Evaluation of anti Epstien-Barr Virus antibodies in female patients with autoimmune hepatitis type-1

تقييم مستوى اضداد فيروس Epstien-Barr في النساء المصابات بالتهاب الكبد المناعي الذاتي النمط الاول

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Abstract

Epstein-Barr Virus (EBV) infection is associated with broad spectrum of clinical manifestations depending on the immune status of the host, To analyze their possible role in the complication of autoimmune hepatitis, we investigated (30) female patients with autoimmune hepatitis type-1 of (10-40)years and 25 healthy female of same ages(control groups). Both groups were carried out to measure the levels of EBV-CA IgM, IgG Ab, EBV-EA IgM, IgG Ab, and EBV-NA IgM, IgG Ab using indirect immunoflourescent assay (IFAT).The prevalence of EBV-CA IgM, IgG Ab were (10%,20%) and EBV-EA IgM, IgG Ab were (10% and20%) respectively, while the prevalence of EBV-NA IgG Ab was(3.33%) and there are no prevalence of EBV-NA IgM Ab. There were significant differences (P≤ 0.05) in percentage of EBV-CA IgG and EBV-EA IgG in patients groups compared to control groups, and no significant differences in percentage of EBV-CA IgM and EBV-EA IgM in patients group compared to control group. This indicates that infection with Epstien-Barr virus plays a role in the pathogenesis of autoimmune hepatitis type-1.

key words: Epstein-Barr Virus, autoimmune hepatitis, Ages, Antibodies

الملخص

إن الإصابة بفيروس Epstein –Barr Virus لها دور بمدى واسع من الإمراض الذاتية المناعة اعتمادا على الحالة المناعية للعا ئل. ولغرض تحليل الدور المحتمل لهذا الفيروس في تعقيدات التهابات الكبد المناعية, تم التحري عن (30) مريضه مصابه بالتهاب الكبد المناعي الذاتي النمط الأول بأعمار تتراوح من (10-40) سنة وتمت المقارنة مع 25 أنثى سليمة. خضعت جميع عينات الدراسة لقيا س مستوى أضداد مستضد الكابسد,أضداد المستضد البدائي, وأضداد المستضد النووي لكلا الصنفين IgG, IgM باستخدام تقنية التالق المناعي الغير مباشر. كانت نسبة انتشار أضداد مستضد الكابسد لكلا الصنفين IgG,IgM 10%و20% والمستضد البدائي لكلا الصنفين IgG IgM 10%و20% على التوالي بينما كانت نسبة انتشار أضداد المستضد النووي للصنف IgG 3.33% ولم يلاحظ أي انتشار لأضداد المستضد النووي IgM كما لوحظ هنالك فرق معنوي P≤ 0.05 بين نسب أضداد مستضد الكابسد الصنف IgG وأضداد المستضد البدائي الصنف IgG في مجاميع المرضى مقارنة بمجاميع السيطرة, و لم يلاحظ هنالك فرقا معنويا بين نسب أضداد مستضد الكابسد الصنف IgM وأضداد المستضد البدائي الصنف IgM في مجاميع المرضى مقارنة بمجاميع السيطرة. تشير نتائج الدراسة بان الإصابة بفيروس Epstein-Barr Virus تلعب دورا في أمراضية التهاب الكبد المناعي الذاتي النمط الأول.

الكلمات المفتاحيه : فيروس Epstein-Barr Virus ا لتهاب الكبد المناعي الذاتي ،المستضدات، العمر، الاجسام المضادة

Introduction

Autoimmune hepatitis(AIH) is an inflammatory liver disease primarily affecting women and is characterized by elevated transaminase levels, The presence of specific auto antibodies, raised IgG, and interface hepatitis on histology, Although the aetiopathogenesis of AIH is unclear, several autoimmune pathways have been proposed which are largely based on auto reactive CD4+ T lymphocytes recognizing a liver-specific auto antigen [1,2]. AIH may present acutely in approximately 40% of cases and may resemble acute viral hepatitis [3, 4]. The aetio pathogenesis of AIH is poorly understood, but there is some evidence to suggest that a numerical and functional impairment of T-regulatory Cells may be involved, in addition, a variety of genetic and environmental factors are involved in the development of the disease, including viruses [5,6]. Hepatitis is a common characteristic of infection by EBV, although severe hepato cellular liver injury is rare and its pathogenesis uncertain [7,8]. Acute or latent EBV infection has already been suggested in autoimmunity process in adults including autoimmune hepatitis AIH , an undeserving, progressive liver disease characterized by hyper gamma globuinaemia, circulating auto antibodies, association with human leukocyte antigens HLA, interface hepatitis on liver histology and a favorable response to immunosuppressant [9,10]. AIH following, an acute viral

infection has been strongly associated with hepatitis A virus (HAV) and hepatitis B virus (HBV), several cases report the development of AIH following EBV Infection [11]. From the pathogenesis point of view, the interplay between viruses and susceptible hosts may result in the clinical development of autoimmunity either by cytokines release which activate auto reactive T- cell and modify antigen processing and activation or by molecular mimicry [12].

The aim of the present study was to determine the relationship between autoimmune hepatitis and Epstein- Barr virus infections.

Material and methods:

The study included 30 female patients with autoimmune hepatitis (AIH) type-1 of (10-40) years old from Gastrointestinal tract and hepatic disease center from period 1/6/2013-30/`12/2013 who are positive to Antinuclear Abs (ANA) and Anti smooth muscle Abs (ASMA) by immunoflourescent test IFAT, and (25) healthy female blood donor as a control group with same ages usually above 18 years old. All groups are from capital of Baghdad and all carried out to measure (EPV-Capsid antigen) EBV-CA IgM and IgG Abs, (EBV-Nuclear antigen) EBV-NA IgM and IgG Abs and (EBV-Early antigen) EBV-EA IgM and IgG Abs by IFAT test. (Euroimmun). Germany.Theses tests were done in educational laboratories/department of clinical immunology according to [13].

1-30 ml of diluted sample was applied to each reaction field of the reagent tray.

2-Reactions were started by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray and incubated Incubate for 30 min at room temperature(25-28 C).

3-The BIOCHIP slides were rinsed with a flush of PBS-Tween using beaker and immediately immersed afterwards in a cuvette containing PBS-Tween for at least 5 min.

4-25ml of fluorescein labeled anti-human globulin was applied to each reaction field of a clean reagent tray. All droplets were added before continuing incubation.

5-One BIOCHIP slides were removed from cuvette. Within five second only the back and the long slides were blotted with a paper towel, the BIOCHIP slides were put into the recesses of the reagent tray, and incubated incubate for 30min at room temperature (25-28C).

6-Cuvette was filled with new PBS-Tween. The BIOCHIP slides were rinsed with a flush of PBS-Tween using a beaker and put into the cuvette filled with the new PBS-Tween for at least 5 min.

7-Embedding medium was placed onto cover glass-drops of max.10ml per reaction field. a polystyrene embedding template was used. BIOCHIP slides were removed from PBS-Tween and dried with a paper towel. The BIOCHIP slides were put with the BIOCHIP facing downwards, onto the prepared cover glass.

8-The fluorescence was read with the microscopic.

Statistical analysis:

Comparison of paired data from the groups of subjects was done using T-test (t), while correlation between groups were analyzed using Person Chi Square. The computer program which used was SPSSv.11.5 [14].

Results and discussion

The results of the present study showed that percentage of EBV-CA IgM, IgG Abs were (10%,20%) and EBV-EA IgM, IgG Ab were(10% and20%)respectively, these indicate that infection with Epstein – Barr virus at early stage of infection or acute infection, while the percentage of EBV-NA IgG Ab was 3.33% and there was negative EBV-NA IgM Ab using IFAT because EBV-NA was present only at late stage of infection. As shown in figure (1, 2 and 3). Both IgM and IgG antibodies to the viral capsid antigen(VAC) peak three to four weeks after primary EBV infection. IgM anti-VCA declines rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking but last indefinitely. However, antibodies to EBV-NA gradually increase in titer and, after three months to one year, reach a plateau level where they persist for life in most individuals therefore, the presence of antibodies to EBV-NA indicates that the EBV infection was not recent [9].

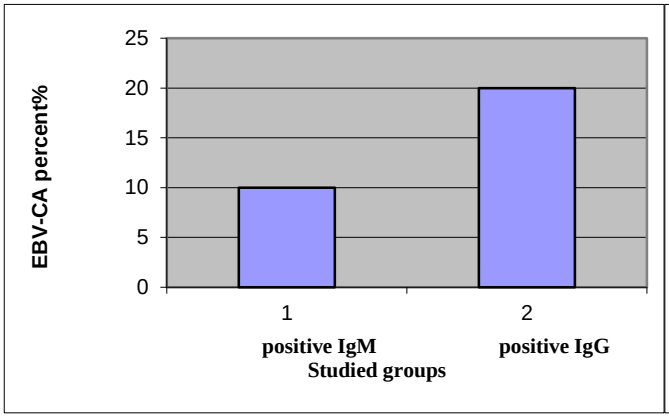

Fig (1): Anti-EBV-CA Ab (IgM,IgG) in sera of patients with AIH Type-1

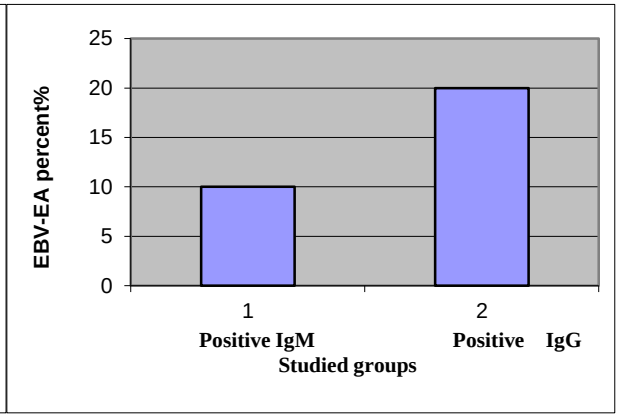

Fig (2):Anti-EBV-EA Ab in sera of patients with AIH Type-1

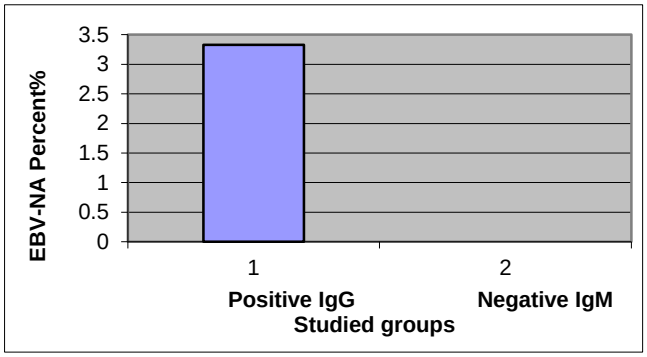

Fig (3): Anti-EBV-NA Ab (IgM,IgG)in sera of patients with AIH Type-1

Also There were significant differences ($P \leq 0.05$) in percentage of EBV-CA IgG and EBV-EA IgG in patients group compared to control group, and no significant differences in percentage of EBV-CA IgM and EBV-EA IgM in patients group compared to control group, figure (4A,4B 4C,). All control group were negative for all testes anti EBV-CA, EBV-EA, and EBV-NA Ab by IFAT test as shown in figure (5).

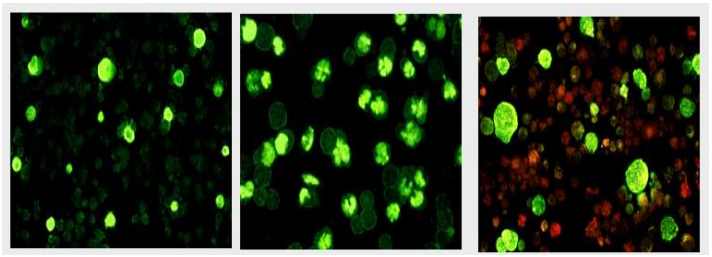

Fig (4A): Positive anti-EBV-CA, (4B): Positive anti-EBV-NA, and (4C): Positive anti-EBV-EA by IFAT test for serum of women with AIH Type-1

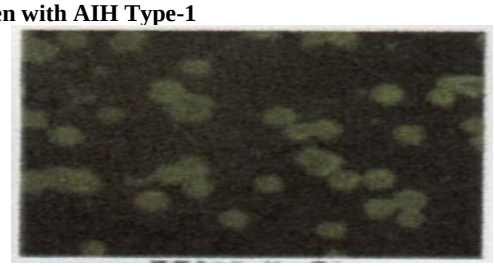

Fig (5): Negative anti EBV-CA, EBV-EA, and EBV-NA Ab by IFAT test for serum of control groups (healthy women).

The results of the present study were agreement with several another studies, one study describes the association of AIH with EBV infection, suggestive of EBV inducing pediatric as well as adults forms of AIH type-1, but it still remains unclear whether there is a true link or it is due to the rarity of these

studies [15,16]. A recent study presents a compelling hypothesis involving immunological deregulation, EBV infection and the development of autoimmunity, CD8+ T-cell deficiency is a characteristic of several autoimmune diseases and also occurs in some relatives of patients with autoimmune disease, suggesting an underlying genetic susceptibility [17]. Others studies suggest that the impairment of CD8+ T cells results in the inability to control EBV infection and thus there is accumulation of EBV-infected, auto reactive B-cells in a variety of target organs, with clonally expansion of these cells, and the development of entopic lymphoid follicles, within the target organ, the EBV infected auto reactive B cells would produce pathogenic auto antibodies and provide co stimulatory signals for the survival of auto reactive T cells. Study also indicated a role for vitamin D deficiency in this model, as vitamin D (and the vitamin D receptor) has been found to be reduced in patients with autoimmune disease, and reduced vitamin D is noted in higher latitudes which have higher rates of autoimmune disease, a role for vitamin D deficiency in autoimmunity is suspected. Another study suggests that decreased vitamin D aggravates the deficiency in CD8+ T cells, therefore causing further impairment of EBV Control [17,18]. Despite the need for extensive investigation, this hypothesis highlights the intriguing interplay between genetic susceptibility and infection which may play a role in the development of autoimmune disease [19]. Because EBV must reactivate from latency to complete its life cycle, this situation is rare, the liver is the most common target organ, and has a very poor prognosis; patients die from hematological or non – hematological disorders within a few years, in these patients, a polymerase chain reaction assay can be used to detect the gene for BZLFI, which can be detected from one copy of EBV DNA [19]. Studies of EBV DNA titers have suggested that it may correlate with disease activity more accurately than do serological data [20,21].The mechanism of liver injury associated with EBV infection is not yet apparent, some studies have recently reported two patients with autoimmune hepatitis caused by EBV infection, in their patients, there was a defect in suppressor- inducer T lymphocyte specifically controlling the immune response to the asialoglycoprotein receptor, the antibodies to this auto antigen persisted and increased after infectious mononucleosis, and autoimmune hepatitis developed, it is suggested that autoantibody to asialoglycoprotein receptor, which is expressed on the hepatocellular membrane as a liver –specific antigen. Contributes to the pathogenesis of autoimmune hepatitis [22, 23]. Some studies reported that children carrying the HLA-DR7 haplo type are more susceptible to AIH and develop more aggressive disease with more severe prognosis, while another study recently proposed that latent EBV infected auto reactive memory B-cell, lodge to the target organ and act as antigen- presenting cells attracting CD4+ T-lymphocytes that fail to undergo apoptosis, as they receive a co-stimulatory survival signal from infected B- lymphocytes [24]. It was also reported that a significant increase in EBV nuclear antigen – positive cells occurred only in B-cells obtained from patients with capsid antigen Epstein-Barr virus (CAEBV) when cells were stimulated with specific antigens [24].

Conclusion: This indicates that Epstein - Barr virus infection plays a role in the pathogenesis of autoimmune hepatitis type-1.

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