Seroprevalence of hepatitis E virus (HEV) infection in blood donors and renal transplant recipients: a retrospective study from central Italy

Sieroprevalenza dell’infezione da virus dell’epatite E (HEV) in donatori di sangue e pazienti trapiantati di rene: uno studio retrospettivo dal centro Italia

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INTRODUCTION

Our knowledge about HEV infection has been dramatically changed over the last years [1-3]. The virus is now well recognized as cause of infection, also in developed countries and its diffusion is largely underestimated [1, 4]. Differently to other areas the spread of the virus in Europe is through a zoonosis involving different genotypes: a documented risk factor is the consumption of undercooked pork [1, 4, 5]. The infection, usually asymptomatic, may cause not only acute hepatitis, but also persistent infection with progressive liver disease in immunocompromised patients [4-6]. Although rare, transmission through blood products or solid organ transplantation has been documented [1, 4, 7]. To date, there are few epidemiological data on the virus circulation in Italy; for this reason, we conducted a study on HEV diffusion in our area.

MATERIALS AND METHODS

We conducted a retrospective study on HEV seroprevalence using 250 stored serum samples collected, after providing informed consent, at the University Hospital of Siena during 2007-2013. Blood samples were obtained from 132 randomly selected blood donors living in Tuscany (Arezzo, Siena and Florence) (M/F=2.3; mean age 43.29, range 19-66 years), who attended the Transfusion Center of the University Hospital of Siena, during June-December 2007; 118 blood samples were also collected from consecutive patients mainly from central Italy (M/F=2.25; mean age 51.35; range 18-72 years) at the time of renal transplantation (T0) during February 2011-February 2013 at the Transplant Center of the same Hospital. Clinical and demographic data were obtained through analysis of donor records and medical records for
transplant recipients. All frozen (-20°C) samples were tested for IgG anti-HEV using a commercial IgG ELISA test (EIAgen HEV IgG kit, Adaltis, Italy). This assay uses synthetic antigens encoding for conservative and immunodominant determinants ORF2 and ORF3 common to the four genotypes of HEV. The specificity and the sensitivity are declared to be both 100%. The results were interpreted according to the manufacturer’s instructions.

For transplant patients were also available blood samples at 1 and 3 months after transplantation.

Statistical analysis
The Chi-square test was used to compare categorical variables, continuous variables were compared for independent samples by ANOVA and Bonferroni test. P values <0.05 were considered significant. Data were analysed by Prism, Version 6.01, GraphPad Software, La Jolla, California, USA.

■ RESULTS

Anti-HEV IgG was positive in 12/132 (9.1%) of the blood donors and 12/118 (10.2%) of the transplant recipients. The overall anti-HEV IgG seroprevalence was 9.6% (n=24/250, M/F=1.67, median age 57, range 26-72 years) (Table 1). All subjects but one were autochthonous and none showed signs of liver disease at the time of sampling. It has been documented a significant association between mean age of patients and the positive serology against HEV especially in the group of blood donors (Table 1). No other studied variable was associated with HEV infection. In 11 of the 12 transplant patients it was possible to analyze blood samples after transplantation; in 36% of cases (n=4/11) the presence of anti-HEV was no longer documented after 1-3 months.

■ DISCUSSION

Hepatitis E virus (HEV) is endemic in many parts of the developing world and globally it is the commonest cause of acute viral hepatitis [1-5, 8]. The virus was not initially thought to occur in developed countries, but recent reports have shown otherwise [4]. Autochthonous HEV is now considered an emerging health issue in developed countries and is thought to be a porcine zoonosis [4, 5]. Molecular characterization of various HEV strains circulating among human beings and animals has led to the recognition of four major genotypes; while HEV 1 and HEV2 are transmitted via contaminated water in developing countries, HEV3 and HEV4 infect also animal species and are responsible for sporadic cases of autochthonous hepatitis E in both developing and developed countries [4, 5]. Although HEV3 and HEV4 infections have been linked to the consumption of raw or undercooked pork or game meat, the full range of species that are reservoirs for HEV is still unknown [4]. An increasing number of studies are confirming the spread of the virus in Europe, especially in the Mediterranean basin [1, 4, 5, 9]. The true prevalence is believed still strongly underestimated due to low sensitivity of tests used and because the infection is often asymptomatic [4, 5]. Overt HEV infection is much more common in middle-aged and elderly men, probably related to host factors [1, 4]. There is little data on the diffusion of HEV infection in Italy. Two recently large studies conducted in South Italy showed a higher circulation of HEV in immigrants and Italian haemodialysis patients, whereas a low prevalence in the remaining Italian population and transplant recipients [9,10]. Most explored is the spread of the virus in the animal reservoir, particularly in domestic pigs and wild boars [11-13]. Our study, although retrospective, confirmed the spread of the virus in central Italy in two populations of native subjects evaluated in two different periods. The HEV seroprevalence documented (9.6%) is similar to that of other studies and it is interesting to note that it appears the same in the two different populations of subjects (healthy donors versus

| Table 1 - Correlation between median age of studied populations and serologic positivity for HEV infection. |
|-------------------------------|---------------------------------|-----------------|-----------------|
| All patients (n=250) | Positive, n=24 | 57 (26-72) | 0.008 |
| Negative, n=226 | 47 (18-70) | |
| Transplant (n=118) | Positive, n=12 | 57.5 (33-72) | 0.447 |
| Negative, n=106 | 52 (18-70) | |
| Blood donors (n=132) | Positive, n=12 | 57 (26-66) | 0.043 |
| Negative, n=120 | 43 (19-65) | |
patients with end-stage renal disease undergoing transplantation), suggesting a common exposure, probably related to dietary habits of this area [9]. Some authors report a greater spread of the virus in haemodialysis patients even if the reasons are not yet clear [5, 9]. The choice of different serological tests significantly affects the prevalence of the infection, since the performance varies greatly; the ELISA test that we used showed in other similar works good sensitivity, however, lower than other methods as Wantai test, especially during acute infection or in immunosuppressed patients [5, 14, 15]. Similar to other studies in our population we observed a relation between age and positivity for HEV antibodies [4, 5, 9]. HEV transmission is thought to be largely via the faeco-oral route, although there are several reports of parenteral transmission via blood transfusion [4, 5, 16]. Since some studies have shown the presence of HEV-RNA in blood donors, the transfusion safety is under evaluation even considering that it is not currently provided a screening test and many infections are asymptomatic [4, 5, 16, 17]. The test used does not allow a distinction between IgM and IgG and therefore we could not demonstrate any acute infections although HEV positive subjects did not show signs of hepatic impairment. The possibility of chronic HEV infection in immunosuppressed patients appears more disturbing; most cases have occurred in solid-organ transplant recipients; several patients fail to clear the virus, and showed rapid progression to cirrhosis [4, 6, 7, 18]. Numerous studies have documented even in Europe acute HEV infection in transplant patients with subsequent chronic liver damage; extra-hepatic manifestations involving the central nervous system or the kidney are also described [9, 16]. In this sense, this paper confirms that patients undergoing transplantation are exposed to the virus, although we have not documented in the subsequent follow up acute or chronic hepatitis and serology may lose effectiveness over time because of immunosuppression. Therefore it is recommended that in transplant patients should be made counseling to avoid risks related to infection, pointing out that screening of the virus may be ineffective with serology in the post transplant period. In conclusion, our study, although limited and retrospective, confirms the circulation of autochthonous HEV in central Italy in different periods; the presence of antibodies against HEV in particular categories of persons as blood donors and transplant patients, which are not screened for the infection, raises questions in terms of transfusion safety and health protection of immunocompromised patients.

**Keywords:** HEV, Italy, blood donors, transplant recipients, autochthonous.

### SUMMARY

Autochthonous hepatitis E virus (HEV) is an emerging health issue in developed countries and is thought to be a porcine zoonosis; its spread is underestimated and there is concern about the possibility of chronic infection in immunosuppressed patients; HEV transmission through blood has also been demonstrated. We conducted a retrospective study (2007-2013) on HEV seroprevalence using stored serum samples from 132 blood donors and 118 renal transplant recipients living mainly in central Italy. Anti-HEV IgG was positive in 12/132 (9.1%) of the blood donors and 12/118 (10.2%) of the transplant recipients. All subjects but one were autochthonous and none showed signs of liver disease at the time of sampling. A significant association was documented between mean age of patients and the serology against HEV especially in the group of blood donors. Our study, albeit limited and retrospective, confirms the circulation of autochthonous HEV in central Italy; the presence of antibodies against HEV in particular categories of persons such as blood donors and transplant patients, who are not screened for the infection, raises questions in terms of transfusion safety and health protection of immunocompromised patients.
RIASSUNTO

L’infezione autoctona da virus dell’epatite E (HEV), sostenuta da una zoonosi porcina, rappresenta un problema emergente di salute pubblica nei paesi industrializzati; la sua diffusione è ampiamente sottostimata e vi sono motivi di preoccupazione sia per la possibilità di cronizzazione dell’infezione nei pazienti immunodepressi, sia per la documentata trasmissione anche attraverso le donazioni di sangue.

Abbiamo condotto uno studio retrospettivo (2007-2013) sulla sieroprevalenza di HEV utilizzando campioni di sangue conservati, appartenenti a 132 donatori di sangue e 118 pazienti trapiantati di rene, residenti prevalentemente nel centro Italia. Anticorpi IgG anti-HEV sono risultati positivi in 12/132 (9,1%) donatori di sangue e 12/118 (10,2%) trapiantati di rene. Tutti i soggetti, tranne uno, erano autoctoni e nessuno presentava segni di malattia epatica al momento del prelievo.

È stata documentata un’associazione significativa tra positività sierologica per HEV ed età media dei pazienti, in particolare nel gruppo dei donatori di sangue. Il nostro studio, seppur limitato e retrospettivo, conferma la circolazione autoctona di HEV nell’Italia centrale; la presenza di anticorpi anti-HEV in particolari categorie di soggetti come donatori di sangue e pazienti trapiantati, che non sono sottoposti a screening per l’infezione, suscita domande in termini di sicurezza trasfusionale e tutela della salute dei pazienti immunodepressi.

REFERENCES