Risk analysis of the exposure to GB virus C/hepatitis G virus among populations of intravenous drug users, commercial sex workers and male outpatients at STD clinic in Chiang Mai, Thailand: a cross-sectional case-control study.

Narufumi Suganuma†††
Da-hong Wang∗∗
Supatra Peerakome§

Satoru Ikeda†
Hideki Yamamoto††
Kriegsak Sitvacharanum¶

Kazuhisa Taketa‡
Kannika Phornphukutkul‡‡
Jaroon Jitiwutlkarn∥

∗Okayama University,
†Okayama University,
‡Okayama University,
∗∗Okayama University,
††Okayama University,
‡‡University of Chiang Mai,
§University of Chiang Mai,
¶Chiang Mai STD Clinic,
∥Northern Drug Dependence Treatment Center,

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Abstract

An exposure to GB virus C/hepatitis G virus (GBV-C/HGV) was studied among populations at risk for blood and sexual exposure to analyze risk factor of the transmission of the virus. Blood samples were drawn from 98 intravenous drug users (IVDU), 100 female high-class commercial sex workers (CSW) and 50 male outpatients (MOP) at a sexually transmitted diseases (STD) clinic in Chiang Mai, Thailand. These blood samples were analyzed for GBV-C/HGV RNA; antibodies against second envelope protein of GBV-C/HGV (anti-E2); anti-hepatitis C virus antibody (HCV-Ab); hepatitis B core antibody (HBcAb); and antibodies against human immunodeficiency virus (HIV-Ab). Prevalences of GBV-C/HGV RNA, anti-E2, HCV-Ab, HBcAb and HIV-Ab were 27.6%, 16.3%, 84.7%, 76.5% and 45.0% in IVDU; 0%, 21.5%, 2.0%, 72.0% and 11.0% in CSW; 6.0%, 13.6%, 0%, 64.0% and 14.0% in MOP. While the prevalence of GBV-C/HGV RNA was higher in IVDU than in CSW and MOP, comparable prevalences of anti-E2 among the three populations were found. Intravenous drug injection showed association with GBV-C/HGV RNA, while history of STD associated with anti-E2. In conclusion, intravenous drug injection and STD were found to be risk factors for the previous exposure to GBV-C/HGV, but STD did not increase the risk of the GBV-C/HGV viraemia.

KEYWORDS: GB virus C/hepatitis G virus, anti-E2 anti-body, sexually transmitted disease, human immunodeficiency virus, hepatitis C virus

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Department of Public Health, Okayama University Medical School, Okayama 700-8558, Japan, Departments of Internal Medicine and Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai STD Clinic and *Northern Drug Dependence Treatment Center, Chiang Mai 50200, Thailand

An exposure to GB virus C/hepatitis G virus (GBV-C/HGV) was studied among populations at risk for blood and sexual exposure to analyze risk factor of the transmission of the virus. Blood samples were drawn from 98 intravenous drug users (IVDU), 100 female high-class commercial sex workers (CSW) and 50 male outpatients (MOP) at a sexually transmitted diseases (STD) clinic in Chiang Mai, Thailand. These blood samples were analyzed for GBV-C/HGV RNA: antibodies against second envelope protein of GBV-C/HGV (anti-E2); anti-hepatitis C virus antibody (HCV-Ab); hepatitis B core antibody (HBCAb); and antibodies against human immunodeficiency virus (HIV-Ab). Prevalences of GBV-C/HGV RNA, anti-E2, HCV-Ab, HBCAb and HIV-Ab were 27.6%, 16.3%, 84.7%, 76.5% and 45.0% in IVDU; 0%, 21.5%, 2.0%, 72.0% and 11.0% in CSW; 6.0%, 13.6%, 0%, 64.0% and 14.0% in MOP. While the prevalence of GBV-C/HGV RNA was higher in IVDU than in CSW and MOP, comparable prevalences of anti-E2 among the three populations were found. Intravenous drug injection showed association with GBV-C/HGV RNA, while history of STD associated with anti-E2. In conclusion, intravenous drug injection and STD were found to be risk factors for the previous exposure to GBV-C/HGV, but STD did not increase the risk of the GBV-C/HGV viraemia.

Key words: GB virus C/hepatitis G virus, anti-E2 antibody, sexually transmitted disease, human immunodeficiency virus, hepatitis C virus

GB virus C/hepatitis G virus (GBV-C/HGV) is a novel flavivirus discovered by two different groups independently in 1995 and 1996 (1-3). A number of published reports have indicated that the recipients of transfused blood or blood products have a high prevalence of GBV-C/HGV infection (4, 5). GBV-C/HGV and hepatitis C virus (HCV), both members of flavivirus, have been shown to be transmitted by a similar parenteral route (6, 7). However, there are a few reports to suggest sexual transmission of GBV-C/HGV; namely, a report of mother-to-child transmission of GBV-C/HGV showing the presence of GBV-C/HGV RNA among sexually active women (8), as well as the interspousal transmission of this virus (9).

In order to know the subjects previously exposed to GBV-C/HGV, newly reported antibodies against the second envelope protein of GBV-C/HGV (anti-E2) (10) were assayed in addition to GBV-C/HGV genome which was detected by reverse transcription polymerase chain reaction (RT-PCR). The prevalences of GBV-C/HGV RNA and anti-E2 were compared with those of anti-HCV antibodies (HCV-Ab), anti-hepatitis B core antibodies (HBCAb) and antibodies against human immunodeficiency virus (HIV-Ab) in different high-risk populations of blood exposure, i.e., intravenous drug users (IVDU), and sexual exposure, i.e., female commercial sex workers (CSW) and male outpatients (MOP) at a sexually trans-

*To whom correspondence should be addressed.
mitted diseases (STD) clinic in Chiang Mai, Thailand. Risk analysis for GBV-C/HGV was done by a case-control study including all the subjects.

**Subjects and Methods**

**Subjects.** Blood samples were drawn from the following individuals after obtaining informed consent for the analysis of the virus markers listed below: 98 IVDU (89 males, 2 females and 7 unidentified) hospitalized in the Northern Drug Dependence Treatment Center, Chiang Mai, Thailand from May 1996 through July 1996; 100 female high-class CSW who visited Chiang Mai STD clinic from September 1996 through December 1996; and 50 MOP at Chiang Mai STD clinic, including patients for evaluation of suspected STD, from December 1996 through June 1997. Their demographic strata and risk factors for blood and sexual exposure were obtained through questionnaire, which was taken by interviewers for risk analysis and given in Table 1. The small number of sex partners of CSW characterizes their feature as high-class CSW. Sera were separated and kept frozen until analysis.

**Study design.** A cross-sectional study was performed to know the prevalence of GBV-C/HGV infection in different populations at risk. Then, a case-control study was done including all the 248 subjects who were tested for GBV-C/HGV markers. Individuals who are positive for GBV-C/HGV (RNA/E2), i.e., GBV-C/HGV RNA or anti-E2, were used as the case to determine the risk of exposure to GBV-C/HGV. Same analysis was applied to subjects positive for GBV-C/HGV RNA or for anti-E2 to know the risk of the GBV-C/HGV viraemia or acquiring anti-E2.

**Analyses for virus markers.** GBV-C/HGV RNA was detected by microplate hybridization technique performed after amplifying combinator DNA from the virus RNA. For the populations of IVDU and CSW, detection was performed by RT-PCR with primers designed from NS3 region of the sequence of GBV-C/HGV genome (SMITEST GBV-C RNA (NS), Sumitomo Metal, Tokyo, Japan). For the population of MOP, we utilized another microplate hybridization kit using different primer set designed from 3' untranslated subgenomic region (3' UTR) of the sequence of the viral RNA (SMITEST GBV-C RNA (3' UTR), Sumitomo Metal). Anti-E2 were determined by enzyme-linked immunosolvent assay using a kit of recombinant E2 protein of GBV-C/HGV as an antigen (Enzymun-Test Anti-HGen, Boehringer Mannheim, Germany). The individuals exposed to GBV-C/HGV, positive for either GBV-C/HGV RNA or anti-E2, were recorded as GBV-C/HGV (RNA/E2).

HCV-Ab were determined with an Abbott HCV EIA 2nd Generation kit (Dainabot Co., Ltd., Tokyo, Japan). HBcAb were assayed with a Hepatitis B Virus Core Antigen (Recombinant) CORAB kit (Dainabot Co., Ltd.) with inhibition rates over 50% being taken as positive. HIV-Ab were assayed with a Genedia HIV-1/2 Mix PA kit (Fujirebio Inc., Tokyo, Japan).

**Statistical evaluation.** Statistical analysis of comparison of prevalences was made by Chi square test or Two Tailed Fischer's Exact test, where appropriate,
on a personal computer with an epidemiological software, EpiInfo Version 6 (CDC, Atlanta, GA, USA and WHO, Geneva, Switzerland). Adjusted odds ratio for the risk factors of GBV-C/HGV were calculated by logistic regression analysis with a computer package SPSS, Version 8.0 (SPSS Inc. Chicago, IL, USA). Behavioral factors entered into the equation for logistic regression analysis were age, blood transfusion, surgical operation, tattoo, intravenous drug injection, bisexual, heterosexual anal sex, STD, genital ulcer, number of sex partners and condom use. Age was categorized into 4 age groups; namely, age younger than 20 years old; 20 to 29; 30 to 39; and older than 39. The number of partners was categorized into 4 groups; less than 5; 6 to 10; 11 to 20; and 21 and more. The condom use was categorized into 3 groups; non-use; inconsistent; and consistent. Sex was not included into predicting variables as it is strongly correlated with intravenous drug injection, which was one of the predicting variables.

Results

Prevalences of GBV-C/HGV, HCV, HBV and HIV markers. Prevalences of GBV-C/HGV RNA, anti-E2, HCV-Ab, HBcAb and HIV-Ab among these populations are shown in Fig. 1. The individuals exposed to GBV-C/HGV, those who were positive for either GBV-C/HGV RNA or anti-E2, were recorded as GBV-C/HGV (RNA/E2), and also given in the figure. The prevalence of GBV-C/HGV RNA in IVDU was significantly higher than those in CSW and MOP, while the prevalences of anti-E2 were 16.3% in IVDU, 21.5% in CSW and 13.6% in MOP, a statistically insignificant difference. Consequently, we determined that the prevalence of GBV-C/HGV (RNA/E2) was the highest in IVDU among the three populations. The prevalence of GBV-C/HGV RNA and anti-E2 were mutually exclusive. Furthermore, the prevalence of HCV-Ab was the highest in IVDU and low or undetectable in CSW and MOP, that of HBcAb was equally high in all the three populations and that of HIV-Ab was the higher in IVDU than in CSW or MOP.

Association among the assayed virus markers. Logistic regression analysis was performed to know the association among the virus markers of GBV-C/HGV and other viruses. The results of the analysis are given in Table 2. Predictive probabilities of GBV-C/HGV (RNA/E2), GBV-C/HGV RNA and anti-E2 with HIV-Ab, HCV-Ab and HBcAb as predicting variables were 73.0%, 87.8% and 82.2%, by which subjects

![Graph](image-url)

Fig. 1  The prevalence of virus markers in different populations at risk in Chiang Mai, Thailand. Subjects positive for GBV-C RNA or anti-E2 were recorded as positive GBV-C/HGV (RNA/E2). □ IVDU; ■ CSW; and ■ MOP. See legends to Tables 1 and 2.
were classified correctly with the logistic models. HIV-Ab showed association not with the exposure to GBV-C/HGV nor GBV-C/HGV RNA but with anti-E2 (OR = 0.4; 95% CI: 0.1, 1.0), which indicated HIV-Ab reduces the risk to acquire anti-E2. Sharing the parenteral route of transmission, GBV-C/HGV RNA and HCV-Ab had strong association (OR = 7.5; 95% CI: 2.9, 19.3), although no significant association was observed.

Table 2  Adjusted odds ratio between virus markers of GB virus C/hepatitis G virus (GBV-C/HGV) and other viruses

<table>
<thead>
<tr>
<th></th>
<th>GBV-C/HGV (RNA/E2)</th>
<th>GBV-C/HGV RNA</th>
<th>Anti-E2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
</tr>
<tr>
<td>HIV-Ab</td>
<td>0.6 [0.3, 1.3]</td>
<td>1.2 [0.5, 2.9]</td>
<td>0.4 [0.1, 1.0]</td>
</tr>
<tr>
<td>HCV-Ab</td>
<td>3.4 [1.8, 6.5]</td>
<td>7.5 [2.9, 19.3]</td>
<td>1.4 [0.7, 4.0]</td>
</tr>
<tr>
<td>HBcAb</td>
<td>0.9 [0.5, 1.7]</td>
<td>0.4 [0.2, 0.9]</td>
<td>1.7 [0.7, 4.0]</td>
</tr>
<tr>
<td>Predictive probabilities</td>
<td>73.0%</td>
<td>87.8%</td>
<td>82.2%</td>
</tr>
</tbody>
</table>

GBV-C/HGV (RNA/E2): Positive for either GBV-C/HGV RNA or anti-E2; RNA: Ribonucleic acid; Anti-E2: Antibodies against second envelope protein of GBV-C/HGV; OR: Adjusted odds ratio; CI: Confidence interval; HIV-Ab: Antibodies against human immunodeficiency virus; HCV-Ab: Anti-hepatitis C virus antibody; HBcAb: Hepatitis B core antibody.

Table 3  Adjusted odds ratio between GB virus C/hepatitis G virus (GBV-C/HGV) virus markers and behavioral factors

<table>
<thead>
<tr>
<th></th>
<th>GBV-C/HGV (RNA/E2)</th>
<th>GBV-C/HGV RNA</th>
<th>Anti-E2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
<td>OR [95% CI]</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 to 29</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>0.7 [0.2, 2.1]</td>
<td>2.9 [0.8, 11.3]</td>
<td>0.1 [0.02, 1.2]</td>
</tr>
<tr>
<td>30 to 39</td>
<td>0.4 [0.1, 1.0]</td>
<td>0.6 [0.2, 2.0]</td>
<td>0.5 [0.2, 1.7]</td>
</tr>
<tr>
<td>&gt; 39</td>
<td>0.4 [0.1, 1.3]</td>
<td>Removeda</td>
<td>1.4 [0.4, 5.1]</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>0.3 [0.1, 1.1]</td>
<td>0.5 [0.1, 2.0]</td>
<td>0.3 [0.05, 1.5]</td>
</tr>
<tr>
<td>Surgical operation</td>
<td>0.4 [0.1, 1.2]</td>
<td>0.1 [0.01, 1.3]</td>
<td>0.8 [0.2, 2.9]</td>
</tr>
<tr>
<td>Tattoo</td>
<td>1.0 [0.4, 2.4]</td>
<td>0.6 [0.2, 1.7]</td>
<td>1.2 [0.4, 3.4]</td>
</tr>
<tr>
<td>Intravenous drug injection</td>
<td>3.3 [1.1, 9.5]</td>
<td>12.3 [2.6, 58.3]</td>
<td>1.0 [0.3, 3.9]</td>
</tr>
<tr>
<td>Bisexual</td>
<td>2.4 [0.8, 6.8]</td>
<td>1.9 [0.6, 6.6]</td>
<td>1.8 [0.5, 6.8]</td>
</tr>
<tr>
<td>Heterosexual anal sex</td>
<td>2.4 [0.5, 12.9]</td>
<td>Removeda</td>
<td>4.7 [0.8, 29.2]</td>
</tr>
<tr>
<td>STD</td>
<td>4.7 [1.7, 13.1]</td>
<td>1.5 [0.3, 7.1]</td>
<td>5.2 [1.7, 16.1]</td>
</tr>
<tr>
<td>Genital ulcer</td>
<td>1.0 [0.3, 3.5]</td>
<td>2.8 [0.5, 14.7]</td>
<td>0.3 [0.1, 1.4]</td>
</tr>
<tr>
<td>Number of sex partners</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>6 to 10</td>
<td>0.5 [0.1, 1.6]</td>
<td>1.4 [0.3, 5.7]</td>
<td>0.3 [0.1, 1.8]</td>
</tr>
<tr>
<td>11 to 20</td>
<td>0.4 [0.1, 2.4]</td>
<td>2.1 [0.4, 12.3]</td>
<td>Removeda</td>
</tr>
<tr>
<td>More than 21</td>
<td>1.8 [0.6, 5.5]</td>
<td>2.9 [0.5, 16.2]</td>
<td>0.9 [0.2, 3.6]</td>
</tr>
<tr>
<td>Condom use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-use</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Inconsistent</td>
<td>1.7 [0.7, 3.0]</td>
<td>2.2 [0.8, 6.5]</td>
<td>1.1 [0.4, 3.1]</td>
</tr>
<tr>
<td>Consistent</td>
<td>0.9 [0.3, 2.4]</td>
<td>0.7 [0.2, 2.8]</td>
<td>1.2 [0.4, 3.7]</td>
</tr>
<tr>
<td>Predictive probabilities</td>
<td>74.2%</td>
<td>89.4%</td>
<td>84.8%</td>
</tr>
</tbody>
</table>

a: "Removed" means that the factor was removed from the equation for logistic regression analysis.

STD: Sexually transmitted diseases. See legend to Table 2 for other abbreviations.
between anti-E2 and HCV-Ab. HBcAb showed negative association with GBV-C/HGV RNA, and the adjusted odds ratio between the pair was 0.4 (95% CI: 0.2, 0.9).

**Association between GBV-C/HGV virus markers and behavioral factors.** Statistical values to show the association between GBV-C/HGV virus markers and behavioral factors are given in Table 3. Predictive probabilities of GBV-C/HGV (RNA/E2), GBV-C/HGV RNA and anti-E2 with selected behavioral factors as predicting variables were 74.2%, 89.4% and 84.9%, by which subjects were classified correctly with the logistic models. GBV-C/HGV (RNA/E2) had positive association with STD (OR = 4.7; 95% CI: 1.7, 13.1) and with intravenous drug injection (OR = 3.3; 95% CI: 1.1, 9.5). Inconsistent condom use had a tendency to increase the risk of exposure to GBV-C/HGV compared to consistent condom use. Bisexual, heterosexual anal sex and number of sex partners more than 21 also tended to associate with GBV-C/HGV (RNA/E2). Blood transfusion, surgical operations and tattoo did not have association with GBV-C/HGV (RNA/E2). Age group of 20 to 29 tended to have higher risk of GBV-C/HGV (RNA/E2) than the other age groups.

GBV-C/HGV RNA had positive association only with intravenous drug injection (OR = 12.3; 95% CI: 2.6, 58.3). Inconsistent condom use, genital ulcer and number of sex partners 11 and more tended to increase the risk of GBV-C/HGV RNA. Blood transfusion, surgical operations and tattoo, did not associate with GBV-C/HGV RNA. Bisexual, number of sex partners less than 11 had no association with GBV-C/HGV RNA, either. Age over 40 and heterosexual anal sex were removed from predicting variables for GBV-C/HGV RNA as they did not modify the result. Age group of 30 to 39 tended to have higher risk for GBV-C/HGV RNA than the other age groups.

Anti-E2 had positive association with STD (OR = 5.2; 95% CI: 1.7, 16.1). Heterosexual anal sex tended to increase the risk of anti-E2. Blood transfusion, surgical operations, tattoo and intravenous drug injection did not have association with anti-E2. Bisexual, number of sex partners and condom use had no association with anti-E2. The number of sex partners from 11 to 20 was removed from predicting variables for anti-E2. Age group of older than 39 tended to have higher risk of anti-E2 than the other age groups.

**Discussion**

Since GBV-C/HGV is a transfusion transmissible virus, we expected that prevalences of both GBV-C/HGV RNA and anti-E2 would be the highest in IDVU among the three populations. Contrary to our expectation, prevalences of anti-E2 were comparably high in both populations at risk for blood and sexual exposure, although the prevalence of GBV-C/HGV RNA was the highest in IDVU. The prevalence of the individuals exposed to GBV-C/HGV remained the highest in IDVU, which confirmed the importance of the parental route for GBV-C/HGV transmission.

In this study, assays using different primer sets were used for the detection of GBV-C/HGV RNA in MOP and the other populations. The prevalence of GBV-C/HGV RNA in MOP was clearly lower than that in IDVU, even though the kit used in MOP had a higher sensitivity (personal communication) than the other kit used for IDVU and CSW.

Case-control studies on GBV-C/HGV (RNA/E2) and GBV-C/HGV RNA also showed similar result that intravenous drug injection increased the risk of previous exposure to GBV-C/HGV and the GBV-C/HGV viraemia. History of STD increased the risk of exposure to GBV-C/HGV, but did not influence the risk of acquiring the GBV-C/HGV viraemia. Strong association was observed between GBV-C/HGV RNA and HCV-Ab as expected.

Most of the reports to support the sexual transmission of GBV-C/HGV are based on the detection of GBV-C/HGV genome (11, 12). A recently published paper on interspousal transmission of this virus assayed both GBV-C/HGV RNA and anti-E2; however, the sexual transmission of GBV-C/HGV was basically determined by the detection of GBV-C/HGV genome alone, and anti-E2 test was subordinate (9). Detecting GBV-C/HGV genome is not sufficient because of the following two reasons. One is that the virus was reported to become undetectable in more than half of the cases of acute infection of GBV-C/HGV, according to the result of a chronological study (13). The other is that the analysis of association between GBV-C/HGV genome and anti-E2 shows that this antibody seems to appear after recovery from the GBV-C/HGV viraemia (14, 15). As may be seen in our study, if we did not assay anti-E2, we would have reached the opposite conclusion.
Higher prevalence of GBV-C/HGV RNA in IVDU than in CSW or MOP may be explained by the influence of the higher prevalence of HIV-Ab in the former population than in the latter ones. Production of anti-E2 was considered to be impaired by the presence of HIV as was shown by the result of logistic regression analysis among the virus markers. It is reported on HCV that the presence of HIV infection itself (16) or immunodeficiency status caused by its infection precipitates the reproduction of the virus or impairs the production of antibodies (17–19). The prevalence of GBV-C/HGV RNA would remain high without the seroconversion since anti-E2 is considered to be a neutralizing antibody.

However, this does not fully explain the highest prevalence of GBV-C/HGV RNA in IVDU among the three populations. Although both parenteral and sexual exposures were important risk factors for previous exposure to GBV-C/HGV, sexual exposure did not increase the risk of acquiring the GBV-C/HGV viraemia. Intravenous drug injection was the only behavioral factor to increase the risk of the GBV-C/HGV viraemia. The strong association found between GBV-C/HGV RNA and HCV-Ab also suggests the importance of parental exposure for the GBV-C/HGV viraemia. There may exist an interaction between GBV-C/HGV and HCV to precipitate the reproduction of each other, although the levels of either virus were reported to be unaffected by the presence of an additional virus (20).

Although the reduction of risk of the GBV-C/HGV viraemia observed in the presence of HBeAb was considered to be due to interaction of GBV-C/HGV with HBV, the prevalence of the surface antigen of HBV was low and did not show the same tendency with HBeAb (unpublished data).

Despite the significant association found between GBV-C/HGV (RNA/E2) and history of STD, the finding that consistent condom use did not reduce the risk of acquiring GBV-C/HGV virus markers suggests that the high prevalence of anti-E2 could not simply be caused by ordinary sexual practice. One of the possible explanations could be seen in the tendency of association found between GBV-C/HGV (RNA/E2) or anti-E2 and some of sexual behaviors; namely, bisexual, heterosexual anal sex, number of partners more than 21 and inconsistent condom use. Exposure to blood due to these risky sexual behaviors might have played a role during sexual practice in exposing the subject to GBV-C/HGV.

In conclusion, the comparable prevalences of anti-E2 and differential prevalences of GBV-C/HGV RNA were found in populations at risk for sexual contact and in population at risk for blood exposure. Case control study revealed that the risk for GBV-C/HGV was significantly increased by intravenous drug injection and the presence of HCV-Ab. History of STD also increased the risk for exposure to GBV-C/HGV, but not for the GBV-C/HGV viraemia.

References

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