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A PILOT STUDY OF THE PREVALENCE OF HEPATITIS C VIRUS ANTIBODIES AND HEPATITIS C VIRUS RNA IN SOUTHERN CAMEROON

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Abstract. Information is lacking on the prevalence of hepatitis C virus (HCV) infection in most African countries. An algorithm based on a combination of enzyme immunoassays (EIAs) with different formats (a commercial test, an HCV antibody [Ab] III test, and an HCV core Ab EIA) was used to estimate the prevalence of HCV infection in different population groups from southern Cameroon. An overall high prevalence was observed, with a significant increasing trend for both sexes with respect to age. A high proportion (67.4%) of HCV-positive sera were viremic as demonstrated by the reverse transcription–polymerase chain reaction. We conclude that the prevalence of HCV is high in southern Cameroon and increases linearly with age.

Serologic assays for the detection of antibodies against hepatitis C virus (HCV) became available soon after the viral genome was characterized.[1] The virus has been shown to be the cause in more than 90% of cases of transfusion-associated non-A, non-B hepatitis. Approximately 45–75% of those infected progress to chronic disease, and about 10% of these will develop cirrhosis.[2] The epidemiology of HCV infection in the developed countries has been well-documented and an overall low prevalence has been observed.[3,4] However, information is lacking on the prevalence of HCV infection in tropical countries. Previous studies carried out in some tropical countries using first-generation enzyme immunoassays (EIAs) were flawed due to the poor sensitivity and specificity of these assays.[5,6] The advent of second- and third-generation HCV EIAs has greatly improved sensitivity, but specificity remains a major concern.[7–9] Classically, confirmation of anti-HCV screening results is done by immunoblotting. However, recent studies have indicated that by applying a combination of EIAs of different formats, reliable but less expensive algorithms could be achieved.[8,9]

In spite of the less expensive algorithm, limited information is available on the epidemiology of HCV infection in subSaharan Africa. Studies on the frequency of HCV infection are essential in these countries to recognize persons at risk for transmitting the virus, thus excluding them from donating blood, and for understanding the epidemiology of HCV, which will assist in the elaboration of adequate preventive strategies.

In this study, we used a combination of three EIAs with different formats to determine the prevalence of HCV infection in sera obtained from southern Cameroon. This algorithm has been demonstrated to be as reliable but cheaper than the classic one, whereby sera reactive in the EIA are confirmed by immunoblotting.[9] In addition, HCV viremia was assessed by reverse transcription-polymerase chain reaction (RT-PCR) on sera confirmed positive for HCV antibodies.

SUBJECTS AND METHODS

Characteristics of the study population. A total of 251 individuals originating from Ebolowa, an urban area in southern Cameroon, were studied. They included five different population groups: 32 blood donors, 78 individuals being treated for various health problems (medical cases), 22 pregnant women, 94 surgical cases, and 25 patients being treated for different sexually transmitted diseases. One hundred twenty-six (50.2%) were males (age range 15–85 years, median age 39 years) and 125 (49.8%) were females (age range 14–70 years, median age 26 years). Individuals gave their informed consent and were consecutively enrolled in the study. Blood samples were collected using sterile syringes, and serum was separated immediately by centrifugation, coded, stored at −20°C, and transported from Cameroon to Belgium on dry ice.

Study design. All 251 sera were initially screened for HCV with a third generation assay (EIA-1 HCV 3.0 enzyme-linked immunosorbent assay test system; Ortho, Emeryville, CA). Reactive sera were retested in two other third generation assays (EIA-2; Innotest HCV antibody [Ab] III prototype assay; Innogenetics, Ghent, Belgium and EIA-3, an in-house HCV EIA). This last assay uses a mixture of two antigens, consisting of immunodominant recombinant fragments of the core (Cf: aa 6-77) and the NS3 (NS3f: aa 1359-1449); it has been previously described elsewhere.[10] Sera reactive in the latter EIAs were considered true positive results, whereas sera nonreactive in the EIA-1 were not retested and reported as negative. Sera with discordant results on the EIA-2 and EIA-3 were reported as indeterminate, and sera nonreactive in both these assays were classified as negative. Previous results with this assay system indicated that a high specificity could be obtained without compromising the sensitivity.[9]

All assays were carried out according to the manufacturer's instructions. Antibody results were expressed quantitatively as an optical density (OD) index, defined as the OD of the test sample divided by the calculated cutoff absorbance value. Sera with OD index values > 1.0 were considered reactive in the tests.

To determine viremia, the RT-PCR was performed as previously described.[11,12]

Statistical analysis. Data were processed using the Epi-Info 5 computer package (Centers for Disease Control and

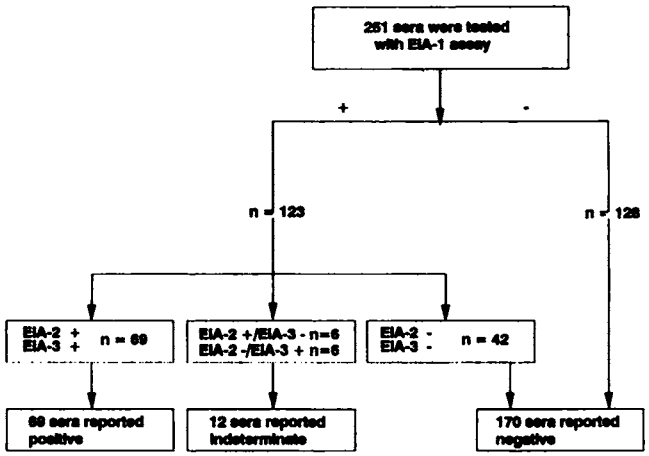

FIGURE 1. Hepatitis C virus testing algorithm using a combination of enzyme immunoassays (EIAs) with different antigenic compositions. Twenty-nine (67.4%) of the 43 sera randomly selected from the 69 positive sera were positive in the polymerase chain reaction. See Subjects and Methods for a description of the three EIAs.

Prevention, Atlanta, GA). Categorical analyses were done using either the chi-square test with Yates' correction or the two-tailed Fisher's exact test; P values less than 0.05 were considered significant.

RESULTS

A total of 123 sera (49%) were reactive in the EIA-1. Sixty-nine (56%) of the 123 sera were simultaneously reactive in the EIA-2 and EIA-3, and thus considered as true positive results according to the testing algorithm (Figure 1). A majority of these sera had high OD index values in the EIA-1. Sixty-one sera (88.4%) had OD index values between 5.2 and 9.8, with a mean ± SD OD index value of 7.33 ± 1.78, and 30 of these sera had a mode OD index value of 8.07. In the EIA-2, 68% (47 of 69) of the sera had OD index values ranging from 4.0 to 10.8, with a mean ± SD OD index value of 6.8 ± 4.0. Twenty-nine of them had a mode OD index value of 10.8. The mean ± SD OD index value of the EIA-3 was 4.4 ± 1.4, with a mode of 5.2.

Overall, 69 sera (27.5%, 95% confidence interval [CI] 21.9–31.1) were considered positive using the testing algorithm (Figure 1). The prevalence of anti-HCV antibodies in the five study groups was generally high and ranged from 5% in the 22 pregnant women to 37% in the surgical cases (Table 1).

No significant difference in HCV reactivity was observed between sexes (33.0% [42 of 126] males versus 25.0% [31 of 125] females; $\chi^2 = 2.48$, $P = 0.115$) (Table 2). However, a significant increasing trend in prevalence was observed for both sexes with respect to age ($\chi^2 = 14.7$ and 30.5, $P < 0.0001$ for both males and females, respectively) (Table 2). The total prevalence increased steadily from 9.0% in the 14–30-year-old age group to 56.0% for those more than 60 years of age.

To determine viremia in the EIA-positive sera, the RT-PCR was done on 43 of the 69 sera that were considered positive; 29 (67.4%) were positive in the PCR assay, while

TABLE 1

*Prevalence of anti-hepatitis C virus antibody in the different study groups**

	n	No. positive	% (95% CI)
Blood donors	32	7	22.0 (7.4–36.6)
Medical cases	78	23	29.0 (18.8–39.2)
Pregnant women	22	1	5.0 (0–14.3)
Surgical cases	94	35	37.0 (27.1–46.9)
STD patients	25	3	12.0 (0–24.9)
Total	251	69	27.5 (21.9–31.1)

* CI = confidence intervals; STD = sexually transmitted disease.

all the negative controls remained negative. Due to a limitation in the quantity of sera, all the positive sera could not be tested in the PCR assay. No correlation was observed between OD index values in the EIAs and PCR-positive results.

DISCUSSION

In this study, an overall prevalence of HCV infection of 27.5% was observed. Fretz and others found a similar prevalence (32.2%) in a rural population originating from the same region of Cameroon with a confirmation strategy based on an immunoblot supplementary test (unpublished data). While the high seroprevalence observed in this study is consistent with what other investigators have reported previously in some African countries,[9] it contrasts with the 2% reported in a recent study in Ethiopia.[13] Our study, however, extends previous independent studies conducted in Africa by providing an estimation of the HCV carrier state in this area of Cameroon. The 67.4% prevalence of PCR-positive samples is in the same range as that reported in HCV-seropositive sujects in Western countries.[10,12] This illustrates that the percentage of carriers among confirmed seropositive cases remains high regardless of whether the prevalence in the population under study is low or high. It further indicates that most of the confirmed seropositive cases are potential transmitters of the virus.

It is not clear why the prevalence of HCV is so high in this population compared with what has been reported in most Western countries.[3,4] Several mutually nonexclusive hypotheses may be entertained. First, ritual scarification and tattooing is a common practice in Cameroon, and this may possibly facilitate transmission of the virus. Second, the reuse of nondisposable or inadequately sterilized skin-piercing medical equipment was customary in Cameroon before 1987, when appropriate measures were implemented to control the spread of the human immunodeficiency virus. This practice undoubtedly contributed to HCV transmission. Third, the role of transmission through an insect vector cannot be formally ruled out. Hepatitis C virus has been classified as a member of the Flaviviridae family,[1] and other infections due to flaviviruses are known to be arthropod-borne.

One salient aspect of this study was the significant increasing trend in seroprevalence that was observed for both sexes with respect to age (from 9.0% in the 14–30-year-old age group to 56% for those more than 60 years of age). This observation in an urban setting is remarkably consistent with

TABLE 2

Prevalence of anti-hepatitis C virus antibody by sex and age group

Age group (years)	Males*		Females†		Total prevalence	
	No. positive/ no. tested	(%)	No. positive/ no. tested	(%)	No. positive/%	(95% CI)‡
14–30	7/47	(15.0)	4/74	(5.0)	11/9.0	(3.8–14.2)
31–45	11/32	(34.0)	12/24	(50.0)	23/41.0	(28.0–54.0)
46–60	11/25	(44.0)	10/17	(59.0)	21/50.0	(35.0–65.0)
>60	13/22	(59.0)	5/10	(50.0)	18/56.0	(38.6–73.4)
Total	42/126	(33.0)	31/125	(25.0)	69/27.5	(21.9–33.1)

* χ^2 for trend = 14.7, degrees of freedom (df) = 1; $P = 0.0001$.
† $\chi^2 = 30.5$, df = 1; $P < 0.0001$.
‡ CI = confidence interval.

previous findings in a rural population in this same region of Cameroon (unpublished data). This same increasing trend in HCV infection with age was also seen in a study conducted in an urban and rural part of Ethiopia.[13] This increase in seropositivity with age is especially notable in those more than 60 years of age, where Fretz and others found a prevalence of 68.4% (unpublished data). A possible implication for these findings is that HCV might be a major problem in the elderly in this population.

Given the high prevalence observed, infection with HCV may actually be a sleeping giant in southern Cameroon. Generally, blood donation in this community relies solely on the friends and relatives of those needing blood and the generosity of the public; thus, most of the subjects enrolled in this study could be potential blood donors. Therefore, the overall high prevalence makes HCV screening imperative.

We are at a loss to explain why the highest rate of HCV infection was found in the groups of surgical and medical cases. This might reflect a sampling bias in that these patients might have had complications related to HCV infection. Alternatively, they could have been transfused with blood infected with HCV, since screening for HCV before transfusion is not a routine activity in Cameroon. The role of sexual transmission of HCV is not yet conclusive. In this study, 12% of sexually transmitted disease clinic attendants had antibodies to HCV; however, it is difficult to draw conclusions based on these observations due to the small number of these patients studied.

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