Detection of hepatitis A, B, and C virus-specific antibodies using oral fluid for epidemiological studies

Luciane A Amado/+, Livia M Villar, Vanessa S de Paula, Adilson J de Almeida*, Ana Maria C Gaspar

Laboratório de Desenvolvimento Tecnológico *Laboratório de Hepatites Virais, Departamento de Virologia, Instituto Oswaldo Cruz-Fiocruz. Av. Brasil 4365, 21040-900 Rio de Janeiro, RJ, Brasil

In this report, we examine the adaptability of commercially available serological kits to detect antibodies markers for viral hepatitis in oral fluid samples. We also assessed the prevalence of hepatitis A, B, and C virus-specific antibodies, and related risk factors for these infectious diseases through sensitivity of the tests in saliva samples to evaluate if oral fluid can be an alternative tool to substitute serum in diagnosis of acute viral hepatitis and in epidemiological studies. One hundred and ten paired serum and saliva specimens from suspect patients of having acute hepatitis were collected to detect antibodies to hepatitis A (total and IgM), hepatitis B (anti-HBs, total anti-HBc and IgM anti-HBc), and hepatitis C (anti-HCV) using commercially available enzyme-linked immunossorbent assay (EIA). In relation to serum samples, oral fluid assay sensitivity and specificity were as follows: 87 and 100% for total anti-HAV, 79 and 100% for anti-HAV IgM, 6 and 95% for anti-HBs, 13 and 100% for total anti-HBc, 100 and 100% for anti-HBc IgM, and 75 and 100% for anti-HCV. The consistency observed between antibodies tests in saliva and expected risk factors for hepatitis A and C suggests that the saliva method could replace serum in epidemiological studies for hepatitis A and C.

Key words: oral fluid - viral hepatitis - diagnostic and epidemiology

A variety of studies have indicated the potential use of oral fluid for clinical diagnosis of infectious diseases and to evaluate immunity levels of important vaccine-preventable virus infections (Parry et al. 1987, Nokes et al. 2001). Although a number of very sensitive and specific serologic tests for viral hepatitis are commercially available, the relative inconvenience of obtaining blood samples and the risk of disease transmission associated with needlestick injuries make serologic testing unattractive.

Due to invasive nature of blood collection, surveys of immunity in representative samples of the general population are difficult to perform. Ease, safety, and the minimally invasive nature of oral fluid collection are the major advantages of this technique. In addition, oral fluid samples can be obtained quicker than blood, it is painless and it can be self-collected. The use of oral fluid samples to detect antibodies to hepatitis viruses makes this a satisfactory alternative, since the possibility to detect acute infection and immunity using body fluids that are easily collected will facilitate the investigation, the follow-up of the outbreak, and the screening of candidates for vaccination against this disease.

Viral hepatitis is considered an important public health problem worldwide. Hepatitis A is one of the most common causes of acute viral hepatitis in the world and different endemicity patterns are found depending on factors such as socio-economic, hygienic, and sanitary conditions. Approximately 1.4 million new hepatitis A virus (HAV) infections are estimated to occur worldwide each year (WHO 2000a). In Brazil, hepatitis A represents more than 60% of reported cases of acute hepatitis (Gaspar et al. 1996). The current global estimate of the number of hepatitis B (HBV) infected individuals is 350 million (Custer et al. 2004). In Brazil, the prevalence rates of hepatitis B increase from south to north of the country (Souto et al. 1999, Tanaka 2000). There are variations from 66.1% (western Amazon) to 1.2% (Fortaleza) (Maddrey 2001, Lewis-Ximenez et al. 2002). The worldwide prevalence of the hepatitis C virus (HCV) infection is estimated to be approximately 3%, corresponding to 170 millions infected persons (WHO 1997). In Brazil, it is estimated that between 2.5 and 4.9% of the general population present anti-HCV antibodies (WHO 2000b). In fact, viral hepatitis assumes alarming proportions in the world, being the major cause of morbidity and mortality in some states of Brazil (Melnick 1995, Moreira-Silva et al. 1998).

Prevention and control of viral hepatitis infections should be goals of public health efforts. To increase the efficacy of the interventions to reduce the exposure to HAV, HBV, and HCV it is important that the main risk factors for viral hepatitis infection in different populations be known. Therefore, the use of oral fluid samples to detect antibodies could help in the surveillance and control of viral hepatitis (Parry 1993, Chaita et al. 1995).

In this report, we examine the adaptability of commercially available serological kits to detect antibodies markers for viral hepatitis in oral fluid samples. In this study, we also assessed the prevalence of hepatitis A, B, and C,

Financial support: Faperj, CNPq
+Corresponding author: l_amado@ioc.fiocruz.br
Received 2 September 2005
Accepted 16 February 2006

and related risk factors for these infectious diseases through sensitivity and specificity of the tests in oral fluid to evaluate if it can be an alternative tool to substitute serum in diagnosis of acute viral hepatitis and in epidemiological studies.

MATERIALS AND METHODS

Study population - One hundred-ten paired serum and oral fluid were collected between August 2002 and March 2003. These samples were obtained from patients with clinical suspect of acute viral hepatitis who were referred to the Brazilian Reference Center for Viral Hepatitis (BRCVH) in the Oswaldo Cruz Institute, city of Rio de Janeiro. The Ethics Committe of Oswaldo Cruz Institute approved this study. Written informed consent was obtained from each patient before entering the study.

Data collection - The survey consisted of a questionnaire to collect information concerning demographic (gender and age), socio-economic aspects (education level, familiar income, and home characteristics), and risk factors for viral hepatitis such as social activities, personal history of jaundice or hepatitis, household contact with hepatitis, occupational exposure, parenteral exposure to blood on previous transfusion, surgery, intravenous drug use, and needle-stick sharing. Patients were also asked about sexual behavior (homosexual/bisexual or heterosexual). All patients were interviewed by health department staff. Data collection took place just before the moment of the samples collection.

Biological samples - Total blood samples were collected by venipuncture. Oral fluid samples were collected by utilizing Orasure® saliva collectors (Epitope Incorporated, Beaverton, Oregon, US), a sponge swab which is rubbed between the teeth and the gums for approximately 2 min. Oral fluid was later recovered by centrifugation at 2500 rpm for 15 min at 10ºC, aliquotted in previously labeled 1.5 ml test tubes and then stored at –20ºC until analysis. All oral fluid and serum specimens were processed for storage on the same day of collection.

Laboratory tests - All serum-oral fluid paired samples collected were analyzed with commercial kits based on enzyme-linked immunossorbent assay (EIA). All viral hepatitis markers were tested by Organon Teknika Kits, excepting anti-HCV antibodies which were tested by using UBI HCV EIA 4.0 (United Biomeical Inc., US). Serum samples antibodies were detected by EIA according to the manufacturer's instructions. To detect total and IgM anti-HAV, IgM anti-HBc, and anti-HCV antibodies in oral fluid samples, no dilutions were made and oral fluids were added to wells corresponding to a total volume of diluted serum samples. To detect total anti-HBc and anti-HBs antibodies, increased the volume of saliva samples added to well (150 µl). These modifications resulted in a more concentrated volume of the oral sample per test than the corresponding serum sample. In positive anti-HCV serum samples it was performed a test Line Immunossorbent assay (INNO-LIA ™ HCV Ab III update. INNOGENETICS N.V) to verify the positivity of these samples. The manufacturer's controls were used to determine the cutoff for both serum and oral fluid samples in all tests above. No modification was done in the cutoff calculation to evaluate the possibility of adapting commercially available serologic kits without doing several changes in manufacturer's instructions and to avoid false-positives results. Since serologic tests are recognized to be 100% sensitive and specific in this capacity, we used them as the "gold standard" for comparison with our salivary antibodies tests (Ochnio et al. 1997).

Data analysis - Data were expressed as mean ± standard deviation (SD). The reliability of the laboratory test using oral fluid was defined by calculating sensitivity and specificity with their respective confidence intervals. The kappa (κ) statistic was used to assess the degree of (inter-rater) agreement between oral fluid and serum antibody status for each marker. The following interpretation of the κ statistic was used: κ < 20% poor, κ = 21-40% fair, κ = 41-60% moderate, κ = 61-80 good, 81-100 excellent agreement (Altman 1991). To compare proportions we used the chi-square (χ^2) test for independence with Yate's continuity correction, χ^2 for trend and Fisher's exact test when appropriate. A two-tailed $p < 0.05$ was considered to be statistically significant.

Epidemiological and clinical data of the patients were included in a logistic regression analysis to identify independent factors associated with detection of total anti-HAV and anti-HCV in oral fluid samples after adjustment for confounding variables. Analysis were conducted using the Statistical Package for the Social Sciences (SPSS for windows, release 8.0; SPSS Inc., Chicago, IL).

RESULTS

Description of the population - The population studied was compounded by 60 female and 50 male, with a mean age of 33 years (SD: 13.9, range: 6-76 years). It has been observed that most of subjects lived under adequate housing sanitary conditions in view of the rate of access to sewage system (86.36%). More than 50% of the individuals reported to live in houses with less than two dormitories, one bathroom or four habitants. Only 30% of the population had complete primary educational level of instruction and 76.36% had monthly familiar income more than three minimum salaries (minimum salary approximately $92.3). We observed some risk factors for hepatitis B and C in the individuals studied, like predominance of previous history of surgery and dental treatment (50.90 and 74.55%, respectively). We also verified that 3.63% of the population was homosexual or bisexual and 52.72% of the sexually active population (n = 99) did not use condoms. Moreover, 20.90% of the patients studied share blades for personal hygiene and 3.64% were intranasal/intravenous drug users. Approximately 23% of the patients reported household contact with hepatitis patients. In relation to hepatitis antecedent, we found 39% with reported previous history of this disease.

Immunoenzymatic tests

Hepatitis A antibodies - Total anti-HAV was detected in 79 (71.81%) serum and 65 (59.09%) oral fluid samples of 110 paired samples, showing sensitivity of 86.67% (95% CI: 76 to 93%). Five oral fluid samples were undetermined and thirty matched samples were negative, representing

specificity of 100% (95% CI: 88 to 100%). The agreement degree of these results was 90.47% (κ=78.80%). IgM anti-HAV was observed in 19 serum samples (17.27%), 15 of them were also positive in oral fluid (13.63%) representing a sensitivity of 78.95% (95% CI: 55 to 94%). Ninety-one paired samples were IgM anti-HAV negative showing specificity of 100% (95% CI: 96 to 100%). The agreement between these results was 96.36% (κ = 86.10%).

Hepatitis B antibodies - For HBV markers, a lower number of samples was tested due to insufficient volume of saliva samples. For total anti-HBc antibody, we tested 105 paired samples. Of 23% (21.90%) total anti-HBc seropositive subjects, 3 (2.85%) were positive for this marker in oral samples, and 1 oral sample was undetermined, representing a sensitivity of 13% (95% CI: 2.7 to 33%). Of 82 total anti-HBc seronegative subjects, 81 were also negative for this marker in oral fluid specimens, demonstrating a specificity of 100% (95% CI: 95 to 100%). The rate of agreement observed between serum and oral fluid samples was 80.70% (κ=18.90%). For IgM anti-HBc, we tested 76 samples, and four (5.26%) paired serum and saliva samples were positive, showing a sensitivity of 100% (95% CI: 39 to 100%). All 72 seronegative subjects were also negative for this marker in oral fluid samples, showing a specificity of 100% (95% CI: 95 to 100%). There was 100% (κ = 100%) of agreement between paired samples. Paired serum and saliva specimens from 60 subjects were tested for anti-HBs. Thirty-seven (61.66%) serum samples were positive for anti-HBs and only 3 (5%) of them were positive for this marker in oral fluid samples, resulting in a sensitivity of 8.33% (95% CI: 1.7 to 22%). One serum and 3 oral fluid samples were undetermined, 22 serum samples were negative for this marker and 54 saliva samples were anti-HBs negative, showing a specificity of 100% (95% CI: 83 to 100%). The agreement between paired results was 37% (κ = 0.30%).

Hepatitis C antibodies - Among 105 paired samples tested for anti-HCV antibodies, 16 (15.23%) serum samples were anti-HCV positive, and 12 of them (11.42%) were also positive in oral fluid samples. This test in oral fluid samples demonstrated a sensitivity of 75% (95% CI: 47 to 92%). Eighty-nine (84.76%) serum samples were anti-HCV negative and 91 (86.66%) were negative in oral fluid samples, resulting in a specificity of 97.75% (95% CI: 92 to 99%) and agreement of 94.28% (κ = 76.70%) between paired samples. Results of specific antibodies tests in paired serum-oral fluid specimens are shown in Table I. Table II shows a summary of sensitivity, specificity and concordance between serum and oral fluid samples according to EIA tests.

Risk factors and the hepatitis A, B and C markers

To analyze the relationship between risk factors and hepatitis A, B, and C we conducted bivariate and multivariate logistic regression.

Hepatitis A - The overall prevalence of total anti-HAV in oral fluid was 59.09%, corresponding to 65 reactive EIA tests out of 110 samples tests. Table III shows the prevalence of total anti-HAV in oral fluid and in serum samples in relation to demographic, socio-economic, and possible risk factors for hepatitis A. Total anti-HAV antibodies prevalence in oral fluid was higher in subjects aging more than 30 years (57%). Age was significantly associated to anti-HAV positivity (p = 0.016). The risk for anti-HAV positivity was associated with familiar income (p = 0.026) and this risk factor became more evident after multivariate re-

TABLE I

Antibodies in paired saliva-serum specimens

Results of antibody tests		Marker (%)					
Serum	Saliva	Total anti-HAV (n = 110)	IgM anti-HAV (n = 110)	Total anti-HBc (n = 105) [a]	IgM anti-HBc (n = 76) [a]	Anti-HBs (n = 60) [a]	Anti-HCV (n = 105) [a]
+	+	65	15	3	4	3	12
+	-	10	4	20	0	33	4
-	-	30	91	81	72	20	87
-	+	0	0	0	0	0	2
IND	+	0	0	0	0	0	0
IND	-	0	0	0	0	1	0
+	IND	4	0	0	0	1	0
-	IND	1	0	1	0	2	0

IND: indeterminate; *a*: total was not 110 due to insufficient volume of saliva samples.

TABLE II

Summary of sensitivity, specificity, concordance, and kappa values in paired samples

	Total anti-HAV	IgM anti-HAV	Total anti-HBc	IgM anti-HBc	Anti-HBs	Anti-HCV
Sensitivity (%)	86.67	78.95	13	100	8.33	75
Specificity (%)	100	100	100	100	100	97.75
Concordance (%)	90.47	96.36	80.70	100	37	94.28
Kappa (%)	78.80	86.10	18.90	100	0.30	76.70

gression analysis (p = 0.022). As the educational level increases (from primary to graduated level) the prevalence of anti-HAV antibodies decreased (p = 0.0006). Previous history of hepatitis, reported by 34 (52%) subjects, was also found to be significantly associated with total anti-HAV antibodies prevalence (p = 0.001). Household contact with hepatitis A cases was not significant associated with the detection of total anti-HAV in saliva (p = 0.953). In relation to the prevalence of anti-HAV antibodies in serum samples, all of the variables studied were considered statistically significant.

Hepatitis B - The overall prevalence of total anti-HBc in oral fluid samples was 2.85%, corresponding to 3 reactive EIA tests, out of 105 samples tested. Total anti-HBc antibody prevalence was higher in subjects aging above 30 years (67%). Of the individuals who related previous hepatitis, 33% were positive for total anti-HBc. Among individuals with surgery antecedent, 67% were total anti-HBc positive and among those with blood transfusion history, 33% had total anti-HBc. We did not perform bivariate and multivariate analysis to evaluate risk factors for hepatitis B due to the low sensitivity of this test in oral fluid.

Hepatitis C - Table IV shows the prevalence of anti-HCV antibodies and some risk factors for hepatitis C by comparing serum and saliva samples. The overall preva-

TABLE III

Total anti-hepatitis A virus (HAV) antibody prevalence in paired samples and risk factors

	Anti-HAV +ve			
	Serum		Saliva	
Variables	%	95% CI	%	95% CI
Age (years)				
0-10	3	0.01 - 1.93	3	0.04 - 2.36
11-20	10	0.05 - 0.97	12	0.15 - 2.35
21-30	28	0.04 - 0.35	28	0.09 - 0.59
> 30	59	0.03 - 0.57	57	0.04 - 0.54
Familiar income (minimum salary)				
< 2	18	0.85 - 10.74	24	0.64 - 15.86
2 - 3	28	0.93 - 22.16	25	0.71 - 9.42
> 3	54	2.02 - 20.52	51	2.07 - 16.02
Educational level				
Primary	38	0.03 - 23.31	38	0.89 - 15.82
Secondary	36	1.34 - 10.74	37	1.64 - 12.90
Graduated	26	2.15 - 21.10	25	1.75 - 10.95
Risk factors				
History of hepatitis	50	2.15 - 21.10	52	–0.13 - 0.27
Household contact	26	0.04 - 0.33	24	–0.21 - 0.22

familiar income was defined as the number of minimum salary that family had monthly; household contact: occurrence of hepatitis cases in the family.

TABLE IV

Total anti-hepatitis C virus (HCV) antibody prevalence in paired samples and risk factors

	Anti-HCV +ve			
	Serum		Saliva	
Variables	%	95% CI	%	95% CI
Age (years)				
0-15	0	0.00 - 5.74	0	0.87 - 7.06
15-30	6	0.00 - 6.88	7	0.00 - 1.04
> 30	94	0.01 - 0.43	93	0.01 - 0.55
Risk factors				
Household contact	23	0.17 - 2.57	21	0.07 - 0.23
History of hepatitis	82	2.70 - 38.03	86	0.16 - 0.46
Blood transfusion	53	5.33 - 73.45	50	0.03 - 0.15
Use of medicine drugs	76	1.79 - 19.89	78	0.13 - 0.42
Pervious surgery	70	0.91 - 8.55	71	1.44 - 21.51
Homo/bisexual behavior	8	0.73 - 42.87	7.1	1.64 - 10.65

household contact: occurrence of hepatitis cases in the family; homo/bisexual behavior

lence of anti-HCV antibodies in oral fluid was 13.33%, corresponding to 14 reactive EIA tests out of 105 samples tested. Anti-HCV antibody prevalence was higher in subjects aging above 30 years (93%) and was significantly associated with anti-HCV positivity ($p = 0.001$). Among individuals with previous history of hepatitis antecedent, 12 (85.71%) out of 14 were positive for anti-HCV, showing that anti-HCV positivity was significantly associated with previous history of hepatitis ($p = 0.0003$). Adjusted prevalence demonstrated that anti-HCV positivity is associated with previous history of hepatitis antecedent ($p = 0.03$). Seventy-one percent of the individuals with surgery antecedent were anti-HCV positive. Among subjects with blood transfusion history 50% were anti-HCV positive in oral fluid. It was observed a significant risk for hepatitis C in subjects who received blood transfusion ($p < 0.0001$) and the effect of this factor became more evident after adjustment for confounding factors ($p = 0.0002$). Sex behavior (homo/bisexual behavior) did not demonstrate significant association with prevalence of anti-HCV antibodies in oral fluid samples. In relation to anti-HCV antibodies prevalence in serum samples, all variables statistically significant using saliva samples were also significant using serum samples. Furthermore, non-significant variables (household contact, previous surgery and contact homo/bisexual) when analyzing saliva samples did not show statistical significance when studying serum samples.

DISCUSSION

In the last two decades, there has been considerable interest in saliva as a safer, more convenient, noninvasive, and more easily obtained specimen than blood. The combination of a simple and rapid testing technology using easily collected oral fluid samples could offer an efficient alternative to conventional serum assays.

Our results demonstrated that the commercially EIA kits can be successfully adapted to the use of oral samples in epidemiological studies for hepatitis A and C.

High sensitivity and specificity were demonstrated for total anti-HAV by using oral samples [86% (95% IC 0.76-0.93) and 100% (95% IC 0.88-1.0), respectively]. The sensitivity was similar to other study testing oral fluid samples with sensitivity of 82% and specificity of 100% (Oba et al. 2000). To IgM anti-HAV, some studies demonstrated greater sensitivity (100%) but lower specificity (100 vs 98%) than that reported in the present study (Piacentini et al. 1993, WHO 2000a). These discrepancies could be related to the larger samples tested here giving more concise results, since previous studies were conducted in populations small in size. In total anti-HAV antibody assay, the kappa coefficient demonstrated a result of 78%, indicating that the agreement between serum and oral fluid was good. To IgM anti-HAV assay, the kappa test demonstrated a result of 86% indicating an excellent agreement between serum and oral fluid results.

The better sensitivity of total anti-HAV when compared to IgM anti-HAV results reported here may reflect higher concentration of IgG than IgM in oral secretion and the capacity of Orasure to concentrate high titers of immunoglobulins of the class IgG (Parry 1993).

In this study we also assessed the prevalence of hepatitis and related risk factors for these diseases, with oral fluid results, which has not been used before in Brazil. In countries, such as Brazil, with improving of living standards, HAV infection is decreasing among children and teenagers, thus making adolescents and adults more susceptible to HAV, as related by Morris-Cunnington et al. (2004) by analyzing the risk factors with oral fluid samples results. The overall prevalence of anti-HAV antibodies was lower in individuals aging less than 30 years old. Socio-economic factors also represent important determinants in the spread of HAV. In accordance with previous studies from all regions of the world, our study also found an association between higher socio-economic status and decreased HAV prevalence rates. In previous studies of age-adjusted prevalence rate and median incomes (World Bank 2003) it was demonstrated a population prevalence rate decrease as median income increases. Our results also showed a higher prevalence of HAV in two monthly income salaries ($p = 0.007$). In addition to household income, others markers of socio-economic status are associated with HAV risk, including household education level. In agreement with Jacobsen (2004), we observed that anti-HAV antibodies prevalence rates increase as the level of education decreases ($p = 0.001$). All of these variables were statistically significant when we used serum samples to study these variables. Thus, these results were very similar since only one discrepancy was observed (household contact), showing that saliva could substitute serum samples for epidemiologic studies for HAV.

The most useful marker to detect immunity against hepatitis B is the antibody against surface antigen (anti-HBs). Our results demonstrated that is possible to detect anti-HBs in oral fluid samples, however this marker was not detected with equal reliability in oral fluid and serum samples. The oral test for anti-HBs presented a low sensitivity, although the specificity was 100%, indicating low false positive results. Agreement between this test and kappa coefficient were considered inappropriate (37 and 0.30%, respectively), showing that more samples should be tested to be used successfully to monitor immunization against HBV. These results may be due to a low concentration of anti-HBs in oral secretions when compared with serum samples or the high number of vaccinated subjects against hepatitis B enrolled in the study giving a high percentage of this marker in serum samples (61.66%).

In relation to total anti-HBc, our results in agreement with previous study (Nokes et al. 2001), demonstrated sensitivity, specificity, and poor agreement between oral fluid and serum results for hepatitis B.

Regarding detection of IgM anti-HBc, Piacentini et al. (1993), in agreement with our results, demonstrated 100% of sensitivity and specificity in samples obtained from hepatitis patients, suggesting that oral fluid might be used in detecting actual or recent HBV infection. IgM anti-HBc, a marker indicative of recent infection, was detected in 5.26% of subjects, with a sensitivity and specificity of 100%. Besides, the kappa coefficient of 100% indicates an excellent agreement of serum and saliva results, suggesting that this marker in oral fluid can be used as a diagnostic tool for recent HBV infection. It would be in-

teresting to test a higher number of samples to confirm these results. The low sensitivity and specificity in anti-HBs and total anti-HBc tests did not enable evaluation of risk factors for hepatitis B using oral fluid samples.

Paired serum and oral fluid samples were assayed for anti-HCV, showing sensitivity and specificity of 75 and 97.75%. Previous studies detecting anti-HCV antibodies in oral fluid resulted in a sensitivity of 72% and specificity 98% using Salivettes collection device with Ortho HCV 3.0 assay (McIntyre et al. 1996). A sensitivity of 84 and 87% and specificity 100% using OraSure collection device with Ortho HCV 3.0 assay was reported previously (Allwright et al. 2000, Judd et al. 2003). De Cock et al. (2004), using Oracol device collection with Ortho HCV 3.0 demonstrated a sensitivity and specificity of 84 and 100%, respectively. A sensitivity of 94 and 98.2% and a specificity of 99% were observed by Bello et al. (1998) and Sherman et al. (1994), using Abott assay. Many factors might explain these different results: the collection devices employed in the various studies; modified antibody detection methods for salivary HCV antibody testing or population studied. All data presented with higher sensitivities are related to the use of the saliva antibody assay in one specific HCV risk group like HIV and prisoners group (Sherman et al. 1994, Bello et al. 1998, Allwright et al. 2000), that in general have high antibody titers in response to repeated antigenic stimulation. On the other hand, the study of McIntyre et al. (1996) demonstrated a lower sensitivity when assay was applied in suspect cases of hepatitis.

The presence of anti-HCV antibodies in oral fluid can result from passive diffusion of serum antibodies. Therein, low serum anti-HCV titers could be associated with false negative results in saliva. This hypothesis is supported by a recent work that demonstrates that subjects with low titers of anti-HCV antibodies and HCV-RNA negative in serum present negative results in oral fluid assay (Alter et al. 1999). Besides, subjects with low levels of IgG anti-HCV cannot be identified by using immunoassays that recognize only immunoglobulins of the class IgG. Recognition of the additional classes (IgM) of anti-HCV antibodies appears to increase the sensitivity of tests on saliva (Lucidarme et al. 2003). Our results demonstrated that the sensitivity observed is appropriate for studies of prevalence in the population, although it has been inappropriate for disease diagnostic. Besides, the diagnostic of hepatitis C should be confirmed with molecular tests because of high rates of false-negative results. The simplicity and reproducibility of this technique make the oral fluid other possible choice for epidemiological surveys of hepatitis C.

The exposure to HCV generally occurs in higher ages (Brandão & Fuchs 2002). Similarly, we observed a high frequency of anti-HCV antibodies in individuals aging more than 30 years (93%) and an increase in the risk for HCV infection between 15 and 30 years old ($p = 0.01$). In terms of environmental exposures, such as household contact with hepatitis, we did not observe an association with anti-HCV positivity. Anti-HCV positivity was associated with personal history of hepatitis ($p = 0.001$). As demonstrated by Brandão and Fuchs (2002), exposure to blood products is considered an important risk factor for HCV infection. Blood transfusion more than 10 years ago was associated with a positive anti-HCV test, and in our study this association became stronger after adjustment for confounding factors in multivariate analysis. All statistically significant variables using saliva samples were also significant using serum samples. Conversely, non-significant variables such as household contact, previous surgery and homo/bisexual behavior when analysing saliva samples did not show statistical significance when studying serum samples These results demonstrated that saliva could substitute serum samples for epidemiologic studies for HCV infection.

In conclusion, the high sensitivity and specificity achieved in the diagnosis of acute hepatitis B (IgM anti-HBc) using oral samples suggest that saliva could be used as a diagnostic tool for this infection, however markers of immunity and past infection had very lower sensitivity and specificity than serum testing. Therefore, saliva testing is not an interesting alternative for HBV screening and epidemiological studies. Testing of saliva samples for hepatitis A and C markers provided a useful alternative to serum-based assays. The convenience, reliability, and minimal non-invasive nature of this method make it an attractive tool for the selection of non-immune candidates for vaccination against hepatitis A and in monitoring of vaccine response, in despite of our preliminary results require further systematic investigation. The consistency observed between expected risks factors for HAV and HCV infections and its corresponding sensitivity rates suggests that saliva method was adequate for epidemiological surveys of the HAV and HCV immune status of selected risk populations, such as children, day care centers, and communities with poor sanitary conditions. Meanwhile, testing saliva for viral hepatitis antibodies has applications in determining the need for immunoglobulin prophylaxis and in epidemiological studies.

ACKNOWLEDGEMENTS

To the Brazilian Reference Center for Viral Hepatitis for the technical support and the staff of the laboratory of technological development, for assistance in obtaining clinical specimens.

REFERENCES

Allwright S, Bradley F, Long J, Barry J, Thornton L, Parry JV 2000. Prevalence of antibodies to hepatitis B, hepatitis C, and HIV and risk factors in Irish prisoners: results of a national cross sectional survey. *BMJ 8:* 78-82.

Alter MJ, Kruszon-Moran D, Nainam OV, Mcquillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS 1999. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *New Engl J Med 341*: 556-562.

Altman DG 1991. *Practical Statistics for Medical Research*, Chapman & Hall/CRC, London, 611 pp.

Bello PY, Pasquier C, Gourney P, Puel J, Izopet J 1998. Assessment of a hepatitis C virus antibody assay in saliva for epidemiological studies. *Eur J Clin Microbiol Infect Dis 17*: 570-572.

Brandão AB, Fuchs SC 2002. Risk factors for hepatitis C virus infection among blood donors in southern Brazil: a case-

control study. *BioMed Central Gastroenterol 8*: 18.

Chaita TM, Graham SM, Maxwell SM, Sirivasin W, Sabchareon A, Beeching NJ 1995. Salivary sampling for hepatitis B surface antigen carriage: a sensitive technique suitable for epidemiological studies. *Ann Trop Paediatric 15*: 135-139.

De Cock L, Hutse V, Verhaegen E, Quoilin S, Vandenberghe H, Vranckx R 2004. Detection of HCV antibodies in oral fluid. *J Virol Methods 15*: 179-183.

Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, Kowdley KV 2004. Global epidemiology of hepatitis B virus. *J Clin Gastroenterol 38*: S158-168.

Gaspar AMC, Oliveira JM, Silva MLP, Yoshida CFT 1996. Orally transmitted acute hepatitis in Rio de Janeiro, Brazil (1989-1995). In Y Buisson, P Coursaget, M Kane (eds), *Enterically Transmitted Hepatitis Viruses*, La Simarre, Jouélés-Tours, France, p. 99-100.

Jacobsen KH, Koopman JS 2004. Declining hepatitis A seroprevalence: a global review and analysis. *Epidemiol Infect 132*: 1005-1022.

Judd A, 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, Nelson M 2003. Evaluation of a modified commercial assay in detecting antibody to hepatitis C virus in oral fluids and dried blood spots. *J Med Virol 71:* 49-55.

Lewis-Ximenez LL, do O KM, Ginuino CF, Silva JC, Schatzmayr HG, Stuver S 2002. Risk factors for hepatitis B virus infection among an Afro-descendents community in Brazil. *BioMed Central Public Health 2*: 26.

Lucidarme D, Decoster A, Delamare C, Schmitt C, Kozlowski D, Harbonnier J, Jacob C, Cyran C, Forzy G, Defer C, Ilef D, Emmanuelli J, Filoche B 2003. An inter-laboratory study of anti-HCV antibody detection in saliva samples. *Gastroenterol Clin Biol 27*: 159-162.

Maddrey WC 2001. Hepatitis B. An important public health issue. *Clinical Laboratory 47*: 51-5.

Melnick JL 1995. History and epidemiology of hepatitis A virus. *J Infect Dis 171* (Suppl. 1): S2-8.

McIntyre PG, Laszlo J, Appleyard K, Ogden GR 1996. Modified enzyme immunoassay to detect hepatitis C virus antibodies in oral fluid. *Eur J Microbiol and Infect Dis 15*: 882-884.

Moreira-Silva SF, Frauches DO, Pereira PE 1998. Hepatite fulminante em crianças em Vitória, ES: observação em hospital pediátrico no período 1992-97. *Rev Soc Bras Med Trop 31*(Supl.): S87.

Morris-Cunnington MC, Edmunds WJ, Miller E, Brown DWG 2004. A population-based seroprevalence study of hepatitis A virus using oral fluid in England and Wales. *Am J Epidemiol 159*: 786-794.

Nokes DJ, Enquselassie F, Nigatu W, Vyse AJ, Cohen BJ, Brown DWG, Cotts FT 2001. Has oral fluid the potential to replace serum for the evaluation of population immunity levels? A study of measles, rubella and hepatitis B in rural Ethiopia. *Bull WHO 79*: 588-593.

Oba IT, Spina AMM, Saraceni CP, Lemos MF, Senhoras RCFA, Moreira RG, Granato CFH 2000. Detection of hepatitis A antibodies by ELISA using saliva as clinical samples. *Rev Inst Med Trop São Paulo 42*: 1-9.

Ochnio JJ, Scheifele DW, HO M, Mitchell LA 1997. New, ultra sensitive enzyme immunoassay for detecting vaccine and disease-induced hepatitis A virus-specific immunoglobulin G in saliva. *J Clin Microbiol* 35: 98-101.

Parry JV 1993. Simple and reliable salivary test for HIV and hepatitis A and B virus diagnosis and surveillance. *Ann NY Acad Sci 694*: 216-233.

Parry JV, Perry KR, Mortimer PP 1987. Sensitive assays for viral antibodies in saliva: an alternative to tests on serum. *Lancet 2*: 72-75.

Piacentini SC, Thieme TR, Beller M, Davidson SL 1993. Diagnosis of hepatitis A, B and C using oral samples. *Ann NY Acad Sci 694*: 334-336.

Sherman KE, Creager RL, O'Brien J, Sargent S, Piacentini S, Thieme T 1994. The use of oral fluid for hepatitis C antibody screening. *Am J Gastroenterol 89*: 2025-2027.

Souto FJ 1999. Distribuição da hepatite B no Brasil: atualização do mapa epidemiológico e proposições para seu controle. *Gastroenterol Endosc Digest 18:*143-150.

Tanaka J 2000. Hepatitis B epidemiology in Latin America. *Vaccine 18*: S57-S60.

World Bank 2003. GNI per capita 2002. World development Indicators Database.

WHO 1997. Hepatitis C: global prevalence. *Wkly Epidemiol Rec 72*: 341-344.

WHO 2000a. Hepatitis A. WHO/CDS/ CSR/EDC, 7.

WHO 2000b. Hepatitis C - global prevalence (update). *Wkly Epidemiol Rec 75*: 18-19.