RESEARCH PAPER

Cardiac Complications and Immunophenotypic Profile of Infectious Mononucleosis Syndrome in Children

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Objective: To investigate cardiac complications in infectious mononucleosis patients and to associate them with biochemical and immunological parameters, as well as with spleen ultrasound findings.

Design: Cross-sectional study with follow-up.

Setting: Tertiary care pediatric unit, in the city of Thessaloniki, Greece.

Participants and Interventions: Twenty-five children (15 boys, aged 1-11.6 years) suffering from infectious mononucleosis were studied during the acute phase and after 3-6 months. Cardiac evaluation comprised of electrocardiogram, echocardiogram, and measurement of creatine phosphokinase, creatine phosphokinase cardiac isoenzyme, and troponin levels. Biochemical and immunological tests included serum transaminases, serum amylase, CD3+/CD8+ T-lymphocytes subpopulation and CD4+/CD8+ T-lymphocytes ratio.

Results: During acute phase, all children had splenomegaly and normal serum amylase values. 17 patients had elevated serum transaminases. Percentages of CD3+/CD8+ T-lymphocytes subpopulation were elevated and CD4+/CD8+ ratio was decreased in all patients. Echocardiography revealed mild pericardial effusion in 13 patients (10/21 with Epstein-Barr infection, 3/4 with cytomegalovirus infection), but none presented with myocarditis. Four out of these 13 patients also had markedly elevated liver enzymes, 10/13 had significant splenomegaly and 12/13 presented very low CD4+/CD8+ T-lymphocytes ratio. Pericardial effusion demonstrated a statistically significant association solely with very low CD4+/CD8+ T-lymphocytes ratio (<0.5). Repetition of laboratory tests 3-6 months post-discharge detected persistent mild pericardial effusion in five patients, along with decreased CD4+/CD8+ ratio in 1/5.

Conclusions: In infectious mononucleosis syndrome, asymptomatic pericardial effusion could be associated with very low CD4+/CD8+ ratio (<0.5). Further studies would extend and confirm such an association.

Key words: *Epstein-Barr virus, Cytomegalovirus, Heart, Complications.*

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Infectious mononucleosis syndrome is a self-limited lymphoproliferative disease that is usually characterized by fever, pharyngitis and lymphadenopathy [1]. Even though the majority of cases are caused by primary infection with the Epstein-Barr virus (EBV) infections due to other agents, such as cytomegalovirus (CMV), adenovirus, herpes simplex virus (HSV), parvo virus B19, hepatitis viruses, human immunodeficiency virus, mycoplasma and toxoplasma present with similar clinical and hematologic findings [1].

The majority of children suffering from infectious mononucleosis recover without any complications. Although many manifestations, have been described in the literature, cardiac complications are rarely reported [2,3]. The aim of this study was to investigate the cardiac complications in children with infectious mononucleosis and to compare them with biochemical and immunological parameters, as well as with the ultrasound findings.

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METHODS

Children reported here had been admitted to our hospital in 2008 and 2009 and fulfilled the diagnostic criteria of infectious mononucleosis syndrome. They both (*a*) presented at least three of the following symptoms: fever, tonsillitis, cervical lymphadenopathy (lymph nodes more than 1 cm in diameter), splenomegaly and hepatomegaly; and (*b*) had IgM antibodies for the causative agents of

infectious mononucleosis syndrome. Written informed consent was obtained from all caregivers.

Twenty-five patients (15 boys, age 1-11.6 years, mean 4.5±2.6 years) fulfilling the diagnostic criteria, were examined. We obtained complete whole blood count, serum transaminases, serum amylase, CD3+/CD8+ T-lymphocytes subpopulation and CD4+/CD8+ T-lymphocytes ratio. We also analyzed the serum for antibodies for EBV, CMV, adenovirus, HSV, Parvo virus B19, mycoplasma and toxoplasma. Ultrasound scanning of the spleen was carried out. Cardiac evaluation comprised of electrocardiogram, echocardiogram, and measurement of serum creatine phosphokinase (CPK), creatine phosphokinase cardiac isoenzyme (CPK-MB) and cardiac troponin levels. All parameters were re-evaluated three to six months later. Only one child out of 25 could not be followed-up.

Transaminase levels were considered as markedly elevated when serum levels of aspartate aminotransferase (AST) and alanine amino-transferase (ALT) were >100 U/L, (normal values <48 and <37 U/L, respectively), while minor elevation was considered if transaminase levels were above normal limits, but up to 100 U/L. The CD3+/CD8+ T-lymphocytes subpopulation and CD4+/CD8+ T-lymphocytes ratio were determined by flow cytometry (Epics Elite ESP Coulter) using monoclonal antibodies (Beckman Coulter Immuno-tech). The CD4+/CD8+ T-lymphocytes ratio was considered to be normal when the value ranged from 0.9 to 2.6, according to age. A ratio between 0.5 and 0.9 was considered as low and ratio less than 0.5 was considered as very low.

The presence of IgG antibodies against CMV, adenovirus and toxoplasma was detected quantitatively, while the presence of IgM antibodies was determined qualitatively using the immunoenzyme method (MEIA, Axsym, Abbott). The presence of IgM and IgG antibodies against the capsid antigen of EBV, HSV, Parvo B19 and of the mycoplasma were detected by indirect immunoassay method (Elisa). Acute phase of infection was determined by the presence of IgM viral antibodies. Recovery of the infection along with the establishment of memory immune response was determined by the detection of IgG viral antibodies.

For the spleen ultrasound, the longitudinal size of the spleen measurement was performed between the most superomedial and the most inferolateral points, while the transverse dimension was measured between the hilum and the most superolateral margin [4]. Values over the 95th percentile, for height and age, were defined as splenomegaly. Mild splenomegaly was defined for cases with values up to 2cm above the 95th percentile, while severe splenomegaly was defined for values more than 2cm over the 95th percentile.

Pericardial effusion upon echocardiography was defined as the existence of pericardial fluid in excess of 5mm. Cardiac findings were correlated with biochemical and immunological parameters (serum transaminases, amylase, CD3+/CD8+ T-lymphocytes subpopulation and CD4+/CD8+ T-lymphocytes ratio), and with the spleen ultrasound findings.

The data were analyzed using the SPSS 16 statistical software. The relation between categorical variables was investigated using chi square test. A difference was considered statistically significant if *P* value was <0.05.

RESULTS

Twenty one out of the 25 children (84%) were diagnosed with acute EBV infection; 4 had acute CMV infection. All patients had splenomegaly and normal serum amylase values. Eight and 9 had markedly and mildly elevated serum transaminases, respectively. Percentages of CD3+/CD8+ T- lymphocytes subpopulation were elevated in all patients according to age (mean 52.05%±10.5%), while CD4+/CD8+ ratio was decreased in all patients (mean 0.45±0.23). None of the patients had any symptoms, electrocardiographic abnormalities or any laboratory findings indicative of cardiac involvement. Echocardiography revealed mild pericardial effusion without any other abnormalities in 13/25 patients (52%). Ten out of these 13 patients were positive for EBV infection and 3 of them were positive for CMV infection.

Markedly elevated liver enzymes were noted in 4/13 patients with pericardial effusion and in 4/12 patients without pericardial effusion. Significant splenomegaly was noted in 10/13 patients with pericardial effusion and in 6/12 patients without pericardial effusion. Very low CD4+/CD8+ T-lymphocytes ratio (<0.5) was seen in 12/13 patients with pericardial effusion, but in only 5/12 patients without pericardial effusion (*P*=0.007). Repetition of laboratory tests 3-6 months post-discharge in 24/25 patients showed normal transaminases levels. We also found decreased percentages of CD3+/CD8+ T-lymphocytes subpopulation (mean 25.75%±5.94%) and increased CD4+/CD8+ ratio (mean 1.48±0.62) compared to initial evaluation.

Echocardiography revealed persistence of mild pericardial effusion in five patients (20.8%). Among these children CD3+/CD8+ percentages were increased compared to CD3+/CD8+ percentages in the 19 patients without pericardial effusion (mean 29.14%±6.02% and 24.85%±5.74%, respectively). Their corresponding CD4+/CD8+ ratio was decreased compared to CD4+/

CD8+ ratio in patients without pericardial effusion (mean 1.16±0.36 and 1.56±0.66 respectively). All patients were followed up with repeated echocardiography until complete recession of the pericardial effusion was observed.

Among the children with persistent pericarditis, 4 suffered from EBV and only 1 from CMV infection. Seroconversion was observed in 23 out of the 24 children (positive IgG, negative IgM antibody). Only one patient presented positive both IgG and IgM EBV antibodies, along with persistent pericardial effusion and low CD4+/CD8+ T-lymphocytes ratio, up to 10 months post-discharge.

DISCUSSION

In this study, cardiac involvement consisting of a mild, asymptomatic and self-limiting pericardial effusion was diagnosed in 52% of the children during the acute phase of the infection, which persisted at follow-up in 20.8% of the patients. This finding was associated with the persistence of high levels of cytotoxic T- lymphocytes (CD3+/CD8+) and low CD4+/CD8+ T-lymphocytes ratio. This could be interpreted probably by the high viral load at the acute phase in these cases that delay the elimination of the infection. The lymphocytes in infectious mononucleosis are activated and they are composed of a mixture of CD8+ cytotoxic-suppressor T cells, Natural Killer cells and CD4+ helper cells. The dominant population by far is the CD8+ T cells, which have a role in the suppression of viral replication and have cytotoxic activity against virally infected B cells [5]. It is referred that patients with infectious mononucleosis who had higher viral load tended to have higher CD3+/CD8+ T cells [6]. In symptomatic infectious mono-nucleosis the characteristic large, virus-driven expansions of CD8+ T cells decrease in parallel with the decrease in viral load [7].

Cardiac complications are not very common in infectious mononucleosis syndrome. The first association of acute pericarditis with EBV infection was reported by Miller, *et al*. [8]. There are few reported cases with pericardial effusion, even less involving children [2]. Two patients mentioned in the literature had sizable pericardial effusion that caused cardiac tamponade, one of a 3-month-old infant and another of an immuno-compromised adolescent [9,10]. Myocarditis due to EBV is particularly observed in immunosupressed patients [11] or in patients suffering from chronic active EBV infection [12], but rarely described in immuno-competent children [13,14], or in combination with other factors [15].

Infectious mononucleosis, resulting from EBV infection, rarely leads to lethal myocarditis [14, 16]. There are also a few cases of CMV-induced fatal myocarditis as demonstrated in postmortem myocardial biopsy samples [17].

Diagnosis of myocarditis is important because it may lead to serious complications such as arrhythmias and dilated cardiomyopathy. These complications can arise suddenly and could become life-threatening. Therefore, clinicians should follow these patients regularly [3]. In the presence of cardiac complications like pericardial effusion, children should be reviewed until complete recovery.

Contributors: KP: conceived and designed the study, evaluated the patients for possible cardiac complications, conducted echocardiography, analyzed the data and drafted the paper. She will act as guarantor of the study. EP: designed the study, followed up with the patients, acquired and interpreted the data. AF: conducted and interpreted laboratory tests. KP, EP, AF, and GV: critically revised the manuscript for important intellectual content. SS: collected data, followed up with the patients, reviewed the literature, and helped in manuscript writing. AP: conducted laboratory tests and interpreted them. GV: advised and supported the study. The final manuscript was approved by all authors.

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REFERENCES

1. Ventura KC, Hudnall SD. Hematologic differences in heterophile-positive and heterophile-negative infectious mononucleosis. Am J Hematol. 2004;76:315-8.
2. Zafrir B, Aviv A, Reichman N, Flatau E. Epstein-Barr virus-associated pericarditis and pericardial effusion: Case report and diagnostic aspects. Eur J Intern Med. 2005;16:528-30.
3. Roubille F, Gahide G, Moore-Morris T, Granier M, Davy JM, Vernhet H, *et al*. Epstein-Barr virus (EBV) and acute myopericarditis in an immunocompetent patient: first demonstrated case and discussion. Intern Med. 2008;47:627-9.
4. Konus OL**,** Özdemir A, Akkaya A, Erbas G, Celic H, Isik S. Normal liver, spleen and kidney dimensions in neonates, infants and children: evaluation with sonography. AJR Am J Roentgenol. 1998;171:1693-8.
5. Weisberger J, Cornfield D, Gorczyca W, Liu Z. Down-regulation of pan-T-cell antigens, particularly CD7, in acute infectious mononucleosis. Am J Clin Pathol. 2003;120:49-55.
6. Sugaya N, Kimura H, Hara S, Hoshino Y, Kojima S, Morishima T, *et al*. Quantitative analysis of Epstein-Barr Virus (EBV)-specific CD8+ T cells in patients with chronic active EBV infection. J Infect Dis. 2004;190: 985-8.
7. Cameron B, Bharadwaj M, Burrows J, Fazou C, Wakefield D, Hickie I, *et al*. Dubbo infection outcome study. Prolonged illness after infectious mononucleosis is associated with altered immunity but not with increased viral load. J Infect Dis. 2006;193:664-71.

8. Miller H**,** Uricchio JF, Phillips RW. Acure pericarditis associated with infectious mononucleosis. N Engl J Med. 1953;249:136-40.
9. Fu YC, Hwang B, Tang RB, Hsing HP, Meng LC, Chi CS. Cytomegalovirus pericarditis with cardiac tamponade in a young infant. Zhonghua Yi Xue Za Zhi. (Taipei) 2000;63:429-32.
10. Razzouk BI, Patrick CC, Marina N. Cytomegalovirus causing pericarditis with tamponade in an adolescent with cancer. Med Pediatr Oncol. 1996;26:70.
11. Fujiwara M, Shimozono H, Ono H, Fujita N, Nishimura S, Ueda K, *et al*. Polyclonal proliferation of lymphocytes containing the Epstein-Barr virus genome in a patient dying of myocarditis in chronic active Epstein-Barr virus infection. J Pediatr Hematol Oncol. 2003;25:85-8.
12. Takano H, Nakagawa K, Ishio N, Daimon M, Daimon M, Cobayashi Y, *et al*. Active myocarditis in a patient with chronic active Epstein-Barr virus infection. Int J Cardiol. 2008;130:e11-3.
13. Ishikawa T**,** Zhu BL, Li DR, Zhao D, Maeda H. Epstein-Barr virus myocarditis as a cause of sudden death: two autopsy cases. Int J Legal Med. 2005;119:231-5.
14. Frishman W, Kraus ME, Zabkar J, Brooks V, Alonso D, Dixon LM. Infectious mononucleosis and fatal myocarditis. Chest. 1977;72:535-8.
15. Talsma MD, Kroos MA, Visser G, Kimpen JL, Niezen KE. A rare presentation of childhood Pompe disease: cardiac involvement provoked by Ebstein-Barr virus infection. Pediatrics. 2002;109:e65.
16. Hebert MM, Yu C, Towbin JA, Rogers BB. Fatal Epstein-Barr virus myocarditis in a child with repetitive myocarditis. Pediatr Pathol Lab Med. 1995;15:805-12.
17. Kytõ V, Vuorinen T, Saukko P, Lautenschlager I, Lignitz E, Saraste A, *et al*. Cytomegalovirus infection of the heart is common in patients with fatal myocarditis. Clin Infect Dis. 2005;40:683-8.