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Epstein - Barr Virus: Correlation of the Presence of Associated Genes and Antibodies with Rheumatoid Arthritis

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Abstract

The study's goal is to find a link between the existence of Epstein-Barr virus nuclear antigens (EBVNA) and rheumatoid arthritis in patients. The present study was conducted on a study population with a sample size of 148 participants, comprising of two groups: the first (74) rheumatoid arthritis patients (case) and the second (74) healthy persons with no symptoms of autoimmunity (control).

The existence of the virus was investigated in both case and control groups. Patients visiting Marjan Medical City in Babil Governorate between November 2017 and May 2018 between the ages of 15 and 75 years were recruited. The patients and control samples were serologically screened using ELISA techniques prior to identification of EBV genes (EBNA1 and EBNA2) was conducted using nested polymerase chain reaction (nPCR).

The results obtained indicated that 3 samples were positive for EBNA1, while 5 were positive for EBNA2 in the patients group. No positive EBNA1 and EBNA2 positive sample was obtained in the control group. This study found that patients with rheumatoid arthritis had detectable levels of EBNA-1, EBNA-2 genes while the control group had none. These findings suggest that the virus has a role in the increased prevalence of rheumatoid arthritis.

Keywords: RA , EBV , PCR, EBNA1 and EBNA2

1. Introduction

Epstein-Barr virus (EBV) is a human herpesvirus that is carried by more than 90% of the adult population globally. EBV has a preference for B cells, and in healthy carriers, latently infected B cells serve as the viral reservoir (1). EBV are very common infectious pathogens that infect around 95 percent of the world's population in a dormant state (2). The majority of EBV infections develop during infancy and might result in a moderate, generally asymptomatic illness. Primary infection in adolescence results in infectious mononucleosis (3). It's also linked to endemic Burkitt lymphoma (4), Hodgkin lymphoma (5), diffuse large B cell lymphoma and two epithelial cancers: gastric carcinoma

and nasopharyngeal carcinoma (6). EBV-associated cancers occur most likely in immunosuppressed persons. Where up to 20% of B-lymphocytes are infected with EBV. (7)

During latent and transitory infections, the first viral protein identified was Epstein–Barr viral NA1 (EBNA 1). (8). The sole protein required for EBV genome persistence is EBNA1, which contributes to replication and histological separation of the viral genome.(9)

This study adds to the evidence that EBNA1 is involved in gene expression control (10). For example, lymphoma is required to avoid cell death.(11)

Epstein–Barr virus NA1 stimulates replication activity via its promoter location (Sp1) and causes protein expression survival. As a result of the suppression of caspase pathways in EBV-positive cells, the regulation of survival in expression will prevent cell death (12). The genome's stability, including DNA damage, chromosomal abnormalities, and double-stranded DNA, can be promoted by EBNA1, (RAG-2) and (RAG-1) and ROS (raised by transcription activity activation) (nX2) / gp91phox, catalytic sensitivity to NADPH oxidase.(13)

EBNA1 has the ability to suppress the production of tyrosine receptors r-kappa, the target gene TGF-b, and aids in the development and survival of HD cells.(14)

EBNA1 can control the malignant tumor cell in nasopharyngeal cancer by boosting expression and nuclear localization (15). EBNA1 stimulates AP-1 transcription activity and enhances vascular endothelial growth factor (VEGF) production in nasopharyngeal cancer cells, indicating that EBNA1 may contribute to blood vessel development and necrosis in nasopharyngeal carcinoma.(16)

Because of the dispersion of PML nuclear bodies, EBNA1 may be crucial for the development of nasopharyngeal cancer and then prevent malignant transformation (17).Through the activation of cellular genes and many viral genes, Epstein–Barr virus NA2 initiated a cascade transcription of primary and secondary targeted genes.(18)

These changes eventually govern B cell activation by triggering cell cycle induction of proliferation in altered growth cells.(19)

EBNA2 has the capacity to express release (c-MYC), which stimulates cell proliferation by regulating cyclin Ds and E and regulates CDK2 inhibitors at the bottom, such as p27KIP1 and p21CIP1.(20)

Epstein–Barr virus NA2 also affects Bcl6 expression, a key regulator of germinal centers difference, and B cells lymphomagenesis has been linked to the involvement of EBNA2.(21)

According to a recent research, the survival of EBNA2 positive DLBCL patients is considerably lower than that of EBNA2-negative DLBCL patients, implying that EBNA2 plays an essential role in lymphoma formation.(22)

Rheumatoid arthritis (RA) is an autoimmune disease that primarily affects the joints. This term (rheumatoid arthritis) is based on the Greek for watery and sore joints. A affects about 24.5 million people of the worlds in 2015 (23).This is between 0.5 and 1% of adults in the urban globe with 5 and 50 per 100,000 new cases is developing the condition each year (24). This condition occurred most frequently during the middle age with the women affected 2.5 times most than the men. In 2013, it resulted in 38,000 deaths up from 30,000 in 1990 (23).

2. Material and methods

Seventy-four patients consisting of 58 females and 16 males which were diagnosed with RA (according to rheumatologist physicians and serological tests) were recruited. The control group consisted of 74 individuals without any symptoms of autoimmune disease. Blood samples were collected at Marjan Medical City in Babil /Iraq, between the periods of November, 2017 to May, 2018. All the samples were diagnosed by ELISA technique, using ELISA kits EBV-VCA (IgM , IgG) antibodies (Euroimmune, Germany).

The control group were 74 case without any symptoms of autoimmune disease. All the samples were diagnosed by ELISA technique, by using ELISA kits EBV-CA (IgM , IgG) antibodies (Euroimmune , Germany).

PCR technique: The DNA was extracted from blood RA patients using extraction kit (Sacase ,USA) .

Primers: EBNA1 Inner and Outer (Lin JC et al ., 2001) ,and EBNA 2(Chen CH et al .,2009).

Nested Polymerase Chain Reaction (nPCR)

Table 1. The PCR profile included

Primer	Sequence	Amplicon
EBNA 1 Inner	F AG.ATGA.CCCA.GG.AGA A.GG C.CC A.AG C	308 bp
	R CA.AAGGG.GA G.ACG.AC T.CA A.TG. GTG T	
EBNA 1 Outer	F GT.AGAA.GGCC.ATT.TTT.CC.AC	609 bp
	R CT.CCAT.CGTC.AAA.GCT.GC. A	
EBNA 2	F AGGCTGCCCACCC.TGAG.GAT	186 bp
	R GCCACCTGGCAGCC.CTA.AAG	

3. Results and discussion

3.1 EBV Specific Primers Detection by PCR According to Age Groups in Whole Blood Sample

The study of the special genes of the EBV (EBNA 1 & EBNA 2) primers was conducted in the blood samples of the RA patients sample by using Nested - PCR technique. The results showed that 5 were given positive results for EBNA1 genes and the most of the positive results were seen in age groups (61-75) and (46-60) years.

Depending on the primer, the second gene that was used in the PCR technique EBNA2 genes from blood samples for the same patients. The results showed that there were 6 samples that gave a positive result results, also all within the same denominator (Table 2).

In case of the control group, the detection of specific primer of EBV genes (EBNA1 & EBNA2) showed that negative results and there were no DNA primer detected in all diagnosed samples. (Table 3).

Table 2. EBV Primers Detection by PCR According to Age Groups in Whole Blood Sample

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	24	0	24
31 – 45	19	0	19	0	19
46 – 60	20	2	18	3	15
61 - 75	11	3	8	3	5
Total	74	5	69	6	63

Table 3. EBV Primers Detection by PCR According to Age Groups in Whole Blood Sample (Control group)

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	0	0	0
31 – 45	19	0	0	0	0
46 – 60	20	0	0	0	0
61 - 75	11	0	0	0	0
Total	74	0	0	0	0

3.2 EBV Primers Detection by PCR According to Age Groups in Serum Sample

The study also involved an investigation of some special genes of the EBV such as (EBNA 1 & EBNA 2) primers were conducted in the blood samples of the RA patients sample by using Nested - PCR technique .

The results showed that (5) samples were given positive results for EBNA1 genes and the most of the positive results were seen in age groups (61-75) and (46-60) years.

Depending on the primer, the second gene that was used in the PCR technique was EBNA2 genes from blood samples for the same patients .

The results showed that there were (6) samples gave a positive result. Table(1)

In case of control group. The detection of specific primer of EBV genes (26) showed that negative results and there were no DNA primer detected in all diagnosed samples. Table (2)

The study investigated the primers of special genes in blood samples of rheumatoid patients in order to study the relationship between some viral genes and the incidence of rheumatism. Several studies have shown that these genes have a direct relationship with rheumatism.

(27)refer to that EBNA-1 has the capacity to cellular chromosome anchoring, for viral DNA replication action and facilitates the evasion of immune response. This process lead to viral maintenance mechanisms such as latency.

The PCR test showed that some positive samples results were observed for the presence of these parameters while the results of the control group were 0 indicating a correlation between these genes and incidence of disease .

The study also included the investigation of the EBV virus genes primers (26) in serum samples of rheumatoid patients using nested PCR.. The results showed that out of 74 serum samples, three samples were given a positive result of a EBNA1 in age groups (61-75) and (46-75).Table (4.3.6 A)

The investigation of the EBV viral primer EBNA2 in the serum sample of RA patients revealed 5 cases were positive results, these positive samples belong to the age groups(61-75) and (46-75).Table: 4.3.6 B.

Table 4. EBV Primers Detection by PCR According to Age Groups in Serum Sample.

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	24	0	24
31 – 45	19	0	19	0	19
46 – 60	20	1	19	2	18
61 - 75	11	2	9	3	8
Total	74	3	71	5	69

Table 5. EBV primers detection by PCR according to age groups in serum sample (Control group).

Age groups	No. of cases	EBNA1 +	EBNA1 -	EBNA2+	EBNA2 -
15 – 30	24	0	0	0	0
31 – 45	19	0	0	0	0
46 – 60	20	0	0	0	0
61 - 75	11	0	0	0	0
Total	74	0	0	0	0

The study also included the investigation of the EBV virus genes primers (EBNA1 & EBNA2) in serum samples of rheumatoid patients using nested PCR. The results showed that out of 74 serum samples, three samples were given a positive result of EBNA1 in age groups (61-75) and (46-75).Table (4)

At the time, the investigation of the EBV viral primer EBNA2 in the serum sample of RA patients revealed 5 cases were positive results. These positive samples belonged to the age groups (61-75) and (46-75).Table (5)

The same special parameters in the viral genes as in the results and the previous tables were also investigated in the serum samples of the disease in order to as certain the presence of these genes in the serum of the patients. The study showed that some serum samples contain the viral gene primers while the results were controlled .

The virus has the high access to serum and not only blood and also indicates the high activity of the virus in patients with rheumatism, and that these viral genes have a close relationship with rheumatism, the same documents did not show these genes in serum samples of healthy people. It is found lost and associated with patients with rheumatism. The same viral genes evaluated in the whole blood samples were also investigated in the serum samples of the diseased and control groups in order to ascertain the presence of these genes in the serum of the patients. The study showed that some serum samples contain the viral gene primers while others do not.

4. Conclusions

According to the results of the study the importance of viral genes are present in the patients' serum. Specific primers EBNA 1 and EBNA2 was detected in some samples of rheumatoid arthritis patients and these primers have a role in the pathogenesis of rheumatoid arthritis. Viral genes EBNA1

and EBNA2 are carcinogenic proteins and play a main role in causing different types of cancers and their presence in patients with rheumatoid arthritis may signal future cancers in these patients.

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