The association of Epstein-Barr Virus Antibodies with Rheumatoid ArthritisamongYemeni patients in Sana'a city

Abstract

Background and objective: Rheumatoid arthritis (RA) is a chronic autoimmune disease that isassociated with progressive disability, systemic complications and early death. Etiology of RA is unknown. It is assumed that environmental factors initiate RA development ingenetically susceptible individuals.Epstein-Barr Virus(EBV) stimulates polyclonal B cell activation and has been suggested to play a role in RA pathogenesis. Our study aimed tostudythe association between EBV and RA.

Methods: One hundred and sixty subjects were enrolled in the study.Eighty individuals were clinically diagnosed to have RA and confirmed by anti-CCP3 test.The remaining 80 individuals were healthy controls matched for age and sex. Serum IgG and IgM antibodies against EBV viral capsid antigen(VCA) weretested by anenzyme-linked immunosorbent assay (ELISA).

Results: The crude prevalence rate of EBV-VCA IgM antibodies among patients was (21.2%)while in healthy individuals was (8.7%) with significant OR equals to 2.8 times for RA patient's. The female prevalence rate of EBV-VCA IgM antibodies was (21.8%) higher than of male(18.7%). Moreover, the crude prevalence rate of EBV-VCA IgG antibodies for RA patients was (91.3%) while in healthy individuals was(76.3%)with significant OR equals to 3.2 times for RA patients'. The female prevalence rate ofEBV-VCA IgGantibodies was (95.3%) higher than of male(75%).

Conclusion:EBV-VCA IgGand IgM antibodiestiters were elevated in RA patients than in healthy controls. However, the causative relationship between EBV and RA is complex and involvesdifferent mechanisms.

Key words: Anti EBV-VCA IgG antibodies, anti EBV-VCA IgM antibodies, Epstein-Barr Virus, Rheumatoid arthritis, Yemeni

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder which is common among females at an older age.Worldwide prevalence of RA is estimated to be about 0.5%-1%.The cause of RA remains unclear though it has been proposedbyprevious studies that both genetic and environmental factors play an important role in RA pathogenesis[1-3].RA genetic susceptibility is carried by HLA-DRB1* alleles containing the QK/RRAA or RRRAA motif in their third hypervariable region. This motif is known as the shared epitope[4]. It is associated with low occurrence of T cells specific for EBV gp110, a replicative phase glycoprotein critical for the control of EBV infection[5]. One environmental trigger may be the Epstein-Barr virus (EBV).

EBV is a double-stranded DNA herpesvirus that is extremely common worldwide, infecting about 98% of the human populationby the age of 40 years[6]. It is transmittedthrough saliva.It infects and replicates in epithelial cellsand B cells. EBV then becomes latent within memory B cells andpersists for the lifetime of the host[7].It causes acute infectious mononucleosis. It also reported to have association with nasopharyngeal

carcinoma, Hodgkin and non-Hodgkin lymphomas, gastric carcinoma, Burkitt lymphoma and other lympho-proliferative disorders in immunocompromised individuals[8].

For long time, EBV has been suspected as a possibleetiology of autoimmune diseases including RAdue to its high prevalence in the population and its lifelonginfection after primary infection[9, 10]. The association between EBV and RA was first reported by Alspaugh and Tan. They reported that sera from RA patients were reactiveagainst a nuclear antigen in EBV-transformed lymphocytes[11]. Association between RA pathogenesis and EBV has been linked to molecular mimicry. Several EBV antigens share similarities with self-antigens; more specifically, glycine/alanine repeats in EBNA-1 resemble synovial proteins.Antibodies againstthis repeat cross-react with a 62kD protein in RA, but not in normal synovium[12, 13].EBV DNA loads are higherin mononuclear cells isolated from active RA patients compared to healthyseropositive individuals as well as EBV serology. Furthermore,antibodies directed against cyclic citrullinated peptides (ACPA) whichare used as confirmatory test for RA diagnosis, were found to react with acitrullinated sequence of Epstein-Barr nuclear antigen-1 (EBNA-1),supporting the association between EBV and RA[14]. Ourstudy aimed to investigate the association between EBV and RA via measuring EBV-VCA IgM and IgG in RA patients compared with healthy controls.

2. Subjects and methods

This study is a case-control study conducted from October 2014 to October 2015. A total number of 160 individuals were included in the study. Eighty persons were clinically diagnosed with RA and confirmed by measuring anti-CCP3. The other 80 were healthy individuals used as controls. A full history from each RA case and healthy control was recorded on a predesigned questionnaire. The study was carried out at Al-Thawra Modern General Hospital and National Center of Central Public Health Laboratories, in Sana'a city, Yemen. Patients with other autoimmune diseases,infectious mononucleosis, Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, or with HIV were excluded from the study.

Five ml of venous blood was collected from each individual into plain vacationer tubes. The specimens were allowed to clot at room temperature and centrifuged at 3500 rpm for five minutes. Serum was separated from each sample into Eppendorf tubes and stored at -20°C until tested.

EBV virologic assays to measure IgG and IgM viral capsid antigen (VCA) were performed using NovaLisaEBV ELISA kits provided by (NOVA TEC, Dietzenbach, Germany). The commercially ELISA test for anti-CCP3 was carried out according to the manufactures instructions (INOVA Diagnostics Kits, San Diego, CA-USA). Statistical analysis of data was performed using the Epi Info statistical program version 6 (CDC, Atlanta, USA).

3. Results

Table 1 shows the characteristics of RA patients and healthy controls. Out of 80 RA cases, 64 (80%) were females while 16 (20%) were males. Their age ranged from 20 to 80 years with mean age 42.3±16.3 years old. Likewise, the control group involved 64 (80%) females and 16 (20%) males. Their age ranged from 20 to 80 years with mean age 39.6±11.2 years old. Most of the cases and controls were at the age group of≥50 years old.

Table 2 shows the prevalence rate of EBV-VCA IgM antibodies in different sex and age groups for RA patients and healthy controls. EBV-VCA IgM antibodies among RA females, 14 (21.8%), were higher than that of RA males, 3 (18.7%), with an OR equals to 2.7 times for females than males whereas in control females, 6 (9%), and males, 1(6%). As regard to the age, the serum EBV-VCA IgM antibodies in RA patients were highest at the age group of 40-49, years in which the rate was (30%), followed by the age group of ≥50 years (25%), then the age group of 30-39 years (18.7%), and finally the age group of 20-29 years (13.6%). Among the controls, corresponding numbers were 1(10%), 3(9.3%), 2(12.5%), and 1(4%), respectively. When we compared the crude prevalence rate of EBV IgM antibodies among cases and controls, we found that the crude prevalence rate among RA patients was 21.2% while among controls was 8.7%. OR of EBV infection for RA cases was 2.8 times, and this association was ranged from 1.01 up to 8.1, with significant $\chi 2$ (4.9) and statistically *p* equals to 0.02.

Table 3 demonstrates the prevalence rate of EBV-VCA IgG antibodies in different sex and age groupsof RA patients and healthy controls. The EBV-VCA IgG antibodies among females, 61 (95.3%), were higher than that of males, 12 (75%), among cases with an OR equal to 5.2 times for females than maleswhereas, in control females, 51(79.6%),and males, 10 (62.5%). As regard to the age, the serum EBV-VCA IgG antibodies were highest at the age group of ≥50 years old, in which the rate was (100%), followed by the age group of 40-49 years (90%), then the age group of 30-39 years (87.5%), and finally the age group of 20-29 years (81.8%).Among the control, corresponding numbers were 28(87.5%), 7(70%), 12(75%), and 14(63.6%), respectively. When we compared the crude prevalence rate of EBVIgG antibodies among cases and controls we found that the crude prevalence rate among RA patients was 91.3% while among controls, was 76.3%. OR of contract EBV infection for RA cases was 3.2 times, and this association was ranged from 1.2 up to 9.2, with significant χ^2(6.6) and statistically *p* equal to 0.01.

4. Discussion

RA is a systemic autoimmune disease of unknown etiology. Both genetic and environmental factorsare suggested to contribute to RA pathogenesis [1, 15]. Epstein-Barr virus was proposed as an environmental triggerof RA.It causes massive polyclonal expansion of resting lymphocytes and becomes latent within memory B cells for the lifetime of the host[16]. Thisstudy aimed to investigate the association between EBV and RA via measuring EBV-VCA IgM and IgG in RA patients compared with healthy controls.

Our study showed a highly significant rate and associated OR of positive EBV-VCA IgM and IgGin RA patients than in healthy controls. Several studies have shown elevation of EBVantibodies in RA patients than healthy controls[14, 17, 18]. So far,there is no decisive theory to explain how EBV is involved in RA pathogenesis. However, several representative hypotheses on the possible mechanisms of EBV's involvement in autoimmune diseases are described[19]. One mechanism by which EBV can trigger RA is molecular mimicry[20]. Studies found antibodies against EBV-encodedproteins cross-react with RA-specific proteins[7, 11, 21]. This finding supports the molecular mimicry hypothesis in RA pathogenesis either by influencing T cell receptor recognition of the HLA 'shared epitope' or through production of autoantibodies against joint antigens[6]. Molecular

mimicry between a major EBV epitope and severalautoantigens might contribute to a breakdown of tolerance and autoimmunity in patientswith RA[22].

Another possible mechanism is through autoreactive B cellstheory. It hypothesizes that in patients with certain autoimmune diseases,EBV infects autoreactive B cells leading to autoantibodies production[23, 24]. Third mechanism is through the mistaken-self theory. EBV-infected B cells together with closely located activated T cells have been demonstrated in the synovial lesions of RA[25, 26].

High titers of EBV antibodies is not attributed to more frequent EBV reactivation in RA patients as a result of immunosuppression therapy. Studies monitored EBV viral load in RA patients under TNF blockers for one to five. EBV load was stable over time, evenwhen TNF blockers were associated with methotrexate[27-29].

In conclusion, high titers of EBV antibodies are associated with RA. However, the causative relationship between EBV and autoimmune diseases is complex and involvesdifferent mechanisms.

Acknowledgments:

The authors thank physicians and specialists at the Department of Rheumatology at Al-Thawra Modern General Hospital and staff at Virology Department atNational Center of Central Public Health Laboratories for their great help during study performance.

Conflict of Interest:

There is no conflict of interest to be declared

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Table 1: Age and sex of rheumatoid arthritis cases and healthy controls

Variable Characteristics	Cases **(n=80)**		Controls**(n=80)**		Total**(n=160)**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Age/ Years						
20-29	22	27.5	22	27.5	44	27.5
30-39	16	20.0	16	20.0	32	20.0
40-49	10	12.5	10	12.5	20	12.5
≥50	32	40.0	32	40.0	64	40.0
Total	**80**	**100**	**80**	**100**	**160**	**100**
Mean/ Years	42.3		39.6		39.9	
SD/ Years	16.3		11.2		14.5	
Min./ Years	20		20		20	
Max./ Years	80		80		80	
Sex						
Females	64	80.0	64	80.0	128	80
Males	16	20.0	16	20.0	32	20
Total	**80**	**100**	**80**	**100**	**160**	**100**

Table 2: Seropositive for EBV-VCA IgM for RA patients and healthy controls

Age and Sex groups	**Seropositive for EBV-VCA IgM**				**OR**	**CI**	χ^2	***P***
	Case		**Control**					
	No.	**%**	**No.**	**%**				
Age/ Years								
20-29	3	13.6	1	4	4	0.26-9.0	1.1	0.29
30-39	3	18.7	2	12.5	1.6	0.17-16.7	0.24	0.62
40-49	3	30	1	10	3.8	0.24-12.1	1.25	0.26
≥50	8	25	3	9.3	3.2	0.66-17.5	2.74	0.09
Sex								
Female **(n=64)**	14	21.8	6	9	2.7	0.8-8.6	3.8	0.05
Males **(n=16)**	3	18.7	1	6	3.46	0.3-9.8	1.14	0.28
Crude rate IgM	17	21.2	7	8.7	2.8	1.01-8.1	4.9	0.02

Table 3: Seropositive for EBV-VCA IgG for RA patients and healthy controls

Age and Sex groups	Case		Control		OR	CI	χ^2	*P*
	No.	**%**	**No.**	**%**				
Age/ Years								
20-29	18	81.8	14	63.6	2.6	0.54-13	1.8	0.17
30-39	14	87.5	12	75	2.3	0.3-22.7	0.82	0.36
40-49	9	90	7	70	3.86	0.24-121	1.25	0.26
≥50	32	100	28	87.5	Undefined		4.2	0.03
Sex								
Female **(n=64)**	61	95.3	51	79.6	5.2	1.28-24.4	7.14	0.007
Males **(n=16)**	12	75	10	62.5	1.8	0.3-10.7	0.58	0.44
Crude rate IgG	73	91.3	61	76.3	3.25	1.2-9.2	6.6	0.01