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Evaluation of HCV RNA in Human Saliva in HCV-Infected Patients and Its Correlation to Treatment Outcome in Egypt

Mohamed AbdAllah, MD[1], Eman M Hassan, MD[2], Eman I Raslan, MD[3*], Tamer Elbaz, MD[4], Yasser Elesnawy MSc[5], Mona A M. Awad, MD[2], Said I Shalaby, MD[1].

[1]Complementary Medicine Department, Medical Research Institute, National Research Centre, Giza, Egypt.
[2]Department of Clinical Pathology, Medical Research Institute, National Research Centre, Giza, Egypt.
[3]Family Medicine Department, Faculty of Medicine, Cairo University, Cairo, Egypt.
[4] Endemic Medicine Department, Faculty of Medicine, Cairo University, Cairo, Egypt.
[5]Hepatitis management and Hepatic Oncology unit, Infectious and Hepatology Department, Ahmad Maher Teaching Hospital – GOTHI, Cairo, Egypt.

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A B S T R A C T

Background: A challenging healthcare concern is the infection caused by the Hepatitis C virus. The Egyptian Ministry of Health adopted many assertive preventive measures to significantly decrease the spread of the disease. These measures focused on all methods of HCV transmission. Human saliva may contain HCV ribonucleic acid (RNA), according to recent research emphasizing the need for further study. **Objective:** to assess the presence of HCV RNA in human saliva and how it relates to serum HCV RNA along with treatment outcomes using direct-acting antivirals. **Methods:** Chronic HCV patients who showed positive HCV viremia and were eligible to be treated according to the Egyptian HCV guidelines enrolled in the study. Assessment of both serum and salivary HCV RNA have been done pre- and post-treatment**. Results:** Fifty (50) patients were enrolled. The mean age was 52.56±12.93 years and the majority (54%) were males. Most of the patients were not cirrhotic either by Fibrosis-4 score (Fib-4) (2.69±1.98) or by ultrasound (Only 18% are cirrhotic). Serum HCV RNA was positive in all cases (100%); however, there are only three positive salivary RNA (6%). Seventy-two percent of patients fall in the easy-to-treat category according to Egyptian protocol for HCV treatment. All patients achieved a sustained virological response (100%) with no positive RNA results in both serum and saliva. **Conclusion:** HCV could be present in human saliva, but its prevalence is low. Saliva has low risk of HCV transmission, and it is not recommended to use salivary RNA to diagnose or follow up HCV.

INTRODUCTION

Hepatitis C virus (HCV) is a worldwide healthcare concern. An estimated 58 million patients have chronic HCV infection with an estimated 1.4 million people worldwide die each year from hepatitis B virus and HCV.[1] The World Health Organization (WHO) unveiled a strategy for the global health sector in 2016 with the intention of eradicating viral hepatitis by 2030. Reduction of HCV incidence is one of the main indicators for viral hepatitis elimination. This indicator can be achieved through a proper understanding of HCV modes of transmission, health education and awareness, and promotion of preventive and infection control measures.[2]

Corresponding Author: Eman Ismail Mohamed Raslan, Family Medicine Department, Faculty of Medicine, Cairo University, Cairo, Egypt. Email: emanraslan@kasralainy.edu.eg

HCV is known as a blood-borne pathogen. The commonest routes via which HCV is transmitted are through the use of unsafe blood products and renal dialysis,[3] transmission among people who inject drugs (PWID), and Vertical transmission has been reported in a small proportion of patients (4-8%).[4] Sexual route of transmission is risky, especially in Human immunodeficiency co-infected patients.[5] However, many cases may present with HCV with no identifiable source of infection. [6]

Many reports revealed that HCV could be presented in salivary secretion resembling a possible route of transmission. Although epidemiological research indicates that salivary HCV viral particles have a low infective capacity, their exact infectious potential remains unknown.[7-9]

The available literature review found a lack of research that explores the possibility of using saliva as a tool for the diagnosis of HCV infection and determining whether the HCV RNA in saliva can be a reliable indicator of HCV replication within the body. Very limited research has compared the results of salivary PCR tests in comparison with the serum PCR results in HCV-infected patients.

The aim of the study was to assess if human saliva contains HCV ribonucleic acid (RNA) in HCV-infected patients and how that relates to serum HCV RNA and treatment outcomes using direct-acting antivirals (DAAs). The study also examined the effect of direct-acting antivirals on HCV RNA levels in saliva and serum. The results of this study could potentially lead to the emergence of non-invasive and cost-effective methods for diagnosing and monitoring HCV infection.

METHODS

This prospective cohort study was carried out by the National Committee for Control of viral hepatitis (NCCVH), Cairo University, and the National Research Center from October 2021 to May 2022.

Fifty adult chronic HCV patients who showed positive serum RNA and were eligible to be treated according to the NCCVH HCV guidelines [10] were enrolled in the study. The NCCVH Protocol of HCV treatment depends on Sofosbuvir and Daclatasvir +/- Ribavirin as a first-line treatment where Sofosbuvir/Ledipasvir and Sofosbuvir/Ledipasvir/Voxilaprevir are reserved as second-line options. All Patients with Positive HCV RNA are eligible for treatment unless they have a recent history of hepatocellular carcinoma, decompensated liver disease, and pregnant females. The selection of patients was carried out according to these inclusion criteria: presence of HCV RNA in plasma detected by serum PCR; absence of additional concurrent liver disorders; the participants did not consume alcohol or use intravenous drugs. Patients with periodontitis were excluded from the study.

Table 1: Demographic and clinical characteristics of the study participants (n= 50)

Characteristics	N (%) *
Age (mean±SD, years)	52.56±12.93
Gender	
Male	27 (54)
Female	23 (46)
Treatment status	
Treatment naive	41 (82)
Treatment experienced	9 (18)
Body mass index, mean±SD	28.5±5.57
Tobacco Consumption	5 (10)
Hypertension	17 (34)
Diabetes mellitus	9 (18)

**Data were presented as number and percentage, unless mentioned otherwise. SD: Standard deviation*

Sample size was calculated using Stata 18. The null hypothesis for the prevalence of positive RNA in Saliva was 84.0%, while the alternative hypothesis was 64.4%. [11] With 80% power and 0.05 error, the sample size was 47 participants. However, the final sample size included in the study was 50 participants.

Data collection: After signing the informed consent, all the enrolled patients underwent careful history taking including age, gender, BMI, smoking status, and chronic diseases such as diabetes and hypertension. Additionally, a clinical examination was done, and a blood sample was obtained for baseline investigations complete blood picture, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, INR, and alkaline phosphatase (ALP) values. The serum PCR detects the levels of HCV RNA in plasma. In addition, salivary HCV RNA testing was done.

Table (2): Laboratory results, ultrasound findings, treatment options, and Fib4 calculation (n=50)

Test	Mean±SD*
Alanine Transaminase U/L	54.97±32
Aspartate Transaminase U/L	55.03 ±28.92
Alpha Fetoprotein IU/L	9.33 ±13.05
Serum Albumin g/dL	4.24±1.62
Total Bilirubin mg/dL	0.81±0.84
White Blood Cells $x10^3mm^3$	5.73±2.04
Absolute Neutrophil Count $x10^3mm^3$	4.67±10.05
Hemoglobin g/L	13.62±1.69
Platelets $x10^3mm^3$	178.18±58.72
International normalized ratio (INR)	1.07±0.13
Creatinine mg/dL	0.78±0.23
Fibrosis-4 Score (Fib4)	2.69±1.98
Ultrasound findings	
Abnormal Echo pattern of liver	35 (70%)
Cirrhotic liver	9 (18%)
Normal echo pattern of liver	6 (12%)
Presence of hepatic focal lesions	0 (0%)
Ascites	0 (0%)

*Data were presented as mean and standard deviation except for ultrasound findings, number and percentage.

After receiving DAAs as per NCCVH guidelines, sustained virological response was assessed in both serum and saliva by HCV RNA testing at 12 weeks following the completion of treatment.

The HCV RNA detection in serum was carried out using The CobasAmpliPrep/ CobasTaqMan HCV Test, Roche Diagnostics quantitative real-time PCR, was the method used for molecular confirmation. The kit contains one negative control [(-) C] and two positive controls, a low positive control [HCV L (+) C] and a high positive control [HCV H (+) C], which are processed with each batch running on the Cobas® 6800/8800 Systems. HCV RNA extraction from saliva: before saliva was collected, the subjects abstained from drinking, eating, smoking, and oral hygiene practices for ninety minutes. Spitting was the method used, and 140 Ul from each sample was used according to the QIAamp viral RNA Mini kit protocol. After measuring their volumes, saliva samples were taken and placed in a sterile container to be kept at -80 °C. The 50 patients with plasma HCV RNA positive had their saliva specimens examined.

The sample is first lysed to inactivate RNase. The sample is loaded onto the QIAamp Mini spin column after buffering conditions are adjusted to allow for optimal RNA binding to the QIAamp membrane in order to guarantee the isolation of intact viral RNA. After the RNA binds to the membrane, impurities are effectively removed in two stages with two distinct wash buffers. Ready for immediate use, high-quality RNA is eluted in a special RNAase-free buffer. RT-q PCR reaction: by using artus HCV RG RT-PCR kit according to manufactural protocol. RT-q PCR detection: by using rotor gene. Hep. C virus RG Master A 12UL, Hep. C virus RG Master B18UL, Hep. C virus RG IC 2UL & 18 UL from each eluted sample to reach a total reaction volume of 50UL. 95 for 30 secs,50 for 60 secs & 72 for 30 secs for 50 cycles.

Table (3): HCV Viraemia status pre and posttreatment (n= 50)

Test	Serum	Salivary
Positive HCV RNA Pre-treatment	50 (100%)	3 (6%)
Positive HCV RNA Post-treatment	0 (0%)	0 (0%)

HCV: Hepatitis C Virus; RNA: Ribonucleic acid

Statistical analysis: Data that had been coded and entered was exported from Microsoft Excel® and then analyzed using the Statistical Package for the Social Sciences (IBM SPSS®) (version 22). Mean, ± Standard Deviation (± SD) or Frequencies (Number of cases), and percentages were used when appropriate to statistically describe the data. The association between levels of viremia and the presence of the virus in saliva was evaluated using Wilcoxon signed-rank test.

RESULTS

The study included fifty patients. The study participants' mean age was 52.56±12.93 years. Twenty-seven (54%) were males. Most of the patients were treatment naïve (82%). The mean body mass index (BMI) of the participants was 28.5±5.57 Kg/m^2. Approximately 34% of the patients had hypertension and 18% had diabetes. The details of the demographic data are shown in Table (1).

Analysis of baseline investigations revealed normal mean values except for liver transaminases, which were less than 2 upper levels of normal. Most of the patients were not cirrhotic either by Fibrosis-4 score (Fib-4) (2.69±1.98) or by ultrasound (Only 18% are cirrhotic). Seventy-two percent of patients (36 patients) fall in the easy-to-treat category according

to Egyptian protocol for HCV treatment, therefore, they received Sofosbuvir (SOF) / Daclatasvir (DCV) regimen, the remaining 14 patients (28%) received SOF/DCV/Ribavirin (RBV) regimen. The rest of the pretreatment assessment results are shown in Table (2).

All patients (100%) showed positive pretreatment serum HCV RNA, but three patients only showed positive results in the salivary sample (6%). All patients achieved sustained virological response (100%) with no positive RNA results in both serum and saliva as shown in Table (3).

The median of HCV viremia in the saliva HCV RNA negative group was 696389 IU/L (IQR: 206000-1370000), while the median in the saliva HCV RNA positive group was 640000 IU/L (IQR: 135000-2670000). The mean difference between the two groups was insignificant (P value =0.9511).

DISCUSSION

A variety of infectious agents can be found in saliva, and although there are several antimicrobial mechanisms in place, it is still possible for infections to be transmitted through saliva. Many studies discussed the existence of HCV antibodies in saliva; Lucidarme et al performed a study that concluded the possibility of using a salivary test as an alternative way to measure HCV antibodies. [12] Many practical, clinical, and therapeutic implications arise from the finding of HCV antibodies in saliva. OraSure Technologies introduced an FDA-approved salivary test for HCV antibodies. According to a study done by Drobnik et al, the OraQuick HCV Rapid Antibody Test results matched the control standard enzyme immunoassay. [13] This test resembles a good choice, especially in PWID who might not have proper venous access or for patients who refused the classic invasive blood sampling technique. [14]

In our study, only 6% showed positive HCV viremia in saliva. Many researchers studied the possibility of the presence of the virus in human salivary fluid. This research came with varying results ranging between 0% to 100% [15]; A study done by Maticic et al found a positive HCV RNA in 35% of whole salivary samples. [16] Another study by Hermida came with a higher HCV prevalence in saliva (52.4%). [15] The cause of the high variation of results may be referred to different techniques of specimen handling and storage, contamination, and efficiency of post-amplification systems.[6]

Periodontal diseases may also act as a predisposing factor for the presence of HCV viremia in saliva through microcapillary leakage in the oral cavity, and migration of gingival crevicular fluid and salivary glands. [17] A study done by Sosa-Jurado et al. came with a contradictory result and showed a high HCV prevalence in saliva (64.4%) in chronic HCV patients without periodontal diseases. [11] It should be noted that the majority of the current patients were in the easy-to-treat category according to Egyptian protocol for HCV treatment. [18]

The viral load in the blood and the presence of the virus in the saliva are directly correlated.[15] Moreover, our study showed that salivary and serum HCV RNA showed the same clearance pattern after treatment with DAAs. That was in concordance with a study done by Diz Dios et al. In patients receiving interferon plus ribavirin therapy for a chronic HCV infection, the HCV clearance in their serum, and saliva was assessed. This study demonstrated that saliva could be used to monitor HCV RNA levels in order to assess the sustained virological response to interferon and ribavirin therapy. [19] Assessment of the use of salivary samples to monitor the response to treatment was also done in a study by Roy et al. in patients who received interferon alpha 2a as a treatment for HCV. Still, they found that there is no correlation between the level of HCV RNA in serum and the presence of HCV RNA in saliva, [20] and as far as we are aware, this is the first study to evaluate the alterations in salivary HCV RNA after treatment with DAAs.

CONCLUSIONS

There is a low prevalence of HCV in human saliva in Egyptian patients denoting a low risk of HCV transmission through saliva. Our results in general act against the use of salivary RNA for HCV diagnosis even though salivary HCV RNA can be found in some cases.

Ethical Approval

The study obtained all required approvals from the Institutional Review Board of the National Research Centre, Giza, Egypt.

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