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Risk factors associated with hepatitis C virus infection in Taiwanese government employees

H. C. CHANG[1], M. W. YU[1]*, C. F. LU[2], Y. H. CHIU[1] AND C. J. CHEN[1]

[1] *Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan*
[2] *Center for Disease Control, Department of Health, Executive Yuan, Taipei, Taiwan*

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SUMMARY

This study evaluated the roles of multiple factors in hepatitis C virus (HCV) infection, with emphasis on the modification of various individual characteristics on the risk associated with percutaneous exposure to blood. Serum samples taken from 4869 men in Taiwan within a cohort study were tested for HCV antibody. The overall positive rate of anti-HCV was 1·6%. In a logistic regression, factors positively associated with anti-HCV positivity were previous blood transfusion (odds ratio [OR] = 7·28; 95% confidence interval [CI] = 4·26–12·45), a history of surgery (OR = 2·06; 95% CI = 1·23–3·46), and lower educational levels (OR = 1·94; 95% CI = 1·14–3·32). The anti-HCV positive rate was significantly lower in hepatitis B surface antigen (HBsAg) carriers than in non-carriers (OR = 0·60; 95% CI = 0·37–0·95). Ageing, lower educational levels, O blood group, and Taiwanese ethnicity enhanced the likelihood of HCV infection through blood transfusion/surgery, whereas HBsAg status, cigarette smoking, and habitual alcohol drinking reduced it.

INTRODUCTION

Most individuals exposed to hepatitis C virus (HCV) develop chronic infection [1]. Infection with HCV is a major cause of chronic liver disease and accounts for a substantial proportion of death worldwide [1–3]. The prevalence of HCV antibody (anti-HCV) among healthy control subjects or unselected blood donors in most countries ranges between 1% and 2% [2]. The risk of transmission of HCV varies according to the routes of infection. Epidemiological studies show that the most efficient transmission of HCV is through the transfusion of blood [4]. Differences in the risk of HCV transmission by the transfusion of blood may be related to the amounts of blood transfusion, the titre of virus and the host susceptibility to infection. There are insufficient data on the threshold concentration of virus needed to transmit or cause infection. Little is known about the host susceptibility factors that may modify the efficiency of HCV transmission.

Variations in immune response to an infectious agent are often associated with age and polymorphism of the major histocompatibility complex. The role of the histocompatibility antigens in the outcome of HCV infection has been examined, but no firm conclusion has been reached [5, 6]. A synergistic effect on the development of hepatocellular carcinoma between anti-HCV positivity and alcohol and cigarette use was observed in our previous case-control study [7]. High rates of HCV infection determined by the status of anti-HCV and/or the level of HCV RNA in serum have been found in alcoholic patients with chronic liver disease [8–10]. Moreover, the titre of viral RNA in serum has been reported to be substantially higher among the patients with chronic hepatitis C who were habitual alcohol drinkers [11]. The effect of cigarette smoking on immunity to HCV

* Author for correspondence: Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Rm. 1550 No. 1 Jen-Ai Rd., Sec. 1, Taipei 100, Taiwan.

has not been stressed in previous investigations. However, there is evidence that cigarette smoking may impair the immune system [12]. These facts arouse concern about whether factors other than the routes of transmission and titre of virus may play a role in the transmission of HCV.

Taiwan is an area hyperendemic for hepatitis B virus (HBV) infection. The prevalence of chronic HBV infection in Taiwan among persons born before the initiation of a nationwide HBV vaccination programme among newborns in 1984 can reach as high as 15–20% [7]. The predominant mode of HBV transmission in Taiwan is perinatal [13]. The high prevalence of chronic HBV infection in Taiwan provides a unique opportunity for investigation of chronic HBV carrier status on the risk of HCV transmission. This study used data on a large number of asymptomatic chronic HBV carriers and non-carriers within a cohort study. We examined the associations between multiple factors and status of anti-HCV, with emphasis on the interactions of various host factors with percutaneous exposure to blood by blood transfusion or surgery in the transmission of HCV.

METHODS

Study population

This study is part of an ongoing prospective study that was undertaken to examine the role of aflatoxin exposure, antioxidant micronutrients, and other factors in the origin of hepatocellular carcinoma (HCC) [14]. The population in the present study consisted of 5375 (2874 HBsAg carriers and 2501 non-carriers) men aged 30–65 years who attended the Government Employee Central Clinics in Taiwan for regular health examination between August 1989 and June 1992. At entry into this cohort study, each study participant was personally interviewed by well-trained research assistants according to a structured questionnaire containing items of various sociodemographic characteristics, habits of cigarette smoking and alcohol drinking, as well as past histories of blood transfusion, surgery and major chronic diseases. A total of 10 ml blood samples were also collected from each study participant. Serum samples were separated on the same day as blood collection and kept frozen at −70 °C until subsequent analyses. Subjects for whom there were no available serum samples for anti-HCV assay (119 subjects) or no data on past histories of blood transfusion or surgery (387 subjects) in the baseline questionnaire were excluded. A total of 4869 study subjects remained available for analysis in this study. Comparisons of data from subjects included in this study with those from subjects excluded showed that the two groups were similar in terms of age, ethnic groups, educational level, habits of cigarette smoking and alcohol drinking, and chronic liver disease.

Serological testing

All study participants were tested for HBsAg by a radioimmunoassay (Abbott Laboratories, North Chicago, IL) at recruitment. Their serum samples were tested for anti-HCV between June and October 1993. Assays for anti-HCV were performed under code using a second-generation enzyme-linked immunoassay (Abbott Laboratories, North Chicago, IL) measuring reactivity to nonstructural HCV antigens (C100-3 and C33c) and the HCV core protein (C22) at the National Institute of Preventive Medicine. It has been reported that the use of anti-HCV enzyme-linked immunoassay in large populations with a relatively low prevalence of infection may yield false-positive results [15]. The non-specificity in the enzyme-linked immunoassay has been attributed to various factors, including hyperglobulinemia and prolonged storage and repeated cycles of thawing and freezing of serum samples [16–18]. Because using a higher enzyme-linked immunoassay ratio of 2·0 or above as the cut-off value was reported to be able to discriminate specific from non-specific anti-HCV reactivity [19, 20], specimens with OD values that were two or more times higher than cut-off limit (usually around 0·42 OD) were considered reactive. Reactive samples were retested in duplicate and were only considered positive if at least 2 of 3 test determinations were reactive.

Statistical analysis

The associations between anti-HCV status and categorical variables were assessed with the unadjusted chi-square statistic. The odds ratio (OR) and its 95% confidence interval (CI) were computed to compare the risk of infection with HCV between levels of categorical variables. A logistic regression model was used in the multivariable modelling of associations. We fitted the model by use of the LOGISTIC

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Table 1. *Detection rate of anti-HCV in 4869 study subjects by various individual characteristics*

Variable	Group	No. tested	Anti-HCV positive No. (%)	Unadjusted OR (95% CI)*
Age (year)	30–39	1594	17 (1·1)	1·00
	40–49	1842	27 (1·5)	1·38 (0·75–2·54)
	50–59	932	21 (2·3)	2·14 (1·12–4·07)
	60–65	501	11 (2·2)	2·08 (0·97–4·47)
Education	College & above	3985	52 (1·3)	1·00
	Senior high school & below	879	24 (2·7)	2·12 (1·30–3·46)
	Missing	5	0	
HBsAg	Negative	2254	44 (2·0)	1·00
	Positive	2615	32 (1·2)	0·62 (0·40–0·98)
History of surgery	No	3056	27 (0·9)	1·00
	One time	1487	39 (2·6)	3·02 (1·84–4·96)
	Two times	257	7 (2·7)	3·14 (1·35–7·29)
	⩾ Three times	69	3 (4·4)	5·10 (1·51–17·23)
History of blood transfusion	No	4590	49 (1·1)	1·00
	Yes	279	27 (9·7)	9·93 (6·11–16·13)
Cigarette smoking	No	3387	59 (1·7)	1·00
	Yes	1482	17 (1·2)	0·66 (0·38–1·13)
Alcohol drinking	No	3819	63 (1·7)	1·00
	Yes	1050	13 (1·2)	0·75 (0·41–1·36)
Blood group	B	1151	12 (1·0)	1·00
	AB	336	4 (1·2)	1·14 (0·37–3·57)
	A	1304	17 (1·3)	1·25 (0·60–2·64)
	O	2078	43 (2·1)	2·01 (1·05–3·82)
Ethnic group	Mainland Chinese	1392	15 (1·1)	1·00
	Fukien Taiwanese	2887	50 (1·7)	1·62 (0·91–2·89)
	Hakka Taiwanese	590	11 (1·9)	1·74 (0·80–3·82)

* OR, odds ratio; CI, confidence interval.

procedure of the statistical package PC-SAS version 6·12. Tests for categorical trend were performed using the Mantel's χ^2 test for a trend. All statistical tests were based on two-tailed probability.

RESULTS

Of the 4869 study subjects, only 198 (4·1%) had a history of clinical chronic liver disease. Among the subjects with a history of clinical chronic liver disease, 88% were positive for HBsAg alone, 2% were positive for anti-HCV alone, and 2% were positive for both HBsAg and anti-HCV. Only 29 (14·6%) of the 198 subjects reported a habit of alcohol drinking. The median quantity of alcohol consumed by these habitual alcohol drinkers was 66·8 g per week. Overall, 1·6% of the study subjects were anti-HCV positive. Table 1 shows the positive rate of anti-HCV by various characteristics of subjects. Approximately 36% of anti-HCV positive subjects reported a history of blood transfusion. A history of transfusion was the most significant risk factor associated with anti-HCV positivity (OR = 9·93, 95% CI = 6·11–16·13). There was also a significant association between a history of surgery and anti-HCV positivity.

As expected, increasing age was a significant risk factor for HCV infection. Lower educational levels were associated with a greater likelihood of anti-HCV positivity (OR = 2·12; 95% CI = 1·30–3·46; for senior high school and below *vs.* college and above). Compared with subjects with B blood group, those who carried the O blood group had a significantly higher detection rate of anti-HCV (OR = 2·01; 95% CI = 1·05–3·82). The anti-HCV positive rate was significantly lower among HBsAg carriers than among non-carriers (OR = 0·62; 95% CI = 0·40–0·98). The anti-HCV positivity rate was lower in cigarette smokers than in nonsmokers, but this association was

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Table 2. *Multivariable analysis of the potential risk factors for HCV infection**

Variable	Group	Multivariable OR (95% CI)†	*P* value
Age (year)	30–39	1·00	
	40–49	1·23 (0·66–2·30)	0·5151
	50–59	1·40 (0·71–2·77)	0·3300
	60–65	1·37 (0·57–3·28)	0·4833
Education	College & above	1·00	
	Senior high school & below	1·94 (1·14–3·32)	0·0153
HBsAg	Negative	1·00	
	Positive	0·60 (0·37–0·95)	0·0306
History of surgery	No	1·00	
	Yes	2·06 (1·23–3·46)	0·0064
History of blood transfusion	No	1·00	
	Yes	7·28 (4·26–12·45)	0·0001
Cigarette smoking	No	1·00	
	Yes	0·59 (0·33–1·06)	0·0761
Alcohol drinking	No	1·00	
	Yes	0·77 (0·41–1·45)	0·4177
Blood group	B	1·00	
	AB	1·35 (0·42–4·28)	0·6123
	A	1·22 (0·57–2·60)	0·6101
	O	1·89 (0·98–3·65)	0·0569
Ethnic group	Mainland Chinese	1·0	
	Fukien/Hakka Taiwanese	1·80 (0·96–3·39)	0·0677

* Five subjects with missing data on educational levels were excluded from the analysis.
† OR, odds ratio; CI, confidence interval.

not statistically significant. Nonsignificantly inverse association with HCV infection was also found for alcohol drinking. The frequency of anti-HCV positivity was about 1·5 times higher among Fukien and Hakka Taiwanese than among mainland Chinese. Despite of a large number of study subjects, the number of anti-HCV-positive study subjects was not large enough to obtain a relatively precise relative risk estimate in stratified analysis. No significant interactions between various factors were found based on multiplicative models.

Results of logistic regression analysis of multiple risk factors with anti-HCV status are shown in Table 2. Adjustment for histories of blood transfusion and surgery substantially attenuated the association between age and HCV infection. As expected, a history of blood transfusion was the risk factor most highly correlated with HCV infection after multivariable adjustment. Other factors significantly associated with HCV infection were a history of surgery, lower educational levels and HBsAg carrier status. O blood group ($P = 0{\cdot}0569$), Taiwanese ethnicity ($P = 0{\cdot}0677$), and cigarette smoking ($P = 0{\cdot}0761$) were associated with anti-HCV positivity at a borderline significance level. In the study subjects, 38% had previous blood transfusion and/or surgery. The OR of HCV infection associated with a history of blood transfusion and/or surgery was estimated as 3·31 (95% CI = 2·03–5·40). Through the computation of population-attributable risk percentage, we estimated that approximately 46·7% of men infected by HCV in this study population could be attributable to blood transfusion and/or surgery.

Table 3 shows the anti-HCV positivity by a history of blood transfusion/surgery and various individual characteristics. In the absence of a history of blood transfusion/surgery, age, blood group O, ethnicity, HBsAg status, and alcohol drinking were not significantly associated with anti-HCV positivity prevalence. The associations of the positivity for anti-HCV with educational levels ($P = 0{\cdot}0502$) and the habit of cigarette smoking ($P = 0{\cdot}0650$) only approached a statistically marginal significance level. However, the ORs suggest an interaction of multiple host factors with a history of blood transfusion and/or surgery in HCV infection. While older ages, lower educational levels, O blood group, and Taiwanese ethnicity increased the likelihood of HCV infection associated

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Table 3. *Joint effects of a history of surgery/blood transfusion with various individual characteristics on the risk of HCV infection*

Variable	No. tested	Anti-HCV positive rate (%)	Unadjusted OR (95% CI)†
Age/history of surgery or transfusion			
30–49/no	2262	0·84	1·00‡
50–65/no	735	0·82	0·97 (0·39–2·44)
30–49/yes	1174	2·13	2·57 (1·41–4·68)
50–65/yes	698	3·72	4·57 (2·51–8·30)
Education/history of surgery or transfusion*			
College and above/no	2486	0·68	1·00‡
Senior high school and below/no	507	1·58	2·33 (1·00–5·43)
College and above/yes	1499	2·33	3·47 (1·94–6·22)
Senior high school and below/yes	372	4·30	6·53 (3·28–13·00)
Blood group/history of surgery or transfusion			
Non-O/no	1703	0·70	1·00‡
O/no	1294	1·00	1·43 (0·65–3·14)
Non-O/yes	1088	1·93	2·77 (1·36–5·66)
O/yes	784	3·83	5·61 (2·86–11·00)
Ethnic group/history of surgery or transfusion			
Mainland Chinese/no	766	0·52	1·00‡
Fukien or Hakka Taiwanese/no	2231	0·94	1·81 (0·62–5·28)
Mainland Chinese/yes	626	1·76	3·41 (1·08–10·74)
Fukien or Hakka Taiwanese/yes	1246	3·21	6·32 (2·26–17·71)
HBsAg status/history of surgery or transfusion			
Positive/no	1624	0·68	1·00‡
Negative/no	1373	1·02	1·51 (0·68–3·34)
Positive/yes	991	2·12	3·18 (1·52–6·61)
Negative/yes	881	3·41	5·17 (2·58–10·36)
Cigarette smoking/history of surgery or transfusion			
Yes/no	890	0·34	1·00‡
No/no	2107	1·04	3·12 (0·93–10·45)
Yes/yes	592	2·36	7·16 (2·05–25·03)
No/yes	1280	2·89	8·80 (2·71–28·63)
Alcohol drinking/history of surgery or transfusion			
Yes/no	635	0·47	1·00‡
No/no	2362	0·93	1·98 (0·59–6·62)
Yes/yes	415	2·41	5·20 (1·43–18·97)
No/yes	1457	2·81	6·10 (1·89–19·72)

* Five subjects with missing data on educational levels were excluded from the analysis.
† OR, odds ratio; CI, confidence interval.
‡ $P < 0·0001$, test for trend.

with a history of blood transfusion or surgery, HBsAg carrier status, cigarette smoking, and alcohol drinking reduced it.

DISCUSSION

Although HCV infection is a major aetiological factor for chronic liver disease worldwide, it is a rare event in most populations [2]. The large sample size of this study allowed us to obtain conclusive evidence about the associations between multiple factors and HCV infection in Taiwan.

The risk factors for exposure to HCV have been studied. These factors were a history of blood transfusion, intranasal cocaine use, intravenous drug use, and sexual promiscuity [4]. Limited data suggest that HCV infection may occur through perinatal transmission or occupational exposure to contaminated blood by needlestick [21, 22]. An approximately 80 times higher anti-HCV prevalence in parenteral

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drug users than in volunteer blood donors has been reported in Taiwan [23]. However, there are no data regarding the proportion of HCV infections attributable to injecting drug behaviours. The subjects in this study were male government employees. They had higher educational status than the general population in Taiwan. The proportion of subjects having a habit of cigarette smoking or alcohol drinking in this study was also observed to be much lower than the general male population in this area [7]. Although the questionnaire used in the study did not include any information on past/current drug behaviour or sexual promiscuity, it is reasonable to assure that the prevalence of such risky behaviours associated with HCV infection were low in our study population. In a recent case-control study involving 272 anti-HCV positive subjects and 282 negative controls identified among 11904 men who were residents in seven townships of Taiwan, multivariable analysis showed that significant risk factors associated with anti-HCV positivity included blood transfusions, medical injections, acupuncture and tattooing. This study estimated that 57% of HCV infections in this population were attributable to medical injections and 25% were attributable to blood transfusions. No data on a history of surgery were collected in this study [24]. In our study, previous transfusion and surgery appeared to be the two risk factors most important for exposure to HCV. A nationwide screening of blood donors for HCV infection began in July 1992 in Taiwan. Blood samples from study subjects were collected between August 1989 and June 1992 in this study. Through the computation of population-attributable risk percentage, we estimated that 46·7% of men infected with HCV could be attributable to blood transfusion and/or surgery.

HCV generally circulates in low titres in infected serum. The outcome of its infection depends on the interplay between the virus and host immune response [25]. A fraction of infected persons develop transient infection despite the high overall rate of chronicity of HCV infection [26, 27]. The precise mechanism of HCV chronicity is not well understood currently. It is likely that certain host factors may define the pathogenesis of HCV. However, little attention has been paid to the potential role of host factors in the HCV infection. Although this study demonstrated that there were statistical interactions between several host factors and a history of transfusion or surgery in the risk of HCV infection, the precise mechanism involving the roles of various individual characteristics in the infection, perhaps the chronicity of this viral infection, remained to be elucidated.

It has been reported that the anti-HCV positivity prevalence increases progressively with age [1]. The significance of ageing on HCV infection may reflect the increased risk for exposure to HCV and/or the repeated exposure to HCV. In this study, increasing age was only significantly associated with anti-HCV positivity in the univariate analysis. This association was no longer statistically significant when adjustment was made for a history of transfusion or surgery. The effect of increasing age on the risk of HCV infection may be simply due to an increased risk for exposure to HCV through blood transfusion or surgery. However, the strong interaction observed between increasing age and a history of transfusion or surgery in the risk of HCV infection suggests that age itself may have an effect on susceptibility to HCV infection.

HCV replication has been reported to be suppressed by active HBV replication among patients with chronic hepatitis B [28]. In our study population, the risk of HCV infection by blood transfusion or surgery depended on the HBsAg carrier status. The HBsAg carriers appeared to have a lower risk of HCV infection associated with blood transfusion or surgery than the non-carriers. HCV core protein has been found to have a trans-suppressing effect on HBV expression and replication in cultured cells [29]. In humans, HCV superinfection may terminate the HBsAg carrier state during chronic HBV infection [30]. Most HBsAg carriers in Taiwan acquire infection through perinatal transmission [13]. Since there may be HBsAg carriers who experience the clearance of HBsAg after superinfection with HCV, the prevalence of HCV infection in the HBsAg non-carriers may be overestimated. However, the degree of the over-estimation is believed to be relatively low because of the infrequency of the dual infection for HBV and HCV (HBsAg carrier rate is 15% and anti-HCV positive rate is about 2% among men $\geqslant$ 30 years according to the data in this study) [7]. In other words, despite the fact that some chronic HBsAg carriers in our study population may have been classified as non-carriers due to the clearance of HBsAg in serum after superinfection with HCV, these carriers might not be enough to explain the significantly negative association observed between HBsAg carrier status and HCV infection.

There was a much higher risk of HCV infection associated with blood transfusion and/or surgery for individuals of blood group O (OR = 5·61) than for

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those who had non-O blood groups (OR = 2·77) in this study. Although the role of ABO blood group in HCV infection has never been stressed in previous investigations, the associations of blood groups with the risk and/or severity of other infectious diseases have been sought. Blood group O has been associated with increased severity of cholera and has been implied as a factor determining the susceptibility to *Helicobacter pylori* infection [31, 32]. It is therefore not unreasonable to assume that blood group O is a susceptibility factor for HCV infection. However, further studies among other populations are needed to confirm our results.

High proportions of anti-HCV/HCV RNA-positive subjects were observed among alcoholic patients with chronic liver disease [8–10]. There was concern about the possibility that alcohol might interfere with the host immune response to HCV. However, the detection rate of both anti-HCV and HCV RNA among alcoholic subjects tended to increase with the magnitude of the severity of chronic liver disease. In a study involving a series of alcoholic subjects with different severities of chronic liver disease, a higher prevalence of anti-HCV was found in alcoholic patients with biopsy-proven chronic active hepatitis and cirrhosis than in healthy subjects and patients with fatty liver or alcoholic hepatitis. Whereas active HCV infection indicated by detectable HCV RNA in serum was found in more than 40% of the subjects with chronic active hepatitis and cirrhosis, none of the 22 healthy alcoholic subjects were positive for HCV RNA [10]. We observed a lower anti-HCV positive rate among habitual alcohol drinkers than among non-drinkers, although the difference was not statistically significant. Furthermore, non-drinkers were observed to have a higher risk of HCV infection associated with blood transfusion or surgery than drinkers. Similar findings were observed in cigarette smokers. In addition, in the absence of a history of blood transfusion and/or surgery, non-smokers were more likely than smokers to be anti-HCV positive, showing an OR of 3·12. Although the mechanism underlying the effect of cigarette smoking and alcohol drinking on the reduction in risk of HCV infection remains a question, our finding might be taken as evidence against the hypothesis that alcohol interferes with the host immune response to HCV. However, the data can not explain our previous observation of a synergistic interactive effect on HCC development for anti-HCV positivity with cigarette smoking and alcohol drinking in a case-control study [7]. HCV exists in heterogeneous quasispecies and has a high rate of mutation [33, 34]. Its heterogeneity may be the result of the selective pressures by the host's immune system [25, 34]. Selection of HCV quasispecies during the treatment with interferon, a known stimulator of the immune response, was also reported [35]. Cigarette smoke contains a number of toxic substances, and alcohol can cause hepatocellular injury. It will be of interest to investigate whether cigarette smoking and the use of alcohol might offer a stress that makes the replication of HCV blocked and so that a small fraction of HCV variants with higher pathogenicity are selected.

More than 80% of the study subjects had college education. We found that an educational level lower than college education was a significant risk factor for HCV infection even after adjustment for multiple potential confounding factors. In addition, it was significantly associated with an increased susceptibility to HCV infection by blood transfusion or surgery. Low educational level and/or low socioeconomic status has also been associated with the prevalence of a number of infectious diseases. This can be interpreted that persons with low educational level may be more likely to be exposed to infectious agents and/or more susceptible to infection. The synergism between ethnicity and a history of blood transfusion or surgery in HCV infection was sufficiently strong that chance is an unlikely explanation. In fact, the independent effect of ethnicity on HCV infection approached a statistically marginal significance level, after adjustment for age, educational level, HBsAg carrier status, cigarette smoking, alcohol drinking, blood group, and histories of blood transfusion and surgery (Table 2). It is therefore likely that there may be other genetic and/or environmental factors more critical to explain the differences in the risk of HCV infection among populations.

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