Increased Prevalence of Carotid Atherosclerosis in Hepatitis B Virus Carriers

Nobukazu Ishizaka, MD; Yuko Ishizaka, MD; Eiko Takahashi, MD; Ei-ichi Toda, RMS; Hideki Hashimoto, MD, DrPH; Minoru Ohno, MD; Ryozo Nagai, MD; Minoru Yamakado, MD

Background—Recent experimental and epidemiological findings suggest that some infectious agents play a role in the development and promotion of atherosclerosis. We have investigated the possible association between hepatitis B virus surface antigen (HBsAg) positivity and carotid arteriosclerosis.

Methods and Results—In this cross-sectional cohort study, we analyzed data from subjects undergoing general health-screening tests, including both high-resolution B-mode carotid ultrasound and assessment of HBsAg status, between 1994 and 2001 at our institute. Of the 4686 study subjects (3137 men and 1549 women; age 22 to 88 years), 1294 (28%) had carotid artery plaque and 40 (0.9%) were positive for HBsAg, indicating they were hepatitis B virus carriers. No HBsAg-positive subjects were positive either for antibodies against the hepatitis C virus (HCV) or for HCV core proteins. Univariate analysis revealed HBsAg positivity was associated with carotid plaque with an odds ratio of 1.58 (95% CI, 1.14 to 2.19, $P<0.05$). When other confounding risk factors for atherosclerosis were included as covariates in the statistical analysis, HBsAg positivity was still positively associated with carotid plaque with an odds ratio of 1.57 (95% CI, 1.10 to 2.24, $P<0.05$).

Conclusions—These findings suggest a possible role of chronic hepatitis B infection in the pathogenesis of carotid arteriosclerosis. **(*Circulation*. 2002;105:1028-1030.)**

Key Words: infection ■ plaque ■ ultrasonics ■ atherosclerosis

The possible role of certain infectious agents in atherogenesis has been suggested by recent observations that viable microorganisms or remnants of them are present in atherosclerotic plaque[1] and that a positive antibody status against some infectious organisms is associated with atherosclerotic diseases.[2] On the other hand, several investigators have reported experimental and epidemiological evidence that suggests that activation of an inflammatory process, instead of specific infectious agents, is responsible for the development and promotion of atherosclerotic diseases.[3,4] Nevertheless, at present, it might still be advantageous to assess possible associations between certain infectious agents and the risk of atheromatous disease to identify persons at higher risk for future cardiovascular and cerebrovascular events.

We recently have reported[5] that seropositivity for the hepatitis C virus (HCV) has a positive association with carotid atherosclerosis. In the present study, we investigated the possible association between hepatitis B virus surface antigen (HBsAg) positivity and carotid arteriosclerosis. We analyzed the data of subjects undergoing general health screening tests. In Japan, a regular health check-up for employees is legally mandated to employers, and the service is available in check-up laboratories affiliated with medical institutes such as ours. Thus, the majority of the study subjects of this large cohort were considered to be healthy.

Methods

Subjects

The study cohort consisted of 4686 subjects (3137 men and 1549 women) aged 22 to 88 years who underwent general health screening tests, including ultrasonographic evaluation of carotid arteries, at the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, between August 1994 and May 2001. The evaluation also included both high-resolution B-mode carotid ultrasound and analysis of HBsAg and other risk factors for atherosclerosis.

Carotid Ultrasound

The status of the carotid arteries was studied by high-resolution B-mode ultrasonography (Sonolayer SSA270A) equipped with a 7.5 MHz transducer (PLF-703ST, Toshiba) as described previously.[6] Plaque was defined as a focal thickening of the intimal-medial layer ≥1.3 mm of thickness.

Risk Factors

Hypertension was defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg. Body mass index

Received December 7, 2001; revision received January 14, 2002; accepted January 14, 2002.

From the Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine (N.I., M.O., R.N.); Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital (Y.I., E.T., E-i.T., M.Y.); and Department of Hygiene and Public Health, Teikyo University School of Medicine (H.H.), Tokyo, Japan.

Correspondence to Nobukazu Ishizaka, MD, PhD, Department of Cardiovascular Medicine, University of Tokyo, Graduate School of Medicine, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail nobuishizka-tky@umin.ac.jp

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/hc0902.105718**

(BMI) was calculated as weight (kg)/height (m^2). Hypercholesterolemia, hypo-HDL-cholesterolemia, and hypertriglyceridemia were defined as a total cholesterol (TC) level of >5.68 mmol/L, an HDL-cholesterol (HDL-C) level of <1.03 mmol/L, and a triglyceride (TG) level of >1.69 mmol/L, respectively. A high hemoglobin A_{1C} level was defined as ≥5.9%.

Laboratory Tests

Subjects fasted overnight before blood samples were obtained. TC, HDL-C, and TG levels were determined enzymatically; HBsAg and hemoglobin A_{1C} levels were determined by latex agglutination immunoassay. Measurement of C-reactive protein (CRP) was performed using a latex-enhanced turbidimetric immunoassay (LPIAACE CRP-L, Dia-iatron).

Statistical Analysis

Statistical analyses were performed using unpaired *t* tests, χ^2 tests, and univariate and multivariate logistic regression analyses using computer software, Statistica version 5J (StatSoft Inc). *P* values <0.05 were considered to be statistically significant.

Results

Study Subjects

Of the 4686 study subjects, 1294 (28%) had carotid artery plaque and 40 (0.9%) were positive for HBsAg, indicating that they were hepatitis B virus (HBV) carriers. No HBsAg-positive subject was found to be positive for anti-HCV antibodies and/or HCV core protein. Baseline clinical characteristics and laboratory data of HBsAg-positive and negative subjects are illustrated in Table 1. Platelet count was lower and HDL-C levels were higher in HBsAg-positive subjects. On the other hand, there was no statistically significant difference in either aspartate aminotransferase or alanine aminotransferase between HBsAg-positive and HBsAg-negative subjects, which supports the notion that the majority, if not all, of the HBV carriers in the present study were not experiencing severe liver damage at the time of the health-screening tests. Notably, an erythrocyte sedimentation rate (ESR) and a CRP level >0.4 mg/dL, both of which are markers for inflammation, were not different between these groups (Table 1). Carotid plaque was positive in 17/40 (43%) and 1277/4646 (27%) in HBsAg-positive and negative subjects, respectively. The difference was statistically significant ($P<0.05$ by χ^2 test).

Relationship Between HBsAg Positivity and Carotid Plaque

Results of the univariate logistic regression analysis are described in Table 2. In addition to conventional risk factors for atherosclerosis (male sex, age, hypertension, hypercholesterolemia hypo-HDL-cholesterolemia, hypertriglyceridemia, increased hemoglobin A_{1C}, and prior smoking), HBsAg positivity was found to be associated with carotid plaque with an odds ratio of 1.58 (95% CI, 1.14 to 2.19, $P<0.05$). In addition, a CRP level >0.4 mg/dL was also positively associated with carotid plaque, as we and others have previously reported.[5,7] A multivariate logistic regression analysis revealed that HBsAg positivity was a statistically significant predictor for carotid plaque with an odds ratio of 1.57 (95% CI, 1.10 to 2.24, $P<0.05$) that was independent of other confounding risk factors for atherosclerosis (Table 2).

TABLE 1. Baseline Characteristics of the Study Subjects

Variables	HBsAg (−) (n=4646)	HBsAg (+) (n=40)	*P*
Clinical characteristics			
Male sex	3108 (67)	26 (65)	NS
Age, y	57±10	58±10	NS
BMI, kg/m^2	23.2±3.0	23.1±2.8	NS
Systolic BP, mm Hg	127±20	134±19	<0.05
Nonsmoker	2222 (48)	23 (58)	NS
Exsmoker	1173 (25)	10 (25)	NS
Current smoker	1251 (27)	7 (18)	NS
Laboratory data			
WBC, $\times10^3/\mu$L	5.6±1.5	5.3±1.4	NS
RBC, $\times10^4/\mu$L	461±43	474±40	NS
Platelet, $\times10^4/\mu$L	22.5±5.2	19.3±3.9	<0.0001
TC, mmol/L	5.4±0.9	5.1±0.8	NS
HDL-C, mmol/L	1.5±0.5	1.8±0.5	<0.001
TG, mmol/L	1.5±1.1	1.1±0.9	NS
AST, U/L	25.3±16.6	26.9±12.2	NS
ALT, U/L	25.4±24.8	26.7±21.5	NS
LDH, U/L	289±109	272±117	NS
Blood sugar, mg/dL	98±21	94±13	NS
Haemoglobin A_{1C}, %	5.3±0.7	5.1±0.6	NS
ESR, mm/h	13.2±10.6	13.0±9.9	NS
CRP >4.0 mg/dL	280 (6)	3 (8)	NS

BP indicates blood pressure; WBC, white blood cell count; RBC, red blood cell count; AST, aspartate transaminase; and ALT, alanine transaminase.

Values are mean±SD or number (percentage).

Discussion

In this cross-sectional cohort, 0.9% of the study subjects were positive for HBsAg and thus considered to be HBV carriers. This percentage is comparable to other epidemiological studies showing the decreasing trend of the prevalence of

TABLE 2. Predictors for Carotid Plaque

	Odds Ratio (95% CI)	
Variables	Unadjusted	Adjusted*
Male sex	1.56 (1.45–1.68)¶	1.42 (1.31–1.54)¶
Age, per 10 y	2.03 (1.96–2.11)¶	1.98 (1.91–2.06)¶
Hypertension	2.10 (1.95–2.25)¶	1.65 (1.52–1.78)¶
Hypercholesterolemia	1.28 (1.18–1.41)§	1.34 (1.22–1.47)§
Hypo-HDL-cholesterolemia	1.20 (1.09–1.32)‡	1.15 (1.03–1.28)‡
Hypertriglyceridemia	1.25 (1.16–1.34)§	1.14 (1.04–1.24)‡
Haemoglobin A_{1C} ≥5.9%	1.35 (1.23–1.49)§	1.10 (0.99–1.22)
CRP >0.4 mg/dL	1.40 (1.23–1.59)‡	1.16 (1.01–1.34)‡
HBsAg positivity	1.58 (1.14–2.19)‡	1.57 (1.10–2.24)‡
Current smoker†	1.03 (0.95–1.11)	1.08 (0.98–1.18)
Exsmoker†	1.22 (1.13–1.32)‡	1.05 (0.96–1.15)

*Odds ratio derived from logistic regression analysis including all the variables listed in the table.

†Nonsmoker was used as reference.

‡$P<0.05$; §$P<0.01$; ‖$P<0.001$; ¶$P<0.0001$.

HBsAg carriers in our country.[8] A multivariate statistical analysis revealed that HBsAg positivity was positively associated with carotid artery plaque with an odds ratio of 1.57 (95% CI, 1.10 to 2.24, $P<0.05$). This relationship was independent of other confounding risk factors for atherosclerosis.

In general, the clinical course of chronic HBV infection is characterized by a series of exacerbations and remissions, but it may lead to hepatic decompensation, progression of liver disease, and the development of cirrhosis. Liver cirrhosis appears to be associated with a decreased risk of atherosclerosis, which may be explained by the decreased coagulation status and the reduction of some of the conventional risk factors for atherosclerosis, such as high TC and lipoprotein(a) (Lp[a]).[9] Therefore, the positive correlation between HBsAg positivity and carotid atherosclerosis observed in the present study was rather unexpected. In the present study, however, TC levels were not significantly different between HBsAg-positive and HBsAg-negative subjects. In addition, Lp(a) data for >90% of the study subjects were available, and Lp(a) levels in the HBsAg-positive (n=39) and HBsAg-negative (n=4317) subjects were 13.9±16.1 mg/dL and 16.5±15.4 mg/dL, respectively, and did not differ significantly. These observations may be explained by the fact that liver dysfunction in the majority of the HBsAg-positive subjects was minor, a possibility that is supported by the finding that serum levels of aspartate transaminase and alanine transaminase were not different between HBsAg-positive and HBsAg-negative subjects. Kiechl et al[3] found no significant association between chronic hepatitis and the carotid atheromatous plaque, although whether each subject had hepatitis B or C was not specified. The different conclusions may be obtained because they analyzed the possible link between chronic active hepatitis and atherosclerosis,[3] whereas most subjects were generally healthy in our study.

Several previous studies have suggested that some microorganisms may contribute to the pathogenesis of atherosclerosis; including *Chlamydia pneumoniae*,[10] cytomegarovirus,[11] *Helicobacter pylori*,[12] and herpes simplex virus.[13] They may cause vascular injury by direct colonization[1] and activation of inflammatory response, which may play a role in the progression and destabilization of atherosclerotic plaques. Similarly, several possible mechanisms may exist to explain the relationship between HBV infection and atherosclerosis. First, HBV may colonize in the vascular tissues,[14] leading to vascular damage. Second, HBV infection occasionally is associated with vasculitis.[15] Third, chronic HBV infection may be associated with increased levels of oxidative stress,[16] which may accelerate atherogenesis. Finally, chronic HBV infection may stimulate inflammatory and immune-mediated responses.

It recently has been recognized that HBV might be detectable in some HBsAg-negative subjects by a more sensitive assay, the HBV DNA detection test.[17] Although this technique is not practical for general health-screening tests from an economic standpoint, the link between HBV DNA-positivity and atherosclerosis should be clarified in future studies.

In conclusion, a general population-based cohort study showed HBsAg positivity to be a risk factor for carotid atherosclerosis that was independent from other confounding risk factors. Whether this association can be observed in other countries where prevalence of HBsAg carrier is greater[18] is a disputed issue.

References

1. Chiu B, Viira E, Tucker W, et al. Chlamydia pneumoniae, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. *Circulation*. 1997;96:2144–2148.
2. Zhu J, Quyyumi AA, Norman JE, et al. The possible role of hepatitis A virus in the pathogenesis of atherosclerosis. *J Infect Dis*. 2000;182: 1583–1587.
3. Kiechl S, Egger G, Mayr M, et al. Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. *Circulation*. 2001;103:1064–1070.
4. Ludewig B, Freigang S, Jaggi M, et al. Linking immune-mediated arterial inflammation and cholesterol-induced atherosclerosis in a transgenic mouse model. *Proc Natl Acad Sci U S A*. 2000;97:12752–12757.
5. Ishizaka N, Ishizaka Y, Takahashi E, et al. Association between hepatitis C seropositivity, carotid artery plaque and intima-media thickening. *Lancet*. 2002;359:133–135.
6. Ishizaka N, Ishizaka Y, Takahashi E, et al. High serum bilirubin level is inversely associated with the presence of carotid plaque. *Stroke*. 2001; 32:580–58
7. Hashimoto H, Kitagawa K, Hougaku H, et al. C-reactive protein is an independent predictor of the rate of increase in early carotid atherosclerosis. *Circulation*. 2001;104:63–67.
8. Matsuo A, Kusumoto Y, Ohtsuka E, et al. Changes in HBsAg carrier rate in Goto Islands, Nagasaki Prefecture, Japan. *Lancet*. 1990;335:955–957.
9. Marchesini G, Ronchi M, Forlani G, et al. Cardiovascular disease in cirrhosis: a point-prevalence study in relation to glucose tolerance. *Am J Gastroenterol*. 1999;94:655–662.
10. Saikku P, Leinonen M, Mattila K, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet*. 1988;2:983–986.
11. Adam E, Melnick JL, Probtsfield JL, et al. High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. *Lancet*. 1987;2:291–293.
12. Mendall MA, Goggin PM, Molineaux N, et al. Relation of *Helicobacter pylori* infection and coronary heart disease. *Br Heart J*. 1994;71: 437–443.
13. Yamashiroya HM, Ghosh L, Yang R, et al. Herpesviridae in the coronary arteries and aorta of young trauma victims. *Am J Pathol*. 1988;130:71–79.
14. Mason A, Wick M, White H, et al. Hepatitis B virus replication in diverse cell types during chronic hepatitis B virus infection. *Hepatology*. 1993; 18:781–789.
15. Guillevin L, Lhote F, Gherardi R. The spectrum and treatment of virus-associated vasculitides. *Curr Opin Rheumatol*. 1997;9:31–36.
16. Sumida Y, Nakashima T, Yoh T, et al. Serum thioredoxin elucidates the significance of serum ferritin as a marker of oxidative stress in chronic liver diseases. *Liver*. 2001;21:295–299.
17. Brechot C, Thiers V, Kremsdorf D, et al. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology*. 2001;34:194–203.
18. Kajiwara E, Akagi K, Ueda K, et al. Prevalences of hepatitis B surface antigen carriers and liver damages in the general population of Hisayama, Japan. *Fukuoka Igaku Zasshi*. 1992;83:257–61.