Rev. Inst. Med. trop. São Paulo
14(2):121-125, março-abril, 1972

UDC 616.988.75

SEROEPIDEMIOLOGIC STUDY OF CORONAVIRUS INFECTION IN BRAZILIAN CHILDREN AND CIVILIAN ADULTS

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SUMMARY

A seroepidemiologic study of infection by coronavirus strain 229E is described. In normal children a measurable CF antibody response was found in 3.2% of the total studied, with no significant differences in relation to sex. In normal civilian adults that percentage was 26.2%, with no significant sex and age group differences. Four cases in a group of 36 non-hospitalized children with respiratory tract illnesses presented fourfold or greater complement fixing antibody response to coronavirus strain 229E.

INTRODUCTION

Using the human embryonic tracheal and nasal organ cultures technique HOORN & TYRRELL [13] demonstrate that it was possible to cultivate a number of respiratory and enteric viruses of human and simian origin. Several investigators [1, 14, 16, 21] using both the organ cultures and the conventional tissue culture systems described the recovery of viruses from humans with upper respiratory tract infections, which resembled morphologically the avian infectious bronchitis virus (IBV) [2, 3] and the murine hepatitis virus (MHV) [10]. The common characteristics of these agents suggested the possibility of classifying them in a new taxonomic group, the "coronaviruses" [9]. Members of the group are medium-sized, ether-labile and have a RNA core; they have petal-shaped surface projections and show a certain degree of antigenic relationship to several strains of MHV [17]. Of the nine human strains isolated in human embryonic tracheal organ cultures, [16, 21, 22] three were adapted to monolayer tissue cultures and two others could be grown in suckling mice. The remaining fourteen human strains were isolated in tissue cultures of diploid human embryonic fibroblasts [11, 15]. BRADBURNE [4] found that the 229 E virus could be isolated from nasal washings in some strains of Hela cells, but the cytopathic effect was poor; this effect was more marked in cultures of a continuous human embryo lung cell line — L132. Seroepidemiologic studies indicate the presence of neutralizing and complement fixing antibodies to the human strains 229 E, OC38 and OC43 and to strains of MHV, in patients with mild upper respiratory tract infections [12, 15, 18]. MCINTOSH et al. [17] studied the complexe relationships between human and animal coronaviruses and found that avian strains are unrelated to the human and murine viruses. On the other hand, several murine strains show a marked antigenic relationship to human strains isolated in organ cultures. The strain 229 E is antigenically very different from the other human strains. These findings demonstrate the possibility of occurring anamnestic responses, as well nondetectable serological res-

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ponses if the antigen used in the survey is very different from the virus responsible for the respiratory infection.

This report describes a seroepidemiologic study developed in an attempt to determine the incidence of 229 E virus infection in normal Brazilian civilian adults and children.

MATERIAL AND METHODS

Serum samples which had been obtained for studies on infectious mononucleosis, from 124 normal children in São Paulo in May and June, 1969, and stored frozen since then and 321 samples obtained from normal civilian adults, in May and June 1971, were tested for complement fixing antibodies to the prototype strain 229E. Serum samples from 36 non-hospitalized children with respiratory tract infections, also obtained for studies on infectious mononucleosis in São Paulo in July 1969, and stored frozen, were included in this study. Acute phase sera were obtained at the moment of medical atendance and convalescent sera four weeks later. Only this last set of sera was obtained by digital puncture and diluted to 1:5 in phosfate buffer saline. For all the other sera twofold dilutions were titrated beginning at 1:4. Dr. M. S. Pereira kindly supplied the 229 E virus and an anti-229 E mouse hyperimmune ascitic fluid.

The coronavirus strain 229 E was passaged in cell strain WI38, obtained from commercial sources, grown in Eagle's basal medium with 10% fetal bovine serum and maintained in Eagle's minimal essential medium supplemented with 2% fetal bovine serum.

The complement fixing antigen employed was prepared in WI38 cultures; 72 hours after the inoculation the cultures were frozen and thawed twice and the fluids used as antigen. The results presented in this study were obtained by complement fixation tests performed several days in succession, using a technique previously described [19]. Serum samples with a complement fixing antibody titre of 4 or greater were considered positives.

RESULTS

Table I shows the number and percentages of normal children distributed by age groups and according to the presence of a complement fixing antibody significant titre of 4 or higher: the proportion of children considered as positive was 3.2%, with no meaningful age and sex pattern distributions (Table II). All the normal children with significant titres are included in the age group of 8-10 years, which is probably occasional. This table also presents the age distribution of measurable complement fixing antibody titres in the group of civilian adults under study, with a global percentage of positives of 26.2% and a discreet age increase from 25.7% to 44.4%. Regarding sex, the distribution of antibodies to coronavirus 229 E strain was similar in both adult groups, with percentages of 26.1% for males and 26.3% for females (Table II).

TABLE I

Distribution of complement fixing antibodies to coronavirus 229E strain in normal children and civilian adults, according to age

Age (years)	Number tested	Positive	
		no.	%
6 — 8	31	0	0
8 — 10	52	4	7.6
10 — 12	38	0	0
12 — 18	3	0	0
sub-total	124	4	3.2
18 — 28	144	37	25.7
28 — 38	102	26	25.5
38 — 48	57	13	22.8
⩾ 48	18	8	44.4
sub-total	321	84	26.2
Total	445	88	19.7

TABLE II

Distribution of complement fixing antibodies to coronavirus 229E strain in normal children and civilian adults, according to sex.

Age (years) \ Sex	Males			Females			Total		
	Number tested	Positive		Number tested	Positive		Number tested	Positive	
		no.	%		no.	%		no.	%
6 — 18	68	3	4.4	56	1	1.8	124	4	3.2
⩾ 18	302	79	26.1	19	5	26.3	321	84	26.2
Total	370	82	22.1	75	6	8.0	445	88	19.7

Table III shows 5 children with measurable complement fixing antibodies (13.8%) from the group of 36 children with respiratory tract infections, 4 of whon presented fourfold or greater complement fixing antibody response to the coronavirus prototype strain 229 E. These children were not examined for evidence of infection by other respiratory viruses.

TABLE III

Complement fixing antibody response to coronavirus 229E strain in children with respiratory tract infection

Patient no.	Age (years)	Reciprocal of CF antibody titre	
		Acute	Convalescent
12824	9	10	<10
13733	4	10	40
17441	1	10	80
17836	5	<10	80
18383	5	10	40

DISCUSSION

The possibility of human coronaviruses being considered as important etiologic agents of acute upper respiratory infections in adults has been already accepted. The study we undertake defines the seroepidemiology of 229 E coronavirus infection in a group of civilian adults in terms very similar to those found by McIntosh et al. [18]. During the period of 1965-1967 they found in adults quite commoly (41.0%) complement fixing antibodies to strain 229 E; in children, on the contrary, these antibodies were rare (0.6%). The global percentage we obtained in civilian adults was lower (26.2%), but in the 48 years or older group our result was approximately the same (44.4%). In relation to the group of normal children we found that complement fixing antibodies to strain 229 E were not so rare (3.2%); considering the non-hospitalized 36 children with respiratory infections included in this study, this percentage was particulary high (13.8%) suggesting that this strain might very well be responsible for the respiratory infections clinically observed. Kapikian et al. [15] in a study on the incidence of 229 E virus in a population of civilian adults, demonstrated that the CF test was more sensitive than virus isolation, as an index of infection. They found 48.0% of a group of 317 adults with CF antibody titres of 4 or higher. In a group of 222 infants only 2.0% had CF antibody titres of 4 or higher to 229 E virus. They consider that infection with this agent is rare in infants and children with lower respiratory tract illness. In a study organized by W. H. O. to gather information on the etiological role of viruses in respiratory tract illness prevailing in developing tropical and semitropical countries, nome of the 528 paired sera obtained from hospi-

talized children up to 5 year of age bore evidence of 229 E infection [8]. Of the 36 children with respiratory tract infection studied in our serologic survey, 31 had clinical signs of upper mild respiratory illness and none showed a measurable CF antibody response to 229 E virus. The 5 remaining children had clinical signs of severe bronchitis and 4 of them developed a significant fourfold rise of complement fixing antibodies to that virus. Considering the antigenic uniformity of the 229 E-like group [17], the serologic response observed in our study might be the result of infection by the 229 E virus. The acute respiratory tract infections represent a complex of diseases caused by a mixture of many different viruses [20] and there is always the possibility, in our samples, of dual infections being present, particulary by type 3 parainfluenza and respiratory syncicial virus, which we found to be very common in Brazilian children [5, 6, 7]. McINTOSH et al. [18] reported dual infections with coronaviruses and mixoviruses or paramixoviruses, in a certain number of children investigated. In our study the four children sera exhibiting a fourfold-rise in complement fixing antibody titre to strain 229 E were drawn in July 1969, during the winter season, which seems to be a period of predominant occurrence of coronavirus infections in adults and children [15, 16, 18].

RESUMO

Estudo sôro-epidemiológico da infecção por coronavírus em crianças e adultos civis brasileiros

Os Autores fizeram um estudo sôro-epidemiológico da infecção pela cepa 229 E de coronavírus, tendo encontrado, em 3,2% das crianças normais estudadas, anticorpos fixadores de complemento para aquêle vírus; não foram observadas diferenças significativas em relação ao sexo.

Em adultos civis normais aquela percentagem foi de 26,2%, sem diferenças significativas em relação ao sexo e grupos etários. De 36 crianças não hospitalizadas com quadros respiratórios agudos, quatro mostraram uma elevação dos títulos de anticorpos fixadores de complemento, para a cepa 229 E, de pelo menos quatro vêzes.

ACKNOWLEDGEMENTS

Thanks are due to Miss Maria Cloniza da Costa Vieira for the technical assistance and to Dr. M. S. Pereira for providing us with reference reagents. Special thanks are also due to Dr. E. Kirchner for the English revision of the manuscript.

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Recebido para publicação em 29/6/1971.