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INDIRECT IMMUNOFLUORESCENCE (IgG AND IgM) TESTS FOR TOXOPLASMOSIS ON 203 PERSONS, WITH NO SYMPTOMATOLOGY SUGGESTING THE DISEASE, LOCATED IN THE CITY OF RIO DE JANEIRO. SEROLOGICAL FOLLOW UP ONE TO TWO YEARS LATER

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Clinical and serological follow up examinations were performed on 203 persons, from three to twenty years of age, from the otolaryngology department of a hospital in the city of Rio de Janeiro, with no symptomatology suggesting toxoplasmosis, but suffering from chronic tonsillitis. According to results obtained during the first indirect immunofluorescence tests, the patients were divided into following groups: Group I (non-reactive IgG and IgM), 98 persons (48.3%); Group II (1:16 ⩽ IgG ⩽ 1:256 and non-reactive IgM), 74 persons (36.5%); Group III (IgG ⩾ 1:1024 and non-reactive IgM), 18 persons (8.8%), and Group IV (IgG and IgM reactive), 13 persons (6.4%). One to two years later, 131 (64.5%) of the 203 persons were reexamined by a second indirect immunofluorescence test. In the case of 66 persons (Group I) whose serum was non-reactive in the IgG and IgM classes during the first indirect immunofluorescence test, serum conversion was observed in aproximately 21.2%. In 65 individuals (49.6%), (Groups II, III and IV), with reactive serum in the IgG classes during the first indirect immunofluorescence test, the second reaction showed an increase in titres in 20% of the cases, a decrease in 67.7% of the cases, or no alterations in 12.3% of the cases. In the IgM class, all 131 sera were non-reactive at 1:16 dilution during the second immunofluorescence test, including the 13 cases that had previously been reactive in the immunoglobulin class. Symptomatology suggesting toxoplasmosis was only observed in one case during the second testing, this patient's principal physical sign being hypertrophied lymph nodes. During this period, the Toxoplasma *antibodies showed titres of IgG 1:32000 and non-reactive IgM, whilst one year previously, during the first test, these titres were IgG 1:1024 and IgM 1:64. Differences in the age, sex and skin coloring of patients were not statistically significant as regards alterations in the indirect immunofluorescence test titres.*

Clinical diagnosis may be very difficult in cases of acquired toxoplasmosis, since cases frequently are oligosymptomatic or asymptomatic and, when clear symptomatology does occur, this may be confused with other infections. Therefore, laboratory diagnosis, principally by serology, can be very important.

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There are several serological tests to diagnosis toxoplasmosis, of which the Sabin Feldman test (Sabin & Feldman, 1948), the indirect immunofluorescence test (Camargo, 1964; Camargo, 1974; Coons, Deduc & Counolly, 1953; Goldman, 1957; Nery Guimarães et al, 1968), the complement fixation reaction (Warren & Sabin 1942), the hemagglutination test (Jacobs & Lunde, 1957) and, more recently, the enzyme-linked immunoabsorbent assay (Walls, Bullock & English, 1977) are outstanding.

Karin & Ludlan 1975; Camargo, Leser & Leser, 1976; Camargo & Leser, 1976, utilizing more than one of these tests, claim it is possible to determine the infection phase by means of serological patterns.

The present paper evaluates the behavior of indirect immunofluorescence test IgG and IgM (IF) in patients with no clinical suspicion of toxoplasmosis when the first blood sample was taken. The majority of these patients could be reexamined, one to two years later, through a second indirect immunofluorescence test, to effect a serological follow up.

MATERIAL AND METHODS

Serological and clinical follow up of 203 persons was carried out, the majority of such persons being children or adolescents, whose ages varied from three to twenty years (96 persons, from three to six years of age; 63, from seven to ten years; 23, from eleven to fourteen years, and 21 persons, aged from fifteen to twenty years), of both sexes (122 females and 91 males) and different skin coloring (166 whites, 25 mulattoes and 12 blacks). They resided in 88 different boroughs and outskirts of the city of Rio de Janeiro. All came from the otolaryngology department of a hospital in Rio de Janeiro and showed no symptomatology suggesting toxoplasmosis, but suffered from chronic tonsillitis. This group of individuals was selected in accordance with plans to simultaneously evaluate the possibility of isolating *T. gondii* from the tonsils of patients with no clinical suspicion of toxoplasmosis (Amendoeira, 1980).

The blood for serological testing was collected a few hours before surgery of the tonsils. A serological follow up was carried out on 131 of these patients, one to two years after the initial testing. The other 72 individuals were not reexamined, either because they could not be located or refused to be reexamined.

The indirect immunofluorescence test (IF) was used to detect *T. gondii* antibodies. The antigen was prepared according to the technique previously used by Coutinho et al, 1970 and Coutinho et al, 1981. Sera to be examined were fourfold diluted, from 1:16 through to 1:4096. Thereafter, the dilutions were doubled. The fluorescein isothiocyanate labelled anti-IgG or anti-IgM human conjugate were manufactured by Travenol Laboratories Inc., and the *Toxoplasma* antigen was prepared in a final concentration of approximately 7×10^{6} per ml. HBO-200 lamp and BG12 filter equipped microscopes were used.

All sera that were IgM class reactive were submitted to the latex agglutination test for the determination of the rheumathoid factor, following instructions in the Bio-Merieux Arthri-test Kit.

RESULTS

The 203 persons, who underwent tonsillectomies and showed no symptoms suggesting toxoplasmosis, were divided into four groups (Table I), according to results found in the first IF.

One to two years after the first test, a second blood sample was taken from 131 individuals (64.5%), from the group of 203 persons who had undergone tonsillectomies, to compare the evolution of antiboby titres of a group of persons not suspected of having the disease (Table II).

TABLE I

Results of indirect immunofluorescence (IgG and IgM) tests for toxoplasmosis of sera of 203 persons who had undergone tonsillectomies and showed no symptomatology suggesting toxoplasmosis

Groups, according to IgG and IgM titres	*Number of sera*	*Per Cent*
I – IgG – NR* IgM – NR*	98	48.3
II – IgG – 1:16 to 1:256 IgM – NR*	74	36.5
III – IgG $\geqslant$ 1:1024 IgM – NR*	18	8.8
IV – IgG $\geqslant$ 1:16 IgM $\geqslant$ 1:16	13	6.4
Total	203	100.0

*Non-reactive serum, at 1:16 dilution.

Of the 98 patients, who had tonsillectomies and were non-reactive in the IgG and IgM immunoglobulin classes of the IF(Group I), 66 persons (67.3%) were reexamined. Of these 66 individuals, 52 patients (78.8%) remained non-reactive in the IgG and IgM immunoglobulin classes, and the remaining 14 persons (21.8%) were non-reative in the IgM class, although reactive in the IgG classes. Of these 14 individuals, six (9.1%) became reactive at the first dilution (1:16) and the remaining eigth persons (12.1 %) were reactive in higher dilutions, the titres ranging between 1:64 and 1:4096.

The individuals pertaining to Groups II, III and IV are further classified, in Table III, in accordance with variations (higher or lower) or showing no alterations in the titres of IgG classes of antibodies during the first and the second IF. However, persons pertaining to Group I were not included in this Table because, since all of them had been non-reactive in the first IF, a decreasing change in titres would be impossible.

Forty three persons (58.1%) were reexamined, from the group of 74 patients who had undergone tonsillectomies and, in the IF, were reactive in the IgG classes ($1:16 \leqslant \leqslant$ IgG $\leqslant$ 1:256) and non-reactive in the IgM class of immunoglobulins (Group II). The second IF of all of them continued to be non-reactive in the IgM class. As regards titres in the IgG immunoglobulin classes (Table II), 29 persons showed lower titres in the second IF, 22 patients being non-reactive at a 1:16 dilution.

Of the 18 persons who had tonsillectomies and reacted in the IgG classes (>1:1024) and were non-reactive in the IgM class of immunoglobulins (Group III), only 10 individuals (55.5%) were submitted to a second IF test. All of those 10 individuals remained

TABLE II

Results of the first indirect immunofluorescence test (when surgery was undergone) and of the second indirect immunofluorescence test (one to two years after the first test) for toxoplasmosis in the IgG immunoglobulin classes, in 131 individuals who had undergone tonsillectomies, pertaining to Groups, I, II, III and IV

Reciprocal titres of lst. immuno-fluorescence test – IgG	*Reciprocal titres of the second indirect immunofluorescence test – IgG*													
	*NR**		*16*		*64*		*256*		*1024*		*4096*		*Total*	
	nº	%	*nº*	%	*nº*	%	*nº*	%	*nº*	%	*nº*	%	*nº*	%
NR*	52	78.8	6	9.1	1	1.5	3	4.6	2	3.0	2	3.0	66	100.0
16	19	90.4	–	–	1	4.8	1	4.8	–	–	–	–	21	100.0
64	2	13.3	1	6.7	5	33.3	4	26.7	3	20.0	–	–	15	100.0
256	1	8.3	4	33.4	3	25.0	3	25.0	1	8.3	–	–	12	100.0
1024	1	11.1	1	11.1	3	33.4	2	22.2	–	–	2	22.2	9	100.0
4096	1	12.5	–	–	3	37.5	3	37.5	–	–	1	12.5	8	100.0
Total	76	58.0	12	9.2	16	12.2	16	12.2	6	4.6	5	3.8	131	100.0

*Serum was non-reactive at 1:16 dilution.

TABLE III

Behavior of serological titres in the IgG immunoglobulin classes during the first and the second indirect immunofluorescence tests of persons with no clinical symptoms of toxoplasmosis, who had undergone tonsillectomies, and who had shown antibody titres in the first indirect immunofluorescence test (Groups II, III, and IV)

Groups according to antibody titres in the first indirect immunofluorescence test	*Decrease in titre, from first to second indirect (immunofluorescence test)*		*No variation in titre from first to second indirect immunofluorescence test*	*Increase in titres from first to second indirect (immunofluorescence test)*		*Total number of reexamined individuals*
	Two or more dilutions	*One dilution*		*One dilution*	*Two or more dilutions*	
II	7	22	7	4	3	43
III	8	2	–	–	–	10
IV	4	1	1	4	2	12
Total	19	25	8	8	5	65

non-reactive in the IgM class, but had lower titres in the IgG classes, and 8 of them (80.0%) in two or more fourfold dilutions (Table III). Two of these persons even became non-reactive to a 1:16 dilution (Table II).

The other 2 patients of the 10 that were reexamined (20.0%) had lower titres in a second IF test in a single fourfold dilution (Table III).

Of the 13 persons who had undergone tonsillectomies and were reactive in the IgG immunoglobulin classes by the IF test (Group IV), 12 patients (92.3%) were submitted to a second IF test, wherein all had become non-reactive in the IgM class. With respect to the IgG classes, 5 persons had lower titres, 4 being lower by two or more dilutions (Table III). Of these 12 individuals, only one person showed a second titre that was equal to that of the first test (Tables III and IV) whereas the remaining 6 patients showed higher titres than in the first IF test. In one such case the first IF test of the patient showed a titre of 1:1024, with no simptomatology suggesting toxoplasmosis. However, the second IF test revealed a titre of 1:32000, with presence of hypertrophied cervical lymph nodes.

TABLE IV

Results of the first and the second indirect immunofluorescence tests for toxoplasmosis in the IgG and IgM classes of immunoglobulin, in 13 individuals who had undergone tonsillectomies pertaining to Group IV (reactive in the IgG and IgM classes during the first indirect immunofluorescence test)

		Reciprocal of Titres First Ind. Immunofl. Test		*Reciprocal of Titres Second Ind. Immunofl. Test*	
Identification	*Age (in years)*	*IgG*	*IgM*	*IgG*	*IgM*
NFS	8	64	16	1024	NR
NRM	7	1024	16	64	NR
DS	11	64	64	64	NR
AEAS	6	1024	64	4096	NR
RSL	16	64	16	256	NR
ALMR	7	4096	16	64	NR
JQS	9	4096	64	8000	NR
SCB	13	256	256	64	NR
FFN	10	16	16	64	NR
WRS	12	4096	4096	64	NR
AAS	7	4096	16	256	NR
OCJ	15	1024	64	32000	NR
CMAM*	8	256	16	--	--

*A second indirect immunofluorescence test could not be made because patient could not be located.

Sera from the first IF-test of patients in Group IV (IgM reactive) were submitted to the latex test for rheumathoid factor, all of them showing negative results.

Of the 203 individuals that were examined, 105 persons (51.7%) were serum reactive in the IgG classes, of which 44 patients were from three to six years of age (45.8%); 33 patients, from seven to ten years (52.4%); 16 patients, eleven to fourteen years (69.6%); and 12 patients, from fifteen to twenty years of age (57.1%).

DISCUSSION

Of the 66 individuals pertaining to Group I (non-reactive to IgG and IgM) (Tables I and II), 52 persons (78.8%) remained non-reactive in IgG and IgM immunoglobulin classes, thus to a certain extent confirming that, at the time of the first testing, they

really had had no contact with the parasite and that, within this period, they also had not been infected. The other 13 individuals (19.7%) must have entered into contact with the parasite during the period between the two tests, since serum conversion was found with antibody titres of the IgG classes varying from 1:16 to 1:4096 on the second testing. Considering that, in eight cases, titres clearly increased by two or more fourfold dilutions, one may infer an incidence of toxoplasmic infection of at least 12.8% of the individuals pertaining to this group within a period from one to two years, the average being of one year and five months. Evidently, this data cannot be extrapolated to the population in general, however, the possibility of a high incidence of toxoplasmosis in a group of young individuals has been demonstrated.

The fact that sera from the second testing continued non-reactive in the IgM class does not invalidate these conclusions, because titres in this class can decrease exceptionally early in dilutions lower than 1:16 within even a few weeks after initial infection, contrary to what usually occurs in the IgG antibody classes (Camargo, Leser & Leser, 1976 and Camargo & Leser, 1976). It should also be mentioned that the tonsils should not be considered responsible for the parasite's entering point in such individuals, since all of them had undergone tonsillectomies immediately after the first blood sample was taken.

Results of the IF test for toxoplasmosis in the 92 individuals pertaining to Groups II and III (reactive in the IgG classes and non-reactive in the IgM classes) indicate that these patients had contact with the parasite previously. They also did not show any symptoms suggesting toxoplasmosis at the time of surgery, and their clinical histories did not permit any definite conclusions in this respect.

Based on the results found in these two groups, nothing can be affirmed as regards the period of infection since low, or even high titres of antibodies in the IgG classes and non-reactive sera in the IgM class, suggest residual antibodies from an infection that was not recent (Camargo, Leser & Leser, 1976; Camargo & Leser, 1976 and Camargo, Leser & Leser, 1977). However, it would be interesting to obtain a comparison with other serological tests, such as hemagglutination and complement fixation, as referred to by Karin & Ludlam, 1975; Camargo, Leser & Leser, 1976; Camargo & Leser, 1976 and Camargo, Leser & Leser, 1977), as well as other authors, to permit a more exact characterization of the infective stages.

Amongst the individuals who were serum reactive, a higher prevalence of titres considered to be low in the IgG classes (1:16, 1:64 and 1:256) was observed. Other authors (Araujo, 1970; Coutinho et al, 1981 and Wall & Kagan, 1967) have demonstrated this fact in their serological surveys. As regards the comparison of antibody titres in the IgG classes, as determined in the first and second IF tests, the differences encountered in more than one fourfold dilution should be more important, since changes in a single dilution may be less significant, as they may be due to variations in the method itself (Coutinho et al, 1970).

According to Apt et al, 1973, such titres may remain high during a period that will vary, according to the intensity of the acute stage of the infection, thereafter decreasing to average (1:256) and low (1:16) titres, and these apparently persist thereafter at such levels, during the patient's lifetime. However, at least amongst the 43 individuals of Group II (low titres in the IgG classes), who were reexamined one to two years after the first IF test, it was observed that 19 of them (44.2%), who had titres equal to 1:16 in the first IF test, became non-reactive at a 1:16 dilution in the second IF test. Three individuals (7.0%) of this Group II, although having titres equal or superior to 1:64 in the first IF test, also became non-reactive at a dilution of 1:16 in the second IF test (Table II). However, both increasing and decreasing titres were observed in these 43 persons, although the former were predominant (Table III).

However, a decrease was found in the case of all ten individuals pertaining to Group III (higher titres in the IgG classes), which, in eight cases, was higher than a four-fold dilution (Table III). Two persons became non-reactive.

A clear increase in titres of the IgG classes was observed, in the second IF test, in three of the 53 individuals pertaining to Groups II and III, who were all non-reactive in the IgM class (Table III). This fact may be ascribed to them either being in a period of increasing titres in the IgG classes, although non-reactive in the IgM class, or due to the possible occurrence of relapse or reinfection between the first and second IF test.

With reference to individuals pertaining to Group IV (IgG and IgM reactive in the IF tests), the presence of IgM class antibodies found during the first IF test, contrary to what was observed during the second test, leads one to consider the possibility of a recent infection during surgery, even through such antibodies may be present in the serum up to about 18 months after the first infection (Camargo, Leser & Leser, 1976 and Camargo & Leser, 1976). In such cases it would therefore be interesting to carry out other serological tests, in order to draw up a profile (Camargo, Leser & Leser, 1976; Camargo & Leser, 1976 and Camargo, Leser & Leser, 1977) that would help to diagnose the acute or subacute stage of the infection.

The majority of individuals pertaining to Group IV had low antibody titres in the IgM class (1:16 and 1:64) (Table IV), which may lead to consideration of possible participation of rheumatoid factor (Hyde, Barnett & Remington, 1975 and Rowe et al, 1971). This could be a possibility since, in addition to others, Remington, Miller & Brownlee, 1968 and Camargo, Leser & Rocca, 1972 found a high prevalence of positive anti-toxoplasma IgM tests in sera that were also positive for rheumatoid factor by the latex agglutination test. In view of the negative results, it was concluded that all 13 sera showed specific *Toxoplasma gondii* antibodies in the IgM class.

All the persons reexamined in the second IF test, had become non-reactive to *Toxoplasma gondii* in the IgM class.

With regards to antibody titres in the IgG classes, during the first and second IF tests, 6 individuals maintained the same titres or showed alterations in one dilution only. Another 4 presented lower titres in two or more dilutions, and were apparently in the decreasing stage of the antibody curve. Higher titres, in two or more dilutions, were observed in the case of 2 individuals, of which, one had a high IgG titre in the first IF test (1:1024) and showed a marked increase in the second IF test (1:32000). No symptomatology suggesting toxoplamosis was noted when surgery was performed but, when the second blood sample was taken, hypertrophied posterior cervical lymph nodes were detected. In view of the serological result and clinical examination, the hypothesis of a sub-acute evolving toxoplasmosis may be raised, as referred to by various authors (Apt et al, 1973), or relapsing or else a reinfection may be considered.

Amongst the 65 individuals who were reactive in the first IF test in the IgG classes (Groups II, III and IV – Table III), and whose examinations were repeated one to two years after the first test, it was observed that 41 patients maintained the same titres as during the first reaction or, at the most, variations of only one dilution occurred.

In 19 individuals, lower titres in two or more fourfold dilutions were observed, whereas in only 5 individuals were titres higher by two dilutions. Such data permit concluding that the majority of these persons had stable titres, or were in the decreasing etage of the IgG classes of antibodies.

As regards age-groups, a smaller percentage of reactive sera was found in the younger group (3 to 6 years old), there being a slight increase in this percentage in the 11

to 14 year group, which decreased again in the 15 to 20 year group. Contrary to what has been observed by other authors (Apt et al, 1973; Coutinho, Frias & Nogueira, 1972; Coutinho, Oliveira & Ferreira, 1972; Deane, 1963; Feldman & Sabin, 1949; Johnson, Roberts & Mc Donald, 1980 and Remington, 1974) no statistically significant differences were found between these groups, possibly due to the small number of individuals per group.

No statistically significant differences were found between the individual sera, which were reactive in the IgG classes in the IF tests, with reference to the patients' sex or skin color, confirming observations made by many authors (Apt et al, 1973; Cantella et al, 1974; Coutinho et al, 1981; Jamra, 1964; Omland, Tonjun & Frentzel-Beyme, 1977; Osorio et al, 1977 and Ricciardi & Sandoval, 1975).

RESUMO

Foi feito o acompanhamento sorológico e clínico de 203 indivíduos de 3 a 20 anos de idade, provenientes de serviços de otorrinolaringologia na cidade do Rio de Janeiro, sem sintomatologia sugestiva de toxoplasmose, mas portadores de amigdalite crônica. Os pacientes, de acordo com os resultados da 1ª RIFI, foram divididos nos seguintes grupos: Grupo I (IgG e IgM não reagente), 98 indivíduos (48,3%); Grupo II (1:16 ⩽ IgG ⩽ 1:256 e IgM não reagente), 74 indivíduos (36,5%); Grupo III (IgG ⩾ 1:1024 e IgM não reagente), 18 indivíduos (8,8%) e Grupo IV (IgG e IgM reagentes), 13 indivíduos (6,4%). Um a dois anos após, 131 (64,5%) dos 203 indivíduos foram reexaminados para uma segunda RIFI. Dentre 66 deles (Grupo I), que eram soro não reagentes nas classes IgG e IgM na primeira RIFI, observou-se soro conversão nas classes IgG em cerca de 21,2%. Em 65 (49,6%) indivíduos (Grupos II, III e IV), que eram soro reagentes nas classes IgG na primeira RIFI, observou-se que na segunda reação, os títulos variaram de modo ascendente em 20% dos casos, descendente em 67,7% dos casos ou não variaram em 12,3% dos casos. Na classe IgM todos os 131 soros apresentaram-se não reagentes à diluição de 1:16 na segunda RIFI, inclusive os 13 casos anteriormente reagentes nesta classe de imunoglobulinas. Apenas em um caso observou-se sintomatologia sugestiva de toxoplasmose na ocasião da segunda coleta, sendo que o paciente apresentava, principalmente, gânglios hipertrofiados. Os títulos de anticorpos para toxoplasmose nesta época foram IgG 1:32000 e IgM não reagente enquanto que na primeira coleta um ano antes eram IgG 1:1024 e IgM 1:64. Quanto à idade, sexo e cor as diferenças nos títulos da RIFI foram estatisticamente não significantes.

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