Research Paper

Relationship Between Immune Parameters and Non-alcoholic Fatty Liver Disease in Obese Children

JUN-QI SHI, WEN-XIA SHEN, XIANG-ZHI WANG, KE HUANG AND CHAO-CHUN ZOU

Shi JQ and Shen WX contributed equally to this article.

From Department of Endocrinology, Children's Hospital, Zhejiang University School of Medicine; Zhejiang Province; China.

Correspondence to: *Dr Chao-Chun Zou, Children's Hospital, Zhejiang University School of Medicine, Binsheng Road, Hangzhou 310051, Zhejiang Province, China. zcc14@zju.edu.cn*

PII: S097475591600073

ABSTRACT

Objective: To investigate the relationship between immune parameters and non-alcoholic fatty liver disease (NAFLD) in obese children.

Design: Case-control study.

Setting: Zhejiang Province, China on July to September 2015.

Participants: A total of 117 obese children and 209 healthy non-obese children were studied as the obese and control groups. Depending on the severity of NAFLD, the obese group was divided into subgroups 1 (without NAFLD), 2 (with simple fatty liver) and 3 (with steatohepatitis).

Main Outcome Measures: Glucose metabolism, lipid metabolism and immune parameters. **Results:** In the obese group, body mass index (BMI), waist-and hip-circumferences, fasting insulin, Homeostasis model of assessment for insulin resistance (HOMA-IR), triglyceride, total cholesterol, low density lipoprotein cholesterol (LDL-C), apolipoprotein (Apo)B/ApoA1, alanine aminotransferase, uric acid, white blood cells, neutrophils percentage, platelet and interleukin (IL)-6 were significantly higher than those in the controls ($P<0.05$), while lower high density lipoprotein cholesterol and lymphocyte percentage were noted ($P<0.05$). IL-10 in the subgroup 3 was higher than those in the control group, subgroup 1 and 2 ($P<0.05$). Logistic regression analysis showed that BMI, LDL-C, HOMA-IR and IL-10 were independent factors of NAFLD ($P<0.05$).

Conclusion: These results support a low-grade chronic inflammation in obese children. Moreover, obesity, dyslipidaemia and IR are risk factors while IL-10 may be a protective factor for NAFLD.

Keywords: *Cytokine, Interleukin-6, Interleukin-10, Metabolic syndrome, Overweight*

Non-alcoholic fatty liver disease (NAFLD) is a severe complication of obesity. It is a kind of hepatic steatosis, inflammation and fibrosis, which is characterized by fatty infiltration in over 5% hepatocytes without a history of alcohol intake, viral infections, autoimmune hepatitis or drug-induced liver disease. According to pathologic changes and liver function, NAFLD is divided into simple fatty liver (SFL), non-alcoholic steatohepatitis (NASH) and cirrhosis [1]. With the increase of childhood obesity, the incidence of NAFLD has increased recently. Studies on NAFLD have suggested that free fatty acid, triacylglycerol and insulin resistance (IR) were involved in pathogenesis of NAFLD [2,3]. However, the pathogenesis of NAFLD is not fully understood till now.

Recently, multitude evidence has revealed that obesity was a low-grade chronic inflammation process. Interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α) may contribute to inflammation [4-6]. TNF-α was reported to be closely related to IR. In addition, some cytokines may play a protective role in the progression of NAFLD, such as IL-4 and IL-10 [7]. However, studies on the cytokines and NAFLD in children were rare. We studied the relationship between immune parameters and NAFLD to explore the risk and protective factors for NAFLD.

METHODS

A total of 117 obese children (81 males), were studied as the obese group aged between 5.2 to 14.8 years (mean (SD) age, 10.8 (2.2)y). They were hospitalized in the Endocrinlogy Department of Children's Hospital, Zhejiang University School of Medicine during July to September 2015 to definite etiology and investigate the complications of obesity, such as disorder of glucose metabolism or lipid metabolism disorders. Their body mass index (BMI) ranged from 22.2 to 42.2 kg/m^2 (mean (SD), 28.5 (3.76) kg/m^2). Children with viral hepatitis, drug-induced hepatitis, autoimmune liver disease, infection, severe heart and kidney disease, or alcohol consumption were excluded.

Another 209 non-obese healthy children aged 6-14 year (mean (SD) age, 10.3 (1.8)y, 149 males) from Children Health Care Department in our hospital were enrolled as the control group. Their BMI ranged from 11.6 to 20.3 kg/m^2 (mean (SD) BMI, 15.6 (1.86) kg/m^2). The age difference between the two group was significantly different (P=0.03) (***Table* I**).

Depending on the severity of NAFLD, the obese group was divided into three subgroups: *Subgroup* 1 (Obesity without NAFLD, N=23), *Subgroup* 2 (Obesity with SFL, N=43) and *Subgroup* 3 (Obesity with NASH, N=51). Subgroup 1 (mean age, 9.8 (2.5)y, 14 males) had BMI from 22.4 to 32.0 kg/m^2 (mean BMI, 26.1 (2.5) kg/m^2). Subgroup 2 (mean age, 10.8 (2.2)y, 30 males) had BMI from 22.2 to 34.9 kg/m^2 (mean BMI, 28.2 (3.1) kg/m^2). Subgroup 3 (mean age, 11.3 (1.9) y, 37 males) had BMI from 22.5 to 42.2 kg/m^2 a (mean BMI, 29.7 (4.2) kg/m^2). The difference of age between the groups was statistically significant (P=0.003).

This study was aproved by the Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine. Informed consent was obtained from the parents of these children.

Obesity was diagnosed according to the criteria that BMI was above 95th percentile compared with the same age and sex children (Height and weight standardized growth charts for Chinese children and adolescents aged 0 to 18 years) [8]. NAFLD was diagnosed according to the revised definition and treatment guidelines for NAFLD by the Chinese Hepatology Association in 2010 [9]. NAFLD was diagnosed as a diffusely echogenic change on liver B-mode ultrasonography (fatty infiltration in liver), with or without elevated liver enzyme (alanine aminotransferase and aspartate aminotransferase). SFL was defined as a diffusely echogenic change with normal liver enzyme while NASH was defined as a diffusely echogenic change with elevated liver enzyme.

Anthropometric indicators were measured by professionals with unified calibrated measurement tools. Body height was measured to the nearest 0.1 cm, after subjects took off their shoes, standing on the measuring instrument. Body weight was measured to the nearest 0.1 kg on a scale. Waist circumference was measured to the nearest 0.1 cm by measuring at the midpoint between the lower rib margin and the iliac crest using a conventional tape in centimeters. Hip circumference was measured to the nearest 0.1 cm by measuring maximum hip circumference. BMI was calculated as weight (kg) divided by height (m) squared.

Blood samples were taken after overnight fasting and placed into tubes at room temperature. Then serum samples were isolated and stored at -70°C until use. Fasting blood glucose, insulin, triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipoprotein A, uric acid, apolipoprotein (Apo) A1 and ApoB were measured in our clinical laboratory center. IR was individually estimated by the homeostasis model of assessment for IR (HOMA-IR) by considering as (fasting insulin×fasting glucose)/22.5.

Serum Th1/Th2 cytokines were measured by flow cytometry. IL-2, IL-4, IL-6, IL-10, TNF-α and interferon (IFN)–γ were quantitatively determined by the CBA kit-BD^{TM} CBA Human Th1/Th2 Cytokine Kit II (BD Biosciences, San Jose, CA). Peripheral blood cell count, including white blood cells, percentage of neutrophils, percentage of lymphocytes and platelet, were measured in our clinical laboratory center.

Statistical analysis: Statistical analyses were conducted by using SPSS software (SPSS Inc. Chicago, USA). The Pearson Chi-square test was used to analyze the difference of categorical data between different groups. Quantitative data with normal distribution were analyzed by the independent t test or One-way ANOVA followed by LSD test when appropriate. Fasting insulin, HOMA-IR, triglyceride, lipoprotein A, ApoB/ApoA1, IL-2, IL-4, IL-6 and TNF-α were analyzed after logarithmic

transformation. Skewness data cannot be converted to normally distributed data were compared with Mann-Whitney U test. Logistic regression analysis was performed to analyze the independent factors of NAFLD (assignment: control group = 1, subgroup 1 = 2, subgroup 2 = 3, subgroup 3 =4). Factors with $P<0.3$ in the comparison between obese and control groups were enrolled in the logistic regression analysis. The differences were considered significant at $P<0.05$.

RESULTS

Compared to the control group, higher BMI, waist circumference, hip circumference, fasting insulin, HOMA-IR, triglyceride, cholesterol, LDL-C, ApoB/ApoA1, ALT and uric acid, and lower HDL-C were found in the obese group ($P<0.05$, respectively). However, fasting glucose, lipoprotein A and AST were not significantly different between the obese and control groups ($P>0.05$, respectively) (***Table* I**). Among the 117 obese children, NAFLD was found in 94 (80.3%), including 43 (36.7%) in subgroup 2 and 51 (43.6%) in subgroup 3. Comparing the control and obese subgroups, BMI, waist circumference, hip circumference, fasting insulin, HOMA-IR, triglyceride, total cholesterol, LDL-C, HDL-C, ApoB/ApoA1, uric acid and ALT were significantly different ($P<0.05$, respectively), although fasting blood glucose and lipoprotein A were not different among these groups ($P>0.05$, respectively). BMI, waist circumference, hip circumference, fasting insulin, HOMA-IR, triglyceride, LDL-C, ALT and uric acid have increased tendency while HDL-C have decreased tendency among the control group and subgroups of obese group.

Higher white blood cells, percentage of neutrophils, platelet and IL-6, and lower percentage of lymphocytes in the obese group were noted ($P<0.05$). The difference of IL-10 was marginal between the control and obese groups ($P=0.07$). IL-2, IL-4, TNF-α and INF-γ were not significantly different between the obese and control groups ($P>0.05$) (***Table* II**). Moreover, white blood cells and platelet in the 3 subgroups were higher than those in the control group ($P<0.05$).

Logistic regression analysis showed that BMI, LDL-C, HOMA-IR and IL-10 were independent determinants for NAFLD ($P<0.001$, $P=0.024$, $P=0.009$ and $P=0.024$) (*Web* ***Table* I**).

DISCUSSION

In this study, 125 obese children were screened and 8 children were excluded as hepatitis B and Prader-Willi syndrome. Also, 221 non-obese children were screened and 12 children were excluded as hepatitis B, atrial septal defect and hypothyroidism. A total of 117 obese children and 209 non-obese children were enrolled in the obese and control groups in the following analysis. We found NAFLD in 94 (80.3%) of the 117 obese children. Logistic analysis revealed that BMI was the most significant independent determinant of NAFLD in these children, which confirmed that obesity was a main risk factor for NAFLD in children [10,11].

There were several limitations in our study. Firstly, the study was not a prospective study, but a

cross-sectional analysis. Secondly, NAFLD, SFL or NASH was diagnosed only by ultrasonic and serum liver enzymes without pathological biopsy of the liver. Thirdly, the sample size in our study was small. Also, we did not investigate the accurate role of IL-10 on the NAFLD.

In our study, we noted higher white blood cells and neutrophils percentages in obese children, which were consistent with studies from adults and mice [12-14]. Moreover, higher levels of IL-6 were noted in obese children, which suggested that IL-6 might play a role in obesity. Although the white blood cells, neutrophils, platelet and IL-6 were not independent factor by the logistic analysis, these data supported systemic low-grade inflammation in obese children and white blood cells may be a cause of increased production of cytokines [15].

Several studies in adults or animal model have found that imbalance between pro- and anti-inflammatory cytokines was involved in the second hit of NAFLD [16]. In this study, we noted that IL-10 and IL-6 levels increased with the severity of NAFLD in obese children. Several studies have reported that IL-10 played a role as a protective factor for liver injury induced by alcoholic, hepatitis C and liver steatosis [17]. Also, animal model studies suggested protective effect of IL-10 against liver steatosis, fibrosis and other metabolic disorders [18,19]. Our results seem to be contrary against the above studies, but consistent with a human study, which found that the IL-10 mRNA was higher in obese patients with NAFLD, and especially in obese patients with NASH [20]. Also, higher IL-10 levels were noted in mice after long-term high fat diet fed, and IL-$10^{-/-}$ mice displayed greater liver inflammatory response after high fat diet feeding [21]. These implied that IL-10 is a protective factor, which may increase to compensate inflammation and NAFLD in obesity.

The effect of IL-6 on the NAFLD reported from different studies was seen to be contradictory. It was reported to play a protective effect in early stage of hepatic steatosis by inhibiting oxidative stress and preventing mitochondrial dysfunction [16]. However, it was reported to mediate inflammatory of hepatocyte, induce apoptosis of hepatocytes and IR in the subsequent progress of fatty liver [22,23]. Animal model found that hepatocytes exposed to exogenous IL-6 accelerated self-repairing in short-term, but aggravated injury in long-term [24]. Another one showed no relationship between IL-6 and NASH [25]. Further studies about the effect of IL-6 on childhood NAFLD is required.

In summary, our data support low-grade chronic inflammation in obese children. Moreover, obesity, dyslipidaemia and IR are risk factors of NAFLD in children. IL-10 may be a protective factor for liver, which increased compensatory in children with NAFLD.

Contributors : CC Zou: concept and design; JQ Shi, WX Shen: data collection and analysis; JQ Shi: drafting the manuscript; XZ Wang, K Huang: help in patient organization and data collection.

Funding : This work is supported, in part, by National Natural Science Foundation of China (81170787 & 81670786), Zhejiang Health and Family Planning Commission (2016KYA128) and Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents (2015).

Competing interest: None stated.

***Acknowledgments*:** Guan-Ping Dong, Wei Wu, and Li Zhang for their patient-care and organization.

WHAT IS ALREADY KNOWN?

- The roles of cytokines in NAFLD in obese children is not well-elucidated

WHAT THIS STUDY ADDS?

- Obesity, dyslipidemia and insulin-resistance are risk factors of non-alcoholic fatty liver disease in children.

REFERENCES

1. Yki-Jarvinen H. Diagnosis of non-alcoholic fatty liver disease (NAFLD). Diabetologia. 2016;59:1104-11.
2. Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). Int J Mol Sci. 2013;14:20704-28.
3. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. Atherosclerosis. 2015; 239:192-202.
4. Boga S, Alkim H, Koksal AR, Bayram M, Ozguven MB, Ergun M, *et al*. Increased plasma levels of asymmetric simethylarginine in nonalcoholic fatty liver disease: Relation with insulin resistance, inflammation, and liver histology. J Investig Med. 2015;63:871-7.
5. Rodriguez-Ramos C, Galan F, Diaz F, Elvira J, Martin-Herrera L, Giron-Gonzalez JA. Expression of proinflammatory cytokines and their inhibitors during the course of spontaneous bacterial peritonitis. Dig Dis Sci. 2001;46:1668-76.
6. Jia G, Di F, Wang Q, Shao J, Gao L, Wang L, *et al*. Non-Alcoholic fatty liver disease is a risk factor for the development of diabetic nephropathy in patients with type 2 diabetes mellitus. PLoS ONE. 2015; 10: e0142808.
7. Das SK, Balakrishnan V. Role of cytokines in the pathogenesis of non-alcoholic fatty liver disease. Indian J Clin Biochem. 2011;26:202-9.
8. Li H, JI CY. Height and weight standardized growth charts for chinese children and adolescents aged 0 to 18 years. Chin J Pediatr. 2009;47:487-92.
9. The Chinese National Workshop on Fatty Liver and Alcoholic Liver Disease for the Chinese Liver Disease Association. Guidelines for Management of Nonalcoholic Fatty Liver Disease:An Updated and Revised Edition. Chin J Hepatol. 2010;18:163-6.
10. Mencin AA, Lavine JE. Nonalcoholic fatty liver disease in children. Curr Opin Clin Nutr Metab Care. 2011;14:151-7.
11. Xanthakos S, Miles L, Bucuvalas J, Daniels S, Garcia V, Inge T. Histologic spectrum of nonalcoholic fatty liver disease in morbidly obese adolescents. Clin Gastroenterol Hepatol. 2006;4:226-32.
12. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis

factor-alpha: Direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.

13. Yang W, Chi LY, Xie XY. Relationship of body mass index and white blood cell count in hypertensive patients. Chin Heart J. 2008; 20:216-8.
14. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, *et al*. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 2005;46:2347-55.
15. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, *et al*. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: Protective effect of vitamin C. Diabetes. 2001;50:159-65.
16. Braunersreuther V, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. World J Gastroenterol. 2012;18:727-35.
17. Paredes-Turrubiarte G, Gonzalez-Chavez A, Perez-Tamayo R, Salazar-Vazquez BY, Hernandez VS, Garibay-Nieto N, *et al*. Severity of non-alcoholic fatty liver disease is associated with high systemic levels of tumor necrosis factor alpha and low serum interleukin 10 in morbidly obese patients. Clin Exp Med. 2016;16:193-202.
18. Li D, Zhang LJ, Chen ZX. Effects of TNFα, IL-6 and IL-10 on the development of experimental rat liver fibrosis. World Chin J Digestol. 2001;9:1242-5.
19. Cintra DE, Pauli JR, Araujo EP, Moraes JC, Souza CT, Milanski M, *et al*. Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. J Hepatol. 2008;48: 628-37.
20. Bertola A, Bonnafous S, Anty R, Patouraux S, Saint-Paul MC, Iannelli A, *et al*. Hepatic expression patterns of inflammatory and immune response genes associated with obesity and NASH in morbidly obese patients. PLoS ONE. 2010;5:e13577.
21. Miller AM, Wang H, Bertola A, Park O, Horiguchi N, Ki SH, *et al*. Inflammation-associated interleukin-6/signal transducer and activator of transcription 3 activation ameliorates alcoholic and nonalcoholic fatty liver diseases in interleukin-10-deficient mice. Hepatology. 2011;54:846-56.
22. Stojsavljevic S, Gomercic Palcic M, Virovic Jukic L, Smircic Duvnjak L, Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20:18070-91.
23. Kumar R, Prakash S, Chhabra S, Singla V, Madan K, Gupta, SD, *et al*. Association of

pro-inflammatory cytokines, adipokines & oxidative stress with insulin resistance & non-alcoholic fatty liver disease. Indian J Med Res. 2012;136:229-36.

24. Jin X, Zimmers TA, Perez EA, Pierce RH, Zhang Z, Koniaris LG. Paradoxical effects of short- and long-term interleukin-6 exposure on liver injury and repair. Hepatology. 2006; 43:474-84.
25. Leite NC, Salles GF, Cardoso CR, Villela-Nogueira CA. Serum biomarkers in type 2 diabetic patients with non-alcoholic steatohepatitis and advanced fibrosis. Hepatol Res. 2013;43:508-15.

TABLE I BASELINE PARAMETERS IN THE CONTROL AND OBESE GROUPS

	Control group N=209	*Obese group N=117*	*P value*
Female/male	149/60	81/36	0.706
Age (y)	10.3 (1.8)	10.8 (2.2)	0.028
Body mass index (kg/m^2)	15.6 (1.9)	28.5 (3.8)	<0.001
Waist circumference (cm)	55.7 (5.6)	91.1 (10.7)	<0.001
Hip circumference (cm)	65.4 (6.2)	95.8 (9.3)	<0.001
*Fasting glucose (mmol/L)	5.3 (5.1-5.5)	5.3 (5.0-5.7)	0.632
*Fasting insulin (pmol/L)	6.9 (4.6-9.6)	17.8 (12.7-26.3)	<0.001
*HOMA-IR	1.6 (1.1-2.2)	4.1 (3.1-6.5)	<0.001
*Triglyceride (mmol/L)	0.8 (0.6-1.1)	1.3 (1.0-1.9)	<0.001
Cholesterol (mmol/L)	4.4 (0.8)	4.7 (0.8)	0.009
*Lipoprotein A (mmol/L)	89.0 (44.0-201.0)	79.0 (47.5-158.0)	0.369
HDL-C (mmol/L)	1.6 (0.3)	1.3 (0.3)	<0.001
LDL-C (mmol/L)	2.7 (0.5)	2.9 (0.5)	<0.001
ApoB/ApoA1	0.3 (0.1)	0.5 (0.2)	<0.001
*Alanine aminotransferase (U/L)	15 (13-20)	34 (23-69)	<0.001
*Aspartate aminotransferase (U/L)	27 (24-30)	28 (22-44)	0.120
Uric acid (μmol/L)	271.2 (65.4)	405.9 (94.2)	<0.001

*HOMA-IR, homeostasis model of assessment for insulin resistance index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; Apo, apolipoprotein; *Median (IQR)*

TABLE II IMMUNE PARAMETERS BETWEEN THE CONTROL GROUP AND OBESE GROUP

	Control group N=209	*Obese group N=117*
*White blood cell (×10^9/L)	7.5 (1.6)	9.0 (2.1)
*Neutrophils (%)	54.2 (9.7)	58.5 (7.5)
*Lymphocyte (%)	37.5 (9.0)	33.2 (6.8)
*Platelet (×10^9/L)	287.8 (63.3)	316.5 (67.4)
#IL-2 (pg/ml)	3.5 (3.1-4.0)	3.4 (3.0-4.0)
#IL-4 (pg/ml)	3.0 (2.6-3.6)	2.9 (2.5-3.3)
*#IL-6 (pg/ml)	2.7 (2.2-3.5)	3.8 (2.9-5.3)
#IL-10 (pg/ml)	2.8 (2.4-3.2)	2.9 (2.4-3.5)
#TNF-α (pg/ml)	2.8 (2.4-3.2)	2.8 (2.4-3.2)
#IFN-γ (pg/ml)	6.4 (5.1-8.0)	6.1 (4.6-8.6)

*IL: interleukin; TNF: tumor necrosis factor; IFN: interferon; *P<0.001; All values in mean (SD) except # in Median (IQR)*

TABLE III IMMUNE PARAMETERS IN THE THREE SUBGROUPS OF OBESE GROUP

	Subgroup 1 N=23	*Subgroup 2 N=43*	*Subgroup 3 N=51*	*P value*
White blood cell ($\times 10^9$/L)	9.4 (2.3)*	9.0 (2.2)*	8.8 (2.0)*	<0.001
Neutrophils (%)	56.7 (6.3)	59.3 (7.8)*	58.8 (7.8)*	<0.001
Lymphocyte (%)	34.6 (5.3)	32.7 (7.2)*	33.0 (7.1)*	<0.001
Platelet ($\times 10^9$/L)	334.0 (74.2)*	318.2 (64.6)*	307.3 (66.1)*	0.001
IL-2 (pg/ml)	3.4 (3.1-4.0)	3.3(3.0-3.9)	3.6 (3.0-4.2)	0.525
IL-4 (pg/ml)	2.9 (0.6)	2.8 (0.6)*	3.2 (1.2)$	0.026
IL-6 (pg/ml)	3.4 (2.5-4.3)	3.8 (3.1-5.1)*	4.0 (3.1-6.0)*,#	<0.001
IL-10 (pg/ml)	2.6 (2.3-3.0)	2.9 (2.4-3.3)	3.2 (2.6-3.7)*,#,$	0.011
TNF-α (pg/ml)	2.8 (2.6-3.1)	2.6 (2.3-3.0)	2.8 (2.4-3.3)	0.079
IFN-γ (pg/ml)	7.2 (5.2-9.5)	5.5 (4.2-7.0)	6.2 (4.8-9.2)	0.068

*Subgroup 1: Without NAFLD; Subgroup 2: With simple fatty liver; Subgroup 3: With steatohepatitis; NAFLD: Non-alcoholic fatty liver disease; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon. *compare to control group (P<0.05); #compare to subgroup 1 (P<0.05); $compare to subgroup 2 (P<0.05).*

WEB TABLE I LOGISTIC REGRESSION ANALYSIS FOR THE INDEPENDENT FACTORS OF NAFLD

Variable	*Estimate*	*S.E.*	*Wald*	*P value*	*OR (95%CI)*
Body mass index	0.430	0.101	18.304	<0.001	0.233-0.627
LDL-C	3.534	1.570	5.067	0.024	0.457-6.612
HOMA-IR	0.635	0.242	6.862	0.009	0.160-1.110
IL-10	0.402	0.178	5.090	0.024	0.053-0.752

R^2=0.820; NAFLD: Non-alcoholic fatty liver disease; LDL-C: low density lipoprotein-cholesterol; HOMA-IR, homeostasis model of assessment for insulin resistance index; IL: interleukin.