Original article

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PREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN BRAIN AND HEART BY IMMUNOHISTOCHEMISTRY IN A HOSPITAL-BASED AUTOPSY SERIES IN DURANGO, MEXICO

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The presence of tissue cysts of *Toxoplasma gondii* has only poorly been investigated in autopsy series. We determined the presence of *T. gondii* cysts in a series of 51 autopsies in a public hospital using immunohistochemistry of brain and heart tissues. The association of tissue cysts with the general characteristics of the autopsy cases was also investigated.

Of the 51 cases studied, five (9.8%) were positive by immunohistochemistry for *T. gondii* cysts in the brain. None of the heart specimens was positive for *T. gondii* cysts. The presence of *T. gondii* cysts in brains did not vary with age, sex, birthplace, residence, education, occupation, or the presence of pathology in the brain. In contrast, multivariate analysis showed that the presence of *T. gondii* cysts was associated with undernourishment (OR = 33.90; 95% CI: 2.82–406.32; $P = 0.005$).

We demonstrated cerebral *T. gondii* cysts in an autopsy series in Durango City, Mexico. Results suggest that *T. gondii* can be more readily found in brain than in heart of infected individuals. This is the first report of an association between the presence of *T. gondii* in brains and undernourishment.

Keywords: *Toxoplasma gondii,* prevalence, postmortem examinations, heart, brain, immunohistochemistry

Introduction

Toxoplasma gondii (*T. gondii*) is a ubiquitous intracellular protozoan parasite [1]. About one-third of the population is infected with *T. gondii* [2], typically via ingestion of food or water contaminated with oocysts shed by *T. gondii*-infected cats [3] or eating undercooked or raw meat containing tissue cysts from *T. gondii*-infected animals [4, 5]. Following dissemination throughout the body, *T. gondii* forms cysts in muscular and central nervous system tissues resulting in latent infection [6]. The latent stage of infection is controlled by the immune system of the host [7]. Although most acute infections with *T. gondii* are asymptomatic, latently infected individuals, i.e., immunocompromised individuals, may develop reactivated disease manifesting in the eye or brain [3, 5, 8]. Infections with *T. gondii* have been associated with a number of mental disorders including memory impairment in seniors [9], schizophrenia [10], and effects on the rate of suicide attempts [11, 12], as well as traffic [10] and work [13] accidents. In addition, *T. gondii* infection may lead to heart disease including myocarditis [14, 15], pericarditis [16, 17], and acute heart failure [18].

The demonstration of *T. gondii* in brain and heart specimens in humans is typically limited to postmortem examinations. While *T. gondii* cysts can be recognized in routine stainings, *T. gondii* can best be demonstrated in tissues using immunohistochemistry [19]. This method has been successfully used for detection of *T. gondii* in AIDS autopsy series [20, 21]. However, to the best of our

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knowledge, the use of this valuable method for the detection of *T. gondii* has not been reported in hospital-based autopsy series of routine postmortem examinations. The presence of *T. gondii* in brain tissue and heart muscle has not been investigated in detail thus far in Mexico. Therefore, we determined the presence of *T. gondii* in brains and heart muscle in an autopsy series in a public hospital in northern Mexico using immunohistochemistry. In addition, the association of the presence of *T. gondii* with general characteristics of the autopsy cases was investigated.

Materials and methods

Autopsy series

We studied a 6-year (2009–2014) hospital-based autopsy series in a public hospital (General Hospital of the Secretary of Health) in Durango City, Mexico. Inclusion criteria for the study cases were: 1) autopsy cases in the Pathology Department of the General Hospital, 2) with brain and heart tissues available, 3) any sex, and 4) any age. During the study period, 94 autopsies were performed. Of these 94 autopsies, only 51 had brain and heart tissues available. In total, 51 cases of postmortem examinations were included. Twenty-seven cases were females and 24 were males. Autopsy cases had an age from 3 days to 78 (mean 35.37 ± 24.62) years.

Immunohistochemistry

Archival formalin-fixed, paraffin-embedded sections of brain and heart from routine autopsy cases were included in the study. All autopsies were performed to clarify the diagnosis. Brain tissues were obtained from the left frontal lobe except one case (No. 21, choroid plexus). Heart tissues were obtained from the left auricular ventricular region. One brain tissue section and one heart tissue section of each of the 51 autopsy cases were analyzed with the aid of the Tinto Detector Immuno DNA System equipment (Bio SB, Santa Barbara, CA, USA) and Digital Pressure Cooker, Model PC-2000 (Bio SB). Immunohistochemistry was performed with the Mouse/Rabbit Immunodetector HRP/DAB (Bio SB). Immunostaining was performed on paraffin-embedded 2 µm tissue sections. The primary antibody "*Toxoplasma gondii*, rabbit polyclonal" (Bio SB) and the positive control "*Toxoplasma gondii* positive control slides" (Bio SB) were used. All assays were performed following the instructions of the manufacturer. A pathologist (LFSA) read the slides.

Statistical analysis

We performed the statistical analysis with the aid of the software: Epi Info version 7 and SPSS version 15.0. Bivariate analysis followed by multivariate analysis were used to examine the association of *T. gondii* infection and the characteristics of the autopsy cases. We used the two-tailed Fisher exact test for comparison of the frequencies among groups. As a strategy to select variables for the multivariate analysis, we included only variables that had P value ≤ 0.10 in the bivariate analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression analysis with the Enter method. We used the Hosmer–Lemeshow test to assess the goodness of fit of our regression model. Statistical significance was set at a P value of <0.05.

Ethical considerations

This study was approved by the Institutional Ethical Committee of the General Hospital of the Secretary of Health in Durango City, Mexico.

Results

Of the 51 autopsy cases studied, five (9.8%) were positive for *T. gondii* in the brain. None of the heart specimens was positive for *T. gondii*. A summary of the clinical and postmortem diagnoses of the 51 cases and their correlation with immunohistochemistry results is shown in *Table 1*.

Table 1. Correlation of clinical characteristics and postmortem diagnoses with the presence of *T. gondii* in brain as detected by immunohistochemistry in an autopsy series

No.	Sex	Age	Diagnosis	Brain histology	Presence of *T. gondii*
1	F	76	Rheumatoid arthritis	Cerebral ischemia	No
2	F	9	Pulmonary embolism	Respirator brain	No
3	M	44	Influenza	Cerebral ischemia	No
4	M	38	Influenza	Cerebral ischemia	No
5	F	67	Cervical cancer	Cerebral ischemia	No
6	F	67	Evans syndrome	Normal	No
7	M	0.4	Battered child syndrome	Hemorrhage	No
8	F	35	Intestinal ischemia	Cerebral ischemia	No

Table 1. *(cont.)*

No.	Sex	Age	Diagnosis	Brain histology	Presence of *T. gondii*
9	M	74	Fibrinopurulent meningitis	Fibrinopurulent meningitis	No
10	M	0.1	Respiratory distress syndrome	Cerebral immaturity	No
11	F	1.8	Hepatitis, liver fibrosis	Normal	No
12	F	38	Pulmonary embolism	Cerebral ischemia	No
13	F	0.5	Choledochal cyst	Normal	No
14	F	34	Sepsis	Cerebral ischemia	No
15	M	17	Bronchopneumonia	Cerebral ischemia	No
16	M	17	Pulmonary embolism	Normal	No
17	M	39	Scorpion envenomation	Normal	No
18	F	63	Morbid obesity	Normal	No
19	M	0.6	Meningoencephalitis	Viral meningoencephalitis	No
20	M	0.2	Intestinal perforation	Normal	No
21	F	7	Undernourishment	Cerebral ischemia	Yes
22	F	67	Diabetes mellitus	Normal	No
23	M	0.2	Thrombocytopenia	Normal	No
24	M	31	Nephrotic syndrome	Acute meningitis	No
25	F	22	Bronchopneumonia	Cerebral ischemia	Yes
26	F	0.03	Prematurity	Cerebral ischemia	No
27	F	38	Pneumonia	Cerebral ischemia	No
28	M	68	Polycythemia vera	Cerebral edema	No
29	F	6	Pulmonary hemorrhage	Cerebral ischemia	No
30	M	66	Diabetes mellitus	Cerebral thrombosis	No
31	M	57	Arterial hypertension	Normal	No
32	M	78	Arterial hypertension	Cerebral ischemia	No
33	M	45	Diabetes mellitus	Normal	No
34	M	51	Adrenal carcinoma	Cerebral edema	No
35	M	26	Miliary tuberculosis	Tuberculosis meningitis	Yes
36	F	32	Miscarriage	Normal	No
37	F	60	Miliary tuberculosis	Tuberculosis meningitis	No
38	F	61	Miliary tuberculosis	Tuberculosis meningitis	No
39	M	66	Fibromuscular dysplasia	Cerebral ischemia and thrombosis	No
40	F	59	Systemic lupus erythematosus	Normal	Yes
41	F	40	Premature rupture of membranes	Normal	No
42	F	29	Meningoencephalitis	Acute and chronic meningitis	No
43	M	44	Fulminant varicella	Cerebral ischemia	No
44	F	38	Miliary tuberculosis	Cerebral ischemia and edema	No
45	F	25	HELLP syndrome	Cerebral ischemia	No
46	M	70	Dermatomyositis	Cerebral ischemia	No
47	F	22	Diabetes mellitus	Cerebral ischemia	No
48	M	13	Thrombocytopenia	Normal	No
49	F	23	Eclampsia	Cerebral ischemia and edema	No
50	M	16	Bronchopneumonia	Normal	Yes
51	F	22	Pyogenic hepatic abscesses	Cerebral ischemia	No

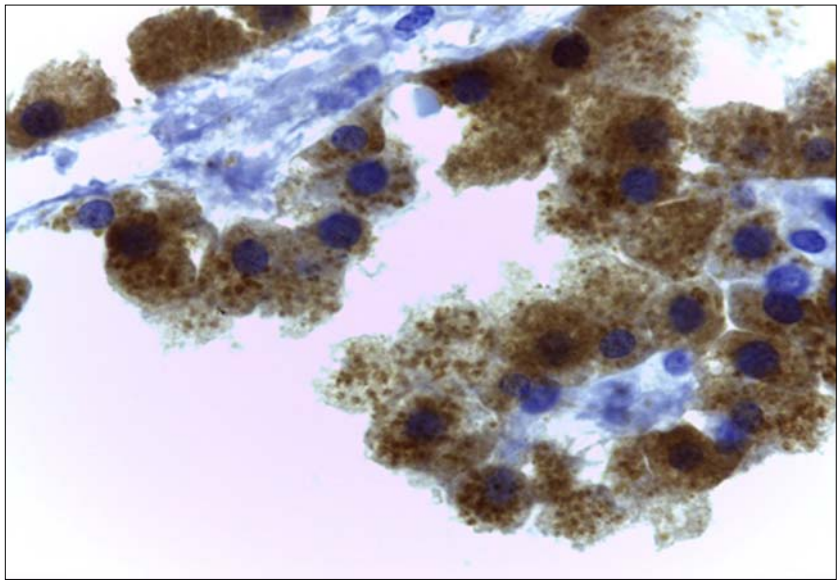

Fig. 1. *T. gondii* in choroid plexus (case No. 21)

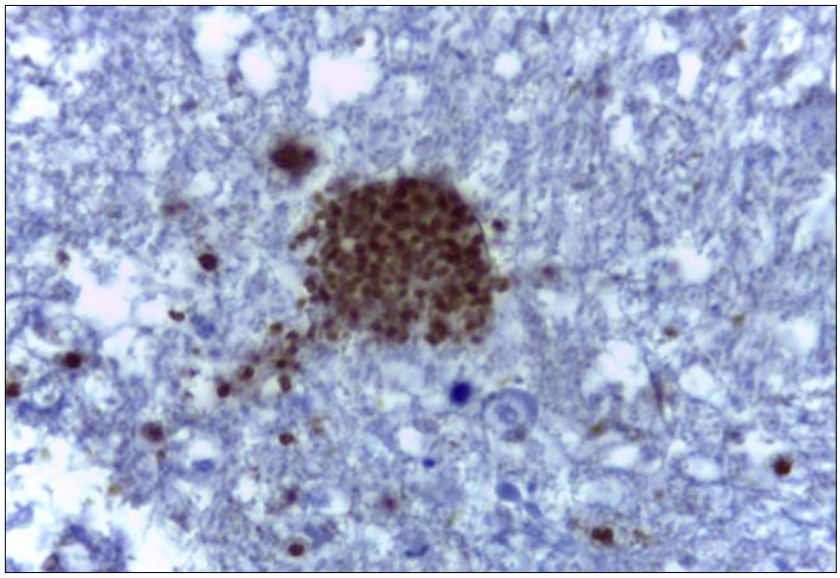

Fig. 2. A cyst of *T. gondii* in the brain (case No. 50)

Figures 1 and 2 show *T. gondii* in choroid plexus (case No. 21) and in frontal lobe (case No. 50), respectively. *T. gondii* was found in astrocytes and neurons. None of the cases had clinical or postmortem diagnosis of toxoplasmosis. *Table 2* shows the correlation of the general characteristics of the autopsy cases and *T. gondii* in brain. Presence of *T. gondii* in brains did not vary with age, sex, birthplace, residence, education, occupation, or the presence of cerebral pathology. In contrast, the presence of *T. gondii* in brains was significantly higher in cases with undernourishment (60%) than in those without undernourishment (4.3%) ($P = 0.005$). In total, two characteristics of cases showed P values of ≤0.10 in the bivariate analysis: age ($P = 0.07$) and undernourishment ($P = 0.005$). Further analysis of these variables by logistic regression showed that the presence of *T. gondii* in brains was only associated with undernourishment (OR = 33.90; 95% CI: 2.82–406.32; $P = 0.005$). The result of the Hosmer–Lemeshow test suggested an acceptable fit of our regression model ($P = 0.45$).

Discussion

The demonstration of *T. gondii* cysts in brain and heart by immunohistochemistry in autopsy series has been scantily reported. In the present study, we determined the presence of *T. gondii* in brain and heart in an autopsy series in a public general hospital in Durango City, Mexico by using immunohistochemistry. Our results showed that 9.8% of the autopsy cases studied had *T. gondii* in their brains. In contrast, none of the heart tissues in the autopsy cases were positive for *T. gondii* by immunohistochemistry. There is poor knowledge on the frequency of *T. gondii* in differ-

Table 2. Correlation of the presence of *T. gondii* in brains and general characteristics of the autopsy cases

Characteristics	No. of autopsy cases tested	Prevalence of infection		*P* value
		No.	%	
Age				
Up to 10 years	11	1	9.1	0.07
11–30 years	11	3	27.3	
>30 years	29	1	3.4	
Sex				
Male	24	2	8.3	1
Female	27	3	11.1	
Birthplace				
Durango State	48	5	10.4	0.84
Other state or abroad	2	0	0.0	
Unknown	1	0	0.0	
Residence				
Urban	23	3	13	0.7
Rural	25	2	8	
Unknown	3	0	0.0	
Education				
Up to 6 years	31	3	9.7	0.61
>6 years	14	2	14.3	
Unknown	6	0	0.0	
Occupation				
Laborer	12	1	8.3	0.64
Nonlaborer	33	4	12.1	
Unknown	6	0	0.0	
Pathology in brain				
Yes	35	3	8.6	0.64
No	16	2	12.5	
Undernourishment				
Yes	5	3	60.0	0.005
No	46	2	4.3	

ent organs and tissues in *T. gondii* infected individuals. Results suggest that *T. gondii* can be more likely found in brain than in heart of infected persons. In the present study, we were able to visualize *T. gondii* for the first time in brain of infected individuals in the region. Results add evidence to previous seroprevalence studies confirming that *T. gondii* infection occurs commonly in people in the region [22–25]. The 9.8% frequency of demonstration of *T. gondii* in brain in autopsy cases is higher than the 6.1% seroprevalence of *T. gondii* infection reported in the general population in Durango City, Mexico [22]. It is not clear why these autopsy cases had a higher frequency of *T. gondii* in their brains than the seroprevalence reported in the general population. However, there are differences in the characteristics of the studied populations. All autopsy cases died because of a number of underlying diseases whereas subjects in the general population included both healthy and ill people. Of note, infection with *T. gondii* in autopsy cases predominated in young cases. Indeed, four (80%) of the five positive autopsy cases were younger than 30 years old. Whereas, only 20 (33.9%) of 59 positive subjects in the survey of the general population were 30 years old or younger [22]. Seroprevalence of *T. gondii* infection increases with age in the region [22, 23]. One wonders whether the inverse "prevalence" pattern observed in autopsy cases points towards a role of infection in the pathology/death of the subjects. None of the autopsy cases had clinical or postmortem diagnoses of toxoplas-

mosis. However, toxoplasmosis in Mexico is a neglected disease and diagnostic examinations are not routinely performed. None of the autopsy cases had serological results for *T. gondii*. The rate of *T. gondii* in brains likely is even higher than 9.8% because a negative immunohistochemistry result cannot exclude presence of cysts in the brain. In autopsy series of AIDS, researchers found *T. gondii* in brains in 15 of 70 autopsy cases in Germany [20] and in heart in 21 of 170 autopsy cases in France [21] by using immunohistochemistry.

In the present study, we found *T. gondii* in the choroid plexus in an autopsy case (No. 21) with cerebral ischemia and undernourishment. Tachyzoites of *T. gondii* have been observed in the choroid plexus in 53% of patients with acquired immunodeficiency syndrome with cerebral toxoplasmosis [25]. No information about serology for human immunodeficiency virus infection was available in any of the autopsy cases in our series. We are not aware of any report of *T. gondii* in choroid plexus in immunocompetent individuals. Remarkably, in the present study, *T. gondii* infection in brain was associated with undernourishment by bivariate analysis, and this association remained significant by multivariate analysis, too. To the best of our knowledge, this is the first report of an association of *T. gondii* infection with undernourishment. It is unclear why autopsy cases with undernourishment had a higher frequency of detection of *T. gondii* than those without undernourishment. Malnutrition profoundly affects immune responses preventing the host from mounting an adequate protective response to infectious agents [26]; it remains to be shown whether malnutrition has a causal association with the dissemination of *T. gondii*. *T. gondii* is located in all brain areas [27], although some studies reported high number of parasites in the amygdala and frontal cortex [27, 28].

Conclusions

We demonstrated the presence of *T. gondii* in brains in an autopsy series in Durango City, Mexico. Results suggest that *T. gondii* can be more readily found in brains than in hearts of infected individuals. We report for the first time an association of *T. gondii* cysts in brains with undernourishment.

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