Anti–HGV E₂ In Relation To Other Viral Hepatitis Agents
In Haemodialysis Patients

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We studied the Anti–HGV E₂ in 50 patients with ESRF on maintenance haemodialysis for at least 12 months and 20 apparently normal individuals as control group to assess their prevalence and liver cell affection in relation to other viral hepatitis agents using ELISA for anti-HGV-E₂. They were 30 men and 20 women with mean age 42.9 ± 4.8 years. Haemodialysis patients were subdivided into subgroup 1 (n: 30) with history of multi-blood transfusion and subgroup 2 (n: 20) without. The study revealed that 22 % (11/50) of the patients group had anti-HGV-E₂ Ab, 30 % (9/30) in the transfused subgroup and 10% (2/20) in the non-transfused while one of the controls was positive (5%). The difference between the patients group and the controls and between the transfused and non-transfused subgroups was insignificant (P > 0.05). Of 11 anti-HGV-E₂ positive patients, 9 (81.8%) had history of blood transfusion, 2 (18.2%) were coinfected with HBs Ag., 9 (81.8%) had HCV Ab and 5 (45.5%) were HCV-RNA positive. However, there was no significant difference between anti-HGV-E₂ positive and negative patients with regard to sex, age, blood transfusion, chronic HBs Ag infection, and frequency of detection of HCV Ab and HCV-RNA and liver transaminases. The prevalence of anti-HGV-E₂ in HD patients coinfected with HBs Ag. 28.6% (2/7) was higher than in those negatives for HBs Ag, 20.9%(9/43) but the difference was insignificant. Also, the rate of detection anti-HGV-E₂ was higher in HD patients coinfected with HCV-RNA 29.4% (5/17) than in those negative for HCV-RNA, 18.2% (6/33) and the difference was insignificant. We concluded that HD patients in our locality are at increased risk for HBV, HCV and HGV infections. Blood transfusion plays an important role in HGV transmission but other routes are suspected for some of the patients. HGV is capable of independent transmission irrespective of HBV and HCV. Hepatitis G infection plays a minor role in liver injury in HD patients.


Hepatitis is the most common cause of morbidity and the most reported communicable disease, especially in tropical and sub-tropical countries¹. Although sensitive and specific tests for detecting the known hepatitis viruses (A–E) are available, the etiology of a significant fraction of post-transfusion and community acquired hepatitis cases remains unclear₂, suggesting the presence of additional agents. Recently, two independent teams³,⁴ described hepatitis agents that were previously designated hepatitis G virus (HGV) and hepatitis GB virus C (HGB-C). Nucleotide sequence as well as amino-acid sequence alignment revealed a homology between HGV and HGB-C of 86% and 95% respectively. Based on this high degree of sequence homology, it has been suggested that they are independent isolates of the same virus⁵. Additional studies showed that HGV and HGBV-C are present among individuals with cryptogenic hepatitis⁶ and individuals with risk factors for exposure to parentally transmitted viruses⁷,⁸. However, HGV can be transmitted sexually and from infected mother to her child⁹. The genomic organization of both viruses is similar to that of HCV and other
members of the Flavivridae family, they have 26% homology at the amino-acid level with HCV indicating that they are distinct agents and not just stereotypes of HCV(6).

Studies carried out so far have reported that HGV prevalence in the general population is around 2%. At the present moment in time, however, very few data exist concerning the presence of HGV-RNA in large-size groups of blood donors and healthy individuals, as detection of HGV infection is restricted to RT-PCR technique, which is very expensive and unsuitable for screening of large groups of individuals. A semipurified E Protein, a putative envelop protein located on the surface of the HGV virion particle, has been recently utilized to develop a prototype enzyme immunoassay to detect HGV anti E 2. Preliminary data report that response to HG env is associated with loss of detectable HGV viremia. HG env-specific antibody as detected by ELISA assay might serve as useful marker for detecting recovery from HGV infection in large-sized population groups(7,10).

Haemodialysis patients have been reported to be at increased risk for hepatitis B and G and are expected to be a high-risk group for exposure to HGV/GBV-C. Since the dialysis procedures offer many possibilities for exposure to blood and these patients are immunologically incompetent(11). However, transmission may occur by erythrocyte concentrate(12) or occur nosocomially with in the dialysis center either by personnel or medical equipment if disinfection procedures are inadequate and contaminated equipment is shared between patients(13).

The aim of this study was to assess anti-HGV-E 2 prevalence and liver cell affection, in relation to other viral hepatitis agents in haemodialysis (HD) patients in Dialysis Unit of Benha University Hospitals.

MATERIAL AND METHODS

Subjects: The current study was conducted in the Microbiology and Immunology Department, Benha Faculty of Medicine, Zagazig University and Dialysis Unit of Benha University Hospitals during the period between July, 1997 and July, 1998. Blood samples were collected from 50 patients (30 men and 20 women) with ESRF who were undergoing maintenance HD for a period of more than 12 months. Their mean age was 42.9 ± 4.8 years. Patients were subdivided into subgroup 1 (n=30) with history of multi- blood transfusion and subgroup 2 (n=20) without. In addition, 20 apparently healthy persons, matched in both age and sex to those of the patient group, with no history of blood transfusion, exposure to blood products or dialysis were chosen as a control group.

Methods: All patients and controls were subjected to the following:
A) Full history was taken as regards the age, sex, history of disease, duration of dialysis, history of blood transfusion, history of jaundice, darkening of urine, history of operation and other risk factors.
B) Laboratory investigations: Blood samples were withdrawn from each subject. Serum samples were separated for performance of:
1- Transaminase enzymes, ALT and AST.
2- Serological assays: ELISA for anti-HGV-E 2, HBsAg, HCV IgG and HIV Ab.
3- Virological methods: RT-PCR for HCV-RNA.

* Serum anti-HGV-E 2 IgG was determined using a microtitre plate assay (μ plate Anti-HGenv) (Boehringer Mannheim, Germany) which is an enzyme immunological test for the qualitative determination of IgG antibodies to HGV-E 2 antigen in serum that based on the method described by Tacke et al. Anti-HGV-E 2 Kit is two-step sandwich assay using streptavidin technology that utilizes recombinant antigen from the envelope E 2 region of this virus. The diluted samples and an incubation solution (containing both anti-HGV-E 2-biotin labeled and HGV-E 2 antigen) are incubated in Streptavidin coated microwells. The anti HGV-E 2 biotin labeled in the incubated solution will bind to the Streptavidin coated wells and any anti-HGV-E 2 antibodies in the sample (if present) will bind to the immobilized anti HGV-E 2 biotin/ HGV-E 2 antigen complex. Following washing to remove unbound materials; the captured anti HGV-E 2 antibodies from the serum samples are incubated with peroxidase (POD)- labeled sheep monoclonal anti-human IgG. During the course of the second incubation, an (antibody/antigen) –human antibody-anti-human antibody conjugated POD enzyme complex will be formed in those microwells, which contained samples with antibodies to HGV-E 2. After removal of excess conjugate, bound enzyme is detected by the addition of substrate solution containing ABTS and hydrogen peroxide (ABTS is the registered trademark for the chromogen di-ammonium 2, 2-azino-bis). A greenish colour, which is read photometrically, will develop in the wells, which contained anti-HGV positive samples. The test was done according to the manufacturer’s instructions.

*Hepatitis B surface antigen (HBsAg) was detected using Murex HBs Ag Kit (Murex
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Diagnostics Limited-UK) according to the manufacturer’s instructions that based on the method described by Krugman and Giles(15)

*Serum Hepatitis C antibodies (HCV IgG) were detected using Murex anti-HCV 3rd generation ELISA Kit (Murex diagnostics Ltd., England) which is an in-vitro qualitative and quantitative enzyme immunoassay that utilizes antigens from the putative core (C, structural), protease/helicase (NS3, non structural), NS4 (non-structural) and replicase (NS5, non structural) regions of the virus to provide a sensitive diagnostic test.

*Serum HIV antibodies was determined using Murex HIV-1-0-2 Detection Kit (Murex Diagnostic Limited, UK) according to the manufacturer’s instructions.

*RT-PCR for detection of HCV-RNA: Serum RNA was extracted using the high pure viral nucleic acid Kit (Boehringer Mannheim, Germany) according to the manufacturer’s instructions that based on the method of Vogelstein and Gillespie(16) cDNA synthesis was performed according to Berger and Kimmel (17). The sequence of the anti-sense primer was: 5'-CGA-GAC-CTC-CCG-GGG-CAC-TCG-CAA-GCA-CCC-3 (synthesized by Biosource, Europe). An HGV segment of 272 bases from the 5 UTR was amplified using PCR in DNA, thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT, USA) according to the method of Bukh et al.119). The PCR products were detected by agarose-gell electrophoresis. In this case florescent bands of products equivalent to the MW of approximately 272 base pairs (bp) were recorded as positive for HCV-RNA.

Statistical analysis: All data entered and analyzed using Epi-Info (Version 6.1) software (Dean et al., 1994). Comparison between group means were done using t-test and comparisons between proportion using Chi-Square test.

RESULTS

Hepatitis B virus surface antigen (HBs Ag) was detected in 14% (7/50) of the patients group, 16.7% (5/30) in subgroup 1 and 10% (2/20) in subgroup 2 while only one of the controls was positive, 5% (1/20). The differences between the patients group and the controls was also highly significant (P < 0.001) and the difference between the two patients subgroups was also highly significant (P < 0.05).

Regarding the RT-PCR for HCV, 34% (17/50) of HD patients were positive (16 out of 17 positive HCV-RNA were also positive for HCV-Ab, while only one was HCV-Ab negative). The prevalence of detectable HCV-RNA was 46.7% (14/30) in subgroup 1 and 15% (3/20) in subgroup 2 while one of the controls 5% (1/20) was HCV-RNA positive. The differences between HD patients and the controls and between the two patients subgroups, regarding HCV-RNA positivity, were significant (P < 0.05).

HIV Ab was not detected in any subject in both the patients and the control groups. Table 1 reports markers of Hepatitis B and C infections in both transfused and non-transfused HD patients and the control.

Anti-HGV-E2 was detected in 22% (11/50) of the patients group, 30% (9/30) in subgroup 1 and 10% (2/20) in subgroup 2 while one (5%) of the controls was positive. The difference between the patients group and the controls was significant (P < 0.05), while the difference between the two patients subgroups was insignificant, regarding anti-HGV-E2 positively. The mean age was 44.2 ± 12 years, 7 were males and 4 were females.

Nine (81.8%) of 11 anti-HGV-E2 positive patients had history of blood requirement. Out of 11 anti-HGV-E2 positive patients, 2 (18.2%) had coinfection with HBsAg, 9 (81.8%) had HCV-Ab, one (9.1%) had coinfection with both HBsAg and HCV Ab, while (45.5%) were HCV- RNA positive. Table 2 shows the characteristics of anti-HGV-E2 positive and negative patients on chronic HD treatment. There was no significant difference between anti-HGV-E2 positive and negative patients with regard to sex, age, transfusion, HBsAg infection, and frequency of detectable HCV Ab and positivity of HCV-RNA. There was also no significant difference between the two groups concerning the levels of ALT, 18.5±6 Vs 16 ±4 and AST, values 20.5 ± 6 Vs 19.5± 10.8.

The prevalence of anti-HGV-E2 infection in HD patients coinfected with HBV, 28.6% (2/7) was higher than in those negative for HBs Ag, 20.9% (9/43) but the difference was insignificant (Table 3). Also, the prevalence of anti-HGV-E2 in HD patients coinfected with hepatitis C (HCV-RNA positive), was higher than in those with negative HCV- RNA
[29.4% (5/17) Vs 18.2% (5/33)] but the difference was also insignificant (Table: 4).

Table (1): Anti-HGV-E2 and markers of other hepatitis viruses infection in the studied subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti-HGV-E2 (%)</th>
<th>HBs-Ag (%)</th>
<th>Anti-HCV (%)</th>
<th>HCV-RNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group (n: 50)</td>
<td>11 (22)</td>
<td>7 (14)</td>
<td>34 (68)</td>
<td>17 (34)</td>
</tr>
<tr>
<td>Subgroup 1 (n: 30)</td>
<td>9 (30)</td>
<td>5 (16.7)</td>
<td>28 (93.3)</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>Subgroup 2 (n: 20)</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td>6 (30)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Control group (n: 20)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>2 (10)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

\( \chi^2 \): \( >0.05 \) (NS) \( \chi^2 \): \( >0.05 \) (NS) \( \chi^2 \): \( <0.001 \) (HS) \( \chi^2 \): \( <0.05 \) (NS)

\( \chi^2 \): Chi-square test for comparison hem ecu patients and control groups. \( \chi^2 \): Chi-square test for comparison between 2 subgroups.

Table (2): Characteristics of Anti-HGV-E2 positive and negative patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Anti-HGV-E2 positive (N=11)</th>
<th>Anti-HGV-E2 negative (N=39)</th>
<th>Test of Significance</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>7/4</td>
<td>23/16</td>
<td>( \chi^2 ): 0.08</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.2 ± 12</td>
<td>40.5 ± 1.5</td>
<td>t: 1.03</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>Transfused/not transfused</td>
<td>9/2 (81.8)</td>
<td>21/18 (53.8)</td>
<td>( \chi^2 ): 2.74</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>HBs Ag positive</td>
<td>2 (18.2%)</td>
<td>5 (12.8)</td>
<td>( \chi^2 ): 0.20</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>Anti-HCV positive</td>
<td>9 (81.8%)</td>
<td>25 (64.1%)</td>
<td>( \chi^2 ): 1.21</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>HCV- RNA positive</td>
<td>5 (45.5%)</td>
<td>12 (30.8%)</td>
<td>( \chi^2 ): 0.81</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>ALT</td>
<td>18.5 ± 6</td>
<td>16.0 ± 4</td>
<td>t: 1.3</td>
<td>&gt;0.05 (NS)</td>
</tr>
<tr>
<td>AST</td>
<td>20.5 ± 6</td>
<td>19.5 ± 10.8</td>
<td>t: 0.06</td>
<td>&gt;0.05 (NS)</td>
</tr>
</tbody>
</table>

Table (3): Relationship between Anti-HGV E2 and HBsAg in HD patients.

<table>
<thead>
<tr>
<th>HBsAg positive</th>
<th>Anti-HGV E2 + ve</th>
<th>Anti-HGV E2 - ve</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Anti-HGV E2 + ve</td>
<td>2</td>
<td>28.6</td>
<td>9</td>
<td>20.9</td>
</tr>
<tr>
<td>Anti-HGV E2 - ve</td>
<td>5</td>
<td>71.4</td>
<td>34</td>
<td>79.1</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Relationship between Anti-HGV E2 and HCV-RNA in HD patients.

<table>
<thead>
<tr>
<th>HCV-RNA positive</th>
<th>Anti-HGV E2 + ve</th>
<th>Anti-HGV E2 - ve</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Anti-HGV E2 + ve</td>
<td>5</td>
<td>29.4</td>
<td>6</td>
<td>18.1</td>
</tr>
<tr>
<td>Anti-HGV E2 - ve</td>
<td>12</td>
<td>71.6</td>
<td>27</td>
<td>82.9</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Viral hepatitis represents a major health problem worldwide. In Egypt, hepatitis is endemic and may be increasing in frequency. Hepatitis G (HGV) is a new discovered virus belonging to Flaviviridae viruses. Current estimates suggest that about 0.9% to 10% of blood donors worldwide, either with normal or elevated ACT level have been or are infected with HGV. Approximately 0.3% of patients with acute viral hepatitis in the United States may be infected with HGV alone. However, it was found that at least 36% of patients with acute hepatitis G virus infection persist and may lead to chronic hepatitis. In addition, HGV infection may be associated with hepatocellular carcinoma and extrahepatic manifestations like aplastic anemia. Little work was published correlating the prevalence of HGV infection in high risk group patients. However, some authors suggested a higher prevalence in multiple transfused patients. Since HGV may be transmitted parentrally, patients on maintenance HD should be at increased risk for such infection, as they are for HCV and HBV infections. Renal failure cases are, also, increasing in frequency, so the importance of studying viral hepatitis agents in patients on maintenance HD. The aim of this study was to assess Anti-HGV E2 prevalence and liver cell affection in relation to other viral hepatitis agents in HD patients.
In this study, the percentage of anti-HGV-E2 IgG in HD patients using ELISA technique was found to be 22% (11/50). Of the patients group, 30% (9/30) in subgroup 1 and 10% (2/20) in subgroup 2. Our results are slightly higher than that of Mohammed who reported 15% prevalence in thalassemic patients and Prati et al. who reported that 18.5% of thalassemic patients had anti-HGV-E2 IgG. This may be attributed to the higher age of our patients, which means more exposure to HGV. Regarding the control group, we find that one (5%) was positive, which suggests previous exposure to HGV. This result is matched with Mohammed who reported that 4% of normal healthy children had anti-HGV-E2 and that of Ross et al. who reported that the prevalence of anti-HGV-E2 in Asian countries; Bhutan, Malaysia and Philippines was 3.9% 6.3% and 2.7%, respectively. However, a prevalence of 10.9% 15.3% 19.5% and 20.3% were reported, respectively, in Germany, Austria, Brazil and South Africa. The difference between the HD patients and the controls and between the two subgroups of HD patients in our study, regarding Anti HGV-E2 –prevalence, did not reach significance. History of transfusion was observed in 81.8% (9/11) of HD patients with positive anti-HGV E2, that is in agreement with studies of Fabriz et al. and Masuko et al. who reported history of transfusion, respectively, in 80% and 75% of HD patients infected with HGV. However, Pujol et al. reported, that none of the HGV positive HD patients had a history of blood transfusion when HGV-RNA was first detected in their sera. The high percentage of anti-HGV-E2, in our study, especially the multiple transfused ones suggests that HD patients are more risky for HGV infection. However, 2 anti-HGV-E2 positive patients and one of the controls, in our study, did not have a history of blood transfusion and we suspect transmission routes other than transfusion route.

Our results showed that the rate of detection of HBsAg, HCV Ab and HCV-RNA, and the ALT and AST levels were higher in anti-HGV-E2 positive than negative patients(Table 2). However, there was no significant difference between anti-HGV-E2 positive and negative patients on chronic HD treatment as regard to age, sex, and number of transfused patients, chronic HBsAg infection, frequency of HCV Ab, detectable HCV-RAN or liver transaminases. These data are in-agreement with many other studies (8, 28). However, Fabriz et al. reported that the difference between HGV positive and negative HD patients was significant regarding HCV-Ab only.

When we studied anti-HGV-E2 infection in patients coinfected with other hepatitis viruses, we find that the prevalence of anti-HGV-E2 infection in HD patients coinfected with HBV, 28.6% (2/7) was higher than in those negative for HBs Ag, 20.9% (9/43) but the difference was insignificant. Also, the prevalence of anti-HGV-E2 in HD patients coinfected with hepatitis C (HCV-RNA positive), was higher than in those with negative HCV-RNA [29.4% (5/17) vs. 18.2% (5/33)] but the difference was also insignificant. These results are in agreement with others (8, 20, 31) in that the prevalence of anti-HGV-E2 infection was higher in patients coinfected with HBV and or hepatitis C. Whether these findings are simply explained by common routes of transmission (blood transfusion), as suggested by others or whether HGV might have any influence i.e. on the replication of HGV remains to be investigated. However, our results suggest that chronic HD patients are a high-risk group for exposure to with HGV. HVB and HCV by means of blood transfusion. Studies based on sequence analysis of HGV isolates are warranted to evaluate the role played by patient to patient transmission in this high-risk group.

Liver transaminases (ALT and AST) were slightly but not significantly different in HGV/HCV coinfected patients versus HCV infected patients after exclusion of one case coinfected with HGV, HBV and HCV (ALT: 19 ± 4 Vs. 17 ± 7.5 and AST: 21 ± 6 Vs. 18 ± 4) which is matched with the results of others. Since patients on maintenance HD are known to have compromised immune responses, it is therefor not certain to know what effect of persistent HGV may have in persons with normal immune responses.

In conclusion, exposure to HGV seems to be prevalent in our locality. Patients on maintenance HD are at increased risk for HGV exposure. HGV is capable of independent transmission irrespective of HBV and HCV. Blood transfusion plays an important role in HGV transmission but other routes are suspected for some of the patients. No specific clinical feature has been associated with positivity of anti-HGV E2 in HD patients. HGV infection seems to play a minor role in liver injury as indicated from the mild elevation of serum transaminases. However, if a clear disease association is established, it will be necessary to develop a practical screening assay to exclude HGV infection from the donor population. The development of such test systems is a matter of utmost injury since mass screening of blood donors using PCR, is impractical at the present time. More studies are necessary in
order to evaluate HGV incidence, other methods of transmission, the role of HGV in the pathogenesis of liver disease and prognosis among HD patients.

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