

Prevalence of Epstein-Barr virus antibodies in healthy children and adolescents in Vitória, State of Espírito Santo, Brazil

Prevalência de anticorpos anti-vírus Epstein-Barr em crianças e adolescentes saudáveis em Vitória, Estado do Espírito Santo, Brasil

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ABSTRACT

The prevalence and age distribution of Epstein-Barr virus infection varies in different populations and there is little information about the epidemiology of this infection in Brazil. We studied the prevalence of EBV antibodies in a sample of 283 children and adolescents between 1 and 21 years old. The sample was taken from two neighborhoods in Vitória (capital city of Espírito Santo, Brazil). The São Pedro (SP) neighborhood represented an area with lower socioeconomic status and the Praias (P) neighborhood represented an area with higher SES. Anti-VCA (Virus Capsid Antigen) antibodies were detected by ELISA and anti-EBNA (Epstein-Barr Nuclear Antigen) antibodies were detected by an anti-complement immunofluorescence method, both using commercial kits. The results showed an overall prevalence rates of anti-VCA and anti-EBNA of 71% and 54% respectively. The prevalence for both anti-EBV antibodies was higher and probably the infection occurred earlier in the SP neighborhood. Among the various socioeconomic factors studied only low family income and maternal education level were significantly correlated with a higher frequency of positive serology for anti-VCA. These results demonstrate that there is a high prevalence of EBV antibodies in children and adolescents living in Vitória, that occurs more frequently at a younger age in children from families with low socioeconomic status. In addition, the results demonstrate an intermediate age distribution pattern between those reported in developed and underdeveloped countries.

Key-words: Epstein-Barr virus. Infectious mononucleosis. EBV antibodies.

RESUMO

O vírus Epstein-Barr tem variações geográficas na prevalência e na idade da soroconversão, e poucos estudos abordam estes aspectos no Brasil. O objetivo deste trabalho foi estudar a prevalência de anticorpos anti-EBV em uma amostra de 283 crianças e adolescentes de 1 a 21 anos de idade, residentes nos bairros São Pedro (SP) e Praias (P) no município de Vitória, ES. A pesquisa de anticorpos anti-VCA foi feita por ELISA e a de anti-EBNA por um método de imunofluorescência anticomplemento, ambos utilizando kits comerciais. Os resultados mostraram 71% de positividade para o anti-VCA e 54% para o anti-EBNA. A frequência do anti-VCA foi significativamente maior e a idade da soroconversão menor na amostra do bairro São Pedro. Maior frequência de sorologia positiva para o anti-VCA foi encontrada entre os grupos de baixa renda e menor escolaridade materna. Esses resultados demonstram que a prevalência de anticorpos anti-EBV é alta na população de Vitória, sendo mais frequente e precoce nas crianças e adolescentes de famílias de baixa renda e menor escolaridade, com curva de distribuição etária intermediária entre a observada em países desenvolvidos e subdesenvolvidos.

Palavras-chaves: Vírus Epstein-Barr. Mononucleose infecciosa. Anticorpos anti-vírus Epstein-Barr.

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Recebido para publicação 28/7/2003

Aceito em 16/7/2004

Although Epstein-Barr virus (EBV) infection is ubiquitous in all human populations, primary EBV infection varies in relation to poverty and crowded living conditions. In Africa, Southeast Asia and Latin America the seroconversion occurs early in childhood and infectious mononucleosis is less frequent^{4 12 15 18 19 27}. In developed countries the seroconversion occurs in adolescence and infectious mononucleosis is more frequent^{11 16 19 20 24 26}. However even in developed countries primary infection occurs earlier and mononucleosis is less frequent among children from impoverished families in crowded living conditions²⁴.

In Brazil there are few reports on the prevalence of EBV infection and antibodies in samples of healthy people: three studies in the city of São Paulo^{6 7 8} and one among indigenous natives from the Amazon region^{5 8}.

EBV infection is closely associated with Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma and B cell lymphoma in immunosuppressed patients¹⁰. Association of EBV infection with Burkitt's lymphoma and Hodgkin's disease in Brazil has been reported in Recife²², Salvador^{2 3} and São Paulo⁹.

Burkitt's lymphoma and Hodgkin's disease were, respectively, 11.1% and 5.7% of all lymphomas diagnosed in children aged 1 to 15 years old at the Oncology Division of Children's Hospital Nossa Senhora da Glória in Vitória (Bortolini et al, 2000, unpublished data). This high frequency of lymphomas that are frequently associated with EBV infection in the state of Espírito Santo lead us to investigate the frequency of EBV antibodies in children living in Vitória, the state capital. The study was carried out by testing anti-VCA (Virus Capsid Antigen) and anti-EBNA (Epstein-Barr Nuclear Antigens) antibodies in two samples of children from two distinct socioeconomic conditions.

PATIENTS AND METHODS

From February 1, 2001 to April 30, 2001 two random samples of children and adolescents aged 1 to 21 years old were studied. The children whose parents consented to participate in the study were selected. One sample (denominated São Pedro) of 145 children and adolescents originated from the Unit for Health Promotion in the neighborhood of São Pedro where families of low socioeconomic conditions live. The other sample (denominated Praias), with 138 children and adolescents, originated from nurseries and schools of the neighborhoods near the beach, including Jardim Camburi, Mata da Praia, Jardim da Penha, Praia do Canto and Santa Lúcia, where families with higher socioeconomic conditions are resident. Vitória has 291,889 inhabitants (100,133 aged 1 to 20 years old)²¹. The location of the neighborhoods is indicated in Figure 1.

All children or adolescents were subjected to a clinical evaluation, and none presented signs or symptoms of acute or chronic disease. For each one we collected information regarding socioeconomic conditions.

After consent was obtained, 5ml of blood was collected by venopuncture from each child and the sera were stored at -20° C. All sera were tested within six months of storage.

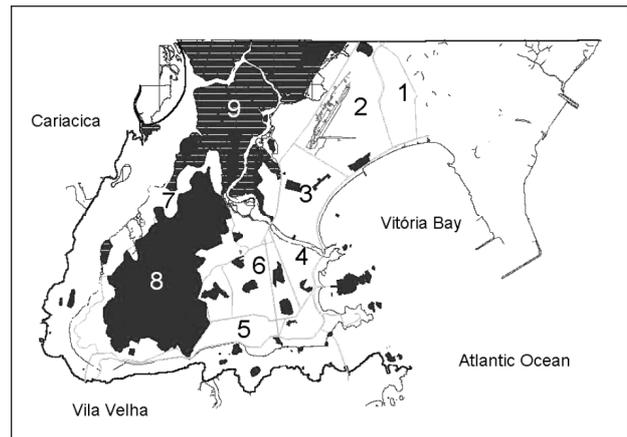


Figure 1 - Map of the City of Vitória showing the neighborhoods in which the anti-EBV antibodies were studied in children and adolescents. The neighborhood named Praias in the text is formed by the following neighbourhoods: 1=Jardim Camburi; 2=Mata da Praia; 3=Jardim da Penha; 4=Praia do Canto; 5= Bento Ferreira and 6= Santa Lúcia. The number 7 indicate the neighborhood of São Pedro. Number 8= Forest reserves. Number 9= Mangrove swamp.

Anti-VCA IgG was detected by ELISA method using commercial kits (DiasorinTM, Italia). Anti-EBNA antibodies were detected by an anti-complement immunofluorescence method using infected and non-infected Raji cells as substrate. (Kits from BiognostTM, Germany). For anti-EBNA antibodies the sera were diluted to 1:80 and results were considered positive for dilutions higher than 1:5. All tests were performed in the same laboratory at the University Hospital. The sensitivity and specificity of the tests used are higher than 87.5%. The main cross reaction in ELISA tests for anti-VCA is with rheumatoid factor, that is more frequent in elderly people^{17 25}.

Statistical analysis was performed using the software SPSS Version 8.0 for Windows. Chi square or Fischer's exact tests were used for comparison of proportions and Student's *t* test for comparison of means. The results were considered significant for *p* values less than 0.05.

This research was approved by the Ethics Committee of the Biomedical Center of the Federal University of Espírito Santo.

RESULTS

The data on age, gender and socioeconomic variables for the two samples studied are shown in Table 1. The results of the serology for anti-EBV antibodies are in Tables 2 and 3. Age distribution of positive serology for anti-VCA antigen is in Figure 2. The comparison of the different variables demonstrates that the two samples did not differ in regard to gender and age distributions, but showed significant differences in respect to income, maternal education, body weight and height, and duration of breastfeeding. The facilities for treated water and sanitary system and the status of immunization were similar in both samples. The frequency of positive serology was significantly higher and the seroconversion occurred earlier in children with family incomes less than twenty minimum salaries and with maternal education level of elementary school or less (Table 4).

Table 1 - Main socioeconomic and demographic variables studied in two samples of children and adolescents from two different neighborhoods in Vitória, ES.

Variable	São Pedro (n = 145)	Praias (n = 138)	P value
Age (mean ± SD)	6.35 ± 4.59	7.42 ± 4.52	0.050*
Gender (M / F)	66 / 79	75 / 63	0.138**
Family income in minimum salaries (n%)			
< 10	145	7	
>10	0 (0%)	131 (94.9%)	0.000**
Maternal education level (n%)			
illiterate	08 (5.5%)	0 (0%)	
elementary education	108 (74.4%)	0 (0%)	
secondary education	29 (20.0%)	19 (13.7%)	
university education	0 (0%)	119 (86.2%)	0.000**
Body weight (mean ±SD of percentile value)	43.56 ± 28.91	58.33 ± 24.52	0.000*
Height (mean ±SD of percentile value)	47.82 ± 27.72	58.62 ± 27.98	0.000*

* T test; ** χ^2 test; SD = standard deviation

Table 2 - Gender distribution of prevalence of antibodies anti-EBV (IgG anti-VCA and IgG anti-EBNA) in a sample of 283 children and adolescents living in Vitória, E. Santo, Brazil.

Serology	Anti-VCA			Anti-EBNA		
	male	female	M + F	male	female	M + F
Positive	95	106	201	75	78	153
Negative	46	36	82	66	64	130
Total	141	142	283	141	142	283

χ^2 test: male VCA x female VCA, p = 0.178 ; male EBNA x female EBNA, p = 0.769; M+EVCA x M+F EBNA, p = 0.000.

Table 3 - Serology for IgG anti-VCA and IgG anti-EBNA to EBV in children from Praias and São Pedro neighborhoods, in Vitória, ES, Brazil.

Serology	Anti-VCA*		Anti-EBNA**	
	Praias	São Pedro	Praias	São Pedro
Positive	88	113	75	78
Negative	50	32	63	67
Total	138	145	138	145

* χ^2 Test: p = 0.009 ** χ^2 Test: p = 0.925

Table 4 - Distribution of anti-VCA antibodies according family income and maternal level of education in children from Vitória, ES, Brazil.

Family income**	Anti-VCA +	Anti-VCA -	p*
< 20 minimum salaries	127	4	
> 20 minimum salaries	74	41	0.040
Maternal level of education			
elementary education	93	23	
above elementary education	108	59	0.004

* χ^2 Test ** Minimum salary is approximately US\$ 86.00.

The sample size was sufficient for this cross-sectional study, if we accept an alpha error up to 5% and a beta error up to 80%, using an estimated prevalence of 70%, as observed in children in São Paulo⁸. The two samples were not different in age and gender but differed in socioeconomic parameters (income under 20 minimum salaries, maternal level of

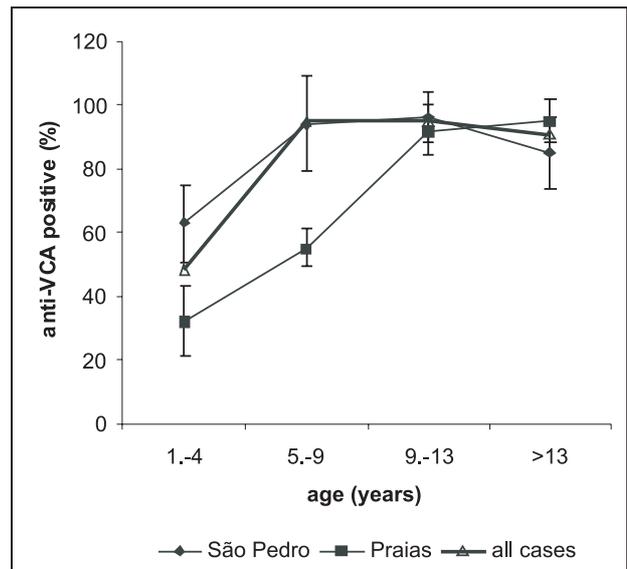


Figure 2 - Age distribution (and 95% confidence intervals) of anti-VCA antibodies in children and adolescents from Vitória, ES, Brazil.

education, lower stature and weight, duration of breastfeeding). Clinical evaluation showed that all children were asymptomatic, with no signs or symptoms of possible recent EBV infection.

DISCUSSION

The overall prevalence of anti-EBNA antibodies in Vitória corresponds well to that which is reported worldwide: progressively higher prevalence of positive serology with increasing age, reaching a peak in the second decade of life, and without sex bias. The pattern of age distribution of positive serology in Vitória is somewhere between the distributions observed in developed countries of Europe, United States and Canada^{11 13 16 19 20} and those observed in underdeveloped countries of the African Continent⁴.

The prevalence of anti-VCA antibodies was similar to that observed in São Paulo⁸ and lower than that observed in the indigenous population of the Amazon region⁵. In addition, seroconversion occurred later in our sample than in both of these groups, in whom 90% had positive serology by five years of age.

The frequency of positive serology for both anti-VCA and anti-EBNA antibodies was higher in children from São Pedro than in children from the Praias neighborhood, confirming that the prevalence of EBV infection is higher and probably the primary infection occurs earlier in children of lower socioeconomic status. In fact our results demonstrate that the prevalence of EBV antibodies and the earlier infection correlates with low family income and low level of maternal education (Table 4). In addition, in the neighborhood of São Pedro the frequency of positive serology was higher in children that are cared for in a nursery (data not shown), supporting the importance of crowding in furthering the spread of EBV.

With regard to the anti-EBNA antibodies the results showed that there were fewer positive tests for this assay than for

anti-VCA antibodies. However all anti-EBNA positive children had anti-VCA antibodies, whereas some anti-VCA positive cases were negative for anti-EBNA. We have not reached a good explanation for these results. Previous studies on EBV prevalence have not measured anti-EBNA antibodies. Andiman et al¹, observed that anti-EBNA antibodies appeared as late as six months after infection. Yadav et al²⁷ also reported a lower frequency of anti-EBNA antibodies among infected children in Malasia. It is accepted that EBNA are less immunogenic and that the anti-EBNA antibodies appear later after infection than anti-VCA. In fact Sumaya & Ench²³ in a follow-up of children with clinical manifestations of infectious mononucleosis demonstrated that anti-EBNA antibodies appeared later and with lower titers than the anti-VCA antibodies. Thus, it is possible that the seroconversion to EBNA occurs slowly in younger children. To further support this theory, a study in adults (blood bank donors) in Cuba reported similar frequencies of anti-VCA and anti-EBNA antibodies¹⁶.

Our results demonstrate that there is a high prevalence of EBV antibodies in children and adolescents living in Vitória, that occurs more frequently at a younger age in children from families with low socioeconomic status, with an intermediate age distribution pattern between those reported in developed and underdeveloped countries.

ACKNOWLEDGMENTS

We thank Latha Nagonna for help in editing. This research was supported by Hospital Universitário Cassiano Antonio Moraes.

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