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Detection of Hepatitis G Virus (GB Virus C) RNA in Human Saliva

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Using PCR and genomic sequencing, we confirmed the presence of and homology between hepatitis G virus (HGV) (also called GB virus C) RNA in six serum samples and that in two saliva samples obtained from 34 patients with chronic hepatitis C virus infections. Thus, HGV may be found outside the circulatory system.

Knowledge of the presence of the newly discovered blood-borne hepatitis G virus (HGV) (also called GB virus C) in human fluids other than serum is limited (4–6). In comparison to other blood-borne viral infections, such as hepatitis B virus and hepatitis C virus (HCV), HGV seems to occur at a higher frequency in the healthy blood donor population, whereas a comparatively lower frequency of HGV is seen in groups with known risk factors for blood-borne viral infections (4–6). This implies that the routes of HGV transmission may differ from the routes of HBV and HCV transmission. The presence of blood-borne viruses in body fluids other than serum, such as saliva (2) and semen (1), may affect the routes of viral transmission. We therefore analyzed whether HGV is present in extravascular compartments.

Paired serum and saliva samples were obtained from 34 patients with chronic HCV infection, of which 19 patients had been included in a previous study on the presence of HCV in saliva (2). Saliva was collected in sterile tubes in an ice bath during 5 min of chewing on paraffin sticks. All patients had been monitored at the Division of Infectious Diseases, Huddinge University Hospital, for at least 2 years. All patients exhibited transiently or persistently elevated alanine aminotransferase (ALT) levels (>42 U/liter), all had HCV RNA-positive serum samples, and six had HCV RNA in saliva (reference 2 and unpublished data). All samples had been aliquoted and were stored at −70°C until the analysis for HGV RNA. Twenty-seven patients were intravenous-drug users, of which five were coinfected with the human immunodeficiency virus type 1 (HIV-1). For the remaining seven patients, no known risk for blood-borne viral infections could be identified and none were coinfected with HIV-1. For 21 of the 34 patients, ALT levels were determined on the day of serum and saliva sampling. Liver biopsies were obtained from 17 patients; 10 of these had chronic active hepatitis and 7 had chronic persistent hepatitis as determined by histology.

HGV RNA was detected by a nested PCR based on primers (outer pair, HGV-1X-OU [5′-TTGTGCCTGCGGCGAGAC-3′] and HGV-2X-OD [5′-AATGCCACCCGCCCTCAC-3′]; inner pair, HGV-3-IU [5′-CGCACGGTCCACAGGTGT-3′] and HGV-4-ID [5′-GGTGGCCCCATGCATTTC-3′]) from the conserved 5′ noncoding region of the HGV genome (4, 6). HGV RNA was extracted from 100 μl of serum or 200 μl of whole saliva by guanidinium extraction as previously described for HCV RNA (2). Reverse transcription and HGV cDNA synthesis was initiated with the HGV-2X-OD primer for 20 min at 43°C followed by inactivation of the reverse transcriptase enzyme for 5 min at 95°C. The first-round PCR consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. The second-round PCR consisted of 30 cycles with the annealing temperature at 55°C. The amplified 245-bp fragment was identified by ethidium bromide-stained gel electrophoresis. A known HGV RNA-positive serum sample and saliva from an HGV RNA-negative patient spiked with the known HGV-positive serum (10 μl of serum-200 μl of saliva) were consistently positive and served as positive controls. Paired serum and saliva samples were always run on different occasions. Sterile water, treated the same way as the serum and saliva samples, was used as a negative control after every fourth sample. The controls were consistently negative for HGV RNA. The detection limit of the PCR used here has not been defined.

Serum HGV RNA was detected in 6 (18%) of the 34 patients (Table 1). Of these six patients, two (33%) also had HGV RNA in whole saliva. None of these six patients had HCV RNA in saliva (reference 2 and unpublished data) or were coinfected by HIV-1. Moreover, none of eight patients negative for HGV RNA in serum had detectable HGV RNA in saliva. There was no difference in ALT levels between the 6 patients coinfected with HCV and HGV and the 15 patients infected only with HCV (74 ± 44 U/liter versus 104 ± 55 U/liter, respectively [mean ± standard deviation]).

To verify that the amplified 245-bp fragment was of HGV origin, sequence analyses were performed. Prior to sequencing, the amplified HGV cDNA fragments were cloned into vector pCR1000 (Invitrogen, San Diego, Calif.). The cloned fragments were then sequenced with M13 forward and reverse primers and T7 polymerase (Pharmacia Biotech, Uppsala, Sweden) by standard protocols. Sequences were compared with one HCV (3) and three HGV (4, 6) full-length genomes by using the GeneWorks 2.3 (Intelligenetics, Mountain View, Calif.) and Phylip software packages, which confirmed an HGV origin for all sequences. In the two subjects with salivary HGV RNA (Fig. 1, patients N and O), the level of sequence homology between serum and salivary HGV cDNA (range, 98.8 to 99.5%) was comparable to the level of homology (99.8%) between two serum-derived HGV cDNA clones obtained from patient H (Fig. 1, clones 8 and 9).

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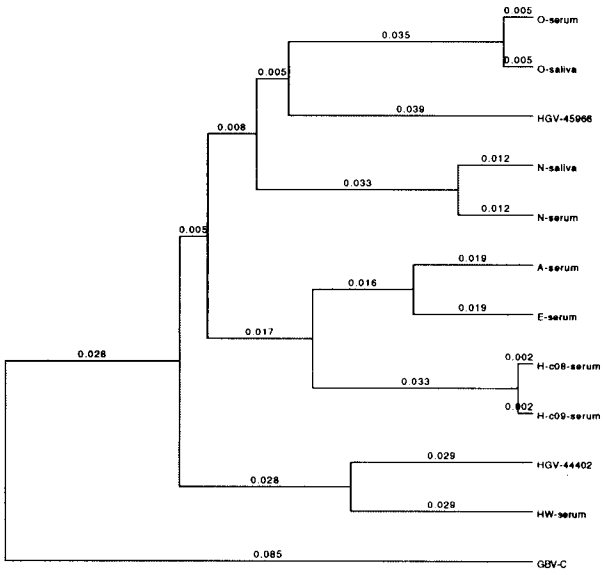
TABLE 1. Presence of HCV RNA and HGV RNA in the serum and saliva of six intravenous drug users with chronic HCV infections

Patient	Age (yr)	ALT (U/liter)[a]	Liver histology[b]	Presence of RNA			
				HCV[c]		HGV	
				Serum	Saliva	Serum	Saliva
A	38	43.1	CAH	+	−	+	−
E	36	65.9	CPH	+	−	+	−
H	43	58.7	CAH	+	−	+	−
N	38	163.5	CAH	+	−	+	+
O	35	58.7	CPH	+	−	+	+
HW	34	52.7	CAH	+	−	+	−

[a] ALT of <42 U/liter is considered normal.
[b] CAH, chronic active hepatitis; CPH, chronic persistent hepatitis.
[c] Some data are from reference 2.

The present study raises some important points. First, saliva from HGV-infected persons may be infectious and should be handled accordingly. Whether salivary HGV RNA transmits HGV infections or has any role in the comparatively high prevalence of HGV in the normal population remains to be investigated. Second, the homology between the two paired serum- and saliva-derived HGV sequences suggests that the virus detected in oral cavities originates from the same source as the virus found in serum. Thus, it is possible that HGV reaches the saliva merely as a contaminant from the circulatory system. Finally, no evidence for more severe liver disease, as determined by mean ALT values or by histology, in persons coinfected with HCV and HGV than in those infected only with HCV was found.



A

B

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Consensus  GRCCCGRCGY YARGCWCGYC RTTAAACCGA GMCCGDHAYY CVCCYGGGCA AACGRCGCYC ACGTACGGTC CACGTCGCCC TTYAATGTCK CTCTTGACCA ATAGGGAYBA YDNMCGGCGA  120
GBV-C      .A....G..C T.G..A..C. G..... .C...TT.CT .C..T..... ....A...C. .......... .......... ..C.....T .......... .....----- CGTA......  115
O-serum    .G....A..T C.G..T..T. G..... .C...TT.CC .A..T..... ....G...C. .......... .......... ..C.....T .......... .......TT- TTAC......  117
O-saliva   .G....A..T C.G..T..T. G..... .C...TT.CC .A..T..... ....A...C. .......... .......... ..C.....T .......... .......TT- TTAC......  117
HGV-45966  .G....A..T C.G..T..T. G..... .C...TA.CC .G..T..... ....A...C. .......... .......... ..C.....G .......... .......CT- TAGC......  117
N-saliva   .G....A..T C.G..T..T. G..... .C...TA.CC .A..C..... ....A...C. .......... .......... ..C.....T .......... .......TC- TATC......  117
N-serum    .G....A..T C.G..T..T. G..... .C...TA.CC .A..C..... ....A...C. .......... .......... ..C.....T .......... .......TT- CATC......  117
HGV-44402  .G....A..T C.G..T..T. G..... .C...TT.CC .A..T..... ....A...C. .......... .......... ..C.....T .......... .......CG- TAGC......  117
HW-serum   .G....A..T C.A..T..T. A..... .C...GT.TC .A..T..... ....A...C. .......... .......... ..T.....T .......... .......CT- TTGC......  117
H-c08-serum .G....A..C C.G..A..T. G..... .A...AC.CC .A..T..... ....A...C. .......... .......... ..C.....T .......... .......CT- TTGC......  117
H-c09-serum .G....A..C C.G..A..T. G..... .A...AC.CC .A..T..... ....A...C. .......... .......... ..C.....T .......... .......CT- TTGC......  117
A-serum    .G....A..T C.G..T..T. G..... .A...AC.CC .A..T..... ....A...T. .......... .......... ..C.....T .......... .......TT. TTCC......  120
E-serum    .G....A..C C.G..T..T. G..... .A...AC.CC .A..T..... ....A...T. .......... .......... ..C.....T .......... .......TT. TTCC......  120

Consensus  GTTGACAAGG ACCAGTGGGG GCCGGRSKBB DNGGRKARGG AYYCCYNDVY BYYGYCCTTC YCGGYGGRVS CGGGAAATGC ATGGGGCCAC C                              211
GBV-C      .......... .......... .....GCGGG AG..GG.A.. .CC..C-ACC GCT.C..... C...-..AGG .......... .......... .                              204
O-serum    .......... .......... .....GGGTC AC..GG.A.. .CC.-TGGAC CCT.C..... C...T-.GGG .......... .......... .                              206
O-saliva   .......... .......... .....GGGTC AC..GG.A.. .CC.-TGGAC CCT.T..... C...T-.GGG .......... .......... .                              206
HGV-45966  .......... .......... .....GGTTT AT..GG.A.. .CC.-CAAAC CCT.C..... C...C-.GAC .......... .......... .                              206
N-saliva   .......... .......... .....GGGCT TG..GT.A.. .CC.-CAAGT CCT.C..... T...T..GGC .......... .......... .                              207
N-serum    .......... .......... .....GGGCT AA..GT.A.. .CC.-CAAGT TCT.C..... T...T..GGC .......... .......... .                              207
HGV-44402  .......... .......... .....GGGC- TT..AG.G.. .CT.-CAAGT CCC.C..... C...T..GCC -......... .......... .                              205
HW-serum   .......... .......... .....AGGC- TT..AG.A.. .TT.-CAAGT CTC.C..... C...T..ACC -......... .......... .                              205
H-c08-serum .......... .......... .....GGGC- AG..GG.A.. .CC.-CCTGT CCT.C..... C...T..GAC -......... .......... .                              205
H-c09-serum .......... .......... .....GGGC- GG..GG.A.. .CC.-CCTGT CCT.C..... C...T..GAC -......... .......... .                              205
A-serum    .......... .......... .....GGGC- AG..GG.A.. .CC.-TTGCC CCT.C..... C...T..GA- .......... .......... .                              208
E-serum    .......... .......... .....GGGC- AG..G-.A.. .CC..CTTGT CCT.C..... C...T..GA- ...-...... .......... .                              207
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FIG. 1. (A) Sequence analyses of cloned HGV 5′ noncoding region fragments from six human serum (subjects A, E, H, N, O, and HW) and two saliva (subjects N and O) samples, corresponding to nucleotides 192 to 397 of the HGV-45699 (4) strain, and their phylogenetic relationship with previously described full-length HGV genomes (GBV-C [6], HGV-44402, and HGV-45966 [4]). From subject H, sequences were obtained from two serum-derived clones (no. 8 and 9). Analyses were performed by using the unweighted pair group method with the arithmetic mean (GeneWorks 2.3; Intelligenetics) algorithm. (B) Alignment of all sequences used in the analyses, with consensus sequences given at the top. Dots and dashes indicate homology and an insertion or deletion, respectively.

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