World Journal of Gastroenterology

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DOI: 10.3748/wjg.v23.i5.876

*World J Gastroenterol* 2017 February 7; 23(5): 876-884
ISSN 1007-9327 (print) ISSN 2219-2840 (online)


ORIGINAL ARTICLE

Clinical Trials Study

# Effects of sex and generation on hepatitis B viral load in families with hepatocellular carcinoma

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Supported by grants from the Chang Gung Memorial Hospital (No. CMRPG3C0701); the National Science Council (No. NSC101-2314-B-182A-025-MY3); and China Medical University (No. CMU103-N-15).

Institutional review board statement: This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taiwan (IRB: 91-124).

Informed consent statement: Written informed consent was obtained from all participants before study participation. All experiments and data comparisons were carried out in compliance with relevant laws and guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available for this study.



Manuscript source: Invited manuscript

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Received: October 12, 2016
Peer-review started: October 14, 2016
First decision: December 1, 2016
Revised: December 14, 2016
Accepted: January 4, 2017
Article in press: January 4, 2017
Published online: February 7, 2017

## Abstract

### *AIM*

To explore factors associated with persistent hepatitis B virus (HBV) infection in a cohort of hepatocellular carcinoma (HCC)-affected families and then investigate factors that correlate with individual viral load among hepatitis B surface antigen (HBsAg)-positive relatives.

### *METHODS*

We evaluated non-genetic factors associated with HBV replication in relatives of patients with HCC. Relatives of 355 HCC cases were interviewed using a structured

questionnaire. Demographics, relationship to index case, HBsAg status of mothers and index cases were evaluated for association with the HBV persistent infection or viral load by generalized estimating equation analysis.

***RESULTS***

Among 729 relatives enrolled, parent generation ($P$ = 0.0076), index generation ($P$ = 0.0044), mothers positive for HBsAg ($P$ = 0.0007), and HBsAg-positive index cases ($P$ = 5.98 × $10^{-8}$) were associated with persistent HBV infection. Factors associated with HBV viral load were evaluated among 303 HBsAg-positive relatives. Parent generation ($P$ = 0.0359) and sex ($P$ = 0.0007) were independent factors associated with HBV viral load. The intra-family HBV viral load was evaluated in families clustered with HBsAg-positive siblings. An intra-family trend of similar HBV viral load was found for 27 of 46 (58.7%) families. Male offspring of HBsAg-positive mothers ($P$ = 0.024) and older siblings were associated with high viral load.

***CONCLUSION***

Sex and generation play important roles on HBV viral load. Maternal birth age and nutritional changes could be the reasons of viral load difference between generations.

**Key words:** Familial generation; Sex; Hepatitis B virus; Perinatal infection; Viral replication



**Core tip:** Familial clustering of chronic hepatitis B infection is identified in this study. Most of the hepatitis B surface antigen (HBsAg) carriers in this cohort are in families of an HBsAg-positive index case. A high prevalence of HBsAg is found in the siblings' generation and in offspring of an HBsAg-positive mother. The HBsAg status of index cases and HBsAg status of the mother are important factors for determining the persistence of hepatitis B virus (HBV) infection in hepatocellular carcinoma families. Sex and generation are factors associated with HBV replication. Perinatal infection has a great influence on male offspring's HBV replication.

Hsieh AR, Fann CSJ, Yeh CT, Lin HC, Wan SY, Chen YC, Hsu CL, Tai J, Lin SM, Tai DI. Effects of sex and generation on hepatitis B viral load in families with hepatocellular carcinoma. *World J Gastroenterol* 2017; 23(5): 876-884 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i5/876.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i5.876

## INTRODUCTION

In the families of hepatitis B virus (HBV)-infected individuals, clustering of chronic hepatitis B surface antigen (HBsAg) carriers and hepatocellular carcinoma (HCC) are common[1-6]. HBV is highly infectious[7,8], and a substantial number of individuals who are exposed to HBV early in life become chronic HBsAg carriers[4,9-11]. Furthermore, intra-familial transmission of HBV could underlie the high incidence of HCC among family members[3,4].

In addition to sex-related behavioral factors[12,13], genome-wide association studies in Japan indicated that the human leukocyte antigen subunits DP and DQ are associated with HBsAg persistence[14,15]. However, the genes identified as being responsible for clinical progression among chronic HBsAg carriers differ among several genome-wide association studies carried out in China and Taiwan[16-20]. Hence, it is possible that non-genetic factors may play a non-negligible role in determining HBV replication. For example, an increased risk of liver cancer among first-degree relatives of HCC patients was shown to be associated with a prolonged HBV replication phase[1,2]. Therefore, before evaluating genetic factors associated with HBV replication, non-genetic factors that may be associated with HBV viral load should be clarified[2-4,6,9-11,21].

Given the familial clustering of chronic HBsAg carriers in HCC families[2,5,6,9,21] with maternal status, those relatives having a similar genetic background may be instrumental in helping clinicians determine any non-genetic factors that may be associated with persistent HBV infection and viral replication. In this respect, we explored factors associated with persistent HBV infection in a cohort of HCC-affected families and then investigated factors that correlated with individual viral load among HBsAg-positive relatives.

## MATERIALS AND METHODS

### *Patients*

Patients with HCC who were diagnosed at Chang Gung Memorial Hospital, Lin-Kou Medical Center were included as index cases. From 2003 to 2007, relatives of these patients were prospectively invited to complete a survey concerning liver diseases. Spouses of index cases or spouses of their relatives were excluded.

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taiwan (IRB: 91-124), and written informed consent was obtained from all participants before the study. All experiments and data comparisons were carried out in compliance with relevant laws and guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

### *Survey*

At entry, basic information that included national citizen identification number, sex, race, alcohol and smoking habits, profession, location of residency at birth, level of education, and family history were obtained through questionnaires and structured interviews.

Each relative that was enrolled in the study underwent liver biochemistry tests for α-fetoprotein and

viral markers, as well as a liver ultrasound. Serum HBsAg and hepatitis C virus antibody (anti-HCV) were measured by enzyme-linked immunosorbent assay (Abbott Diagnostics, Chicago, IL, United States). Maternal HBsAg was assayed at enrollment or obtained by reviewing our hospital records.

### HBV viral load and HBV genotyping

A quantitative HBV DNA assay was carried out initially with the Digene Hybridization System (Digene Diagnostics, Inc., Beltsville, MD, United States; lower limit of detection, $1.4 \times 10^5$ cps/mL). Those with HBV DNA lower than the detectable limit were further assayed using the COBAS Amplicor HBV Monitor Test (Roche Diagnostics, Branchburg, NJ, United States; lower limit of detection, 200 cps/mL). Our previous long-term follow-up study revealed that nearly 40% of HBsAg carriers with persistent normal alanine aminotransferase levels have a level of HBV DNA of $> 1.0 \times 10^4$ cps/mL[22]. Therefore, relatives with HBV DNA levels of $\geqslant 1.0 \times 10^5$ cps/mL were considered as having high HBV replication, and those with levels $< 1.0 \times 10^5$ cps/mL were considered as having low HBV replication.

HBV genotype was initially determined with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method[23], but we later changed to a more sensitive SMITEST HBV Genotyping kit (Medical and Biological Laboratories Co., Ltd., Nagoya, Japan) for all subjects. For those subjects with low HBV DNA level, the S region of the genome was amplified by nested PCR followed by direct sequencing (CEQ 8000 Genetic Analysis System; Beckman Coulter, Brea, CA, United States).

### Body height in relation to birth year

Thomas *et al*[24] reported that body height at adulthood may predict the nutritional status of a population in a particular birth year. Hence, we estimated the nutritional status of Taiwan based on body height data according to birth year for subjects who received a general checkup between year 2000 and 2004 at Chang Gung Memorial Hospital[9] and in the cohort of HCC families.

### Statistical analysis

The analysis of cohort data was divided into two stages. In the first stage, we searched for factors associated with chronic HBsAg carriers. In the second stage, we examined factors associated with HBV viral load in HBsAg-positive relatives only.

The relatives included in the study were individuals from the same household. Because both individual and familial responses from the same household should be evaluated, we used the generalized estimating equation (GEE) method to determine correlations between the data and each binary response (*e.g.*, for HBsAg status or HBV DNA level) using the exchangeable working correlation structure[25,26] in our first and second stages of the analyses. Univariate and *multivariate analyses* in the two stages were assessed using the GEE method with the PROC GENMOD procedure in SAS 9.3 (SAS Institute Inc., Cary, NC, United States).

The role of sex hormones in the development and progression of HBV-associated HCC has been reported[12,13]. Therefore, we added a new familial view on HBV replication status in this cohort. We examined intra-familial HBV replication among HBsAg-positive siblings of the same sex in each family. A sex difference with respect to HBV viral load in families clustered with HBsAg-positive siblings. We used logistic regression to explore the sex effect for families in which the mother was positive for HBsAg as well as in all families.

## RESULTS

### Index cases

A total of 355 families participated in this study. Of the 330 index cases with data on HBV, 203 (61.5%) were seropositive for HBsAg, 29 (8.8%) were seropositive for both HBsAg and anti-HCV, 75 (22.7%) were seropositive for anti-HCV, and 23 (7.0%) were seronegative for both HBsAg and anti-HCV. The diagnosis of HCC was based on cytology or histology for 180 (50.7%) patients. The others were diagnosed clinically based on a serum $\alpha$-fetoprotein level and/or imaging studies[27].

### Relatives

There were 806 relatives and 205 spouses in the study. Twenty-five relatives were diagnosed with liver cirrhosis by ultrasound at screening. None of the study relatives had HCC detected on initial screening. Three siblings and three children of the indexed HCC patients developed HCC during the subsequent follow-up study.

### First-stage: Persistent HBV infection analysis

Of the 806 relatives who participated in this study, 77 were born after 1984 when the nationwide vaccination program against HBV started in Taiwan; these 77 subjects were excluded from the first-stage analysis (Figure 1). The dataset used for the first-stage analysis thus contained 729 individuals.

The risk factor of chronically expressing HBsAg was examined in the first stage. The following factors were evaluated: sex, index case sex, age, relation to the index case, HBsAg status of the mother (maternal HBsAg), and HBsAg status of the index case (index HBsAg). Index HBsAg, maternal HBsAg, and index generation were significantly associated with persistent HBV infection ($P < 0.0001$; Table 1). After controlling for sex, these associations remained statistically significant ($P < 0.0001$; Table 1).

In the multivariate GEE analysis, persistent HBV infection was lower for parents of index cases (OR = 0.24, $P = 0.0076$; Table 2). The risk was higher for subjects in the index generation (OR = 2.25, $P =$

**Table 1 Association between demographics and hepatitis B surface antigen status among relatives of patients with hepatocellular carcinoma *n* (%)**

| Category | HBsAg | | OR (95%CI) | Adjusted OR (95%CI)[1] |
|---|---|---|---|---|
| | Positive | Negative | | |
| Total family members | 314 | 415 | | |
| Sex | | | | |
| Male | 171 (54.46) | 196 (47.23) | 1.25 (0.97-1.61) | |
| Female | 143 (45.54) | 219 (52.77) | | |
| Index sex | | | | |
| Male | 229 (72.93) | 302 (72.77) | 1.07 (0.70-1.62) | 1.25 (0.97-1.60) |
| Female | 85 (27.07) | 113 (27.23) | | |
| Age, mean ± SD | 40.49 ± 10.89 | 37.87 ± 11.69 | 1.01 (1.00-1.03) | 1.28 (1.00-1.64) |
| Relation to index | | | | |
| Parent | 10 (3.18) | 20 (4.82) | 0.78 (0.37-1.64) | 0.81 (0.38-1.71) |
| Index generation | 86 (27.39) | 36 (8.67) | 3.89 (2.32-6.51)[a] | 3.97 (2.38-6.63)[a] |
| Child | 206 (65.61) | 347 (83.61) | | |
| Grandchild | 12 (3.82) | 12 (2.89) | 1.43 (0.66-3.13) | 1.39 (0.65-3.00) |
| Maternal HBsAg | | | | |
| Negative | 86 (27.38) | 244 (58.80) | | |
| Positive | 129 (41.08) | 53 (12.77) | 5.03 (3.16-8.01)[a] | 5.00 (3.13-7.97)[a] |
| Unknown | 99 (31.53) | 118 (28.43) | 2.01 (1.30-3.38)[a] | 2.04 (1.33-3.13)[a] |
| Index HBsAg[2] | | | | |
| Negative | 48 (15.43) | 203 (49.03) | | |
| Positive | 263 (84.57) | 211 (50.97) | 5.57 (3.56-8.71)[a] | 5.51 (3.53-8.61)[a] |

[1]Adjusted by sex; [2]Four index cases. HBsAg status unknown. [a]$P < 0.0001$. HBsAg: Hepatitis B surface antigen.

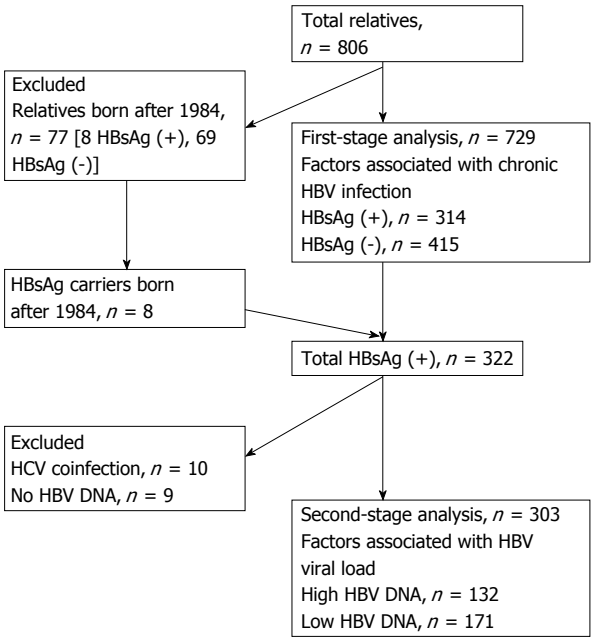


**Figure 1 Flow chart depicting the collection and potential exclusion of subjects for our cohort and the stages of analysis.**

0.0044; Table 2), those who had an HBsAg-positive mother (OR = 2.65, $P = 0.0007$; Table 2), those related to an HBsAg-positive index case (OR = 4.19, $P = 5.98 \times 10^{-8}$), and those of older age (OR = 1.03, $P = 0.0037$; Table 2).

### *Second-stage: HBV viral load association analysis*

Among the 314 HBsAg-positive relatives born before 1984 and 8 relatives born after 1984, for this second-stage analysis we excluded 10 relatives with dual HBV and HCV infections and 9 relatives who did not have an HBV DNA assay (Figure 1). A total of 303 individuals were thus included in the HBV viral load association analysis.

**Table 2 Multivariate analyses using generalized estimating equation to find predictive factors for hepatitis B surface antigen status**

| Factor | Item | OR (95%CI) | *P* value |
|---|---|---|---|
| Sex | Male | 1.26 (0.94-1.70) | |
| Index sex | Male | 1.28 (0.78-2.10) | |
| Age | | 1.03 (1.01-1.05) | 0.0037 |
| Relation to index | Parent | 0.24 (0.09-0.69) | 0.0076 |
| | Index generation | 2.25 (1.29-3.94) | 0.0044 |
| | Grandchild | 2.06 (0.78-5.45) | |
| Maternal HBsAg | Positive | 2.65 (1.51-4.67) | 0.0007 |
| | Unknown | 1.21 (0.72-2.03) | |
| Index HBsAg | Positive | 4.19 (2.50-7.04) | $5.98 \times 10^{-8}$ |

HBsAg: Hepatitis B surface antigen.

The associations between HBV DNA level and sex, index sex, age, relation to index case, maternal HBsAg, index HBsAg, and HBV genotype were examined. A positive association was found between high HBV DNA level and male sex (OR = 2.12, $P = 0.0013$; Table 3). A significant association with HBV viral load was noted between parents of index cases and child plus grandchild generations (OR = 4.77, $P = 0.0348$; Table 3). Index HBsAg status was significantly associated with HBV DNA level (OR = 2.32, $P = 0.0221$; Table 3). A significant association with HBV viral load was also noted between HBV genotype C and HBV genotype B (OR = 1.71, $P = 0.008$; Table 3); after controlling for sex, however, the association was of marginal statistical

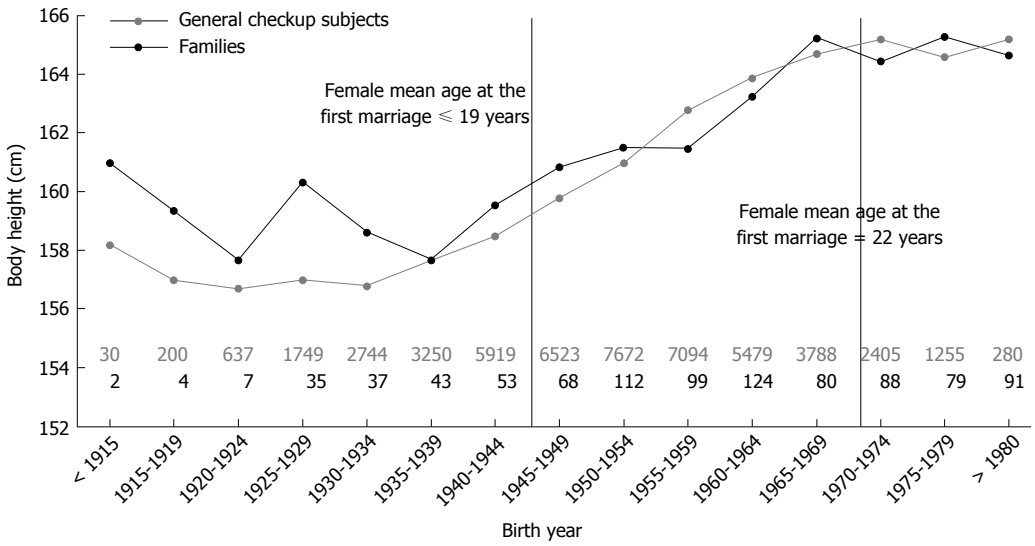


**Figure 2 Body height changes according to birth year for subjects of our cohort who underwent a general checkup (gray line) and hepatocellular carcinoma families (black line).** The two horizontal lines indicate the female mean age at first marriage for each birth-year period. The mean age at first marriage before 1945 was ≤ 19 years and was 22 years in 1970.

**Table 3 Association between demographics and hepatitis B virus viral load in 303 hepatitis B surface antigen-positive relatives *n* (%)**

| Factor | HBV DNA | | OR (95%CI) | *P* value | Adjusted OR (95% CI)[1] | *P* value |
|---|---|---|---|---|---|---|
| | ≥ 100000 cps/mL | < 100000 cps/mL | | | | |
| Total family members | 132 | 171 | | | | |
| Sex | | | | | | |
| Male | 84 (63.64) | 79 (46.20) | 2.12 (1.34-3.39) | 0.0013 | | |
| Female | 48 (36.36) | 92 (53.80) | | | | |
| Index sex | | | | | | |
| Male | 99 (75) | 121 (70.76) | 1.83 (0.69-2.04) | | 1.17 (0.68-2.01) | |
| Female | 33 (25) | 50 (29.24) | | | | |
| Age, mean ± SD | 40.51 ± 12.18 | 39.15 ± 10.55 | 1.01 (0.99-1.03) | | 1.02 (0.99-1.04) | |
| Relation to index | | | | | | |
| Child and grandchild | 83 (62.88) | 128 (74.85) | | | | |
| Parent | 7 (5.30) | 2 (1.17) | 4.77 (1.12-20.31) | 0.0348 | 4.57 (1.15-18.14) | 0.0307 |
| Index generation | 42 (31.82) | 41 (23.98) | 1.51 (0.87-2.62) | | 0.64 (0.36-1.14) | |
| Maternal HBsAg | | | | | | |
| Negative | 33 (25) | 51 (29.82) | | | | |
| Positive | 61 (46.21) | 64 (37.43) | 1.55 (0.84-2.87) | | 1.57 (0.84-2.92) | |
| Unknown | 38 (28.79) | 56 (32.75) | 1.08 (0.57-2.06) | | 1.20 (0.62-2.33) | |
| Index HBsAg | | | | | | |
| Negative | 12 (9.16) | 32 (18.93) | | | | |
| Positive | 119 (90.84) | 137 (81.07) | 2.32 (1.13-4.76) | 0.0221 | 2.47 (1.19-5.15) | 0.0158 |
| HBV genotype[2] | | | | 0.0017 | | |
| N[3] | 2 (1.53) | 21 (12.88) | 0.11 (0.03-0.44) | | 0.09 (0.02-0.39) | 0.0011 |
| B | 97 (74.62) | 120 (73.62) | | | | |
| C | 31 (23.85) | 22 (13.50) | 1.71(0.94-3.14) | 0.008 | 1.80 (0.97-3.36) | 0.0640 |

[1]Adjusted by sex; [2]There are 10 missing HBV genotypes; [3]Genotyping failed due to low HBV DNA. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen.

significance (*P* = 0.064; Table 3).

In the multivariate GEE analysis, HBV viral load was independently associated with sex (OR = 2.65, *P* = 0.0007; Table 4) and being the parent of an index case (OR = 6.49, *P* = 0.0359; Table 4).

### *Body height in relation to birth year*

Figure 2 presents data for body height change according to birth year in general checkup subjects and HCC families. The body height of the general checkup subjects and of HCC families increased similarly according to birth year.

### *Intra-family comparison of HBV viral load among HBsAg-positive siblings*

Forty-six families were found to have at least two HBsAg-positive siblings of the same sex. Among them, 28 were male sibling families and 18 were female

**Table 4 Multivariate analyses using generalized estimating equation to find predictive factors for hepatitis B virus viral load**

| Factor | Item | OR (95%CI) | *P* value |
|---|---|---|---|
| Sex | Male | 2.65 (1.51-4.64) | 0.0007 |
| Index sex | Male | 1.47 (0.73-2.95) | |
| Age | | 1.01 (0.98-1.03) | |
| Relation to index | Parent | 6.49 (1.13-37.27) | 0.0359 |
| | Index generation | 1.19 (0.60-2.37) | |
| Maternal HBsAg | Positive | 1.50 (0.71-3.17) | |
| | Unknown | 1.02 (0.49-2.15) | |
| Index HBsAg | Positive | 1.51 (0.68-3.38) | |
| HBV genotype | N | 0.12 (0.03-0.56) | 0.0066 |
| | C | 1.22 (0.59-2.51) | |

HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen.

sibling families (Table 5). All siblings had a high HBV viral load in 13 (28.26%) families, and all siblings had a low HBV viral load in 14 (30.43%) families. These two groups (58.69%) revealed a familial trend of HBV replication status; among those siblings, male sibling families generally had a high HBV viral load, whereas female sibling families had a low HBV viral load (OR = 29.96, $P$ = 0.007; Table 5). Maternal HBsAg positivity had a large influence on male offspring in that most of male offspring were in the high HBV viral load group; on the other hand, female offspring were generally in the low HBV viral load group (OR = 21, $P$ = 0.024; Table 5).

For 11 families (23.91%), older siblings had a higher level of HBV DNA than their younger siblings; this trend was opposite for only 5 families (10.87%). Older siblings tended to have a higher HBV DNA level than their younger siblings, but the difference was not statistically significant owing to the small number of cases. Because all siblings were generally infected at an early stage of life[4,9-11], this phenomenon contradicts the general trend that HBV replication declines with increasing age[28,29].

## DISCUSSION

This study reveals a familial clustering of chronic HBV infection. As shown in Table 1, most of the chronically HBV-infected carriers (84.57%) in this cohort were families of an HBsAg-positive index case. A high prevalence of HBsAg was apparent for the siblings' generation (86/122 or 70.49%, $P$ < 0.0001) and for offspring of an HBsAg-positive mother (129/182 or 70.88%, $P$ < 0.0001). These findings remained significant in the multivariate analysis. Notably, the majority of index cases were male (72.93%), indicating that both vertical and horizontal infections were present in HCC families.

HBV replication phase or viral load plays roles in determining the prognosis of chronic persistent HBV infection[2,30]. In our study, we found that sex and generation played independent roles in determining HBV DNA level (Tables 3 and 4). HBV viral load was higher for subjects with HBV genotype C than genotype B in the univariate analysis ($P$ = 0.008; Table 3), but this difference was not statistically significant in the multivariate analysis (Table 4).

Sex is a well-known factor associated with chronic HBV infection[9]. We therefore added a new family view on HBV replication status in this cohort, and we identified a sex difference with respect to HBV viral load in families that had HBsAg-positive siblings (Table 5). HBV viral load was generally higher in male than female siblings (OR = 29.96, $P$ = 0.007). In addition, male siblings in families of an HBsAg-positive mother tended to be in the high HBV DNA group, whereas female siblings were generally in the low HBV DNA group (OR = 21, $P$ = 0.024). Male offspring are more vulnerable to the influence of maternal HBsAg status, whereas female offspring may overcome the maternal influence of persistent HBV replication.

Relatively high HBV replication in older generations has not been well documented in the literature. A study of pregnant women between 1990 and 1995 revealed a progressively decreasing prevalence of hepatitis B e antigen (HBeAg) among chronically HBV-infected carriers[31]. This finding was confirmed in a longer study spanning 1985 to 2000[32], in which the prevalence of HBsAg remained nearly the same, but the prevalence of HBeAg declined progressively from 40% in 1986 to 18% in 2000. This difference between HBsAg and HBeAg prevalence remained apparent even when the ages of the pregnant women were considered[32].

In our previous study of HCC families, we found that older siblings frequently cleared HBeAg later than did their younger siblings[21], and an HBV phylogenetic study yielded similar findings[33]. Among 13 families with an HBsAg-positive mother, the 11 oldest siblings were HBeAg positive whereas only 3 of the youngest siblings were HBeAg positive. These observations provided a clue that maternal age at birth might influence HBV replication in offspring.

The mean age of women entering their first marriage in Taiwan was 18 years before 1917 and remained at about 19 years between 1918 and 1945 (Figure 2)[34]. In the 1970s, however, this mean age had risen to 22 years (http://nccur.lib.nccu.edu.tw/handle/140.119/34632) and increased rather rapidly to 29.2 years by 2010 (http://www.moi.gov.tw/stat/news_content.aspx?sn=5261). Thus, mothers in younger generations of this period between 1918 and 2010 may be 3-5 years older than mothers of the older generations.

A 2014 review article by Bertoletti *et al*[35] presented an interesting viewpoint that immune responses change during the life of an individual, based on the observed higher mortality of influenza infection at age 30 than at age 20. This implies that a more vigorous immune response produces a more fulminant disease by age 30, whereas a weaker immune response produces a

**Table 5 Intra-family comparison of hepatitis B virus viral load among hepatitis B surface antigen-positive siblings *n* (%)**

| HBV DNA level[1] | Maternal HBsAg | | | Total |
|---|---|---|---|---|
| | Positive | Unknown | Negative | |
| Total male siblings | 12 | 9 | 7 | 28 |
| All high level | 7 (58.33)[2] | 2 (22.22) | 2 (28.57) | 11 (39.3)[3] |
| All low level | 1 (8.33)[2] | 2 (22.22) | 1 (14.29) | 4 (14.3)[3] |
| Older > younger | 3 (25.00) | 3 (33.33) | 3 (42.86) | 9 (32.1) |
| Younger > older | 1 (8.33) | 1 (11.11) | 1 (14.29) | 3 (10.7) |
| Other | 0 (0.00) | 1 (11.11) | 0 (0.00) | 1 (3.6) |
| Total female siblings | 11 | 3 | 4 | 18 |
| All high level | 2 (18.18)[2] | 0 (0.00) | 0 (0.00) | 2 (11.1)[3] |
| All low level | 6 (54.55)[2] | 1 (33.33) | 3 (75.00) | 10 (55.6)[3] |
| Older > younger | 1 (9.09) | 1 (33.33) | 0 (0.00) | 2 (11.1) |
| Younger > older | 1 (9.09) | 0 (0.00) | 1 (25.00) | 2 (11.1) |
| Other | 1 (9.09) | 1 (33.33) | 0 (0.00) | 2 (11.1) |

[1]Low HBV DNA level, < $1 \times 10^5$ cps/mL; high HBV DNA level, ≥ $1 \times 10^5$ cps/mL. [2]OR (95%CI) = 21 (1.50-293.25), $P = 0.024$; [3]OR (95%CI) = 29.96 (2.54-353.17), $P = 0.007$; logistic regression. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen.

self-limited infection at age 20. A similar situation can be found for chronic HBV infection in that such patients usually enter the immune clearance phase by age 30. We suspect that generational differences might be associated with differences in maternal immunity at the time of an offspring's birth[36]. Further study will be needed.

Better nutrition is another potential reason for reduced HBV replication in younger generations, and long-term follow-up studies revealed that hepatic steatosis is a good prognostic indicator for chronic HBsAg carriers[28,29]. Hepatic steatosis correlated with a lower risk of HCC, lower mortality rate, and higher chance of spontaneous HBsAg clearance. A recent PNPLA3 polymorphism study on non-alcoholic fatty liver disease found that those SNP genotypes favoring hepatic steatosis development were associated with lower HBV DNA level[37].

During the time frame of our study, we did not have data on the nutritional habits of individuals, but for most participants we obtained body height data, which may reflect long-term nutritional status during the major growth period of humans[24,38]. In our cohort, the mean body height remained < 159 cm for individuals born before 1945. From about 1955 to 1965, however, mean body height increase rapidly to > 164 cm (Figure 2). These findings indicate a significant change in socioeconomic status of the Taiwanese population after the Second World War. Hence, increased food consumption and decreased physical activity may have contributed to the observed increase in the prevalence of hepatic steatosis[39]. Therefore, lifestyle and nutritional habits are factors that may have contributed to our observed shortened HBV replication phase in the younger generation.

We conclude that the generation of the family member, index HBsAg, and maternal HBsAg are important factors for predicting HBV persistence in HCC families. Sex and generation are factors associated with HBV replication. Perinatal infection substantially influences the duration of HBV replication in male offspring.

## ACKNOWLEDGMENTS

We are grateful to the National Science Council and the Institute of Biomedical Sciences and Academia Sinica of Taiwan for their support.

## COMMENTS

### Background

Hepatitis B virus (HBV) replication is critical for disease progression. Multiple inconsistent genetic factors have been identified to be involved in the disease progression. Therefore, the non-genetic factors concerning persistent HBV replication should be clarified.

### Research frontiers

Among 729 relatives enrolled, parent generation, index generation, maternal hepatitis B surface antigen (HBsAg), and index cases HBsAg status were factors associated with persistent HBV infection. Factors associated with HBV viral load were evaluated among 303 HBsAg-positive relatives. Generation and sex were independent factors associated with HBV viral load. The intra-familial HBV viral load was evaluated in families clustered with HBsAg-positive siblings. An intra-family trend of similar HBV viral load was found for 27 of 46 (58.7%) families. Male offspring of HBsAg-positive mothers and older siblings were associated with high viral load.

### Innovations and breakthroughs

Based on the finding that older generation and older siblings have higher viral load, the authors suspect that maternal age at birth and nutritional status might be related to generational differences on viral load. HBsAg-positive mothers usually associated with high viral load on male offspring, but not on female offspring.

### Applications

Sex, generation, maternal age at birth and maternal HBsAg status are factors that should be taken into consideration when genetic factors associated with HBV-related outcome are evaluated.

### Peer-review

The manuscript from Hsieh *et al* reported the sex and generation associated with HBV load in hepatocellular carcinoma family. And perinatal infection is a major effect factor for male offspring's HBV replication. The entire sets of data

are nicely presented, and highly supportive of the conclusion.

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**P- Reviewer**: Liu XL **S- Editor**: Qi Y **L- Editor**: Filipodia
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ISSN 1007-9327