 and positively correlated with OPN and resistin. The findings of the logistic regression analysis show that PTX3 is an independent protective factor affecting steatosis in patients with NAFLD (P<0.05) and that OPN and resistin are PTX3 independent risk factors affecting steatosis in patients with NAFLD (P<0.05). This suggests that PTX3, OPN, and resistin levels are closely related to the severity of steatosis and the related fibrosis of patients with NAFLD.

In summary, the levels of PTX3, OPN, and resistin in the serum of patients with NAFLD are abnormally expressed and vary according to the severity of steatosis in patients. They are correlated with the related fibrosis indicators of patients with NAFLD and may provide support for the diagnosis and assessment of NAFLD.

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