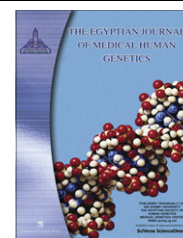




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ORIGINAL ARTICLE

Pilot study for assessment of prevalence of intrafamilial hepatitis C transmission in relation to salivary viral load among infected patients with and without chronic renal failure

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Abstract HCV-RNA in saliva of HCV patients provides a biological basis for its potential transmission. HCV viremia is particularly high in HCV patients on hemodialysis. This study aimed to evaluate the prevalence of HCV in saliva of HCV patients with and without renal failure, and the possible role of intrafamilial transmission of the virus.

Twenty HCV patients were enrolled in this study. They were divided into two groups: 10 HCV-infected patients without renal failure (Group I) and 10 with renal failure (Group II). Detection of HCV-RNA by quantitative RT-PCR in serum and saliva of both groups was done. Thirty-eight family members of both groups were included for the detection of serum HCV antibody. The percentage of the saliva-positive patients for HCV was significantly higher in the renal failure group (70%) than the other group (40%) ($p < 0.05$). There was insignificant statistical difference between the two groups as regards infectivity to their family contacts.

Also there was insignificant correlation between the level of viremia and the intra familial transmission with a mean + SD (9,33,250 + 24,501) in negative relatives and a mean + SD(79,912 + 26,879)

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in positive relatives ($p > 0.05$). But a significant correlation was revealed between the level of viremia and saliva positivity, with a mean \pm SD(12,95,666 \pm 1792) in saliva-positive patients and a mean \pm SD (3,74,465 \pm 2150) in saliva-negative patients ($p < 0.05$). There was a highly significant difference between infectivity of HCV saliva-positive patients and saliva-negative patients to their family contacts ($p < 0.001$).

Conclusion: Increased percentage of HCV detection in saliva of HCV patients with renal failure on HD may cause spreading of HCV in HD units among RF patients. Also there was increased percentage of interfamilial infectivity among the saliva-positive patients to their relatives and this suggests that saliva might have an infective role.

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1. Introduction

Hepatitis C virus (HCV) infection represents a major public health problem in the world today. Egypt has possibly the highest HCV prevalence in the world; 10–20% of the general populations are infected and HCV is the leading cause of hepatocellular carcinoma (HCC) and chronic liver disease in the country [1–3]. Approximately 90% of Egyptian HCV isolates belongs to a single genotype, 4a, which responds less successfully to interferon (IFN) therapy than other genotypes [1].

Epidemiological surveys demonstrate that body fluids other than blood, including saliva, might be the potential sources of HCV infection. Several studies have demonstrated HCV-RNA in the saliva of hepatitis C patients by reverse transcription (RT)-nested PCR. However, the detection rates of viral RNA within saliva have varied widely since the efficiency of HCV transmission is likely related to its viral load. It is important to quantitate viral RNA levels within body fluids in order to properly evaluate the possible non-parenteral routes of HCV infection. [4].

Although sexual and vertical transmissions have also been reported, there remains a large number of HCV carriers in whom no route of infection has been identified [5]. The potential infectivity of body fluids in HCV-positive-infected patients has been identified by the detection of HCV-RNA in saliva, ascites, breast milk, urine, feces, semen and cervico-vaginal secretions [6].

Poor oral hygiene, with gingivitis and oral mucosal lesions can cause the exudation of serum into the saliva and increase the shedding of potentially infected mononuclear cells into the salivary pool, but interestingly neither factor correlated with the presence of HCV-RNA in saliva [7].

Some HCV-RNA particles could appear in the saliva as a result of active HCV replication in the salivary glands. This could explain the HCV-RNA sequences found in serum and oral tissues in some patients, the detection of different genotypes in serum and in saliva of a single patient [7], and the existence of patients who are serum negative but saliva positive for HCV [8].

Dialysis patients have an increased risk of exposure to parentally transmitted hepatitis viruses. The prevalence of HCV among hemodialysis patients is highly variable between different countries and between different centers in the same locality [9].

Duration of hemodialysis, past history of blood transfusions, and alanine aminotransferase (ALT) elevations correlate with higher rates of anti-HCV positivity. Molecular virological studies have clearly shown the nosocomial transmission of HCV

to hemodialysis patients, but the exact modes of transmission remain unclear. Studies suggest several risk factors, including transmission through blood components; patient-to-patient transmission through shared equipment, devices, or multidose vials; and between patients treated on the same shift but not sharing equipment [10].

Possible contamination by HCV was studied by collecting environmental samples in a hemodialysis unit. Samples and controls were tested for HCV-RNA, by qualitative transcription-mediated amplification assay. The HCV-RNA-positive samples were found in the dialysis unit on the external surface of the dialysate (inlet–outlet) connector of a dialysis machine used for HCV-negative patients.[11].

2. Aim of the study

The aim of the present study was to evaluate the correlation of HCV load in saliva of HCV patients with and without renal failure and its effect on intrafamilial transmission of the virus.

3. Subjects and methods

Twenty patients with HCV were enrolled in this study. They were attending in Ain Shams University Hospitals outpatient clinic or admitted in internal medicine department in the period from March 2007 to July 2007. Thirty-eight of their family members were included in this study after their verbal consent.

3.1. Patient group

The patients were classified into two groups as follows:

- Group I: This group included 10 patients with hepatitis C virus positive without renal failure (RF).
- Group II: This group included 10 patients with hepatitis C virus positive with chronic renal failure on hemodialysis for 3–5 years in 80% of them and over 5 years in 20%.

3.2. Family group

Included 38 family members, their ages ranged between 7 and 73 years with a mean \pm SD (37.97 \pm 15.4). Rapid chromatographic immunoassay for qualitative detection of antibody to HCV in serum or plasma using one step Test Device was done to this group.

All patients were subjected to:

1. Full detailed history including risk factors.
2. Full clinical examination.
3. Laboratory investigations include: liver function tests [total and direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), serum albumin, total protein], renal function tests [serum creatinine and blood urea nitrogen (BUN)], complete blood count (CBC), prothrombin time (PT) and partial thromboplastin time (PTT), serum electrolytes [sodium and potassium (Na&K)].
4. Abdominal ultrasonography.
5. Detection of HCV-RNA: patients spit into a cup to obtain saliva samples. Whole saliva samples (approximately 2 ml) were then transferred into sterile containers. All samples were macroscopically observed to exclude samples contain blood. Detection of HCV-RNA by using Quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in serum and saliva of the patient group.

4. Extraction of viral RNA

Viral RNA was extracted from the sera of patients using viral RNA extraction kit supplied by (QIAGEN) according to manufacturer's instruction. All RNA preparation and handling steps took place in a laminar flow hood, under RNase-free conditions. The isolated RNA was resuspended in RNase-free water and stored at -80°C until assay. The RNA concentration was assessed by absorbance reading at 260 nm with UV spectrophotometry (Beckman; Du series 650, Inc., USA).

4.1. Amplification by one step RT-PCR

RT-PCR reactions were performed using Qiagen one step RT-PCR kit. A total amount of 50 μl reaction solution contained 16 μl RNase-free water, 10 μl 5 \times Qiagen one step RT-PCR buffer, 2 μl dNTP mix, 5 μl of forward primer, 5 μl of reverse primer, 2 μl of Qiagen one step RT-PCR Enzyme mix, 10 μl of template RNA.

Primers sequence were: forward primer (5'-CTT T CG CGACCCAAC ACT AC-3') and reverse primer (5'-AGC GCC ATA GTG GTC TGC GG-3').

Thermocycling was carried out in an MJ Research PTC 200 (MJ Research, Inc., Boston, MA) according to the following cycling profiles; 50 $^{\circ}\text{C}$ for 30 min, 95 $^{\circ}\text{C}$ for 15 min, then 40 cycles of 94 $^{\circ}\text{C}$ for 1 min, 55 $^{\circ}\text{C}$ for 1 min, 72 $^{\circ}\text{C}$ for 1 min, then 72 $^{\circ}\text{C}$ for 10 min, finally hold on 4 $^{\circ}\text{C}$.

The expected PCR product for HCV is 270 base pair on 2% agarose gel.

Quantification of PCR products of both serum and saliva samples was performed using UVP GEL DOCUMENT System.

4.2. Exclusion criteria

1. Toxic habits as alcohol consumption and recreational drugs.
2. Concomitant hepatic diseases.

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12) as follows:

- Description of quantitative variables as mean, SD and range.
- Description of qualitative variables as number and percentage.
- Chi-square test was used to compare qualitative variables between groups.
- Fisher exact test was used instead of chi-square test when one expected cell or more are less than or equal to 5.
 - P value > 0.05 was insignificant.
 - $P < 0.05$ was significant.
 - $P < 0.001$ was highly significant.

5. Results and discussion

In the present study, the patients were divided into two groups: Group I included 10 patients with hepatitis C virus positive without renal failure (RF) of whom 5 (50%) were males and 5 (50%) were females. Their ages ranged between 26 and 53 years with a mean + SD (40.4 + 10). Four of them (40%) were married for more than 15 years and 6 (60%) were married for less than 15 years. Also 9 (90%) were nonsmokers while 1 (10%) was a smoker and Group II which included 10 patients with hepatitis C virus positive with chronic renal failure on hemodialysis for 3–5 years in 80% of them and over 5 years in 20%. They were 4 (40%) males and 6 (60%) females. Their ages ranged between 30 and 74 years with a mean + SD (48.7 + 13). Six (60%) were married for more than 15 years and 4 (40%) were married for less than 15 years. Also 7 (70%) were nonsmokers and 3 (30%) were smokers. Another group which is a family group included 38 family members, their ages ranged between 7 and 73 years with a mean \pm SD (37.97 \pm 15.4).

There was insignificant difference ($P > 0.05$) as regards age, gender, duration of marriage and smoking between the two groups. Abdominal ultrasound showed 3 (30%) were normal, 5 (50%) had mild hepatomegaly and 2 (20%) had cirrhosis in Group I (HCV +ve only) while in Group II (HCV + RF) 7 (70%) had mild hepatomegaly and 3 (30%) had cirrhosis.

The results of our study were shown in Tables 1–9 as follows: Table 1 showed a highly statistically significant difference between both groups as regards bilharziasis and blood transfusion. Table 2 showed highly significant difference in viral load in group one patients with pcr positive saliva. Table 3 showed highly significant difference in viral load in group two patients with pcr positive saliva. Table 4 showed comparison as regards PCR titer in HCV patients in relation to positive family members and showed insignificant difference. Table 5 showed that the number and percentage of relative affected are same in both groups. Table 6 showed a highly statistical significant difference between HCV +ve relatives and HCV -ve relatives as regards the saliva positivity of the infected patients by using chi square test. Table 7 showed a highly statistical significant difference between the two groups with as regards the positivity of their relatives for HCV by using Fisher exact test. Table 8 showed a statistically significant difference between both groups as regards AST, and a highly statistically significant difference between both groups as regards ALT by using chi-square test. Table 9 showed a statistically significant difference between both groups as regards saliva positivity by using Fisher exact test.

In this study the percentage of the saliva-positive patients for HCV by RT-PCR was significantly higher in the renal fail-

Table 1 Comparison between both groups as regards past history and exposure risk factors.

Variables	HCV(10)	HCV(10) + RF	P
<i>Bilharziasis (B)</i>			
Yes	5(50%)	9(90%)	< 0.05*
No	5(50%)	1(10%)	
<i>Injection treatment for B</i>			
No	6(60%)	8(80%)	> 0.05
Yes	4(40%)	2(20%)	
<i>Tablets treatment for B</i>			
No	7(70%)	9(90%)	> 0.05
Yes	3(30%)	1(10%)	
<i>Operations</i>			
No	0	1(10%)	> 0.05
Yes	10(100%)	9(90%)	
<i>Blood transfusion</i>			
No	9(90%)	3(30%)	< 0.01**
Yes	1(10%)	7(70%)	
<i>Tooth extraction</i>			
No	2(20%)	2(20%)	> 0.05
Yes	8(80%)	8(80%)	
<i>Pinprick injury</i>			
No	7(70%)	10(100%)	< 0.05*
Yes	3(30%)	0	
<i>Sharing house tools</i>			
No	1(10%)	0	> 0.05
Yes	9(90%)	10(100%)	
<i>Tooth brush sharing</i>			
No	10(100%)	10(100%)	> 0.05
Yes	0	0	

* Significant test.

** Highly significant test.

Table 2 Comparison as regards PCR titer in HCV patient (10 pts) without renal failure in relation to presence of HCV in saliva.

Variable	Mean PCR titer (IU)	± SD	P
Negative saliva	2,75,366	± 2305.51	< .001
Positive saliva	12,15,206	± 1700.22	

Table 3 Comparison as regards PCR titer in HCV patient on HD in relation to presence of HCV in saliva.

Variable	Mean PCR titer (IU)	± SD	Z	P
Negative saliva	37,465	± 2150	2.2	< 0.05*
Positive saliva	12,95,666	± 1792		

* Significant test.

ure Group II (70%) than Group I (40%) (P value > .05) (Table 9), taking into consideration that the patients did not suffer from bleeding per gums and there was no RBCs detected in the saliva samples, which may indicate the effect of hemodialysis and renal failure as a type of immuno-suppression to secrete HCV in saliva.

Table 4 Comparison as regards PCR titer in HCV patients in relation to positive family members.

Relatives	Mean (IU)	± SD	Z	P
Negative (12 relatives)	9,33,250	± 24501	1.8	> 0.05
Positive (3 relatives)	79,912	± 26879		

In comparison to a study done by Patrícia et al. [12] where HCV-RNA was detected in saliva samples from 8 out of 32 (25%) patients with detectable plasmatic HCV-RNA. The genotype found in the saliva samples was the same as that observed in the plasma samples. No correlation was found between the presence of HCV-RNA in the saliva, and gender, risk factors or clinical presentation of HCV infection.

This also agrees with a study done by Lock et al. [13] where in nine patients (30%), the saliva before tooth brushing was positive for HCV-RNA, and in 11 patients (36.7%) HCV-RNA was detected in the saliva after tooth brushing. Five of these 11 patients tested negative for saliva samples before tooth brushing.

In this study it was found that 8 familial contacts out of 38 total familial contacts were positive to HCV with over all percentage (21%) with insignificant difference between the studied groups as regards intrafamilial transmission (Table 5).

This is also confirmed by Fabris et al. [14] where HCV-RNA was detectable in the cell fraction of saliva in a high proportion of highly viremic patients with chronic hepatitis C.

In this study 6 out of 26 (children and brother and sisters) were positive with the prevalence of 23% which shows importance of intrafamilial transmission by the non sexual route and possibility of genetic factor or other unknown risk factors.

This agrees with a study done by Mostafa et al. [15] which revealed that Egyptian children from rural communities whose parents had antibodies to HCV (anti-HCV) were at higher risk for having anti-HCV than children whose parents did not. The association was greater with mothers than fathers and when the parent had positive HCV-RNA.

There was insignificant correlation between the level of viremia and the intrafamilial transmission (Table 4) and this was in agreement with a study done by Keiserman et al. [16] who concluded that there was low prevalence of intrafamilial transmission of HCV, independent of HCV/HIV co infection and the viral load of HCV did not affect to spread in family.

There was a significant correlation between the level of viremia and saliva positivity in these patients but there was no cut-off value predicting the shedding of the virus in saliva where one patient with low viremia had the virus positive in her saliva while some other patients who had high to moderate viremia were saliva negative.

Regarding the genotype of HCV, it was 4a in serum and saliva of patients and their positive relatives.

This was compared with another study done by Michael Carter [17] where the strongest predictor of shedding was HCV serum viral load. No patients shed HCV in their saliva if they had a serum HCV viral load below 1 million copies/ml. Having an HCV viral load 1-log higher increased 40-fold the likelihood of the virus being shed in the saliva ($P < .0001$).

In comparison to another study done by Liliane Lins et al. [18] there was no correlation found between HCV-RNA in saliva, oral health, and viral load. These results suggested that

Table 5 Comparison between both groups as regards the positivity of the relatives.

Relatives	HCV patients without RF	HCV patients with RF
Positive relatives	4(21%)	4(21%)
Negative relatives	15(79%)	15(79%)
Total	19	19

Table 6 Relationship between saliva-positive patients and HCV +ve relatives.

Saliva by PCR	HCV +ve relatives	HCV -ve relatives	P
Saliva +ve pts	6	17	< 0.01**
Saliva -ve pts	2	13	

** Highly significant test.

Table 7 Relationship between saliva-positive pts and positive HCV relatives in both groups.

Variable	Saliva +ve HCV pts	Saliva +ve RF pts	P
Positive relatives	2	4	< 0.01**
Negative relatives	5	12	

** Highly significant test.

Table 8 Relation of abnormality of liver enzymes (ALT, AST) with HCV spread in family contacts.

Variable	AST in positive relatives	AST in negative relatives	ALT in positive relatives	ALT in negative relatives
Mean	76 IU	42.7 IU	133	34.9
SD	25.7	11.7	75.1	10.2
P	< 0.05*		< 0.01**	

* Significant test.

** Highly significant test.

Table 9 Relationship between saliva-positive patients in the two groups.

Saliva	HCV pts	HCV + RF pts	P
Saliva positive	4	7	< 0.05*
Saliva negative	6	3	

* Significant test.

HCV-RNA presence in saliva was independent of the viral load and the oral pathology of HCV positive individuals.

In this study, there was a high significant difference between infectivity of HCV saliva-positive patients and saliva-negative patients to their family contacts, 11 saliva-positive patients had 6 out of 23 relatives were positive, i.e. 26% were infected, 9 saliva-negative patients had 2 out of 15 relatives were positive, i.e.

13% were infected ($P < 0.01$), which shows the possible role of saliva as an important route of infection. (Tables 6 and 7).

In this study we found a high statistical significance between the degree of elevation of liver enzymes (ALT, AST) and the liability of infectivity to family contacts, being higher in positive HCV relatives than negative one. This may show a correlation between the virus activity in the patient, and the infectivity of that patient to his family members, also the importance of different pathogenic factors as well as patient factors (Table 9).

5.1. Bilharzial infection as a risk factor for HCV infection

In this study there was a high statistically significant difference between both groups as regards bilharzial infection. This signifies the importance of bilharzial infection as a source of HCV infection here in Egypt.

This agrees with Abdollah et al. [19] where the history of parenteral treatment for Schistosomiasis was observed to cluster within households, OR for clustering: 2.44 (95% CI: 1.47–4.06). Overall, HCV sero-positivity was 40% (321/796) and was observed to cluster within households that had members who had received parenteral treatment for Schistosomiasis (Table 1).

5.1.1. A case study

In this study there was a case of saliva-positive HCV by RT-PCR, although the serum was negative for PCR (taking into consideration that it was positive 4 months ago) which shows the fluctuation of the HCV viremia and the possibility of the virus to remain dormant in the salivary glands causing infectivity to other family members in spite of being serum HCV negative, and that HCV can directly infect the salivary glands.

6. Conclusion

In this study we found increased percentage of HCV detection in saliva in HCV-infected patients with renal failure on HD possibly due to the effect of immuno-suppression in these patients, which may cause spreading of HCV in HD units among RF patients in spite of taking the standard precautions. Also we found that there is increased percentage of infectivity among the saliva-positive patients to their relatives than saliva-negative patients and this suggests that saliva might have an infective role and additional way of intrafamilial spread of the virus.

Conflict of interest

No one of the authors have conflict of interest regarding this paper.

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