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Juvenile Rheumatoid Arthritis in Children with *Ebstein barr virus* Infection

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Abstract: Juvenile Rheumatoid Arthritis (JRA) is a disease of unknown etiology. A total of 50 patients with JRA who were hospitalized in the Pediatrics Rheumatology Ward of Imam Khomeini Hospital in Tehran during the years 2001-2002, were assessed serologically (IgM and IgG specific viral capsid antigens) for EBV infection and their response to therapy was studied. Minimum age of the patients was at least 6 months and mean age was 60.96 plus/minus 43.46 months. EBV infection was seen in 44 (88%) patients 24 of whom were girls and 20 boys. Ninety two percent of girls and 83% boys were infected with the virus. *Ebstein barr virus* (EBV) infection was seen in 33 cases, 6 cases, 4 cases and 1 case in the polyarticular, pauciarticular, systemic and spondylitis group, respectively. Fifty four percent of EBV-positive patients with JRA did not respond to the classic therapy. EBV virus is involved in the pathogenesis of JRA and patients with EBV are in greater risk of developing JRA.

Key words: JRA, EBV, infectious mononucleosis, children

INTRODUCTION

JRA is the most common rheumatic disease among children and is one of the most common causes of chronic disability. Annually, around 13.9 per 100,000 children develop this disease by age 15. JRA is characterized by synovitis of peripheral joints and is associated with soft tissue inflammation and accumulation of fluid in the joints. Its etiology is unknown but at least two factors are worthy of attention for immunogenic susceptibility and probable environmental factors.

At the top of the probable factors initiating this disease are specific viruses including: EBV, *Parvovirus* B_{19} and *Rubella virus* (Modarress and Modarress, 1998; Miller and Cassidy, 2004; David, 2001; Mehraein *et al*., 2004). Amongst these factors, the EBV, a herpes virus which affects immune cells, has a special role. Other factors include increased host response to autoantibodies such as collagen type II and increased reaction of T-lymphocytes to specific proteins related to bacteria and mycobacteria (Miller and Cassidy, 2004; David, 2001). On assessment of patients with JRA, EBV has a major role in the pathogenesis of disease and the load of EBV was higher in T-lymphocytes. Patients with JRA have a higher EBV load in their peripheral blood lymphocytes (Balandraud *et al*., 2004). In the studies performed in Iran, more than 70% of children up to 14 years of age, develop EBV (Modarress and Modarress, 1998). Now researchers believe that EBV plays the primary role in the pathogenesis of JRA (Cassidy and Petty, 1995; Blaschke *et al*., 2000) After entering the body, viremia develops and peripheral blood B-lymphocytes and the lymphoreticular system, including the liver and spleen, become infected with the virus. Atypical lymphocytes, which are characteristic of infectious mononucleosis are actually T CD_8^+ cells which are produced in response to B-lymphocytes infected with EBV. The primary goal of EBV is the B-lymphocyte, which stimulates T-lymphocytes after becoming infected with the EBV. Immune response has also been confirmed in synovial T-lymphocytes in JRA (Miller and Cassidy, 2004). Recent publications provide evidence for an altered Epstein-Barr virus host balance in patients with rheumatoid arthritis, who have a relatively high Epstein-Barr virus load. Overall, patients with rheumatoid arthritis have two-fold increased risk of developing lymphoma. Some, but not all, of this higher risk reflects an increase in EBV-associated lymphomas. This in turn may be influenced by the elevated EBV load found in JRA patients and may reflect subtle impairment of antiviral immunity in this group of patients (Callan, 2004).

Present study we want to search if EBV plays the primary role in the pathogenesis of JRA?

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MATERIALS AND METHODS

Children with JRA who were hospitalized in the Pediatrics Rheumatology Ward of Imam Khomeini Hospital in Tehran during the years 2001-2002, were entered into the study.

The students were enrolled in the study voluntarily and with full knowledge about the procedure of the study only after the Health Authority of each school had obtained parent consent. Before starting treatment, 2 mL of venous blood was taken from each child and studied for presence of EBV capsid antigen (EBVCA), for which the sera was separated and stored at a temperature of -20°C. In the next stage, the antibody was tested for using the special anti EBV Enzygnost kit and conjugated serum with I gG and I gM human specific antigens and alkaline phosphatase (Behring, Germany, Marburg Company). The patients were then treated by conventional methods.

Different groups were defined based on probable time of infection (Table 3).

Group 1: Patients with positive IgM and IgG titers.
Group 2: Patients in whom only serum IgG is positive.
Group 3: Patients in whom only serum IgM is positive.
Group 4: Patients with negative IgM and IgG titers (Table 3).

All patients were given Tolmetin and Metotrexate and steroids were added in case of systemic type of disease in the presence of pericardial or pleural effusion. After at least 6 months of treatment, expected changes in the general health of the patient including: body temperature, improvement of joint pain or swelling, probable involvement of a new joint and feeling of wellness were assessed. All changes were compared After at least 6 months in both groups.

- Patients in whom antibody titer was positive as compared to EBV antigen.
- Patients in whom antibody titer was negative as compared to EBV antigen.

Pulse therapy was used in cases with failure to respond to classic treatment (intravenous methylprednisolone injection 30 kg day^{-1} for three consecutive days).

RESULTS

A total of 50 patients with JRA were studied. The age of onset of disease ranged from 6-144 months and mean age was 60.96±43.46 months. The patients were divided into four groups according to onset of disease:

0-2 years: Eleven patients (22%) with minimum age 6 months,
2-4 years: Twelve patients (24%),
4-10 years: Ninteen patients (38%) and
10-12 years: Eight patients (16%).

Among the different types of JRA, most patients had the polyarticular type, which comprised 74% of cases. The number of polyarticular cases clearly increased with advancing age. Pauciarticular (5 cases, 10%) and monoarticular (2 cases, 4%) JRA were seen in 14% of cases and systemic JRA comprised 5 cases (10%). Among the patients with polyarticular JRA, 43% were aged above 10 years. In present study, the frequency of polyarticular disease was more in subjects aged less than 10 years. Among the pauciarticular cases, 40 and 60% were aged less than and more than two years, respectively. In the systemic group, 60 and 40% were aged less than and more than two years, respectively. There was a significant statistical relationship between age group and type of JRA ($p = 0.021$) (Table 1). Overall, 26 cases were girls (52%) and 24 (48%) were boys. There was no significant relationship between type of JRA and gender ($p = 0.853$). In most cases (46%) arthritis was first seen in the wrist and ankle joints and in 24% of cases it was seen in the knee joint. Forty one cases (82%) had fever in the onset of disease. Thirty percent patients had skin rash which appeared at the time of fever and it was seen in 11 (22%) boys and 4 (8%) girls. According to laboratory findings, leukocytosis, polynucleosis and thrombocytosis were seen in 21 (42%), 35 (70%) and 21 (42%) of cases, respectively. Polynucleosis was seen in 27 (54%) cases with polyarticular JRA and all patients with systemic JRA (100%) and 3 cases (60%) with pauciarticular JRA. There was a significant relationship between type of disease and polynucleosis JRA ($p = 0.049$). Ninty four percent and 76% of cases had positive ESR (above 20 mmHg) and CRP, respectively. Specific Antibody level, EBV IgG was positive in 22 cases (44%) ($p = 0.475$) and IgM specific antigen, was positive in 41 case (82%) ($p = 0.300$) and none had a significant relationship (Table 2). Among the 28 IgG-negative patients, 22 had positive IgM antibody titers (Table 2). Out of the 50 patients under study, 44 (88%) were infected with the EBV (Table 3).

The number of infected patients increased with advancing age. There was a significant relationship between infected and non-infected subjects in different age groups ($p = 0.031$) (Table 4). Among the 44 cases with EBV infection, 24 (48%) and 20 (40%) were girls and boys, respectively. On the whole, in our study 92% of girls and 83% of boys were infected. Patients were assessed based on type of antibody and according to response to therapy (Table 5).

Table 1: Types of juvenile rheumatoid arthritis based on age

	Age groups									
	0-2 years		2-4 years		4-10 years		10 years		Total	
JRA	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Polyarticular	2	4	4	8	15	30	16	32	37	74
Paucyarticular	2	4	2	4	-	-	1	2	5	10
Monoarticular	-	-	1	2	1	2	-	-	2	4
Systemic	3	6	1	2	-	-	1	2	5	10
Spondylitis	-	-	-	-	-	-	1	2	1	2
Total	7	14	8	16	16	32	19	38	50	100

Table 2: Specific antibodies against EBVCA in JRA

	JRA									
	Polyarticular		Paucyarticular		Systemic		Spondylitis		Total	
Antibody	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
IgG positive	17	34	4	8	1	2	-	-	22	44
IgG negative	20	40	3	6	4	8	1	2	28	56
IgM positive	32	64	4	8	4	8	1	2	41	82
IgM negative	5	10	3	6	1	2	-	-	9	18

Table 3: Types of JRA based on probable time of EBV infection

	JRA									
	Polyarticular		Paucyarticular		Systemic		Spondylitis		Total	
Groups	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
1	14	28	2	4	3	6	1	2	20	40
2	33	66	6	12	4	8	1	2	44	88
3	1	2	2	4	-	-	-	-	3	6
4	4	8	1	2	1	2	-	-	6	12

Table 4: EBV infection in children with JRA

	Age groups									
	0-2 years		2-4 years		4-10 years		10 years		Total	
JRA	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
IgG +, IgM +	-	-	2	4	6	12	11	22	19	38
IgG -, IgM +	3	6	4	8	7	14	8	16	22	44
IgG +, IgM -	-	-	-	-	1	2	2	4	3	6
IgG -, IgM -	3	6	1	2	1	2	1	2	6	12
Total	6	12	7	14	15	30	22	44	50	100

Table 5: Response to therapy based on EBVCA Antibody

	Response					
	Good response (Conventional therapy)		Bad response (Pulse therapy)		Total	
Antibody	n	(%)	n	(%)	n	(%)
IgG positive	16	32	25	50	41	82
IgG negative	3	6	6	12	9	18
IgM positive	11	22	11	22	22	44
IgM negative	8	16	20	40	28	56

DISCUSSION

In this study, a total of 50 patients with JRA were enrolled (age range = 6 months to 12 years). Sixty four percent of patients (22 persons) were in the 4-10 years age group (Table 1). 26 (52%) were girls and 24 (48%) were boys. However, in some studies the number of girls is twice that of boys (Siegel, 1994). The systemic type of disease was seen in 3 (60%) girls and 2 (40%) boys while gender does not count in the systemic form of disease. The most common clinical finding in our study was polyarticular JRA (74%) (Table 1) whereas it was reported

to be 30% in other studies (Siegel, 1994; Jenson, 2004a, b; Sumaya, 2000; Mandell and Calabress, 1998) Pauciarticular JRA was seen in 5 (10%) patients and monoarticular type was seen in only 2 (4%) patients, making a total of 14% of cases, whereas the pauciarticular type was reported as 60% in data from different regions (Siegel, 1994; Jenson, 2004; Sumaya, 2000; Mandell and Calabress 1998).

One of the causes of this difference was a difference in the period of time that the patients were studied; many first present with pauciarticular JRA which may later develop into polyarticular form. Systemic JRA comprised 5 persons (10%) which is in agreement with other references (Siegel, 1994; Jenson, 2004; Sumaya, 2000; Mandell and Calabress, 1998). Forty three percent of subjects with polyarticular disease were aged above 10 years, whereas the most prevalent age is 10 years or more in other studies (Table 1) (Sumya, 2000). There was no significant relationship between type of disease and gender ($p = 0.853$). Forty one subjects (82%) had fever. There was a significant statistical relationship between age of onset of disease and fever ($p = 0.000$, t-test), which is in agreement with other studies. Eighty nine percent of patients in the polyarticular group, 80% of patients in the systemic group and 57% of patients in the pauciarticular group developed fever. There was a significant statistical relationship between different types of JRA and fever ($p = 0.032$). While according to references, fever is more common in the systemic form of disease (Balandraud *et al*., 2004; Sumaya, 2000). Fifteen percent of patients had skin rash which appeared at the same time as fever. A significant statistical relationship was not found between rash and type of disease ($p = 0.09$). However, 80% of subjects in the systemic group developed skin rash, which is in agreement with other studies (Balandraud *et al*., 2004; Sumaya, 2000). Among the 15 patients with skin rash, 9 (60%) were aged above 2 years. There was a significant relationship between age of onset of disease and skin rash ($p = 0.000$, t-test). Other studies have not clearly discussed this relationship but it has been found to be associated with the systemic form of disease which is usually seen in younger ages (Balandraud *et al*., 2004; Sumaya, 2004). There was also a significant relationship between skin rash and gender ($p = 0.019$) and this condition was more frequent among males. Other studies have not pointed to this relationship. Leukocytosis was seen in 21 subjects (42%) but polynucleosis was present in 35 cases (70%). Other references have only pointed to leukocytosis (Balandraud *et al*., 2004; Sumaya, 2000). Thrombocytosis was seen in 21 cases (42%). There was no statistical relationship between leukocytosis or thrombocytosis and age groups, gender, or type of disease ($p = 0.05$). There was a significant relationship between type of disease and polynucleosis ($p = 0.049$). ESR>20 mm in 1st h and positive CRP (as an indicator of inflammation) were present in most cases. ESR was negative in only two cases and simultaneous CRP was around 3^+. In 12 cases, CRP was negative, all of which had ESR>20 mm. there was no significant relationship between CRP and type of disease ($p = 0.271$). All patients were studied serologically for EBV capsid specific antigens. All sera were tested with a minimum titer (1/10) so that EBV infected cases can be identified. In order to eliminate false positive cases in RF^+ subjects, a dilution of (1/20) was also used. Among the 50 cases with JRA, 41 (82%) had IgM antibodies and IgG antibodies were positive in 22 (44%) cases. Three patients lacked IgM antibodies (6%) but IgG antibodies were present in their sera which indicated previous infection. Twenty subjects (40%) were only IgM-positive. On the whole, those who showed IgM antibody or IgG antibody or both, were subjects with EBV infection. Forty four patients (88%) were infected with EBV (Table 2). In the previous study, EBV infection in healthy 0-14 year old children in Tehran was reported to be around 70%. Therefore, infection with this virus among children with JRA is more than the normal population (Modarress and Modarress, 1998) which indicates the relationship between EBV and JRA and it has been pointed to in other studies as well (Blaschke *et al*., 2000). The patients were divided into four different groups based on the probable time of infection:

- Patients in whom IgM is only present in their serum which is the initial infection with EBV.
- Patients in whom both IgG and IgM are positive and four months have elapsed since their onset of disease (IgM gradually disappears after this time).
- Patients in whom IgG is only present in their serum in which probably more than four months have elapsed since onset of disease.
- Patients in whom both IgG and IgM are negative) (Table 3).

Thirty three of 37 (89%) patients with polyarticular JRA were infected with the virus ($p = 0.532$). Other studies have shown the involvement of this virus in the pathogenesis of disease but the type of disease has not been mentioned (Cassidy and Petty, 1995; Blaschke *et al*., 2000). Regarding the role of the virus in stimulating the immune system and since the primary goal of EBV is the

B lymphocyte, which stimulates T-lymphocytes once it is infected with the virus and immune system stimulation has also been confirmed by synovial T-lymphocytes in JRA (Mehraein *et al*., 2004; Balandraud *et al*., 2004; Callan, 2004). The rate of infection with EBV, which is higher than normal in these patients, indicates the relationship between this virus and JRA and has been referred to in other studies as well (Balandraud *et al*., 2004). EBV infection is different in different age groups (Table 4). With advancing age, people with EBV infection also increase in number, which is in agreement with the study performed by the Pasteur Institute (Modarress and Modarress, 1998). Infection rate is higher in females and out of 88% of infected cases, 48 and 40% occurred in girls and boys, respectively. Regarding that the cases of JRA were higher among girls (52% vs. 48%) there is a probable relationship between EBV and JRA. Till today, there has been no study on the relationship between response to therapy and EBV infection. By focusing on the type of antibody, we reach the fact that a large proportion of the patients under study were IgM EBVCA positive and that the majority of these cases were subjects who were hospitalized after at least 6 months of disease due to failure to respond to conventional therapy and the progressive role of the disease (based on physical examination, observation of the patient's general condition, involvement of new joints and limitation of movement which had worsened since the onset of disease) so that we could alter the mode of treatment and perform pulse therapy. In addition, in the EBV-positive group, response to conventional therapies were not very satisfactory and most of them required a change in the mode of treatment and use of pulse therapy (Table 5).

CONCLUSIONS

Regarding the prevalence of infection with EBV (annually 100,000 cases of EBV infection is diagnosed, (Callan, 2004) about 1/1,000/year (Jensen, 2004a, b) and considering that this virus is involved in the pathogenesis of JRA, as well as the fact that patients with EBV are twice at risk of developing JRA (Blaschke *et al*., 2000; Callan, 2004) and since antiviral drugs are not capable of destroying EBV (Beaulieu and Sullivan, 2002), the best option is to prevent infection with this virus.

EBV vaccination in endemic areas results in a decrease in Burkitt's lymphoma and prevents infectious mononucleosis in young adults in developed countries.

Gp 350 is one of the viral extra-cellular membrane proteins which is present in infected lysed cells. Gp 350 subunit vaccine is currently being used experimentally (Callan, 2004). We recommend that more widespread studies be performed in order to find the relationship between this virus and JRA so that we could help patients by decreasing the prevalence of this disease by using the vaccine so that we could at least decrease the intensity of disease and prevent its progression thereby decreasing morbidity and disabilities.

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