Expression of Anti- Apoptotic Gene (BARF1) and Immunomodulatory Gene (BCRF1) For Epstein Barr Virus in Chronic Active EBV Patients

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KEYWORDS
Apoptotic Gene (BARF1) and Immunomodulatory Gene (BCRF1)

ABSTRACT

Epstein Barr virus (EBV) is a herpes virus with double-stranded DNA enclosed by proteins. The envelope of the virus has glycoproteins, which are important for attachment and entry into the host cells (B cells and epithelial cells). EBV targets B cells by utilizing their molecular machinery to replicate the viral genome. The virus causes B cells to differentiate into memory B cells, which then can move into the circulatory system, or become latent until a trigger causes reactivation. The transmission of the Epstein Barr virus occurs in several ways, such as deep kissing or food-sharing. Increased levels of viral DNA are found in salivary secretions after the initial infection. Children can be infected after eating food that has already been chewed by an EBV infected individual. The transmission has occurred through stem cell and organ transplantation, as well as blood transfusion. EBV has several associated complications, One dangerous complication is splenic rupture due to infectious mononucleosis. Aim of the Study: To Estimation the immunemodulatory and Oncogenes for Epstein Barr Virus Associated with Viral Tumorigenesis in Chronic Active EBV Patients By Detection of EBV IgG in all samples , Estimation the expression of antiapoptotic gene (BARF1) and immunomodulatory gene (BCRF1). Result: The study showed there are high significant between the patient group with different inflammation disease in addition infected with virus compare with have not disease When detection genetically about the virus in sample of infected by EBV the presence of genes has been diagnosed BARF1 and BCRF1 in patient's group.

1. Introduction

Epstein-Barr virus (EBV or human herpesvirus 4 (HHV-4)) is a member of the γ-herpesvirus family. It was the first human oncovirus to be identified. It is one of the most successful viruses, infecting up to 95% of the adult population worldwide and continuing to infect B cells asymptomatically throughout life [1,2,3,4]. Natural infection with EBV is remarkably widespread in humans [2,5]. Studies have demonstrated its role as a causal factor in the development of a variety of diseases, including benign diseases (infectious mumps (IM)) [2], oral diseases [6], diseases associated with functional immune abnormalities and multiple sclerosis (MS) [7], systemic autoimmune diseases (SAD) [8], various malignancies (hematological malignancies, epithelial cancers) [3,9] and Epstein-Barr virus-associated hematopoietic lymphoma (EBV-HLH). Epstein-Barr virus (EBV) is a herpesvirus that contains double-stranded, protein-penetrating DNA. The viral envelope contains glycoproteins, which are important for cell formation and entry into proliferating cells (B cells and epithelial cells) [11]. EBV is transmitted in several ways, such as through deep kissing or sharing food. High levels of viral DNA have been found in salivary secretions after initial infection. Children can become infected after eating food that has already been chewed by a person infected with EBV. The disease is transmitted through organ and stem cell transplants, as well as blood transfusions. [11][12] Late genes account for more than one-third of the herpesvirus genome. The functions of many of these genes are essential for the viral life cycle. Late genes encode structural proteins that form the viral capsid and glycoproteins that mediate virus attachment, fusion, and entry during primary infection. Other late proteins also mediate essential events during virus assembly and maturation, such as cleavage and packaging of viral nucleic acid into preformed capsids, capsid encapsidation, and egress of infectious particles. Furthermore, late proteins play a key role in suppressing the immune system of infected cells. Expression of late genes in Epstein-Barr virus

(EBV), an oncogenic gammaherpesvirus, has been associated with several forms of cancer, including Burkitt lymphoma [13], nasopharyngeal carcinoma [14, 15], Hodgkin lymphoma [16], gastric cancer [17, 18], and post-transplant lymphoproliferative disease [19, 20]. Furthermore, late proteins play a key role in suppressing the immune response of infected cells. Here, we investigated the expression of late genes in Epstein-Barr virus (EBV), a gammaherpesvirus associated with several forms of cancer, including Burkitt lymphoma [21], nasopharyngeal carcinoma [22, 23], Hodgkin lymphoma [24], gastric cancer [25, 26], and post-transplant lymphoproliferative disease [27, 28]. The BARF1 gene is located in the BamHI-A segment of the Epstein-Barr virus (EBV) genome, encodes 221 amino acids and has oncogene activity. Several reports have shown that BARF1 is expressed in tissues of several EBV-associated epithelial malignancies. However, BARF1 is thought to be a lytic gene, as its expression is induced upon induction of the lytic cycle in Burkitt's lymphoma cell lines. Therefore, the possibility that BARF1 expression in EBV-associated epithelial-like malignancies reflects the spontaneous induction of the lytic cycle in cancer cells cannot be excluded. Epstein-Barr virus (EBV) encodes two immune evasion gene products, BCRF1 (viral IL-10) and BPLF1 (deubiquitinase/deneddylase); both proteins suppress antiviral immune responses during primary infection. BCRF1 and BPLF1 genes are expressed during the late phase of the lytic cycle, an essential but poorly understood stage of viral gene expression. Several recently identified late gene regulators in betaviruses and gammaherpesviruses form a viral transcriptional precursor complex. It is not known whether each of these late gene regulators is required for transcription of all late genes.[30]

2. Materials and Methods

Diagnosis of Epstein Barr Virus

Human Epstein Barr Virus early antigen (EBEA) antibody, IgG ELISA Kit Cat.No:ED0291Hu.

Identification of Anti- apoptotic gene (BARF1) and immunomodulatory gene (BCRF1) for Epstein Barr Virus

This study was included two hundred male and female with Chronic Active EBV inflammation Patients or cancer symptoms were used in this case study and with no inflammation or cancer symptoms were used as control. Amount of blood was collected from all participants and preserve in EDTA blood collection tube that used to diagnosis BARF1 and BCRF1 gene for Epstein Barr Virus by PCR methods. The procedure of Genomic DNA extraction was done according to manufacturer's kit (Genomic Fast DNA Kit, (product number: DNK1001). The primers were design by Primer3plus. Primers sequence (Forward and Reveres) that were used for identification of Virus genes in this study, are shown in table (1):-

Table (1): Show the Primer sequence (Forward and Reveres)

Oligo	Nucleotide sequences (5 ' ' 3') (20mer)
BARF1F	CTTTCTTGGGTGAGCGAGTC
BARF1R	CTGGGAACTGAGACCTTTCG
BCRF1F	GGTAGGCCTGCACACCTTAG
BCRF1R	TCCTTTTTCCTGCAGCTTGT

Statistical analysis

Data was analyzed by using SPSS version 17. T test used to get the results. Data was presented as mean ± SD, with P value ≤ 0.05.

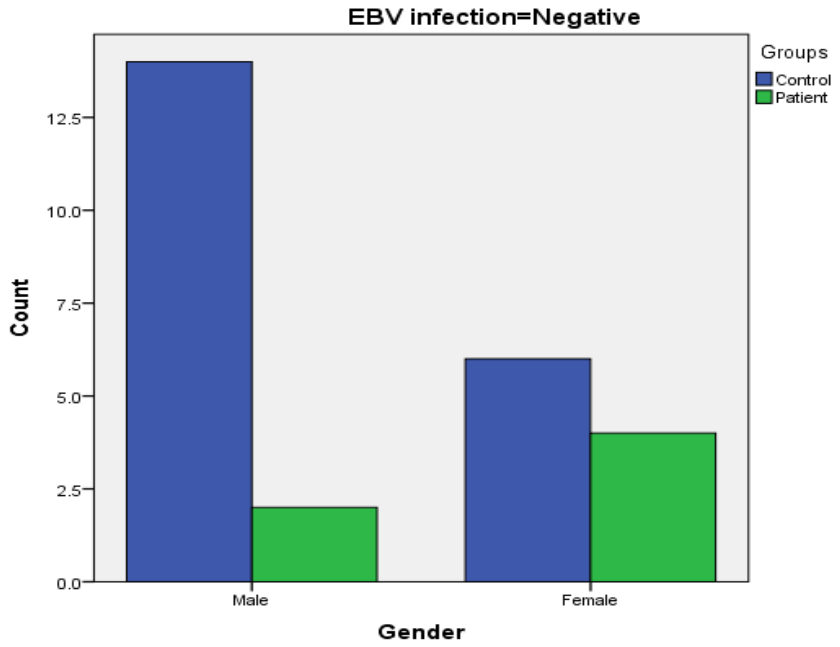
3. Result and Disscusion

EBV IgG in infection relation with gender:

In this study the results showed that the control group showed 20 people, males and females, who did not show symptoms and were not infected, out of A total 96 samples, while 76 people were infected with EBV and did not show symptoms of the virus.

While in the patient group, 6 females and males out of a total of 104 patients were not infected with the virus, and 98 females and males were found to be infected with the virus and had symptoms. The results of current study reveals the female and male with no statistically significant difference were found p value total 0.321. Table (1).

In Table (1) the study included investigating the positivity of EBV IgG infection between both the sexes, and when comparing, it was noted that there were not significant differences between IgG at p_value > 0.05 between the gender.



Detection of* BCRF$_1$ *and* *BARF$_1$* *gene

The results showed the expression of the immunomodulation BCRF1 gene and anti-apoptotic BARF1 gene in the all patients with positive EBV as presented in the figures (1&2)

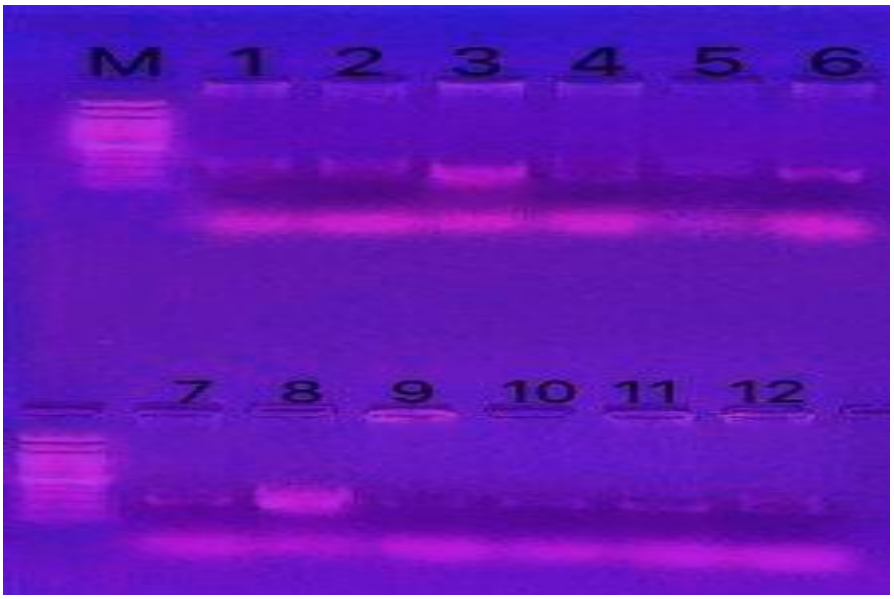


Figure (1) Gel electrophoresis of BARF1 gene polymorphism (product 345) amplify with a specific pair of primer by using conventional PCR. Using red safe as fluorescent dye, voltage 70 for 40 minutes, agarose 1.5g. Wild type on 345bp. L: ladder ,N.C : non-template negative control , Lanes (1,2,3,4,5,6,7,8,9,10,11,12) successful amplification with 345bp.

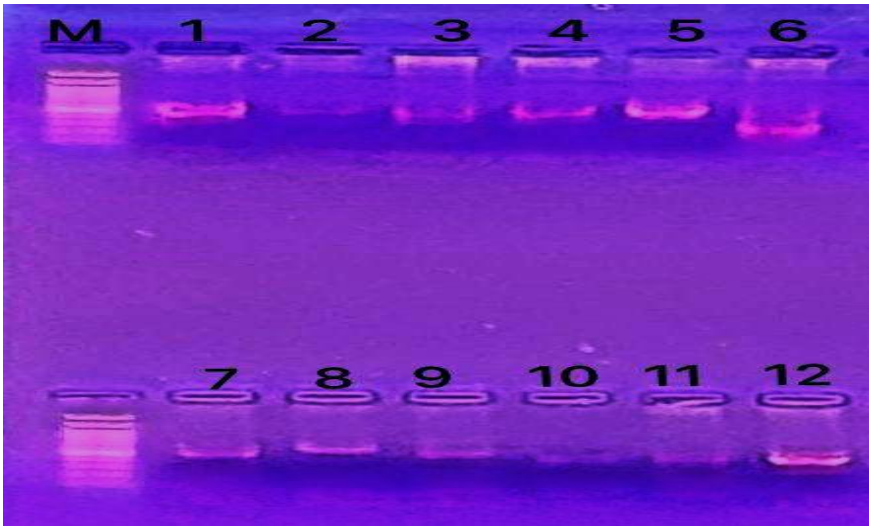


Figure (2) Gel electrophoresis of BCRF1 gene polymorphism (product 345) amplify with a specific pair of primer by using conventional PCR. Using red safe as fluorescent dye, voltage 70 for 40 minutes, agarose 1.5g. Wild type on 222bp and mutant type on 190 bp. L: ladder ,N.C : non-template negative control , Lanes (1,2,3,4,5,6,7,8,9,10,11,12) successful amplification with 222bp.

Discussion

Gel electrophoresis findings of EBV genetic material

The gene that we assayed with electrophores was the antiapoptotic BARF1 gene. To investigate the impact of BARF1 on EBV-induced transformation, we introduced recombinant BARF1 to B cells along with EBV. The addition of BARF1 did not enhance the transformation of B cells by EBV in a laboratory setting. Despite previous findings indicating BARF1's oncogenic properties in various cell lines, the deletion mutant of EBV BARF1 successfully transformed B cells and initiated latent infection. Furthermore, B cells transformed with the BARF1 mutant virus-induced tumors in SCID

mice with a similar efficiency to the wild-type recombinant virus. Given that human CSF-1 triggers the release of alpha interferon from mononuclear cells and BARF1 encodes a soluble CSF-1 receptor, we investigated whether recombinant BARF1 or BARF1 from EBV-infected B cells could impede alpha interferon secretion. The B cells transformed with mutant BARF1 EBV exhibited a decreased inhibition of alpha interferon secretion by human mononuclear cells compared to those infected with wild-type recombinant viruses. To summarize, cells were exposed to phorbol myristate acetate to induce EBV replication, irradiated with 90 Gy, and then incubated with B cells in 96-well plates; the number of wells containing transformed cells was subsequently tallied[32]. The last gene was one immunomodulatory gene, which was the BCRF1 gene.

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