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SEROLOGY IN EARLY DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS (*)

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SUMMARY

The early serological recognition of clinically inapparent congenital toxoplasmosis is handicapped by the frequent occurrence of passive transfer antibodies in infants. The possible emergence of a distinctive serologic pattern in congenital infection was investigated by comparing results of IgG- and IgM-immunofluorescence (IgG-IF and IgM-IF), complement fixation (CF) and hemagglutination (HA) tests in congenital cases and in non-infected newborn children. Serologic patterns in mother's serum were also investigated and found helpful for screening purposes.

INTRODUCTION

Congenital toxoplasmosis is marked many times by evident clinical manifestations, but not rarely the infection remains unsuspected because it is less characteristic or even inapparent. Consequences of a silent infection may arise only much later as visual problems or as a retarded neuro-psychomotor development of the child.

Early diagnosis is necessary to open opportunity for anti-parasitic therapeutics and its possible effect in interrupting any further organic impairment.

Although in most congenital cases toxoplasma can be isolated from the placenta through inoculation in mice [7], this method is not fit for screening purposes; the possibility of a standard serological diagnosis should thus be welcome. However, in newborns the evaluation of results of toxoplasmosis tests is hindered by the frequent presence of passive transfer maternal antibodies in infant serum. The progressive clearance of such maternal antibodies leads to negativation of the tests, titers usually being reduced to one half in a month, which corresponds to the half-life of passive transfer immunoglobulins [8]. In this way, the serological persistence of a positive test beyond the expected period, frequently even with increasing titers, ensures a diagnosis of congenital toxoplasmosis. However, an early serological diagnosis finds serious limitations. Presence of a positive toxoplasmosis IgM-IF test in newborn serum could indicate infection in the child [14], but should false results occur its actual diagnostic meaning could be impaired. Other serologic characteristics have been proposed as indicating congenital infection, such as titers observed in different tests, including immunofluorescence, hemagglutination [9] and complement fixation [10] tests.

In the present study, we investigated the significance of different tests for the diagnosis of congenital infection, and the possibility of distinctive serologic patterns to characterize infected children from those presenting only passive transfer maternal antibodies.

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MATERIAL AND METHODS

Cases — The group studied consisted of 23 children 9 days to 18 months old, presenting a diagnosis of congenital toxoplasmosis. Diagnosis was based on persistence of anti-toxoplasma antibodies in serum beyond clearance periods of passive transfer antibodies, for 17 cases. These included children exhibiting no clinical signs through severe evidence of the disease, as displayed in Table I. For cases no. 2, 5, 6, 7, 13, 14, high anti-toxoplasma antibody titers and clinical signs usually observed in congenital toxoplasmosis as referred by ALFORD et al.[1], were considered as sufficient evidence of congenital toxoplasmosis. For 8 of the children serologic tests could be performed for rubella, cytomegalovirus, herpes virus, hepatitis B, syphilis, listeria and **Trypanosoma cruzi** infections, with negative results.

A further group of 30 infants a few days to 3 months old, presenting positive toxoplasmosis IgG-IF test and selected at random was studied for comparison. Several of them were normal children but others showed different clinical signs of disease, as jaundice, hepatomegaly, splenomegaly, fever, etc. but in these cases toxoplasmosis could be excluded as the test was found negative in blood samples later collected.

Serum samples — Serum was collected from every patient when first examined. Follow-up samples could be obtained after varying periods from several children a total of 51 sera being studied. Samples were kept at -20°C for periods of days to a few weeks before testing.

Serologic tests — IgC - and IgM - immunofluorescence (IgG-IF, IgM-IF), complement fixation (CF) and hemagglutination (HA) tests were performed as described[5]. Positive results were expressed as reciprocal of serum dilutions. For immunofluorescence tests, anti-IgG and anti-IgM fluorescent conjugates were obtained from Hyland, Travenol Laboratories USA. The WHO International Standard Serum for Toxoplasmosis was used as a reference serum and a titer: unit 0.15 ratio was found for the IgG-IF test, in a similar way as referred by NIEL et al.[12]. Sera presenting positive IgM-IF tests were re-assayed after a tentative removal of anti-globulin antibodies through absorption with heat-aggregated or polymerized IgG, as previously described[6,11].

TABLE I

Supporting data for diagnosis of congenital toxoplasmosis, in 23 children aged 9 days to 18 months when firstly observed

Cases	Age	Clinical and/or serologic data
1 — FRL	9 d.	crt, jdc, hpmg, splmg, PTS, NSD
2 — CAS	10 d.	crt, jdc, hpmg, splmg
3 — AM	11 d.	ncs, PTS
4 — FGR	12 d.	hpmg, PTS
5 — AG	1 mo.	hpmg, splmg, moph, crt, hcph, NSD
6 — RCB	1 mo.	crt, jdc, hpmg, splmg
7 — RM	2 mo.	crt, icc, mcph
8 — KS	2 mo.	ncs, PTS
9 — CBS	2 mo.	ncs, PTS
10 — RTS	2 mo.	crt, splmg, PTS
11 — IBF	2 mo.	ukn, PTS
12 — RCM	2 mo.	jdc, hpmg, splmg, mcph, icc, crt, PTS NSD
13 — RbSM	3 mo.	jdc, hpmg, splmg, crt, NSD
14 — RnSM	3 mo.	jdc, hpmg, splmg, crt, NSD
15 — ERC	3 mo.	jdc, hpmg, splmg, crt, NSD, PTS
16 — DCP	5 mo.	rnpd, PTS
17 — LRM	6 mo.	hpmg, splmg, crt, PTS
18 — EPT	6 mo.	hpmg, splmg, crt, icc, PTS
19 — DHC	6 mo.	mcs, PTS
20 — Ed0C	10 mo.	hpmg, crt, PTS, NSD
21 — E10C	10 mo.	hpmg, crt, PTS, NSD
22 — RP	18 mo.	crt, mcph, rnpd, PTS
23 — ARS	18 mo.	crt, hcph, rnpd, PTS

crt = chorioretinitis
hcph = hydrocephalus
hpmg = hepatomegaly
icc = intracranial calcifications
jdc = jaundice
mcph = microcephaly
moph = microophtalmia
ncs = no clinical signs
rndp = retarded neuro-psychic development
splmg = splenomegaly
ukn = unknown
NSD = negative serological data for other perinatal infections
PTS = persistent positive toxoplasmosis serology

RESULTS

In the group of children presenting congenital infection, IgG-IF test was positive in every case, showing high titers for most patients (Table II), with a geometric mean titer of 29,000 for the group. A low titer (1,024) occurred only for case no. 3, an asymptomatic girl with persistently positive serology, whose mother's serum exhibited an acute toxoplasmosis serologic pattern.

IF-IgM test was positive in 13 cases (56.5%) with titers ranging from 16 to 1,024. However, in 3 cases (no. 2, 5, 18) negative results followed serum absorption with polymerized IgG, indicating a false positive test due to IgM anti-globulin antibodies (Table III). A false positive IgM test was seen to develop lately in case no. 17 and in cases no. 20 and 21 a false positive IgM followed a supposedly specific positive test.

CF showed titers from 40 through 2,560 (Table IV), but was negative in two few days old infants, cases no. 3 and no. 4. Geometric mean titer for the group was 200.

HA test was positive in all cases, except for initial samples in cases no. 3 and no. 4 (Table V), with titers up to 128,000. However, a striking difference in titers occurred between samples from children up to about 6 month old and older children. While low in the first months, with a geometric mean titer of 138, very high titers were the rule after this period, with a geometric mean titer of 15,600. For the other tests, such a large difference was not observed and IgG-IF geometric mean titers for younger and older children were respectively 16,384 and 49,505. For CF test such geometric mean titers were 112 and 352.

For the control group of children with no toxoplasma infection, IgG-IF titers varied from 16 to 8,000, with a geometric mean titer of 277. CF test was negative for 26 children (87%) and titers varied from 20 to 80 whenever positive. HA test was positive for 90.0% of the children, with titers from 64 to 8,000 and a geometric mean titer of 99.

Toxoplasmosis serologic tests could be performed for 15 mothers of children with congenital toxoplasmosis (Table VI). A serologic pattern I of a recent infection, as previously defined[3] was observed in the 6 cases from which samples could be obtained within 2 months after delivery. The remaining mothers showed a transitional pattern II between recent and ancient infection, indicating as highly probable a toxoplasma infection acquired during the last pregnancy.

DISCUSSION

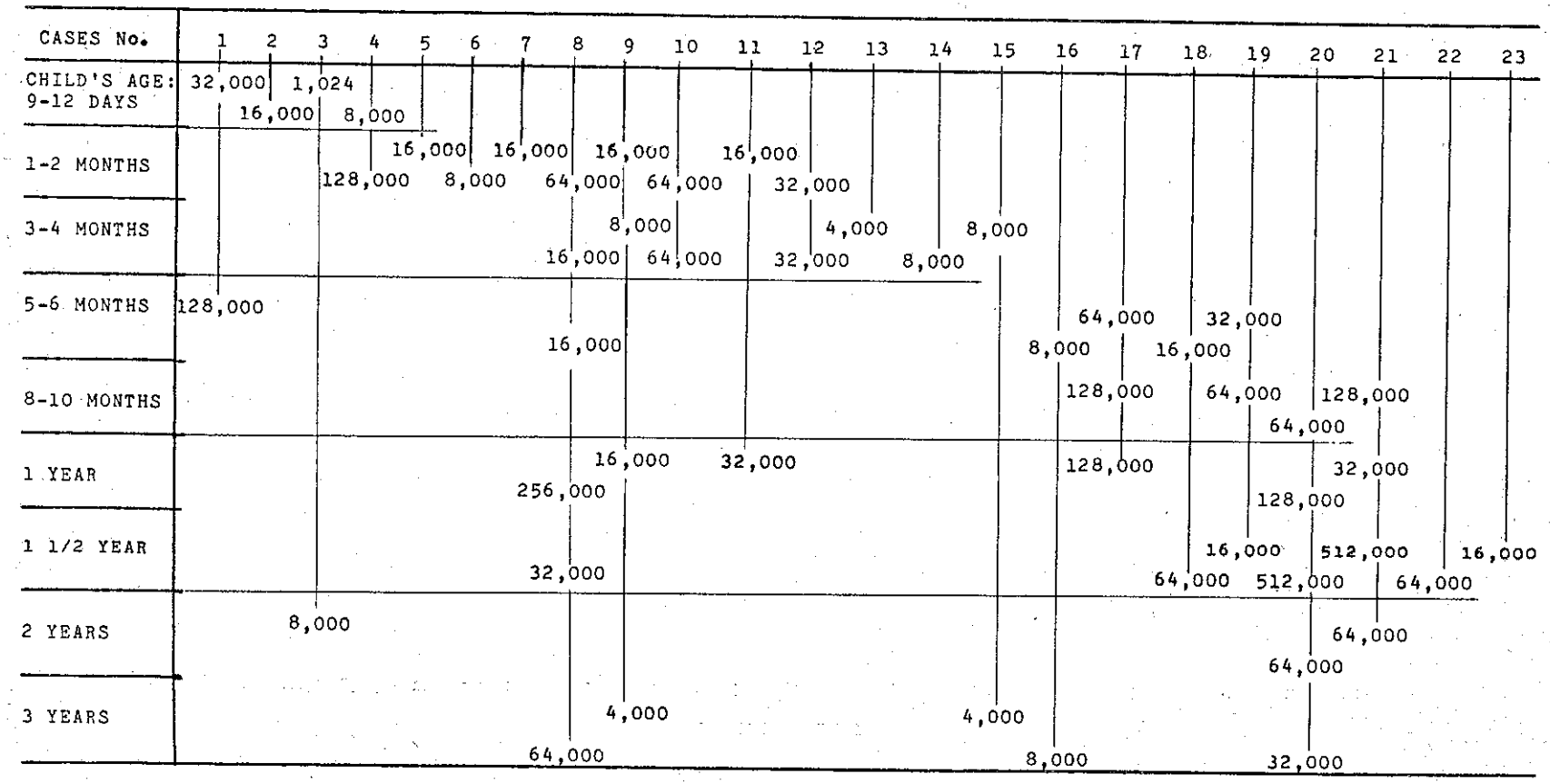
Our results indicate IgM-test as of limited value for screening congenital toxoplasmosis in newborn children, at least when performed with the currently available anti-IgM reagents and techniques, as already reported by others [8,9,10]. Thus, only 56.5% of our cases showed a positive IgM-IF test.

Another cause for the limited diagnostic value of toxoplasmosis IgM-IF tests can derive from false positive results in non-infected children. One case recently observed deserves special reference. It was an infant with clinical and necroscopical evidences of congenital syphilis, serum showing positive cardiolipin and FTA-ABS tests as well as a clear-cut positive fluorescent treponemal test for IgM antibodies. However, positive results were seen also for IgM-IF tests with **Toxoplasma gondii** (titer 1,024) and **Trypanosoma cruzi** (titer 160) antigens. Infection with both these agents could not be revealed through careful "post-mortem" investigation. Positive tests for syphilis, toxoplasmosis and Chagas'disease were found in the mother's serum. In this case negativation of IgM tests did not occur after absorbing serum with insolubilized IgG. However, it is to be noticed that specificity of toxoplasmosis IgM-IF tests cannot be taken as granted when negativation does not occur after serum absorption with polymerized IgG, because of allotypic characteristics of IgG and possible antigenic differences between batches of the insolubilized reagents. Thus, mother's polymerized IgG should be a more efficient reagent to determine specificity of IgM-IF tests in infant sera while in the case of older children and adults, sera should be treated with polymers from their own globulins.

False positive results for IgM-IF tests are occasionally seen also for toxoplasma-infected children. In the present investigation, it was observed for a one month old infant (case no.

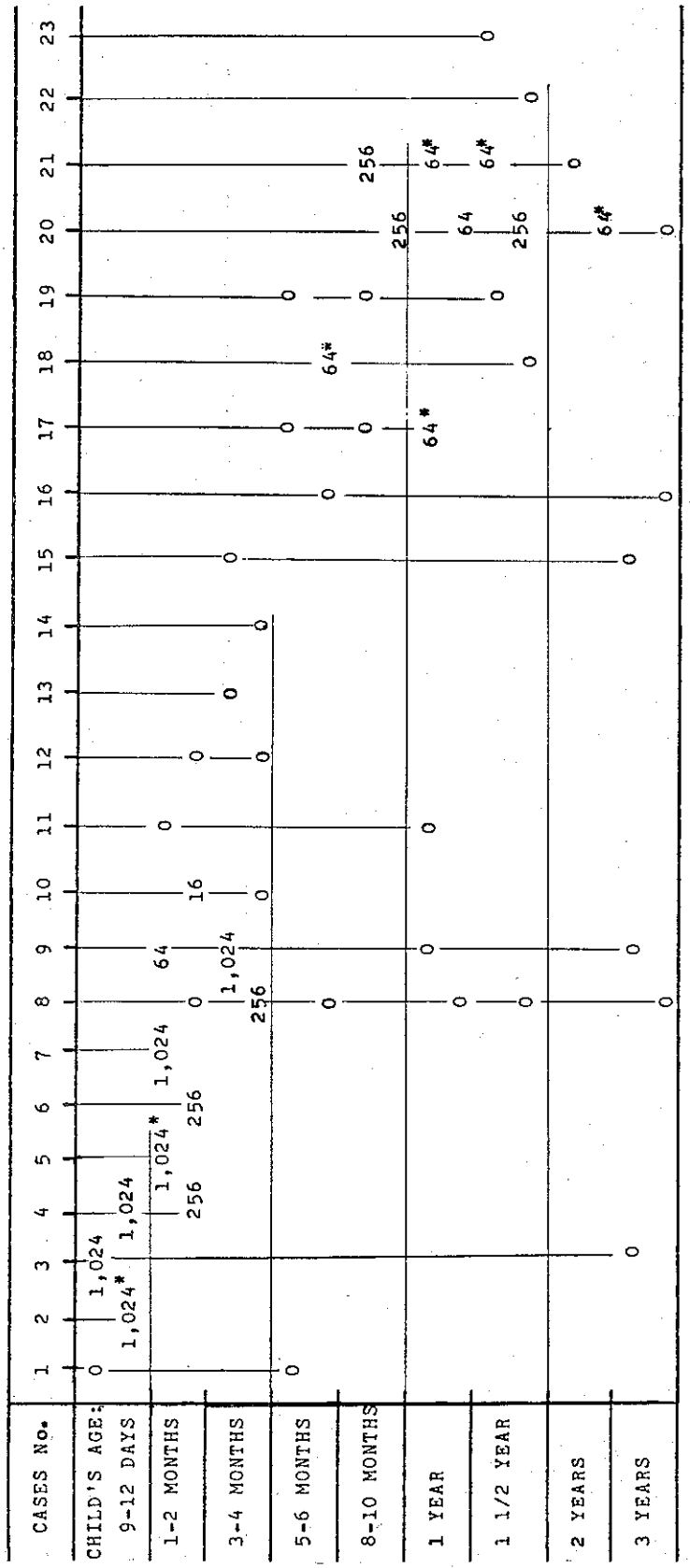
T A B L E I I

Toxoplasmosis IgG-immunofluorescence tests for 23 cases of congenital toxoplasmosis. Results referred according to child's age



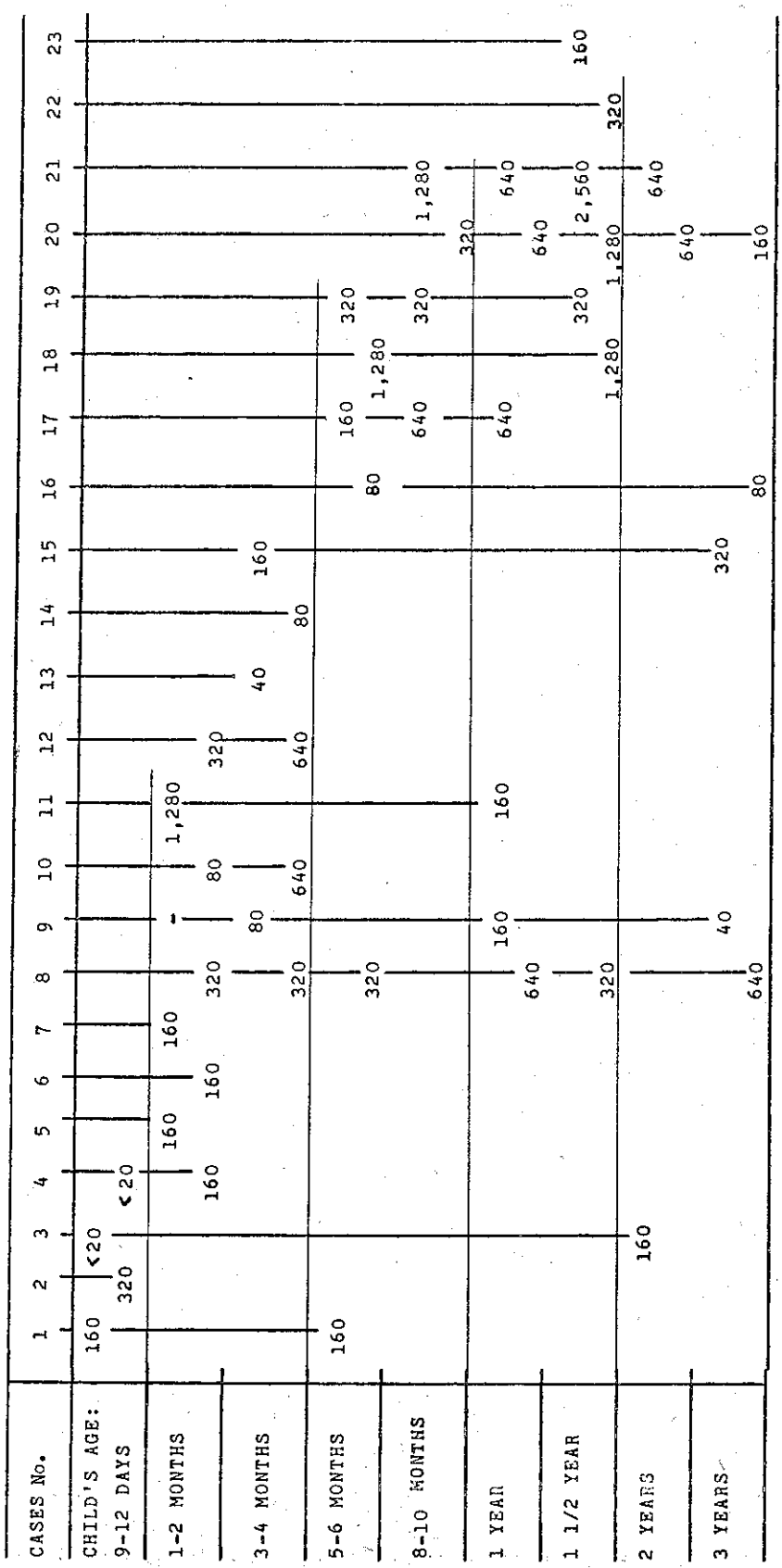
T A B L E I I I

Toxoplasmosis IgM-immunofluorescence test for 23 cases of congenital toxoplasmosis. Results referred according to child's age



T A B L E I V

Toxoplasmosis complement fixation test for 23 cases of congenital toxoplasmosis. Results referred according to child's age



T A B L E V

Toxoplasmosis hemagglutination test for 23 cases of congenital toxoplasmosis. Results referred according to child's age

CASES No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
CHILD'S AGE																							
9-12 DAYS	256	512	<64	64	4,000	128	512	64	-	2,000	1,024	8,000	1,024	4,000	2,000	8,000	512	16,000	8,000	16,000	16,000	16,000	4,000
1-2 MONTHS			<64		4,000		512				1,024												
3-4 MONTHS								256	512	1,024	64,000		1,024	4,000		8,000		16,000		64,000		64,000	
5-6 MONTHS	8,000						8,000																
8-10 MONTHS									16,000	32,000					8,000		8,000		16,000		32,000		
1 YEAR							128,000												64,000				
1 1/2 YEAR								32,000															
2 YEARS			16,000						2,000						8,000				128,000				
3 YEARS								16,000							4,000					32,000			

TABLE VI

Results and serologic patterns of tests for toxoplasmosis for 14 mothers of congenital cases, after delivery

Mother of cases no.	Time after child's birth	IgG-IF	IgM-IF	CF	HA	Toxoplasmosis serologic pattern
1	9 d.	16,000	0	160	2,000	I
3	11 d.	8,000	4,000	320	1,000	I
4	12 d.	8,000	256	160	128	I
6	1 mo.	8,000	64	640	8,000	I
10	2 mo.	16,000	1,024	640	4,000	I
13, 14	3 mo.	8,000	0	80	8,000	II
15	3 mo.	8,000	0	320	8,000	II
2	5 mo.	8,000	0	160	4,000	II
16	5 mo.	32,000	0	160	8,000	II
8	6 mo.	32,000	0	320	16,000	II
17	6 mo.	64,000	0	1,280	8,000	II
18	6 mo.	8,000	0	160	16,000	II
20, 21	10 mo.	16,000	0	80	4,000	II
22	18 mo.	16,000	0	320	16,000	II

5) showing a toxoplasmosis meningo-encephalitis and characteristic clinical evidences of congenital toxoplasmosis, which could be histologically confirmed in "post-mortem" examination. A latex test for rheumatoid factor was positive in the child's serum, with a 2,560 titer. As suggested by REIMER et al. [13] child's IgM antibodies against maternal IgG are frequently observed in young infants and should be responsible for such false positive results.

False positive IgM-IF tests were observed also in older children (Table III), sometimes following a negative IgM-IF test, other times preceded by a positive one.

As we have remarked in previous papers [6, 11], false positive toxoplasmosis IgM-IF tests due to IgM antiglobulin antibodies are frequently observed not only in patients with rheumatoid arthritis, but also in those presenting a long-lasting parasitic infection, as we have observed both for Brazilian Indians living in holoendemic malaria regions, and in the course of acquired toxoplasmosis, as well [4].

As our results indicate, much higher titers in IgG-IF and CF tests are usually observed for congenital cases than for passive transfer antibodies in non-infected infants. However, not only high titers are eventually observed for passive transfer antibodies but low titers can be seen for infected infants. Recently we attended a one month old child with hepatomegaly, splenomegaly, cerebral calcifications, microcephaly and chorioretinitis, exhibiting low initial titers in IgG-IF, CF and HA tests and a negative IgM-IF test. Titers decreased for even lower values during the first 3 months, but at the 5th month very high IgG-IF and CF titers were present. In the mother's serum a toxoplasmosis serologic pattern II was patent one month after delivery.

Mother's titers are also a useful index for screening possible cases of congenital infection. Thus, it seems to us that, when serologic follow-up cannot be done during pregnancy in mothers presenting negative tests, serological screening of possible congenital infection cases is a complex problem which should be based on detection of high titers of anti-toxoplasma antibodies in infant's and/or mother's sera, at delivery. However, diagnosis will depend on clinical, serological and parasitological follow-up of the child. For such purposes, although IgG-IF is a sensitive test, other tests could be more practical for routine, as our experience with immuno-enzymatic tests (ELISA) [2] and even with radio-immuno tests (to be published) indicates.

RESUMO

Diagnóstico sorológico precoce da toxoplasmose congênita

A presença freqüente de anticorpos maternos, de transferência passiva, no recém-nascido, dificulta o diagnóstico sorológico da

toxoplasmose congênita clinicamente inaparente. Investigou-se a possibilidade de um perfil sorológico característico, comparando-se resultados de testes de imunofluorescência IgG e IgM (IF-IgG e IF-IgM), fixação do complemento (FC) e hemaglutinação (HA), em casos congênitos e em récem-nascidos não infectados, portadores de anticorpos anti-toxoplasma.

A sorologia materna mostrou-se útil para triagem diagnóstica, com perfis de toxoplasmose recente em todos os casos examinados.

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