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Hepatitis G virus infection in Egyptian children with chronic renal failure (single centre study)

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Published: 16 December 2009

Received: 30 September 2009

Annals of Clinical Microbiology and Antimicrobials 2009, **8**:36 doi:10.1186/1476-0711-8-36

Accepted: 16 December 2009

This article is available from: <http://www.ann-clinmicrob.com/content/8/1/36>

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Abstract

Background: Hepatitis G virus (HGV) is an RNA virus. It is mainly transmitted through exposure to contaminated blood although other routes may also exist. Patients with chronic renal failure (CRF) are at high risk of acquiring HGV because they require frequent blood transfusions. Ongoing HGV infection can be only diagnosed by demonstrating viremia in patient sample by reverse transcriptase (RT) PCR. Antibodies to the envelop protein E₂ (anti E₂) of HGV is an indicator of virus clearance and testify past HGV contact. This cross sectional study was done to assess the frequency of HGV exposure (ongoing and past infection) in Egyptian children with CRF and to study the possible risk factors of infection.

Methods: This study included 100 children with CRF [34 on regular haemodialysis (HD) and 66 before the start of dialysis (predialysis)]. All patients sera were tested for HGV RNA by RT-PCR, anti E₂, hepatitis C virus (HCV) antibody, hepatitis B surface antigen (HBsAg), and hepatitis B core antibody (HBcAB). Twenty five healthy children of matched age & sex were used as controls.

Results: HGV RNA was positive in 9 (26.5%) of HD and 9 (13.6%) of predialysis children. Anti E₂ was positive in 14 (41.2%) of HD and 19 (28.8%) of predialysis children.

In comparison to controls; CRF (n = 100); HD and predialysis children had significantly higher prevalence of anti E₂ [4% VS 33% for all CRF cases; (p = 0.002)& 41.2% (p = 0.002) and 28.8% (p = 0.01); for HD and predialysis groups; respectively]. HGV RNA was significantly more prevalent only in HD children in comparison to controls (p = 0.03). HD and predialysis children did not have significant difference in the prevalence of HGV RNA (p = 0.16) or anti E₂ (p = 0.26).

HGV exposure was not correlated with positivity of anti HCV (p = 0.32), HCV RNA (0.09), HBsAg/HBcAB (p = 1), age (p = 0.06), or gender (p = 0.83). It was significantly correlated with duration of the disease (p < 0.001). Ongoing HGV infection was significantly more prevalent with frequent blood transfusion (p < 0.001). There were no significant differences in serum levels of ALT (p = 0.09), total bilirubin (p = 0.1) and albumin (p = 0.06) in children with ongoing infection in comparison to healthy controls.

Conclusions: The frequency of HGV exposure in Egyptian children with CRF appears to be high and is mainly related to frequent blood transfusions and longer disease duration. HGV infection in these children is not associated with significant changes in hepatic biochemical parameters.

Background

Infections are one of the important causes of morbidity and mortality in patients with end stage renal failure [1]. Chronic hepatitis is a major complication of chronic haemodialysis (HD). Initially; hepatitis B virus (HBV) infection was the most common etiologic agent of chronic hepatitis in patients on chronic HD. Later; after HBV vaccines became available and measures for screening and exclusion of hepatitis B surface antigen (HBsAg) positive blood were routinely used, HBV infection dropped significantly. Subsequently; however, hepatitis C virus (HCV) emerged as a new problem. In USA; rates of positive anti HCV reached up to 36% in HD patients in 1990s [2].

Two different laboratories in the USA isolated a new flavivirus-like RNA virus in the years 1995 and 1996. The first laboratory named it "G B virus-C (GBV-C)" and the other "hepatitis G virus (HGV)". Both viruses were subsequently considered different genotypes of the same virus because they were found to share most of the nucleotide and amino acid sequences [3]. Both viruses have a single stranded RNA genome of approximately 9.4 kb. It encodes a single poly protein of 2900 amino acids in which the non structural proteins are located at the C terminal end and the structural proteins at the N terminal end [4]. The HGV genome encodes an open reading frame coding for two envelop proteins (E1 & E2) [5].

The ongoing HGV infection can be diagnosed by demonstration of viremia in patient blood by reverse transcriptase (RT)-PCR. An assay detecting antibodies to the envelop protein E2 (anti E2) of HGV has been developed and this serological marker is considered to be an indicator of the virus clearance [6,7]. Thus; the presence of anti E2 seems to indicate past HGV exposure and is associated with immunity and protection from reinfection [8].

Blood transfusion is the main risk factor for HGV transmission [9]. Patients with chronic renal failure (CRF) usually require frequent blood transfusions which make them more vulnerable to HGV infection [10,11]. The present data on the prevalence of HGV anti E2 in HD patients is conflicting, with studies showing rates of 7% in Japan [12] up to 29% in Germany [13]. Up to our knowledge there are no published data on the prevalence of HGV infection in Egyptian children with CRF.

HGV associated hepatitis runs with normal biochemical parameters in 75% of patients [14]. Although HGV infection appears to be not associated with severe hepatic damage; however a possible link between HGV infection and acute [15-17], fulminant hepatitis [18-21], chronic (mild and moderate) hepatitis [22,23] or even hepatic fibrosis [24] has been suggested. This study was done to assess the frequency of HGV exposure (ongoing & past infection) in

Egyptian children with CRF and to correlate viral exposure with age, sex, duration of disease, frequency of blood transfusion, and co infection with HBV & HCV and to study the effect of ongoing viral infection on hepatic biochemical parameters in these children.

Methods

This cross sectional study was carried out at Mansoura University Children's Hospital Nephrology Unit, Egypt in the period from January till June 2008. The study involved 100 children (67 males & 33 females) with CRF. Their ages ranged from 2 to 18 years [median (Interquartile range; IQR) = 9 (5-14) years]. Written consents were obtained from the parents of children and the study was approved by the ethical committee of the university.

Among the studied patients; 34 children (19 males & 15 females) were on regular HD for a mean duration of 5.1 ± 2.1 years and the remaining 66 children (48 males & 18 females) were on medical therapy without a need to receive dialysis with mean disease duration of 2.4 ± 0.94 years.

A questionnaire was used to collect sociodemographic data such as age, sex, number of previous blood transfusions and duration of the disease. A group of 25 randomly chosen apparently healthy children of matched age and sex served as a control group.

Five mls. of blood samples were withdrawn from patients and controls. They were centrifuged and serum was separated. Serum was stored in aliquots at -70°C . Repeated freezing and thawing was avoided. All the biochemical parameters were done by routine laboratory methods unless otherwise mentioned. HBsAg, Total hepatitis B core antibody (HBcAB) and Anti HCV antibody were detected with commercially available enzyme linked immunosorbent assay (Equipar, 21047 Saronno VA, Italy). Serum alanine aminotransferase (ALT) values were measured by Hitachi 902 auto analyzer (Roche, Switzerland). HGV Anti E2 was detected by ELISA and HGV RNA by RT - PCR.

HBsAg

This kit uses double antibody (sandwich) ELISA method, in which polystyrene microwell strips are pre-coated with antibody to HBsAg. Patients samples are added to the microwells together with a second antibody; horseradish peroxidase (HRP-Conjugate). During incubation the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing, chromogen solution containing Tetramethylbenzidine (TMB) is added to the wells in presence of the antibody -antigen -antibody (HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns

yellow after the reaction is stopped with sulfuric acid. The intensity of color is proportional to the amount of antigen captured in the wells, and to the sample respectively.

HbcAb

HbcAb test uses the principle of competitive immunoassay technique for the detection of antibodies to Hbc in human serum. During the assay, the test specimen and HRP-HbcAb conjugates are incubated simultaneously with the coated microwells. HbcAb if present in the specimen will compete with HRP-HbcAb for the constant amount of Hbc antigen coated on the microwells surface. Thus the amount of HRP-HbcAb bound to the well is inversely proportion to the concentration of HbcAb in the specimen. Unbound conjugated are then removed by washing. The presence of the HRP-complex is shown by a blue color upon additional incubation with TMB substrate.

HCV-Ab

This kit employs solid phase ELISA method for detection of antibodies to HCV in two step incubation procedure. Polystyrene microwell strips are precoated with recombinant antigens corresponding to the core and the non-structural regions of HCV. During the first incubation step, specific HCV-Ab, if present, are captured on the solid phase. The wells are washed and anti-human IgG antibodies conjugated to the enzyme (HRP - Conjugate) are added. During the second incubation step, these HRP Conjugate antibodies bind to any antigen antibody complexes previously formed and the unbound HRP Conjugate is then removed by washing. Chromogen solution containing TMB is added to the wells in presence of the antigen-antibody-anti-IgG (HRP) immunoconplex which is hydrolyzed to a blue colored product. The blue color turns yellow after the reaction is stopped with sulfuric acid. The intensity of color can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

Hitachi 902

A random access fully automated chemistry analyzer with a throughput up 200 tests per hour and up to 300 tests with optional ion selective electrode. The Roche/Hitachi 902 test menu comprises clinical chemistry, protein profiling and therapeutic drug monitoring. It is able to consolidate routine testing and support special testing assays and a choice of over 100 different assay applications.

HGV Anti E2 Testing

All of the samples were tested with a commercial Enzyme-linked Immunosorbent Assay (Anti-H Genv EIA; Boehringer, GmbH, Mannheim, Germany) for the detection of specific IgG antibodies against HGV E2 protein. Antibodies to the E2-protein of HGV were detected by a two-

step sandwich enzyme immunoassay [7]. Serum samples or controls were diluted in sample buffer and were added to coated microtiter wells. The microplate was incubated and washed then a solution containing peroxidase-conjugated anti-human IgG antibody was added. After incubation at room temperature and the addition of chromogen substrate, the optical density was measured at 405 nm within 10 minutes. All the incubation steps were performed using a shaker. Results were interpreted according to the manufacturer's instruction.

RT-PCR for HGV RNA

Total RNA was extracted from 100 µL of serum using an RNA easy Mini kit (Qiagen, Valencia, California, USA). The isolated RNA was used for reverse transcription (RT) and first round PCR. RT-PCR was performed in a single tube using RT-PCR System (Promega, Madison, WI). Both first and second-round PCR were carried out using primers that hybridize to 5' non-translated regions of an infectious GBV-C clone (GenBank accession no. [AF121950](#), nt 54 to 389). Primers for the first-round RT-PCR were GBVF1 5'-CCGACGCCTATCTAAGTA GACGC and GBVR1 5'-TCAACTCGCCGATAAA-CCTATTGG. Primers for the second-round PCR were GBVF2 5'-GTGACAGGGTTGTAGG and GBVR2 5'-GACATTGAA-GGG-CGACGTGG. PCR products were detected on 1.5% agarose gels containing 0.5 µg/ml ethidium bromide. The expected band sizes were 336 and 231 bp for the first- and second-round PCR; respectively. A reaction was considered positive if either the first- or second-round PCR produced a band of the expected size [25].

Qualitative HCV RNA detection (Cobas Amplicor, Roche Diagnostics)

The amplicor HCV RNA detection is based on five major processes; specimen preparation, reverse transcriptase of target RNA to generate complementary DNA, PCR amplification of target DNA using HCV complementary primers, hybridization of the amplified products to oligonucleotide probes specific to the targets and detection of the probe - bound amplified products by colorimetric determination. The master mix reagent has been developed to insure equivalent detection of all known genotypes of HCV at concentration of 50 Iu/ml.

Statistical analysis

Data was analyzed by SPSS (Statistical Package for Social Sciences) under Windows (version 10). Numerical data were found to have a non parametric distribution by Kolmogorov Smirnov test. Data were expressed as median and interquartile range (IQR) or when indicated as absolute numbers and percentages. Tests used included Chi square and Mann Whitney test. P value less than 0.05 was considered significant.

Results

Table 1 gives the frequency of HGV, HCV and HBV markers in the two groups of patients and controls. Among the 100 CRF patients; HGV exposure (ongoing and past infection) was found in (51/100) 51% of children. HGV RNA and Anti E2 were not detected simultaneously in any patient. Among the HD group: HGV RNA, anti E2 antibodies, HBsAg; HbcAB, HCV RNA and anti HCV were positive in (9/34) 26.5%; (14/34) 41.2%; (2/34) 5.9%; (2/34) 5.9%; (33/34) 97% and (32/34) 94.1% of patients; respectively.

In the predialysis group: (9/66) 13.6%; (19/66) 28.8%; (3/66) 4.5%; (3/66) 4.5%; (25/66) 37.9% and (20/66) 30.3% of patients tested positive for HGV RNA, Anti E2, HBsAg, HbcAB. HCV RNA and anti HCV; respectively.

HGV RNA was detected in significantly more children on HD (26.5%) than in controls (4%) ($p = 0.03$). On the other hand; in comparison to controls; all CRF, HD and predialysis children had significantly higher prevalence of anti E2 [4% vs. 33% for all CRF cases; ($p = 0.002$), 41.2% ($p = 0.002$) and 28.8% for HD and predialysis groups ($p = 0.01$); respectively].

HD and predialysis children did not have significant difference in the prevalence of HGV RNA ($p = 0.16$) or anti E2 ($p = 0.26$).

Demographic and virological markers were compared between children with HGV exposure (ongoing infection or past infection) and those who were not exposed. HGV exposure was not related to age ($p = 0.06$), sex ($p = 0.83$), history of blood transfusion ($p = 0.15$), co infection with HCV ($p = 0.09$) or HBV ($p = 1$). HGV exposed patients had significantly longer disease duration in comparison to none exposed patients ($p < 0.001$), (Table 2).

The number of blood transfusions was studied in relation to ongoing HGV infection. Patients with ongoing infection had significantly more frequent blood transfusion in the last 12 months preceding sampling in comparison to patients without ongoing infection [median (Interquartile range; IQR) = 9 (7-24) Vs 2 (0-5) times; $p < 0.001$; respectively].

The significance of HGV viraemia in the liver biochemistry was also studied. There were no significant differences in serum levels of ALT ($p = 0.09$), total bilirubin ($p = 0.1$) and albumin ($p = 0.06$) in children with ongoing infection in comparison to healthy controls, (Table 3).

Discussion

In the current work, the frequency of HGV exposure was (28/66) 42.4% in predialysis children with CRF and increased to (23/34) 67.6% in HD children. It was 51% (51/100) in the total group of CRF children and only 8% in healthy controls.

Patients with chronic renal failure have a great risk to acquire HGV infection because they usually need frequent blood transfusions [26]. HGV RNA prevalence in chronic dialysis adult patients has been studied in different countries with somehow conflicting results. HGV RNA prevalence rates in HD patients ranged from 11.5 to 27% in the USA [27,28], from 6-57.5% in Europe [11,16] and in Asia, from less than 2.2% in India [29] to 55% in Indonesia [30]. In contrast, few reports are available on HGV prevalence in children. HGV RNA is prevalent in 6.3% of healthy American children [31], only in 0.5% of healthy Japanese children [32], in 21% of Pakistani children with Beta thalassaemia [33], in 13% of Italian children with thalassaemia [34] and in 18.5% of American children with CRF [35]. As Egypt is one of the countries world wide suffering from high prevalence of hepatotropic viruses [36]; it is not surprising to find a high frequency of HGV exposure in the studied children.

Although a positive correlation between the prevalence of HGV infection and the history and/or amounts of blood transfusion have been reported in some studies [37,38]; other studies have found no such connections [16,39]. In our study; HGV exposure was not significantly different in children with and without history of blood transfusion; however patients with ongoing infection have significantly more frequent blood transfusions in the last 12 months preceding sampling in comparison to patients without ongoing infection. These apparently opposing findings can be explained easily if we took into consideration that a large number of our patients had history of blood transfusion. 49/51 (96%) of HGV exposed patients

Table 1: Frequency of HGV RNA, Anti E2, HBsAg, HbcAB, HCV RNA and Anti HCV in patients and controls.

	HGV Exposure	HGV/RNA	Anti E2	HbsAg	HbcAB	HCV RNA	Anti HCV
Haemodialysis(34)	23(67.6%)	9 (26.5%)	14 (41.2%)	2 (5.9%)	2 (5.9%)	33 (97%)	32 (94.1%)
Predialysis (66)	28(42.4%)	9 (13.6%)	19 (28.8%)	3 (4.5%)	3 (4.5%)	25 (37.9%)	20 (30.3%)
Total (100)	51 (51%)	18 (18%)	33 (33%)	5 (5%)	5 (5%)	58 (58%)	52 (52%)
Controls (25)	2 (8%)	1 (4%)	1 (4%)	0	0	0	0

Table 2: Comparison of different parameters in patients with and without HGV exposure.

	<i>HGV Exposed</i>	<i>HGV None Exposed</i>	<i>P</i>
Number of patients	51	49	-
Age (years)	7 (5-13)	9 (7-15)	0.06*
Sex (M : F)	35 : 16	32 : 17	0.83**
HBsAg (+)	3	2	1.0**
HCV RNA (+)	26 (50.1%)	32 (65.3%)	0.09**
History of blood transfusion (+)	49 (96%)	43 (87.7%)	0.15**
Duration (years)	3 (3-5)	2 (2-3)	< 0.001*

Values expressed as percentages or median and IQR

* Mann Whitney test

**Chi Square test

and 43/49 (87.7%) of HGV non exposed patients had history of blood transfusion. Our centre is a tertiary referral facility and most of our patients present to us during late stages of renal diseases after they have received blood transfusion for treatment of anemia of CRF in their primary health care facilities. Thus when we studied HGV exposure in relation to history of blood transfusion; we did not find significant difference between patients with and without history of blood transfusion. It is logic to think that every blood transfusion session carries a potential risk of getting blood borne infection and that the number of blood transfusions should be considered in evaluating the potential risk of getting any blood borne infection; so when we studied the relation between ongoing HGV infection and the number of blood transfusions in the last 12 months before sampling; we found that HGV infection was significantly more common with frequent transfusions.

Several other non parenteral routes of infection have been suggested for HGV infection such as percutaneous contamination from saliva [40], maternal-fetal vertical transmission [41] and nosocomial infection through patient to patient transmission [42]. In our study, HGV exposed patients had significantly longer disease duration in comparison to none exposed patients. We believe that longer disease duration could increase the chance to get HGV infection through exposure to increasing numbers of

Table 3: Comparison of liver biochemistry in children with positive HGV RNA and controls.

	<i>Patients</i>	<i>Controls</i>	<i>p*</i>
Albumin (Gm/dl)	4 (3.8-4)	4 (3.9-4.2)	0.06
Total bilirubin (mg/dl)	0.9 (0.8-1)	0.8 (0.8-0.9)	0.1
ALT (U/L)	27 (21.7-33)	23 (20.5-28)	0.09

Values expressed as median and IQR

* Mann Whitney test

blood transfusion and/or more exposure to the above mentioned suggested non parenteral modes of infection.

Numerous authors have studied the association between HCV and HGV infection with variable results [39,43,44]. HGV co infection has been reported in up to 21% [45] of patients with chronic HCV infection. Fabrizi et al., reported that the frequency of anti HCV antibody was significantly higher in HGV positive than in HGV negative patients on chronic HD treatment and that the rate of co infection was 82% [46]. On the other hand, none of HGV positive HD patients were found to be co infected with HCV in an Italian [47] and Iranian study [48]. In our study the prevalence of HGV in the HCV RNA positive group was not significantly different than in the HCV RNA negative group. Therefore HGV and HCV appear to be transmitted independently as previously reported [47,48].

Some studies have reported coinfection between HGV and HBV in dialysis patients with rates varying from 2.3 to 13% in HD patients [39,49,50]. Other studies have not found any association between these viral infections [51]. In our study; only 5% of CRF children were positive for HBsAg and HBcAB. Again there was no significant difference in the prevalence of HGV infection between HBsAg/HBcAB positive and negative children.

The simultaneous detection of HGV RNA and anti E2 is uncommon and appears just before clearance, probably in the form of immunocomplex [52]. In the current work; none of the anti E2 positive patients tested positive for HGV RNA. Similarly none of the HGV RNA positive patients was serologically positive. This confirms that the appearance of the antibody is accompanied by clearance of HGV RNA. Similar findings have been previously reported [7,53].

In the current study, serum ALT, total bilirubin and albumin levels were not significantly elevated in children with

ongoing infection in comparison to controls. Although children with ongoing infection had slightly elevated serum ALT levels in comparison to controls [median (IQR) 27 (21.7-33) Vs 23 (20.5-28) U/L; respectively]; this was not statistically significant. HGV infection was reported to be associated with acute [15-17] up to fulminant hepatitis [18-21]. On the contrary; other researchers have suggested that in most instances HGV infection does not cause an elevation of ALT levels [37]. Histopathologic examination of liver biopsies revealed no differences between patients with HCV infection alone and patients who were co infected with HGV [54]. Pessoa et al; reported that the hepatic to serum median ratio of HGV RNA was less than 1, in contrast to HCV which is present in high ratio [55]. The current work suggests that HGV infection in children with CRF does not cause significant elevation in ALT levels and therefore there is no strong indication to consider HGV as an etiologic agent of symptomatic hepatitis in these children. Another possible explanation is the small number of children with ongoing infection in our study (18 patients) which may have affected the relevance of ALT values in comparison to controls. A study of larger number of children with ongoing HGV infection is required.

Conclusion

In summary, the frequency of HGV exposure in Egyptian children with CRF appears to be high and is mainly related to frequent blood transfusions and longer disease duration. HGV infection in these children is not associated with significant changes in hepatic biochemical parameters.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AMH was responsible for the choice of cases and the clinical data consultation, statistical analysis and drafted the manuscript. MHZ is the one who suggested the idea and participated in the design of the study, carried out the molecular biology study and helped to draft the manuscript. All authors read and approved the final manuscript

Acknowledgements

We acknowledge all technicians who contributed towards the study by making the practical part of the study. We also acknowledge the team that was responsible for the acquisition of data. Also we should acknowledge persons who was involved in revising the manuscript critically. The funding of the whole work was provided by the Mansoura faculty of medicine. Also we acknowledge anyone who contributed materials essential for the study.

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