

Detection of HCV-RNA in saliva of patients with chronic hepatitis C

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Abstract

Previous studies have provided conflicting results on the presence of hepatitis C virus-RNA in saliva. In this study, 23 (62%) of 37 patients tested positive for hepatitis C virus-RNA in saliva, using polymerase chain reaction analysis. A slightly greater proportion had a sporadic rather than a parenteral origin of chronic hepatitis C. These results provide a biological basis for saliva as a possible source of hepatitis C virus (HCV) infection, but do not necessarily imply transmission by this route.

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We carried out a study using the polymerase chain reaction (PCR) test to determine whether HCV-RNA is present in the saliva of patients with chronic HCV infection.

Patients

Thirty seven patients (mean age 49 years, range 26-72) with chronic HCV proved histologically were included in the study. Twenty three had known parenteral exposure to HCV, while 14 had HCV of unknown origin (sporadic). All were positive for antibodies to HCV (anti-HCV) on second generation ELISA and there was no other cause of liver disease.

Methods

SALIVA COLLECTION

Saliva was collected before antiviral treatment and stored immediately at -20°C. In samples testing positive on PCR, absence of blood in

saliva was controlled by Labstix test strip (Ames, Miles Company, Puteaux, France).

RNA PREPARATION

HCV-RNA was extracted from 200 µl of saliva using guanidinium isothiocyanate and phenol-chloroform isoamylalcohol, precipitation by isopropanol, and purification with 70% ethanol.

RETROTRANSCRIPTION

Retrotranscription was carried out using 200 IU of Moloney murine leukaemia virus reverse transcriptase and 10 µl RNA.

NESTED PRIMER TECHNIQUE

Two rounds of PCR were performed to amplify the highly-conserved 5' nontranslated region of the HCV genome: outer primers: 5' TGC GGG CGA CAC TCC ACC ATA GAT 3' (sense) and 5' CGT GCT GGT GCA CGG TGT ACG AGA CCT 3' (antisense); inner primers: 5' CCA CCA TAG ATC ACT CCC CTG T 3' (sense) and 5' CAC TCG CAA GCA CCC TAT CAG GCA GT 3' (antisense).

The presence of a 286 bp band in the second amplification was considered a positive result. Negative and positive controls were included during the extraction and PCR processes. Statistical analysis was carried out using non-parametric tests.

Results

Of the 37 patients tested, 23 (62%) were found to be positive for HCV-RNA in saliva (Table I). A slightly, but not significantly, higher proportion of patients with sporadic infection tested positive compared with those with known parenteral exposure.

Discussion

Previous studies have provided conflicting results on the presence of HCV-RNA in saliva (Table II). The difference between the studies may be because of differing time intervals between collection and storage and different methodology, or both, especially in the separation/non-separation of cells in the saliva. In our laboratory, preliminary data indicate a reverse from positive to negative results when samples are frozen after three hours at room temperature rather than immediate freezing. In

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TABLE I HCV-RNA in saliva

	Parenteral exposure n (%)	Unknown transmission n (%)	Total n (%)
HCV-RNA positive	12 (52)	11 (78)	23 (62)
HCV-RNA negative	11 (48)	3 (22)	14 (38)
Total	23	14	37

TABLE II Published data on HCV-RNA in saliva

	Takamatsu 1990 ¹	Wang 1991 ²	Komiya 1991 ³		Hsu 1991 ⁴	Fried 1992 ⁵
			Treated	Untreated		
HCV-RNA positive	5	3	0	2	0	0
HCV-RNA negative	0	0	7	4	16	14
Total	5	3	7	6	16	14

contrast with the studies of Fried⁵ and, possibly, Hsu,⁴ in our study cells are not removed before extraction of RNA. Presence of HCV-RNA in cells, especially mononuclear cells, has recently been described by Artini⁶ and Romeo.⁷

Conclusions

The presence of HCV-RNA in saliva provides a biological basis for saliva as a possible source of HCV infection, although it does not necessarily imply transmission. To date, no undisputed case of HCV saliva transmission has been documented.

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