Biochemical characteristics and risk factors in non-alcoholic fatty liver

Ying Wang[1], Jin Dong[1], Hong-ying Liu[1], De-qing Wang[1], Ya-ping Tian[1]

[1]*Department of Clinical Biochemistry, Chinese PLA General Hospital, No.28, Fuxing Road, Beijing, China, 100853*

TABLE OF CONTENTS

1. ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a condition associated with metabolic disturbances. The aim of the present study was to investigate the biochemical characteristics and risk factors for NAFLD. The study was carried out in a total of 881 subjects diagnosed by ultrasonography. NAFLD occurred mainly in middle-aged individuals, with a prevalence of 63.1%. Multiple logistic regression analysis showed that male gender, presence of obesity, hypertension, dyslipidemia, or Type 2 diabetes and elevated serum alanine transaminase activities were independent risk factors for the development of NAFLD. Subjects with NAFLD were obese, hypertensive and often had signs of disturbances in lipid and glucose metabolism and abnormalities in hepatic functions. These findings show risk factors in the development and offer opportunities for prevention of NAFLD.

2. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common pathological state in association with metabolic syndrome. Some of the affected individuals develop fibrosis, which may eventually progress to cirrhosis and hepatocarcinoma (1, 2). NAFLD is becoming a major public health problem due to the increasing prevalence of obesity and Type 2 diabetes (T2DM) worldwide (3). Large population-based surveys in Western industrialized countries showed that NAFLD affects 20-40% of the general population (4, 5). The prevalence of NAFLD currently among Asians is 12-24%, however, that in China has nearly doubled in the past 10-15 years (6), a trend which can not be overlooked. The present study was undertaken to investigate the biochemical characteristics and risk factors for NAFLD in a Chinese physical examination population.

3. MATERIALS AND METHODS

3.1. Demographics of the study population

A total of 881 Chinese subjects (73% males, at the age of 24 to79 years) with either no past history of alcohol intake or weekly alcohol consumption below 40 g were enrolled between June 1 and October 30, 2006, in the Chinese PLA General Hospital Health Science Center. This was an urban population with about 60% of them had average income above median level. Approximately 70% of subjects were urban government employees and take their general yearly physical examination in the Health Science Center. Among them, 172 subjects (75% males) were aged less than 40 years, 401subjects (77.8% males) and 308 subjects (66.9% males) were aged 40 to 50 years and 50 to 60 years or greater. A brief interview, physical examination including blood pressure (BP) and anthropometric measurements, blood biochemical assessments and ultrasound liver scan were performed for each subject on the same day. The study protocol was approved by the Research Ethics Committee of the Chinese PLA General Hospital.

3.2. Clinical and biochemical assessments

On the day of the assessment, subjects attended the center after at least 8 hours of fasting. They underwent physical examinations by physicians. For the calculation of body mass index (BMI), body weight and height were measured in subjects, who wore light-weight clothing and no shoes. Sitting BP was measured in the right arm after at least 5 minutes of rest. Venous blood samples were collected from forearm vessels for biochemical assays, including fasting plasma glucose (FPG), glycated hemoglobin (HbA_{1c}), glycated serum protein (GSP), full lipid profile and liver function. A 2h plasma glucose (PG-2h) samples were collected following a challenge of 75 g oral glucose for non-diabetics or 100 g steamed bread for diabetics. The samples were centrifuged at 2000g for 10 min at $25^{o}C$ immediately and specimens were then frozen and stored at $-20 \, ^{o}C$ until analyses. All measurements were performed in the laboratory of the Health Science Center. Plasma glucose and lipid profiles were measured with an automatic chemistry-immuno-abalyzer (Olympus Corporation, Tokyo, Japan). Serum alanine transaminase (ALT), aspartate transaminase (AST) and Gamma-glutamyl transpepidase (Gamma-GT) levels were measured by standard laboratory methods. An ultrasound liver scan was performed by an experienced ultrasonographist using a Simens Sonoline-SI450 unit with a 3.5-MHz probe.

3.3. Diagnostic criteria

The diagnosis of NAFLD requires fatty changes in the liver confirmed by ultrasonography, the absence of a history of alcohol intake or weekly alcohol intake less than 40 g, and exclusion of alcoholic liver disease, viral hepatitis and other chronic liver diseases (7). Obesity was defined as BMI ≥ 25 kg/m^2, based on the revised WHO criteria for Asians (8). Hypertension was defined as systolic BP of 140 mmHg or greater or diastolic BP of 90 mmHg or greater or in subjects taking antihypertensive medications (9). Type 2 diabetic mellitus (T2DM) was defined as 2 abnormalities in asymptomatic subjects (FPG $\geq$ 7.0 mmol/l and/or PG-2h $\geq$

11.1 mmol/l and/or random plasma glucose $\geq$ 11.1 mmol/l) or 1 abnormal value in the presence of typical symptoms (10). Impaired fasting glucose (IFG) was defined as 6.1 mmol/l $\leq$ FPG < 7.0 mmol/l and PG-2h < 7.8 mmol/l. Impairment of glucose tolerance (IGT) was defined as 7.8 mmol/l $\leq$ PG-2h < 11.1 mmol/l and FPG < 7.0 mmol/l. Fasting plasma total cholesterol (TC) $\geq$ 5.7 mmol/l, triglyceride (TG) $\geq$ 5.7 mmol/l and low density lipoprotein cholesterol (LDL-C) $\geq$ 3.64 mmol/l were regarded as hypercholesteremia, hypertriglyceridemia and high LDL cholesteremia, respectively. Low HDL cholesteremia was defined as high density lipoprotein cholesterol (HDL-C) < 0.91 mmol/l (11).

3.4. Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (Version 11.0, SPSS Inc.) and expressed as means (means±SD) or percentages. Bonferroni's ANOVA and Chi-square tests were used where appropriate. Multiple logistic regression was used to obtain an odds ratio (OR) with 95% confidence intervals (CI) of independent risk factors for NAFLD. A *P* value less than 0.05 (2-tailed) was considered significant.

4. RESULTS

4.1. General profile of the study population

In this study population, the mean age was 46.4 (46.4±9.0) years and 58.8% of subjects had NAFLD. All subjects were divided into non-NAFLD, light and mild or severe NAFLD groups. Compared with the subjects without NAFLD, those with light, mild or severe NAFLD had higher BMI, systolic BP and diastolic BP. Male subjects were more likely to develop NAFLD (Table 1). The highest total prevalence of NAFLD (63.1%) was found in subjects aged 40 to 49 years (Figure 1).

4.2. Characteristics of lipid metabolism

Subjects with light and mild or severe NAFLD had higher TC, TG and LDL-C levels, but lower HDL-C levels than those without NAFLD (Table 1). Hypertriglyceridemia (N=284) combined with low HDL cholesteremia (N=97) and the combination of hypertriglyceridemia, hypercholesteremia and high LDL cholesteremia (N=56) were common lipid disorders and accounted for 50% of total subjects. Subjects with any type of these lipid disorders had a significantly higher total prevalence of NAFLD than subjects with normal lipids (N=316) (70.4% in subjects with hypertriglyceridemia, 81.4% in subjects with hypertriglyceridemia combined with low HDL cholesteremia, 62.5% in subjects with a combination of hypertriglyceridemia, hypercholesteremia and high LDL-cholesteremia vs. 36.4% in subjects with normal lipids, P<0.001) (Figure 2)

4.3. Characteristics of glucose metabolism

Subjects with light and mild or severe NAFLD had higher FPG, PG-2h, and HbA1c, but comparable GSP levels compared with subjects without NAFLD (Table 1). Subjects with IFG (N=56), IGT (N=36) or T2DM (N=146) had a significantly higher prevalence of mild or severe NAFLD than subjects with normal FPG (N=643) (19.6%

Characteristics of NAFLD

Table 1. Clinical and biochemical characteristics of NAFLD in a physical examination population

Items	Non- NAFLD	Light NAFLD	Mild/Severe NAFLD
N	363	299	219
Male (%)	53.2	87.0[1]	88.6[1]
Age (years)	46.6±9.9	46.2±8.6	46.3±8.1
BMI (kg/m^2)	23.4±2.7	26.6±2.7[1]	28.6±3.5[1, 3]
SBP (mmHg)	114±18.3	120±15.3[1]	124±17.7[1, 3]
DBP (mmHg)	76±10.9	82±10.2[1]	84±10.7[1, 3]
TC (mmol/l)	4.9±1.0	5.2±1.1[1]	5.3±1.3[1]
TG (mmol/l)	1.6±1.1	2.4±1.7[1]	3.2±2.2[1, 3]
HDL-C (mmol/l)	1.3±0.3	1.2±0.4[1]	1.0±0.2[1, 3]
LDL-C (mmol/l)	2.7±0.8	2.9±0.8[1]	3.0±0.6[1]
FPG (mmol/l)	5.4±1.2	5.9±1.4[1]	6.4±1.7[1, 3]
PG-2h (mmol/l)	7.0±3.1	8.2±3.2[1]	9.7±3.2[1, 3]
HbA1c (%)	5.6±0.9	5.8±1.0[2]	6.2±1.1[1, 3]
GSP (μmol/l)	170.2±43.7	172.0±45.0	173.8±47.9
ALT (U/L)	20.9±12.5	30.5±21.8[1]	43.5±30.8[1, 3]
AST (U/L) [a]	20.3±7.6	23. 7±12.5[1]	28.4±14.2[1, 3]
Gamma-GT (U/L)[b]	33.7±22.3	58.3±51.8[1]	65.0±54.8[1]
TP (g/l)[c]	73.9±5.0	73.9±3.9	75.1±4.0
ALB (g/L)[d]	42.9±2.5	43.3±2.6	43.5±2.1[1]

Abbreviations: [a]aspartate aminotransferase, [b]Gamma-glutamyl transpepidase, [c]total protein, [d]albumin, [1]P<0.01 for patients with light or mild/severe NAFLD vs. patients were free of NAFLD, [2]P<0.05 for patients with light NAFLD vs. patients were free of NAFLD, [3]P<0.01 for patients with mild/severe NAFLD vs. patients with light NAFLD

Table 2. Multiple logistic regression analysis to examine risk factors for NAFLD

Independent Risk factors	Risk for having NAFLD		
	OR	95% CI	P Value
Obesity	7.8	5.5-11.00	<0.001
Male gender	2.7	1.8-4.1	<0.001
T2DM	2.3	1.3-3.8	<0.01
Dyslipidemia[1]	1.9	1.0-3.6	<0.05
Hypertension	1.6	1.1-2.3	<0.05
Elevation of ALT	1.1	1.0-1.1	<0.001

[1]Dyslipidemia was defined as hypertriglyceridemia combined with low HDL cholesteremia

in subjects with IFG, 25.0% in subjects with IGT and 43.2% in subjects with T2DM vs. 2.7% in subjects with normal FPG, P<0.001). Among subjects with abnormal glucose metabolism, the total prevalence of mild or severe NAFLD was significantly higher in subjects with T2DM compared with subjects with IFG (P=0.002) or IGT (P=0.046) (Figure 3).

4.4. Characteristics of liver function

Subjects with light and mild or severe NAFLD had increased alanine transaminase (ALT), aspartate transaminase (AST) and Gamma-glutamyl transpepidase (Gamma-GT) levels than subjects without NAFLD. The subjects with mild or severe NAFLD also had a higher level of serum albumin (ALB) compared with subjects without NAFLD (Table 1).

4.5. Risk factors for NAFLD

Multiple logistic regression analysis showed that male gender, the presence of obesity, T2DM, dyslipidemia, or hypertension and elevated levels of ALT were independent risk factors for NAFLD (Table 2).

5. DISCUSSION

NAFLD is not only emerging as one of the common liver disorders in Western countries but also highly prevalent in China due to the improvement in quality of life and changes in life style (6). NAFLD was previously believed to be a benign, non-progressive condition, but subsequent evidence showed that some subjects with NAFLD can develop advanced fibrosis, cirrhosis and hepatocellular carcinoma (12). However, NAFLD is essentially an asymptomatic condition. Therefore, we performed the present study to understand the biochemical characteristics and risk factors for NAFLD in a Chinese physical examination population in hopes of better prevention and management.

In this cohort, we observed that up to 58.8% of subjects were diagnosed as having NAFLD by ultrasonography, which is higher than the previously reported prevalence of NAFLD in the general population of China, because the subjects in this study were mostly obese. The mean BMI of non-NAFLD subjects was 23.4 kg/m^2, which suggests that obesity has become a major reason for people to obtain a physical examination. The prevalence of NAFLD in obese Chinese individuals has been reported to be 70-80% (6). Multiple logistic regression analysis revealed that the obese subjects had a 7.8-fold higher risk of having NAFLD than non-obese subjects.

Although a number of studies showed that females were more likely to have an increased risk of NAFLD (13, 14), we found that male gender was an independent risk factor for the disease. This is supported by epidemiologic data from the National Health and Nutrition Examination Survey including 12241 adults. The survey indicated NAFLD was more prevalent in men than in
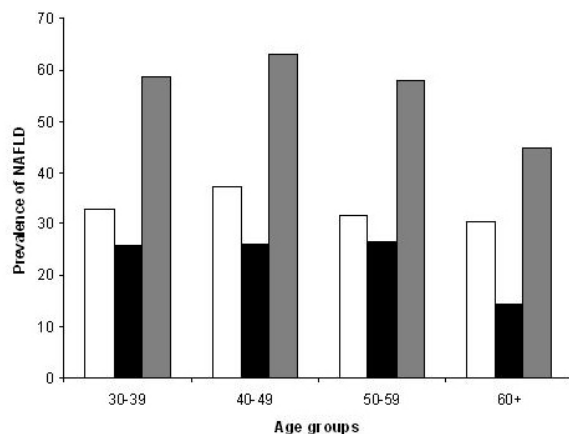
Figure 1. The prevalence of NAFLD stratified by age. White bar: prevalence of light NAFLD, Black bar: prevalence of mild/severe NAFLD, Gray bar: total prevalence of NAFLD.
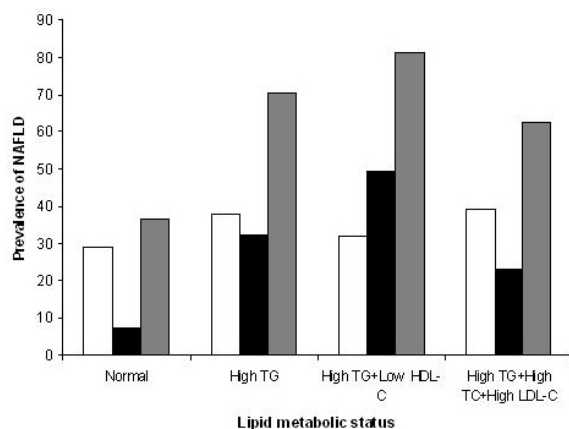

Figure 2. The prevalence of NAFLD stratified by lipid metabolic status. White bar: prevalence of light NAFLD, Black bar: prevalence of mild/severe NAFLD, Gray bar: total prevalence of NAFLD.
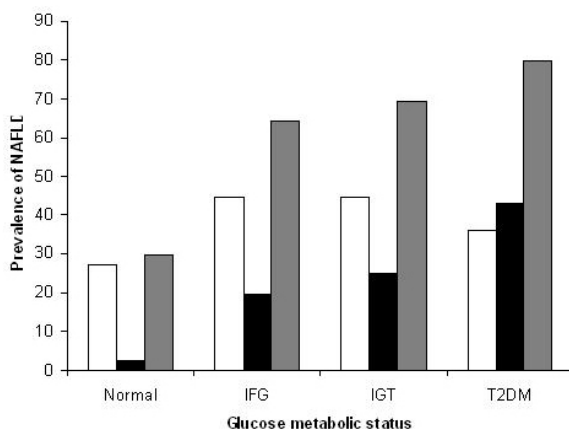

Figure 3. The prevalence of NAFLD stratified by glucose metabolic status. White bar: prevalence of light NAFLD, Black bar: prevalence of mild/severe NAFLD, Gray bar: total prevalence of NAFLD.

women in every age group (15). Furthermore, additional evidence confirmed this observation in both Caucasians (16, 17) and Asians (18). This gender predilection might be attributed to the influence of sex hormones. Sex hormone binding globulin produced by the liver has been reported to be strongly correlated with insulin sensitivity (19). There have been associations between sex hormone binding globulin and hyperandrogenism (20). Women with polycystic ovary syndrome, especially related to hyperandrogenism, have been shown to be at high risk for NAFLD (21). In addition, a number of studies have demonstrated a relationship between the amount of visceral adipose tissue and the presence of fatty liver (22, 23). Males are more likely to have a distribution of excess body fat in the intra-abdominal compartment.

In agreement with previous reports (14, 18), other risk factors for NAFLD in our study included the presence of dyslipidemia, T2DM, obesity, hypertension and elevated levels of ALT. A recent review stated that dyslipidemia characterized by hypertriglyceridemia, often accompanied by low HDL-C, was commonly found in patients with NAFLD (24). In the present study, the most common type of lipid disorder was hypertriglyceridemia, accounting for 50.3% of total dyslipidemia subjects. This type of hypertriglyceridemia, combined with low HDL-cholesteremia, was found in 17.2% of subjects. However, multiple regression analysis showed that subjects who presented the latter type of dyslipidemia had a 1.9-fold increased risk for NAFLD.

Insulin resistance is a "common soil" connecting multiple metabolic disorders including dyslipidemia, obesity, T2DM and hypertension (25) Insulin resistance is a universal element in the pathophysiology of NAFLD (2, 26). A follow-up study showed that poor glycemic control preceded the onset of steatohepatitis (27). Marchesini *et al.* (2) noted that the presence of metabolic syndrome carried a high risk of fibrosis and was associated with potentially progressive, severe liver disease in Caucasian subjects. We found that subjects with abnormal glucose metabolism (IFG, IGT and T2DM) had a significantly higher prevalence of mild or severe NAFDL than subjects with normal FPG. The prevalence increased significantly with the severity of glucose metabolic disorders.

The most common abnormal liver function tests were elevated ALT and AST. Gamma-GT may also be elevated. However, subjects with NAFLD may have normal transaminase levels. The ratio of AST/ALT is usually less than 1; but in alcoholic liver disease, the ratio will typically be greater than 2 (28). In the present cohort, the mean level of ALT in subjects with mild or severe NAFLD was higher than the upper limits of normal (40U/L); however, the ratio of AST to ALT was less than 1. Elevated level of ALT was an independent risk factor for NAFLD. Gamma-GT might also be elevated in subjects with NAFLD, but only limited data on its occurrence and degree of elevation are available. Of these liver enzymes, ALT is the most closely related to liver fat accumulation. Consequently, ALT has been used as marker of NAFLD (29).

In conclusion, the present study revealed that the prevalence of NAFLD among this urban population is 58.8%. The risk factors included age (40 to 49 years), high BMI or BP, poor glycemic control and abnormalities in lipids metabolism or hepatic functions. Since most of these risk factors are still reversible, early prevention of when these warning signs appear is important to ameliorate the disease process and reducing the overall risk of more severe complications.

6. REFERENCES

1. Cuadrado A, Orive A, Garcia-Suarez C, Dominguez A, Fernandez-Escalante JC., Crespo J and Pons-Romero : Non-alcoholic Steatohepatitis (NASH) and Hepatocellular Carcinoma. *Obesity Surgery* 15, 442-446 (2005)

2. Marchesini G, Bugianesi E , Forlani G , Cerrelli F, Lenzi M , Manini R , Natale S, Vanni E, Villanova N , Melchionda N and Rizzetto M: Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37, 917-923 (2003)

3. Yoon Kun-Ho, Lee Jin-Hee, Kim Ji-Won, Cho Jae Hyoung, Choi Yoon-Hee, Ko Seung-Hyun, Zimmet Paul and Son Ho-Young: Epidemic obesity and type 2 diabetes in Asia. *The Lancet* 368, 1681-1688 (2006)

4. Browning JD, Szczepaniak LS , Dobbins R, Nuremberg P, HortonJ D , CohenJ C , Grundy SM and Hobbs HH.: Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology* 40, 1387-1395 (2004)

5. Clark JM: The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 40, S5-10 (2006)

6. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ and Goh KL: How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? *J Gastroenterol Hepatol* 22, 788-93 (2007)

7. Fatty Liver and Alcoholic Liver Disease Study Group of Chinese Liver Disease Association: Diagnostic criteria of nonalcoholic fatty liver disease. *Chin J Hepatol* 11, 71 (2003)

8. The Asia-Pacific Perspective: Redefining obesity and its treatment, WHO (2000)

9. 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Sub-Committee. *Blood Press* Suppl 1, 9-43 (1999)

10. American Diabetes Association. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 20, 1183-97 (1997)

11. Study Group of Prevention and Treatment Strategy on Abnormal Serum Lipid. Advice on prevention and treatment of abnormal serum lipid. *Chin J Cardiol* 25, 169-75 (1997)

12. Bugianesi E: Non-alcoholic Steatohepatitis and Cancer. *Clinics in Liver Disease* 11, 191-207 (2007)

13. Angulo Paul, Keach JC, Batts KP and Lindor KD : Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 30, 1356-1362 (1999)

14. Loguercio C, De Girolamo V, de Sio I , Tuccillo C, Ascione A, Baldi F, Budillon G, Cimino L , Di Carlo A , Di Marino MP, Morisco F, Picciotto F, Terracciano L, Vecchione R, VerdeV and Del Vecchio Blanco C : Non-alcoholic fatty liver disease in an area of southern Italy: main clinical, histological, and pathophysiological aspects. *Journal of hepatology* 35, 568-574 (2001)

15. Clark J M., Brancati F.and Diehl A M: The prevalence and etiology of elevated aminotransferase levels in the United States. *American Journal of Gastroenterology* 98, 960-967 (2003)

16. Arun J, Clements RH, Lazenby AJ, Leeth RR and Abrams GA: The Prevalence of Nonalcoholic Steatohepatitis is Greater in Morbidly Obese Men Compared to Women. *Obesity Surgery* 16, 1351-1358 (2006)

17. Papatheodoridis GV, Goulis J, Christodoulou D, Manolakopoulos S, Raptopoulou M, Andrioti E, Alexandropoulos N, Savvidou S, Papachristou A, Zervou E, Seferiadis K, Kousidou P, Vogiatzakis E and Tsianos E: High prevalence of elevated liver enzymes in blood donors: associations with male gender and central adiposity. *Eur J Gastroenterol Hepatol* 19, 281-287 (2007)

18. Fan JG, Saibara T, Chitturi S, Kim BI, SungJJ and Chutaputti A: What are the risk factors and settings for non-alcoholic fatty liver disease in Asia-Pacific? *Journal of Gastroenterology and Hepatology* 22, 794-800 (2007)

19. Galloway PJ, Donaldson MDC and Wallace AM: Sex hormone binding globulin concentration as a prepubertal marker for hyperinsulinaemia in obesity. *Arch Dis Child* 85, 489-491 (2001)

20. Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B and Hammond G.: Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. *J Clin Invest* 109, 973-981 (2002)

21. Schwimmer JB, Khorram O , Chiu V and Schwimmer WB : Abnormal aminotransferase activity in women with polycystic ovary syndrome. *Fertility and sterility* 83, 494-497 (2005)

22. Kelley DE, McKolanis TM, Hegazi RA, Kuller LH and Kalhan SC: Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab* 285, E906-916 (2003)

23. Nguyen-Duy TB, Nichaman MZ, Church TS, Blair SN and Ross R: Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab* 284, E1065-1071 (2003)

24. Malnick SDH, Beergabel M and Knobler H: Non-alcoholic fatty liver: a common manifestation of a metabolic disorder. *QJM* 96, 699-709 (2003)

25. Saltiel AR: The molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases. *J Clin Invest* 106, 163-164 (2000)

26. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough A J, Natale S, Forlani G and Melchionda N: Nonalcoholic Fatty Liver Disease: A Feature of the Metabolic Syndrome. *Diabetes* 50, 1844-1850 (2001)

27. Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW and Powell LW: The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 11, 74-80 (1990)

28. Sorbi D, Boynton J and Lindor KD: The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol.* 94, 1018-1022 (1999)

29. Song Hye Ryoung, Yun Kyung Eun and Park Hye Soon: Relation between alanine aminotransferase concentrations and visceral fat accumulation among nondiabetic overweight Korean women. *Am J Clin Nutr* 88, 16-21 (2008)

Abbreviations: NAFLD: non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus, BMI: body mass index, BP: blood pressure, FPG: fasting plasma glucose, PG-2h: 2h plasma glucose, HbA_{1c}: glycated hemoglobin, GSP: glycated serum protein, IFG: impaired fasting glucose, IGT: impairment of glucose tolerance, TC: total cholesterol, TG: triglyceride, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, OR: odds ratio, CI: confidence intervals, ALT: alanine transaminase, AST: aspartate transaminase, Gamma-GT: Gamma-glutamyl transpepidase, TP: total protein, ALB: albumin

Key words: non-alcoholic fatty liver disease, characteristics, risk factors, urban population, Chinese

Send correspondence to: Ya-ping Tian, Department of Clinical Biochemistry, Chinese PLA General Hospital, No. 28, Fuxing Road, Beijing, China, 100853, Tel: 86-010-66939374, Fax: 86-010-88217385, E-mail: tianyp@301hospital.com.cn