

COMPARATIVE STUDY OF SPECIFIC EBV ANTIBODIES BETWEEN CHILDREN MANIFEST CLASSIC TRIAD OF MONONUCLEOSIS WITH UNAFFECTED CHILDREN IN HAZRAT RASOOL AKRAM HOSPITAL (1998-2000)

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ABSTRACT

Epstein barr virus (EBV) is one of seven known herpes virus pathogenic for humans. Since it is ubiquitous, it infects nearly 95% of individuals worldwide by adulthood. EBV is the etiologic agent of infectious mononucleosis (IM) and is implicated in Burkitt lymphoma, nasopharyngeal carcinoma and x-linked lymphoproliferative syndrome. Diagnosis of IM based upon clinical manifestations in conjunction with hematologic evidence for lymphocytosis; and serological changes such as heterophil antibody and or antibodies to EBV specific proteins.

The purpose of this study was to determine the frequency of acute and chronic infections by examining the levels of antibodies against viral capsid (VCA-IgG and VCA-IgM) and Epstein Barr nuclear antibody (EBNA-IgG) in the serum of children with IM syndrome (patient group) and the serum of unaffected children (control group). This longitudinal case-control study was performed on thirty one children between 1 to 14 years old who were admitted to the pediatric ward of Rasool Akram hospital; based on diagnostic parameters for IM within two years (1998-2000). Fourteen patients were eliminated due to other diagnosis. The average age of remaining 17 patients was 6.9 ± 3.3 , male/female ratio = 9/8. The results of this study showed a significant difference ($p = 0.038$) between the amount of EBNA-IgG but no significant difference in the amount of VCA-IgG, VCA-IgM between case and control groups. There is no difference between case and control groups in negative values for VCA-IgM, VCA-IgG and EBNA-IgG.

Keywords: EBV, Infectious mononucleosis, Elisa, EBNA-IgG, VCA-IgG, VCA-IgM.

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INTRODUCTION

Infectious mononucleosis (IM) is the best-known clinical syndrome caused by Epstein-Barr virus (EBV). It is characterized by systemic somatic complaints consisting primarily of fatigue, malaise, fever, sore throat, and generalized lymphadenopathy.^(1,2) Other infectious agents may cause infectious mononucleosis-like illnesses.

During childhood, primary infection with EBV is often asymptomatic. After primary infection EBV remains latent for life.^(2,3,4,5)

Diagnosis of EBV is based upon clinical, hematological evidence for lymphocytosis and serological evidence for the presence of heterophil antibody and/or antibodies to EBV specific proteins. Confirmation of an acute diagnosis of EBV infectious mononucleosis is generally sought by a positive heterophil antibody test. However, difficulties in diagnosis arise when the heterophil test is negative or clinical manifestation are atypical. In this circumstance diagnosis may be confirmed by identification of specific antibodies to EBV proteins such as VCA-IgG; VCA IgM; EBNA-IgG. The presence of IgM to VCA is specific and instrumental for diagnosis of acute EBV infection. However, verification should be sought by assaying other antibodies such as VCA-IgG and EBNA-IgM.^(3,4,5) In recent years; EBV load measurement by PCR methods in peripheral blood lymphocytes is used as a marker of EBV related disease.^(7,8,9,10) EBV is the etiologic agent of infectious mononucleosis (IM) and is implicated in hemophagocytic syndrome; Burkitt lymphoma, nasopharyngeal carcinoma; acquired immune deficiencies and x-linked lymphoproliferative syndrome.^(11,12,13,14,15,16,17,18)

The epidemiology of IM is related to the epidemiology and age of acquisition of EBV infection. EBV infects more than 95% of the world's population.^(3,5)

There is no comprehensive study performed on Iranian children except for study conducted by Moddarres and his coworkers.^(4,1) Previous study in Iranian children determined prior EBV infection in 52% of 4 year old.

The purpose of this prospective study was to compare the frequency of specific EBV antibody (VCA-IgM and VCA IgG: acute infection); EBNA-IgG: chronic infection) in children whom manifest by classic triad of IM with unaffected ones.

Identification of the role of this virus in Iranian children with mononucleosis and its differentiation from other microbial agents causing Infectious mononucleosis (IM) like syndrome can help to determine the importance of this infection and its consequences.

METHODS AND MATERIALS

This longitudinal case-control study was performed on children between 1 to 14 years old who were admitted to the pediatric ward of Rasool Akram hospital affiliated to Iran University of Medical Sciences; based on diagnostic parameters for IM within two years (1998-2000).

The inclusion criteria of this study included patients with classical triad (fever, exudative pharyngitis, lymphadenopathy) accompanied by one of the signs of skin rashes and/or hepatosplenomegaly.

Exclusion criteria on the other hand, consisted of positivity of microbial culture of different body tissues or fluids (pharynx, urine, blood and CSF), one of the: streptococcal infection (positive ASOT), positive Widal test, or final diagnosis of other diseases such as leukemia, lymphoma, Kawasaki etc.

The control group was in the same range of age without febrile disease or classical triad.

Initially from each patient with IM, a questionnaire was completed by the authorized physician, followed by clinical exams in existence of clinical triad; Two ml blood was drawn from each patient. Blood samples were centrifuged and transferred to Research laboratory. The sera were stored in -20°C freezer until the serologic Elisa tests were performed on them. On the serum of control group which had been drawn in the past for other reason, serologic tests were done as discussed above.

Serological test: Qualitative determination of specific antibodies in the serum were accomplished with ELISA method. The evaluation of VCA-IgM, VCA-IgG and EBNA-IgG were carried out with commercial kits (Biochem Immuno Systems Italy, S.P.A.) as suggested by the manufacturer.

Based on the ELISA technique; measurement of enzyme activity was performed by production of a color. The plates were read on an Elisa reader in 450 and 620 nm. The results were interpreted based on positive, negative and cut off controls values.

Statistical analysis; In this study, descriptive statistics (Mean, Standard Deviation), comprehensive statistics including Chi square for comparing positive results with confidence interval of 95% (CI=95%) were used. All the above statistical tests were used via SPSS 9 software and EPI6.

RESULTS

Thirty one patients studied in two years. However 14 of them were excluded from the study because of other final diagnosis (leukemia, Kawasaki, scarlet fever etc...). 17 remaining patients were studied.

The age of patients were 6.8 ± 3.24 (Range 1.5-12.5 years). The male/female ratio was 9.8. (Fig. 1,2,3).

The serologic test results are shown in tables (Fig. 4,5,6)

There is no significant difference in VCA-IgM (29.4% of patients vs to 8.3% of control groups, $p=0.354$) and VCA-IgG (17.6% vs 50%, $p=0.195$), but significant difference in EBNA IgG antibodies (11.8% vs 50%, $p<0.038$, $\chi^2 = 5.148$) between case and control groups.

Four cases (23.5%) in comparison 1 (8.3%) to control group were positive for both VCA- IgM and VCA-IgG. There is no difference between case and control groups in negative values for VCA-IgM, VCA-IgG and

EBNA-IgG (Table I).

DISCUSSION

Although Infectious mononucleosis is a known clinical syndrome caused by EBV, but it can also be induced by a number of other pathogens including CMV; toxoplasma Gondii; hepatitis virus; HIV and other agents.⁽¹⁻⁵⁾ This virus like other herpes viruses, is oncogenic and results in malignant proliferation of lymphatic system such as Burkitt lymphoma,^(20,21,22,23,24) leukoplakia, nasopharyngeal carcinoma, Hodgkin disease^(25,2526,27,28,29) or benign form of disease like hemophago-

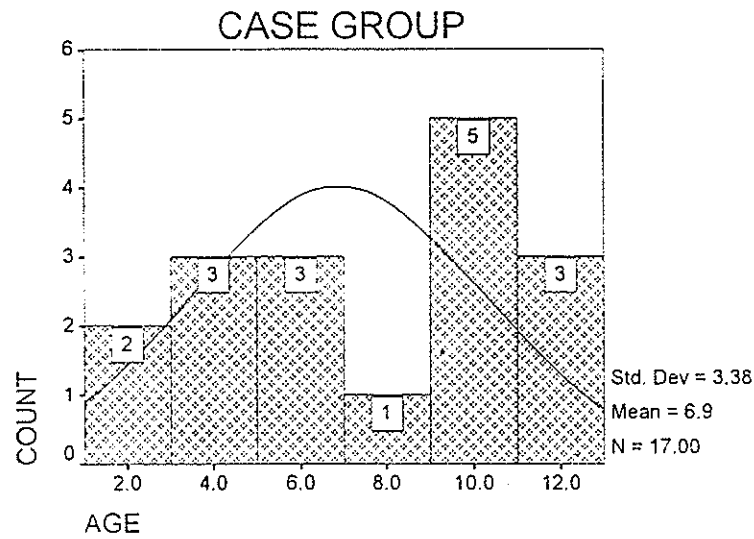


Fig. 1. Frequency of Age in Case Group.

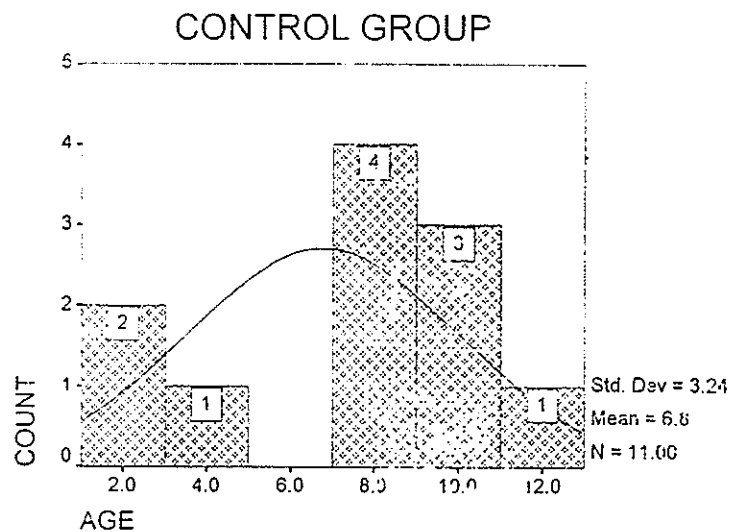


Fig. 2. Frequency of Age in Control Group.

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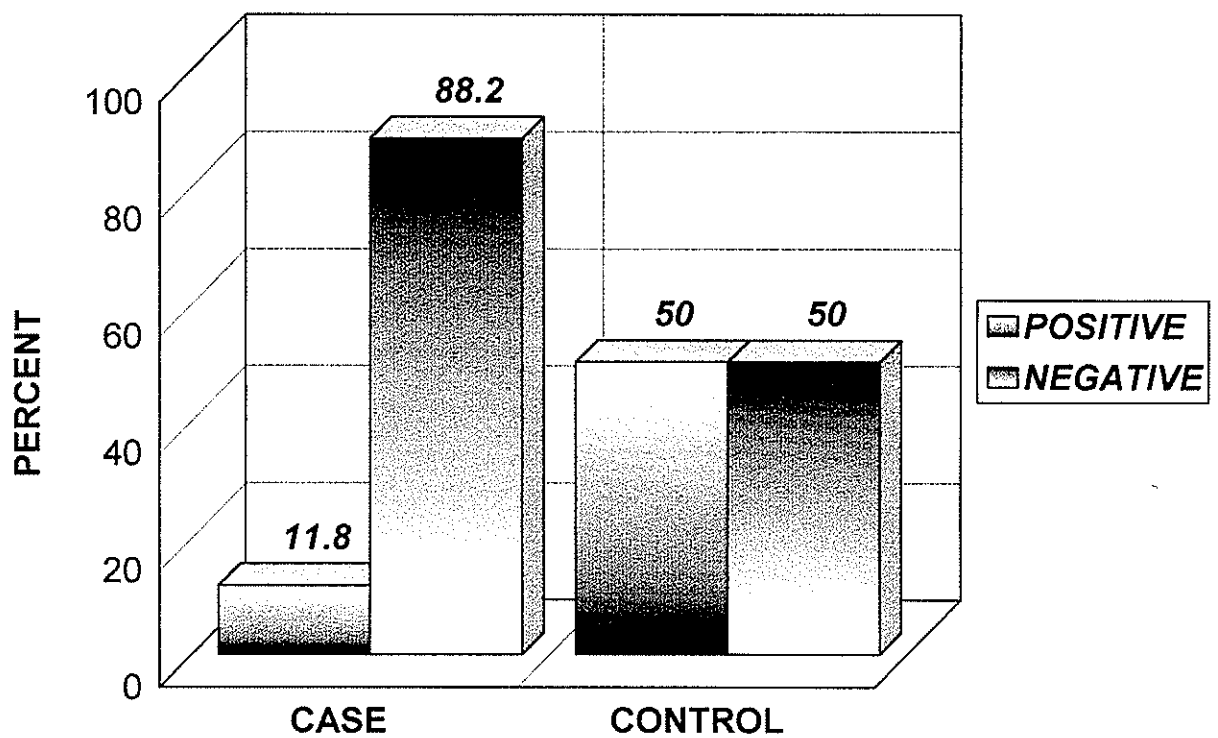


Fig. 3. Frequency of Sex Between Groups.

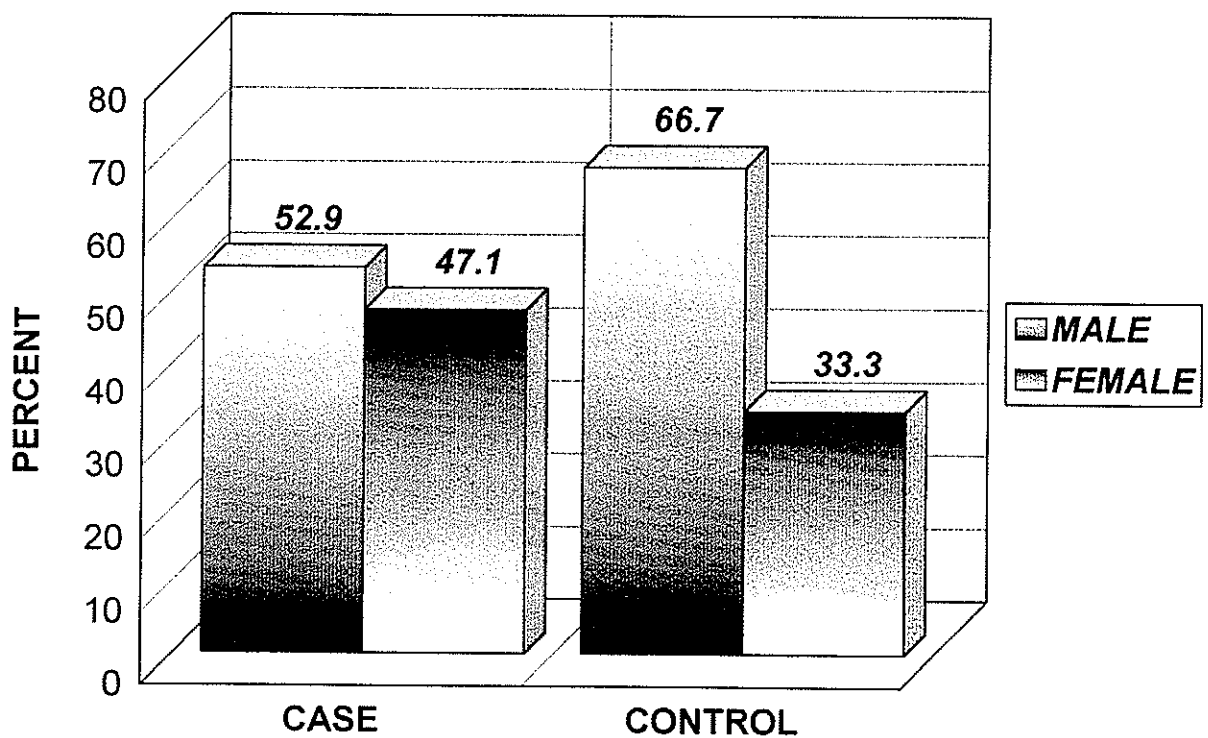


Fig. 4. Frequency of "Epstein Barr Nuclear Antibody" Between Groups.

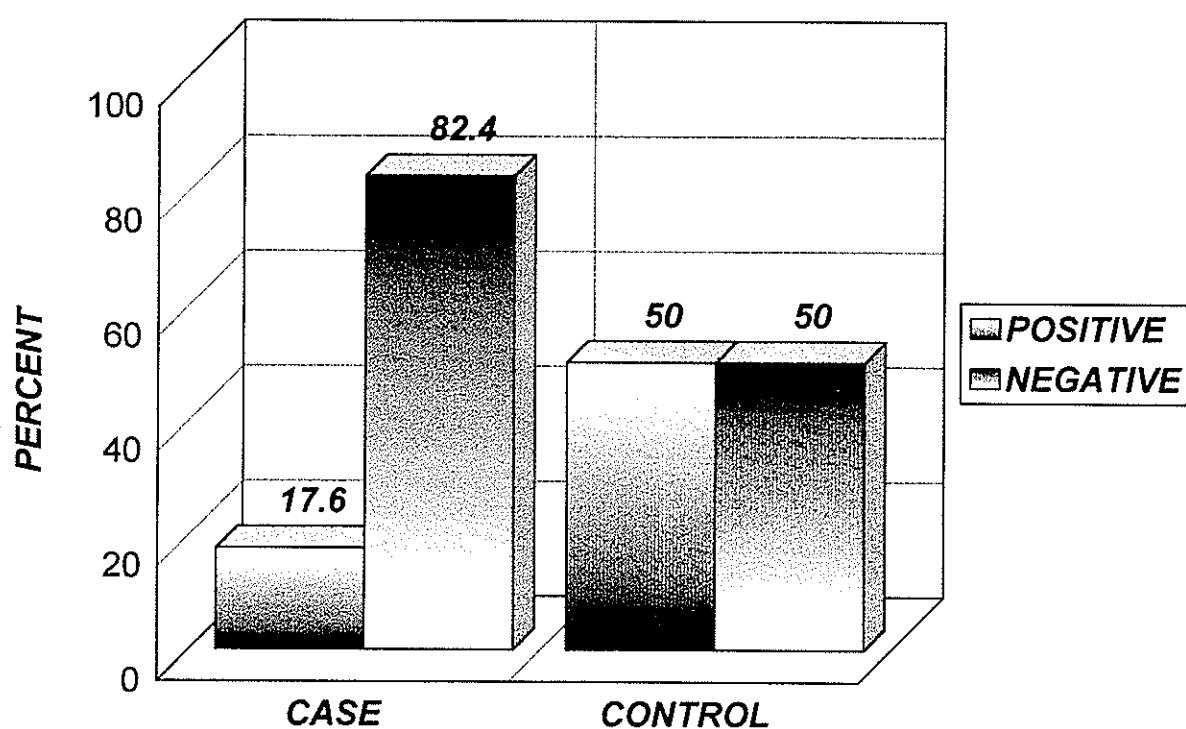


Fig. 5. Frequency of "Viral Capsid Antibody-IgG" Between Groups.

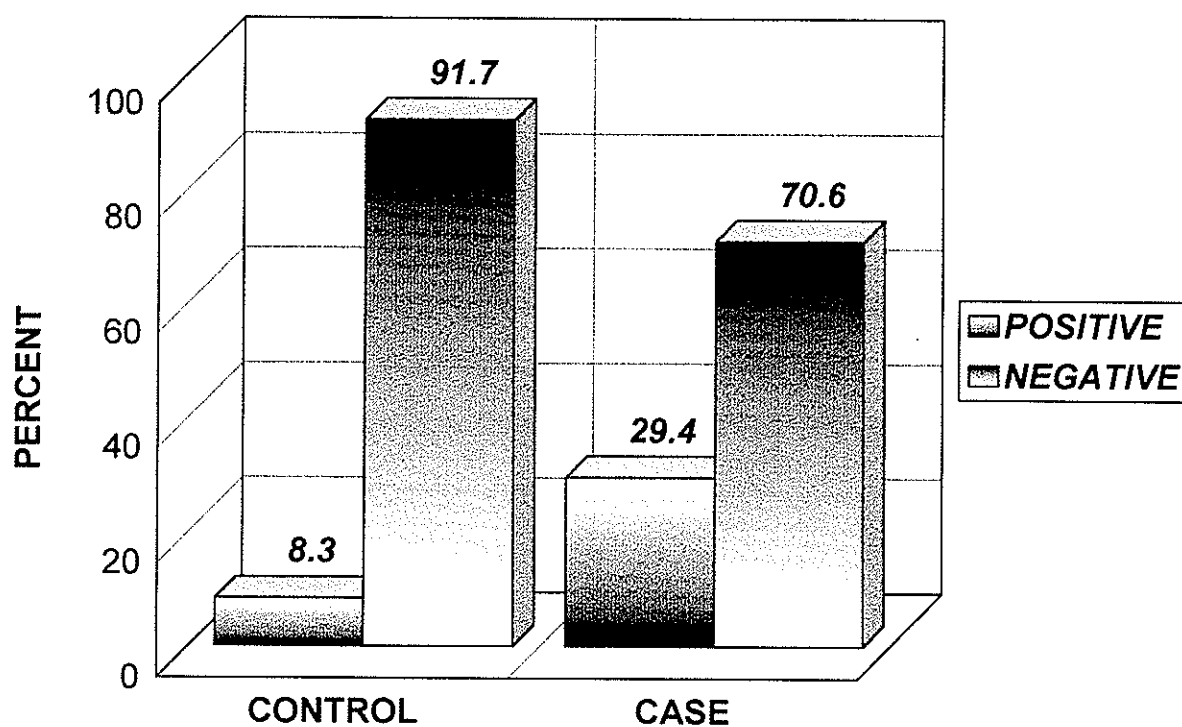


Fig. 6. Frequency of "Viral Capsid Antibody-IgM" Between Groups.

cytic syndromes,^(11-12,15,24) probably in chronic fatigue syndrome;lupus erythematosus;rheumatoid

arthritis,acute cerebêlitis, myeloradiculitis.^(6,30,31,32,33)

The importance of IM in children relies on clinical

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Table 1: Comparison of different stages of EBV infection in case and control groups

Disease stage response	Serologic of case	Number of control	Number
Negative reaction (susceptible)	EBNA=neg VCA-IgG=neg VCA-IgM=neg	10	6
Acute primary infection	EBNA=neg VCA-IgG=neg VCA-IgM=pos	3	0
Recent primary infection	EBNA=neg VCA-IgG=pos VCA-IgM=pos	11	
Previous infection	EBNA=pos VCA-IgG=pos VCA-IgM=neg	3	5
Total		17	12

Neg= Negative

Pos= Positive

variation. Primary infection with EBV during childhood is usually inapparent or indistinguishable from hemophother childhood infections.^(3,5) Primary EBV infection in adolescents and adults is manifested in >50% of cases by the classic triad of fatigue, pharyngitis, and generalized lymphadenopathy, which constitute the major clinical manifestations of IM. This syndrome may be seen at all ages but rarely apparent in children <4yr of age, when most EBV infections are asymptomatic, or in adults >40yr of age, when most individuals have already been infected by EBV. The true incidence of the syndrome of IM is unknown but is estimated to occur in 20-70/100,000 persons/yr. In young adults the incidence rises to about 1/1,000 persons/yr. The prevalence of serologic evidence of past EBV infection increases with age: almost all adults in the USA are seropositive.^(3,5) Infection with EBV in developing countries usually occurs in infancy and early childhood, in central Africa, almost all children are infected by 3 years of age.^(3,5,34,35,36,37)

Due to similarity between symptoms of EBV infection and streptococcal or staphylococcal infections as well as some malignancies (lymphoma, Hodgkin).

In most cases, a definite diagnosis is possible via detection of specific serum antibodies against EBV.^(1,3,4,5) Heterophil antibody detection which is in almost 90% of adults, is only 50% positive in children below 4 years. Acute phase of infection shows high level of VCA-IgM and VCA-IgG. The increase in VCA-IgM is

temporary and lasts for 4 weeks to 3 months. In this phase VCA-IgG increases later and it drops in a few weeks to a month. However, it stays stable for the entire of life. EBNA-IgG is the antibody which raises within 3 to 4 months and stays for life long.^(3,5,34,35,36,38) Recently, routine use of real time quantitative PCR for laboratory diagnosis of EBV is performed.⁽³⁹⁻⁴⁰⁾

Streptococcal pharyngitis which is accompanied by sore throat and lymphadenopathy and easily treated by antibiotics; is almost indistinguishable from EBV infection, except for the presence of hepatosplenomegaly, EBV infection will be documented by heterophil antibody or specific antibodies tests.^(1,3,4,5,34,35,36,38) On the other hand in 50% of individuals with IM, throat culture is positive for streptococci infection which makes the harder.^(1,3,4,5) If patients with streptococcal infection do not respond to antibiotic therapy within 48 hours, they are suspected for IM.^(1,3,4,5) In severe types of IM which is accompanied by thrombocytopenia, leukopenia or hemolytic anemia to exclude leukemia, it is required to perform bone marrow aspiration.^(9,10,11,12,13,15,16,17,18,20)

According to Dr Moddares's Study (41); out of 1100 children between 1-14 years old age in Tehran, 40% were infected with EBV (EBNA-IgG) in first year; 52% in fourth and 70% in 14th year of life ($p < 0.0001$), and it reaches more than 85% in individuals above forty years old.

We were determined to investigate the role of EBV

in children hospitalized with IM manifestation. This study shows that there is a significant difference ($p=0.038$) in EBNA IgG antibodies between children with mononucleosis and children without it. This finding indicates that individual in control group had previous infection as opposed to patients. These results are similar to Moddares study in Iran⁽⁴¹⁾ and in other developing countries.^(3,5,35,37)

However, there were no statistically difference between case and control group in terms of serological changes towards primary acute or recent infections (VCA-IgG, VCA-IgM). There is no difference between case and control groups in negative values for VCA-IgM, VCA-IgG and EBNA-IgG (susceptible to infection). In addition, no increase in total antibodies in some of patients may be due to either weak antibody response in less than 5 years old children, or early sampling before the antibody titer reaches its optimum level to be detected. On the other hand; the occurrence of IM triad by some other infectious agents could be another factor in seronegativity of patient group.

ACKNOWLEDGEMENT

The authors wish to thank Hamid Reza Monavari Ph.D student in virology, Hazrat Rasool Hospital.

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