ARTIGO ORIGINAL

Prevalence of Hepatitis E virus antibody in a non endemic population - prospective study

Prevalência da Hepatite E em população não endémica - Estudo prospectivo

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RESUMO | INTRODUÇÃO: O vírus da Hepatite E (VHE) ocorre, geralmente, em grandes surtos em países endémicos. Na Europa, os casos de VHE estão associados a viajantes de países endémicos. No entanto, estudos recentes descrevem casos esporádicos de VHE em indivíduos sem história de viagens a áreas endémicas.

Objectivo: identificar a taxa de prevalência do VHE na nossa população, como país não-endémico.

MATERIAL E MÉTODOS: Foram seleccionados 237 indivíduos (152 doentes do departamento de gastrenterologia e 85 dadores de sangue saudáveis): para colheita de amostras para doseamento do Ac total VHE. Foi preenchido um questionário com dados pessoais, epidemiológicos e laboratoriais

RESULTADOS: 10 doentes foram positivos para o Ac-VHE (7 do grupo de doentes – 4,6% e 3 do grupo de dadores saudáveis – 3,5%). Dois dos sete doentes do primeiro grupo tinham história de viagens a países endémicos. Não se encontraram outros factores de risco.

Um estudo paramétrico para identidade de proporções mostrou que as populações eram idênticas e tinham um valor de seropositividade semelhante.

Conclusão: Enquanto país não endémico 4,2% da nossa população era seropositiva para VHE. Não havia factores de risco epidemiológicos ou história de viagens na maioria dos casos (3,4%)

Perante o aumento de resultados em toda a Europa, similares os deste estudo, coloca-se a questão da necessidade de despistar VHE nos doentes com icterícia ou hepatite aguda.

ABSTRACT | *BACKGROUND hepatitis E virus (HEV) infection occurs as large outbreaks in endemic countries. In Europe, it is generally associated with travelling to those countries. However, sporadic cases of HEV infection without previous history of travelling have been appearing.*

AIM: To identify the prevalence of HEV antibodies in our population as a non endemic country.

METHODS: 237 patients were selected (152 patients from the gastroenterology department and 85 healthy blood donors). Blood samples were collected to measure total HEV-antibody (HEV-ab).

A questionnaire that included personal, epidemiologic and biochemical data was completed.

RESULTS: 10 individuals (7 patients – 4.6% and 3 healthy donors – 3.5%) were positive for anti-HEV. Two patients from the first group had a travelling history but the other 5 didn't. No other risk factors were found.

A parametric test for identity of proportions was used: there was no differences between the characteristics of the two groups and the positivity for anti-HEV.

CONCLUSION: As a non-endemic country, we found that 4.2% of our population have anti-HEV virus antibodies. The majority (3.4%) of them weren't related to travelling or to other epidemiologic risk factors.

The results are similar to that found around Europe. According to that, in Portugal we may need to start considering testing for HEV in patients with jaundice or acute hepatitis.

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INTRODUCTION

Hepatitis E virus (HEV) is a non enveloped RNA virus[1], which is endemic in Central Asia, Middle East, Africa and Mexico. HEV infection is transmitted predominantly through the faecal-oral route and inter-individual transmission is extremely rare[1,2]. In endemic countries it occurs as large outbreaks or as sporadic cases, being rare in non-endemic areas.

In Europe and other industrialized countries, acute hepatitis E has been associated with travellers from endemic regions, with a 1 to 3% seroprevalence, with a tendency to increase in the last years. However, some sporadic cases of HEV hepatitis have been reported in individual without a history of travelling to HEV-endemic areas[1,2,3]. Because we are also a non-endemic country the goal of our study is to determine the prevalence of HEV infection in our population.

PATIENTS AND METHODS:

Patients:

Serum samples were collected consecutively from 237 patients, between October and December 2007.

We divided the patients in two groups: 152 patients, from the Gastroenterology department (62 women, 102 men; mean age 57; range 23-85) and 85 healthy blood donors (32 women, 53 men, mean age :38; range 25-60).

We did not divide the patients with gastrointestinal pathology into further subgroups because we were analysing total IgG-HEV(prevalence of HEV infection, with or without past disease) and not acute disease (disease's incidence).

Serological methods for detection of anti-HEV

A chemiluminescence's enzyme immunoassay of third generation was used (EIAgen HEV IgG Kit ®) to do a qualitative determination of IgG antibodies of HEV.

The results were considered positive to a cut-off value > 1.1, equivocal 0.9-1.1 and negative for a cut-off value < 0.9.

The results considered positive or between 0.9-1.1 were tested twice one or two weeks later and we only considered a positive result if >1.1. The results <1.1 at second analyses were considered negative. Performances' evaluation had been conducted on negative and positive samples, in an external clinical center with reference to a FDA approved kit and a specificity of > 99.5% and a sensitivity of 100% were found.

Other data

At the same time we also collected a blood sample to test seric levels of aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT) and bilirrubin.

Before the samples collection the patients fulfilled a questionnaire with personal (age, sex, personal priors), epidemiologic (recent travellers, any traveller to endemic areas, blood transfusion) data. In the same questionnaire the last part was completed by the investigators if any biochemical results was not normal.

Any patient who was positive for anti-HEV was called after to explain the meaning of an anti-HEV positive sample and for a personal interview to make sure that no epidemiologic data had been missed.

RESULTS

Prevalence of anti-HEV

Ten cases (4.2%) of positive anti-HEV were detected. Seven (4.6%) belonged to the group from gastroenterology department and 3 (3.5%) from the group of healthy blood donors. (See table 1 for patients' features).

From the first group, 2 patients had been in an endemic country and 5 patients (3.3%) had no risk factors. Adding the patients without endemic risk from both groups we found 8 patients (3.4%) with positive anti-HEV antibodies.

Risk factors

Among the patients with HEV infection and based on the questionnaire and the personal interviews we found 2 patients with an epidemiologic risk in the first group and no epidemiologic risk in the group of healthy blood donors (see table 1).

Both patients with epidemiological risk had travelled to an endemic area. The first patient, while travelling in Paquistan, the year before, had had an acute episode of jaundice and increase of aminotransferases value (with ALT>AST). The laboratory samples for hepatitis A, B, C (HAV-IgM, HBsAg, HBcAc, HBeAg, HBeAc, HBsAc, HBV-DNA, HCV-IgM and IgG and HCV-RNA, respectively) were negative as well as other biochemical (anti mitocondrial antibody-AMA, anti smooth muscle antibody-ASMA, anti nuclear antibody-ANA, antibodies to liver-kidney microsome type 1- LKM 1, antibodies to soluble liver antigen/liver pancreas – SLA/LP, perinuclear antineutrophyl cytoplasmic antibodies –pANCA, serum and urinary cooper, ceruloplasmin, iron, alfa-fetoprotein, serum immunoglobulin G concentration and *Epstein-Barr*, *Herpes simplex* and cytomegalovirus) and radiological exams (ultrasonography with Doppler technology) in order to try to find out the cause for the acute clinical picture but nothing was found. The biochemical analyses were normal 3 months later, after her return. The other patient has been followed due to alcoholic cirrhosis Child-Pugh B. As risk factor he had lived in Angola 20 years ago, where he had had an auto-limited jaundice. Serologies for hepatitis A, B or C were negative but the patient maintained sporadic periods of increased aminotransferases, GGT and AP.

No patient from both groups had had any blood transfusion, haemodialysis or organ-recipient transplant.

Other data

Three patients positive for anti-HEV antibodies from the gastroenterology department group had increased aminotransferases or cholestatic jaundice. Two of these patients has alcoholic cirrhosis Child-Pugh B and were hospitalized at the time of the blood sample collection due to upper digestive haemorrhage and the other had a terminal pancreatic cancer, compressing the biliary system. (see table 1).

Two patients were females and the rest were males. All of them were Caucasian.

All the non-caucasian patients (30%) in our sample were negative for HEV infection.

Statistical analysis

Because we might had a selection bias since a group of patients had gastrointestinal pathology, where there is an increase incidence of hepatic biochemistry, blood transfusions and hepatitis, although they had no scientific reason for having a higher risk of HEV, we studied the two groups separately (4.6% vs. 3.5% to Ac-HEV positivity). Then, we performed a parametric test for proportions' equality, with a 5% of significance level, based on obtained estimative from samples values, to evaluate if both groups could be considered similar for seropositivity.

The observed statistic test value was 1.77. Because this value belong the acceptance region]-1.96,1.96[, to a 5% significance we were able to say that both groups seems to have a similar value of seropositivity.

DISCUSSION

By an IgG assay we found a HEV's prevalence of 4.2% in our population and 3.4% without any risk factor or any trip to an endemic country.

The HEV was identified for the first time in 1983[4]. In endemic regions HEV can occurs as a large outbreak or as sporadic cases (50 to 70% to the latter)[4].

The majority of outbreaks in endemic areas follow the seasons of heavy rain and floods, due to the contamination of water flows by infected faecal matter. Although the consumption of fecally contaminated water is the commonest way of transmission, the ingestion of non cooked

infected meat it is another way.[2,4,5]. Others routes of transmission have been described in the literature, like blood borne, perinatal transmission or animals[2,4,5] but they are too rare.

Although there are some reports of higher detection of HEV infection (past or subclinical)[6] in multitransfused patients or with chronic haemodialysis[7] we didn't observe that. None of our positive anti-HEV antibodies patients had received any blood transfusion or haemodialysis before they were tested and those with blood transfusions or haemodialysis were negative for HEV infection.

Its high prevalence in habitants of endemic countries without any history of acute hepatitis may suggest that the infection can be asymptomatic[1,4,8,9] and could represent a past or subclinical infection[1].

Until recently, HEV prevalence in non-endemic countries was very low and any infection was related to travelling from endemic regions[4,10]. However, all over the Europe, are being related clinical cases of sporadic infection with any association to travellers[10,11,12] or dates from analyses from healthy blood donors[3,13,14,15]. Furthermore, In some of these studies the HEV infection was becoming more prevalent than HAV infection[10,16]

Our results were similar from other European countries[2,3,14,15]. We also found sporadic cases of infection non-related to travelling in 3.4% and 2 additional cases were related to travellers.

Until now 4 genotypes have been identified, each one with several subtypes[2,5]. and were named accordingly to the country where they were first found (type 1 – Asian strain found in developing countries in Asia and Africa; type 2 is the Mexican strain, found in Mexico and West Africa; type 3 – strain from the United States (EUA), is distributed through the world (Europe, Japan) but has been isolated is sporadic cases in the EUA and type 4 – found in outbreaks in China and Taiwan and included human and pig strains[2,5]. By the time this article has been written probably new strains are been identified.

Most of these cases from non-endemic areas are caused by genotypes 3 and 4 which are less frequent in endemic regions[10]. Unfortunately we weren't able to identify the genotypes. At this time we might hypotise that because genotypes 3 and 4 are those found in animals like pigs, cats and dogs[2,5,10] that might be the cause of HEV location in non endemic areas.

The laboratorial diagnosis of HEV can be done by the presence of viral RNA or by the presence of antigens or antibodies. The viral RNA can be detected 2 weeks after the disease although this test is still unavailable in most countries. The most used laboratory test is the one that confirms the presence of antibodies. In spite of the value of aminotransferases don't have any correlation with the severity of the disease[4], the presence of HEV-antibodies occurs at the same time as the ALT increases, suggesting that is an immune-mediated lesion, especially because most lymphocytes that are seen in the liver have a cytotoxic phenotype[2,4].

Anti-IgM antibody means acute infection, appears in the beginning of the disease and last, in average, 5 months. Anti-IgG antibody appears few days later and can be present several years.

In our study we use a qualitative determination of IgG anti-HEV because we only wanted to know HEV prevalence.

We first separated our groups because we considered that patients from a gastroenterology department could be a selection bias since those patients may have more epidemiologic risk factors or liver pathology that could aggravate HEV infection when present but after excluding the 2 patients with travelling to endemic countries we could found any other risk or bias.

It' s also curious that one of our patients had had travelling to the endemic area 20 years ago which could mean that IgG anti-HEV can last all that time.

In the patient that had travelled to Paquistan the fact that inter-personal transmission

Table 1 | Features of anti-HEV antibodie s positive patients

Gender	Age(years)	Risk Factors	Main disease	Increased laboratory values	Race
Male	79	Ø	Alcoholic Cirrhosis Child-Pugh B	AST > ALT, GGT, AP	Caucasian
Male	65	Ø	Terminal pancreatic cancer	Aminotransferases AP, GGT, Bilirrubin	Caucasian
Male	56	Trip to Angola	Alcoholic Cirrhosis Child-Pugh B	AST > ALT, GGT, AP	Caucasian
Female	45	Trip to Paquistan	Elevated aminotransferases	ALT>AST	Caucasian
Male	36	Ø	Peptic ulcer	Ø	Caucasian
Female	32	Ø	Gastric band	Ø	Caucasian
Male	45	Ø	Chronic Hepatitis	Ø	Caucasian
Male	37	Ø	Blood donor	Ø	Caucasian
Male	47	Ø	Blood donor	Ø	Caucasian
Male	31	Ø	Blood donor	Ø	Caucasian

is rare may justify why there wasn't a small HEV outbreak inside her family.

The same way as HAV, the HEV is not associated to cirrhosis or hepatocarcinoma and has a low mortality rate (0.5 to 4%)[4,7,18]. The exceptions are endemic areas and pregnant women; in the latter it can cause fulminate hepatitis with mortality rate of 25%[4,10]. However, there are recent studies where HEV infection where responsible for chronic hepatitis and cirrhosis in organ-transplant recipients[18] or T-cell lymphoma patient[19]. All our patients with HEV infection with cirrhosis had a known cause for it and none of them had had any organ-transplant. Our patients with aminotranferases increase or cholestatic jaundice (table 1) were hospitalized due to decompensate cirrhosis or to terminal pancreatic cancer. However, RNA HEV weren't measured therefore we can´t garantee that none of our patients had chronic HEV infection.

There are also some reports published referring workings in pigs, or even cats and dogs, as a possible risk factor[2,5,10,20]. We didn't consider that risk factor and that could be other selection bias. After asking their job

in our questionnaire and because we considered that we were dealing with persons living in a big city we excluded creating pigs or other animals as a hobby. It was also impossible to determine if our Anti-HEV positive patients have the habit of eating under-cooked pig meat.

Because of its mild severity and lower prevalence HEV does not belong to our clinical practise. Reports of small outbreaks continue to appear in non endemic countries and HEV transmission in that countries is still not fully understood. Could it be also due to contaminated water or food? Should we start giving more attention to interpersonal transmission? Could it be a zoonose? Until all the questions are answered we may need to rethink our practise.

CONCLUSION

Like other non-endemic countries we also found a 4.2% evidence of anti-HEV virus antibodies in our population and healthy blood donors. The majority (3.4%) of them weren't related to travelling or to other epidemiologic risk fact.

Due to the increasing amount of reports of HEV infection (clinical or asymptomatic) we may need to start considering HEV in patients with jaundice or hepatitis.

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