Seroprevalence of Epstein-Barr Virus in North-Eastern Bulgaria

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Abstract

Epstein-Barr Virus (EBV) is a widely spread member of the *Herpesviridae* family. Data show that in more than 90% of the adult population, specific anti-EBV can be found. In different Bulgarian studies the seroprevalence was set between 60% and 80%. The primary infection early in life is often asymptomatic or with nonspecific clinical symptoms, while in teenagers infectious mononucleosis develops. EBV is a proven agent of malignant, autoimmune and lymphoproliferative diseases, especially in immunocompromised patients. Tracking and analyzing the seroepidemiological status of the population in every region is of utmost importance. In our study, 5016 single serum samples from patients referred for EBV testing for various reasons were analyzed for the period 2010-2015. People aged between 1 month and 89 years old were divided into sixteen age groups. The samples were tested using indirect enzyme-linked immunosorbent assay (ELISA) for detection of anti-EBV (VCA) IgM and IgG. The proportion of EBV seropositive individuals and their corresponding confidence intervals (CI), chi-squared distributions and p-values were calculated. Our data indicated high, age-dependent EBV (VCA) IgG dissemination with 90% seroprevalence after age 26. Primary infections detected by anti-EBV (VCA) IgM occur in a bimodal model with peaks in age groups 1 – 5 and 11 - 20 years old.

Key words: Epstein-Barr virus (EBV), seroprevalence, anti-EBV (VCA) IgM, anti-EBV (VCA) IgG, infectious mononucleosis (IM).

Резюме

EBV е широко разпространен представител на семейство *Herpesviridae*. По литературните данни повече от 90% от възрастното население притежава специфични антитела срещу вируса. Според различни проучвания в България серопревалентността се движи между 60% и 80%. Първичната инфекция в ранното детство често е асимптомна или протича с неспецифична клинична симптоматика, докато в юношеска възраст се развиват симптоми на инфекциозна мононуклеоза. EBV е доказан причинител на малигнени, автоимунни и лимфопролиферативни заболявания, особено при имунокомпрометирани пациенти. Проследяването и анализирането на сероепидемиологичния статус на населението във всеки регион е от изключителна важност. Нашето проучване се основава на анализ на 5016 единични серумни проби от пациенти, насочени по различни причини за изследване на EBV-инфекцията към Лаборатория по клинична вирусология в УМБАЛ "Света Марина" – Варна в периода 2010 – 2015 г. Пациентите бяха разделени в 16 възрастови групи. Използвани са стандартизирани ELISA китове за доказване на специфични anti-EBV (VCA) IgM и IgG. Статистическият анализ беше направен с помощта на McCallum Layton calculators (www.mccallum-layton.co.uk) и Social Science Statistics (www.socscistatistics.com). Нашите данни показват високо, възрастово свързано разпространение на заразеността с EBV, според наличието на anti EBV (VCA) IgG. Серопревалентността след 26-годишна възраст е над 90%. Първичната инфекция според наличието на anti-EBV (VCA IgM) има бимодален модел на разпространение с пикове във възрастови групи 1 – 5 и 11 - 20 години.

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Introduction

Epstein-Barr Virus (EBV) is a widely spread member of the family Herpesviridae. The unique properties of the viruses of this family are their lifetime persistence in latent stage after primary infection and reactivation capability (Middleton, 1974; Tan et al., 1999; Rickinson and Kieff, 2007). More than 90% of the adult population possesses specific anti-EBV (VCA) IgG, and according to various studies in Bulgaria, seroprevalence is 60-80% (Dundarov, 1998; Genova et al., 2013). A correlation between the time of the primary infection and the socio-economic status of the population has been established (Henle et al., 1979; Straus et al., 1993). Previous studies in northeastern Bulgaria suggest that the infection occurs mostly by age 4-7 and marks a peak in the age group 15-22 (Ivanova, 2007). Most commonly a major mechanism of transmission is through kissing, but infection from blood transfusions, organ and bone marrow transplantation and sexual contact is also possible (Niederman et al., 1976; Craig et al., 1993; Cohen, 2000; Thomas et al., 2006). The virus replicates in epithelial cells of the macro-organism and establishes latency in B-lymphocytes (Tan et al., 1999; Rickinson and Kieff, 2007). Primary infection occurs as infectious mononucleosis (IM) mainly in adolescence and later. Infection during early childhood is more often asymptomatic or with non-specific symptoms (Dowd et al., 2013; Straus et al., 1993). In later life, EBV is associated with a variety of malignancies, autoimmune and lymphoproliferative disorders (Craig et al., 1993; Alexander et al., 2003; Magrath, 2012).

In recent years, the diagnosis of EBV infection is mainly performed by serological methods based on enzyme-linked immunosorbent assay (ELISA). Specific serological markers for acute infection are IgM and IgG antibodies of VCA (viral capsid antigen), anti-EA (D) IgG (early antigen) (Klutts et al., 2009). Specific serological markers for past infection are anti-EBNA1 IgG and anti-EBV (VCA) IgG, which persist for life (Linde, 1996; Hess, 2004). The purpose of this study is a retrospective analysis to clarify the seroepidemiological situation in northeastern Bulgaria and to define the time of primary infection with EBV.

Material and Methods

Tested population

In the period 2010 - 2015, 5016 hospitalized and outpatients were tested for various reasons, in a single serum sample in the Laboratory of Clinical Virology, University Hospital „St. Marina" - Varna. Patients were divided into 16 age groups: 1 month-1 year old, 1-5 (years old), 6-10 (years old), 11-15 (years old), 16-20 (years old), 21-25 (years old), 26-30 (years old), 31-35 (years old), 36-40 (years old), 41-45 (years old), 46-50 (years old), 51-55 (years old), 56-60 (years old), 61-65 (years old), 66-70 (years old) and over 71. The average age of patients was 29.84 (SD±23.85). Minimal age was 1 month, the maximal – 89. In the studied sample, the proportion of male individuals was 54.7% (95% CI: 53.32% -56.08%), and females were 45.3% (95% CI: 43.92% - 46.68%) (Table. 1).

Methods

Standardized serological ELISA tests for the detection of specific anti-EBV (VCA) IgM (Anti-EBV-CA ELISA IgM, EUROIMMUNE- Germany) and IgG (Anti-EBV-CA ELISA IgG, EUROIMMUNE - Germany) were performed according to the producer's recommendations.

Statistical analysis

The proportion of EBV seropositive individuals and their corresponding confidence intervals (CI), chi-squared distributions and p-values were calculated with SPSS, v.19.0. A p-value <0.05 was considered significant for all statistical tests.

Results

The highest share of tested individuals was in the age group 1-5 (15.9%, 95% CI: 14.79% - 16.81%), possibly due to the need for more frequent consultation and laboratory diagnostics of atypical clinical manifestations in young children. The proportion of tested hospitalized patients was higher (86.04%, 95% CI: 85.08% -87%) compared

Table 1. Distribution of tested individuals by sex for the period 2010-2015

Sex	Total number of tested individuals (N)	Proportion (95% CI)
Men	2744	54.7% (53.32%-56.08%)
Women	2272	45.3% (43.92%-46.68%)
In total	5016	100%

to outpatients (13.96%, 95% CI: 13% -14.92%). This shows that tests for EBV are predominantly appointed in individuals during their hospital stay and in conjunction with concomitant unspecified illness.

Anti-EBV (VCA) IgG immune status:

From a total of 5016 participants in this study, 5012 were tested for the presence of anti-EBV (VCA) IgG (99,9%, 95% CI: 99.81% -99.99%) (Table. 2). Hospitalized patients were 86.1% (95% CI: 85.14% -87.06%) of the sample compared with outpatients (13.9%, 95% CI: 12.94% -14.86%).

4135 of the tested individuals showed positive results (82.5%) (95% CI: 81.45% -83.55%). We found differences in seroprevalence between outpatients (88.2% - 95% CI: 85.8% -90.6%) and hospitalized patients (81.6% - 95% CI: 80.44% -82.76%).

The proportion of positive men (n = 2216) was 80.8% (95% CI: 79.33% -82.27% and the positive women were 1919 - 84.5% (95% CI: 83.01% -85.99%). The difference is statistically significant (p <0.05) (Cramer's V = 0.048, p <0.05).

There is a statistically significant relationship between the age of tested individuals and anti-EBV (VCA) IgG seropositivity, established with chi-squared test (p <0.01) and the fact that the strength of this relationship is weak (Cramer's V = 0.414, p <0.01).

Anti-EBV (VCA) IgM antibody status:

For the presence of anti-EBV (VCA) IgM 4984 people were tested (99.4%, 95% CI: 99.19%-99.61%) (Table. 3). Hospitalized patients had a higher percentage (86.6%, 95% CI: 85.65% -87.55%) compared with the relative proportion (13.4%, 95% CI: 12.45% -14.35%) of outpatients. A positive result was confirmed in 876 (17.6% - 95% CI: 16.54%-18.66%). The proportion of positive outpatients was 15.8% (95% CI: 13.04% - 18.56%) and 17.8% (95% CI: 16.66% - 18.94%) for hospitalized patients. The positive males (n = 482) were 17.6% (95% CI: 16.8% - 19.02%) and females (n = 394) were 17.3% (95% CI: 15.74% - 18.86%). The difference was not statistically significant (p> 0.05).

The age of the tested people is associated with anti-EBV (VCA) IgM seroprevalence (p <0.05), and the power is not strong (Cramer's V = 0.307, p <0.01).

Discussion

According to the presence of anti-EBV (VCA) IgG in our study, 82.5% (95% CI: 81.45% -83.55%) of the population in Northeastern Bulgaria aged between 1 month and 89 years old shows positive evidence of infection with the EBV. In another study (Genova *et al.*, 2013), 79% of the population

Table 2. Age-related distribution of anti-EBV (VCA) IgG seroprevalence in northeastern Bulgaria in the period 2010-2015

Age	Persons (N)	Anti- EBV (VCA) IgG – seropositive	
		Anti-EBV (VCA) IgG seropositive by age	Proportion (95%CI)
under 1 yr.	314	215	68.5% (63.36%-73.64%)
1-5 yr.	793	404	50.9% (47.31%-54.29%)
6-10 yr.	408	308	75.5% (71.53%-79.87%)
11-15 yr.	316	262	82.9% (78.75%-87.05%)
16-20 yr.	355	294	82.8% (78.87%-86.73%)
21-25 yr.	265	237	89.4% (85,69%-93.11%)
26-30 yr.	280	261	93.2% (90.25%-96.15%)
31-35 yr.	320	298	93.1% (90.32%-95.88%)
36-40 yr.	300	286	95.3% (92.91%-97.69%)
41-45 yr.	226	216	95.6% (92.93%-98.27%)
46-50 yr.	231	215	93.1% (89.97%-96.43%)
51-55 yr.	223	208	93.3% (89.54%-96.26%)
56-60 yr.	248	235	94.8% (92.04%-97.56%)
61-65 yr.	237	223	94.1% (91.1%-97.1%)
66-70 yr.	231	223	96.5% (94.13%-98.87%)
Over 71 yr.	265	250	94.3% (91.15%-96.85%)
Total	5012	4135	82.5% (81.45%-83.55%)

Table 3. Age distribution of anti-EBV (VCA) IgM seropositivity in northeastern Bulgaria in the period 2010-2015

Age	Persons (N)	Anti-EBV (VCA) IgM – seropositive	
		Anti-EBV (VCA) IgM seropositive	Proportion (95%CI)
under 1 yr.	314	41	13.1% (9.37%-16.83%)
1-5 yr.	794	254	32.0% (28.7%-35.25%)
6-10 yr.	408	121	29.7%(25.07%-33.93%)
11-15yr.	314	100	31.8% (26.65%-36.95%)
16-20 yr.	354	126	35.6% (30.61%-40.59%)
21-25 yr.	263	42	16.0% (11.57%-20.43%)
26-30yr.	272	32	11.8% (7.97%-15.63%)
31-35 yr.	317	38	12.0% (8.42%-15.58%)
36-40 yr.	297	29	9.8% (6.42%-13.18%)
41-45 yr.	224	15	6.7% (3.43%-9.97%)
46-50 yr.	229	17	7.4% (3.95%-10.65%)
51-55 yr.	220	9	4.1% (1.77%-7.23%)
56-60 yr.	246	14	5.7% (2.8%-8.6%)
61-65 yr.	237	12	5.1% (2.3%-7.9%)
66-70 yr.	231	12	5.2% (2.34%-8.06%)
over 71 yr.	264	14	5.3% (2.91%-8.49%)
Total	4984	876	17.6% (16.54%-18.66%)

was found to be infected with the virus. A previous study for Northeastern Bulgaria determined an average prevalence of 60.83% with immunofluorescent test (Ivanova, 2007). Our data show increased prevalence compared to previous studies, which is possibly due to the higher number of investigated individuals and more sensitive methods. We affirm the prevalence of the virus, which is in line with the global tendency. In a study performed in Bangladesh and Taiwan across a wide age range population, the mean seroprevalence was 81.27% and 88.5% respectively (Haque *et al.*, 1996, Chao *et al.*, 2015). The data in our study confirm the global trends of increasing prevalence with age. Similarly, in Denmark, the proportion of people with antibodies increases from 15% in 1 year olds to 60-65% in the age group 4-6, reaching up to 95% in late adulthood (Hesse *et al.*, 1983). In the United States for the period 2003-2010, among the age group 6-19 the average seroprevalence was 66.5% and also was increasing with age (Dowd *et al.*, 2013).

The analysis of the age distribution of seroprevalence and primary infection with EBV in our survey shows 68.5% seropositivity in children under 1 year old. Anti-EBV (VCA) IgM antibodies were found in 13.1% (9.37% - 16.83%) of them. In this age range,

236 of the tested individuals were up to 6 months of age with 76.3% (95% CI: 72.17% - 82.38%) seropositivity to anti-EBV (VCA) IgG. For comparison, 92.4% (95% CI: 87.86% - 91.68%) of tested women of childbearing age defined by WHO (15-49 years old), were positive for anti-EBV (VCA) IgG, but according to literature, in pregnant women anti-EBV (VCA) IgG antibodies were detected in 94% to 100% (Dundarov, 1998; Crawford *et al.*, 2002). Therefore, in infancy, IgG antibodies probably are passively passed from the mother. This determines protection from infection and therefore the lowest share of early infected (12.7%) as shown by the test of anti-EBV (VCA) IgM. During early childhood (1-5 years) seroprevalence is the lowest - 50.9% (47.31% - 54.29%). Serological evidence of primary infection in this age group is 32.0% (28.7% - 35.25%). Seropositivity after this age group gradually increases and reaches 93.2% (90.25% -96.15%) in the age group 26-30, then continues to grow reaching 94.3% (91.15% - 96.85%) in age over 71. The highest proportion of anti-EBV (VCA) IgM seropositive people is observed in the age group 16-20 - 35.6% (30.61% -40.59%) and with age it gradually declines, reaching values between 12% and 4.1% in the age groups over 31.

The distribution of seropositivity by sex in our study shows a higher degree of infection in women (84.5%) compared to men (80.8%) (p < 0.05). These data do not correlate with the findings of Genova *et al*, indicating male/female ratio 2:1 Genova *et al*., 2013), but correlate with data from other studies (Biggar *et al*.,1981; Crawford *et al*., 2006; Higgins *et al*., 2007; Dowd *et al*., 2013), where the proportion of women was also higher.

According to available literature, EBV infection begins in childhood or adolescence and identified risk factors for the duration of infection are mainly socio-economic (Cecília *et al.,* 2004). The analysis of our data from Northeastern Bulgaria shows that primary infection with EBV is attributed to early childhood and pre-school and school period. About 1/3 of children are infected by age 5, possibly with nonspecific clinical signs, and only the awareness of clinicians in our region refers patients to specific serological testing for EBV. Our data indicate a bimodal model of primary infection with a higher proportion in the age groups 1-5 and 11–20. According to earlier studies for the same region, the peaks of infections are observed in age groups 4-7 and 19-22 (Ivanova, 2007). Infection in early childhood is associated with the main route of transmission of the virus through oropharyngeal secretions in the family or in organized children's collectives. Our study did not test the impact of the household income, education level or other factors which could also have importance in determining the risk of infection in this age group. We think the higher prevalence is due mainly to the wide distribution of EBV and periodic transmission of the virus with oropharyngeal secretions from asymptomatic carriers. Periodic transmission of the virus in saliva was found in 15% - 20% of healthy seropositive youngsters and in immunosuppressed patients (Straus *et al*., 1993, Ling *et al*., 2003a). The second prominent peak during adolescence is due to the addition of sexual contact and the risk is mainly determined by the number of sexual partners. Accordingly, the virus is often confirmed in genital secretions. In surveys amongst young people not using protection during sexual intercourse, a higher incidence of infection was found, but further studies are needed, since the infection through kissing cannot be ignored (Crawford *et al*., 2002; Higgins *et al*., 2007). The high frequency of infection during adolescence suggests more frequent occurrence of infectious mononucleosis in this period.

Conclusion

In our survey we found:

1. EBV infection is highly prevalent in Northeast Bulgaria (82.5% - 95% CI: 81.45%-83.55%) and increased in comparison with a previous study, probably due to the more sensitive modern ELISA tests, and the larger number of tested individuals.
2. EBV infection is highly prevalent in women (84.5%) compared to men (80.8%) (p<0.05).
3. Our data indicate a bimodal model of primary infection with a higher proportion in the age groups 1 - 5 and 11 – 20.
4. Serological screening and viral load testing should be performed for immunocompromised patients and patients with different malignancies with respect to reactivation and/or reinfection with possible fatal complication.

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