ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JANUARY 2005 ISSN 1595-689X VOL 6 NO 1
AJCEM/2004020/2508

SERO-PREVALENCE OF HEPATITIS C VIRUS AMOUNG PATIENTS ATTENDING STD CLINIC IN IBADAN, NIGERIA

[1]Oni, A. A., [2]Odaibo, G. N., [3]Ola, S. O., [2]Olaleye, O. D., [1]Bakare, R. A.,

[1]Special Treatment Clinic, Department of Medical Microbiology
Departments of [2]Virology and [3]Medicine,
College of Medicine, University College Hospital, Ibadan, Nigeria

Correspondence to: Dr. A. A. Oni

In the tropics, hepatitis C virus (HCV) seroprevalence ranges from < 0.2% in whole Africa. A strong association between HCV and hepatitis B surface antigen (HBsAg)-negative chronic liver disease and hepatocellular carcinoma has been described. Hepatocellular carcinoma (HCC) is one of the most common cancers among Africans, and in Nigeria by 1970 the estimated rate was 6.6 per 100,000 populations per annum. Sexual transmission was regarded as a minor cause of HCV, the degree of which has not been properly evaluated in most environments. Since it has been established that sexual transmission is an important mode of acquisition of the infection, we therefore set out to find the seroprevalence of HCV among 95 patients attending sexually transmitted diseases (STD) clinic in University College Hospital, Ibadan, Nigeria with a view to recommending preventive and control measures of HCV in our community. The sera collected from these respondents were used for screening for syphilis using the VDRL test, and for HCV antibodies using the MONOLISA anti-HCV (Sanofi, Pasteur France). Mid-stream urine was collected from all participants, and urethral swabs from all male participants while endocervical and high vaginal swabs were collected from female participants. Ulcer swabs were collected from those with genital ulcers. The prevalence of HCV infection was found to be 37.9% in patients presenting with STDs. This comprised 38.9% of males and 61.1% females. This prevalence rate is very high compared with the rate in the general population and other "high-risk" groups in previous studies in the same environment. Factors associated with HCV infection in this environment include high heterosexuality, high level of education, and previous instrumentations such as in scarifications and termination of pregnancy. Prevention and control of STDs will definitely reduce HCV infection and hence the attendant consequences, particularly hepatocellular carcinoma, in our environment.

INTRODUCTION

Hepatitis C virus (HCV) is a positive strand RNA virus (1). A strong association between HCV and hepatitis B virus has been demonstrated. They have the same route of transmission and these include sexual, parenteral and vertically from mother to child (2). In various studies all over the world, the HCV seroprevalence among heterosexual groups was 1.5% in Amsterdam, 0.4% in Germany, 0.5-1% among UK blood donors, 0.68% among French blood donors and 1.4% in United States (3). In the tropics, HCV seroprevalence ranges from < 0.2% in whole Africa (4).

Studies on the prevalence of antibodies against HCV revealed several high-risk groups, such as poly-transfused patients, haemophiliacs, patients treated by haemodialysis or surgery, and intravenous drug abusers (2). A strong relationship between HCV and hepatitis B surface antigen (HBsAg)-negative chronic liver disease and hepatocellular carcinoma has been described (3).

Although it has been established that sexual transmission is an important mode of acquisition of the infection (4), the degree of which has not been properly evaluated in most environments. It is therefore important to document the seroprevalence of antibodies against HCV in the at-risk group in our community. We therefore set out to find the seroprevalence of HCV among patients attending sexually transmitted diseases clinic in University College Hospital, .Ibadan, Nigeria. In addition, the risk factors for the transmission of HCV will be evaluated.

PATIENTS, MATERIALS AND METHOD

Subjects

The participants were recruited from patients attending the clinic for Sexually Transmitted Diseases, Special Treatment Clinic (STC) of the College of Medicine, University College Hospital, Ibadan, Nigeria between September 1997 and August 1998. Criteria for enrolment in the study were age of 18 years and above who gave consent voluntarily, after a detailed explanation of the purpose and procedure of the study. Each participant completed a structured questionnaire to assess demographic characteristics, medical history and sexual and social behaviour. The participants were examined for STDs using a standard protocol.

Specimen collection

Five milliliters of venous blood was collected from each participant. The serum was separated from each blood sample and stored at -20°C until analyzed. The sera were tested for syphilis and anti-HCV antibodies. Mid-stream urine was collected from all participants; urethral swabs from all male participants while endocervical and high vaginal swabs were collected from female participants. Ulcer swabs were collected from those with genital ulcers.

Laboratory work

Direct microscopy was carried out for gonorrhea (men and women), trichomoniasis, candidiasis and bacterial vaginosis (women only). Routine cultures were done for gonococci, *Haemophilus ducreyi* and *Candida albicans*. Non-specific genital infections due to *Chlamydia trachomatis* and *Ureaplasma urealyticum* was by exclusion.

HCV ELISA serology

Antibody to HCV was detected using the MONOLISA anti-HCV (Sanofi, Pasteur France) (3). Testing was carried out according to the manufacturer's instructions. This test is based on the use of a solid phase prepared with solid antigens; two recombinant proteins produced by *Escherichia coli* from clones selected in the nonstructural area of the hepatitis C virus genome (NS3 and NS4), two peptides coded by capsid area of the HC virus genome. Detection is with the goat ant-human IgG antibody purified by affinity chromatography and coupled to peroxidase.

Steps in the performance of the test include; i) the test sera and the control sera were added to the wells. If the antibodies to HCV were present, they would bind to the antigens fixed on the solid phase, ii) the peroxidase labeled antibodies to human IgG was added after a washing step. They in turn bound to the specific antibodies captured on the solid phase, iii) after removal of the unbound enzymatic conjugate, the antigen-antibody complex was revealed by addition of substrate, iv) after the reaction has been stopped, the absorbance values were read using a microplate reader at 492/620nm. The absorbance measured for a sample allowed the presence or absence of antibody to HCV to be determined. The colour intensity is proportional to the quality of antibody to HCV bound on the solid phase. The absorbance of the positive control was □ 0.900 □ 2.500, and the individual negative control was < 0.200. The presence or absence of antibodies to HCV is determined by comparing for each sample the recorded absorbance with that of the calculated cutoff

value. Samples with an optical density less than the cutoff value are considered to be negative with the MONOLISA anti-HCV (new antigens) test. Samples with an optical density higher than or equal to the cutoff value are considered to be initially positive. We considered a specimen positive only if all three tests results were greater than the cutoff.

Serological test for syphilis

Screening for syphilis was carried out using the VDRL (Welcome) assay and reactive or borderline results were confirmed by using the TPHA test.

Statistical analysis

The data were analyzed using EPI INFO Version 6.0 computer software and results expressed in tables.

RESULTS

The distribution of age, sex and marital status of the respondents is shown in Table 1. Of the 95 respondents, 46 (48.4%) were males and 49 (51.6%) females (M: F ratio of 1:1.1); 47 (49.5%) were single and 48 (51.5%) married; 73.7% were in age range 21 - 35 years. The sexual contacts and HCV status of the respondents is shown in Table 2. Of the respondents, 37.9% had sexual intercourse with spouse alone; 49.5% with other partners (these were single men); 8.4% with spouse and other partners (this include 13% of males and 4.1% of female); 4.3% of men had sexual intercourse with both spouse and Commercial Sex Workers (CSW). These differences are statistically significant. (P value = 0.0000036). Of these respondents, 36 (37.9%) were positive for HCV. This comprised of 14 (30.4%) males and 22 (44.9%) females.

Of the respondents, 14 (38.9%) of the 36 of those who had sexual intercourse with spouse alone were positive for HCV, these were all females; 22 (37.9%) of the 58 respondents with multiple partners were positive for HCV, (P value = 0.9506), 3 of 4 men (75%) who had relation with CSW had HCV. This difference is significant statistically (P value = 0.0271)

Of the respondents, 61.1% did not use any precaution; 31.6% used condom; 2.1% used pills. Six point three percent of the women were on IUCD (Table 3). These findings are statistically significant (P value = 0.0278). Of the 95 respondents, 38 (40%) had needle prick, 18 (47.4%) of which were HCV positive, 18 of 57 (31.6%) respondents without positive history of needle prick were HCV positive, a difference that is not statistically significant (P value = 0.2562).

Considering scarification, 32 of 69 (46.4%) respondents who were scarified were HCV positive; whereas only 5 of the 26 (19.2%) without scarification were HCV positive. This difference is statistically significant (P value = 0.0451). The history of abortion was given by 23 (47%) of the females, 6 (26.1%) of whom were HCV positive, a finding that is statistically significant (P value = 0.0164). The history of instrumentation was offered by 22 (45%) of these females, 6 (27.3%) of whom were HCV positive, whereas only one male had instrumentation. These differences are significant statistically (P value = 0.0322).

The analysis of level of education, sexual contacts and HCV status revealed that of the 47 who had sexual intercourse with other partners, 43 (91.5%) had secondary and tertiary education. Of the 36 who had with spouse alone, 4 (11.1%), 12 (33.3%), 6 (16.7%) and 14 (38.9%) had no formal, primary, secondary and tertiary

education respectively. The higher the educational level, the higher the HCV infection rate. These differences are statistically significant (P value <0.05) (Table 4). The diagnosis of the respondents is shown in Table 6.

Table 1: Age, sex and marital status

Age (Years)	Sex		Marital status		Total number
	Male	Female	Single	Married	
15 – 20	2	2	4	0	4
21 – 25	10	19	23	6	29
26 – 30	12	9	14	7	21
31 - 35	10	10	5	15	20
36 – 40	9	5	1	13	14
41 – 45	2	2	0	4	4
46 – 50	1	2	0	3	3
Total	**46**	**49**	**47**	**48**	**95**
%	48.4	51.6	49.5	51.5	100
P value	0.595	0.00000087			

Table 2: Sexual contacts and HCV status of respondents

HCV status	Sexual contacts						
	Husband alone	Wife alone	Partners	CSW	Spouse and Partner	Spouse and CSW	Total
Positive (%)	14 (56.0)	0	16 (34.0)	2 (100)	3 (37.5)	1 (50)	36 (37.9)
Negative (%)	11 (44.0)	11 (100)	31 (66.0)	0	5 (62.5)	1 (50)	59 (62.1)
Total	**25**	**11**	**47**	**2**	**8**	**2**	**95**
%	26.3	11.6	49.5	2.1	8.4	2.1	100

Table 3: Marital status, precaution and HCV

Marital status	Types of precaution used						HCV		
	Nil	Condom	Pills	IUCD	Others	Total	Positive	Negative	Total
Single	25	21	1	0	0	47	17	30	47
Married	33	9	1	3	2	48	19	29	48
Total	**58**	**30**	**2**	**3**	**2**	**95**	**36**	**59**	**95**
%	61.1	31.6	2.1	3.2	2.1	100	37.9	62.1	100
P value	0.02778						0.580		

Table 4: Education, sexual contacts and HCV

Level of education	Sexual contacts							HCV		
	Husband Alone	Wife alone	Other Partner	CSW	Spouse & other partner	Spouse & CSW	Total	Positive	Negative	Total
None	2	2	2	0	1	0	7	3	4	7
Primary	9	3	2	1	3	1	19	8	11	19
Secondary	5	1	20	0	4	0	30	11	19	30
Tertiary	9	5	23	1	0	1	39	14	25	39
Total	**25**	**11**	**47**	**2**	**8**	**2**	**95**	**36**	**59**	**95**
%	26.3	11.6	49.5	2.1	8.4	2.1	100	37.9	62.1	100
P value	0.03480284							0.04233256		

Table 5: HCV results of respondents

Specimen/Result		Specimen/Result		Specimen/Result		Specimen/Result	
1	+ +	25	+	49	+	73	-
2	-	26	-	50	+	74	+
3	-	27	-	51	+	75	+ +
4	-	28	-	52	-	76	+ +
5	+ +	29	-	53	+	77	-
6	-	30	-	54	-	78	-
7	-	31	-	55	+ +	79	+
8	-	32	-	56	-	80	+
9	-	33	-	57	-	81	-
10	-	34	-	58	-	82	+
11	+	35	+ + +	59	+	83	+
12		36	-	60	+ + +	84	+
13	-	37		61	-	85	
14	-	38		62	+	86	+
15	-	39	-	63	+	87	-
16	-	40	-	64	-	88	+
17	-	41	+	65	+	89	-
18	-	42	+	66	+ + + +	90	+
19	+	43	-	67	+ +	91	-
20	-	44	-	68	-	92	-
21	-	45	-	69	+	93	-
22	+ + +	46	-	70	+	94	-
23	+ + +	47	+	71	-	95	-
24	-	48	-	72	-		
Controls		**i) Positive**	+ + +	**ii) Negative**			

Of the 95 specimens 36 (37.9%) had antibodies against HCV.

Table 6: Diagnosis of the respondents

Diagnosis	H C V		Total
	Positive	**Negative**	
Non-Specific Urethritis	6	12	18
Non-Specific Cervicitis	5	5	10
Gonococcal Urethritis	3	6	9
Gonococcal Cervicitis	0	1	1
Bacterial Vaginosis	5	8	13
Vaginal Trichomoniasis	1	3	4
Vaginal Candidiasis	2	4	6
Tinea cruris	1	0	1
Genital warts	1	3	4
Genital herpes	2	0	2
UTI	0	1	1
Chancroid	0	3	3
Schistosomiasis	0	1	1
Vaginal Candidiasis & Gonococcal Cervicitis	0	2	2
Non-Specific Urethritis & Tinea cruris	0	2	2
Bacterial Vaginosis & Genital warts	0	1	1
Bacterial Vaginosis & Vaginal Candidiasis	4	2	6
Bacterial Vaginosis & Bartholin cyst	1	0	1
Bacterial Vaginosis & Gonococcal cervicitis	1	1	2
Bacterial Vaginosis & Genital ulcer	1	0	1
Bacterial Vaginosis & Genital herpes	0	1	1
Bacterial Vaginosis & Genital warts & Trichomoniasis	0	1	1
Gonococcal urethritis & Conjunctivitis	1	0	1
Non-Specific Urethritis & Genital Ulcer	0	1	1
Venerophobia	1	1	2
HIV/AIDS	1	0	1
Total	**36**	**57**	**95**
%	37.9	62.1	100

DISCUSSION

People in the age range 21 - 35 years form the greatest percent of attendees in STD clinic (73.7%) a finding that is similar to those of previous workers (6, 7). This is the age range when sexual activity is highest. In this study, the seroprevalence of HCV infection was found to be 37.9% in

patients attending STD clinic in Ibadan, Nigeria. This comprised 38.9% of males and 61.1% females. This prevalence rate is very high compared with the rate in selected risk groups in previous study in the same environment in which an estimated prevalence of 11% of anti-HCV antibodies was found in doctors and dentist; 10.9% of non-hepatic patients and 18.7% of patients with hepatocellular carcinoma (8). Data on the HCV infection in Africa are still incomplete and somewhat contradictory, some sero-epidemiological studies have claimed a prevalence of anti-HCV antibodies in healthy subjects ranging from 4% to 12.5%; others have found it to be as low as that reported from western Europe or North America, where serological evidence of contact with HCV is found in < 1.5% of blood donors or general population (9).

Our study population is that with high sexual exposure, a fact that supports sexual route as an important mode of transmission (2). Of these respondents, 37.9% had sex with spouse alone, while 62.1% had with multiple partners. Those with multiple partners had high prevalence rate than those with single partners. Hence heterosexuality is an important predator of HCV infection, a finding that occurs is HIV infection. Therefore avoidance of heterosexual behaviour will help to reduce HCV infection. Of the respondents, only 31.6% used barrier method during sexual intercourse, and these are likely to be protected from STI including HIV and HCV infections. The remaining 68.4% are likely to be exposed. Scarification, a common practice in our environment was found to be an important factor in the transmission of HCV. During scarification, non-sterile equipments are often used thereby aiding transmission. Hence scarification is a very strong factor in this environment.

The history of abortion was given by 23 (47%) of the females, 6 (26.1%) of whom were HCV positive, a finding that is statistically significant (P value = 0.0164). Just like scarification, instrumentation as in termination of pregnancy (TOP) is an important factor associated with HCV infection. The instruments used are either not sterilized or inappropriately sterilized by the quacks, who are the main actors of TOP in our environment. Studies on the prevalence of antibodies against HCV revealed several other "high-risk" groups, such as polytransfused patients, haemophiliacs, patients treated by haemodialysis or surgery and intravenous drug abusers (10).

The higher the educational level, the more likely the sexual adventure. This adventure is likely to involve multiple partners, and may explain the higher rate of HCV infection with increasing level of education. The higher level of education is prone to sophistication involving sexual exposure and hence HCV infection]

Since a strong association has been established between HCV chronic liver disease and hepatocellular carcinoma, preventive and control measures for STDs/HIV/AIDS will reduce the incidence of these liver diseases in our environment. These steps include 1) Primary prevention activities, the only strategies that can have an effect on those presently incurable STDs resulting from viral infections and these involve safer sexual behaviour (abstinence, being faithful to one faithful sexual partner, use of condom for penetrative sexual acts).

2) Secondary prevention activities, which involves adequate management of cases since "one person treatment and cure for STD is primary prevention for a potential contact" (11).

CONCLUSION

The prevalence of HCV infection was found to be 37.9% in patients presenting with STDs. This comprised 38.9% of males and 61.1% females. This prevalence rate is very high compared with the rate in the general population and other "high-risk" groups in previous studies in the same environment. Factors associated with HCV infection in this environment include high heterosexuality, high level of education, and previous instrumentations such as in scarifications and termination of pregnancy. Prevention and control of STDs will definitely reduce HCV infection, and hence the attendant consequences, particularly hepatocellular carcinoma in our environment

ACKNOWLEDGEMENT

We wish to appreciate the cooperation and assistance of the Resident Doctors, Nursing Staff and other workers of the Special Treatment Clinic, College of Medicine, and other laboratory staff in Department of Virology. This work was partly sponsored by the University of Ibadan Senate Research Grant.

REFERENCES

1. Choo QL, Weiner AJ, Overby LR, Kuo G, Houghton M, Bradley DW. Hepatitis C Virus: The major causative agent of viral non-A, non-B hepatitis. *Br. Med. Bull.* 1990; **46**: 423-441
2. Alter MJ, Coleman PJ, Kramer E, *et al.* Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. *JAMA.* 1989; **262**: 1201 - 1205
3. Chen DS, Kuo GC, Sung JL, *et al.* Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic liver disease: The Taiwan experience. *J. Infect. Dis.* 1990; **162**: 817 822
4. Doll R, Muir C, Waterhouse J. Cancer Incidence in Five Continents. Springer Verlag (for Union Internationale contre le Cancer), New York. 1970: 110 - 111.
5. Sanofi, Pasteur, France.
6. Oni AA, Adu FD, Ekweozor CC, Bakare RA. Genital Herpes simplex virus infection in females in Ibadan, Nigeria. *West Afr. J. Med.* 1996; **15(5)**: 107 - 110.
7. Bakare RA, Oni AA, Umar US, *et al.* Non-gonococcal urethritis due to *Chlamydia trachomatis;* the Ibadan experience. *Afr. J. Med. med. Sci.* 2002; **31**: 17 - 20
8. Olubuyide IO, Ola SO, Aliyu B, *et al.* Hepatitis B and C in doctors and dentists in Nigeria. *Qt. J. Med.*1997; **90**: 417- 422
9. Tibbs CJ, Palmer SJ, Coker R, *et al.* Prevalence of hepatitis C virus in tropical communities: the importance of confirmatory assays. *J. Med. Virol.* 1991; **34**: 143 -147
10. van Doornum GJJ, Hooykaas C, Cuypers MT, van der Linden MMD, Coutinho RA. Prevalence of Hepatitis C virus infections among heterosexuals with multiple partners. *J. Med. Virol.* 1991; **35**: 22 - 27
11. World Health Organization: HIV/AIDS: Key documentation and strategic information products. May 2003