IN THE NAME OF GOD

Serum levels of soluble CD26, a novel prognostic marker for acute hepatitis E infection

Alireza Rafiei, Araz Mohammad Mirabi, Mohammad Jafar Safar, Abulghasem Ajami

Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
Introduction

- Hepatitis E virus (HEV) is the major cause of acute viral hepatitis in developing countries.

- HEV virions are small, spherical, non-enveloped, positive-sense single-stranded RNA viruses belong to the genus hepevirus of the family hepeviridae.

- It transmits predominantly through the fecal-oral route, which is profoundly responsible for epidemics in tropical and subtropical countries.
Introduction

- Hepatitis E usually causes self-limiting disease with low mortality; however, acute liver failure, chronic infection, or extrahepatic symptoms are possible.
- The infection is rarely progressed into the severe form, this can occur during pregnancy and in immunosuppressive conditions such as solid organ transplantation.
- Both humoral and cellular immune responses, can play a role in the pathogenesis of HEV infection.
- Both IgM and IgG antibodies appear at the time of onset of illness such as jaundice and persist for variable periods of time.
Introduction

- Although hepatitis E is associated with a robust antibody response and this is the basis of HEV vaccine designing strategy, cellular immune responses to HEV proteins develop in HEV infected patients.

- CD30 is a member of the tumor necrosis factor (TNF) family, and appears to be preferentially expressed and released by human CD4+ and CD8+ T cells.
Introduction

- CD26 is a cell surface glycoprotein with dipeptidyl peptidase IV (DPP IV) enzyme activity in the extracellular domain, correlating with production of Th1-like cytokines in several diseases.

- After cellular activation, the soluble forms of CD30 (sCD30) and CD26 (sCD26) release into the bloodstream. Therefore, analysis of CD26 and CD30 and their soluble forms has been proposed to be useful in discriminating Th1 and Th2 responses.
Materials and Methods

Study population

Serological Enzyme Immunoassays

Statistical analysis
Study population

- 35 anti-HEV IgM positive patients and 35 age and sex matched anti-HEV negative controls.

- patients presenting clinical signs of hepatitis such as icterus, dark-colored urine, elevated alanine aminotransferase (ALT) and bilirubin levels in the serum, bile salts and pigments in the urine.

- control group had the same epidemiological conditions as patients, and had no history of an illness resembling viral hepatitis indicating that they had been exposed to HEV in the past

- Individuals with acute infection, pregnancy, receiving blood or blood products within the last 3 months and chronic renal and liver diseases were excluded
5 ml blood sample was drawn and serum was obtained by centrifugation at 1450g for 15 minutes at 4 ºC and stored at –80 ºC until further processing.

Serum levels of CD26 and CD30, IFN-γ, and IL-4 were detected using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits with paired cytokine-specific monoclonal antibodies according to the manufacturer’s recommended procedure.

The detection limit for CD26 and CD30 were 7.26 pg/ml and 1.9 U/ml, respectively.
Analysis of significance was performed for comparison differences between HEV positive and non HEV-positive subjects using Mann-Whitney U test and independent t-test.

Relationships between clinical variables and levels of soluble markers were evaluated by determining the Pearson correlation coefficient. A p_value < 0.05 was considered to be significant.

Linear regression analyses were carried out to determine a possible correlation between values of sCD30 and sCD26 and several biological markers such as IFN-γ and IL-4.
Results

Table 1. Basic characteristics of acute HEV infected patients and HEV negative controls.

<table>
<thead>
<tr>
<th></th>
<th>Acute HEV patients (n=35)</th>
<th>Controls (n=35)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (±SD)</td>
<td>19.7 ± 3.3</td>
<td>18.4 ± 3.2</td>
<td>0.119</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>17/18</td>
<td>14/21</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban area</td>
<td>14 (40)</td>
<td>16 (45.7)</td>
<td>0.8</td>
</tr>
<tr>
<td>Rural area</td>
<td>21 (60)</td>
<td>19 (54.3)</td>
<td></td>
</tr>
</tbody>
</table>

There was no significant differences between patients with acute HEV infection and HEV negative controls with respect to age and gender (p=0.119 and p=0.6, respectively).
Table 2. Comparison of cytokines and sCD26 and sCD30 in acute hepatitis E patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Anti-HEV positive (n=35)</th>
<th>Anti-HEV negative (n=35)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>23.7 (6.3-73)</td>
<td>7.2 (4.3-48)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>7.1 (2.3-17.2)</td>
<td>5.5 (2.1-24)</td>
<td>0.354</td>
</tr>
<tr>
<td>sCD26 (pg/ml)</td>
<td>248 (104-536)</td>
<td>91 (16-324)</td>
<td>0.001</td>
</tr>
<tr>
<td>sCD30 (U/ml)</td>
<td>48.0 (12.8-116.25)</td>
<td>40.9 (5-99)</td>
<td>0.159</td>
</tr>
<tr>
<td>sCD26/sCD30</td>
<td>5.17 (1.6-30)</td>
<td>2.22 (0.28-10.8)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note. Data are represented as median (range). All statistical comparisons were determined by the Mann-Whitney U test.
Most of the patients exhibit higher sCD26 serum levels (104-536 pg/ml) than the controls (16-324 Pg/ml) which gained significant difference (p=0.001)

The median serum levels of sCD30 was 48 and 40.9 U/ml in patients with HEV infection and HEV negative subjects and not significant(p=0.159)

IFN-γ production was higher in acute HEV patients than HEV negative controls (23.7 vs. 7.2 pg/ml, p<0.0001)

Acute HEV patients produced more serum IL-4 than controls differences were not statistical significance (7.1 vs. 5.5 U/ml, p=0.354).
Association between sCD26 and IFN-γ in sera from patients with acute HEV infection

- a positive correlation found only between the serum levels of sCD26 and IFN-γ in acute HEV patients (r=0.64, p=0.001)
Association between sCD30 and IL-4 in sera from patients with acute HEV infection

✓ Levels of sCD30 did not have any significant correlation with serum levels of IL-4 (r=0.25, p=0.37)
Discussion

- Evaluation of correlation between serum levels of sCD26 and sCD30 corresponding to Th1 and Th2-type immune responses in acute HEV infected patients in a HEV endemic area.

- Increased serum levels of IFN-γ and sCD26 in acute HEV patients compared to HEV negative controls.

- T cell activation occurs in acute HEV infected patients compared to controls as shown by an increased in IFN-γ production in acute HEV patients.

- T cell–mediated immune responses play a critical role in eliminating viral infections and virus-infected cells.
IFN-γ, a key cytokine of Th1-type immune response, plays an important role in protective immune response against viral infections.

- It increases antigen presentation by antigen-presenting cells and to enhance MHC class I.

- It enhances antiviral state by increasing cytotoxic activity of NK, NKT, and CD8 T cells on virus-infected cells.

- It inhibits development of Th2 type immune response by decrease production IL-4.
Th1-type response correlated with the hepatic inflammatory activity of viral hepatitis, which favors clearance of virus, while Th2 cells may be associated with the persistence of those infection.

Increased the levels of IFN-γ in acute hepatitis E infected patients compared to the controls might contribute to the successful clearance of the virus.

Presence of a direct close correlation between serum levels of sCD26 and IFN-γ in acute HEV patients suggests that sCD26 might be a surrogate indicator of Th1 immune response and a valuable factor for predicting the disease progression.
THANKS FOR YOUR ATTENTION