



DISTRIBUTION OF EPSTEIN - BARR VIRUS AMONG WOMEN OF REPRODUCTIVE AGE AND CHILDREN UP TO 1 YEAR IN THE VARNA REGION

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ABSTRACT

Purpose: The Epstein-Barr virus (EBV) is widespread in the human population and is the major cause of infectious mononucleosis. Also, the virus is associated with the development of Hodgkin's and non-Hodgkin's lymphomas and nasopharyngeal carcinoma. The evidence of its role in neonatal pathology is contradictory and not well known. The aim of this study is to evaluate the EBV serostatus of women of reproductive age in the Varna region (2010-2016) to determine the risk of intrauterine and early postnatal EBV infection.

Materials/Methods: We analyzed the results of a total of 1126 women of reproductive age and 360 children up to 1 year tested for anti-VCA IgM (viral capsid antigen) and anti-VCA IgG. An indirect ELISA of Euroimmun – Germany was used.

Results: The proportion of positive anti-VCA IgG women in the reproductive age (76.8%; 95% CI: 74.2 %-79.3%) correlates with that of children up to 6 months - 68.0% (95% CI: 62.1% -73.6%), Pearson's = 8.395, p = 0.004.

Conclusion: We found high anti-VCA IgG seropositivity among women of reproductive age, which reduces the risk of infection during pregnancy and intrauterine infection of the fetus, respectively. The presence of seronegative women (around 6.0%) and of women with serological evidence of primary infection or reactivation (17%) assumes a group of babies at risk of early infection. Despite the little evidence of virus involvement in neonatal pathology, contamination should be considered and sought after excluding the most common infectious agents.

Keywords: Epstein-Barr virus, reproductive age, anti-VCA IgM, anti-VCA IgG, newborns,

INTRODUCTION

Epstein-Barr virus (EBV) is widespread in the human population. Over 90% of the adult population is infected with it. EBV causes infectious mononucleosis, especially when infected during the adolescent period. Also, the virus is associated with the development of Hodgkin's and non-Hodgkin's lymphomas in Europe and North America, and nasopharyngeal carcinoma mainly among the Chinese population. In Africa, EBV is the cause of Burkitt's lymphoma, the most common cancer in this region in childhood. To detect EBV infection, ELISA is used to demonstrate anti-VCA IgM and anti-VCA IgG. Anti-VCA IgM is a major marker for acute infection and disappear as it progresses, but may also occur during reactivation. Anti-VCA IgG appears almost simultaneously with IgM and persist for life, serving to determine the prevalence of EBV [1]. EBV infection in the Varna region, according to our previous study, occurs most often at ages 1 to 5 and 16 to 20 years with a continuing infection after 20 years regardless of gender. The most frequent infection among girls is in the teenage age, and then it decreases in the older ages [2]. Consequently, the risk of primary infection in the majority of women getting pregnant is low. However, the possibility of reactivation of the virus in the context of the changes in the immune system in this physiological state is not neglected [3,4]. The available literature data provide conflicting results with regard to the intrauterine transmission of the virus and the effect on the development of the fetus [3,5]. It is assumed to be a rare phenomenon, but at the same time, there is insufficient data on the role of EBV infection and its outcome. Therefore, the pathophysiology and clinical consequences of primary infection and EBV reactivation during pregnancy require further investigation.

MATERIALS/METHODS

We analyzed the results of the studies on a total of 1126 women of reproductive age (15-49 years, as defined by the WHO). The women were studied in the period 2010-2016 in the virological laboratory at the University Hospital "St. Marina"-Varna. The mean age was 30.7 years (SD \pm 10.2). The performed anti-VCA IgM tests were 1113 and anti-VCA IgG tests – 1122. We analyzed the results of 360 children up to 1 year tested in the same period and in the same laboratory. All were tested for anti-VCA IgM and 358 for anti-VCA IgG. An indirect ELISA of Euroimmun-Germany was used. The statistical processing was with the SPSS vs23 statistical package, and the assessment of dependencies was done with the Chi-square test.

RESULTS

Positive for anti-VCA IgG were 76.8% (95% CI: 74.2% - 79.3%, n = 862) of the women tested (n=1122), excluding those with anti-VCA IgM in combination with IgG. The lowest seropositivity has been demonstrated in individuals below 20 years of age. After this age, the proportion of positive women increased, with most of the ages above 77.0%. We divided the women tested into three groups according to the age (the results are presented in Figure 1).

Fig. 1. Distribution of positive anti-VCA IgG women by age group

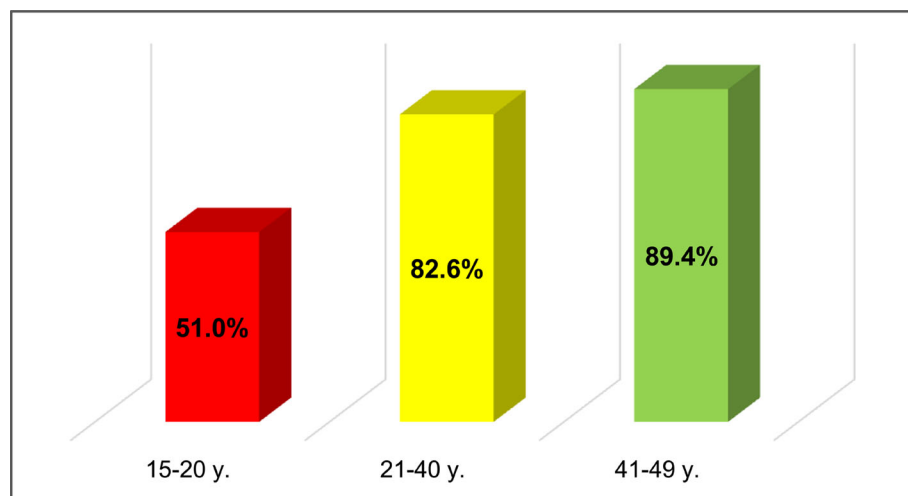


Table 1. Number and proportion of the anti-VCA IgM and anti-VCA IgG positive newborns

Age (months)	N*	N**	Proportion IgG positive (95% CI)	N***	Proportion IgM positive (95% CI)
1-6 m.	269/271	183	68.0%(62.1%-73.6%)	31	11.4%(7.9%-15.8%)
7-11 m.	89	32	36.0%(26.1%-46.8%)	12	13.5%(7.2%-22.4%)
All	358/360	215	60.1%(54.8%-65.2%)	43	11.9%(8.8%-15.8%)

N* -all newborn (the second number is the number of the IgM tests);

N** - number of anti-VCA IgG seropositivity;

N*** - number of anti-VCA IgM seropositivity

In 17.1% (95% CI: 14.9% - 19.4%, n = 190) we found anti-VCA IgM and therefore they were susceptible to primary infection or reactivation. The highest proportion of positive results is logical at the age of 20 years. Of all the women surveyed, 6.1% had no serological evidence of EBV infection, so the possibility of primary infection should be considered during pregnancy. According to our data, about 23% of women of reproductive age are at risk of infecting the fetus and the newborn.

In children up to 1 year (n = 360), the proportion of children up to 6 months of age is 75.3%, n = 271. During this period, studies were mostly done to detect intrauterine and/or early postnatal infection.

In children up to 6 months of age, the proportion of seropositive anti-VCA IgG was 68.0% and decreased to 36.0% in those between the age of 7 and 11 months. Children with serological evidence of primary infection were excluded from these numbers.

The highest proportion of anti-VCA IgG seropositivity was found in children up to 1 month (83.5%, 95% CI: 75.6% - 89.6%), followed by a significant decrease, and at the age of 6 months, the proportion was 25.0% (95% CI: 7.3% -52.4%).

The positive results for anti-VCA IgM in children up to 1 year were 11.9% (95% CI: 8.8% - 15.8%), and the cases were considered as probable primary infection (Table 1). In this age group, those tested up to 1 m predominated, and we found evidence of specific IgM antibodies in 8.3% (95% CI: 4.6% - 15.6%).

The highest proportion of IgM positive infants was at 8 months - 23.5% (95% CI: 6.8% - 49.9%), confirming the fact that primary infection started as soon as passive maternal antibodies were exhausted.

The difference in IgG positivity of children up to 1 year was significantly different from IgM positivity (p <0.05).

DISCUSSION

In our country, it is not a routine practice for pregnant women to be tested for EBV contamination. To our knowledge, this study is not included in the preventive measures package during pregnancy in most of the countries. Additionally, there are not many reports about the role of EBV in fetal pathology, but there are data, based on molecular-biological methods, that prove the risk of birth defects and even fetal death [3,5,6].

Antibody class IgG that passes through the placenta persists 6 to 8 months after birth. At this time, these antibodies provide protection for neonates from infection [7]. The more women of reproductive age have IgG class antibodies, the lower the risk of intrauterine and early postnatal infection.

The proportion of positive anti-VCA IgG women in reproductive age in our study (76.8%) correlates with that of children up to 6 months (68.0%) (Pearson's = 8.395, $p = 0.004$). Therefore, in the majority of cases, children receive at birth 100% of maternal antibodies. According to the literature, IgG antibodies to the capsid antigen are found in 94% to 100% of the pregnant women examined [8,9]. In another study, seropositive women defined in the immunofluorescence method in Northeastern Bulgaria were 82.5% [10]. Data from the UK show higher proportions than ours (93.6%) in pregnant women from three ethnic groups [11]. Increasing seropositivity with age and the observed tendency to increase the birth age among the Bulgarian population are factors that limit primary infection during pregnancy. At the same time, there are ethnic groups in which births are more frequent in the teenage age when we have demonstrated lower seropositive IgG and a higher percentage of IgM positive cases.

In our study, we found 68.0% seropositive for anti-VCA IgG infants up to 6 m with a decrease to 36.0% after this age. In a previous study in the same region, higher seropositivity (80%) in newborns was determined by the immunofluorescence method, which rapidly declined and reached 15% at 6 months of age [10]. In Thailand, the prevalence of anti-VCA IgG was relatively lower (47.1%), with a gradual decrease to 34.9% in children aged 6-24

months [12]. The reason for decreasing the positivity for anti-VCA IgG is the exhaustion of passive maternal antibodies, which depends on the levels of placental passages. With maternal antibody exhaustion, children may become susceptible to infection, which is recorded with seroconversion.

We found women with detectable anti-VCA IgM (17.1%). In a large-scale study of pregnant women in Norway, primary infection was found in 1.5% of cases and reactivation – in 25%. Studies were conducted only up to 10 gestational weeks in pregnant women, with no follow-up until the end of pregnancy and after the birth of babies [3]. A more recent study found evidence of reactivation in 35% of those surveyed [13]. The reasons for reactivation during pregnancy are still debatable. Probable risk factors include other infections, chronic diseases, environmental factors, stress and pregnancy itself as an immunosuppressed condition [3,4]. It is believed that EBV reactivation during this period leads to a shorter duration of pregnancy and the birth of babies with a lower weight. Further evidence suggests that the reactivation of maternal EBV infection is associated with a significantly higher risk of developing acute lymphoblastic leukemia in the offspring [14]. There is evidence that primary EBV infection during pregnancy may cause damage to the heart, eyes and liver. It is also believed that EBV can cause placental infection [3,5].

CONCLUSION

High anti-VCA IgG seropositivity among women of reproductive age reduces the risk of primary infection during pregnancy and intrauterine fetal contamination. The presence of seronegative women and women with serological evidence of primary infection or reactivation in our study suggest that further studies are needed in this direction. Despite the little evidence of virus involvement in neonatal pathology, contamination with it should be considered and sought after excluding the most common infectious agents. When the maternal antibodies are depleted after 6 months, children become susceptible to primary infection, and this requires training of young mothers to prevent early infection.

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