ANALYSIS OF CLINICAL, HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN PATIENTS WITH INFECTIOUS MONONUCLEOSIS

Petar Canović¹, Aleksandra Vranic², Sara Petrovic³, Ivana Rakovic³, Biljana Popovska Jovicic³, Nedim Hamzagic⁴

¹ Department of Biochemistry, Faculty of Medical Sciences, University of Kragujevac, Serbia

² Faculty of Medical Sciences, University of Kragujevac, Serbia

³ Department of Infectious Diseases, Faculty of Medical Sciences, University of Kragujevac, Serbia

⁴ Th e General Hospital, Department of Nephrology, Tutin, Serbia

ANALIZA KLINIČKIH, HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA KOD BOLESNIKA SA INFEKTIVNOM MONONUKLEOZOM

Petar Čanović¹, Aleksandra Vranić², Sara Petrović³, Ivana Raković³, Biljana Popovska Jovičić³, Nedim Hamzagić⁴

¹ Katedra za biohemiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija

² Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija

³ Katedra za infektivne bolesti, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija

⁴ Opšta bolnica, Odeljenje za nefrologiju, Tutin, Srbija

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ABSTRACT

Primary infection with Epstein-Barr virus (EBV) usually occurs in early childhood and often does not present clinical symptoms. More than 90% of adults are infected with this virus. A primary infection that occurs in adolescence or adulthood is usually clinically presented as infectious mononucleosis with a triad of symptoms: fever, lymphadenopathy and pharyngitis. Our retrospective study included 51 patients with a median age of 17 (9-23) years and serologically confirmed infectious mononucleosis. All patients with infectious mononucleosis were treated at the Clinic for Infectious Diseases at the Clinical Center in Kragujevac during 2013. We analysed the clinical, haematological and laboratory parameters of patients. The aspartate-aminotransferase levels were increased in 40 patients, with a mean value of 116.24 (± 93.22) ; the alanine-aminotransferase levels were increased in 44 patients, with a mean value of 189.24 (±196.69). Lymphadenopathy was the most common clinical feature upon admission in 49 patients (96%); 38 patients (74.5%) had splenomegaly, and 20 (39%) had hepatomegaly. Twenty-six patients (51%) had leukocytosis with lymphocytosis, while 15 (75%) of the 20 who had a normal leukocyte count also had lymphocytosis. In the present study, we updated the clinical, haematological and laboratory parameters, which may lead to the establishment of an accurate diagnosis and promote further treatment of the patients.

Keywords: Epstein-Barr virus, Infectious mononucleosis, Aspartate-aminotransferase, Alanine-aminotransferase, Reticuloendothelial system, Young adults

SAŽETAK

Primarna infekcija Epstein-Barr virusom (EBV) uglavnom nastaje u ranom detinjstvu i najčešće protiče klinički inaparentno. Više od 90% odraslih osoba je inficirano ovim virusom. Ukoliko se primarna infekcija odigra u adolescentnom ili odraslom dobu, najčešće se klinički prezentuje kao infektivna mononukleoza, u vidu povišene telesne temperature, limfadenopatije i angine. Naša retrospektivna studija je obuhvatila 51 pacijenta prosečne starosti 17 (9-23) godina sa serološki potvrđenom infektivnom mononukleozom. Svi pacijenti su lečeni na Klinici za infektivne bolesti Kliničkog centra u Kragujevcu, tokom 2013. godine. Analizirani su klinički, hematološki i laboratorijski parametri. Enzimska aktivnost aspartat-aminotrasferaze povećana je kod 40 pacijenata, srednja vrednost iznosila je 116.24 (±93.22); aktivnost alanin-aminotrasferaze povećana je kod 44 pacijenta, srednja vrednost iznosila je 189.24 (±196.69). Limfadenopatija je najčešći klinički znak koji je na prijemu bio prisutan kod 49 pacijenata (96%), 38 pacijenata (74,5%) imalo je splenomegaliju i 20 pacijenata (39%) imalo je hepatomegaliju. Dvadeset šest pacijenata (51%) imalo je leukocitozu sa limfocitzom dok je 15 pacijenata (75 %) od 20 koji su imali normalni broj leukocita takođe imalo limfocitozu. U ovoj studiji prikupili smo kliničke, hematološke i laboratorijske parametare koji mogu da pomognu u uspostavljanju tačne dijagnoze i daljeg adekvatnog tretmana pacijenata.

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Ključne reči: Epstein-Barr virus, Infektivna mononukleoza, Aspartat - aminotrasferaza, Alanin - aminotrasferaza, Reticuloendotelni sistem, Mladi

ABBREVIATIONS

 $egin{aligned} \mathbf{ALT} - & \text{alanine aminotransferase} \\ \mathbf{AST} - & \text{aspartate aminotransferase} \\ & \mathbf{EA} - & \text{early antigen} \\ & \mathbf{EBV} - & \text{Epstein-Barr virus} \end{aligned}$

$$\label{eq:local_local_local} \begin{split} \mathbf{IM} - & \text{Infectious mononucleosis} \\ \mathbf{LYDMA} - & \text{lymphocyte-determined membrane antigens} \\ \mathbf{NA} - & \text{nuclear antigen} \end{split}$$

VCA – viral-capsid antigen

 $\pmb{ELISA}-enzyme\text{-linked immunosorbent assay}$



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INTRODUCTION

Primary infection with Epstein-Barr virus (EBV) usually occurs in early childhood and often does not present clinical symptoms. More than 90% of adults are infected with this virus, which is demonstrated by serological reactions (1). Primary infections that occur in adolescence or adulthood are usually clinically presented as infectious mononucleosis (IM) (1, 2) with a triad of symptoms: fever, lymphadenopathy and pharyngitis (3, 4). However, splenomegaly, hepatomegaly and palatal petechiae are each present in more than 10 percent of patients. Less common complications include haemolytic anaemia, thrombocytopenia, aplastic anaemia, hepatitis, splenic rupture and rash (3). As a member of the Herpesviridae (5, 6) family, EBV possesses the ability to establish a latent infection with the possibility of later reactivation, which may be clinically manifested as recurrent parotitis, uveitis or interstitial pneumonia (5). EBV also leads to an aetiological relationship with some carcinomas such as nasopharyngeal carcinoma, Burkitt's tumour, Hodgkin's disease and B-cell lymphoma in HIV-infected patients (7).

EBV has a specific affinity for B-lymphocytes and epithelial cells in the oropharynx that bind to the CD21 receptor. Infection is most often transmitted by the saliva. The antigenic structure of EBV is quite complex. It possesses capsid antigen (EBV-VCA), nuclear antigen (EBV-NA), early antigen (EBV-EA) and lymphocyte-determined membrane antigens (LYDMA) (7).

Haematological analysis is a characteristic test for diagnosing IM with leukocytosis and lymphocytosis (more than 10% atypical lymphocytes). Biochemical analyses in approximately 90% of cases show increased aminotransferase and alkaline phosphatase activity in the serum as a result of liver damage (8, 9). The Paul-Bunnell-Davidson test is used in the diagnosis of IM to identify the heterophilic antibodies in the patient's serum but the test is not specific. In the first week of the disease, the test is positive in 40% of patients, and it is positive in the third week in 80-90% of patients (7). The most important analyses for acute IM diagnosis assess the IgM class of antibodies that bind to VCA, which is present in the first two months of the illness. The IgG class of antibodies that binds to VCA is mainly used for diagnosing IM that persists for life (8).

IM is a self-limiting disease with a good prognosis. Fatal cases are extremely rare and are usually caused by complications in the central nervous system, spleen rupture or obstruction of the upper respiratory tract.

The aim of this study was to highlight the most important clinical, haematological and biochemical abnormalities in patients with IM and to determine the frequency of these abnormalities in young adults.

PATIENTS AND METHODS

Participants

Our retrospective study included 51 patients with serologically confirmed IM. The study was conducted at the Clinic for Infectious Diseases at the Clinical Center in Kragujevac and included all patients with IM treated in the period from 1 January 2013 to 31 December 2013. The subjects had a median age of 17 (9-23) years and included 24 (47%) males and 27 (53%) females. In all cases, the diagnosis was established by the detection of EBV-VCA IgM antibodies using an enzyme-linked immunosorbent assay (ELISA).

We analysed the following clinical parameters: activity of the liver enzymes in the blood (aspartate aminotransferase - AST, alanine aminotransferase - ALT), the number of leukocytes and lymphocytes, body temperature, the reticuloendothelial system (liver, spleen, lymph nodes), pharyngitis, edema of the eyelid, and rash.

Statistical analysis

The experimental data were analysed using basic descriptive statistics: the mean value $(X) \pm \text{standard deviation (SD)}$. The chi-squared test was performed to compare categorical variables between groups. A database analysis of the results was performed using the SPSS version 18 software package (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical characteristics

The study was performed in the Clinic for Infectious Diseases at the Clinical Center in Kragujevac. The records of 51 patients hospitalized with a documented EBV infection over a 1-year period were retrospectively reviewed.

Sore throat was noted in 37 patients (72.5%). Twenty-six patients (51%) suffered from fever, 5 (10%) from edema of the eyelid, and 3 (6%) from rash.

Lymphadenopathy was the most common clinical feature upon admission and was detected in 49 patients (96%). Thirty-eight patients (74.5%) had splenomegaly and 20 (39%) had hepatomegaly. The median enlargement of the spleen, identified by ultrasonography, was 13.3 cm (10.2–20), while the median enlargement of the liver was 14.1 cm (9.6-16.5). The triad of symptoms (fever, lymphadenopathy, and sore throat) was noted in 21 patients (41%) (Table 1).

Laboratory findings

From the total number of patients, 31 (61%) had leu-kocytosis (mean value: 11.3; range: $4.1-24.8 \times 10^9/L$), while 41 (80.5%) had lymphocytosis (mean value: 54.4;



















range: 24.2-81%). Twenty six patients (51%) had leukocytosis with lymphocytosis. while 15 (75%) of the 20 who had a normal leukocyte count also had lymphocytosis (Table 2).

The AST levels were increased in 40 patients (78.4%), and the ALT levels were increased in 44 patients (86.3%) (Table 3).

Treatment and outcome

All patients received supportive care. Empirical antimicrobial regimens were given to 38 (74.5%) patients before the establishment of the diagnosis. No cases of fulminant hepatitis or liver failure were observed. None developed chronic liver disease. Finally, no cases of EBV-induced hepatitis-related mortality were noted among the study patients. All patients fully recovered, as confirmed by follow up visits in the outpatient clinic.

DISCUSSION

As previously mentioned, IM manifests with characteristic clinical, haematological and serological parameters after an incubation period, which may last up to 50 days (8). The manifestation usually begins with fever, pharyngitis and enlargement of the liver, spleen and lymph nodes (10). IM is generally a self-limiting disease with a good prognosis. Complications are rare, but they can be very serious. In less frequent cases, the complications may be the only clinical manifestation of the disease (11).

Due to the various clinical, haematological and biochemical manifestations, in this study, we wanted to determine the frequency and level of these abnormalities.

Regarding the hepatic complications, it is known that 80 - 90% of patients with IM have moderately increased aminotransferase levels, which indicates liver lesions. In cases when there are no clinical signs of liver damage, there is always a characteristic histopathological change (6, 8, 12). Consistent with those studies, our results are similar in that the liver enzymes were increased. The AST levels were increased in 40 (78.4%) patients, while the ALT levels were increased in 44 (863%) patients. During EBV infection, transaminases are typically elevated less than five-fold compared with the normal levels (13). Our data showed that the AST and ALT levels were elevated approximately 5-fold above the normal limits, a finding that is consistent with the existing literature (6, 14, 15). Elevations greater than 10-fold over the normal levels are less likely (16), but our results have shown that the AST levels in 3.9% of patients and ALT levels in 9.8% of patients were greater than 10-fold the normal levels, which correlates with the results of Yang et al (12). The incidence of serum ALT levels greater than 1000 IU/L was similar to the result of a previous study (17). It is believed that EBV does not have a di-

Table 1. Frequency of the associated signs and symptoms in patients^a with EBV infection.

Signs and symptoms	No of patients (%)
Sore throat	37 (72.5)
Fever	26 (51)
37-38°C	14 (54)
38-39°C	11 (42)
39-40°C	1 (4)
>40°C	0
Edema of the eyelid	5 (10)
Rash	3 (6)
Lymphadenopathy	49 (96)
Splenomegaly	38 (74.5)
Hepatomegaly	20 (39)
Triad of symptoms ^b	21 (41)

^a Total number of patients: 51.

Table 2. Laboratory values on admission of the 51 patients with EBV infection.

Laboratory value	Mean (±SD)
Leukocytes, cells x109/l	11.29 (4.47)
Lymphocytes, in %	54.36 (12.32)
aÁSŤ (U/Í)	116.24 (93.22)
^b ALT (U/I)	189.24 (196.69)

^aAST: aspartate-aminotransferase.

Table 3. Number of patients (%) with different levels of enzyme elevation (AST, ALT) during EBV infection.

	ASTa (%)	ALT b(%)
Normal	11 (21.6)	7 (13.7)
< 2x	11 (21.6)	(17.6)
2-3x	8 (15.7)	9 (17.6)
3-5x	15 (29.4)	9 (17.6)
5-10x	4 (7.8)	12 (23.5)
>10x	2 (3.9)	5 (9.8)

^a AST: aspartate-aminotransferase.

rect cytocidal effect on hepatocytes, but that damage is caused by free radicals (8). It is believed that another possible cause of liver damage during EBV is type II (cytotoxic) hypersensitivity reactions (18).

Leukocytosis was noted in 61% of patients, while Yang et al noted leukocytosis in 86.1% of children who had IM (12). Furthermore, leukocytosis with lymphocytosis occurred in 51% of patients, which is similar to the results of Kofteridis et al, who investigated the same aged population (6).

The reticuloendothelial system is usually affected, as evidenced by generalized splenomegaly, hepatomegaly and lymphadenopathy (19).

^bThe triad of symptoms includes patients who had a fever, lymphadenopathy and a sore throat.

^bALT: alanine-aminotransferase.

^b ALT: alanine-aminotransferase.



















When we analysed the size of spleen, we found similar results as the study by Kofteridis et al, who found that approximately two-thirds of the patients developed splenomegaly (6). Regarding the clinical and laboratory manifestations, our results differed somewhat from earlier studies (12, 20). Hepatomegaly in young adults has been found in 36.7% of patients with diagnosed IM (20), which is similar to the results in our study, where we had 39% of patients with an enlarged liver.

Lymphadenopathy is most common in the anterior and posterior cervical lymph nodes, the submandibular lymph nodes, and occasionally the axillary and inguinal lymph nodes. Traditionally, epitrochlear lymphadenopathy is suggestive of IM because this finding is uncommon in generalized lymphadenopathy (19). In our study, this was the most common symptom. A difference was noted in rate of lymphadenopathy (96% in our cases vs. 78-89% in earlier reports) (6, 20, 21), while our result was similar with the percentage of patients with lymphadenopathy in a study of preschool children (21).

The onset of illness was defined as the first day of sore throat and fever. Fever was the most common feature in previous studies (6, 12, 21), while in our study, 51% of patients had a body temperature higher than 37°C. The physical examination typically reveals pharyngitis that is often is accompanied by moderate-to-marked tonsillar enlargement, occasionally with exudates that cannot be distinguished from streptococcal pharyngitis (19). Our results have shown that fewer patients had pharyngitis compared to previous studies (20, 21).

Rash occurs in 3% to 15% of patients and is usually maculopapular (19). In our study, the percentage of patients who had this symptom was less than has been observed in previous research (6, 12, 20, 21).

Tanner has logically divided the ocular manifestations of IM into two groups: 1) those due to direct EBV infection of the eye and ocular adnexa and 2) those affecting vision and the neuroophthalmologic apparatus from a remote focus, particularly the central nervous system. Symptoms of pain upon rotation of the eyes, deep orbital pain, photophobia, and an epiphora of short duration have all been noted (22). Edema of the eyelids and periorbital tissue, which may be pronounced, is observed in 25 to 40% of patients (23). Only 6% of patients had this symptom in our study, which was similar to the adult patients in another study and six times higher than that observed in preschool children with IM (21).

CONCLUSIONS

In conclusion, the present study, despite its limitations, namely the small number of patients and its retrospective nature, provides updated clinical, haematological and laboratory parameters, which may lead to the establishment of an accurate diagnosis and promote further treatment of IM patients.

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