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Elevation of Serum Levels of Advanced Glycation End Products in Patients With Non-B or Non-C Hepatocellular Carcinoma

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Background: The prevalence of non-B or non-C hepatocellular carcinoma (NBNC-HCC) has been increasing all over the world. Advanced glycation end products (AGE) play a role in the pathogenesis of alcoholic liver injury or nonalcoholic steatohepatitis (NASH). *Methods:* We examined here whether serum levels of AGE were elevated in NBNC-HCC patients compared with NASH subjects without HCC and investigated which anthropometric and clinical variables were independent determinants of AGE. *Results:* Ninety NBNC-HCC, 56 NASH, and 27 control subjects underwent a complete history and physical examination, determination of blood chemistries, including AGE levels. Serum levels of AGE were significantly higher in NBNC-HCC patients compared with NASH and control subjects [9.1 $\pm$ 2.7, 5.2 $\pm$ 1.7, 3.5 $\pm$ 1.2 (U/ml), respectively, $P < 0.05$]. Univariate analysis showed that AGE levels were associated with male ($P < 0.05$), age ($P < 0.01$), aspartate aminotransferase ($P < 0.05$), γ-glutamyl transpeptidase (GGT) ($P < 0.01$), HDL-cholesterol (inversely, $P < 0.01$), fasting plasma glucose ($P < 0.01$), and HbA1c ($P < 0.05$). By the use of multiple stepwise regression analysis, age, GGT, and HDL-cholesterol (inversely) remained significant and were independently related to AGE levels ($R^2 = 0.406$). *Conclusion:* The present results suggest that AGE might be involved in the pathogenesis of NBNC-HCC, thereby being a biomarker that could discriminate NBNC-HCC from NASH. J. Clin. Lab. Anal. 29:480–484, 2015.

Key words: AGE; NASH; Non-B or non-C hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies and causes of cancer-related deaths in the world (1,2). Although most cases of HCC are attributable to chronic liver disease resulting from chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, a substantial proportion of HCC patients are negative for markers of HBV surface antigen (HBVAg) or HCV antibody (HCVAb), being diagnosed as non-B or non-C HCC (NBNC-HCC). The frequency of NBNC-HCC has been reported to be 5–15%, and number of NBNC-HCC has been increasing gradually all over the world (3–8). Alcoholic or metabolic derangement-related liver damage could contribute to the pathogenesis of NBNC-HCC (3–8). However, in contrast to HBV- or HCV-related HCC, clinical characteristics of NBNC-HCC are not fully examined. Therefore, carcinomas in these patients were

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often detected at an advanced stage, and NBNC-HCC patients were reported to have a poorer prognosis than hepatitis virus-related HCC subjects (3–5, 9, 10).

Nonalcoholic fatty liver (NAFL) is the most common chronic liver disease in the world. According to the annual health-check reports in Japan, 9–30% of Japanese adults have suffered from NAFL due to the Westernization of lifestyles and increasing rates of obesity and diabetes (11–15). NAFL is characterized by hepatic steatosis in the absence of significant alcohol intake or other known liver diseases. NAFL includes a wide spectrum of liver diseases, ranging from fatty liver, a benign and nonprogressive condition, to nonalcoholic steatohepatitis (NASH), which can progress to liver cirrhosis and HCC (16–19). Indeed, several case series of NASH-associated HCC have been actually reported (20, 21).

Advanced glycation end products (AGE) were originally characterized by a yellow-brown fluorescent color and an ability to form cross-links with and between amino groups, but the term is now used for a broad range of advanced products of the glycation process, also called the Maillard reaction (22–24). The formation and accumulation of AGE in various tissues have been known to progress during normal aging, and at an extremely accelerated rate in diabetes mellitus (22–24). Recent studies have suggested that AGE can be formed not only from reducing sugars, but also from carbonyl compounds derived from the auto-oxidation of sugars and the other metabolic pathways (25, 26). There is accumulating evidence to show that AGE play a role in the pathogenesis of a variety of disorders such as diabetic vascular complications, alcoholic liver injury, NASH, Alzheimer's disease, osteoporosis and cancer growth, and metastasis (27–34). However, the clinical significance for measuring serum AGE levels as a biomarker of NBNC-HCC remains unknown. Therefore, in this study, we examined whether serum levels of AGE were elevated in NBNC-HCC patients compared with NASH subjects without HCC or controls and further investigated which anthropometric and clinical variables were independent determinants of AGE.

PATIENTS AND RESEARCH DESIGN

Ninety patients with treatment-naïve NBNC-HCC were enrolled between April 2008 and March 2012. In addition, 27 volunteers and 56 NASH without HCC were enrolled as controls and comparators for NBNC-HCC, respectively. For the enrollment of volunteers, steatosis, liver diseases, and metabolic complications were excluded by ultrasound scan and by clinical laboratory examination. All had normal physical examinations and liver function tests were negative for serology and viral hepatitis and had no history of liver diseases. In NASH patients, current and past daily alcohol intake was less than 20 g/week. None of the patients had received any medication that could cause NASH. Among these patients, those with the following disorders were excluded: secondary cause of steatohepatitis and drug-induced liver disease, alcohol liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, α_1-antitrypsindeficiency, hemochromatosis, Wilson's disease, and biliary obstruction (29). Histological diagnosis for NAFLD was performed according to the methods of Matteoni et al. (18). NBNC-HCC was defined as negative for both HBsAg and HCVAb. HBsAg in sera were assayed using the HBsAg-EIA Cobas Core Test (Hoffmann La Roche). Anti-HCV in sera was assayed using the Anti-HCV-EIA Cobas Core Test (Hoffmann La Roche, Basel, Switzerland). Patients with autoimmune hepatitis or with primary biliary cirrhosis were also excluded from NBNC-HCC group. Heavy alcohol consumption was defined when alcohol intake was more than 80 g/day for 5 years (35). Etiology of NBNC-HCC patients with heavy alcohol consumption was estimated to be alcohol. Informed consent was obtained from all patients, and the study was conducted in conformity with the ethical guidelines of the seventh revision of the Declaration of Helsinki (36) and was approved by the ethics and research committees of Hiroshima University Hospital.

The body mass index was calculated as the weight (kg) divided by height (m)-squared. Blood pressure was measured in the sitting position using an upright standard sphygmomanometer. Venous blood samples were taken in the morning following overnight fasting for 12 h. Blood chemistries such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), and lipid parameters were measured using the standard methods as described previously (29). Insulin resistance was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (μU/ml) $\times$ plasma glucose (mg/dl) /405. For the measurements of AGE, serum was stored at -30°C until use. Serum levels of AGE was measured with a competitive enzyme-linked immunosorbent assay system as described previously (29). All measurements were made in triplicate, and the average values were used in the statistical analyses.

Data are presented as mean $\pm$ standard deviation (SD) or as number. All analysis was performed using the R statistical package (http://www.r-project.org). Statistical differences in quantitative data were determined using the Mann–Whitney *U*-test, the Kruskal–Wallis test or Chi-squared test. Differences were considered statistically significant at *P*-values less than 0.05.

TABLE 1. Characteristics of Patients

Characteristics	NBNC-HCC ($n = 90$)	NASH without HCC ($n = 56$)	Control ($n = 27$)
Sex (male/female)	71/19*	39/17	13/14
Age (year)	69.3 ± 9.7*,**	49.9 ± 12.9	48.4 ± 12.0
Body mass index (kg/m^2)	24.1 ± 3.6*,**	27.1 ± 3.7*	21.8 ± 2.2
AST (U/l)	59.1 ± 71.2*	53.4 ± 35.3*	20.5 ± 4.8
ALT (U/l)	49.5 ± 105.3**	93.9 ± 72.4*	17.1 ± 6.9
GGT (U/l)	157.7 ± 187.8*,**	88.4 ± 98.9*	28.9 ± 16.3
LDL-C (mg/dl)	103.9 ± 33.7**	142.3 ± 27.4*	109.0 ± 15.8
TG (mg/dl)	107.5 ± 60.1**	184.7 ± 86.5*	80.1 ± 37.4
HDL-C (mg/dl)	48.5 ± 14.8*	49.0 ± 12.8*	65.2 ± 17.4
FPG (mg/dl)	125.6 ± 42.3*,**	107.9 ± 18.7*	96.7 ± 7.5
HbA1c (%)	6.0 ± 1.3*	5.7 ± 0.9	5.3 ± 0.4
HOMA-IR	3.4 ± 2.5*	3.2 ± 2.0*	1.8 ± 1.2
AGE (U/ml)	9.1 ± 2.7*,**	5.2 ± 1.7*	3.5 ± 1.2
Systolic blood pressure (mmHg)	118.4 ± 8.0*	125.0 ± 14.5*	110.4 ± 13.4
Diastolic blood pressure (mmHg)	72.0 ± 7.8	74.8 ± 11.2	69.4 ± 7.2

*$P < 0.05$ compared with control. **$P < 0.05$ compared with NASH.

RESULTS AND DISCUSSION

Clinical and laboratory characteristics of 90 patients with treatment-naïve NBNC-HCC, 56 biopsy-proven NASH and 27 healthy controls are shown in Table 1. Among NBNC-HCC patients, 10 were NASH-associated HCC, 49 alcoholic liver disease-associated one, and the etiology of rest of them ($N = 31$) was unknown. As shown in Table 1, serum levels of AGE in NBNC-HCC patients were significantly higher than those in NASH subjects without HCC or controls (9.1 ± 2.7 vs. 5.2 ± 1.7 or 3.5 ± 1.2 U/ml, respectively, $P < 0.05$). There was no significant difference in serum AGE levels among NASH-, alcohol-associated and unknown-etiology HCC (9.1 ± 2.1 vs. 8.6 ± 1.9 vs. 9.8 ± 3.7 U/ml). Compared with NASH patients without HCC, age, GGT, and FPG were significantly higher in NBNC-HCC, while body mass index, ALT, low-density lipoprotein-cholesterol (LDL-cholesterol), and triglycerides (TG) were lower. As the case of NASH subjects without HCC, compared with controls, age, GGT and FPG were significantly elevated in NBNC-HCC patients. Although there was no significant difference of ratio of male to female, AST, high-density lipoprotein-cholesterol (HDL-cholesterol), HbA1c, and HOMA-IR between NBNC-HCC and NASH subjects without HCC, ratio of male to female, AST, HbA1c, and HOMA-IR were significantly higher, whereas HDL-cholesterol was lower in NBNC-HCC patients compared with controls.

Univariate analysis revealed that male ($P < 0.05$), age ($P < 0.01$), AST ($P < 0.05$), GGT ($P < 0.01$), HDL-cholesterol (inversely, $P < 0.01$), FPG ($P < 0.01$), and HbA1c ($P < 0.05$) were significantly associated with serum levels of AGE (Table 2). Because these significant parameters could be closely correlated with each other, we performed multiple stepwise regression analysis in order to determine the independent correlates of serum AGE levels. As shown in Table 2, age, GGT, and HDL-cholesterol (inversely) remained significant and were independently related to AGE levels ($R^2 = 0.406$).

We have previously shown that AGE could play a role in the pathogenesis of NASH and alcoholic liver injury in humans (28–30, 37). Indeed, in vitro, AGE could stimulate the proliferation and activation of hepatic stellate cells, thereby causing hepatic inflammation and fibrosis (37). In humans, serum levels of AGE were significantly elevated in NASH patients compared with simple steatosis (29). Furthermore, chronic ethanol ingestion has been shown to stimulate acetaldehyde-induced AGE formation in the liver and correlate with the severity of alcoholic liver disease in rats (30). In this study, we found for the first time that serum levels of AGE were significantly higher compared with NASH subjects without HCC or controls. The present findings have extended our previous observations showing that (1) AGE not only enhance the angiogenic potential of HCC by upregulating vascular endothelial growth factor expression, but also stimulate the proliferation of HCC in vitro (38). AGE might contribute to the development and progression of NBNC-HCC in humans, thereby being a biomarker of NBNC-HCC.

In the present study, besides age, GGT and HDL-cholesterol (inversely) were independent correlates of serum AGE levels in all subjects. GGT levels have been shown to be a biomarker that could predict outcomes and overall survival in HCC patients treated with or without transarterial chemoembolization or operation (39–42). These findings suggest that increased AGE levels could partly explain the link between high GTT values and shorter survival in HCC patients. Further, since HDL-cholesterol levels were reported to decrease in HCC

TABLE 2. Univariate and Multivariate Stepwise Regression Analysis for the Determinants of AGE

	Univariate		Multivariate	
Characteristics	β	*P*	β	*P*
Sex (male/female)	−0.161	***P* < 0.05**		
Age (year)	0.550	***P* < 0.01**	0.518	***P* < 0.01**
Body mass index (kg/m^2)	−0.036	0.638		
AST (U/L)	0.170	***P* < 0.05**		
ALT (U/L)	0.052	0.508		
GGT (U/L)	0.271	***P* < 0.01**	0.206	***P* < 0.01**
LDL-C (mg/dl)	−0.095	0.232		
TG (mg/dl)	−0.070	0.377		
HDL-C (mg/dl)	−0.235	***P* < 0.01**	−0.253	***P* < 0.01**
FPG (mg/dl)	0.257	***P* < 0.01**		
HbA1c (%)	0.193	***P* < 0.05**		
HOMA-IR	0.006	0.951		
Systolic blood pressure (mmHg)	0.013	0.900		
Diastolic blood pressure (mmHg)	−0.024	0.826		

Male = 0, Female = 1. $R^2 = 0.406$

subjects (43), AGE might decrease HDL-cholesterol levels, which could lead to poor prognosis in these patients.

CONCLUSIONS

In this study, we found that circulating AGE levels were significantly increased in NBNC-HCC patients compared with NASH subjects without HCC or controls. The present results suggest that AGE could be involved in the pathogenesis of NBNC-HCC, thereby being a biomarker that might discriminate NBNC-HCC from NASH.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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