Detection of Epstein-Barr virus in autoimmune and Thalassemia patients using new Immunoblot assay

التحري عن فايروس الابشتاين- بار في مرضى المناعة الذاتية وفقر دم البحر المتوسط بتقنيه اللطخة المناعية

Alaa Younis Mahdy Al-Hamadany **Basima A. Abdullah***

Technical Institute/ Mosul

* College of Science/ University of Mosul

علاء يونس مهدي الحمداني **باسمة احمد عبدالله***

المعهد التقني/ الموصل

*كلية العلوم/ جامعة الموصل

Abstract

A new immunoblot assay, composed of five Epstein-Barr virus (EBV)-encoded recombinant proteins virus capsid antigen [VCA] gp125, p19, p22, early antigen [EA], and EBNA-1 IgG, was used to manifest the EBV infection and look at the antibody pattern to EBV proteins in the serum of both autoimmune disorders and Thalassemia patients and compare the observations with those in normal healthy controls. Serum samples from 35 rheumatoid arthritis patients, 20 SLE, 20 autoimmune hypothyroid diseases, 35 Thalassemia patients and 20 healthy controls were tested for EBV IgG antibodies by an immunoblot assay (Euroline). The results showed that the high percentage recorded was 50% in acute infection. Followed by 30% at late infection, while late phase with loss EBNA-1 and reactivated infection were 10% compared to the normal healthy controls. Our study showed an increased EBV activation among the autoimmune patient groups compared to the normal healthy controls. Further studies are required to delineate the association between the etiology of autoimmune disorders and EBV.

Key wards: PCOS, Thyroid disorders, TPO, Mutations, Hormonal disturbances

الملخص

استخدمت طريقه جديده اللطخة المناعية (Immunoblot) تحوي خمس بروتينات مشفره لمستضد المحفظة لفايروس الابشتاين- بار (VCA)هم gp125,p19,p22 والمستضد المبكر EA و EBNA-IgG لتقدير الإصابة بفايروس EBV والتعرف على نمط الاجسام المضادة لبروتينات الفايروس في مصول مرضى المناعة الذاتية وفقر دم البحر المتوسط ومقارنة هذه المشاهدات مع مصول الاصحاء. المواد وطرق العمل: جمعت 35 عينه من مرضى التهاب المفاصل الرثواني و20 من مرضى الغدد المناعية و35 من مرضى فقر الدم البحر المتوسط و10 من مرضى ذوات الذئب الاحمراري و20 من الاصحاء واختبرت عن وجود اجسام مضادة نوع EBV IgG بطريقة اللطخة المناعية من شركة Euroimmune. النتائج: اوضحت النتائج ان اعلى شده تواجد gp125 VCA كانت 108\+++ في مرضى ذوات الذئب الاحمراري يليها مرضى الغدد المناعية 100\+++ واقل شده في 43\++ في مرضى التهاب المفاصل الرثواني. اعطى مستضد EA-D, EBNA-1 اعلى شده بلغت 52\++ و40\++ على التوالي في مرضى المناعة الذاتية, واقل شده بلغت 19\+ و13\+ في مرضى فقر دم البحر المتوسط. علما ان معدل الموجب + هو 25-10, و++ 50-26 و+++ ≥51. الاستنتاج: اوضحت دراستنا ان اعلى نسب الإصابة هي الإصابة الحاده بالفايروس وكانت 50% اعقبها الإصابة المتأخرة وكانت 30% بينما بلغت نسبه الإصابة المتأخرة مع فقدان مستضد EBNA-1 والإصابة المنشطة 10% مقارنة بالأصحاء. لذا يتطلب اجراء دراسات لاحقه لتوضيح العلاقة بين مسببات امراض المناعة الذاتية و EBV.

الكلمات المفتاحية: متلازمة تعدد الاكياس المبيضية، اضطرابات الغدة الدرقية، الطفرات، الاضطرابات الهرمونية

Introduction

Epstein-Barr virus (EBV), or human herpes virus 4 (HHV4), belongs to the herpes virus family. A typical EBV virion consists of a linear double-strand DNA genome packaged into an icosahedra capsid, which is surrounded by a proteinaceous structure called the tegument, and an envelope composed of several viral glycoproteins embedded in a lipid bilayer [1]. It is replicated during each cell division by the host DNA polymerase together with the host chromosomes. The EBV genome encodes over 85 open reading frames ORFs [2].

Two types of EBV exist; type 1 is the most commonly occurring while type 2 is equally as common as type 1 in equatorial Africa and New Guinea. The difference between the two types comes from slight variation in the genes that encode EBV nuclear proteins [3], and mainly due to variations in the C-terminal repeat regions, and thus allowing strain typing [4].

EBV has been associated with a number of diseases, particularly autoimmune diseases, cancer such as Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, conditions associated with human immunodeficiency virus HIV [5] and multiple transfused beta-Thalassemia [6], also associated with some non-B cell malignancies like extra nodal T-cell and NK cell lymphomas, nodal T-cell lymphomas, nasopharyngeal carcinoma (NPC), gastric carcinoma and some others [4] indicating the capacity of EBV to infect non-B cells as well.

The aim of the present study was to investigate the antibody pattern to EBV proteins in the serum of systemic autoimmune diseases and Thalassemia patients compared to normal healthy controls to ascertain the association of EBV with these two common autoimmune disorders.

Materials and Methods

Patients

A cohort of 130 serum samples has been collected of different autoimmune diseases as (RA, SLE, &ATD), Thalassemia and controls. Among those samples have been 35 RA, 20 SLE, 20 ATD, 35 Thalassemia and 20 control samples, which were pay a visit Al-Salam Teaching, Ibn-Atheer Teaching and Nuclear medicine hospitals from date of 15\8\2012 to 15\2\2013 and diagnosed by the treating physicians and confirm the diagnosis by specific tests for each disease. Their ages are ranging from 3-84 years.

Methods

Primary detection of EBV in all samples was done by using Anti-EBV-CA ELISA (IgM) kit (Euroimmune, Germany) then the antibody pattern was analyzed by testing the positive samples with an EBV IgG immunoblot (EBV-profile 2 EUROLINE, Euroimmune, German). This provides a qualitative *in-vitro* assay for human antibodies to five different EBV antigens: VCA gp125, VCA p19, EBNA-1, p22, and EA-D. The manufacturer's instructions were followed while carrying out the assay. In brief pre-treated strips with block buffer then incubated at room temperature with 1:51 diluted serum samples for 30 minutes on a rocking shaker followed by washing and addition of enzyme conjugate (alkaline phosphatase conjugated anti-human IgG). After 30 minutes of incubation at room temperature the strips were washed again followed by the addition of substrate (NBT/BCIP) and incubation for 10 minutes at room temperature. The reaction was stopped by washing the strips with the addition of distilled water. The membrane strips were dried and automated evaluations of these strips for the analysis of different bands and examined with EUROL ineScan system provided by the manufacturer. The reading was taken by keeping the strips on a flatbed scanner (Canon) which enables the EUROL ineScan to recognizes the position of the strips, identify the bands and measure its intensity.

Results

According to ELISA technique, the results revealed that the highest EBV infection percent was in SLE 15% followed by ATD 10% and RA 8.6% while the lowest infection percent was in Thalassemia 5.7%. On other hand, the results according to gender showed that the highest infection percentage recorded in females with rheumatoid arthritis 30 %, followed by females with SLE, autoimmune thyroid diseases and males of Thalassemia patients 20% and the lowest infection percentage in male with SLE 10%, while no infection in males with rheumatoid arthritis and autoimmune thyroid disease and females of Thalassemia patients Table (1).

Table (1): Percentage seropositivly EBV infection in relation to gender

Study groups / Gender	Thala	RA	SLE	ATD	Total
			N0. (%)		
Males	2(20%)	0(0%)	1(10%)	0(0%)	3(30%)
Females	0(0%)	3(30%)	2(20%)	2(20%)	7(70%)
Total	2(20%)	3(30%)	3(30%)	2(20%)	10(100%)

The overall immunoblot results with the classical serological markers VCA gp125, VCA p19, P22, EBNA-1, and EA-D showed the highest intensity of VCA gp125 was 108 /+++ in SLE, followed by autoimmune thyroid disease was 100/+++ while the lowest intensity was 43/++ in rheumatoid arthritis. The EBNA-1antigen and EA_D showed highest intensity were 52/+++, 40/++ respectively in autoimmune thyroid diseases while the lowest intensity were 19/+, 13/+ in thalassemia Table (2)

Table (2): Maker of IgG antibodies associated with EBV infection in patients groups under study

EBV Ab / Study groups	VCA gp125	VCA p19	P 22	EBNA-1	EA-D	IgG
Thalassemia	44 / ++	71 / +++	16 / +	19 / +	13 / +	87 / +++
RA	43 / ++	72 / +++	26 / ++	24 / +	15 / +	98 /+++
SLE	108 / +++	48 / ++	88 / +++	47 / ++	29 / ++	84 / +++
AT	100 / +++	90 / +++	38 / ++	52 / +++	40 / ++	94 / +++

Thala= Thalassemia, R.A= Rheumatoid arthritis, ATD= Autoimmune thyroid disease, SLE= Systemic Lupus Erythromatosis, (No.) indicate mean of intensity, + (10-25), ++ (26-50), and +++ (≥ 51) indicate class

According to data in this study, the results showed that the high percentage recorded was 50% in acute infection apportioned as 40% at rheumatoid arthritis, 20% at Thalassemia, autoimmune thyroid

diseases, and SLE. Followed by 30% at late infection, while late phase with loss EBNA-1 and reactivated infection were 10% Table (3).

Table (3): infectious status associated diseases

study groups / Infection status	Thala	RA	SLE	ATD	Total
	No. (%)				
Acute infection	1(10%)	2(20%)	1(10%)	1(10%	5(50%)
Late infection	1(10%)	-	2(20%)	-	3(30%)
Reactivated infection	-	-	-	1(10%)	1(10%)
Late phase with loss EBNA-1	-	1(10%)	-	-	1(10%)
Total	2(20%)	3(30%)	3(30%)	2(20%)	10(100%)

Discussion

An association of autoimmune diseases with EBV infection has been found [7]. Immunoblot (Euroline) for EBV consists of various markers that help to differentiate EBV infection as acute, latent infection, latent infection with lose of EBNA1 or reactivation of latent infection. The presence of VCA IgG,VCA IgM and EA in the absence of EBNA-1 IgG indicates acute infection, while the presence of VCA IgG and EBNA-1 IgG in the absence of VCA IgM is typical of past infection [8]. A significantly higher number of rheumatoid arthritis patients in our study group had immune responses against these proteins especially VCA and EA-D suggestive of acute EBV infection. Recent study observed among 53% of rheumatoid arthritis patients is positive for EBNA1 antibodies [9]. Possibly that may association between significant increase in the EBV IgM and IgG and rheumatoid factor, which can induce signalling from B-cells and in turn lead to the activation of persistent EBV [10]. Other study showed the relative risk for RA was not increased in patients with higher levels of anti-EBNA-1 and demonstrated anti-EBNA-1 by serological methods cannot evaluate the interactions between EBV and the RA risk factors [11].

Antibodies against the capsid antigen IgG (VCA IgG) typically appear at the time of the onset of the clinical symptoms of acute infection, and remain positive for life [12], whereas IgM antibodies (VCA IgM) usually appear at the same time as VCA IgG and disappear within a few weeks [13], although they may persist for several months. Children and adults with primary infection are not always positive for VCA IgM [12] Anti-EA antibodies (EA IgG) reflect two patterns, a diffuse (D) and a restricted pattern (R). Although not always present, EA (D) IgG increases during the first 3-4 weeks and is no longer detectable after 3-4 months, approximately 85% of the patients with acute infection are positive for up to 3 months after symptom onset [14] even though in some cases they can still be detected years after a primary infection. Approximately, 20%-30% of healthy subjects who have previously been infected by EBV have EA (D) IgG [14]. High titers have also been seen during reactivation and in patients with nasopharyngeal carcinoma [15] who also has high titers of VCA and EA IgA [16]. EA (R) IgG levels may remain high for up to 2 years and, EA (R) IgG has been found in children aged less than 2 years with silent infection, in patients with Burkitt's lymphoma, and also in the previously infected subjects at low levels. High levels of EA (D) and/or EA (R) IgG can also be seen in cases of reactivation and in immunocompromised patients [16]. Anti-EBNA-2 IgG (EBNA-2 IgG) appear early, and may be present in up to 30% of the patients in the course of disease [14], whereas anti-EBNA-1 IgG (EBNA-1 IgG) is usually undetectable during the first 3-4 weeks after the onset of clinical symptoms [12] and is therefore indicative of past infection. Furthermore, most patients with chronic infection and immunosuppressed patients are negative for EBNA-1 IgG or have only low levels of it [17]. Generally by using the parameters VCA IgG, VCA IgM, EA and EBNA-1 IgG can easy to distinguish acute, past and reactivated infections in autoimmune diseases and Thalassemia patients.

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