Seroprevalence of HEV antibodies in a sample of pregnant women in the city of Messina

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Parole chiave: HEV, gravidanza, sieroprevalenza, prevenzione

Abstract

Background. Hepatitis E virus (HEV) is widespread in developing countries and the disease is also increasing in the developed ones. This infection in pregnancy can cause spontaneous abortion and neonatal death in 56% of newborns.

Study design. The study was conducted on a sample of 352 pregnant women, 326 Italian and 26 foreign, in order to confirm the presence of HEV in our territory, to analyze wrong habits of the population and to suggest preventive actions against the risk to contract the infection during pregnancy.

Methods. We asked all women under study to fill an anonymous questionnaire immediately before taking a venous blood samples to determine the presence of anti-HEV antibodies. The questionnaire contained a set of questions to gain information about lifestyles and risk factors to contract HEV.

Results. The questionnaire revealed that a portion of the tested women have risk behaviours, as consumption of raw or undercooked food, eating unwashed food and traveling to endemic areas. The percentage of women positive for HEV antibodies was 3.4%, in agreement with national data; all the women were Italian.

Conclusions. This study confirms the circulation of HEV in the city of Messina. For this reason, it is highly recommended to disseminate hygienic and appropriate behaviours and feeding habits in order to prevent the risk to contract the infection.

Introduction

Despite the remarkable results achieved in the last few decades, infections caused by hepatitides viruses are one of the major causes of acute hepatitis worldwide and still constitute one of the greatest public health problems (1, 2). Based on WHO figures, around 20 million new cases of HEV infections take place every year worldwide with over three million cases of acute hepatitis and 56,600 fatalities (3, 4). This disease is widespread in the rural communities of Egypt, in the Middle East, in Central America and, above all, in the Indian Subcontinent (5). In Italy, records of acute HEV cases have been kept since 2007 via the system SEIEVA: 145 cases of Hepatitis E were reported between 2007 and 2014. The epidemiological branch (Epicentro) of the Italian National Institute of Health has produced estimates of the percentage of positive HEV antibodies for different parts of Italy as follows: 1-3% in Northern and Central section and 3-6% in

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the South and the islands (6). A study on samples of wastewater from treatment plants of Messina University Hospital and Messina City Council found positive rates of 8.33% on samples entering the Hospital wastewater treatment plant and 4.5% on samples of the City Council plant (7). In Europe, figures relating to seropositivity vary widely, ranging from 0.26% in Greece (8) to 52.5% in France (9).

HEV is a RNA virus with four genotypes. Genotypes 1 and 2 are transmitted via the fecal-oral route but also via vertical transmission, especially the Genotype 1 (10); furthermore, there is a risk of transmission through blood transfusions (11, 12). These viruses are endemic mostly in countries with poor sanitation (Africa, Asia and South America); however, cases of hepatitis E in people with no history of recent travel to endemic countries have been reported in developed regions such as North America, Europe, Japan, New Zealand, and Australia (13). Genotypes 1 and 2 infect only humans and are often associated with waterborne outbreaks (14). Genotypes 3 and 4 are considered zoonotic strains because they infect both humans and many animal species (e.g. pig, deer, wild boar, and rabbit), and cause sporadic cases in developed countries (15); pigs and, less frequently, other animal species (wild boar, deer) are considered reservoir of these viruses (16). The first genotype found in animals was the genotype 3 that has been detected in pigs since the mid ‘90s (17). Recently, also the genotype 4 has been detected in Europe in both pigs and humans (18, 19).

Infection rates in endemic regions are closely linked to the level of economic development, access to drinking water and facilities for hygiene and sanitation (20). The main sources of infection are drinking water contaminated by infected waste water and consumption of raw or undercooked shellfish. Bivalve mollusks are a significant risk factor, being marine organisms capable of concentrating, via filtration, toxins and microorganisms, i.e. bacteria, viruses and parasites. A three-year study by Le Guyader (2000) demonstrated that mollusks represent an important vehicle of transmission of hepatitis viruses (21). It is therefore advisable to avoid eating raw or undercooked shellfish (22, 23) or to cook them at high temperatures (71° for 20 minutes), ensuring that the heat reaches the body of the shellfish in order to kill the HEV (24).

Although HEV is the major cause of acute hepatitis in developing countries, the disease is also increasing in developed countries, where cases have been reported not only in subjects who have travelled to endemic regions but also in non-travelers (25-27). In France, this disease is currently regarded as emergent (9). The infection seriously affects pregnant women, particularly infections caused by genotype 1 (28, 29), with reported death to case rates of 15-25% (30), occurring above all during the second or third trimester (31, 32). This is probably due to immunological changes (suppression of cell-mediated immunity) induced by hormones produced during pregnancy (33). The vertical transmission rate is reported to be 50% (34, 35). A study carried out in India, where the disease is endemic, showed HEV infection in pregnancy to be linked to spontaneous abortion, stillbirth and neonatal death in 56% of newborns (34). Another recent study (36) highlighted HEV infection to be the likely cause of 2,400-3,000 stillbirths annually in developing countries (29).

The aim of this study was to assess the seroprevalence of total antibodies against the HEV in a sample of pregnant women, native Italian and foreign, resident in the Italian city of Messina and surrounding province, to confirm the circulation of the virus in our territory, to verify the immunological status against this virus during pregnancy and the risk to contract the infection.
Materials and methods

The study was conducted at the University Hospital of Messina during a period of four months (September-December 2015) and included a sample of 352 pregnant women, 326 Italian and 26 foreign; 54% of the latter were from endemic areas (Sri-Lanka), while the remainder came from a range of different countries. All the foreign women were in Italy for at least five years. The pregnant women attended the hospital to carry out the common pregnancy screening. A questionnaire was completed immediately before blood samples were taken to detect HEV antibodies, after signing an informed consent.

The questionnaire included personal details (full name, date and place of birth), a first section regarding vaccination records (Hepatitis B, polio, tetanus, diphtheria, MMR, HPV, tetravalent meningococcaus) and a second about risk factors for HEV infection during the last two years, such as eating habits (raw/undercooked food, especially the eating of meat and mussels) and information about trips to endemic areas. Samples were taken during the first or second trimester of pregnancy. The serological tests were conducted using 5 mL venous blood samples centrifuged at 4000 rpm for 10 min. The sera obtained were assayed using a third generation ELISA to determine total HEV antibodies (DIA. PRO) by using microplates coated with HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from Mexican and Burma viral strains. (37).

Results

The 352 tested women were grouped according to country of origin - Italy or abroad - by age, trimester and number of pregnancies (Tab. 1).

Analysis of the replies to the questionnaire revealed that the two groups had similar habits, regarding consumption of raw/undercooked food and the eating of unwashed food. Moreover, 10% of the Italian women stated they had travelled to endemic regions in the last 2 years and, of these, 67% had eaten food with a potential risk of HEV transmission. The percentages of all risk factors in tested women are shown in Fig. 1, for only Italian women in Fig. 1a and for only foreigner women in Fig. 1b.

The second stage of the study revealed the prevalence of HEV antibodies to be 3.4%. All the positive subjects were Italian pregnant women, while none of the women from overseas origin tested positive, despite many of them come from endemic areas. The women testing positive were all in their second trimester; they had been vaccinated in compliance with the Italian vaccination schedule and all were resident in the coastal areas of the city and province; 33% of them were from coastal areas of Messina while 66.6% were from coastal areas of the province.

<table>
<thead>
<tr>
<th>Age</th>
<th>Trimester of pregnancy</th>
<th>Number of pregnancy</th>
</tr>
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<tbody>
<tr>
<td>18-25</td>
<td>26-30</td>
<td>31-35</td>
</tr>
<tr>
<td>Italian</td>
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<td>Second</td>
</tr>
<tr>
<td>17%</td>
<td>31%</td>
<td>36%</td>
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<tr>
<td>(56)</td>
<td>(101)</td>
<td>(117)</td>
</tr>
<tr>
<td>Foreign</td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>31%</td>
<td>39%</td>
<td>23%</td>
</tr>
<tr>
<td>(8)</td>
<td>(10)</td>
<td>(6)</td>
</tr>
</tbody>
</table>
Fig. 1 - The figure shows the percentages of exposure to risk factors in tested women.

Fig. 1a. The figure shows the percentages of exposure to risk factors in Italian women

Fig. 1b. The figure shows the percentages of exposure to risk factors in foreign women
Fig. 2 shows the percentages regarding dietary habits and trips of subjects who tested positive, these being the major risk factors according to Seieva (Integrated Epidemiological System of Acute Viral Hepatitis) (6).

Discussion and conclusions

Although the responses given to the questionnaire regarding dietary habits did not reveal any notable differences between the two groups, serological positivity emerged only in the group of native Italian women. Many of positive women reported exposure related to risk factors, including consumption of raw or undercooked food, particularly shellfish (mussels) and unbottled drinking water, and also they reported having travelled to endemic areas. The serological tests conducted showed a rate of 3.4% testing positive for HEV, in line with that reported for the nation as a whole (3-6%) (6). The highest percentage of seropositive subjects were in the age group 26-35 years.

This result confirms previous studies showing that the virus is circulating in the province of Messina (7, 38). Our territory is a coastal region where raw mussels are eaten in abundance. This habit could be one of the causes of the circulation of the virus. Therefore, it is important to suggest correct hygienic behaviours to women to avoid the risk of contracting the infection: a correct hand hygiene, particularly after touching raw food and/or surfaces in contact with them, always eating well-cooked and washed food, drinking bottled water should the piped water be declared unsafe and avoiding risky behaviours during travel in endemic areas.

HEV is still a little-known or underestimated infection, but is nevertheless an emergent disease in industrialized countries. Both the US Center for Disease Control and Prevention as well as the WHO have turned their attention to this disease, in the hope that a realistic worldwide epidemiological profile may soon be drawn up. In December 2011, a vaccine was approved only in China, the first against this virus (39) and this currently constitutes there one of the major means of prevention.
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Ethical Approval. All patients gave an informed consent.
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Competing Interests. None declared.

Riassunto
Sieroprevalenza di anticorpi anti-hev in un campione di donne in gravidanza nella città di Messina

Background. Il virus dell’Epatite E (HEV) è molto diffuso nei paesi in via di sviluppo e la malattia è in aumento nelle aree industrializzate. Questa infezione può essere particolarmente grave in gravidanza poiché è in grado di causare aborto spontaneo e morte nel 56% dei neonati.

Disegno dello studio. Lo studio è stato condotto su un campione di 352 donne in gravidanza, 326 italiane e 26 straniere, per confermare la diffusione di HEV nel nostro territorio, analizzare i comportamenti errati e prevenire il rischio di contrarre l’infezione durante questo periodo.

Metodi. A tutte le donne in studio è stato preventivamente somministrato un questionario anonimo a cui ha fatto seguito un prelievo ematico per la ricerca degli anticorpi anti-HEV. Il questionario comprendeva una serie di domande su stili di vita in relazione ai potenziali fattori di rischio che potrebbero causare infezione da HEV.

Risultati. Il questionario ha rivelato che una parte delle donne presentava fattori di rischio comportamentali quali il consumo di alimenti crudi o poco cotti, il consumo di cibi non lavati e precedenti viaggi in zone endemiche. La percentuale di donne positive per gli anticorpi anti-HEV è stata del 3,4%, in linea con i dati nazionali; tutte le donne risultate positive erano italiane.

Conclusioni. Questo studio riconferma la circolazione del virus nella città di Messina. È, quindi, necessario consigliare l’adozione di idonee misure igieniche e adeguati comportamenti alimentari per ridurre il rischio di contrarre questa infezione.

References


