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Detection of Epstein-Barr virus in synovial fluid of rheumatoid arthritis patients

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Abstract

Introduction: Rheumatoid arthritis (RA) is one of the most common chronic inflammatory disorders. Genes and environmental factors contribute to RA. Epstein–Barr Virus (EBV) has been considered as one the RA pathogeneses. The aim of this study was to detect of the EBV genome in patients with RA.

Methods: In this cross-sectional study, 50 samples of synovial fluid were obtained from patients with RA from 2010–2012. Using a standard of the EBV genome and EBNA-1-specific primers, the method of PCR was set up. Then, all of the samples of synovial fluids separately were subjected to DNA extraction and Polymerase Chain Reaction (PCR) amplification. Data were analyzed using SPSS version 18.0. The statistical analysis was performed by the t-test.

Results: The demographic and laboratory characteristic assay revealed that the mean age of patients was 49, and the patients were 60% males and 40% females. In addition, in all cases, the mean rheumatoid factor (RF) levels of the patients were below the normal level. The results of this study showed that the PCR was able to detect EBV DNA in > 60% of the cases.

Conclusion: The results of this study indicated that EBV was frequently detected in the synovial fluid of RA patients. Thus, EBV may be a strong candidate that can act at several levels of the pathophysiology of RA. However, these findings also indicated that EBV may play a role in the pathogenesis of RA. However, the possible relationship between RA and EBV must be determined by further research.

Keywords: rheumatoid arthritis, EBV, EBNA-1, PCR

1. Introduction

Epstein-Barr virus is one of the human herpes viruses with a 184 kbp-sized, double-stranded DNA genome. It is categorized in the gamma 1 or lymphocryptovirus (LCV) genus, gamma herpes virus subfamily, and herpesviridae family. Epstein-Barr virus is a ubiquitous virus that infects 95% of the world's population (1). In 1964, EBV was the first virus detected from human neoplastic cells (2). EBV is known to have a relationship with Burkitt lymphoma, nasopharyngeal carcinoma (NPC), Hodgkin's lymphoma, and gastric carcinoma. In addition, the association

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between EBV and some autoimmune diseases, such as systemic lupus erythematous (SLE) and RA, have been documented (2). The association between EBV and rheumatoid arthritis was first reported by Alspaugh and Tan (3). This study showed that sera from RA patients contain antibodies that precipitate antigens associated with EBV-infected B cell lines (3). The antigen was named RA nuclear antigen (RANA), and it is identical to Epstein-Barr nuclear antigen 1 (EBNA-1) (4). Many other studies have shown that patients who have rheumatoid arthritis have higher levels of serum antibodies against early antigen (EA), Epstein-Barr nuclear antigen (EBNA), and viral capsid antigen (VCA) than normal people (5-11). There is a similarity in the sequence and the cross reaction between Epstein-Barr virus proteins and joint proteins (12). However, several studies have shown that Epstein-Barr virus was present in the synovial tissue obtained from RA patients (13-19). Scotet et al. showed that synovial tissues from RA patients contain cytotoxic T lymphocytes specific for EBV-encoded proteins (20). The synovial tissue of an RA patient has a fibroblast-like cell line that contains EBV (21). However, some studies have failed to detect EBV DNA in synovial tissue from RA patients (22), but the expression of the latent membrane protein 1 (LMP1) and the small EBV-encoded RNAs (EBERs) of EBV have been reported in synovial cells from eight out of 34 RA patients (17). This study was designed to evaluate the presence of the EBV genome in the synovial tissue of RA patients.

2. Material and Methods

2.1. Patient Population

This cross sectional study was performed between 2010 and 2012 on 50 patients. The samples of synovial fluids that were used for this study were taken from 50 patients with rheumatoid arthritis who were admitted to Baqiyatallah Hospital in Tehran, Iran. For each case, information of age, gender, and laboratory parameters was collected. These samples were related to patients with confirmed rheumatoid arthritis from 2010–2012, and the mean RF factor of all patients was below the normal level. This study was approved by the Research Ethics Committee at Baqiyatallah University of Medical Sciences, Tehran, Iran.

2.2. Extraction of DNA

DNA was isolated from samples of the synovial fluids using QIAamp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Briefly, a 500-µl aliquot of synovial fluids was added to 6 µl of mutanolysin, and the mixture was incubated at 37 °C for 30 min. Then, 50 µl of Proteinase K with a concentration of 20 mg/ml and 500 µl of AL buffer were added, and the sample was incubated at 56 °C for 30 min. After this, 500 µl of ethanol were added, and the DNA was purified by using the columns provided in the kit (Qiagen, Valencia, CA).

2.3. Polymerase chain reaction (PCR)

The PCR was carried out with forward primer (5′-GGGCCAAGACATAGAGATGGTGTCC-3′) and reverse primer (5′-GCGGTGGAGACCCGGATGATGATGA-3′) (23). The PCR for the amplification of the EBV EBNA-1 was performed by adding 10 µL of the extracted DNA to 40 µL of the reaction mixture that contained 26 µL distilled water, 5 µL 10X PCR buffer, 4 µL dNTP, 2.5 µL MgCl2, 1 µL forward primer, 1 µL reverse primer, and 0.5 µL of Taq DNA polymerase. Amplification conditions consisted of 5 min at 95 °C, 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 10 min. All PCRs were run with positive and negative controls. The PCR amplicons were 226 bp. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel and viewed under UV light.

2.4. Statistical methods

The data were analyzed using SPSS statistical package version 18.0 (SPSS, Inc, Chicago, IL, USA). The statistical analysis was performed by the chi-squared test and Fisher's exact test. The t-test was performed to compare the means of the continuous variables. All P values were two-sided and considered significant at $p < 0.05$.

3. Results

Out of the synovial fluid samples from the 50 patients with confirmed rheumatoid arthritis, 30 cases (60%) had EBV DNA in their synovial fluids. This represented a significant difference with the control group ($p \leq 0.04$). Among the 50 patients, 30 samples were from men (60%) and 20 samples were from women (40%). The statistical analysis of the patients with rheumatoid arthritis and the mean changes of the laboratory parameters, including rheumatoid factor, Alanine Transaminase, Alanine Aminotransferase, White Blood Cell Count in Blood, Platelets, C Reactive Protein, Erythrocyte Sedimentation Rate, Alkaline Phosphotase, anti-cyclic citrullinated peptide antibody, White Blood Cell Count in Synovial fluid, and Polymorphonuclear leukocytes in the synovial fluid, are shown in Table 1. The mean age for all patients was 49. The mean levels of the laboratory parameters RF, ALT, AST, and WBC in

blood; PLT, CRP, ESR, ALP, Anti-CCP, and WBC in the synovial fluid; and PMN in the synovial fluid were as 1.9167, 28.9048, 21.4286, 707.2222, 247.7600, 1.5217, 31.2000, 174.1250, 138.22424, 336.9767, and 68.9512 per ml, respectively. The mean duration of disease (years) and duration of treatment (years) for all patients were 5.8068 and 3.6667, respectively.

Table 1. Statistical analysis of patients and laboratory parameters

Variable	n	Minimum	Maximum	Mean	SD
Age	60	16.00	70.00	49.0000	13.24249
Duration of disease (year)	44	1.00	30.00	5.8068	4.75592
Number of Injection	22	0.00	10.00	3.4091	2.71958
Duration of treatment (year)	3	2.00	6.00	3.6667	2.08167
Cumulative drug Dosage	23	0.20	2.00	.6609	0.52374
WBC in Blood	60	1020.00	13500.00	7072.2222	2237.98092
PLT	60	126.00	451.00	247.7600	86.42881
CRP	60	1.00	4.00	1.5217	0.89796
ESR	60	4.00	96.00	31.2000	21.28628
RF	60	1.00	3.00	1.9167	0.66856
AST	21	10.00	72.00	21.4286	13.61092
ALT	21	6.00	145.00	28.9048	29.77399
ALP	8	88.00	258.00	174.1250	61.48969
ANTICCP	19	0.00	500.00	138.2242	142.21142
WBC in Synovial fluid	43	1000.00	10000.00	4336.9767	2731.18306
PMN in Synovial fluid	41	25.00	95.00	68.9512	16.87891

4. Discussion

Rheumatoid arthritis (RA) as an inflammatory disorder that involves synovial destruction. The etiology of RA still is unknown, but genetic and environmental factors may contribute to the disease (24). Among environmental factors, viruses play important roles for triggering this disease, and, among them, Epstein-Barr virus (EBV) was first suggested as a causative agent (3). This study investigated EBV DNA genomic in RA patients' synovial fluids using PCR methods for the EBV EBNA-1 gene. Our results showed that EBV DNA was detected in ⩾ 60% of rheumatoid arthritis patients' synovial fluids. The high prevalence of EBV that was detected in this study was similar to a previous study. For example, Takeda et al. evaluated the existence of EBV DNA in the synovial tissue of patients with RA using Southern blot hybridization and PCR methods. Epstein-Barr virus DNA was detected by PCR in 15 of the 32 samples from the RA patients (47%), but it was not detected in any of the 30 osteoarthritis patients. This study demonstrated that EBV was detected frequently in the synovial tissue of rheumatoid arthritis patients (25). Synovial tissue from 84 patients with RA and 81 patients with non-RA were investigated for EBV DNA/RNA using PCR method, and the results indicated that the EBV DNA and/or RNA were significantly higher in RA patients than in non-RA patients. Thus, the results suggested the EBV is related to RA pathogenesis (26).

Chiu and et al.'s study showed that all synovial fluid samples from patients with non-resolving inflammation of rheumatoid arthritis showed positive expression of Epstein-Barr virus-encoded small RNA1 (EBER1), whereas none of the synovial samples from patients with osteoarthritis showed expression of EBER1. They suggested that non-resolving rheumatoid arthritis inflammation is related strongly to the presence of EBER1and Epstein-Barr virus. EBER1 is responsible for synovial fibroblast interleukin-6 production (27). Balandraud and et al.'s study results showed that patients with rheumatoid arthritis have higher EBV load in peripheral blood lymphocytes than healthy controls. This study recommended anti-EBV antibody responses should be considered as one of the chronic autoantibody responses that are most relevant to the development of rheumatoid arthritis (28). One study produced results that showed elevated EBV load in their peripheral blood mononuclear cells (PBMCs), which demonstrated in patients with RA, the Epstein-Barr virus DNA load was increased almost 10-fold. This study showed that patients with rheumatoid arthritis had elevated EBV DNA viral load in their peripheral blood (29). This study showed a high prevalence of EBV DNA detection in synovial fluid samples. So, this study suggested that EBV could play a role in the pathogenesis of rheumatoid arthritis. The EBV viral particles may act as potent superantigens that are involved in triggering inflammatory diseases, such as RA. It should be noted that this is not the only superantigen that contributes to RA disease, since recent research has indicated that several other bacterial superantigens, including

Staphylococcal (30-32) and Mycoplasma superantigens (33, 34), were reported in the synovial fluids of RA patients. In addition, the existence of Staphylococcal superantigens in CSF of meningitides patients were reported (35). However, the similarity point of the individual multiple superantigens in pathophysiology of the disease is unknown, and more research will be required to clarify their roles.

5. Conclusions

The main findings of this study indicate that the EBV DNA gene were amplified in 30 cases (60%) of synovial fluids of rheumatoid arthritis patients. However, the results of this study suggested a possible role for the EBV in the pathophysiology of RA as an autoimmune disease and specific antiviral treatment may be recommended for RA patients. More research is needed to define the pathophysiology of RA disease.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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