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A Case-Control Study on the Risk Factors of Hepatitis C Virus Infection among Koreans

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In order to identify the risk factors for hepatitis C virus(HCV) infections, a case-control study was conducted from September 1993 to April 1994. HCV infection was confirmed by the second generation of recombinant immunoblot assay. Sixty-four cases and 128 controls matched for age and sex with a 1 : 2 ratio of cases to controls were enrolled. Exposure data were obtained from all participants by self-administered questionnaire and the odds ratios of possible risk factors of HCV infection analysed. Sixty-four cases consisted of forty-two patients with chronic hepatitis, nine with cirrhosis, one with hepatocellular carcinoma, and twelve with normal liver function. History of acute hepatitis(OR 3.9) and transfusion(OR 2.4) were associated with an increased risk of HCV infection. Operation, acupuncture, endoscopy, tooth extraction, tattooing, ear piercing, needle sharing and family history of hepatitis were not associated with an increased risk of HCV infection. In conclusion, transfusion remains the major route of transmission of HCV in Korea.

Key Words : *Hepatitis C virus, Risk factor, Recombinant immunoblot assay*

INTRODUCTION

Chronic liver disease is the most common cause of non-surgical morbidity for inpatients in Korea(KMIC, 1993). An estimated 11,000 deaths occur each year as a result of chronic hepatitis and cirrhosis, and more than 9,000 occur as a result of liver cancer(NSO, 1992). Seroprevalences of anti-HCV are different according to the author ; 0.9 %(Kim et al., 1990), 0.5 %(Jung et al., 1991), 0.6 %(Kim et al., 1993) in blood donors and 1.7 % in healthy adults without symptomatic liver disease(Kim et al., 1992). In patients with chronic liver disease, 15-30 % were positive for anti-HCV(Chi et al., 1990 ; Park et al., 1991 ; Chang et al., 1992).

Since 1990 blood donors and patients with liver disease have been systematically tested for hepatitis C virus antibody(anti-HCV) with enzyme linked immunoabsorbent assay(ELISA). However, anti-HCV ELISA test has shown low specificity and low positive predictive value in several studies(Van Der Poel et al., 1991 ; Kim et al., 1993), especially in blood donors and low risk populations. As a result, early studies of HCV infection on the basis of anti-HCV ELISA were

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probably biased by the proportion of false positive results, which led to false increase in the prevalence of HCV infection and null effect in the risk factor analysis.

History of acute hepatitis, transfusion, and acupuncture were associated with an increased risk of HCV infection in a recent study(Kim et al., 1994), which was not confirmed by any confirmatory tests.

The purpose of this study was to identify the risk factors for HCV infection which was confirmed by the second-generation recombinant immunoblot assay(RIBA-2) test results.

MATERIALS AND METHODS

Case group

From September 1993 to February 1994 serum samples from 180 anti-HCV positives who were tested with the second generation anti-HCV ELISA at Asan Medical Center were stored at −60℃. All the serum samples were tested for confirmation with RIBA-2. One hundred and fourteen(63.3 %) were positive, 8(4.4 %) were indeterminate and 58(32.2 %) were negative for RIBA-2 test. All RIBA-2 positive and indeterminate patients were informed of their test results by mail and were invited to participate in this study. Sixty-four(52.5 %) responded to our invitation between March and April 1994. Sixty-one of the responders were RIBA-2 positive and three had indeterminate results. Three patients with indeterminate RIBA-2 results were confirmed by Polymerase chain reaction(PCR). HCV-RNA was detected in all three patients with indeterminate results. Finally, 64 subjects were included in this study as a case group(Fig. 1).

Control group

Of the 1,000 health examinees, 766 responded to our routine self administrative questionnaire survey at the department of Family Medicine, Asan Medical Center from September, 1993 to February, 1994. Out of which, 703 were selected for a control base because they were both negative for HBsAg and anti—HCV with normal liver function. Finally, 128 controls matched for age(within ± 3 years) and sex with a 1 : 2 ratio of cases to controls were selected-(Fig. 2).

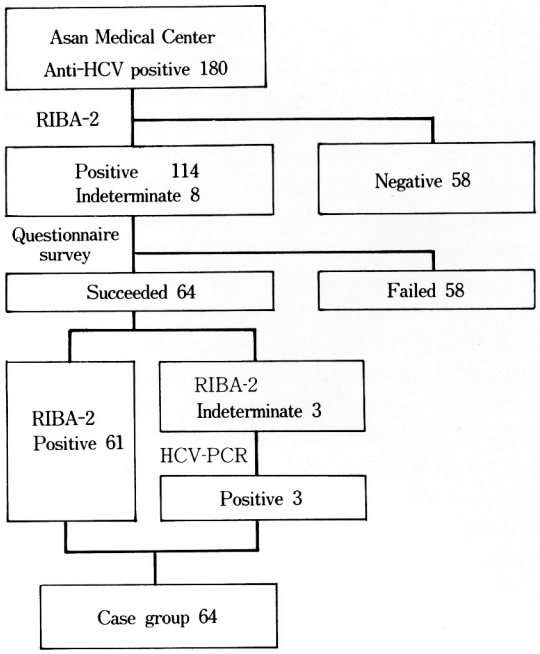

Fig. 1. Schematic presentation of cases selection.

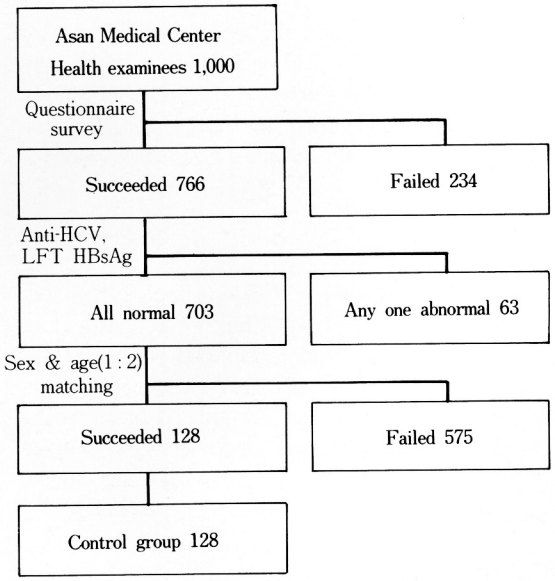

Fig. 2. Schematic presentation of controls selection.

Laboratory Tests

All 64 cases and the 703 control base were tested for serum aspartate aminotransferase(AST) and alanine aminotransferase(ALT). AST and ALT activities were considered elevated when both values were greater than 40 IU/L. HBsAg was tested by radioimmunoassay(AUSRIA, Abbott Laboratories). All 64 cases were tested for second generation anti-HCV ELISA(Abbott Laboratories USA) and RIBA-2(Ortho Diagnostics USA). Anti-HCV was considered positive when the ELISA ratio was greater than 1.0. RIBA-2 test was completed under the manufacturer's instructions. Sera reacting with more than two of the four antigens were taken as positive, those reacting with just one antigen as indeterminate, and sera that did not react were negative. Also, sera reacting with one or more of the four antigens and superoxide dismutase were taken as indeterminate, and sera that reacted only with superoxide dismutase were negative. Three cases with indeterminate RIBA-2 results were confirmed by reverse transcribed polymerase chain reaction(RT-PCR). For the detection of hepatitis C viral sequences serum RNA was extracted, reverse-transcribed, and amplified by nested PCR method. PCR amplication was first performed using sense primer 5'-CTGTGAGGAACTACTGTCTT-3' (nucleotides 28-47) and antisense primer 5'-AACACTACTCGGCTAGCAGT-3'(nucleotides 229-248), and second by sense primer 5'-TTCACGCAGAAAGCGTCTAG-3' (nucleotides 46-65) and antisense primer 5'-GTTGATCCAAGAAAGGACCC-3' (nucleotides 171-190).

Exposure data

Data were obtained from all participants by self-administered questionnaire. The questionnaire included questions on demographic and socioeconomic characteristics such as education, income level, marital status, alcohol and smoking, and on past medical histories such as operations, blood transfusions, acute hepatitis, dental procedures, acupuncture use, endoscopy, tattoos, ear piercing and needle sharing.

Statistical Analysis

We examined the odds ratios of possible risk factors for HCV infection confirmed by RIBA-2 with X^2 test or Fisher's exact test. Factors that were significant on univariate analysis were examined with Mantel Haenszel method and Logistic Regression using the Statistical Analysis System for personal computers(6.04).

RESULTS

Positive predictive value of anti-HCV ELISA

Out of 180 anti-HCV positives, 114 were positive and 8 were indeterminate for RIBA-2 test. Overall positive predictive value of ELISA including indeterminate case was 68 %.

Characteristics of cases and controls

Sixty-four cases who were diagnosed clinically consisted of forty-two patients with chronic hepatitis, nine with cirrhosis, one with hepatocellular carcinoma, and twelve with normal liver function tests(Table 1). Controls were comparable to cases for several variables as seen in Table 2, but the former had higher levels of education and income than the latter.

Risk factors associated with HCV infection

History of acute hepatitis(OR 3.9) and transfusion(OR 2.4) were associated with an increased risk of HCV infection(Table 3). Operation, acupuncture, endoscopy, tooth extraction, tattooing, ear piercing, needle sharing and family history of hepatitis were not associated with an increased risk of HCV infection.

DISCUSSION

By selecting the cases and susceptibles to HCV infection from a group of patients during a defined period who are assigned to same hospital, we designed a hospital based case-control study. Although the controls were higher socioeconomic status than the cases in the aspects of education and income, no significant differences exist between the two groups with respect to demographic and other general char-

Table 1. Clinical diagnoses of the cases.

Diagnosis	No.
Chronic hepatitis	42
Normal Liver Function	12
Cirrhosis	9
Hepatocellular carcinoma	1
Total	64

Table 2. Distribution of demographic variables by case and control groups.

Variables		Cases No.(%)	Controls No.(%)
Sex	male	40(62.5)	80(62.5)
	female	24(37.5)	48(37.5)
Age	<40	9(14.1)	18(14.1)
	40-49	9(14.1)	18(14.1)
	50-59	28(43.8)	56(43.8)
	≧60	18(28.1)	36(28.1)
Marriage	married	63(98.4)	111(95.7)
	never married	1(1.6)	5(4.3)
Years of education	<12	28(43.8)	34(28.1)*
	12	10(15.6)	38(31.4)
	>12	26(40.6)	49(40.5)
Income/ month	<1 mill.#	21(36.2)	20(17.0)*
	1-3 mill.	28(48.3)	68(57.6)
	>3 mill.	9(15.5)	30(25.4)
Smoking	smoking	15(23.4)	41(34.2)
	non-smoking	49(76.6)	79(65.8)
Alcohol	drinker	21(32.8)	64(53.8)
	non-drinker	43(67.2)	55(46.2)

million won
* P<0.05

acteristics. As such, these groups are validly comparable to each other. The advantage of this study was to exclude the false positive cases, which led to a nullifying effect in the risk factor analysis.

All controls were anti-HCV ELISA negative, therefore HCV-infected subjects who were in the window period of 30 days to 90days(Dienstag et al., 1994) might be included. Because the false-negative rate of second generation enzyme linked immunoassay is only 0-2 %(Silva et al., 1994), it is unlikely that this had a significant effect on these results. HBsAg positive subjects were excluded from the control group in order to rule out the confounding effects of HBV infection.

According to our data on the test results of anti-HCV ELISA, the positive predictive value of the test method is low, 68 % in this study. But the rate was higher than in blood donors(Kim et al., 1993 ; Serfaty et al., 1993). The ELISA-positive, RIBA-2 negative donors should be considered to have false-positive results for a number of reasons. Van Der Poel et al.(1991) found that none of the ELISA-positive, RIBA-2 negative donors had posttransfusion hepatitis and that all were negative for viral RNA in a PCR-based assay. It therefore appears that a negative RIBA-2 test in an ELISA-positive donor is indicative of false positivity. An indeterminate RIBA-2 test result can have several meanings. In this study, all three indeterminate cases were positive results for PCR-based testing of HCV RNA.

Intrafamilial transmission of HCV is still controversial. Intrafamilial transmission of HCV was reported by Ideo et al.(1990) and Kiyosawa et al.(1991) but not by Everhart et al.(1990) and Kim et al.(1994).

Although Ideo et al.(1990) and Kiyosawa et al.(1991) found intra-familial transmission of HCV the differences between cases and controls were much smaller than those of HBV infection. Perinatal transmis-

Table 3. Distribution of potential risk factors by case and control groups.

Variables	Cases No.(%)	Controls No.(%)	Crude OR	Adjusted OR•	95% CI
Total	64(100.0)	128(100.0)			
Operation	32 (50.0)	44 (35.8)	1.8		
Transfusion	22 (34.4)	20 (17.1)	2.5*	2.4	(1.1—5.2)
History of acute hepatitis	15 (24.6)	8 (7.3)	4.1**	3.9	(1.4—10.7)
Endoscopy	45 (70.3)	72 (59.0)	1.6		
Tooth extraction	45 (71.4)	85 (70.3)	1.1		
Acupuncture	46 (73.0)	97 (79.5)	0.7		
Tattooed	9 (14.3)	15 (12.8)	1.1		
Pierced ear	15 (23.4)	20 (16.8)	1.5		
F.H.# of hepatitis	9 (14.5)	22 (18.6)	0.7		
Needle sharing	3 (4.7)	3 (2.6)	1.8		

*P<0.05 **P<0.01
Family History
• Mantel Haenszel estimates

sion of HCV was found in the studies by Wejstal et al.(1990) and others(Kamitsukasa et al., 1989 ; Kuroki et al., 1991). However, Reinus et al.(1992) and Novati et al.(1992) suggested passive transfer of anti-HCV rather than vertical transmission as the explanation for this finding. While mother to child transmission of HCV may be possible(Kuroki et al., 1991) it may not be as important a route as in the transmission of HBV(Kim et al., 1994). Spouse to spouse transmission of HCV was reported by Kamitsukasu et al.(1989) but not by Everhart et al.(1990) and Kiyosawa et al.(1991). Having multiple heterosexual partners was reported as a risk factor for sexual transmission of HCV(McHutchison et al., 1992) but homosexuality was not(Melbye et al., 1990). Such controversial results might be due to the low possibility of sexual transmission of HCV in view of the report by Hsu et al.(1991) in which HCV was not found in semen or saliva. Thus, intrafamilial transmission of HCV which includes all forms of close contacts(i.e. mother to child and sexual), is not as common as in HBV infection and does not manifest in familial clustering. Therefore the parenteral route remains the major route of transmission of HCV.

In our study, transfusion, as the classic form of parenteral transmission, has an odds ratio of 2.4 and is consistent with findings by Esteban et al.(1991) and Serfaty et al.(1993). History of acute hepatitis is found to be a significant risk factor, which is consistent with the findings of Diodati et al.(1991) and Serfaty et al.(1993).

Acute hepatitis is a non-specific risk factor for HCV infection and may be affected by recall bias. It is, however, useful to suggest HCV infection which is diagnosed only by ELISA without confirmatory test in the clinical practice. Acupuncture is not found to be a risk factor of HCV infection. This finding was different from various reports in the literature(Alexis et al., 1988 ; Kim et al., 1994) which considered acupuncture as a significant route of transmission of hepatitis C virus. It deserves further study in the future. Lee et al.(1991), McHutchison et al.(1992) and Serfaty et al.(1993) report intravenous drug use as a significant route of transmission. Our study was not able to adequately evaluate needle sharing as a risk factor due to the very low incidence of needle sharing in both cases and controls. We also attempted to evaluate tattooing as a possible risk factor as reported by Ko et al.(1992). However tattooing was not found to be a risk factor.

Some limitations of our study are ; first, the present control population may not be comparable to the case group in the aspects of education and economic status. Second, the study was not designed to examine homosexuality, although the size of this population is known to much smaller in Korea. Even with these limitations, we are able to conclude that transfusion remains the major route of transmission of HCV in Korea.

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