



ANALYSIS OF INFECTIOUS MONONUCLEOSIS CLINICAL CASES IN VARNA REGION (2010-2016)

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ABSTRACT

Purpose: Infectious mononucleosis (IM) is an acute and self-limiting lymphoproliferative disease, and according to literature data in 90% of cases is associated with primary Epstein-Barr (EBV) viral infection. However, the mononucleosis-like syndrome is also caused by a number of other pathogens such as HHV-6, CMV, HIV, adenoviruses, etc., which makes the laboratory diagnosis necessary to identify the etiology of the disease. We compared clinical with serological IM data and defined serological profiles requiring further laboratory investigation.

Materials/ Methods: We investigated 746 patients with clinical symptoms for IM who had been tested in a virological laboratory at the „St.Marina“ University Hospital in the period 2010-2016, 57.6% (95% CI: 54.0% - 61.2%, n = 430) were men. The average age of the subjects was 9.56 years (SD ± 8.26), lower in the boys. We used an indirect ELISA to detect anti-VCA IgM/IgG (Euroimmun, Germany).

Conclusion: The serological markers used in our laboratories (anti-VCA IgM/IgG) in combination with symptoms and other laboratory results are in most cases sufficient to confirm primary EBV infection, but there are cases where additional studies are needed to accurately determine the stage of the infection. Serological profiles requiring ongoing studies are the isolated anti-VCA IgM and anti-VCA IgG models.

Keywords: Infectious mononucleosis, Epstein-Barr virus, ELISA, anti-VCA IgM/IgG,

INTRODUCTION

According to the literature, 90% of cases of IM are due to primary EBV infection. Viral etiology is determined by the high levels of EBV replication in the oropharynx in subjects with clinical manifestations of the disease, which results in high levels of infectious virus in the sa-

liva, lack of specific antibodies prior to clinical symptoms, and the appearance of anti-VCA IgM at the onset of symptoms [1]. It is found to occur in more than 50% of cases (in different studies the range is 25% to 77%) when the primary EBV infection is in adolescence and post-adolescence [2]. This is the pattern of infection in developed societies.

IM varies from region to region. In the United States, 500 cases per 100,000 population are reported annually, with a higher prevalence amongst 15-24-year-olds [3]. A lower annual average - 130 cases per 100,000 population was established in Israel [4].

However, the mononucleosis-like syndrome is also caused by a number of other pathogens, such as HHV-6, CMV, HIV, adenoviruses, and others [5,6], which makes the laboratory diagnosis necessary to identify the etiology of the disease. Laboratory confirmation of IM is performed by serological methods. Mass tests used to detect heterophile antibodies (Paul and Bunnell test) are non-specific and can be found positive in other conditions. They are probably due to polyclonal activation and are not directed against specific viral antigens [7]. These tests have proven low specificity in young children under 5 years of age. In about 5% of adults, they can give a false positive and in a 10% - 20% false negative result [8]. The specific tests, the main markers of which are viral antigens (VCA and EBNA), have much better informative value. The main method of diagnosis is ELISA, although the immunofluorescence method is the gold standard. In order to detect a primary infection in the ELISA, antibodies against IgM and IgG viral capsid antigen (VCA) are determined, the first occurring in the beginning, and the second remaining persist for life. In the world literature, there is also a third marker to use – anti-EBNA 1 IgG that is lacking in primary infection and appears later. The combination of the three antibodies may in most cases determine the stage of the infection. In the diagnosis of primary EBV infection, the variability in the appearance of these antibodies should be considered, and additional mark-

ers and methods should be used [6,9]. This study aims to compare clinical with serological data (anti-VCA IgM/ IgG) for infectious mononucleosis and to define serological profiles that require further laboratory investigation.

MATERIALS AND METHODS

We investigated 746 samples of patients with clinical symptoms on IM who had been tested in the virological laboratory at the University Hospital “St. Marina” in the period 2010-2016. The male patients were 57.6% (95% CI: 54.0% -61.2%, n = 430) and 42.4% (95% CI: 38.8% -46.0%, n = 316) were women. The average age of males was 8.87 (SD ± 8.19) and was lower than that of female subjects 10.49 (SD ± 8.27). For the purpose of our analysis, patients were divided into 10 age groups at regular intervals of 5 years except for first and last group.

We used:

1. Serological methods - indirect ELISA for detection of anti-VCA IgM/IgG (Euroimmun, Germany). Samples were tested according to the standard manufacturer’s instructions.

2. Statistical methods - The results were processed with the statistical program SPSS, vs 23. The average age of the sample, the relative proportions and the confidence intervals were determined. We used the chi-square test for correlation with $p < 0.05$ as statistically significant.

RESULTS

Data on acute infection based on ELISA studies for anti-VCA IgM, alone or in combination with anti-VCA IgG, were found in 43.2% (95% CI: 39.6% -46.8%, n = 322) of the patients – 41.9% (95% CI: 37.2% - 46.7%, n = 180) of the males and 44.9% (95% CI: 39.4% -50.6%, n = 142) of the females.

The highest proportion of the laboratory confirmed cases with IM was registered in the age group 16-20 years - 62.2% (95% CI: 52.5% - 71.2%, n = 69), followed by age group 11-15 - 56.0% (95% CI: 44.1% - 67.7%, n = 42). Although according to our analysis, the clinical diagnosis of IM was more frequent in early childhood and early school age, the laboratory confirmed cases were in the period of adolescence (Table1).

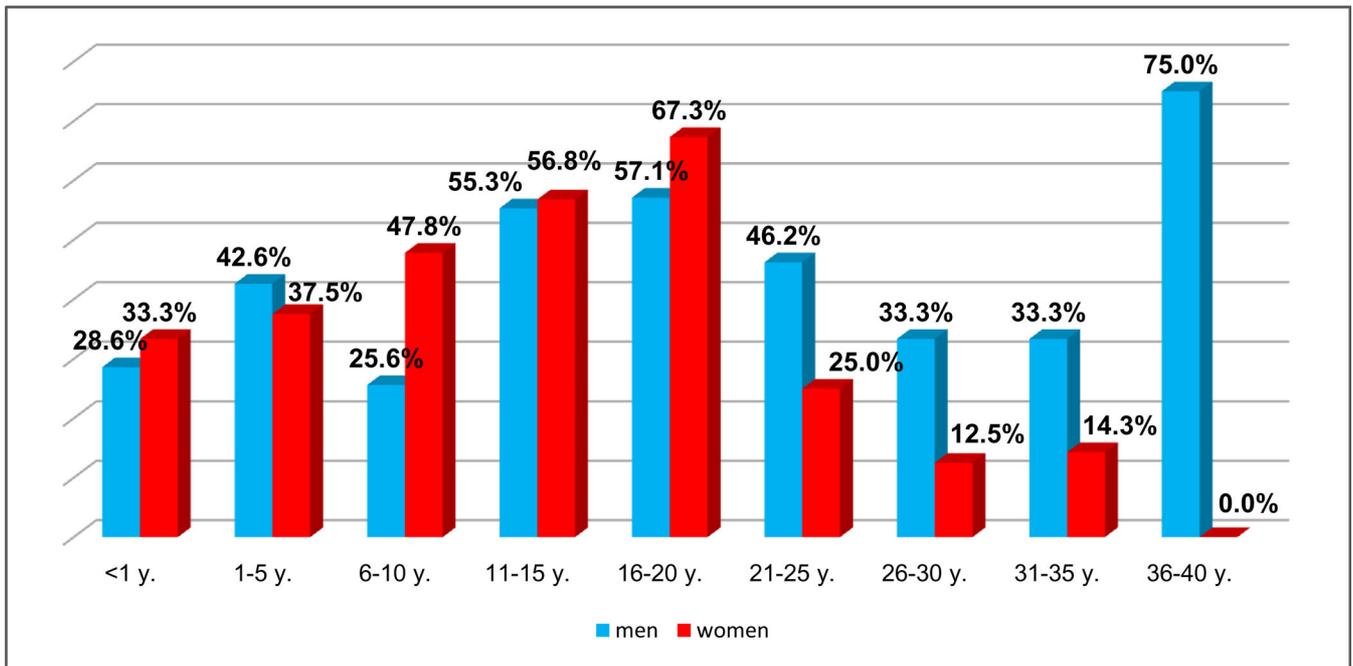
Table 1. Number and proportion of the patients with diagnosis IM and patients positive in ELISA anti-VCA IgM

Age	N*	Proportion (% ,95% CI)	N**	Proportion (%, 95% CI)
< 1 y.	10	1.3% (0.6-2.5)	3	30.0% (6.7-65.2)
1-5 y.	322	43.2% (39.6-46.8)	131	40.7% (35.3-46.3)
6-10 y.	157	21.0% (18.2-24.1)	55	35.0% (27.6-43.0)
11-15 y.	75	10.1% (8.0-12.4)	42	56.0% (44.1-67.7)
16-20 y.	111	14.9% (12.4-17.6)	69	62.2% (52.5-71.2)
21-25 y.	29	3.9% (2.6-5.5)	10	34.5% (17.9-54.3)
26-30 y.	20	2.7% (1.6-4.1)	5	25.0% (8.7-49.1)
31-35 y.	13	1.7% (0.9-3.0)	3	23.1% (5.0-53.0)
36-40 y.	6	0.8% (0.3-1.7)	3	50% (11.8-88.2)
41 +	3	0.4% (0.1-1.2)	1	33.3% (0.6-2.5)
All	746	100%	322	43.2%(39.6-46.8)

N*- patients with diagnosis IM; N**- patients positive in ELISA anti-VCA IgM

The average age of anti-VCA IgM positive patients diagnosed with IM was 9.99 (SD ± 7.78), lower in men (9.7 years (SD ± 8.57)). Statistically significant differences ($p < 0.05$) in the proportions of anti-VCA IgM positive patients were found in all age groups where the primary infection most commonly occurs. During adolescence, the laboratory confirmed cases of IM in women were higher than men. Boys with laboratory- confirmed the diagnosis of IM predominated in the age group 1-5 years (Figure 1).

Fig. 1. Proportion of anti-VCA IgM-positive by age and sex

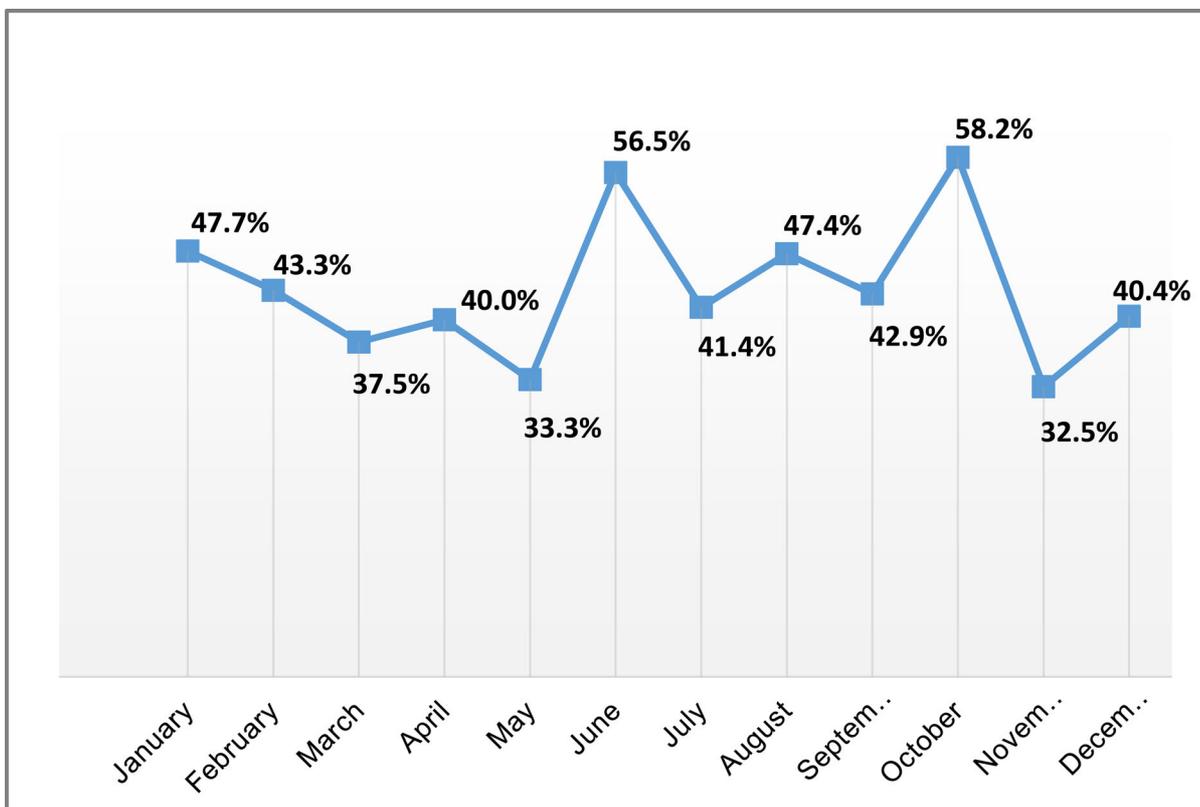


When analyzing anti-VCA IgM negative subjects with clinical diagnosis of IM, 33.9% (95% CI: 54.8% - 64.4%, n = 253) were positive in anti-VCA IgG. This was one of the groups that require the use of additional methods or markers to identify isolated IgG models.

Our data showed that the most common diagnosis of IM was during the colder months of the year when many

other respiratory and influenza viruses were circulating. The highest was the incidence in December - 16.8% and in November - 15.4% and at least in August (8.5%) and September (7.7%). Laboratory confirmed cases (anti-VCA IgM positive) were more frequent in October and June, with no distinct seasonality (Figure 2).

Fig. 2. Laboratory confirmed cases of IM by months



DISCUSSION

When analyzing ELISA results for the presence of anti-VCA IgM, fewer than half of the patients showed a positive result. A lower proportion was found in a Pleven region study of 37 hospitalized patients in the 2008-2012 period where EBV serological evidence was found in 38% [10]. In a study of 330 patients in the age group 18- 23, other authors found a positive result in 55.9% of those surveyed [4]. Possible causes are an inaccurate or guideline diagnosis or the presence of a profile with isolated anti-VCA IgG, which is observed in about 7% according to literature data [11]. A high degree of variability in the serologic response to EBV has been identified, particularly in the positivity of IgM antibodies against VCA. This requires careful interpretation in cases only positive for anti-VCA IgG with clinical data for primary infection where additional serological tests such as the IgG antibody avidity test should be used [6]. Although IM is a self-limiting disease, various studies indicate an increased risk of developing HL [7,12]. More recent data indicate a link with some NHL subtypes [13]. IM is more common in primary infection in the teenage period. Upon monitoring of seronegative students (n = 510) from Edinburgh, Scotland, 110 of them were seroconverted within the university stay, and 27 (25%) developed IM symptoms [2]. In another study among seronegative students in Minnesota, United States, observed for seroconversion during their university stay, 77% of the primary infected have developed IM symptoms [14]. In a retrospective analysis for our region, we found that 1/3 of the primary infections were in an age range of 10 years to 20 years, which we associate with adolescence [15]. The increasing proportion of cases of IM during adolescence is explained by the beginning of sexual contacts and the so-

called “deep kissing”, whereby a greater amount of virus is acquired. This leads to rapid colonization of B lymphocytes and consequent induction of a more potent T cell immune response [2,14]. The development of clinical symptoms of IM is considered largely determined by the high viral load and the high number of NK and CD8 + T cells. Several studies have found a correlation between viral load and symptom development [14,16]. Our data showed more frequent diagnosis at earlier ages, but serological confirmation was obtained in adolescent patients.

There is currently no recognized seasonal pattern in the manifestation of IM. According to a 12-year observation in one university, it peaked in October, and in a study conducted in Israel during the summer months [4]. We also cannot claim seasonality in the disease.

CONCLUSION:

1. Detection of anti-VCA IgM/IgG in most cases is sufficient to confirm primary EBV infection. This is most likely due to the presence of both classes of antibodies
2. No antibodies to EBV were found in 22.9% of IM patients tested in our study. In these cases, the role of other viruses involved in the etiology of the disease may be presumed.
3. In 33.9% of people diagnosed with IM, we found only anti-VCA IgG, and this is a group that has to be tested for other markers to be confirmed in future laboratory practice.
4. Patients with isolated anti-VCA IgM models should also be eligible for further studies, given the possibility of false positive results. In our study, this was approximately 11.0%, predominant in younger ages.

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Please cite this article as: Kostadinova T, Ivanova L, Stojkova Zh, Todorova T, Tsaneva D. Analysis of infectious mononucleosis clinical cases in Varna region (2010-2016). *J of IMAB.* 2019 Jan-Mar;25(1):2332-2336. DOI: <https://doi.org/10.5272/jimab.2019251.2332>

Received: 09/06/2018; Published online: 22/01/2019



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