

EBV INFECTIONS IN BRAZIL III — INFECTIOUS MONONUCLEOSIS

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SUMMARY

Following strict hematologic and serologic criteria for the diagnosis of infectious mononucleosis including the presence of heterophile antibodies, 87 patients from three laboratories in the city of São Paulo were studied retrospectively regarding age, season and sex distribution. Analysis of results showed that in São Paulo city, where infections by Epstein-Barr virus (EBV) are prevalent in the first years of life, age distribution of the disease seems to be different from that found in developed countries. A bimodal distribution was seen, with a peak in the 6-10 years age group (28.7%) and another in the 16-20 years age group (34.5%); 55.1% of all cases observed were 16 years old or less, the mean age of cases increasing with socio-economic level. There was no difference regarding seasonal or sex distribution. The limitations of EBV anti-capsid IgG antibody test for the diagnosis of acute EBV infections was once more demonstrated.

UNITERMS — Infectious mononucleosis. Epidemiology

INTRODUCTION

The discovery of the Epstein-Barr virus (EBV) in 1964⁶ and its association with the etiology of Infectious Mononucleosis (IM)¹⁶ helped to explain certain poorly understood aspects of this entity which had long puzzled researchers. It is believed today that no other agent besides EBV can lead to Paul-Bunnell-Davidsohn positive infectious mononucleosis⁸. On the other hand, tests for specific antibodies have shown that the infection with this virus, especially in children, is often sub-clinical^{15,24,25} but may sometimes give rise to a typical clinical and hematologic picture without heterophile antibodies^{12,19,20,23}.

The frequency of clinical infectious mononucleosis with positive heterophile antibody seems to depend on the number of individuals

missed by the infection until age 15 to 25; thus, in developing countries and in lower socio-economic level groups where EBV infection occurs early in life, heterophile positive IM would be uncommon⁹.

This paper is part of a general investigation on EBV in Brazil. Its purpose was to study the occurrence of acute infections with this virus and the influence of age, sex and season of the year on the distribution of heterophile positive infectious mononucleosis in the city of São Paulo.

METHODS

This research involved two phases: 1) A prospective study of high socio-economic level

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school children (Jardim Escola São Paulo) that was carried out to evaluate the EBV infection rate and the associated clinical manifestations. In 1969 and 1970, 164 blood samples were initially collected from children between 6 and 14 years of age. Anti EBV antibodies were measured and, one year later, it was possible to collect a new sample from 26 of those who were originally negative. All children were carefully observed during the period referred, in order to detect any clinical abnormality. 2) Retrospective study — Patients of this group were selected on the basis on heterophile antibodies and hematologic alterations suggestive of infectious mononucleosis. The following patients were studied: a) 169 Sera, from patients referred to a public health laboratory (Instituto Adolfo Lutz) for heterophile antibody assay from September 1969 to March 1970; b) 3,353 Sera, from patients of a government employee hospital (Hospital do Servidor Público Estadual "Francisco Morato de Oliveira"), were tested between January 1973 and June 1977; c) 1,988 Sera, from patients referred to a private laboratory ("Laboratório de Patologia Clínica Fleury", which serves high economic level groups) were studied from April 1975 to December 1976.

Heterophile antibody test

At the government employee hospital and the private laboratory the original method of Paul-Bunnell and Davidsohn with sheep red blood cells⁵ was employed. Sera were considered positive for infectious mononucleosis when the titer was $\geq 1:56$ after absorption with guinea-pig kidney. The microtiter technique with sheep red blood cells was applied to the Instituto Adolfo Lutz sera. Titers $\geq 1:40$ after guinea-pig kidney absorption were considered positive¹⁰.

Indirect fluorescent anti-EBV antibody test (IFAT)

IFAT EBV-specific was performed according to the method of HENLE & HENLE¹⁴, employing the Jijoye cell line 2PHR1, as the antigen source. In the prospective study sera were also tested with the EB3 cell line. All sera from the selected cases were submitted to the IFAT with exception of 24 of the 57 sera from the government employee hospital.

WBC analyses

These analyses were performed by several hematologists, and the criteria for considering the results as indicative of infectious mononucleosis was 50% or more lymphocytes with 10% or more atypical cells.

RESULTS

1) Prospective study

When EB3 cell line was used as the antigen source, anti-EBV-VCA antibodies were detected in 52.4% of the 164 school children tested in the beginning of the study (geometric mean titer: 1:24.4). In addition, when 151 of these sera were tested using the Jijoye cell line as the source of antigen, a 68.9% positivity was observed (geometric mean titer: 1:44.2).

In children whose initial sera lacked EBV antibody there was serological conversion in only 2 (7.7%) of the 26 serum samples collected after one year of observation. These 2 children were 5 and 8 years old and both titers changed from negative ($< 1:5$) to 1:160 (EB3 cells) during this interval. Titer variations from negative to 1:20 were observed in 2 other children, and if these were also taken into consideration, the seroconversion rate would be 15.4%. These children developed no known clinical symptoms nor did other children in the entire study group have a clinical syndrome resembling infectious mononucleosis.

2) Retrospective Study

An analysis of the records on 169 patients with suspected infectious mononucleosis in a public health laboratory, 3,353 in a government hospital, and 1,988 in a private laboratory are shown in Table I. Only 87 patients or 1.58 percent of the 5,507 patients analyzed fulfilled the heterophile antibody and hematological criteria set forth under materials and methods. The highest percent of confirmed patients was seen in the public health laboratory material. The distribution by age of the 87 confirmed cases is shown in Table II. Over half of the patients (55.1 percent) were under age 16. Furthermore, a double peak was seen: at age 6-10 and age 16-20 (Fig. 1). Further analysis indicated that the double peak was observed in the middle socioeconomic government hospital patients but only a peak at age 16-20 was seen

in patients tested by a private laboratory dealing with high socio-economic patients. The 4 confirmed cases in the lowest socio-economic group in the public health laboratory were all in the 6-10 year age group. The mean age of

cases according to socio-economic status were: lowest, age 7.7 g middle, age 13.3 and highest, age 15.1. The mean age of all the 87 cases was 13.6.

GRAPHIC I: AGE DISTRIBUTION OF 87 INFECTIOUS MONONUCLEOSIS CASES IN SÃO PAULO - BRAZIL

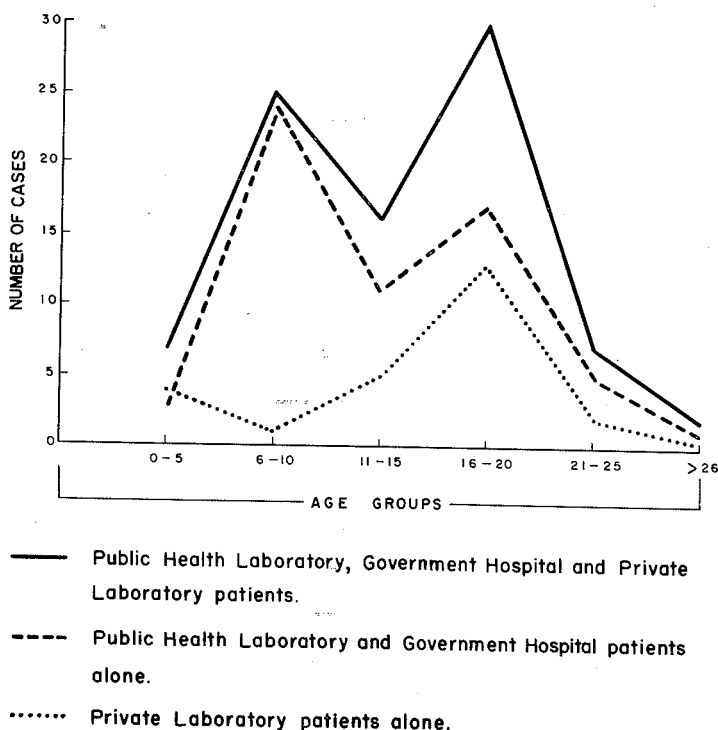


TABLE I
Number of Patients with Suspected Infectious Mononucleosis that Fulfilled Heterophile Antibody and Hematological Criteria in Three Socioeconomic Groups

Source of Material	Socio-Economic Level	Period	No. Tested	No. Confirmed	Percent Confirmed
Public Health Laboratory	Lower	9/69-3/70	169	4	2.4
Government Hospital	Middle	1/73-6/77	3,350	57	1.7
Private Laboratory	High	4/75-12/76	1,988	26	1.3
Total			5,507	87	1.6

An analysis of 72 patients by month of diagnosis shows no clearcut distribution, although, September and May had the highest percent of patients. The illness in the middle income group of patients from the government hospital occurred primarily in the last half of the year.

There was no difference in the sex distribu-

tion of the patients (42 males and 45 females).

The distribution of antibody to the viral capsid antigen of EBV in the sera of 63 patients are given in Table II. Only 2 patients lacked antibody at the time of testing. Most antibody titers (79 percent) lay between 1:10-1:320 and 17.5 percent were at 1:320 or higher.

T A B L E II

Age group distribution of 87 heterophile positive infectious mononucleosis patients — São Paulo-Brazil

Age	Public Health Laboratory	Government Hospital	Private Laboratory	Total	%
0-5	0	3	4	07	8.0
6-10	4	20	1	25	28.7
11-15	0	11	5	16	18.4
16-20	0	17	13	30	34.5
21-25	0	5	2	07	8.0
≥ 26	0	1	1	02	2.3
Total	4	57	26	87	100.0
Mean	7.7	13.3	15.1	13.6	

T A B L E III

Anti-EBV viral capsid antigen (VCA) antibody titers in sera from 63 patients with heterophile positive infectious mononucleosis

	1:10	1:10 — 1:60	1:320	Total	Geometric mean titer (*)
Public Health Laboratory (***)	0	3	1	4	1:95.1
Government Hospital (**)	0	30	3	33	1:62.2
Private Laboratory (**)	2	17	7	26	1:89.8
Total	2	50	11	63	1:73.9

(*) These calculations are based only on those with antibody

(**) Jijoye cell line

(***) EB3 cell line

DISCUSSION

In a prospective study of children, age 6-14, of upper socio-economic level in a private school in São Paulo, 68.9 percent had EBV antibody at the start of the study, confirming findings reported in earlier papers^{2,3}. The antibody prevalence in this 6-14 age group is equal to or even somewhat higher than that found in entering College and in University students, age 17-19, in England¹⁸ and in the United States^{13,22}. On the other hand, the seroconversion rate of 15.4% in one year is similar to the 12-13 percent infection rate in College students^{13,18,22}. However, no clinical manifestation of infectious mononucleosis could be detected in our cases, showing the tendency of EBV infections to be mild or asymptomatic in children.

A retrospective analysis of 5,507 patients tested for heterophile antibody because of suspected infectious mononucleosis in a public health laboratory, a government hospital, and a private laboratory revealed only 87 (1.6 percent) persons that fulfilled strict hematologic and serologic criteria. This is in contrast to the experience of public health laboratory in the United States such as the Wisconsin State Laboratory of Hygiene where 11.1 percent of 3,480 suspected cases were heterophile positive⁷. The difference is probably due to the earlier age of the suspected infectious mononucleosis cases in São Paulo at a time when the majority is heterophile negative⁹. The mean age for the 87 cases was 13.6 years, and 55 percent occurred under age 16. This age is lower than that found in the United States, France and Denmark

where the peak frequency is in the 15 to 25 year age group¹⁰. In addition to the low mean age, the 87 heterophile positive cases in São Paulo showed bi-modal peaks at age 6-10 and at age 16-20, with a dip in between. A reasonable explanation for the surprisingly high incidence of heterophile positive infectious mononucleosis in children could be the great number of infections in childhood in the city of São Paulo, because despite the fact that clinical expression of EBV infection in this age group tends to be non-characteristic, the low percentage of cases with positive Paul-Bunnell-Davidsohn test is still sufficient to represent a significant absolute number. The opposite should probably occur in EBV infections in late childhood and young adult life, for there would be a small number of infections but a lot of them would be clinically and serologically characteristic.

The number of heterophile positive cases, especially in childhood, would probably have been much higher if horse rather than sheep red blood cells had been used^{11,21}. On the other hand, the use of the EBV-IgM test would have permitted positive identification of heterophile negative cases^{11,21,23}. In the 26 patients of the highest socio-economic group, from the private laboratory, no bimodal distribution was observed, showing a pattern most closely resembling more developed countries.

The geometric mean titer of EBV-VCA antibody was quite similar in the cases of infectious mononucleosis from the 3 laboratories, ranging from 1:62.2 to 1:95.8. Variable results have been reported for this test by others^{1,4,11,17,20}. The limitation of the EBV-VCA test as a diagnostic tool is emphasized by the fact that only 17.5 percent of the sera were positive at a "diagnostic level" of 1:320. The frequency of EBV-VCA antibody increases has also been of only 15-20 percent in most studies^{8,10}. In future studies the absorbed horse cell agglutination microtest should be performed first, and if negative followed by the EBV-IgM test. The latter is of particular importance in children with the infectious mononucleosis syndrome. The possibility that agents other than EBV were responsible for the syndromes seen in this study was not tested but is investigated in another recent study in the São Paulo area by our group²¹, in which the improved diagnostic techniques for infectious mononucleosis were also employed.

RESUMO

Infecções pelo vírus EB no Brasil. III — Mononucleose infecciosa

Adotando critérios hematológicos e sorológicos bem definidos para o diagnóstico de mononucleose infecciosa com anticorpos heterófilos presentes, foram estudados retrospectivamente, em relação à distribuição etária, sazonal e de sexo, todos os pacientes (87 no total) que se apresentaram com este diagnóstico em três instituições médicas da cidade de São Paulo, durante o período do estudo. Analisando os resultados obtidos, concluem os Autores que em São Paulo, onde a infecção pelo vírus de Epstein-Barr (VEB) se dá principalmente nos primeiros anos de vida, a distribuição etária desta doença seria diferente da encontrada em populações de países desenvolvidos, pois a porcentagem de casos observados no grupo etário de 6-10 anos (28,7%) aproximou-se bastante da porcentagem de casos observados no grupo etário de 16 a 20 anos (34,5%) e além disso 55,1% do total de casos tinha menos de 16 anos de idade. A análise de pacientes por mês de diagnóstico não mostrou nenhuma distribuição sazonal característica, não tendo também sido notada nenhuma diferença quanto ao sexo. Além disso, ficou mais uma vez comprovada a limitação da dosagem de anticorpos IgG anti-cápside do VEB para o diagnóstico de infecções agudas por este vírus.

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