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Epidemiology of hepatitis E virus infection during pregnancy in Benin

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Abstract

OBJECTIVES Hepatitis E virus (HEV) is the cause of enterically transmitted non-A, non-C hepatitis (an infection that is particularly severe during pregnancy) in tropical and subtropical countries. As there are no published data concerning the prevalence of HEV antibodies in Benin, their presence was investigated in pregnant women undergoing routine HIV screening in a rural area in northern Benin and in pregnant women with acute non-A, non-C hepatitis.

METHODS A total of 278 serum samples were collected from asymptomatic pregnant women in 2011 were tested for HEV and hepatitis A virus (HAV) antibodies, and the HEV IgM-positive samples were further tested for HEV-RNA. A further seven samples of pregnant women with acute non-A, non-C hepatitis collected during episodes of acute hepatitis in 2005 were also analysed.

RESULTS Of the 278 samples collected in 2011, 16.19% were positive for HEV IgG and 1.44% for HEV IgM (none positive for HEV-RNA), and 99.64% were positive for total HAV antibodies (none positive for HAV IgM). Six of the seven samples collected in 2005 were positive for HEV IgG and IgM, and two were also positive for HEV-RNA.

CONCLUSIONS The circulation of HEV infection is significant among pregnant women in Benin, in whom the consequences may be fatal.

keywords hepatitis E virus, Africa, pregnant women, immunoblotting, viral Hepatitis

Introduction

Hepatitis E virus (HEV), the main aetiological agent of enterically transmitted, non-A, non-C hepatitis, is highly endemic in the tropical and subtropical countries of Asia, Africa and South America [1]. The infection is mainly transmitted by means of contaminated water, but may also be transmitted via food or blood transfusions, or vertically from mother to foetus [2]. In most cases, the infection is asymptomatic, the virus is spontaneously cleared and only a few cases develop jaundice [3, 4], although this can be severe and accompanied by acute fulminant hepatitis.

In developed Western Countries, HEV seroprevalence is generally <5% [5, 6], and HEV-induced acute hepatitis is sporadic and attributable to travellers from endemic areas. However, many widespread epidemics involving hundreds of thousands of people have been described in Asia, Africa, the Middle East and South America [7–9], with mortality rates varying from 0.2% to 4%. For some still unknown, but possibly immunological or hormonal reason [10], mortality is more frequent in pregnant women (10–20%), especially those in the third trimester of gestation [11, 12]. HEV genotypes appear to have a different geographic distribution and different clinical severity [1, 13]: genotypes 1 or 2 are usually seen in developing countries and cause epidemic outbreaks. Genotype 1 is associated with vertical transmission [14]. Genotype 3 is usually seen in developed countries; it does not cause outbreaks and the infection resolves without transmission to the infant [15, 16]. Genotype 4 was found in sporadic cases of acute hepatitis E from China, Taiwan, Japan and Vietnam [13].

The first serologically confirmed HEV epidemic occurred in New Delhi (India) in 1955–56. The first confirmed outbreak in Africa occurred in Côte d'Ivoire in 1986 [8]. During the course of an epidemic, 90% of patients produce specific HEV antibodies [17], whereas in

non-epidemic periods, antibody prevalence varies widely from country to country or among different groups in the same area, and may also be age-related. The reported prevalence rates in Africa range from 0% to 94% [18–28]. Among pregnant women, HEV antibody prevalence is 11.6% in Burkina Faso and 84.3% in Egypt [28–32].

As there are very few published data on the prevalence of HEV antibodies in West Africa (and none at all relating to Benin), the aim of this study was to evaluate their prevalence in a rural area of northern Benin during the course of an anti-HIV screening programme routinely undergone by pregnant women. The same population was also tested for antibodies against hepatitis A virus (HAV), another enterically transmitted virus whose prevalence in Benin is unknown (it is about 90–95% in other African countries) [33], in order to compare infections due to the two viruses. Moreover, the availability of serum samples collected from pregnant women of the same area with acute hepatitis of unknown aetiology in 2005 made it possible to investigate whether HEV was involved the epidemics of the time.

Methods

Hôpital St. Jean de Dieu of Tanguiéta is located in the north of Benin on a large communication route that allows the regions of sub-Saharan Africa access to the Ocean. It is situated in Atacora district, a rural area in northern Benin with close contacts with neighbouring Burkina Faso, Niger and Nigeria. The population accessing the hospital comes from a rather extensive and heterogeneous region, unlike the catchment population of the maternity unit, which comes from a more circumscribed territory with wells drawing from a shared aquifer and surface waters from the Natural Park of Pandjari.

Between July and September 2011, 278 serum samples from pregnant women (mean age 26.2 years, range 15–41; 12.4% in the first trimester, 29.0% in the second and 58.6% in the third) were collected and frozen at the time of the HIV screening usually undertaken at Saint Jean de Dieu di Tanguiéta Hospital. Eight women (2.88% 95% CI: 0.91–4.85) screened ELISA positive for HIV-1 (confirmed by Western blotting).

Other available data were area of residence, occupation, religion, ethnicity, marital status and number of pregnancies (Table 1). At the time of blood sampling, none of the women showed clinical symptoms of hepatic disease, and hence did not undergo any specific biochemical tests.

All of the samples were retrospectively analysed for the presence of markers of HEV infection. Immunoenzymatic

Table 1 Age, place of residence, occupation, religion, ethnicity, marital status and number of pregnancies of the study population

	Number of pregnant women
Age	
15–20 years	51 (18.3%)
21–30 years	172 (61.9%)
>30 years	55 (19.8%)
Total	278
Place of residence	
Unknown	40
Known	238
Atacora district	
Tanguiéta	168 (70.6%)
Boukoumbe (south of Tanguiéta)	4 (1.7%)
Cobly (north-east of Tanguiéta)	14 (5.9%)
Materi (north-west of Tanguiéta)	28 (11.8%)
Natingou (south of Tanguiéta)	8 (3.4%)
Toucoutouna (south of Tanguiéta)	5 (2.1%)
Other districts in Benin	5 (2.1%)
Other African countries	6 (2.5%)
Occupation	
Unknown	33
Known	245
Dressmakers	28 (11.4%)
Hairdressers	11 (4.5%)
Housewives	148 (61.4%)
Students	17 (6.9%)
Tradeswomen	30 (12.2%)
Others	11 (4.5%)
Ethnicity	
Unknown	57
Known	221
Bariba	3 (1.4%)
Biali	67 (30.3%)
Dendi	13 (5.9%)
Ditamari	7 (3.2%)
Fon	14 (6.3%)
Fulfulde	17 (7.7%)
Gourmanchemà	10 (4.5%)
Hausa	9 (4.1%)
Ibo	3 (1.4%)
Kabiye	4 (1.8%)
Lupka	7 (3.2%)
Mossi	3 (1.4%)
Nateni	21 (9.5%)
Pila	3 (1.4%)
Youruba	6 (2.7%)
Waama	20 (9.0%)
Zarma	8 (3.6%)
Others	6 (2.7%)
Religion	
Unknown	71
Known	207
Animist	13 (6.3%)

Table 1 (*Continued*)

	Number of pregnant women
Christian (non-Catholic)	29 (14.0%)
Christian (Catholic)	73 (35.3%)
Muslim	92 (44.4%)
Marital status	
Unknown	63
Known	215
Married monogamously	150 (69.7%)
Married polygamously	56 (26.0%)
Unmarried	9 (4.2%)
Number of pregnancies	
Unknown	31
Known	247
One pregnancy	47 (19.0%)
Two-three pregnancies	96 (38.9%)
Four-five pregnancies	45 (18.2%)
More than five pregnancies	59 (23.9%)

assays were used to identify HEV IgG and IgM antibodies (HEV IgG, HEV IgM, DIA.PRO, Sesto San Giovanni, Milan, Italy) and total HAV and IgM antibodies (ETI-AB-HAVK PLUS, ETI-AB-IGMK PLUS, DiaSorin, Saluggia, Vercelli, Italy). If a sample tested positive, the test was repeated. The repeatedly HEV IgG and/or IgM-positive samples were confirmed using an immunoblotting assay (RecomLine HEV IgG/IgM; MIKROGEN, Neuried, Germany). In case of HEV IgM positivity, a search was made for HEV-RNA using two nested real-time polymerase chain reactions (RT-PCRs) as previously described [34].

The serum samples of seven HIV-negative pregnant women with acute hepatitis of unknown aetiology collected and frozen in 2005 were tested for the presence of HBsAg, HCV and HAV IgM with negative results, and then for HEV IgG and IgM antibodies and HEV-RNA using the RT-PCR. These women lived in the same area as the women whose serum was collected in 2011 and were hospitalised in the same hospital when some episodes of hepatitis of unknown aetiology occurred in the population of Tanguiéta.

To assess the differences between HIV-positive and negative women, Fisher's exact test was used. The prevalence of HEV and HAV markers in relation to age, residential area, occupation, religion, ethnic group, trimester of pregnancy, marital status or the number of pregnancies was analysed using logistic regression and SPSS software (Version 16.0; SPSS Inc. Chicago, IL). Also the confidence interval was calculated by SPSS software.

Results

Sixty-two of the 278 samples taken from asymptomatic pregnant women (22.30%; 95% CI: 17.41–27.19) were repeatedly HEV IgG positive. Forty-five of these (72.58%; 95% CI: 59.55–85.61) were confirmed as positive by means of immunoblotting: the prevalence of HEV IgG was therefore 16.19%; 95% CI: 11.86–20.52 (45/278).

Seven (2.52%; 95% CI: 0.68–4.36) samples were HEV IgM positive, four of which (57.14%; 95% CI: 20.48–93.80) were confirmed as positive by immunoblotting, giving a prevalence rate of 1.44%; 95% CI: 0.04–2.84 (4/278). All samples were obtained from women in the first trimester of pregnancy, and two were also HEV IgG positive (Table 2). None of the samples was positive for HEV-RNA. A total of 277 (99.64%; 95% CI: 98.94–100.00) women were also positive for total HAV antibodies, but none were HAV IgM positive.

All eight women who were HIV-1 positive were positive for total HAV antibodies, and 2 (25.00%; 95% CI: 00.00–55.01) were also positive for HEV antibodies (one for IgG and the other for IgM). There was no significant statistical difference between the prevalence of HEV or HAV antibodies in the HIV-positive and HIV-negative women; neither were there differences in the prevalence of HEV and HAV markers in relation to age, residential area, occupation, religion, ethnic group, trimester of pregnancy, marital status or the number of pregnancies.

Six of the seven samples taken from pregnant women affected by acute hepatitis in 2005 were HEV IgG and IgM positive, two of which were also HEV-RNA positive.

Discussion

This study found that 16.19% of pregnant women in Benin had come into contact with HEV and had IgG antibodies, a prevalence that is similar to that (11.6%) found among pregnant women in the neighbouring country of Burkina Faso [28], but very different from that (94%) found among healthy adults in bordering Nigeria [26] or other African countries (0–84%) [8]. The reported seroprevalence of HEV IgG antibodies in Africa during non-epidemic periods of acute and symptomatic hepatitis varies widely between countries and studied groups: among pregnant women, it reaches 84.3% in Egypt [28–32]. The differences may be due to differences (in addition to the geographical area) in the composition of studied populations (blood donors, residents in rural area, children with chronic haematological diseases, haemodialysis patients, medical students, pig handlers,

Table 2 Prevalence of hepatitis E virus (HEV) IgG and IgM, and total hepatitis A virus (HAV) antibodies in 278 pregnant women (North Benin)

	Antibodies							
	Against HEV				Against HAV			
	IgG		IgM		Total		IgM	
TEST	No.	95% CI	No.	95% CI	No.	95% CI	No.	95% CI
ELISA	62 (22.30%)	17.41–27.19	7 (2.52%)	0.68–4.36	277 (99.64%)	98.94–100.00	0 (0.00%)	0.00–0.00
Immunoblotting*	45 (16.19%)	11.86–20.52	4 (1.44%)	0.04–2.84	–	–	–	–

*Performed only on repeatedly ELISA-positive samples.

etc.) or differences in the type of assay (for total Ig or IgG) used. Commercially available assays can vary in terms of sensitivity and specificity: sensitivity and specificity of tests for IgM range between 72% and 98% and between 78% and 100%, respectively [35, 36].

The results of the HEV IgG screening test used in this study were verified by means of immunoblotting, which did not confirm the positive screening of about one-fifth of the samples, thus underlining the importance of confirmatory tests not only at diagnostic level, but also during epidemiological studies [37]. The presence of IgM antibodies (confirmed by immunoblotting) allowed the identification of cases of acute infection, even though HEV-RNA was not detected because of the short viraemic period [38]. No clinical follow-up data were available.

Nonetheless, the detection of these cases shows that HEV is present in Benin and circulating among pregnant women, and the confirmed HEV IgM positivity in almost all cases of acute hepatitis observed during pregnancy in 2005 suggests that HEV played a significant aetiological role in the epidemics of that period. Because genotypes 1 or 2 are commonly seen in developing countries and cause epidemic outbreaks while genotype 3, usually seen in the developed countries, does not (and genotype 4 is has been found in sporadic cases of acute hepatitis E from far East) [1, 13], it can be hypothesised that women with liver disease in Benin had genotype 1 or 2. Unfortunately, the samples of women in 2005 were not sufficient to perform genotyping, so we cannot verify this hypothesis.

There is no evidence of higher susceptibility to HEV in HIV-positive than HIV-negative women. It should be emphasised, however, that the number of HIV-positive women was very small. HIV prevalence in Benin is low (0.4–2.1%), so in screening campaigns, the expected quota of HIV-positive women is small and the data reported in this study are in line with those reported by the World Health Organization [39].

It is well known that the acute symptomatic form of HAV infection is very rare in developing countries [40, 41]. The prevalence of HAV antibodies among the people living in sub-Saharan Africa is as high as 90–95% [33], which means that most develop immunity in childhood and few adults are at risk of infection. Only one of the women in this study was HAV antibody negative, which indicates that the seroprevalence of antibodies against HAV is much higher than that of antibodies against HEV, as has been previously found in other African countries [42].

In conclusion, the high concentration of population in the village of Tanguiéta (and the surrounding villages), the associated difficulties for the implementation of a proper water supply, and the precariousness of the system for the disposal of organic waste, lead to high risk of contamination of aquifer and surface waters, thus increasing the risk of transmitted feco-oral diseases. This especially occurs at the end of the dry season, when water resources are minimal, and water contamination reaches the highest levels. In fact, there have been cases of infection with HEV in both 2005 and in 2011 with severe clinical consequences when the infection was contracted during pregnancy, as seen in 2005. Consequently, preventive measures must be taken, and as the main route of transmission is contaminated water, priority should be given to improving the country's drinking water supply.

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