

SECONDARY DENGUE INFECTION IN SCHOOLCHILDREN IN A DENGUE ENDEMIC AREA IN THE STATE OF RIO DE JANEIRO, BRAZIL.

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SUMMARY

A seroepidemiologic survey was carried out in schoolchildren from public schools of the Niterói municipality, state of Rio de Janeiro, Brazil, after a period of sequential epidemics by dengue virus type 1 and 2 (DEN-1 and DEN-2). 450 blood samples were obtained by fingertip puncture and collected on filter paper discs. The hemagglutination inhibition (HAI) test was carried out using DEN-1 and DEN-2 antigens. HAI titres were demonstrated in 66% (297/450) of the sera and the geometric means of the titres were 1/182 and 1/71 for DEN-1 and DEN-2, respectively. Secondary infections were observed in 61% (181/297) of positive cases. Among these, 75% (135/181) were under fifteen years old. No dengue haemorrhagic fever (DHF) was reported in these children. Asymptomatic or oligosymptomatic infections were detected in 56% of the studied population. The absolute and relative frequencies of positive tests by age group and sex did not evidence statistically significant difference. The number of individuals infected probably produced a immunologic barrier responsible for the non occurrence of dengue epidemic in the latter years.

KEYWORDS: Dengue; secondary infection; Epidemiology; Brazil.

INTRODUCTION

In the last 20 years the frequency of dengue fever epidemic has increased and hyperepidemic transmission has been established over an expanding geographic area. The risk of secondary infection and consequently the incidence of dengue haemorrhagic fever (DHF) risen first in Asia and later reach the American region¹³. Undoubtedly, the DHF outbreak due to dengue virus type 2 (DEN-2) in Cuba in 1981 was the most significant event of the decade, as it was responsible for the worst

human impact ever observed in the dengue history in the Americas²¹.

Dengue fever was described in Brazil in the last century on clinical grounds^{10,11,20,23}, but the first dengue infection confirmed by laboratory tests, occurred in 1982 in Roraima, Amazonian area¹⁹. In 1986 an extensive classical dengue fever epidemic caused by dengue virus type 1 (DEN-1) was recognized in the state of Rio

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de Janeiro²⁴. During 1986-1987 more than one million people were estimated to be infected¹⁷. After that the disease became endemic with discrete elevation of the number of notified cases during summer time. In April 1990, DEN-2 was isolated in the state causing a widespread epidemic during 1990-1991 where both dengue serotypes co-circulated^{15, 16}. In this period more severe forms of disease associated with secondary infection were observed and dengue haemorrhagic fever (DHF) was reported in about 3% of the notified cases^{16, 27}. The majority of DHF cases (94.9%) occurred in persons with age equal or greater than 15 years old⁷, differing from the age groups early described by several authors in other parts of the world^{2, 8, 14, 25, 26, 28}.

This lack of DHF in children lead us to carry out an immunological evaluation of schoolchildren residents in an endemic area of dengue fever in the state of Rio de Janeiro, Brazil. The serological survey was carried out in the summer of 1991/1992, after a period of sequential epidemics by dengue virus type 1 and 2.

MATERIAL AND METHODS

Population studied

Blood samples from 450 schoolchildren with age ranging from 5 to 19 years old were obtained in public schools of Niterói by fingertip puncture and collected on filter paper discs (Whatman N° 1). The sample size was based on the prevalence of DEN-1 antibodies detected in previous inquiries (62% in 1987 and 55% in 1988) carried out in the same municipality⁵. The confidence level of 95%, a sampling error of 10% and a refusal possibility of 25% were considered. Questionnaires were filled out by parents or responsables for the children which gave also a formal consent for the blood collection.

Haemagglutination Inhibition test (HAI)

The haemagglutination inhibition test (HAI) was carried out according to the method of CLARKE & CASALS⁴. Blood samples were treated with kaolin (Sigma) and goose erythrocytes for the remotion of non-specific inhibitors and natural haemagglutinins respectively.

Following WHO orientation¹⁴, the geometric mean titre of 1/42 (standard deviation = 38.5), obtained through serological survey carried out in 1988, during a inter epidemic period (data not published) it was adopted as primary serologic response pattern; for the serologic

pattern of secondary infection, two standard deviation (SD=38.5 x 2) were added to the value of geometric mean titre adopted as a primary serologic response pattern (1/42). As the value of 1/119 was found (42+(2x38.5)=119), titres equal or greater than 1/160 were accepted as secondary infection for a post-epidemic period.

Antigens

DEN-1 (Mochizuki strain) and DEN-2 (New Guinea strain) antigens were prepared by intracerebral inoculation of suckling mice and extracted by the sucrose-acetone method⁴.

RESULTS

HAI antibody titres equal or greater than 1/20 to DEN-1 and/or DEN-2 were detected in 66% (297/450) of the tested samples. In 80.8% (240/297) of the samples, cross reaction for DEN-1 and DEN-2 by HAI test was observed. In addition, 270 (60%) presented HAI titres for DEN-1 and 267 (59.5%) for DEN-2. The geometric mean of the antibody titres was 1/182 and 1/71 to DEN-1 and DEN-2 respectively (Table 1).

TABLE 1

Absolute and relative frequencies of HAI antibody titres in schoolchildren of Niterói city.

ANTIBODY TITRES	DEN-1*		DEN-2**	
	N°	%	N°	%
1/20	22	8.1%	69	25.9%
1/40	35	13.0%	51	19.0%
1/80	32	11.9%	56	21.0%
1/160	57	21.0%	49	18.3%
1/320	62	23.0%	29	10.9%
1/640	37	13.7%	13	4.9%
1/1280	20	7.4%	-	-
1/2560	03	1.1%	-	-
1/5120	01	0.4%	-	-
1/10240	01	0.4%	-	-
TOTAL	270	100.0%	270	100.0%

* Geometric mean = 1/182

** Geometric mean = 1/71

Secondary infection was demonstrated in 61% (181/297) of positive tests. Among these, 75% (135/181) were under fifteen years old.

The absolute and relative frequencies of positive tests by age group and sex did not show statistically significant differences; nevertheless the total result shows a slight predominance of the positivity for the

female group (Table 2). The incidence of dengue infection by borough ranged from 40.3% (Center of the city) to 100% (Badu and Caramujo).

TABLE 2

Absolute and relative frequencies of HAI antibody by age group and sex in schoolchildren of Niterói city.

SEX/ AGE (years)	FEMALE		MALE		TOTAL	
	N°	%	N°	%	N°	%
<= 10	80	57.5	60	51.7	140	55.0
11 - 14	115	74.8	103	64.1	218	69.7
>= 15	41	70.7	51	76.5	92	73.9
TOTAL	236	68.2	214	63.5	450	66.0

The serologic results confirmed the presence of dengue antibody in 88% (117/133) of the schoolchildren whose parents confirmed that their children have had dengue fever. No case of dengue haemorrhagic fever was reported. Negative serology was found in 43.7 (124/284) of the schoolchildren whose parents affirmed that their children have no dengue infection before. Predictive values were 88% and 43.7% for the affirmative and negative answers respectively. Asymptomatic or oligosymptomatic were detected in 56% of the studied cases.

DISCUSSION

The adopted sampling plan for the present study limits the generalization of the results to the whole population of the Niterói municipality; nevertheless the results obtained are acceptable to determine the serological feature of dengue infections in the studied places. On the other hand, if we consider that the introduction of dengue virus in this municipality is relatively recent (1986), being therefore all age groups susceptible to the virus, we can expect a prevalence of dengue infections in the other age groups close to that of the examined schoolchildren. The low prevalence of antibodies for flavivirus in the state of Rio de Janeiro before the introduction of dengue viruses²², contributed for the reduction of the error margin in the interpretation of the results related here.

Considering the seropositivity of 66% and that the population of the Niterói municipality reach approximately 435,000 inhabitants⁶, about 290,000 persons may have been infected in this municipality, since the introduction of dengue until the time that the present

study was done. In Cuba, a serological survey carried out after DEN-1 epidemic in 1977-78 showed 44.5% of positivity¹.

The high prevalence of antibodies suggests that the decline of the DEN-2 epidemic (1990/1991) and its no recurrence in the further years was due to the immunologic barrier. Since the domestic infestation indexes of *Aedes aegypti* in Niterói were sufficient for the persistence of virus transmission⁷, we think that those antibody levels were also responsible by an immunological barrier, resulting in the autolimitation of the epidemic.

Between 1986 and 1992 only 14.3% of the notified classical dengue fever cases in Niterói occurred in persons with age less than fifteen years old, giving an average incidence rate for the period of 50.5/10,000 inhabitants against 98/10,000 inhabitants for those with age equal or greater than fifteen years old. In epidemics years, like 1987 and 1991, the incidence rate reached respectively 143.7 and 87.0 for 10,000 inhabitants for persons under fifteen years old and 328.8 and 187.7 for 10,000 inhabitants respectively for persons with fifteen years old and over (data not published). Such data allow to assess the occurrence of a major percentage of the asymptomatic infections in the younger age groups, since the domestic transmission of this virus would have equal chance of infection in the different age groups. The predictive values (88% and 43.7% for the affirmative and negative answers, respectively) suggests that asymptomatic or oligosymptomatic infections may have been occurred in 56% of the studied cases.

The geometric mean of the observed antibody titres (1/182 and 1/71 for DEN-1 and DEN-2, respectively), were higher to those found before the introduction of DEN-2 (1/80 and 1/42 in 1987 and 1988 respectively). The increase of the geometric mean of the antibodies titres for DEN-1 is due to an anamnestic response caused by a sequential infection. In the state of Rio de Janeiro, Niterói was one of the countries where high dengue activity was reported during both epidemics^{12, 16}.

Secondary infection was demonstrated in 61% (181/297) of the positive cases (or 40.2% (181/450) of studied cases). Among these, 75% (135/181) were detected in schoolchildren with age under fifteen years old. During DEN-2 epidemic in the state of Rio de Janeiro, NOGUEIRA et al.¹⁶ found 72.2% (130/180) of secondary infections. In the most of the cases the individuals were age equal or greater than fifteen years old. There-

fore we can accept that secondary infections were frequent in both children and adult populations.

According to MONATH¹³, the relative risk of develop the most severe form of the disease is 100-fold higher after secondary than primary infection. In Asia DHF is predominantly a child disease, representing the third or fourth cause of hospitalization in children, in some areas⁹.

The lack of DHF in Niterói in schoolchildren studied with documented secondary infection is puzzled, but our data confirm the recorded incidence of DHF in Niterói city (Table 3). It is clear that clinical profile of dengue infection in Rio de Janeiro was altered after DEN-2 introduction, when DHF occurred in adults and fatal cases were observed in the same group^{16,18,27}. The influence of the other factors in the etiopathogenesis of the DHF, besides secondary infection, Niterói must be studied, in order to better understand this peculiar presentation of dengue infections in Brazil⁴.

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RESUMO

Infecção secundária por dengue em escolares numa região endêmica para o dengue no Estado do Rio de Janeiro, Brasil.

Após um período de epidemias sequenciais pelos vírus dengue tipo 1 e 2 (DEN-1 e DEN-2), foi realizado um estudo soropidemiológico em uma amostra de escolares da rede pública de ensino do município de Niterói; 450 amostras de sangue foram obtidas através de punção da polpa digital, coletadas sobre discos de papel de filtro e testadas para a detecção de anticorpos inibidores da hemaglutinação (IHA) para DEN-1 e DEN-2.

Das amostras testadas, 66,0% (297/450) apresentaram títulos de anticorpos IHA e as médias geométricas dos títulos de anticorpos foram de 1/182 e 1/71, para DEN-1 e DEN-2, respectivamente. Cerca de 61,0% (181/297) daqueles com anticorpos IHA tiveram infecção secundária. Destes, 75% (135/181) tinham idade igual a ou menor do que 15 anos. Nenhum caso de dengue hemorrágico foi relato entre os participantes do estudo. Cerca de 56,0% dos casos com anticorpos IHA tiveram infecção assintomática ou oligossintomática. As frequências absoluta e relativa das sorologias positivas por idade e sexo não evidenciaram diferenças estatisticamente significativas.

Acredita-se que a barreira imunitária formada pelos indivíduos infectados pelo DEN-2 seja a responsável pela não ocorrência do dengue de forma epidêmica nos anos imediatamente posteriores ao presente estudo.

TABLE 3

Incidence of dengue haemorrhagic fever (DHF) by age group and sex. Niterói - 1990/91.

SEX/ AGE (years)	FEMALE		MALE		TOTAL	
	Nº	%	Nº	%	Nº	%
0 - 4	-	-	-	-	-	-
5 - 9	5	1.3	4	1.1	9	2.4
10 - 14	5	1.3	5	1.3	10	2.7
15 - 49	116	31.4	103	27.9	219	59.3
> 49	44	11.9	69	18.7	113	30.6
UNKNOWN	6	1.6	12	3.2	18	4.8
TOTAL	176	47.7	193	52.3	369	100.0

Source: Secretary of Health - Niterói city

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