Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University. Microbiology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the research across the whole spectrum of the subject. These including bacteriology, virology, mycology and parasitology. In addition, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem.....etc

www.eajbs.eg.net
Prevalence of Epstein - Barr virus infection in Hepatitis C Patients

Hussam Ghanem¹, Sahar Shoman¹, Mohamed Nabil¹, Ashraf Tabl²
1- Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt.
2- Department of Biomedical Technology, National Research Center, Giza, Egypt.

ARTICLE INFO

Article History
Received: 5/1/2014
Accepted:21/3/2014

Keywords:
Epstein - Barr virus (EBV)
Hepatitis C virus

ABSTRACT

Background: Epstein - Barr virus (EBV) may be an omnipresent, common infective agent of severe illness in patients with impaired immune functions. Reactivation of Epstein-Barr virus in immunocompetent host is typically symptomless, however could deteriorate the prognosis of patient with chronic illness.

Objectives: This study was conducted to detect EBV infection in patients with chronic hepatitis C virus (HCV) infections and to point out the effects of EBV-HCV co-infections on liver enzymes activity.

Study design: Expression of EBV-DNA was determined in Serum samples by nested polymerase chain reaction (nested-PCR) method. There were 79 chronic HCV within the study group. Control group consisted of 52 cases without viral hepatitis.

Results: EBV-DNA infection was demonstrated in 29% of chronic HCV patients. Although alanine aminotransferase (ALT) and (AST) levels of EBV-infected HCV patients were increased.

Conclusion: We conclude that EBV infection is common in chronic HCV patients, who can be regarded as patients at high risk for EBV disease.

INTRODUCTION

Epstein-Barr virus (EBV) is a ubiquitous, worldwide pathogen that is harbored persistently by virtually all adults, regardless of geographic location (Anne, 2005). EBV is a member of the Gammaherpesvirinae subfamily of herpes viruses. It infects more than 90% of the adult population all over the world (Henry et al., 2013). EBV shares the tendency of establishing latency in the host with other herpes viruses (Petrova et al., 2010). Primary infection leads to transitional viremia, followed by a strong T-cell adaptive immune response, which actually holds the infection latent in immunocompetent individuals (Cohen et al., 2009). Short episodes of spontaneous reactivation and consequent viral replication normally occur in healthy individuals (Paschale et al., 2012).
In the immunocompetent individual the occurrence of EBV reactivation leading to immortalization of B-lymphocytes is strongly regulated by cytotoxic T lymphocytes (CTLs) specific for lytic and latent antigens (Petrova et al., 2010). The role of EBV in the evolution of chronic hepatitis from hepatotropic viruses is considered. Chronic EBV associated hepatitis is suspected in immunocompetent adults with compatible serology, suggestive histology and detection of the viral genome in the liver and/or increase of specific circulating cytotoxic T-lymphocytes (Dejcinov et al., 2011). Co-infections with EBV or CMV in patients infected with HCV have been proven to accelerate the course of chronic hepatitis C thus leading to a more severe histological picture and facilitating the disease progression to fibrosis, cirrhosis and hepatocellular carcinoma (Julio et al., 2007).

The present study aimed to investigate the incidence of co-infection of Epstein - Barr virus (EBV) in sera samples from patients (cases with positive HCV infection) and controls (cases with negative HCV infection). Study the effect of EBV pathogenicity on the changes in the liver functions (like liver enzymatic activity of ALT and AST).

**MATERIALS AND METHODS**

**Study population**

The study consisted of a patient group ($n = 79$) with chronic viral hepatitis C and a control group ($n = 52$) without viral hepatitis. We evaluated 79 consecutive patients with chronic hepatitis C (mean age 44.6±11.3; range: 17–68; 44 males, 35 females). All of the chronic HCV patients were positive for antibodies against hepatitis C virus (anti-HCV) and serum HCV-RNA. The control group ($n = 52$; mean age 31.34±8.7 range: 18–58; 32 males, 20 females) consisted of individuals without viral hepatitis. All of the control patients were negative for anti-HCV and HCV-RNA. The age, sex, alanine aminotransferase (ALT) levels, antibodies against EBV (anti-EBV-IgM, anti-EBV IgG), EBV-DNA of the serum samples of the cases were assessed and recorded.

**Serological analysis of EBV infection**

EBV-IgM and EBV-IgG antibodies were determined by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available EBV-IgM and IgG Kits. EBV-IgM antibodies were determined by commercially available Diagnostic Automation EBV-IgM Kit (Diagnostic Automation, INC 23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302, USA). EBV-IgG antibodies were determined by commercially available ATLAS Medical, William James House, Cowley Road, Cambridge, CB4 0WX, UK. Tests were done according to the instructions of the manufacturer.

**Detection of HCV RNA**

RNA was isolated from serum samples as described by Lohr et al., (1995) and El Awady et al., (2006). Reverse transcription-nested PCR was carried out according to Chomczynski and Sacchi (1992). Primers used for detecting HCV in clinical samples were purchased from Promega and their sequences were as following: P1: 5′ GGTGCACGGTCTACGAGACCTC 3′ – P2: 5′ AACTACTGTCTTCACGCAGAA 3′ - P3: 5′ TGCTCATGGTGACGTCG 3′ -P4: 5′ ACTCGGCTAGCAGTCTCGCG 3′ -P5: 5′ GTGCAGGCTCCAGGACC 3′ (Madison, WI, USA).

**Detection of EBV-DNA**

Viral nucleic acid DNA was extracted from serum sample using Wizard® DNA purification mini kit, Promega (Madison, USA). Nested PCR of serum samples for detection of EBV-DNA was carried according to (Kapranos et al., 2003). The reaction mixture of the qualitative PCR contained, in total volume of 25 μl, 5 μl 10x buffer ( 10mM Tris-HCl pH 8.0, 50mM KCl, 25mM MgCl$_2$), 0.5 μl 50mM dNTP mix, 0.25 μl of primers E2P1: 5′ ATCCGGACTTAGGCA 3′ and E2P2: 5′ CAGCAGGCTCCAGGACC 3′ (Bioneer, Atlantic Avenue, Alameda, USA)
Prevalence of Epstein - Barr virus infection in Hepatitis C Patients

for amplification of 556 bp. Except for internal primers Ap1: 5’ CCAGTAGCATCTCTGTCCTGG 3’ and AP2: 5’ GAACCATCCTCGTCCTCATC 3’ (Bioneer, Atlantic Avenue, Alameda, USA) for amplification of 190 bp.

**Agarose gel electrophoresis and analysis of nested-PCR product.**

Analysis of nested-PCR products were performed according to Aaij and Borst, (1972); Ergazaki et al. (1994). Amplification products of both HCV RT-nested PCR and EBV nested-PCR were visualized after electrophoresis on 2% agarose gel stained with ethidium bromide.

**Biochemical Analysis:**

Biochemical tests, including Alanine amino transferase (normal range, 40 U/L) and Aspartate amino transferase (normal range, 38 U/L) levels were done on all collected samples with commercially available Flex ALAT (GPT) and ASAT (GOT) Kits (Siemens Healthcare Diagnostic Inc., USA). Tests were done according to the instructions of the manufacturer.

**RESULTS**

Detection of anti-EBV antibodies in study and control groups

From the study group, 45 out of 79 (56.9%) chronic HCV were positive for EBV-IgG antibodies (Table 1). Sera from 18 out of 52 (34.6%) individuals from the control group were positive for EBV-IgG antibodies (Table 2).

Three patients out of 79 (3.8%) chronic HCV were positive for EBV-IgM antibodies (Table 1). One case out of 52 (1.9%) from the control group was positive for EBV-IgM antibodies (Table 2).

### Table 1: Detection of EBV-IgG and IgM antibodies among patient group

<table>
<thead>
<tr>
<th></th>
<th>Positive EBV-IgG samples</th>
<th>Positive EBV-IgM samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Sex</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>45/79 (56.9%)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Detection of EBV-IgG and IgM in Control group

<table>
<thead>
<tr>
<th></th>
<th>Positive EBV-IgG samples</th>
<th>Positive EBV-IgM samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>sex</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>18/52 (34.6%)</td>
<td></td>
</tr>
</tbody>
</table>

**Detection of EBV-DNA by nested PCR**

In chronic HCV patients, EBV-DNA was positive in 29% (23/79) of the serum samples. EBV-DNA was detected in 4 out of 52 (7.7%) samples obtained from the control group (Fig 1). The difference between the presence of EBV-DNA in patients with chronic HCV infection and the control group was statistically significant (p < 0.01).
Serum levels of the liver transaminases in the study groups

In positive HCV-RNA patient group, serum ALT activity levels was detected as 81.89±16.2 IU/L which means that, it was higher than that of control group 32.98±12.7 IU/L. Also serum AST activity level of HCV patient group was 80.18±15.8 IU/L and higher than that of control group 31.7±12.46 IU/L (Table 3 and Fig. 2).

Table 3: ALT& AST activity levels in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>patients group</th>
<th>Normal control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ALT (IU/L)</td>
<td>81.89±16.2</td>
<td>32.98±12.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean AST (IU/L)</td>
<td>80.18±15.8</td>
<td>31.7±12.46</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In HCV-positive group (patient group), serum ALT levels (mean: 96.6±10.4 U/l) in EBV-positive patients were slightly higher than that of EBV-negative patients (mean: 75.3±15.2 IU/l). Serum AST activity level of EBV-positive patients (mean: 93.9±11.3 IU/L) was slightly higher than that of EBV negative patients (mean: 73.4±14.6 IU/L) (Table 4 and Fig. 3).
Prevalence of Epstein - Barr virus infection in Hepatitis C Patients

Table 4: ALT& AST activity levels in EBV Patient group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive EBV patients group</th>
<th>Negative EBV patients group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ALT (IU/L)</td>
<td>96.6±10.4</td>
<td>75.3±15.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean AST (IU/L)</td>
<td>93.9±11.3</td>
<td>73.4±14.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 3: The activity level of ALT and AST liver enzymes in EBV patient group

► In Control group, serum ALT levels (mean: 46.1±3.4 U/l) in EBV-positive patients were slightly higher than that of EBV-negative patients (mean: 31.8±12.6 U/l). Serum AST activity level of EBV-positive patients (mean: 44.2±2.9 IU/L) was slightly higher than that of EBV negative patients (mean: 30.6±12.4 IU/L) (Table 5 and Fig. 4).

Table 5: ALT& AST activity levels in EBV Control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive EBV Control group</th>
<th>Negative EBV Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ALT (IU/L)</td>
<td>46.1±3.4</td>
<td>31.8±12.6</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mean AST (IU/L)</td>
<td>44.2±2.9</td>
<td>30.6±12.4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Fig. 4: The activity level of ALT and AST liver enzymes in EBV control group
DISCUSSION

Epstein-Barr virus infection is characterized by alternating periods of latency and reactivation. Replication of EBV in the absence of an effective immune response is central to the pathogenesis of the disease (Ning, 2011). Therefore, reactivation of the virus is seen during periods of down-regulation of the immune system, such as drug treatment and illness-related stress, or during on-going activation of the immune system such as inflammatory diseases, or co-infection with other pathogens (IARC, 2011). Virus–virus interactions have been demonstrated to modify the pathogenesis of human viral infections (Palma et al., 2010).

In this study, we investigated the incidence of EBV infection in Egyptian HCV patients who progressed to chronic HCV infection and we examined the potential role that EBV plays in HCV progression. The present data showed that the percent of positive EBV Abs were significantly higher (P < 0.001) in chronic HCV patient than those in Control group. Also, the EBV DNA was detected in 29% of chronic HCV infected patients compared with 4 out of 52 (7.7%) Control group cases. The difference between the presence of EBV DNA among chronic HCV patients and the control group was statistically significant (p < 0.05). Moreover, the results confirmed that the detection of EBV DNA by PCR in peripheral blood leukocytes is a sensitive and reproducible procedure for detecting viral infection. As the serological methods reported to be insensitive and can’t distinguish between EBV infection and EBV disease as IgM antibodies may persist for months or years and may be detected during reactivation of latent virus infections (Shibuya et al., 2003).

Due to the fact that EBV has immunomodulating properties, Gallegos-Oroza et al., (2010) it was presumed that reactivation of EBV could accelerate HCV pathogenesis in critically ill patients.

In the two study groups (Patient group and Control), we study the activity levels of ALT and AST liver enzymes. In HCV-RNA positive cases, serum activity levels of ALT and AST enzymes illustrated in this study showed a highly significant (p<0.001) elevation in positive EBV-DNA than negative individuals. These findings indicated to the active EBV infection in chronic HCV patients that had high influence on activity of ALT and AST enzymes by increasing their levels in sera of EBV patients. These findings indicated to primary and reactivated EBV infections in chronic HCV patients had high effect on liver enzymes, which seems to be attributed to the immune system of the chronic HCV patient. The primary and reactivated EBV infections were interacted with HCV and raised the influence on the liver enzymes, as with other herpes viruses (Petrova and Kamburov, 2010).

Latency follows all primary infections and is considered to be lifelong, where EBV can reactivate periodically under the influence of exogenous and endogenous factors and cause immunosuppression (Hinedi TB et al., 2003). So there was high effect on serum activity levels of ALT and AST by EBV infection on HCV patients and immunosuppressed individuals, these findings are revealed with other studies, DNA of some types of HHVs (CMV, EBV and HHV-6) are more frequently encountered in specimens from patients with HCV hepatitis than from subjects without liver hepatitis (Claudio et al., 1999). HCV replication was promoted by EBV and that EBNA1 was responsible for supporting HCV replication (Sugawara et al., 1999; Cacopardo et al., 2003).

In HCV negative group (Control group), ALT and AST activity levels in positive EBV-DNA cases were slightly higher than that in negative cases. All the previous results indicated that the pathogenesis of HCV is strongly influenced by its interaction with EBV. These findings are in agreement with other studies donated that, Epstein- Barr virus infection can cause liver function test abnormalities without
pharyngitis or lymphadenopathy (Dogan et al., 2007). Liver involvement usually causes mild elevation of transaminases and this abnormality resolves spontaneously. Jaundice might develop rarely during the clinical course of Epstein-Barr virus infection. It reflects either more severe hepatitis or Epstein-Barr virus infection-associated hemolytic anemia (Hinedi and Koff, 2003).

The result of this study indicated that infection with EBV was prevalent in HCV patients. This may be attributed to several reasons: Differences in exposure to EBV infection may be excluded because this is ubiquitous virus infecting almost the whole population since infancy (Claudio et al., 1999). EBV viruses may exert an immunomodulatory effect resulting in enhanced immunosuppression (Anne et al., 2007).

These previous findings are in agreement with other studies (Li et al., 2004; Petrova et al., 2010) reported that, in some patients with chronic liver disease caused by a major hepatotropic virus, an infection with other viral agents may be discovered. We previously evaluated patients with chronic hepatitis B and C regarding their EBV serology. Petrova et al. (2010) reported that, The patients with reactivated EBV infection had lower levels of HBV DNA and higher mean values of serum hepatitis C virus (HCV) RNA respectively, compared to EBV-seropositive patients without reactivation.

REFERENCES
Hussam Ghanem et al.


ARABIC SUMMARY

 مدى انتشار فيروس الإبشتاين في المرضى المصابين بالالتهاب الكبدي الوبائي سي

حسبم غانم 1 - ماهر نبيل - أشرف طبل

1- قسم الميكروبيولوجي - كلية العلوم - جامعة عين شمس - القاهرة - مصر.
2- قسم التكنولوجيا الطبية الحيوية - المركز القومي للبحوث - الجليزة - مصر.

يعتبر فيروس الإبشتاين شائع في الفيروسات واسعة الانتشار في عالم البشر وهو من الفيروسات التي تظل كامنة داخل خلايا الجسم إلى أن يحدث خلل في الجهاز المناعي مما يسبب في نشاط هذا الفيروس مما يؤدي إلى ظهور امراض خطيرة. ولقد صممت هذه الدراسة لتقصي مدى انتشار فيروس الإبشتاين في المرضى المصابين بالتهاب الكبد الوبائي (سي). ونتجت هذه الدراسة مجموعة الألواح والحالات المصابة بالالتهاب الكبدي الوبائي سي وعددهم حوالي 79 حالة أما المجموعة الثانية وتضمنت 52 حالة وهي الحالات غير مصابا بفيروس الألتهاب الكبدي الوبائي سي. ومن خلال الدراسة تبين أن فيروس الإبشتاين ينتشر بين الحالات المصابة بالالتهاب الكبدي الوبائي سي. وهذه الدراسة المزودة أدت إلى ارتفاع معدل انزيمات الكبد في هؤلاء المرضى.