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Low seroprevalence of hepatitis E virus in pregnant women in an urban area near Pretoria, South Africa

Omphile E. Simani[a], Tshegofatso P. Seipone[a], Gloria Selabe[a], L. Mapaseka Seheri[a], M. Jeffrey Mphahlele[a], Simnikiwe H. Mayaphi[b,c], A. Duncan Steele[a,1,*]

[a] Department of Virology, School of Medicine, Sefako Makgatho Health Sciences University, PO Box 173, Medunsa, Pretoria, South Africa
[b] Department of Medical Virology, University of Pretoria, City of Tshwane, South Africa
[c] National Health Laboratory Service — Tshwane Academic Division (NHLS-TAD), City of Tshwane, South Africa

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ABSTRACT

Objectives: Hepatitis E virus (HEV) infection is a globally neglected health problem with a high burden in resource-poor communities. Pregnant women are at increased risk of complications. This pilot study sought to assess the seroprevalence of HEV infection in pregnant women at Dr George Mukhari Academic Hospital, South Africa.
Methods: Stored serum samples from 384 HIV-uninfected pregnant women attending the antenatal clinic were initially screened for HEV total antibody. Positive samples were further evaluated for the presence of IgG and IgM antibody isotypes, using commercial ELISA assays. HEV RNA was assessed in antibody-positive samples utilizing qRT-PCR assay.
Results: The sample consisted of women with a median age of 31 years (interquartile range: 28–35 years). Total HEV antibody was detected in 12/384 (3.13%, 95% CI: 1.80–5.38) of these pregnant women. All 12 samples were IgG HEV antibody positive, but none tested positive for IgM antibody or for HEV RNA, demonstrating a lack of current or recent exposure.
Conclusions: Our study revealed a low seroprevalence of HEV among pregnant women from an urban area north of Pretoria. This observation warrants further attention to the circulation of HEV in this population, and a greater understanding of the epidemiology of the infection in South Africa.

Introduction

Hepatitis E virus (HEV) infection is a globally neglected health problem (Azman et al, 2019). Although information on its morbidity and mortality has improved, there is much we do not understand of the epidemiology, natural history, and pathogenesis of this virus. The World Health Organization (WHO) has reported that more than 20 million people worldwide are infected every year with HEV, resulting in approximately 44 000 deaths annually (WHO, 2015). Due to the sparsity of data, this is likely to be an underrepresentation. Pregnant women are at an increased risk of complications with HEV infection, with the risk increasing as the pregnancy progresses, often leading to fulminant hepatic failure and adverse outcomes, including stillbirths and maternal mortality (Shinde et al., 2014; Labrique et al., 2012).

Data from sub-Saharan Africa are limited, but show high burdens in several settings. A recent review of HEV epidemiology in Africa described the widespread occurrence of the virus in at least half of the countries across all corners of the continent, although many countries had no specific HEV surveillance data (Kim et al., 2014). Seroprevalence studies indicated a very broad range of prevalences for anti-HEV antibodies, as might be expected. Ranges varied widely across the continent, but also within the same country in different settings, and across different time periods. The review included studies on patients with acute or chronic hepatitis disease, highlighting the widespread distribution of HEV as a clinical disease in Africa (Kim et al., 2014).

HEV is regularly found in settings that lack basic resources. It is shed in feces, with risk factors for infection including those relating to unsanitary environmental conditions, including contaminated water and undercooked meat products (Khuroo et al., 2016). HEV exposure in human communities occurs sporadically, although it can occasionally occur as large outbreaks in crowded environments, such as camps for internally displaced populations (IDPs). Prevalence seems to be based on local conditions, including access to water sources and domesticated animals, as well as socio-economic factors (Aggarwal, 2013). WHO reports that HEV

* Corresponding author: Prof AD Steele, Department of Virology, Sefako Magatho Health Sciences University, PO Box 173, Medunsa 0204, Pretoria, South Africa, Tel: +27 12 521 3631; Fax: +27 12 521 5794.
E-mail address: duncan.steele@gatesfoundation.org (A.D. Steele).
[1] Present address: Enteric and Diarrheal Diseases, Bill and Melinda Gates Foundation, PO Box 23350, Seattle, WA 98102, USA

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infection has been identified in at least 63 countries, of which about half have reported large outbreaks, particularly in IDP communities living in camps in African countries, such as Sudan, Chad, Uganda, Kenya, and Somalia (WHO, 2015). Recent outbreaks have been documented in Niger, Chad, and Namibia (Browne et al., 2015; Lagare et al., 2018; Vernier et al., 2018). A recent HEV outbreak in an IDP camp in Uganda was associated with high attack rates, with mortality caused by HEV as high as 64% (Amanya et al., 2017).

There has been a limited number of seroprevalence studies in pregnant women in Africa, with a recent systematic review pooling these data together (Dagnew et al., 2019). In this meta-analysis, HEV seroprevalence in pregnant women ranged from 6.6% in Gabon to 84.3% in Egypt. The overall pooled seroprevalence for the continent was ~29% (Dagnew et al., 2019). Interestingly, the highest rates were consistently found in Egypt and other countries in northeast Africa (i.e. Sudan, Ethiopia, and Eritrea) (Kim et al., 2014; Dagnew et al., 2019). Whether this is a true representation of the virus circulation and exposure is unknown. Seroprevalence in pregnant women in other African countries tended to be lower by at least two-fold. Nevertheless, there were several trends that are worth noting — for instance, high overall mortality rates were observed among HEV-infected pregnant women from Ghana (28.7%); these differed according to gestational age, with higher mortality (75.8%) in the third trimester (Adjei et al, 2009). In addition, seroprevalence in pregnant women was higher than in the general population in Ghana (12.2–28.7% vs 4.6%) (Adjei et al., 2009; Obiri-Yeboah et al., 2018) and in Gabon (14.2% vs 0%) (Caron et al., 2008; Lenoble et al., 1995). There are currently no data for HEV seroprevalence in pregnant women in South Africa.

Results from seroprevalence studies in South Africa in the 1990s ranged from 1.8% to 10.7% in samples collected from healthy adults, including competitive canoeists and medical students (Grabow et al., 1994; Tucker et al., 1996). A more recent finding from healthy populations and blood donors in the Western Cape Province reported an overall seroprevalence of 27.9%, with a rapid and significant increase in acquisition of infection by age (Madden et al., 2016). The much higher rate found in their study may well be related to more sensitive and specific testing, which is now available, including the commercial ELISA utilized in our study. Consumption of pork products was also significantly associated with increased seroprevalence in the Madden et al. study. Finally, HEV infection was shown to be associated with patients with acute hepatitis in Cape Town (Korsman et al., 2019).

The aim of our small pilot study was to investigate the seroprevalence of HEV in pregnant women from part of the greater Pretoria metropolitan area.

Materials And Methods

Subjects

This was a descriptive pilot study that used a convenience sampling strategy to select stored serum samples. Samples had been collected in 2017 for a study investigating hepatitis B virus infections in HIV-infected patients at Dr George Mukhari Academic Hospital, Pretoria, South Africa. Our pilot analysis involved only samples from the control group — HIV-uninfected women — and included approximately half (384/800) of these control samples. Dr George Mukhari Academic Hospital is a tertiary care hospital situated in Ga-Rankuwa, which is located approximately 35 km northwest of Pretoria. A 2011 population census (the last conducted) indicated that over 80% of households had access to electricity, over half had flushing toilets connected to sewerage, and almost 75% had access to piped water in or adjacent to their dwelling. However, given the date of this last census, most of these parameters are likely outdated. The next national census is planned in 2022. In 2011, there were over 800 000 people living in this area, with 58% classified as low income and 20% as unemployed; only 17% of the population had completed secondary school education (https://www.tshwane.gov.za/sites/regions/Pages/Region-1.aspx).

Informed consent was obtained for analysis of the serum samples for exposure to HIV and hepatitis viruses. The study was approved by the Sefako Makgatho Health Sciences University Research and Ethics Committee (SMUREC number: SMUREC/M/74/2018:PG). The samples were stored at −80°C in the Department of Virology until testing. Pregnant women residing in the Tshwane Metropolitan area north of Pretoria, aged ≥ 18 years, and attending the antenatal clinic of the hospital, were included in this study.

HEV antibody testing

The commercial enzyme-linked immunosorbant assay (ELISA) kits manufactured by Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China were used according to the manufacturer's instructions. First, samples were screened for the presence of HEV total antibody (HEV-Ab) by Wantai HEV-Ab ELISA. Samples that were positive for total antibody were then assessed for the presence of HEV-IgG and/or HEV-IgM. Samples were considered positive if the absorbance cut-off ratio was > 1.2; a ratio of < 0.9 was taken as negative, while ratios between 0.9 and 1.1 were regarded as borderline for all the assessed antibody isotypes, as per the manufacturer's instructions. Automated extraction (NucliSENS EasyMag; bioMérieux, USA) was run according to the manufacturer's instructions. Patients were stratified by age in order to assess the distribution of HEV seroprevalence by age.

For the IgG-positive specimens, HEV RNA screening and viral load quantification using a qRT-PCR assay (Fast Track Diagnostics HEV RNA, Luxembourg) was conducted according to manufacturer's instructions.

Data analysis

All analysis was conducted using the STATA statistical package (STATA Corporation, College Station, TX, USA). For categorical variables, frequencies were calculated for total anti-HEV, anti-HEV-IgG, and anti-HEV-IgM. For continuous variables, such as age, median (25–75% interquartile range [IQR]) was calculated. Any missing data were excluded from all comparison analyses. Logistic regression was used to determine differences between the various age groups, and a p-value of < 0.05 was considered significant.

Results

In total, 384 pregnant women were recruited from Dr George Mukhari Academic Hospital ANC in 2017. The median age was 31 years (IQR 28–35), with most women falling in the 30–39 years range. The women included in the study were negative for HIV infection and showed HBsAg seropositivity of 2.4%.

The prevalence of total anti-HEV was 12/384 (3.13%, 95% CI: 1.80–5.38). The presence of anti-HEV-IgG and -IgM was then tested in all 12 positive samples. All samples (12/12) were anti-HEV-IgG positive, while none of the specimens tested was positive for the marker of acute infection — IgM. Moreover, none of the 12 samples was HEV-RNA positive, so no genotyping was conducted in this study.

The majority of the samples tested were from patients aged 30–39 years (53.91%; 206/384), followed by 19–29 years (32.85%; 130/384) and > 40 years (12.24%; 47/384) (Table 1). Total anti-HEV was highest in the > 40 age group (6.38%, 95% CI: 1.34–17.54; 3/47); this was not statistically different when compared with the other age groups (p = 0.800) (Table 1).

Discussion

Our study investigated the seroprevalence of anti-HEV antibodies among a small cohort of pregnant women in an urban area north of

Table 1
Prevalence of HEV antibody by age in pregnant women

Age (yrs)	Total tested*n* (%)	HEV-Ab*n* (% [95% CI])*	HEV-IgG	HEV-IgM
19–29	130 (33.85)	2 (1.54 [0.19–5.45])	2/2	0
30–39	206 (53.64)	7 (3.40 [1.39–6.88])	7/7	0
> 40	47 (12.24)	3 (6.38 [1.34–17.54])	3/3	0
Total	384	12 (3.13 [1.80–5.38])	12/12	0

HEV-Ab: hepatitis E virus total antibody
* Number positive (percentage positive [95% confidence interval])

Pretoria, the catchment area for Dr George Mukhari Academic Hospital, showing an overall seroprevalence of 3.13%. This was much lower than found by similar studies conducted in other African countries — for instance, seroprevalences of approximately 30% in Ghana and Ethiopia (Adjei et al., 2009; Abebe et al., 2017), and around 14% in Gabon (Caron et al., 2008). A recent meta-analysis of HEV seroprevalence in pregnant women in Africa yielded a pooled figure of 29.1% (Dagnew et al., 2019), although rates varied between different settings. In both Ghana and Gabon, markedly different rates were seen in different communities within the country, emphasizing the need to better understand the natural history of HEV (Adjei et al., 2009; Obiri-Yeboah et al., 2018; Caron et al., 2008; Lenoble et al., 1995).

Seroprevalence rates have been observed to be higher in pregnant women than in the general population (Kim et al., 2014), which may be due to the fact that women are generally carrying water and preparing food for the household — contaminated water is a known source of transmission (Khuroo et al., 2016; Aggarwal, 2013). In one study in Uganda, HEV transmission was recorded within households (Amanya et al., 2017). The fact that most of the South African population lives in urbanized areas — with access to piped water and improved sanitation — probably accounts for the lower rates seen in our study. Furthermore, two studies, including one from South Africa, reported that HEV seroprevalence was higher in rural than in urban communities (Tucker et al., 1996), which again might explain the low seroprevalence observed in our study. Finally, and in line with other reports, the seroprevalence observed in our study seemed to rise with increasing age, with the highest levels noted in the oldest age group, although our numbers were small (Dagnew et al., 2019; Adjei et al., 2009; Abebe et al., 2017).

Recently, a healthy population group in the Western Cape, South Africa showed a seroprevalence rate of 27%, with no differences between genders or ethnic groups (pregnant women were not included in the study) (Madden et al., 2016). The same increasing rate of IgG antibody prevalence with age was observed, with a rapid increase in the 20–30 years age bracket (Madden et al., 2016).

In our small study, neither HEV-IgM nor HEV-RNA was detected in any of the samples tested. Our results were similar to those obtained in a South African study of blood donors in the Western Cape, which screened 250 samples that were negative for HEV-IgM but positive for HEV-IgG; no HEV-RNA was identified in the tested samples in the presence of HEV-IgG ([Lopes et al., 2017).

Finally, the burden carried by pregnant women is well known. Importantly, pregnant women are a specific high-risk group for HEV infection, with case fatality rates of 25–45% (Labrique et al., 2012; Kim et al., 2014). In an HEV outbreak in Uganda, the case fatality rate in the general population was 2.2%, but 65.2% in pregnant women (Amanya et al., 2017). The ongoing protracted outbreak in neighboring Namibia should serve as a reminder that it is important to understand the risk factors and comorbidities for the local population in South Africa. In addition to socio-environmental conditions, such as contaminated water sources and undercooked meat products, hepatic disease caused by other hepatitis viruses, as well as HIV infection, are both associated with greater severity and poorer outcomes for HEV infection (Kim et al., 2014).

Our pilot study had some limitations. For example, samples were collected from only one local, albeit large, tertiary-care hospital, which limits the generalizability of the data. HIV-infected women were excluded, but should be examined in future studies, given the higher risk posed in HIV-infected individuals. In addition, our data lacked robust demographic information, such as gestational age, a full socio-economic description of the women's living conditions (including food consumption and sanitation), economic status, and educational status. Future studies should be designed to evaluate these sociological risk factors associated with HEV seropositivity or acute disease. The lack of detection of HEV RNA in the small number of IgG samples might have been due to degradation of the viral RNA during storage and repeated freeze–thaw cycles, or indicate that the patients has cleared the HEV infection.

Conclusion

Based on this pilot study, future studies will need to include: (i) examination of outlying communities with higher risk factors, including better-defined demographic information about the pregnant women and their families; (ii) evaluation of acute clinical hepatitis cases to assess clinical symptoms of liver infection; (iii) investigation of HEV seroprevalence in HIV-infected pregnant women, since some studies in African settings have shown increased prevalence when compared with HIV-uninfected pregnant women; and (iv) genotyping of local strains when found.

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Conflicts of Interest

The authors declare no conflicts of interest.

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