Studying Of HCV and Its Specific Antibody in Oral Fluid

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Abstract: Background & Aims The possibility of the non-parenteral Hepatitis C Virus (HCV) transmission is supported by the demonstration that the actual virus is present in several body fluids, including oral fluid. From a review of the literature, many investigators have found the presence of HCV-RNA in oral fluid; however, widely contrasting results emerge, with detection rates ranging from 0-100%. This study aimed to evaluate oral fluid as a possible alternative to serum for the detection of HCV-Ab, and to determine the correlation between detection of antibodies against HCV in oral fluid and HCV-RNA positivity in the same fluid. Reported here also is the correlation between HCV-RNA positivity in serum and the detection of antibodies against it in oral fluid by testing paired serum/oral fluid samples. **Methods:** Paired OF and serum samples were collected from 100 patients attending the outpatient clinics in the National Institute of Liver and endemic diseases at Cairo, Egypt. For the 100 serum samples found positive for HCV-RNA, using the Real-time RT-PCR technique *(ANALYTIC JENA HCV 2.0 ASSAY)*, 20 of the corresponding oral fluid samples tested positive for HCV-Ab using the *AXIOM* Anti – HCV ELISA – Version 4 (SAV). **Results** Our findings indicate that the HCV-Ab in oral fluid occur in about one fourth of HCV infected patients, and blood leakage into the oral cavity may possibly the main source of the oral HCV-RNA as we rejected any oral fluid sample contaminated with blood. Our findings also suggest a weak positive correlation between HCV-RNA serum Level and HCV-Ab Titer in Oral Fluid (r = 0.15). On the other hand, results showed a perfect positive correlation between HCV-Ab serum Level and HCV-Ab Titer in Oral Fluid (r = 1**)**. In the current study, the patients were subdivided into two groups "positive & Negative" based on presence or absence of HCV-Ab in oral fluid. There was a statistically significant difference between both groups as regards ALT and Albumin by using Welch's t-test ($P< 0.05$). In addition, there was a highly statistically significant difference between both groups as regards AST also by using Welch's t-test ($P<0.01$), while insignificant difference between both groups as regards ALP and Bilirubin ($P> 0.05$)**. Conclusion** It could be concluded that HCV-Ab may present in oral fluid, which may be an effective alternative to serum antibody testing for surveillance of hepatitis C infection. Presence of HCV-RNA in oral fluid could not be demonstrated.

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Keywords: HCV-RNA - HCV-Ab - Oral Fluid - Serum.

1. Introduction

Hepatitis C is an insidious disease in that it does not usually cause a clinically evident acute illness. Instead, its first manifestation (in 25% of those infected) may be the presence of smoldering chronic hepatitis that may ultimately lead to liver failure (Champoux et al., 2004). Globally, an estimated 130–170 million persons (2%–3% of the world's population) are living with hepatitis C virus (HCV) infection. About 80 percent of those who become infected are asymptomatic (Averhoff et al., 2012).

HCV belongs to the family Flaviviridae, whose genome consists of a positive-stranded ribonucleic acid (RNA) molecule of about 9.6 kilobases and encodes a large polyprotein precursor (about 3000 amino acids) (Kato, 2000). It is known for its genetic heterogeneity (Nakano et al., 2012).

Saliva can contain a range of infectious agents and, despite several antimicrobial mechanisms; transmission of these can occur (Ferreiro et al., 2005). HCV is transmitted by unknown routes as well as by the percutaneous route and sexual contact. HCV-RNA can be detected by the polymerase chain reaction (PCR) which also shows that HCV-RNA may be present in the saliva of HCV-infected patients. This might provide an argument for the possible transmission of HCV via contaminated saliva (Gonçalves et al., 2005).

Epidemiological studies suggest that the infective capacity of hepatitis C viral particles in saliva is low, but it has not been possible to determine their infective potential. Moreover, HCV-specific receptors have not been defined on oral epithelial cells (Ferreiro et al., 2005).

The role of oral fluid (OF) in HCV transmission remains controversial (Açıkgöz et al., 2009). There was great variability between many studies in HVC-RNA detection frequency in saliva from zero to 100%. It was noted that the presence of HCV-RNA in OF was associated with oral pathology of the patients, as the studies reported 100% HCV-RNA detection in saliva samples were done on HCV-RNA seropositive patients suffered from sialoadenitis (Gonçalves et al., 2005).

OF is the liquid present in the oral cavity. It is a mixture of saliva and oral mucosal transudate. It contains both pathogens and antibodies (Prickett et al., 2008).

IgG is the most abundant class of immunoglobulin found in the plasma. The ability of IgG antibodies to diffuse easily throughout the extracellular fluid and their high affinity make these the principal neutralizing antibodies for toxins found in tissues (Janeway et al., 2001). Although the salivary gland secretions contain mostly IgA, The Cervicular fluid probably represents a transudate of plasma; Where IgM and IgG are predominant (Patil and Patil, 2011).

Aim of the study

The ultimate goal of this study is to evaluate the possibility of analyzing the HCV-Ab in OF by the means of HCV antibody assay routinely used for serum testing (ELISA), and to assess whether there is a relationship between the presence of HCV-Ab and HCV-RNA in the OF.

2.Subjects and Methods

The current study included a group of 100 HCV-RNA seropositive patients; 41 males (41%) and 59 females (59 %) with a mean age of 45.62 ± 10.7 years (range 25 – 64 years), attending the outpatient clinics in the National Institute of Liver and endemic diseases at Cairo, Egypt. over a period of Two months; from May 2014 to July 2014. All patients provided informed consent and the following information was recorded: (Age, Gender, History of blood transfusion, surgical operations, Family history of HCV infection, Bilharziasis and its Parenteral treatment, and Dental treatment history). The patients with a history of alcoholism, exposure to hepatotoxic drugs or presence of hepatitis B surface antigen were excluded from the study. None of the patients was receiving any specific antiviral treatment.

Each participant gave paired blood and oral fluid samples. Blood samples were collected in sterile tubes, and non-stimulated oral fluid samples were obtained by asking the participant to spit into a sterile plastic cup.

Oral fluid samples were examined for the presence of red blood cells by naked eyes. In case of visible blood contamination, the samples were rejected and another was taken.

Blood samples were centrifuged immediately at 2000 rpm, for 5 minutes at room temperature, while oral fluid samples were handled and tested without centrifugation.

Table (1): Baseline demographics of the studied population.

Demographics Characteristics	All Patients (n =100)
Age (Years)	45.62 ± 10.7
Gender	Male 41 (41%) Female 59 (59%)
Race	Egyptian 100 (100%)
History of patients	
Blood Transfusion	20 (20%)
Surgical Operations	33 (33%)
Family History of HCV Infection	31 (31%)
Bilharziasis	62 (62%)
Parenteral anti-bilharzial	46 (46%)
Dental Treatment	43 (43%)

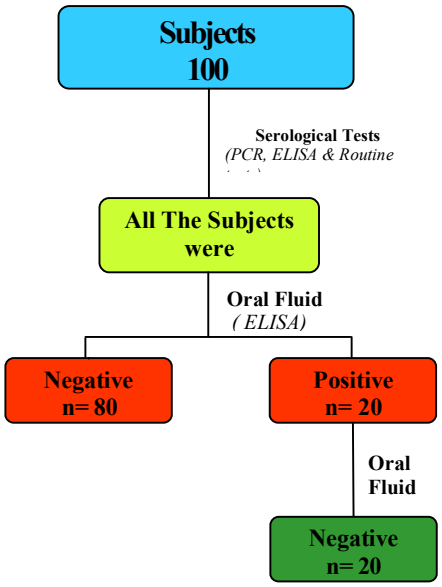

Figure (1): Design of The work.

Paired serum and oral fluid specimens were transferred in icebox to El Sayed Galal University Hospital Lab where tests were done.

Serum specimens were screened first for routine tests (ALT, AST, ALP, Total bilirubin and albumin) as well as HCV-RNA.

Oral fluid specimens of only HCV seropositive patients were examined for detection of HCV Ab with the *AXIOM* Anti – HCV ELISA – Version 4 (SAV) without any modification of the manufacturer's protocol. Positive Anti HCV oral fluid samples were stored at-20° C until tested by real time PCR next day.

Serum HCV-RNA was tested by using the *ANALYTIC JENA HCV 2.0 ASSAY (AJ INNUSCREEN, BERLIN, GERMANY)*, according to the manufacturer's

instructions to assess active viral replication. The lower detection limit of this assay was 100 IU/mL.

Methods

1- HCV-Ab detection by (ELISA)

Principle

In *AXIOM* Anti-HCV Version 4, the kit employs solidphase, indirect ELISA assay for detection of antibodies to HCV in two-step incubation procedure (Engvall and Perlmann, 1971).

2-HCV-RNA detection by (Real-time RT-PCR).

Principle

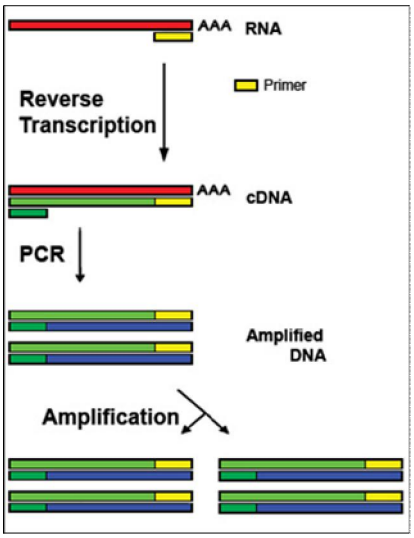

Figure (2)

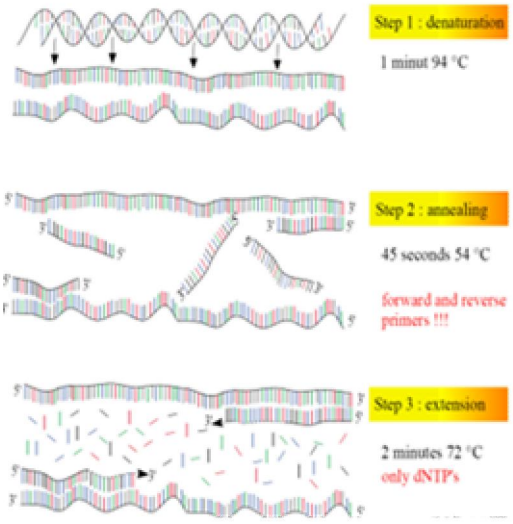

Figure (3)

Reverse Transcription PCR (Witoyo, 2014).

The basic steps of PCR (Vierstraete, 1999).

In RT-PCR, the RNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR as illustrated in figure (2). There are three major steps in a PCR, Which are repeated for a number of cycles as shown in figure (3). In the present study, the quantification of mRNA using RT-PCR was achieved as one-step reaction as shown in figure (4).

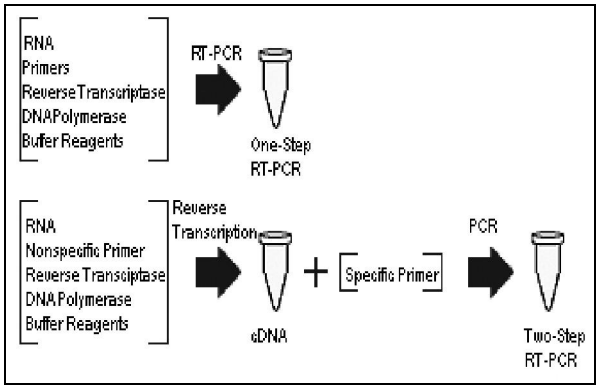

Figure (4): One-step vs Two-step RT-PCR (Wong and Medrano, 2005).

Calculation & Interpretation of Results:

Each RNA amplification is associated with generation of a fluorescence signal measurable in FAM channel (for HCV-RNA) or in JOE/VIC channel (for Internal Control) resulting in a sigmoid growth curve (log scale). The data analysis is performed according to manufacturer's instructions *(ROTOR-GENETM 3000/6000, CORBETT RESEARCH)*.

Statistical Analysis:

Analysis of data was done by personal computer using Microsoft Excel (version 2003) 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.
- t-Test: Two-sample assuming unequal variances used to compare quantitative variables between groups of unequal variances.
- Welch's test was used to compare quantitative variables between groups of unequal sizes.
- For interpretation of results;

P value > 0.05 was insignificant.

P value < 0.05 was significant.

P value < 0.001 was highly significant.

3. Results

The 100 patients enrolled in the current study were all HCV-RNA seropositive. The serum viral load in the study group was ranging from 381 IU/ml to 2.75 X 10^7 IU/ml.

For the 100 serum samples found positive for HCV-RNA, using the Real-time RT-PCR technique

(ANALYTIC JENA HCV 2.0 ASSAY) (AJ INNUSCREEN, BERLIN, GERMANY), 20 of the corresponding oral fluid samples tested positive for HCV-Ab using the *AXIOM* Anti – HCV ELISA – Version 4 (SAV) as illustrated in Figure (5).

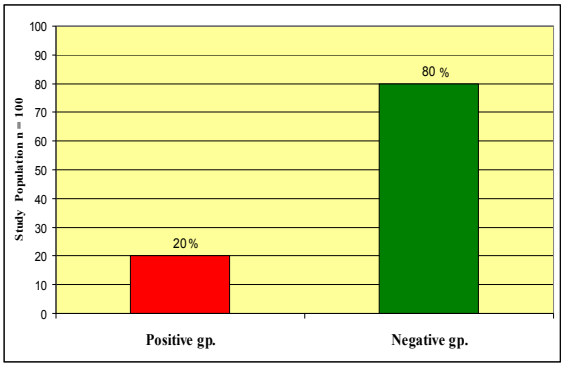

Figure (5): Percentage of positive and negative gp within the whole study population (n=100).

For 20 of the 100-oral fluid samples found positive for antibodies against HCV, the HCV-RNA had not been detected as shown in Figure (6).

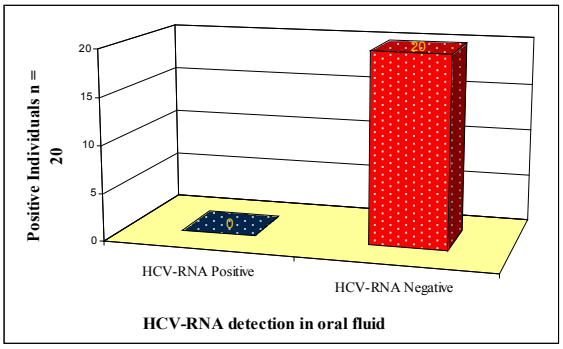

Figure (6): Frequency of HCV-RNA in oral Fluid of the positive gp.

Table (2) showed the range and median ELISA absorbance values of oral fluid and serum samples within positive and negative groups.

Table (2): The range and median absorbance values of OF and serum samples within positive and negative gp.

Parameter	ELISA absorbance in OF		ELISA absorbance in serum	
	Range	Median	Range	Median
Positive gp (n=20)	0.136 - 0.765	0.265	1.20 - 6.90	3.936
Negative gp(n= 80)	0.002 - 0.129	0.049	0.06 - 7.10	1.980

Results showed a weak positive correlation between HCV-RNA serum level and HCV-Ab titer in oral fluid (r = 0.15) as illustrated in Figure (7).

On the other hand, results showed a perfect positive correlation between HCV-Ab serum level and HCV-Ab titer in oral fluid (r = 1) as shown in Figures (8) & (9).

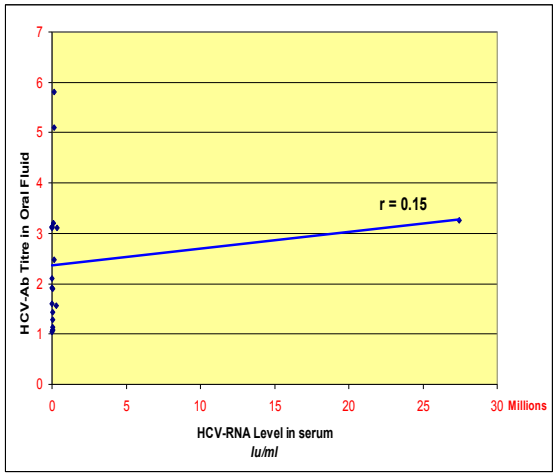

Figure (7): Correlation between HCV-RNA serum level and HCV-Ab titer in oral fluid.

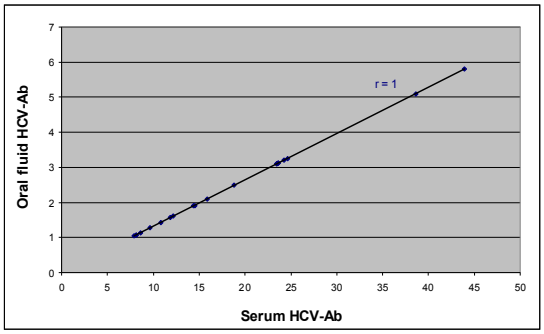

Figure (8): Correlation between HCV-Ab serum level and HCV-Ab titer in oral fluid.

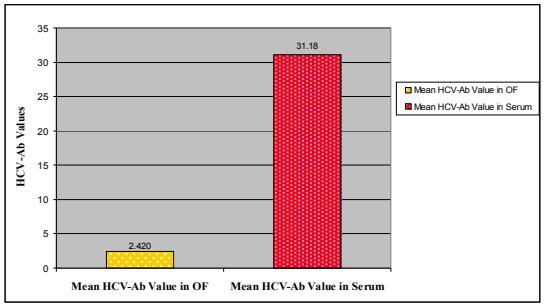

Figure (9): Mean ±SD of HCV-Ab values in OF and serum of positive gp.

Table (3) showed a highly statistically significant difference between both groups as regards surgical operation, family history of HCV infection, bilharziasis and parenteral anti-bilharziasis by using Welch's t-test.

Table (3): Comparison between both groups as regards age, gender and exposure to probable risk factors.

Variables	Anti HCV-Ab in Oral fluid		
	Positive n=20	Negative n=80	*P*
Age (years, mean ± SD)	47.75 ± 9.6	45.08 ± 10.9	> 0.05
Gender			
Male	8	33	
Female	12	47	
Risk Factors			
Blood Transfusion	5	15	< 0.05
Surgical Operations	11	22	< 0.01
Family History of HCV Infection	4	27	< 0.01
Bilharziasis	16	46	< 0.01
Parenteral anti-bilharzial	10	36	< 0.01
Dental Treatment	6	37	< 0.05

Table (4) showed insignificant difference between both groups as regards HCV-RNA serum level by using Welch's t-test.

Table (4): Serum HCV-RNA of Negative and Positive gp.

Variable	HCV-RNA in Negative gp	HCV-RNA in Positive gp
Mean(IU/ml)	4.395×10^5	1.442×10^6
SD	1.275×10^6	6.1223×10^6
Range	381- 1.07×10^7	3244 - 2.745×10^7

Table (5) showed a highly statistically significant difference between both groups as regards serum HCV-Ab titre by using Welch's t-test.

Table (5): Serum HCV-Ab titre of negative and positive gp.

Variable	Serum HCV-Ab in Negative gp	Serum HCV-Ab in Positive gp
Mean	15.19	31.10*
±SD	9.823	11.54
Range	0.455 - 53.79	9.091 - 52.23

*: Significant from negative gp at $p < 0.01$

Table (6) also showed a highly statistically significant difference between both groups as regards OF HCV-Ab titre by using Welch's t-test.

Table (7) showed a highly statistical significant difference between HCV +ve relatives and HCV –ve relatives as regards the OF positivity of the infected patients by using Welch's t-test.

Table (6): HCV-Ab titre of Negative and Positive gp in OF.

Variable	OF HCV-Ab in Negative gp	OF HCV-Ab in Positive gp
Mean	0.419	2.420*
±SD	0.278	1.323
Range	0.012 - 0.989	1.043 - 5.8

*: Significant from negative gp at $p < 0.01$

Table (7): Comparison as regards HCV-Ab titre in OF of positive group in relation to positive family members.

Relatives of Positive gp	Mean (Ab)	±SD	*P*
Positive Relative members	1.213	0.236	< 0.01
Negative Relative members	2.722	1.312	

Table (8) showed significant difference between both groups as regards ALT by using Welch's t-test.

Table (8): Serum ALT of negative and positive gp.

Variable	ALT in Negative gp	ALT in Positive gp
Mean (IU/L)	42.4	57.7*
SD	28.5	28.8
Range	8-137	22-99

*: Significant from negative gp at $p < 0.05$

Table (9) showed a highly statistically significant difference between both groups as regards AST by using Welch's t-test.

Table (9): Serum AST of negative and positive gp.

Variable	AST in Negative gp	AST in Positive gp
Mean (IU/L)	40.6	61.9*
SD	28.6	27.2
Range	7-129	30-109

*: Significant from negative gp at $p < 0.01$

Table (10) showed insignificant statistical difference between both groups as regards ALP by using t-Test: Two-sample assuming unequal sizes and unequal variances.

Table (10): Serum ALP of negative and positive gp.

Variable	ALP in Negative gp	ALP in Positive gp
Mean (IU/L)	105.7	127.6
±SD	46.54	68.62
Range	34.0 – 240.0	65.0 – 287.0

Table (11) showed insignificant difference between both groups as regards total bilrubin level by using Welch's t-test.

Table (12) showed a statistically significant difference between both groups as regards Albumin by using Welch's t-test.

Table (11): Serum total bilirubin of negative and positive gp.

Variable	Bilirubin in Negative gp	Bilirubin in Positive gp
Mean (mg/dl)	0.97	1.66
SD	0.72	1.45
Range	0.35 – 6	0.39 - 7.3

Table (12): Serum albumin of negative and positive gp.

Variable	Albumin in Negative gp	Albumin in Positive gp
Mean	3.65	3.13*
SD	0.72	0.79
Range	1.8 - 4.9	1.7 - 4.7

*: Significant from negative gp at $p < 0.05$

4. Discussion

The present work showed 20/100 patients having HCV Ab in their oral fluid, giving a sensitivity of 20%. This finding was in agreement with Açıkgöz *et al.* (2009) who collected saliva samples by asking the patient to spit into a sterile plastic cup and got sensitivity 15.4% in gingival crevicular fluid (GCF) and 12.8% in saliva. They observed HCV Ab more commonly in the GCF than the saliva of HCV-seropositive hemodialysis patients.

On the other hand, Moorthy *et al.* (2008) collected saliva and plasma sample (sample pair) from 142 patients included in their study. Saliva samples were collected using a commercial collection device – OmniSal and tested with an in-use ELISA for the detection of antibodies to HCV (HCV-Ab), with a minor modification in the manufacturer's protocol.

Van Doornum *et al.* (2001) evaluated hepatitis C antibody testing in saliva specimens collected by two different systems (Salivette and Omni-Sal) in comparison with HCV antibody and HCV-RNA in serum and found that the Salivette/Mono-Lisa combination gave the greatest proportion of HCV antibody positive saliva specimens obtained from the 102 HCV serum antibody positive participants, 88% and 79%, respectively.

De Cocka *et al. (*2004) detected HCV-Ab in oral fluid of 61/73 anti-HCV seropositive samples and got a sensitivity of 83.6%. They suggested that the modified ELISA method for anti-HCV detection in oral fluid can be used for epidemiological surveys.

In addition, Judd *et al. (*2003) evaluated a modified commercial assay in detecting antibody to hepatitis C virus in oral fluids and dried blood. The optimal sensitivities of the modified assay on OraSure, Salivette, and dried blood spots were 92%, 83%, and virtually 100%, respectively. Oral fluid samples were collected by two different oral fluid collection devices (the Epitope OraSure trade mark and Sarstedt Salivette).

Although the frequency of anti HCV Ab that we found in oral fluid samples was 20%, lower than that reported by other authors, we could not find an explanation for the discrepancies in the observed anti HCV Ab frequency in oral fluid samples from anti-HCV seropositive patients. It is possible that the collection method, sampling and centrifugation used, as well as the presence of blood may have contributed to these differences.

In addition, a weak positive correlation was found between anti HCV-Ab in saliva and viral load in serum in the present study.

These results suggest that HCV-Ab presence in saliva is independent of the viral load of HCV positive individuals. This finding was in agreement with Moorthy *et al. (*2008) who found no correlation between salivary positivity and HCV viral load in plasma or infecting genotype.

In the current study, the hepatitis C viral genome was not detected in any oral fluid sample. These findings suggest that oral fluid of patients with chronic hepatitis C is rarely, if ever, contaminated with the hepatitis C virus. This may help to explain the infrequent transmission of this disease by sexual or close physical contact. This finding was in agreement with Hsu *et al. (*1991); Fried *et al. (*1992); Van Doornum *et al.* (2001).

On the other hand, few studies reported 100% HCV-RNA detection in saliva samples from HCV-positive patients; however, only patients with sialoadenitis were considered in these studies (Gonçalves *et al.,* 2005).

HCV-RNA has been also detected in saliva and in salivary glands from patients with sialadenitis by Arrieta *et al. (*2001), who have shown that HCV infects and replicates in the epithelial cells from salivary glands of patients with Sjögren's syndrome or chronic sialadenitis.

Significant variability in HVC-RNA detection frequency in saliva samples was found in a careful review of the HCV literature from 1990 to 2003. Data from 38 published papers indicated a frequency of HCV-RNA in saliva samples from 0 to 100% (Gonçalves *et al.,* 2005).

Absence of HCV-RNA in oral fluid samples observed in this study may be due to the oral fluid sampling method used or the sensitivity of real-time reverse transcriptase polymerase chain reaction (Real-time RT-PCR) which indicates that, if hepatitis C virus were in secretions, it would be present in amounts less than 100 IU/ml.

These findings support seroepidemiological studies, which indicate that nonparenteral transmission of hepatitis C through secretions is uncommon (Hsu *et*

al., 1991; Fried *et al.,* 1992; Van Doornum *et al.,* 2001; Terrault, 2002; Hahn, 2007).

Puchhammer-Stockl *et al. (*1994) used oropharyngeal wash as a sampling method and got a result of 20% HCV-RNA positivity.

In the current study, HCV-RNA was not detected in oral fluid even in patients with high viral load in the serum. This finding was in agreement with Lins *et al. (*2005) who suggested that HCV-RNA presence in saliva is independent of the viral load and the oral pathology of HCV positive individuals.

On the other hand, Sosa-Jurado *et al. (*2014) proved that HCV-RNA in saliva was associated with the level of serum viral load but not with periodontal or liver disease severity.

Suzuki *et al. (*2005) found that most patients (14 of 18; 78%) whose saliva specimens were negative had HCV-RNA in their GCF. In addition, most patients (20 of 26; 77%) had higher HCV-RNA levels in their GCF. Although there was not a statistically significant correlation between the serum viral load and HCV-RNA level in saliva or GCF, patients with low serum HCV loads were less likely to have detectable HCV in their saliva.

Shafique *et al. (*2009) found that, in addition to the blood, HCV-RNA can also be found in oral secretions as well as urine of chronic HCV patients.

Conclusion:

From the results of this study, we can conclude the following:

HCV-Ab may be present in oral fluid of patients infected with HCV.

Oral fluid testing may be an effective alternative to serum antibody testing for surveillance of hepatitis c infection.

Presence of HCV-RNA in OF could not be demonstrated.

The accuracy indices indicate that the assay must be optimized further before it can be recommended for routine use in epidemiological surveys for HCV-Ab.

Presence of HCV-RNA in oral fluid could not be demonstrated.

Nonparenteral transmission of hepatitis C through secretions is uncommon.

Body fluids of patients with chronic hepatitis C are rarely, if ever, contaminated with the hepatitis C virus. This may help to explain the infrequent transmission of this disease by sexual or close physical contact.

References

1. Açıkgöz G., Cengiz M., Keskiner I., Açıkgöz S., Can M. and Açıkgöz3 A. (2009): Correlation of Hepatitis C Antibody Levels in Gingival Crevicular Fluid and Saliva of Hepatitis C Seropositive Hemodialysis Patients. International journal of dentistry 2009: 121 – 126.
2. Arrieta J., Rodríguez-Iñigo E., Ortiz-Movilla N., Bartolomé J., Pardo M., Manzarbeitia F., Oliva H., Macías D. and Carreño V. (2001): In Situ Detection of Hepatitis C Virus RNA in Salivary Glands. The American Journal of Pathology 158(1): 259–264.
3. Averhoff F., Glass N. and Holtzman D. (2012): Global Burden of Hepatitis C: Considerations for Healthcare Providers in the United States. Clinical Infectious Diseases 5(suppl 1): S10-S15.
4. Champoux J., Neidhardt F., Drew L. And Plorde J. (2004): Sherris medical microbiology; An introduction to infectious diseases. 4th edition. McGraw-Hill– United States of America.
5. De Cocka L., Hutseb V., Verhaegena E., Quoilinb S., Vandenbergheb H. and Vranckxa R. (2004): Detection of HCV antibodies in oral fluid. Journal of Virological Methods 122(2): 179 – 183.
6. Engvall E. and Perlmann P. (1971): Enzyme linked immunosorbent assay (ELISA): qualitative assay of IgG. Immunochemistry 8: 871-874.
7. Ferreiro MC., Dios PD. and Scully C. (2005): Transmission of hepatitis C virus by saliva? Oral diseases 11(4): 230-235.
8. Fried MW, Shindo M, Fong TL, Fox PC, Hoofnagle JH, Di Bisceglie AM (1992): Absence of hepatitis C viral RNA from saliva and semen of patients with chronic hepatitis C. Gastroenterology 102(4):1306-1308.
9. Gonçalves P., Cunha C., Busek S., Oliveira G., Ribeiro-Rodrigues R., EL Pereira F. (2005): Detection of hepatitis C virus RNA in saliva samples from patients with seric anti-HCV antibodies. The Brazilian journal of infectious diseases 9(1): 28 – 34.
10. Hahn J. (2007): Sex, Drugs, and Hepatitis C Virus. The Journal of Infectious Diseases 195(11): 1556-1559.
11. Hsu HH., Wright TL., Luba D., Martin M., Feinstone SM., Garcia G. and Greenberg HB. (1991): Failure to detect hepatitis C virus genome in human secretions with the polymerase chain reaction. Hepatology 14(5): 763-767.
12. Janeway C., Travers P. and Walport M. (2001): The distribution and functions of immunoglobulin isotypes: The Immune System in Health and Disease. (5th edition). Immunobiology 4(9):14-15.
13. Judd A1., Parry J., Hickman M., McDonald T., Jordan L., Lewis K., Contreras M., Dusheiko G., Foster G., Gill N., Kemp K., Main J., Murray-Lyon I. and Nelson M. (2003): Evaluation of a modified commercial assay in detecting antibody

to hepatitis C virus in oral fluids and dried blood. Journal of medical virology 71(1): 49-55.
14. Kato N. (2000): Genome of human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. Microb Comp Genomics 5(3):129-151.
15. Lins L., Almeida H., Vitvisk L., Carmo T., Paraná R. and Reis M. (2005): Detection of hepatitis C virus RNA in saliva is not related to oral health status or viral load. Journal of medical virology 77(2): 216–220.
16. Moorthy M., Daniel1 HD., Kurian G. and Abraham1 P. (2008): An evaluation of saliva as an alternative to plasma for the detection of hepatitis C virus antibodies. Indian journal of medical microbiology 26(4): 327-332.
17. Nakano T., Lau G., Lau G., Sugiyama M. and Mizokami M. (2012): An updated analysis of hepatitis C virus genotypes and subtypes based on the complete coding region. Liver International 32(2): 339–345.
18. Patil P. and Patil B. (2011): Saliva: A diagnostic biomarker of periodontal diseases. Journal of indian society of periodontology 15(4): 310-317.
19. Prickett J., Simer R. and Christopher-Hennings J. (2008): Detection of porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: A longitudinal study under experimental conditions. Journal of veterinary diagnostic investigation 20: 156-163.
20. Puchhammer-Stockl E., Mor W. and Kundi M. (1994): Prevalence of hepatitis-C virus RNA in serum and throat washings of children with chronic hepatitis. Journal of medical virology 43:143- 150.
21. Shafique M., Ahmad N., Awan FR., Mustafa T., Ullah M. and Qureshi JA. (2009): Investigating the concurrent presence of HCV in serum, oral fluid and urine samples from chronic HCV patients in Faisalabad, Pakistan. Archives of virology. 154(9):1523-1527.
22. Sosa-Jurado F., Hernández-Galindo V., Meléndez-Mena D., Mendoza-Torres M., Martínez-Arroniz F., Vallejo-Ruiz1 V., Reyes-Leyva J. and Santos-López G. (2014): Detection of hepatitis C virus RNA in saliva of patients with active infection not associated with periodontal or liver disease severity. BMC Infectious Diseases 14: 72.
23. Suzuki T., Omata1 K., Satoh T., Miyasaka T., Arai C., Maeda M., Matsuno T. and Miyamura T. (2005): Quantitative Detection of Hepatitis C Virus (HCV) RNA in Saliva and Gingival Crevicular Fluid of HCV-Infected Patients. Journal of clinical microbiology 43(9): 4413-4417.
24. Terrault NA. (2002): Sexual activity as a risk factor for hepatitis C. Hepatology 36: S99- S105.
25. Van Doornum GJ., Lodder A., Buimer M., van Ameijden EJ. and Bruisten S. (2001): Evaluation of hepatitis C antibody testing in saliva specimens collected by two different systems in comparison with HCV antibody and HCV-RNA in serum. Journal of medical virology 64(1):13-20.
26. Vierstraete A.(1999): The basic steps of PCR. Quotedfrom: http://www.academia.edu/5695176/Polymerase_Chain_Reactiom. (Access-ed on 26/11/2014 at 8:00 AM).
27. Witoyo J. (2014): Tugas dasar bioteknologi: PCR. Quoted from: http://blog.ub.ac.id/jatmikoekotbp/category/biologi/. (Accessed on 25/ 11/ 2014 at 9:50 PM).
28. Wong ML. and Medrano JF. (2005): Real-time PCR for mRNA. BioTechniques 39 (1): 75-85.

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