HERPESVIRUS INFECTIONS IN HUMAN POPULATION IN NORTH / EASTERN BULGARIA

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ABSTRACT

Herpesvirus infections are among the commonest human viral infections. In most cases the primary infection is asymptomatic but remains for a lifetime. In the last years, a tendency of increasing the frequency and severity of clinically manifested cases was observed. The most useful indicator for available infection is a specific serum antibody. The aims of the present study are to determine the antibody prevalence to 5 Herpesviruses: Herpes simplex virus type 1 (HSV1), Herpes simplex virus type 2 (HSV2), Varicella zoster virus (VZV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV) in a randomly chosen population of North/Eastern Bulgaria by different methods, depending on the age, and to determine the diagnostic capacity of each of them in common medical practice. Study population: 1846 single serum samples of randomly chosen persons divided into age groups were tested. Methods: CFT, ELISA, and IFA for detection of IgG specific antibodies. Results: Depending on the used methods, mean positivity to HSV1 was 66% - 70%, to HSV2 - 56%, to VZV - 50% - 62%, to CMV - 65% - 70% and to EBV - 45% - 61%. We found age depending dynamic in seropositivity rate. The prevalence of sera that reacted with all 5 Herpesviruses in reproductive age groups is 51%. Our study indicated different results in age related seropositivity rate to VZV and EBV depending on performed tests. Conclusions: Herpesvirus infections are widespread in our region. The different serological tests permit to determine the diagnostic capacity of each of them in common medical practice.

MATERIAL AND METHODS

Study population: 1846 single serum samples of randomly chosen persons (from newborns to 60 years), divided into age groups as follow: newborns, 1 month, 2 months, 3 months, 6 months, 1 yr, 2 yrs, 3 yrs, 4-7 yrs, 8-10 yrs, 11-14 yrs, 15-19 yrs, 19-22 yrs, 23-25 yrs, 26-30 yrs, 31-40 yrs, 41-50 yrs, 51-60 yrs.

Laboratory methods: Complement-fixation test (CFT) performed by micromethod (according to S. Bradstreet and S. Taylor), modification of I. Dobrev (4). Antigens, produced by the National Center of Infectious and Parasitic Diseases - Sofia, Herpesvirus Laboratory (HSV1, HSV2, VZV, CMV, and EBV), Behring Germany (HSV, VZV, CMV) and Dynatec Virion Germany (EBV) were used. Titors of specific antibodies (to each virus) < 1:4 were assessed as negative. Titors of specific antibodies >= 1:4 were determined as evidence for immune response to experienced infection. Enzyme linked immunosorbent assay (ELISA) for detection of specific IgG antibodies to HSV, VZV and CMV in the serum samples dilution 1:44 (Behring Diagnostics, Germany) was performed, according to the manufacturer’s recommendations. The results were calculated according to the detection limit and cut off value. Immunofluorescent assay (IFA) for detection of specific...

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Fig. 1. Age related distribution of seropositivity to herpesviruses in CFT

![Graph showing the age-related distribution of seropositivity to herpesviruses in CFT.](image)

Fig. 2. Age related distribution of seropositivity to herpesviruses in ELISA and IFA

![Graph showing the age-related distribution of seropositivity to herpesviruses in ELISA and IFA.](image)

IgG anti EBV (VCA) antibodies in the serum samples dilution 1:10, performed according to the manufacturer’s recommendations.

**Statistical methods:** Variation and Alternative analysis.

**RESULTS**

Figure 1 presents age-related seropositivity to 5 herpesviruses by CFT. The mean seropositive rate to HSV1 was 66%, to CMV - 65%, to HSV2 - 56%, to VZV - 50%,
and to EBV - 45%. The mean seropositive rate in reproductive age groups (15 – 50 years) was 79% to HSV1, 76% to HSV2, 72% to CMV, 60% to VZV and 57% to EBV. The seropositive rate in the newborn population corresponds to the seropositive rate in the reproductive age groups to all 5 herpesviruses. CF antibodies are predominantly IgG type and pass through the placenta. The maternal antibodies, received by the newborn babies at delivery, decrease on half every next month. Maternal antibodies gradually decrease and lose in the period from 2 months to 6 months of life for the different herpesviruses (Fig.1).

Our findings for CMV indicated, that seropositive mothers probably infected intramurally some of the newborns. We observed delayed decreasing of the mean antibody geometrical titers in one-month babies. We observed also early postnatal CMV infected babies (probably by the milk of their seropositive mothers) after the 6th months of age (Fig.1). The HSV1 and CMV seropositivity rates increase after 6 months to 1 year of age. More than 50% of the children investigated till 3 years of age were seropositive. These findings reflect the similar epidemiological factors of acquisition – close contact with positive parents and kissing of the babies. The large number of infected individuals with VZV we observed after 3 years of age, with EBV – after 4 – 7 years of age. We found a significant increasing of HSV2 and EBV seropositivity in the group of 15 - 18 years old persons and older. These findings reflect horizontal infectivity after close intimate contact and after sexual debut of adolescents and young people. EBV seropositivity in this age group may be explained with infectious mononucleosis developing also. We observed high titers of HSV2 antibodies in 6% - 10% of the investigated persons, older than 20 years, which probably corresponds to the group of persons with clinically apparent genital herpes. After 20 years of age, most of the persons already had antibodies to herpesviruses, and 51% had antibodies to all 5 herpesviruses. We observed about 15% - 20% persons, older than 20 years without specific antibody to different herpesviruses.

Figure 2 shows age-related herpesviruses seroprevalence detected by ELISA and IFA. The mean seropositive rate to HSV was 70%, to CMV - 70%, to VZV - 62%, and to EBV - 61%. The mean seropositive rate in reproductive age groups (15 – 50 y.) was 86% to HSV, 86% to CMV, 83% to VZV and 82% to EBV (IFA). As has been described before there is a clear age dependency of the seropositivity rate to Herpesviruses. The seroprevalence rises at older age.

**DISCUSSION**

The tendency of the herpesviruses to cause latent infections may contribute to persistence of their CF, ELISA and IF antibody, since CF antibody to other viruses does not appear to be as long lasting as detectable neutralizing antibody. The CFT was chosen for these studies because it is simpler, less expensive and less dependent upon strain specificity (12). ELISA is contemporary method, which is performed in the world seroepidemiological and diagnostic practice. IFA is a gold standard for EBV seroepidemiological investigations and for diagnostic purposes. In our study, we investigated seroprevalence to 5 herpesviruses comparatively by the two methods for each virus except HSV2. We found that there is serological data for earlier and widespread HSV1 and CMV infectivity in the North/Eastern Bulgaria people. Over 50% of the tested children up to 3 years of age were seropositive. The prevalence of herpesvirus infections overall and by age varies markedly by country, regions within country and population subgroup (9). A recent study in the general populations of eight European countries including Bulgaria (7) reported of marked intercountry variation in the median age of HSV1 and HSV2 acquisition. Median age related acquisition of HSV1 in Bulgaria, depending above cited authors (7) is 5-9 years of age. Using ELISA and CFT we found similar results to HSV1 and CMV age related epidemiology. CMV seroprevalence increases significantly after 6 months of age and gradually after 1 year of age. There are no statistical differences by two methods performed, but in ELISA we prove more reliable early postnatal CMV infected newborns. Acquisition of HSV2 is frequently cited as a behavioral marker with HSV2 antibody status correlated to previous sexual activity (3). In our study age-related distribution of seropositive HSV2 rate in CFT to great extend repeats the age-related distribution proved to HSV1 because of cross-reactions between these two types herpes simplex viruses. But from adolescents onwards an increasing proportion was HSV2 seropositive with increasing age. Whichever asymptomatic genital shedding of HSV2 plays an important role in the transmission including vertically to the newborns. The performed ELISA test in the present study can't determine the differences between HSV1 and HSV2 serostatus. Type-specific serological tests for HSV1 and HSV2 have become available. These tests specify the relative deal of infected persons and of patients with genital herpes. Age depending distribution of VZV seroprevalence was performed for the first time in Bulgaria. Till 10 years of age more than 60% of children were infected with VZV probably experienced varicella. There are statistically significant differences in mean seropositivity rate obtained by the two methods. Using ELISA we can prove more reliable infectivity with VZV till 3 years of age and after 18 years of age (probably resulting of herpes zoster or varicella). Using two different methods we confirm that ELISA is more reliable method for sereopidemiological studies and for diagnostic purposes, because VZV is closely cell-related virus and induced CF antibody in low titters. We observed about 15% - 20% persons after 20 years of age lacking VZV specific antibodies. VZV seropositivity rate after 15 – 18 years of age is important marker, because varicella in older age may reflects serious disease and may leads to increasing number of neonatal varicella cases. One study of Swedish children aged 9-12 yeas (11) shows, that seroprevalence to VZV increased markedly for over 30 years period (from 1967 till 1997), but smaller increase in the CMV seroprevalence, while seroprevalence to HSV and EBV re-
mained relatively stable. Age related distribution of EBV seroprevalence was performed for the first time in our region. IFA detects statistically significant differences in EBV seroprevalence in the individuals from 4 – 7 years of age and older. This fact may be explained with the different type of EBV antibodies, detected by IFA (to viral capsid antigen - VCA), and the antibodies, detected by CFT (to viral nuclear antigen - EBNA). EBV VCA antibodies persist for a lifetime, similar to EBNA in the all infected individuals, and were more sensitive marker for diagnostic purposes. In our study there are differences between the time of acquisition of CMV and this for EBV. Depending on other authors from tropical countries (14), the age-specific prevalence rate of IgG antibodies to EBV and CMV rise rapidly after birth, reaching a value of over 90% by the fourth year of life.

CONCLUSIONS

The results of this study provide baseline information concerning the epidemiology of human herpesvirus infections in North/Eastern Bulgaria. These data confirm the widespread infectivity, because of similar epidemiological factors of acquisition. We determine the age of the beginning of contraction and the age of common acquisition of the infection for each herpesvirus investigated. Generally, HSV1 has been associated with oro-labial disease, with most infections occurring during childhood and HSV2 with genital disease with infection following sexual debut. Widespread CMV infectivity reflects intranatal and early postnatal infectivity of the newborns.

The relative deal of seropositive persons to all 5 herpesviruses rises with the age. We detect 15% - 20% persons older than 20 years that lack specific antibodies to different herpesviruses. These may lead to severe diseases in older age and to severe neonatal diseases after primary maternal infection during pregnancy.

Testing one and the same serum sample by different methods, gives reliable information about their diagnostic capacity. The well-informed medical stuff on the problem of distribution of different Herpes viruses in our region is of great importance for quick and correct diagnosis and adequate etiological treatment.

REFERENCES