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研究報告の概要

血液製剤諮問委員会(BPAC)はHBVコア(HBc)抗体検査による偽陽性結果のために供血延期となったドナーのリエントリーに関するアルゴリズムを策定するようFDAに勧告した。現在、繰り返し陽性(RR)となったドナーのための確認検査プロトコルや標準的な確認アルゴリズムは存在しない。HBc抗体検査は特異性に問題があり、陽性結果の約65%は誤りであることが判明している。最近承認されたUltraQUAL PCRや近々上市予定であるPRISMシステム、B型肝炎に対するNATの認可など、特異性及び感度とも高い検査法の導入が予定されていることから、リエントリーに関するアルゴリズムを策定する好機であるとBPACでは判断した。FDAの提案では、8週後に他の血清マーカーとともにRRを示したHBc抗体検査の結果を個別NATにより否定された場合、供血延期となっていたドナーをリエントリーすることを認めるとしている。米国赤十字社によると、2000年から2003年のドナーを追跡調査したところ、HBc抗体検査がRRとなった初回ドナーの81.5%、リピートドナーの80%がその後3年以内に供血を行っていないことが明らかになった。HBc抗体のRR率は0.4～1.6%で、これは50万人以上のドナーが供血延期となったことを意味し、1987年以降の総数は100万人にものぼる可能性があるとのことである。

報告企業の意見	今後の対応
米国FDAの血液製剤諮問委員会はHBVコア(HBc)抗体検査による偽陽性結果のために供血延期となったドナーのリエントリーに関するアルゴリズムを策定するようFDAに勧告したとの報告である。	日本赤十字社は、HBc抗体検査の検査結果が陽性の献血者について、次回以降の献血を遠慮していただくよう通知しており、当面の間、HBc抗体検査が陽性の献血者のリエントリーは行わないこととしている。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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vCJD Risk to Plasma Recipients (continued from page 7)

FDA has not approved any manufacturing claim that the production process for any plasma-based coagulation product eliminates the risk of vCJD transmission. However, to date, no cases of vCJD are known to have been transmitted by any plasma product, NHF said, adding that the UK health authorities have said their actions are "precautionary" and "the actual risk to individuals is very low."

Canadians Address Potential Risk. Canadian Blood Services is working with hospitals and physicians to notify between 30 and 40 Canadians who may have received the BPL Factor XI between 1992 and 1998, CBS said this week in a statement (10/18/04). In addition, Health Canada is undertaking a risk assessment for Canadian patients treated with the product.

A search of Canadian Blood Services' records and those inherited from its predecessor, the Canadian Red Cross Society, found that between 1992 and 1998, Canada received small quantities of BPL Factor XI made from UK plasma and no other products. Furthermore, BPL has confirmed that none of the Factor XI sent to Canada was from the implicated donors (those who had gone on to develop vCJD).

"While the news that Canada did not receive any implicated products is reassuring and the actual risk is considered very low, we are working with Health Canada, Héma-Québec, the Canadian Hemophilia Society and the Association of Hemophilia Clinic Directors of Canada to ensure maximum safety and openness for patients and the public," said CBS CEO Graham Sher, MD. ♦

FDA Blood Panel Encourages Development of New Anti-HBc Re-entry Algorithm

The Blood Products Advisory Committee (BPAC) yesterday encouraged the Food and Drug Administration (FDA) to continue developing an algorithm to re-enter donors who have been deferred because of false positives from anti-hepatitis B core (HBc) assays. The tests have had specificity problems since they were introduced in the late 1980s, speakers told the panel..

Currently, there is no confirmatory testing protocol or standard confirmatory algorithm for repeat reactives, and current tests are so nonspecific that about 65 percent of positive test results turn out to be false. With a more specific and sensitive assay, UltraQUAL PCR, recently approved, the expected rollout of a more sensitive and specific assay with Abbott Diagnostics' PRISM system on the horizon, and the licensure of nucleic acid tests for hepatitis B, it is the right time to consider a uniform algorithm, said several presenters during the meeting in Gaithersburg, Maryland.

Under the FDA's proposal, donors who tested positive would be permitted to donate again if individual donation NAT assays administered in addition to other serological markers eight weeks later showed that the repeat reactive HBc tests were false. FDA asked the panel for guidance only; no vote was taken.

> **"I'd like to support the concept of the FDA algorithm and would encourage them to do whatever possible to get a more specific core antibody test," said Harvey G, Klein, MD, of the National Institutes of Health.**

Susan Stramer, PhD, executive scientific officer of the American Red Cross Blood Services presented data showing a low yield of return among first-time and repeat donors who have been deferred because they tested repeatedly reactive to for HBc antibodies. Repeatedly reactive (RR) rates for HBc range from 0.4 percent to 1.6 percent, with more than 500,000 deferred donors, and possibly as many as 1 million donors, deferred since 1987. "It's clearly the highest marker of deferral," Dr. Stramer said, and the measure that could reap the most re-entered donors.

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Anti-HBc Re-Entry (continued from page 8)

The Red Cross tracked donors from 2000 until 2003, finding that about 81.5 percent of first-time donors who tested RR for anti-HBc did not return to donate within three years. Among repeat donors testing anti-HBc RR, 80 percent did not return within three years. Of those who did return, about a third had tested RR for anti-HBc for a second time, she said.

Another problem is how donors who test repeatedly reactive for HBc antibodies are notified. Currently, there is no uniformity from center to center regarding how donors are told of positive results or the implications of a false positive test or their deferral. Some centers defer based on the first RR result rather than waiting for a second RR result.

Steve Kleinman, MD, chairman of AABB's Transfusion-Transmitted Diseases Committee, also speaking on behalf of America's Blood Centers and ARC, said that the three organizations believe the proposed re-entry algorithm will help reassure deferred donors and increase the blood supply without compromising blood safety.

Dr. Kleinman also urged that new, more specific assays be hurried to the market. Most blood collection centers would like to move forward with donor re-entry once the PRISM anti-HBc assay is licensed and implemented and a NAT assay is available, he said.

The ambiguous implications of repeatedly reactive results from faulty tests that lead to deferrals "fly in the face of [donors'] own self-assessment of what [constitutes] good health," Merlyn Sayers, MD, PhD, CEO of Carter BloodCare in Dallas-Forth Worth, told the panel. He also called it a "disincentive to others in the community when deferred donors relate their experiences" to their friends and families. ♦

CBER Issues Final Guidance on HIV-1/HCV NAT Testing

The Center for Biologics Evaluation and Research yesterday issued final guidance entitled *Use of Nucleic Acid Tests on Pooled and Individual Samples from Donations of Whole Blood and Blood Components (including Source Plasma and Source Leukocytes) to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV.* The draft guidance was issued in March 2002.

In the final guidance, FDA recommends that blood and plasma collection facilities meet the testing requirements in 21 *CFR* 610.40(b) for conducting adequate and appropriate testing for HIV and HCV by using FDA-licensed NAT assays for units that are not reactive on an antibody test.

CBER does not specifically recommend testing reactive units that are to be discarded or used to manufacture non-injectables – but notes that blood and plasma collectors may elect to perform NAT to obtain information about the donor's infectious status.

However, if a donation that is reactive on a test for the detection of antibodies to HIV-1 will be used for autologous transfusion or for further manufacturing into injectable products, CBER recommends that it be tested using an FDA licensed HIV-1 NAT.

HIV-1 p24 Antigen Testing. The guidance states: "When HIV-1 NAT is used in accordance with these recommendations, the use of the HIV-1 p24 test (previously recommended by FDA) is not necessary to reduce adequately and appropriately the risk of transmission of HIV-1."

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別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
一般的名称	加熱人血漿たん白		研究報告の公表状況	Khuroo M-S et. al. Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. J Gastroenterology and Hepatology 2004; 19: 778-84.	公表国 サウジアラビア	
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研究報告の概要

E 型肝炎ウイルス感染が、この風土病を持つといわれる地域において輸血により生じる可能性：
目的：E 型肝炎ウイルスが非経口経路で伝播するか否か調べる。
方法：輸血を何回か受けたことのある患者 145 人を、健常人 250 人を対照としてレトロスペクティブに調査した。また、50 人の入院患者を対象にプロスペクティブ試験も行った。このうち 25 人は総計 107 単位の輸血を受けており、残る 25 人は輸血を受けたことがまったくない患者であった。
結果：レトロスペクティブ試験では、急性 HEV 感染のマーカー［HEV 抗体（IgM）及び HEV-RNA］は、輸血患者群（145 人中 13 人）において、対照群（250 人中 2 人）に比べて有意に多い人数に検出された（P<0.001; OR=12.21［95% 信頼区間: 2.71-54.70］）。HEV 感染が認められた 13 人の患者はすべて、マーカー検査以前、少なくとも 3 ヶ月以内に一度輸血を受けていた。全体的に、HEV マーカー［HEV 抗体（IgG）、HEV 抗体（IgM）及び HEV-RNA］のいずれかについて陽性だった患者は、輸血回数も多く、黄疸の発症頻度も高く、また血清アラニンアミノトランスフェラーゼ値も高かった。プロスペクティブ試験においては、HEV 抗体（IgG）が検出されたものは、供血者からの血液検体 107 中 11、被輸血患者 25 人のうち 3 人の輸血前血液検体（うち 1 人の血液検体は HEV 抗体（IgM）についても陽性であった）、および対照群 25 人中 2 人の患者であった。輸血後 HEV 感染が、HEV 抗体（IgG）陰性の被輸血患者 22 人のうち 3 人に生じた。この感染を遡ると、供血者 4 人が無症状ではあったが HEV-RNA 陽性（4/4 人）または HEV 抗体（IgM）陽性（3/4 人）であった。これに対し、輸血を受けていない患者群は観察期間中 1 人も HEV に感染しなかった。
結論：輸血による HEV 感染の頻度が高いことは被輸血者にとって危険を意味し、特に本疾患がその地域の風土病である地域においては供血者のスクリーニング方針を見直す必要があることを示している。

報告企業の意見	今後の対応
輸血後 E 型肝炎が認められた 3 例では供血者由来の血液を輸血されたことにより HEV が感染した可能性が高いと考えられる。 本剤の原材料である人血漿の原産国の米国での発生ではないこと、血漿分画製剤による HEV 伝播が疑われる症例は報告されていないこと、並びに、血漿分画製剤では分画精製におけるウイルス不活化／除去工程により、HEV のモデルウイルスとして想定し得る HAV は不活化あるいは除去できることより、血漿分画製剤による HEV 伝播の可能性は極めて低いと考えている。	当該感染症に関し、引き続き、情報の収集を行っていく。 また、同様に同一生物種等から人に感染すると認められる疾病に関する情報情報の収集に努める。

使用上の注意記載状況・その他の参考事項等

2．重要な基本的注意（1）本剤の原材料となる血漿については、FDA で認可された方法で HBs 抗原、抗 HCV 抗体、抗 HIV-1 及び HIV-2 抗体陰性であることを確認し、かつ ALT（GPT）値でスクリーニングを実施している。さらに、プールした試験血漿については、HBV-DNA、HCV-RNA、HIV-1-RNA、HIV-2-RNA 及び HAV-RNA について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。同様に、パルボウイルス B19-DNA についてはプールした試験血漿で核酸増幅検査（NAT）を実施し、10^5IU/mL 以下であることを確認した健康人血漿を用いている。本剤の製造工程である、Cohn 低温エタノール分画法及び 60±0.5℃ 10 時間の液状加熱処理は、HIV をはじめとする各種ウイルスに対し、除去・不活化効果を有することが確認されているが、投与に際しては、次の点に十分注意すること。

血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。

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HEPATOLOGY

Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area

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Abstract

Aim: To address the issue of whether or not hepatitis E virus (HEV) is transmitted parenterally.

Methods: We conducted a retrospective study which involved 145 multiple transfused patients and 250 healthy controls. A prospective study was also undertaken involving 50 hospitalized patients, 25 of whom were transfused with 107 blood units, while the other 25 did not receive any transfusions.

Results: In our retrospective study, markers of acute HEV infection (IgM anti-HEV and HEV RNA) were detected in a significantly higher number of multiple transfused patients (13 of 145) compared to controls (two of 250) ($P < 0.001$; OR = 12.21 [95% confidence interval: 2.71–54.70]). All 13 HEV-infected patients had been transfused at least once in a 3-month period before testing. Overall, patients positive for any of the HEV markers (IgG, IgM or HEV RNA) had received more blood transfusions, had higher occurrence of icteric disease and higher serum alanine aminotransferase levels. In our prospective study, IgG anti-HEV was detected in 11 of 107 donor samples, three of 25 patients in their pre-transfusion samples (one sample was positive for IgM anti-HEV as well) and two of 25 control patients. Post-transfusion HEV infection developed in three of 22 susceptible (IgG anti-HEV negative) transfused patients; the infection was traced to their four respective donors who were asymptomatic, HEV RNA positive (4/4) and IgM anti-HEV positive (3/4). In contrast, none of the non-transfused patients developed HEV infection during the follow-up period.

Conclusion: Frequent transmission of HEV by blood transfusion places recipients at risk and warrants redefining of the donor screening policy by blood banks, especially in endemic areas.

Key words: blood donors, hepatitis E virus, hepatitis E virus RNA, parenteral transmission, seroprevalence.

INTRODUCTION

Hepatitis E is an enterically transmitted, self-limiting acute viral hepatitis, caused by an unclassified RNA virus, the hepatitis E virus (HEV). The disease causes large-scale epidemics of viral hepatitis in several developing countries and is the most common cause of acute sporadic hepatitis and fulminant hepatic failure in such countries.[1] Hepatitis E has been reported in developed countries as an imported disease to travelers to endemic areas and recently, as a cause of a small percentage of sporadic and fulminant hepatic failure in persons without history of travel to endemic areas.[2] Seroprevalence data reveal a global distribution of the infection.[1]

Hepatitis E has peculiar and as yet unexplained epidemiological characteristics, including repeated waves of large scale epidemics in the endemic areas, occurrence of disease in adult populations, and high incidence and severity of disease in pregnant women in whom the mortality rate reaches as high as 20%.[3] Recently, superinfection with HEV in chronic liver disease patients was shown to cause severe hepatic decompensation, leading to increased morbidity and mortality.[4]

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During epidemics of hepatitis E, the fecal-oral route is the predominant mode of transmission of the disease.[5] Following epidemics, secondary waves of hepatitis usually do not occur, suggesting that person-to-person transmission is not a major factor in the evolution of the outbreaks. During sporadic infections, secondary attack rates among household members are only 0.7–2.2%; in contrast, 50–75% of susceptible household contacts with hepatitis A are known to become infected.[1] Vertical transmission of HEV is known to occur.[6] Nosocomial spread of HEV to health care workers in a hospital setting has also been reported.[7]

Factors pointing towards the possibility of parenteral transmission of HEV are the presence of viremia during the prodromal phase of the disease,[8,9] substantial proportion of subclinical infections in endemic areas[1,10] and recent reports of the existence of symptom-free carriers of the virus.[11] The predilection of HEV for young adults, who are also eligible donors, further increases the risk of transmission of the virus through blood transfusion. Parenteral transmission of hepatitis A, another enterically transmitted disease, is known to occur.[12] Based on higher anti-HEV prevalence in at-risk patient populations, parenteral transmission of HEV has been suggested in some studies,[13–16] however, these data are preliminary and inconclusive and there are no prospective studies published to establish such a mode of transmission. In the present study we report on transfusion-associated HEV infections from an endemic area of hepatitis E.

METHODS

Retrospective study

Initially we compared the prevalence of markers of HEV infection (IgM anti-HEV, IgG anti-HEV and HEV RNA) in multiple (3 or more than 3) transfused patients and controls. From January 1993 to December 1994, serum samples were collected from 145 patients (males 88, females 57; mean ± 1 SD age: 30.7 ± 17.3 years, range 4–75 years) who had received multiple blood transfusions (mean ± 1 SD units of blood transfused per patient: 7.44 ± 10.3; range: 3–60) in the past. Indications for transfusions were hemophilia ($n = 29$), hypoplastic anemia ($n = 22$), gastrointestinal cancers ($n = 33$), chronic renal failure ($n = 19$), gastrointestinal bleeding ($n = 8$) and major surgical procedures ($n = 34$). In addition to blood transfusions, 29 hemophiliacs had received 696 pooled plasma preparations and 38 units of fresh frozen plasma were administered to patients in other groups to correct abnormal prothrombin time before invasive procedures. The median time period between the last units of blood transfused to serum collection was 17.4 (range 1–72) months. Thirty patients had been transfused at least one unit of blood in the 3-month period before serum collection. For each patient, we selected one or more age (± 5 years) and sex-matched relatives of the index case as controls. However, in 12 female patients we were not able to select age and sex-matched relatives as controls so age-matched spouses were used as control subjects. In all, 250 healthy subjects (males 202, females 48; mean ± 1 SD age 27.1 ± 20.4 years, range 2–76 years) formed the control group. All controls were asymptomatic and had never received blood transfusions in the past.

Prospective study

From December 1994 to November 1995, we studied 25 patients (17 males and 8 females; mean age 31.5 ± 16.4 years) who were transfused a total of 107 blood units (mean units per patient 4.3 ± 2.0, range 2–8 units) during the hospital stay. The indication for hospital admissions and transfusions was elective surgical procedures in seven and upper gastrointestinal bleeding in 18 patients. Patients had been transfused all blood units within a period of 48 h of a hospital stay. Sera from 107 voluntary donor units transfused to 25 patients were also available for study.

During the same period, another 25 hospitalized patients (15 males and 10 females; mean age 29.5 ± 15.9 years), who did not receive any transfusions, were included as controls. Patients were admitted for elective surgery ($n = 10$) and upper gastrointestinal bleeding ($n = 15$). The length of hospital stay in the transfused patients (mean ± 1 SD 4.4 ± 2.4 days) and those not transfused (3.8 ± 2.5 days) did not differ significantly ($P = 0.47$; 95% CI −0.75 to 1.95). All patients in the prospective study were consecutive patients who fulfilled the criteria for inclusion and were prospectively followed-up for 3 months following hospitalization. Sera from the patients in the study group were collected before transfusions and at 1, 2 and 3 months post-transfusion, and from the control group during the hospital stay and at 1, 2 and 3 months after discharge from the hospital.

During the period of the present study there was no epidemic of hepatitis E reported from anywhere in Kashmir, India. Informed consent was taken from each patient included in the study and the study protocol was approved by the Scientific and Ethical Committees of our institution.

Methods

Liver function tests (serum bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST]) and alkaline phosphatase [ALP]) were performed on all sera, including 107 donor sera. All sera were stored at −70°C and tested under code and in duplicate by enzyme immuno assays (EIA) for IgG and IgM antibodies to HEV by a kit using two recombinant HEV antigens corresponding to the structural region of the HEV (Diagnostic Biotechnology, Singapore). Sera were also tested for HEV RNA by polymerase chain reaction (PCR), as previously described.[3] Confirmed positive and negative controls were run with all PCR amplification reactions to ensure faithful amplifications. Strict application of containment measures was used to avoid false positives. Results of any PCR reactions were

considered valid only if they were consistent in at least two independent experiments, that also included a RNA extraction step.

Serology for hepatitis A virus (IgM anti-HAV), hepatitis B virus (HBsAg and IgM anti-HBc) and hepatitis C virus (anti-HCV second generation) was performed by EIA using commercially available kits from Abbott Laboratories (North Chicago, IL, USA). The assays were performed strictly according to manufacturer's instructions.

The criteria for the diagnosis of icteric hepatitis included clinically detectable icterus with serum bilirubin of 2.0 mg/dL or more and an increase in transaminases two and a half times above the upper limit of normal (serum AST 6–18 IU/L, serum ALT 3–26 IU/L). Anicteric hepatitis was diagnosed when serum aminotransferases were elevated two and a half times above the upper limit of normal with serum bilirubin within normal limits.

Statistical methods

Comparisons of categorical variables were analyzed using either Fisher's exact test when any of the expected values were less than 5 or the chi-squared test for all others. Comparisons of continuous variables were analyzed using Student's *t*-test for normally distributed variables and the Mann–Whitney *U*-test for non-normally distributed variables. Variables with skewed deviation (serum bilirubin, ALT, AST and ALP) were normalized using log transformation for analysis. Odds ratios were computed from the coefficients and their 95% confidence intervals were calculated. All values are expressed as mean ± 1 SD. A *P*-value of <0.05 was considered significant.[17,18]

RESULTS

Retrospective study

The prevalence of markers of HEV infection in multiple transfused patients and controls is shown in Table 1. Twenty-two transfused patients had HEV markers compared to 12 control subjects ($P < 0.001$). Acute HEV markers (IgM anti-HEV and HEV RNA) were seen only in the patient group which had received transfusions in the 3-month period before testing (recent transfusion), while IgG antibodies to HEV alone were detected among patients who were transfused more than 3 months before testing (remote transfusion). IgG antibodies to HEV were detected in nine (7.8%) out of 115 patients with remote transfusions compared to six (3%) of the 200 corresponding control subjects ($P = 0.05$). Acute markers of HEV infection were detected in 13 (43.3%) out of 30 patients with recent transfusions compared to one (2%) out of 50 corresponding controls ($P < 0.001$). Thirty patients with recent transfusions had received a significantly higher number of units of blood than those with remote transfusions (12.5 ± 6.5 *vs* 6.12 ± 4.2, respectively; $P < 0.001$).

Table 2 shows the characteristics of patients positive and negative for HEV markers. Patients positive for HEV markers had received more than 10 units of blood transfusions, had higher occurrence of icteric disease and had higher serum ALT levels. Only two of the eight patients with icteric disease were coinfected with hepatitis C virus infection. However, the two groups did not differ significantly from each other in age, sex, underlying disease, and number of blood transfusions per patient, serum bilirubin, and occurrence of coinfections with other hepatitis viruses.

Prospective study

Of the 107 donor samples tested, IgG anti-HEV alone was detected in 11 and HEV RNA in four (three of which were also IgM anti-HEV reactive) patients. Two of the 25 controls had IgG anti-HEV alone detected in the initial and follow-up samples. None of the remaining 23 non-transfused susceptible patients had HEV infection in the follow-up and had normal liver function tests. Among the study group, two patients had IgG anti-HEV alone in the pre-transfusion and follow-up samples. Another patient had IgM anti-HEV in the initial sample and IgG anti-HEV in the follow-up samples, suggesting that this patient had HEV infection before receiving blood transfusions. He did not develop symptoms of acute hepatitis and had normal serum ALT

Table 1 Hepatitis E virus (HEV) markers in multiple transfused patients and controls

Group	HEV markers	Transfused No./total (%)	Control No./total (%)	*P*-value	Odds ratio (95% CI)
All patients	Any	22/145 (15.2)	12/250 (4.8)	<0.001	3.55 (1.70–7.41)
	IgG anti-HEV alone	9/145 (6.2)	10/250 (4)	0.32	1.59 (0.63–4.00)
	IgM anti-HEV	13/145 (9)	2/250 (0.8)	<0.001	12.21 (2.71–54.70)
	HEV RNA	8/145 (5.5)	2/250 (0.8)	0.004	7.24 (1.52–34.52)
Recent transfusion†	IgM anti-HEV	13/30 (43.3)	1/50 (2.0)	<0.001	37.5 (2.18–183)
Remote transfusion‡	IgG anti-HEV	9/115 (7.8)	6/200 (3.0)	0.05	2.6 (1.09–7.30)

†Patients transfused in the 3-month period before testing; ‡patients transfused earlier than 3-months before testing. CI, confidence interval.

Table 2 Comparison of demographic, clinical and biochemical characteristics of anti-hepatitis E virus (HEV)-positive and anti-HEV-negative patients from the retrospective study

Characteristic	Anti-HEV positive	Anti-HEV negative	*P*-value	95% confidence interval
Number (%)	22 (15.2)	123 (84.7)	-	-
Age (years ± 1 SD)†	26.31 ± 18.46	31.58 ± 17.13	0.20	−2.6 to 13.1
Sex (%)	-	-	0.10	-
Male	10 (45.5)	78 (63.4)	-	-
Female	12 (54.5)	45 (36.6)	-	-
Underlying disease state (%)	-	-	0.64	-
Hemophiliac	5 (22.7)	24 (19.5)	-	-
Anemia	5 (22.7)	17 (13.8)	-	-
Cancer	4 (18.2)	29 (23.5)	-	-
Chronic renal failure	1 (4.6)	18 (14.6)	-	-
Gastrointestinal bleeding	2 (9.1)	6 (4.9)	-	-
Surgical procedure	5 (22.7)	29 (23.5)	-	-
Mean number of units transfused/ patient mean ± 1 SD (range)†	8.68 ± 11.33 (1–55)	7.22 + 8.39 (1–60)	0.40	−2.6 to 5.5
Number of units transfused/patient	-	-	0.02	-
<10 units	13	99	-	-
>10 units	9	24	-	-
Clinical disease (%)			<0.001	-
Icteric hepatitis	8 (36.4)	9 (7.3)	-	-
Anicteric hepatitis	8 (36.4)	11 (8.9)	-	-
Normal liver tests	6 (27.2)	103 (83.8)	-	-
Liver tests‡				
Serum bilirubin (mg/dL)	1.55 ± 1.29	1.15 ± 2.28	0.40	−0.6 to 1.4
Serum ALT (U/L)	138.27 ± 255.83	6.48 ± 129.54	0.03	7.2 to 148.4
Serum AST (U/L)	59.59 ± 47.23	47.31 ± 58.94	0.41	−13.8 to 38.3
Serum ALP (U/L)	497.38 ± 462.25	358.65 ± 329.65	0.09	−21.6 to 299.1
Co-infections (%)	5 (22.7)	22 (17.9)	0.72	-
Anti-HCV positive	5	15	-	-
HBsAg positive	0	9	-	-

†Mann–Whitney *U*-test was used as data were non-normally distributed; ‡Student's *t*-test was used after data were normalized using log transformation. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBsAg, ; HCV, hepatitis C virus.

levels. Twenty-two patients were susceptible to HEV before transfusions (IgG anti-HEV negative). Three (13.6%) of these 22 patients had evidence of HEV infection following transfusions (Table 3). None of these three patients had evidence of infection with other hepatitis viruses (HAV, HBV and HCV), cytomegalovirus or Epstein-Barr virus. The remaining 19 patients transfused showed normal liver function tests in the pre-transfusion and post-transfusion samples.

DISCUSSION

The present study addressed the issue of post-transfusion hepatitis E by determining the prevalence of HEV markers in multiple transfused patients and healthy controls in a retrospective study. A prospective study, involving following-up hospitalized patients receiving none or multiple transfusions for any evidence of HEV infection and then tracing the infections to their respective donors, was also conducted. Hepatitis E causes repeated episodes of massive water-borne epidemics in endemic areas and is the most common cause of acute sporadic hepatitis and acute liver failure in such countries.[1,19,20] However, antibody prevalence in healthy adults ranges from 4 to 20%, rendering the majority of the adult population in endemic areas susceptible to HEV infection.[10,19] In our retrospective study, markers of acute HEV infection (IgM anti-HEV and HEV RNA) were detected in a significantly large number of susceptible patients (43.3%, 13/30) with recent transfusions compared to corresponding susceptible healthy controls (2%). IgG antibodies to HEV were detected in only 7.8% (9/115) patients transfused in the remote past (> 3 months before testing). This seroprevalence was higher than in the corresponding control group (3%), yet was much lower than the prevalence of acute markers in the recently transfused patients. Hepatitis E infection was detected in only 13.6% (3/22) of susceptible patients in the prospective study. The higher rate of acute hepatitis E markers in the recently transfused patients was most likely related to the higher number of

Table 3 Donor and patient details of three transfused patients who developed post-transfusion hepatitis E in the prospective study

No.	Donor samples details	Patient details			Patient samples details			
		Age/sex	Indication for transfusion	Clinical status after transfusions	Pre-transfusion	1 month	2 months	3 months
1	Five units transfused, donors no symptoms, normal liver tests, one donor unit positive for IgM anti-HEV and HEV RNA positive	25 years/female	Mitral valve replacement	No symptoms	Normal liver tests, all HEV markers negative	Normal liver tests, IgG and IgM anti-HEV and HEV RNA reactive	Normal liver tests, IgM anti-HEV reactive, HEV RNA negative	Normal liver tests, IgG and IgM anti-HEV reactive, HEV RNA negative
2	Two units transfused, donors no symptoms, one donor sample with ALT 60 U/L and IgM anti-HEV and HEV RNA reactive	35 years/female	Splenectomy for hemolytic anemia	No symptoms	Normal liver tests, all HEV markers negative	Normal liver tests, all HEV markers negative	ALT 120 U/L, IgG and IgM anti-HEV and HEV RNA reactive	Normal liver tests, IgG and IgM anti-HEV reactive, HEV RNA negative
3	Seven units transfused, donors no symptoms, two samples HEV RNA reactive, one had ALT 60 U/L	45 years/male	Massive GI bleed	Icteric hepatitis at 2 months, recovered in 4 weeks	Normal liver tests, all HEV markers negative	Normal liver tests, all HEV markers negative	Serum bilirubin 3 mg/dL, ALT 607 U/L, IgM anti-HEV negative, HEV RNA negative	Normal liver tests, IgG anti-HEV reactive, IgM anti-HEV and HEV RNA negative

transfusions received by these patients than those in the remote transfusion and prospective groups. Long-term antibody status of antibodies to HEV may also partly explain the low prevalence of antibodies in patients with remote transfusions. In an earlier study we have shown that antibodies disappear in a significant proportion of the population with time.[20] Antibodies were detected in only 47% (21/47) patients infected with HEV 14 years earlier.

Hepatitis E virus infection is a self-limiting acute viral hepatitis and commonly occurs among young adults who are also eligible blood donors. Viremia is detected in the late incubation period and for at least 2 weeks after onset of illness.[8,9,22] A subgroup of patients has biphasic enzyme elevation, prolonged fecal shedding and viremia.[23] Majority of infections in humans and experimental animals are subclinical and a substantial proportion of infections occur in the absence of elevated serum enzymes.[1,24] Thus asymptomatic viremia may occur in healthy adults in endemic areas. Four of the 107 voluntary donors and two of the 250 healthy controls in the present study had viremia and were asymptomatic. Viremia and fecal shedding has also been reported in symptom-free carriers in another study.[11] In the present study we found that HEV infection developed in three of 22 susceptible patients following blood transfusions. The infections were traced to infected donor samples and occurred within the incubation period of HEV infection. In contrast, susceptible patients who were hospitalized and not transfused had no evidence of HEV infection. A number of studies from endemic regions have revealed high prevalence of HEV antibodies in post-transfusion patients, hemophiliacs, renal dialysis patients and chronic HCV patients.[13–16] High prevalence of antibodies in patients with HCV infection may point to a common route of acquiring both infections.

Hepatitis E virus infection is spread through contaminated water, causing large-scale epidemics in endemic areas.[5] Vertical transmission commonly occurs from mother to fetus[6] and human infections may have a zoonotic origin.[25] Person-to-person transmission of infection is uncommon and the mode of spread of sporadic infections is not known. In the absence of virus inactivating treatment, HEV containing blood or blood products collected from asymptomatic donors during the prodromal phase or with asymptomatic infection has the potential to transmit HEV infection. Infectivity experiments conducted in cynomolgus macaques confirmed the viability and transmission potential of the virus excreted by animals with subclinical HEV infection.[24] The results of the present study provide sufficient evidence that HEV can be transmitted by viremic blood units and may explain one of the possible modes of transmission of sporadic HEV infections in endemic areas. Further evidence of transmission of HEV through transfusions needs sequence analysis of the HEV genome of the donor and the recipient.

Sporadic HEV infections exist in the community in endemic areas and cause subclinical infections. Infection might be transmitted from one person to another through a number of parenteral routes. The practice of reusing unsterile needles, syringes and other appliances for drug and intravenous therapy, tattooing, nose and ear piercing and circumcision are common practices in such regions. Transfusion of HEV viremic blood may be particularly devastating for patients with underlying chronic liver diseases, as it was recently reported that superinfection with HEV in such patients can cause severe hepatic decompensation leading to increased morbidity and mortality.[4]

In summary, HEV RNA contaminated blood donations present a challenge to virus safety of blood and its products in endemic areas and has important implications on blood transfusion practices in such regions. Further prospective data need to be collected in endemic areas to define the magnitude of transfusion-associated HEV infections. Such data will help blood banks in these countries to define policy for donor screening for HEV.

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