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Hepatitis E virus infection as a promoting factor for hepatocellular carcinoma in Cameroon: Preliminary Observations

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ABSTRACT

Objectives: To determine the seroprevalence of hepatitis E virus (HEV) infection in patients with chronic hepatitis and/or hepatocellular carcinoma (HCC) and to assess its potential consequences for disease progression.
Methods: We conducted a prospective case-control study on patients with HCC hepatitis B or C related and non-HCC patients including patients with CLD and patients without clinical evidence of liver disease. Anti-HEV IgG and IgM were tested by ELISA using commercially available kits. Liver damage was assessed by alanine aminotransferase, aspartate aminotransferase, platelets and prothrombin measurements.
Results: We observed a significant anti-HEV IgG carriage in HCC patients compared to non-HCC subjects with CLD (41.8% vs 12.6%; P = 9.1 E-6; OR = 4.8, 95%CI: 2.3-10.6). HCC patients with HEV infection display more profound alterations of circulating liver enzymes, platelets count and prothrombin time than HCC patients without sero-reactivity to HEV.
Conclusion: Overall, this study indicates a high prevalence of HEV infection in Cameroonian patients with CLD and HCC. These data suggest either that patients with liver tumors are more susceptible to hepeviral infection or that, in a tropical context, HEV might promote the progression of liver diseases towards tumor.

Introduction

Hepatitis E is a global health problem, and WHO reported that approximately 3.7 million people are infected each year by HEV with an associated mortality of 70 000 persons (WHO, 2017). Currently, infection with HEV represents the most common cause of acute viral hepatitis among adults in Southern Asia and sub-Saharan Africa (Coursaget et al., 1998; Das et al., 2000). In Cameroon, serological data concerning hepatitis E are scarce, however, a recent report indicate a higher HEV frequency in the adult compared to children (Feldt et al., 2013).

HEV infection is usually a self-limiting disease in otherwise healthy individuals, but it can trigger a fulminant hepatitis in pregnant women where it causes 20-25% of death (Boccia et al., 2006; Mushahwar, 2008). Infections with HEV can also progress and become either rapidly fatal or chronic in immunosuppressed individuals (Hamid et al., 2002; Ramachandran et al., 2004; Hoan et al., 2015). In addition, it has been shown that cirrhosis can also result from HEV infection under certain circumstances (Khuroo et al., 2016). Thus, its capacity to generate acute or persistent infections makes of HEV a novel candidate in the landscape of agents participating to liver disease progression.

In sub-Saharan Africa region, hepatitis B and C virus infections are highly endemic (Amougou et al., 2016). HCC is very frequent in Central Africa and represents a leading cause of cancer-related death (Kew, 2013; Kew, 2010). In this part of the world, large

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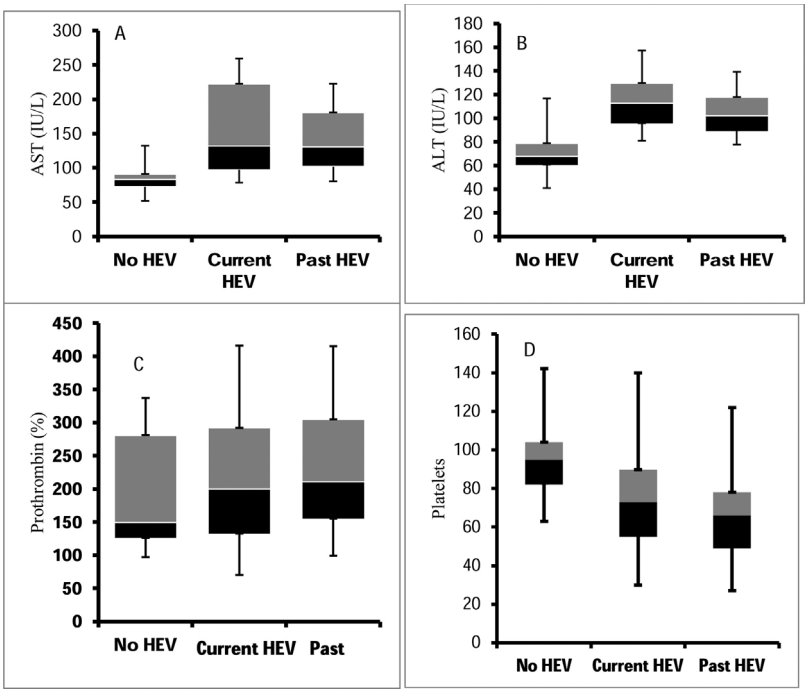

Figure 1. Biological outcome of HEV infection in patients with HCC. Patients were stratified in three groups according to anti-HEV serology ie absence of serological scar (no HEV), current infection (anti-HEV IgM(+)) and past infection (anti-HEV IgG(+)). A. Levels of aspartate aminotransferase (AST), B. Levels of alanine aminotransferase (ALT), C. prothrombin time in % of control, D. Platelet counts. Box-plots represent medians with 25 and 75 percentiles.

segments of the population are presumably vulnerable to additional causes of liver damage. Worryingly, there are still limited therapeutic options to treat liver cancer in Africa, most of them with negligible clinical benefit (Farazi and DePinho, 2006).

We made the hypothesis that the final steps of tumorigenesis might be promoted, at least in some regions of the world, by an intercurrent infection on a liver tissue already initiated for cancer. Mild liver insult, even subclinical forms, results in the production of growth factors and subsequent regeneration (Forbes and Newsome, 2016). Thus, moderate damage by HEV could be in certain circumstances responsible of the progression of the chronic liver disease toward HCC. Commonly described as “acute-on-chronic liver diseases”, cases of liver decompensations and subsequent mortality associated with a super-infection by HEV has been observed in patients with pre-existing hepatitis B or C in several geographical settings (Hoan et al., 2015; Singh et al., 2016; Atiq et al., 2009). However, so far, a link between HCC and a more a less recent hepeviral infection was not made. On a broader epidemiological standpoint, until now, the circulation and the role of HEV infection in local liver pathologies is poorly known in Central Africa. This study aims to assess the prevalence of HEV infection in patients with and without HCC and to assess the consequences of this infection for patients with chronic hepatitis B or C.

Methods

Study population and design

A case-control study was conducted. The blood samples from all the patients included in this study has been tested for hepatitis B and C viruses (HBV; HCV) infections in a previous case-control study aimed to investigate their relationships with HCC in Cameroon (Amougou et al., 2016). The study population consisted with 67 HCC cases (47 HBV-related and 20 HCV-related) and non-HCC patients composed of 67 patients with CLD (47 HBV and 20 HCV) and 67 patients without HBV or HCV. All the patients were recruited in the Gastroenterology and Radiology Units of Yaounde Central Hospital between February 2013 and January 2014. Cases and controls were paired-matched and diagnose as previously described (Amougou et al., 2016). HBV and HCV infected-individuals were all respectively carrying hepatitis B surface antigen, anti-HCV and all characterized for viral loads. Our study protocol conformed to ethical guidelines of the 1975 declaration of Helsinki was approved by the National Ethics Committee and the Ministry of Health of Cameroon.

Serological and hematological analyses

Antibodies against HEV infection were analyzed usingcommercial kits for enzyme-linked immunosorbent assay HEV IgM ELISA 3.0 and HEV ELISA IgG respectively (MP Biomedicals Asia Pacific Pte Ltd, Singapore, formerly Genelabs Diagnostics Pte Ltd) according to the manufacturer’s instructions. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes levels were estimated by a multiple point rate reflectance spectrophotometry using an automated Vitros 250 autoanalyzer dry slide from Ortho-Clinical Diagnostics (Johnson and Johnson Company U.S.A) whereas platelets and prothrombin were analyzed using Beckman Coulter LH 780 hematology Blood Analyzer (Beckman Coulter, Miami, FL, USA).

Statistical analysis

Data were presented as mean ± SD. Prevalence are given as percentages. Categorical variables were compared applying Fisher Exact or Chi square tests. Kruskal-Wallis test was used to compare quantitative variables. The odds ratios were calculated using a conditional logistic regression analysis. The level of significance was $P < 0.05$. All the analyses were performed using SPSS 16.0 statistical software.

Results

The prevalence of the marker for past HEV infection (anti-HEV IgG) was significantly higher in HCC patients (41.8%, n = 28/67) than in controls with CLD (14.9%, n = 10/67, P = 0.0009) or in individuals without HBV or HCV infections (10.4%, n = 7/67, P = 5.8 E-5). This observation clearly indicates that Cameroonian patients with HCC are at increased risk to have been formerly infected with HEV when compared either with CLD patients (OR = 4.0, 95%CI 1.7-10.4) or with healthy control subjects (OR = 5.8, 95%CI 2.3-18.1).

The serum marker for recent or ongoing of HEV infection (anti-HEV IgM) was, by contrast, more prevalent in non-HCC patients with CLD (25.4%, n = 17/67) than in HCC patients (11.9%, n = 8/67). Regarding healthy controls, a low rate of IgM carriage (8.9%, n = 6/67) was found (See Table 1). The prevalence of anti-HEV IgM between CLD and HCC patients displayed a trend for difference albeit without reaching the significance threshold (P = 0.07). This difference was, however, significant between CLD group and patients without hepatitis (P = 0.020, OR = 3.4, 95%CI 1.1-11.4). HCC patients were, thus, apparently at mildly lower risk of being affected by an ongoing/recent HEV infection (anti-HEV IgM seroreactivity) than individuals in the non-HCC CLD group (OR = 0.40, 95%CI 0.13-1.08, ns). A subset of HCC patients (n = 17.9%, 5/28) with anti-HEV IgG was also carrying anti-HEV IgM suggesting a recent HEV infection. In the CLD group, 82.3% of the subjects (n = 14/17) were simultaneously carrying IgM and IgG suggesting an ongoing or a very recent infection (Table 1). Overall, our data suggest that the two potentially sequential pathological conditions, CLD and HCC are, at least in a subset of patients, associated with different patterns of anti-HEV immunity.

We next wondered whether past or ongoing HEV infections were somehow associated with alterations of liver functions. To answer this question, HCC patients were stratified into three groups according to their serological status for HEV: HCC patients without any hallmark of HEV infection, HCC patients with current/recent infection (IgM anti-HEV (+)) and HCC patients with past HEV infection (IgG anti-HEV (+)). As a result, we found higher levels of ALT and AST in HCC patients with current or past HEV infection compared with HCC patients without previous infection (P = 0.001, Figure 1a and b).

In addition, we observed that prothrombin time was significantly longer in HCC patients currently or formerly infected with HEV compared to HCC patients without HEV infection (p = 0.013, Figure 1c). Finally, platelet count was lower in HCC patients with anti-HEV IgG (P = 0.032, Figure 1d). Taken together, our data suggest that anti-HEV seroconversion (past or recent) is associated with a deterioration of liver functions in Cameroonian patients. The patho-physiological bases of this observation are currently unknown.

Discussion

This is the first study that investigates the role of HEV infection in patients with chronic hepatitis B or C and/or HCC in Cameroon and more largely in sub-Saharan Africa. HEV infection is known to be involved in a significant subset of liver disease decompensations and in subsequent increased mortality of patients with chronic HBV and HCV infections (Ramachandran et al., 2004; Kc et al., 2006). By contrast, the potential role of an intercurrent hepatitis E in the progression of liver disease toward cancer is poorly documented (Ramachandran et al., 2004; Kc et al., 2006).

In agreement with other studies, we found a higher seroprevalence of HEV infection (anti-HEV IgM and/or anti-HEV IgG) in patients with hepatitis B or C viral infections compared to healthy patients without hepatitis (Hamid et al., 2002; Hoan et al., 2015; Atiq et al., 2009). The distributions of HEV infection markers among HCC cases with HBV or HCV infection and CLD patients chronically infected with the same viruses were, however, different. Our results show a higher prevalence of anti-HEV IgM, in CLD patients compared to uninfected healthy controls patients whereas HCC patients are more often anti-HEV IgG carriers.

Several hypotheses could be made to explain this situation. A simple explanation would be that HEV and other hepatitis viruses share some routes of transmission. This is, however, improbable regarding HBV, a virus generally horizontally transmitted in the first years of lifespan in sub-Saharan Africa. Another hypothesis regarding the higher prevalence of anti-HEV among patients with pre-existing chronic liver disease could be linked to defects of the intestinal mucosal barrier, that might become more vulnerable to both bacterial and viral translocations (Krain et al., 2014). We observed above 4-fold odds ratios to be seroconverted for HEV in

Table 1
Seroprevalence of hepatitis E virus markers by socio-demographic characteristics in HCC patients and the different controls.

Features	HCC cases n (%)	CLD controls (G1) n (%)	No Liver disease (G2) n (%)
Anti-HEV (%)			
IgG	28 (41.8)	10 (14.9)	7 (10.4)
IgM	8 (11.9)	17 (25.4)	6 (8.9)
Gender			
Males, n = 43 (%)	21 (48.8)	12 (27.9)	4 (9.3)
Females, n = 24 (%)	7 (29.2)	5 (20.8)	3 (12.5)
Viruses			
HBV(+), n = 47	17 (36.2)	12 (25.5)	/
HCV(+), n = 20	11 (55.0)	8 (40.0)	/
Age groups			
<20	0/5 (/)	1/5 (20.0)	1/4 (25.0)
20-39	12/29 (41.3)	5/28 (17.8)	3/30 (10.0)
40-59	6/16 (37.5)	2/16 (12.5)	2/17 (11.8)
≥60	10/17 (58.8)	2/18 (11.1)	1/16 (6.2)

HCC: hepatocellular carcinoma; HBsAg: hepatitis B surface antigen, Anti-HCV: Antibodies against hepatitis C virus infection; Anti-HEV: Antibodies against hepatitis E virus infection; IgG: Immunoglobulin G, IgM: immunoglobulin M; (G1): Non-HCC patients without hepatitis B and C viral infections; (G2): Non-HCC patients with chronic hepatitis B and C viral infections.

case of HCC with 41% of these patients anti-HEV (+). In this regard, a high prevalence of anti-HEV IgG has been recently reported in Egyptian patients with CLD (30%) as well as in HCC albeit to a lesser extent (13%) (El Sayed and Othman, 2011).

Our results show increased levels of liver enzymes and prothrombin time but a lower platelets count in HCC patients currently or pastly infected with HEV compared with HCC patients without HEV infection. This observation suggests that HEV infection contributes to worsen liver inflammation and apparently increases the severity of infections with HBV or HCV (Hoan et al., 2015; Sotoodeh and Pourahmad, 2013; Gotanda et al., 2007). It is, therefore, tempting to hypothesize that in such circumstances HEV might hasten the pathological progresses toward HCC. In Cameroon, infection with HEV could represent a significant co-carcinogenic risk in patients with prior HBV or HCV infections. In this regard, the differing distribution of IgM and IgG in CLD and HCC patients suggests a kinetic of this process. Indeed, anti-HEV IgM signal a recent infection in a time frame insufficient to allow tumor development whereas IgG, hallmark of an older infection, indicate that patients at risk enter in a more distant time window suitable to observe tumor development.

Our work has, of course, some limitations and should be now replicated in other countries and on larger cohorts. If it turns out to be at least partly true, it implies that the development of anti-HEV vaccine will be especially useful to protect from liver cancer the large segments of African populations chronically infected with HBV or HCV. In addition, our data open some perspectives regarding the monitoring of patients with CLD at risk to develop HCC. The serological surveillance of anti-HEV in these patients might, indeed, help clinicians to focus on those at imminent risk and lead to the detection of a larger subset of early tumors.

Conclusion

Overall, we observe a high seroprevalence of anti-HEV in Cameroonian patients with HCC developed in a context of chronic hepatitis B or C. These data suggest either that patients with liver tumors are for unknown reasons more exposed to hepeviral infections, that a defective digestive mucosal barrier may be leaky to HEV in the final stages of liver disease or that HEV promotes, at least in some cases, the final progression of liver diseases towards tumors. An effective surveillance of HEV is therefore crucial in patients with a history of persistent viral infection of the liver.

The main limitation of the present study is that hepatitis E RNA levels were not checked. As a consequence we cannot exclude that there is no substantial contribution of HEV load to the disease severity of Hepatitis B or C and HEV superinfection. Despite this limitation, our study provides preliminary support to the hypothesis that hepatitis E virus superinfection might promote the progression of liver diseases towards tumor.

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Declaration of interests

All the authors declare no competing interest. The authors are responsible for view expressed in this publication, which do not necessary represent the decision policy or view of WHO.

The role of funding Source

The funders of this study had no role in disign, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the study data and had final responsibility for the decision to submit for publication.

Ethics approval and consent to participate

The study protocol conformed to the ethical guidelines of the 1975 declaration of Helsinki was approved by the National Ethics Committee (Number 199/CNE/SE/2011) and the Ministry of Health of Cameroon (Number 631-01.12). Written informed consent was obtained from all the patients (or from parents, in the case of children).

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