Journal of Clinical Virology 106 (2018) 44–48

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Diagnostic utility of hepatitis E virus antigen-specific ELISA versus PCR testing in a cohort of post liver transplant patients in a large university hospital

G. Soothill[a,*], S. Hessey[a], M. Erotocritou[b], P. Griffiths[a], S. Ijaz[c], D. Thorburn[a], M. Ankcorn[c,d], D. Irish[a]

[a] Royal Free Hospital, Pond St, London, NW3 2QG, UK
[b] University College London, Gower St, Bloomsbury, London WC1E 6BT, UK
[c] Blood Borne Virus Unit, Virus Reference Department, National Infection Service, Public Health England, London, UK
[d] Transfusion Microbiology, National Health Service Blood and Transplant, London, UK

ARTICLE INFO

Keywords:
Hepatitis E virus
Antigen-specific ELISA
Liver transplant patients
Screening
Positive predictive value
Negative predictive value

ABSTRACT

Background: Hepatitis E virus (HEV) is an important infectious agent causing acute and chronic disease. Chronic hepatitis E affects immunocompromised people and serological testing is neither reliable nor sufficient to infer whether a patient has infection; therefore HEV RNA testing is the only reliable diagnostic test presently available. An HEV antigen-specific ELISA test is commercially available but is not yet in clinical use.

Objectives: 1) determine the prevalence of HEV infection in the Royal Free Hospital (RFH) liver transplant cohort; 2) compare the diagnostic utility of HEV antigen-detection against the current gold standard; 3) consider screening strategies for HEV infection in immunocompromised groups.

Study design: The serum samples of 490 post liver transplant patients visiting the outpatient clinic at the RFH over an eight-month period were tested for HEV with both an HEV antigen-specific ELISA and HEV RNA test.

Results: The prevalence of HEV infection was 0.20% (95% CI 0.0%–1.1%). The specificity of the ELISA was 98.2% with a positive predictive value of 10.0%. There was one true positive HEV case, which was picked up correctly by the antigen-specific ELISA. These results were improved by incorporating a neutralisation step into further ELISA tests.

Conclusions: The antigen-specific ELISA test gave no false negative results, supporting its utility as a screening tool. There was one true antigen positive result. Further investigation including cost analysis is indicated to determine the efficacy of HEV antigen-specific ELISA testing in a screening context and in the clinical investigation of HEV infection in immunocompromised patients.

1. Background

Hepatitis E virus (HEV) was first isolated by Mikhail Balayan in 1983 and is a positive sense single-stranded non-enveloped RNA virus of the *Hepeviridae* family [1]. It is recognised as a leading cause of acute infectious hepatitis worldwide affecting around 20 million people a year and responsible for over 50,000 deaths [2]. It is currently the most common cause of enteric acute hepatitis in the UK [3].

HEV was previously considered a disease of developing countries but now an increasing number of human infections, mainly of genotype 3, are being reported in Europe and North America [4]. Four genotypes are well recognised: 1 and 2 (human viruses that infect humans via the faeco-oral route) and 3 and 4 (animal viruses that infect humans zoonotically). Genotype 3 can affect animals such as pigs and deer and transmission to humans is usually acquired from the diet, with pork products carrying the greatest risk [4,5].

The incubation period is 2–8 weeks and may be followed by acute hepatitis; however, the majority of cases are asymptomatic [6]. Infection is usually self-limiting and the overall case-fatality for HEV-induced acute hepatitis is around 0.5–4% [6]. Fulminant hepatic failure

Abbreviations: HEV, Hepatitis E virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HSCT, haematopoietic stem cell transplant; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RFH, Royal Free Hospital; PPV, positive predictive value; NPV, negative predictive value; PHE, Public Health England; VRD, Virus Reference Department; CI, confidence interval

* Corresponding author.
E-mail address: germander.soothill@nhs.net (G. Soothill).

https://doi.org/10.1016/j.jcv.2018.07.007
Received 7 May 2018; Received in revised form 8 July 2018; Accepted 20 July 2018

in pregnant women is a feature of genotype 1 infection and carries up to 25% mortality [7]. A diagnosis of chronic HEV infection requires persistence of the virus for over three months and is a feature of genotype 3 virus infections [8], with some cases of genotype 4 persistence also reported [9]. This manifests with symptoms of fatigue, diarrhoea and arthralgia and persistent moderately elevated transaminases [10]. In solid organ transplant patients it may result in progressive liver injury and cirrhosis in around 10% of cases [11]. Lack of awareness and a largely asymptomatic infection means that chronic HEV infection can remain undiagnosed [12]. The main risk factor for persistence of HEV is being immunocompromised and it is well recognised in transplant recipients (renal, liver and haematopoietic stem cell (HSCT)), HIV-infected patients with low CD4 counts and patients with haematological malignancies receiving chemotherapy [13].

The prevalence of HEV infection in Europe is higher than expected, with some studies showing around 25% of adults in the sixth and seventh decades of life to be seropositive [14]. Data from English blood donors indicates 0.04% to be positive for active HEV infection [15]. The prevalence has been found to be higher amongst at-risk patient populations such as transplant recipients [16]. Morbidity and mortality is high in transplant populations and up to 60% of patients do not clear the virus [17].

Current diagnostic testing for HEV includes the detection of HEV antibodies (IgM and IgG) and HEV RNA. As antibody detection may be unreliable in immunocompromised patients, HEV RNA testing must be used for the screening and diagnosis of HEV infection in this patient group [8].

An enzyme-linked immunosorbent assay (ELISA), which tests for HEV antigen has been developed by Wantai and is now commercially available. Testing carried out by the manufacturers determined the test to perform with a sensitivity of 66.7% and a specificity of 99.9% (Wantai). This test has also been trialled in a transplant cohort with reported sensitivity of 65% for detecting acutely infected HEV RNA-positive patients and sensitivity of 100% for detecting those chronically infected, with an overall specificity of 92% [18].

The aims of this study were threefold: 1) determine the current prevalence of HEV infection in the Royal Free Hospital (RFH) liver transplant cohort; 2) compare the diagnostic utility of HEV antigen-detection against the current gold standard of HEV RNA detection; 3) use this data to consider screening strategies for HEV infection in immunocompromised groups.

2. Study design

2.1. Setting and population

The study was conducted at the RFH, which is a large university teaching hospital situated in North London serving a population of about 1.6 million. The hospital has specialist tertiary referral hepatology and liver transplant services. The study group included patients who had undergone a liver transplant (at RFH or elsewhere) and were being followed up at the liver transplant outpatient clinic.

2.2. Ethics

Ethical review was not required for this study since it was undertaken as a clinical audit to establish the prevalence of HEV and validity of an HEV ELISA test in liver transplant patients at the hospital. The liver transplant patients at RFH are given the opportunity to consent for their clinical samples to be tested to improve clinical services. The patient serum samples were assigned a number and all patient identifying information was removed prior to antigen and PCR testing. In the case of a positive PCR test, the protocol was to de-anonymise the sample and inform the clinical team, in the best interest of the patient and to review potential treatment options.

2.3. Data collection

As part of routine care, post liver transplant patients attend the hepatology clinic at RFH for regular follow-up. Time interval between follow-up visits varies depending on time from transplant and/or any hepatological complications. Serum samples are taken on these visits for assessment of liver biochemistry and graft function. Once all requested biochemistry tests have been performed by the laboratory any surplus samples would usually be discarded. These residual samples were taken to the Virology department and tested as part of this audit. Blood samples from 490 patients who visited the outpatient clinic between 31st January and 10th August 2017 were collected. Samples were aliquoted into two tubes, one for the HEV antigen ELISA and another for HEV RNA detection, which were stored separately at −80 °C, prior to analysis.

A liver transplant database, which included data on 458 of the patients included in the study (patients who had transplants at other hospitals were not included in the database), was used to determine the demographics, baseline serology and the use of blood products perioperatively for the patients.

2.4. Laboratory methods

HEV antigen was detected using the Fortress anti-HEV antigen ELISA assay (Fortress Diagnostics, Antrim, United Kingdom, BXE0903 A) which is a two-step, solid phase antibody sandwich ELISA. The ELISA uses micro-well strips pre-coated with anti-HEV antibodies. In the first step, the test serum is placed onto the strips and incubated to allow for immune-complex formation and capture onto the solid phase. In the second step, anti-HEV antibodies conjugated to horseradish peroxidase are added, binding to the immobilised immune-complexes. After adding a peroxidase substrate the wells with antibody-antigen-antibody 'sandwich' immune-complexes form coloured products, which can be visualised and measured. The colour intensity or light absorbance of each sample is proportional to the amount of antigen present. The absorbance value is read at wavelength 450/630 nm when a dual filter instrument is used; the Biochrom ASYS Expert 96 plate reader was used in this study.

The results from all the samples were expressed as a ratio of the individual absorbance (S) to the cut-off (CO) (S/CO). Samples with a ratio < 1, were classified as negative. Samples with a ratio of > 1 were considered to be *initially reactive*. The manufacturer's definition of a positive result is at least two reactive tests. Hence, *initially reactive* samples were subsequently retested and if they repeatedly (at least twice) produced a ratio > 1, they were considered to be *repeatedly reactive* and classified as positive. Initially equivocal results (S/CO = 0.9–1.1) were also retested. Initially reactive or equivocal samples, which were not reactive on repeat testing, were classified as negative.

All samples were tested individually for HEV RNA at the Virus Reference Department (VRD), Public Health England (PHE), Colindale, using a method as previously described [19,20]. Nucleic acid was extracted from 200 µl of each sample on the MagNA Pure 96 (Roche Diagnostics Ltd. Burgess Hill, UK; virus-specific cell-free protocol) before HEV RNA detection and quantitation using an internally controlled validated quantitative HEV PCR (limit of detection 22 IU/ml) [20].

To confirm the specificity of reactivity in the HEV antigen assay the reactive samples were referred for neutralisation, which was performed at VRD. This technique, recently developed to differentiate between true reactivity and non-specific reactivity, was performed on samples that were HEV antigen reactive a third time at the reference laboratory and is described in detail elsewhere [21]. Samples were considered as truly positive in the HEV antigen assay when neutralisation exceeded 50%.