MEDICAL SCIENCE MONITOR

META-ANALYSIS

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 1547-1552
DOI: 10.12659/MSM.897155

Received: 2015.12.17
Accepted: 2016.01.05
Published: 2016.05.07

Is *Toxoplasma Gondii* Infection a Risk Factor for Leukemia? An Evidence-Based Meta-Analysis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: Departmental sources

Background: Possible associations of parasite infection with cancer risk have recently attracted much attention. Published studies concerning the association between *Toxoplasma gondii* (*T. gondii*) infection and leukemia risk have generated inconsistent results. In the present study, we aimed to address this topic by conducting a quantitative meta-analysis.

Material/Methods: Relevant publications were searched in electronic databases and eligible studies were rigorously screened and selected. Essential information was extracted and the data were pooled. Subgroup analysis on source of controls and detection target was also performed.

Results: A total of 6 studies that met the inclusion criteria were selected. The overall data show that *T. gondii* infection might have an association with increased leukemia risk (OR=3.05; 95%CI=1.83–5.08). Similar results were shown in the subgroups regarding source of controls and detection target.

Conclusions: Our results suggest that *T. gondii* infection might be a risk factor for leukemia, providing new insight into the etiology of leukemia. Future studies with large sample sizes in different geographic areas are needed to confirm this conclusion.

MeSH Keywords: Disease Susceptibility • Leukemia • Meta-Analysis • Toxoplasma

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/897155

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Background

Leukemia is a malignant tumor of the hematopoietic system, which often has a poor prognosis. The mechanisms are not clear. Several factors, such as radiation [1] and chemical carcinogens exposure [2], have been reported to be risk factors for leukemia, and internal factors, such as gene variation [3], have also been regarded as important factors. In our previous study, XRCC1 polymorphism was found to confer leukemia susceptibility [4]. However, despite findings in the literature, the etiology of leukemia is still poorly understood.

For years, the roles of parasitic infection in the genesis of tumors have attracted much attention. For example, the parasites *Clonorchis sinensis, Opisthorchis viverrini*, and *Schistosoma haematobium* are associated with a number of cancers, such as nasopharyngeal carcinoma, leukemia, and hepatocellular carcinoma [5]. *Toxoplasma gondii* (*T. gondii*) is a wide-spread parasite reported to infect about one-third of the world population, mainly in low- or middle-income countries [6]. Human infection results from accidental ingestion of oocysts from unsanitary food or water that contains *T. gondii* tissue cysts [7]. Toxoplasmosis in immunocompetent individuals is generally asymptomatic, but it often leads to serious pathological effects in immunocompromised patients (e.g., people with HIV/AIDS or transplant patients) [8]. Usually, infection with *T. gondii* is regarded as an established risk factor for poor obstetric history and is one of the major causes of congenitally acquired infections [9]. Moreover, *T. gondii* infection has also been implicated in the development of several disorders, such as liver cirrhosis [10], epilepsy [11], and even schizophrenia [12].

Recently, reports have indicated a possible association of *T. gondii* infection with cancer risk [13,14]. For leukemia, a number of studies on this subject have generated conflicting results. Whether *T. gondii* infection has a relationship with leukemia risk has been uncertain. Therefore, we aimed to conduct a meta-analysis addressing this issue to obtain a reliable result. This may help promote understanding of leukemia etiology in cancer research.

Material and Methods

Literature search strategy

A systematic literature search was conducted in the biological databases Medline, ScienceDirect, and Chinese National Knowledge Infrastructure (CNKI), without language restriction. Papers published up to December 2015 were covered. The following keywords were used for searching: *Toxoplasma, toxoplasmosis, leukemia, hematology, malignancy, neoplasm, and cancer*. All relevant studies found in the search were retrieved and their bibliographies were checked for other relevant publications.

Selection of studies

Papers were screened by reading their titles and abstracts. The potentially eligible articles were read in full, but only those that met the inclusion criteria were selected. The criteria for the literature selection were: first, leukemia as a disease and toxoplasmosis as an exposure; second, presence of a control group, with details of techniques and relevant targets used to diagnose *T. gondii* infection; and third, data on sample size, odds ratios (ORs), and their 95% confidence intervals (CIs) or information from which these could be inferred.

Articles that met the following criteria were excluded: first, the designs were obviously different from other selected papers; second, lack of controls and other essential information; third, reviews and repeated articles.

Data extraction and quality assessment

Two of the authors carefully read the full texts and selected the papers according to the criteria mentioned above. Essential information was extracted from the articles. For conflicting evaluations, a discussion was conducted. If we did not reach a consensus, another author of the present study was consulted to resolve the dispute and a final decision was made by majority vote.

The Newcastle-Ottawa Scale (NOS) was used to evaluate the quality of the included studies, which generated scales ranging from 0 to 9 stars [15]. Studies with less than or equal to 3 stars were indicated to be low-quality and were excluded.

Statistical analysis

The odds ratio (OR) of leukemia risk associated with presence of *T. gondii* was estimated for each study. The OR and its 95% confidence interval (CI) in each study was plotted against the number of participants for detection of any possible sample size biases. The chi square-based Q statistic test was performed to assess heterogeneity. If the result of the Q test was $P>0.1$, ORs were pooled according to the fixed-effects model (Mantel-Haenszel); otherwise, the random-effects model (DerSimonian and Laird) was used. The significance of the pooled ORs was determined by the Z test.

Sensitivity analysis was assessed by changing the effects models and using one-way sensitivity analysis [16] to assess stability of the results. Publication bias was evaluated by creating funnel plots, in which the standard error of the log (OR) of each study was plotted against its log (OR). An asymmetric plot indicates a possible publication bias. To minimize the potential subjective effects on the results, Egger's linear regression test [17] was used to assess the symmetry. The fail-safe number for $P=0.05$ ($Nfs_{0.05}$) [18] was also used for assessment

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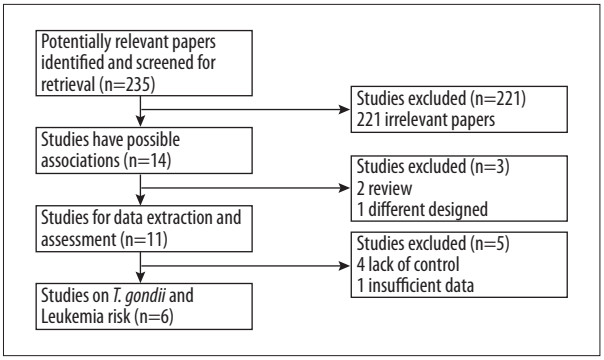

Figure 1. Flow diagram of included/excluded studies.

of possible publication bias. Statistical analysis was performed using Excel 2003 (Microsoft) and STATA 11.0 software (Stata Corporation, TX).

Results

Study characteristics

A total of 235 publications were identified, of which 221 irrelevant papers were excluded after a review of their titles and abstracts. As shown in Figure 1, 14 publications were preliminarily eligible, of which 2 reviews [19,20] and 1 study [21] whose selection of cases obviously led to selection bias were excluded. Then, after a careful review of the texts, 4 studies without controls [22–25] and 1 study with insufficient data [26] were further excluded. Finally, 6 studies [27–32] were selected for analysis.

Of the selected publications, 1 was written in Turkish [29] and the other 5 were in Chinese. No papers in English or other languages were included because relevant papers did not meet the inclusion criteria. Relevant information was extracted and listed in Table 1. The quality assessment scales of these included studies were all over 3 (data not shown); thus, they were all selected for analysis.

Meta-analysis results

As shown in Table 2, we first analyzed the heterogeneity of the pooled data. No marked heterogeneities were observed (Q=8.18; P=0.147). Thus, the fixed-effects model was used for data pooling.

For the overall data, a significant association was observed (OR=3.05; 95%CI=1.83-5.08; P=0.147 for heterogeneity), indicating that the infection rate of *T. gondii* in the leukemia cases was significantly higher than that in the controls (Figure 2).

Considering the potential effect of confounding factors on the results, we conducted subgroup analysis according to source of controls and target of detection. The data showed that the results of the subgroups regarding these 2 factors were in line with the overall data, suggesting that both the source of controls and the detection targets exerted little influence on the overall data.

Table 1. Characteristics of studies included in the meta-analysis.

First author	Year	No. and type of cases	Cases		No. and type of controls	Controls		Method for diagnosis of *T. gondii*	Target of detection	Area
			Positive	Negative		Positive	Negative			
Peng	2004	10 leukemia	4	6	75 healthy controls (PB)	4	71	IHT*	Toxoplasma IgG	China
Cheng	2001	93 leukemia (47 acute; 26 chronic; 20 recurrent)	14	79	151 Non-cancerous controls (HB)	5	146	PCR probe	Toxoplasma DNA	China
Gulesci	2005	25 leukemia	16	9	40 Non-cancerous controls (HB, age-, sex-matched)	25	15	IFA**	Toxoplasma IgG	Turkey
Yang	2005	46 leukemia	8	38	20 healthy controls (PB)	0	20	PCR***	Toxoplasma DNA	China
Chang	2007	58 leukemia	4	54	20 healthy controls (PB)	0	20	PCR	Toxoplasma DNA	China
Tian	2015	150 leukemia	24	126	110 healthy controls (PB)	7	103	ELISA****	Toxoplasma IgG	China

* IHT – indirect hemagglutination test; ** IFA – indirect immunofluorescence assa; *** PCR – polymerase chain reaction; **** ELISA – enzyme-linked immunosorbent assay.

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Table 2. Main results of the meta-analysis.

Factors	Overall OR (95%CI)	z	P	Heterogeneity test		Number of studies	Model
				Q	P		
Total (case *vs.* control)	3.05 (1.83, 5.08)	4.29	0.000	8.18	0.147	6	Fixed-effect
Stratification by source of controls							
PB*	3.85 (1.84, 8.08)	3.57	0.000	2.69	0.442	4	Fixed-effect
HB**	2.38 (1.17, 4.84)	2.38	0.017	4.37	0.037	2	Fixed-effect
Stratification by target of detection							
Toxoplasma IgG	2.32 (1.26, 4.28)	2.69	0.007	6.23	0.044	3	Fixed-effect
Toxoplasma DNA	5.39 (2.08, 14.01)	3.46	0.001	0.22	0.895	3	Fixed-effect

* PB – population-based; ** HB – hospital-based.

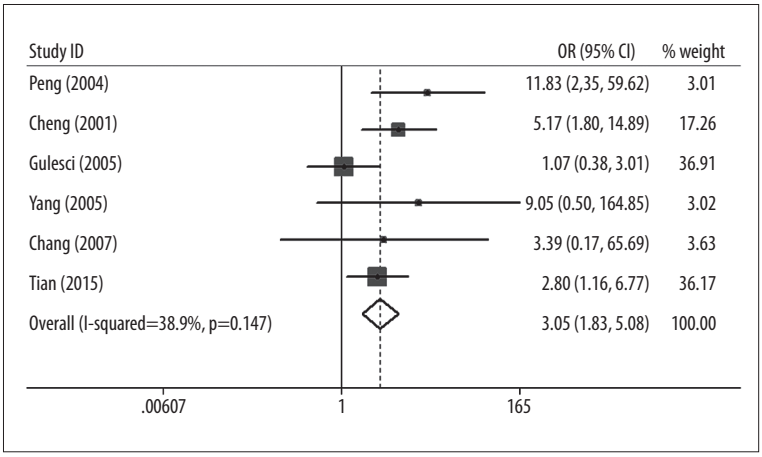

Figure 2. Meta-analysis of the association of leukemia risk with *T. gondii* infection (case *vs.* control).

Sensitivity analysis and bias diagnostics

To test whether the results were stable, we changed the effects model for pooling the data. As expected, the significance of the random-effects model (OR=3.39; 95%CI=1.64–7.03) was in accordance with the fixed-effects model. Then, we repeated the analysis by sequentially omitting each study, and found that the results were not significantly changed (data not shown). The results suggest that the results of the present meta-analysis were stable.

For detection of possible publication bias, a funnel plot was created, which appeared to be visually symmetrical. The funnel plot was further estimated by Egger's linear regression test, in which 95%CI (–2.512, 4.964) of the point of intersection between the regression curve and Y axis included the original point, with t value of 0.91 and P value of more than 0.05, indicating the symmetry of the funnel plot (Figure 3). In addition, $Nfs_{0.05}$ was also calculated to assess publication bias. The result was 53, which is more than 8 times the number of included studies. These data suggest that publication bias did not exert an evident influence on the results.

Discussion

Whether infection with *T. gondii* is a risk factor for leukemia has been uncertain and only a few reports have addressed this topic, with inconsistent results. In the present meta-analysis, we found that *T. gondii* infection appears to be associated with increased leukemia susceptibility.

Recently, the association of parasite infection with cancer risk has attracted much attention. For instance, parasitization by liver flukes, such as *Opisthorchis viverrini* and *Clonorchis sinensis,* has been suggested to confer cholangiocarcinoma susceptibility [33,34]. Infection by another parasite, *Anisakis*, may increase gastric or colon adenocarcinoma risk [35]. *Schistosoma haematobium* infection has been reported to play a role in genesis and development of bladder cancer [36]. Thus, parasite

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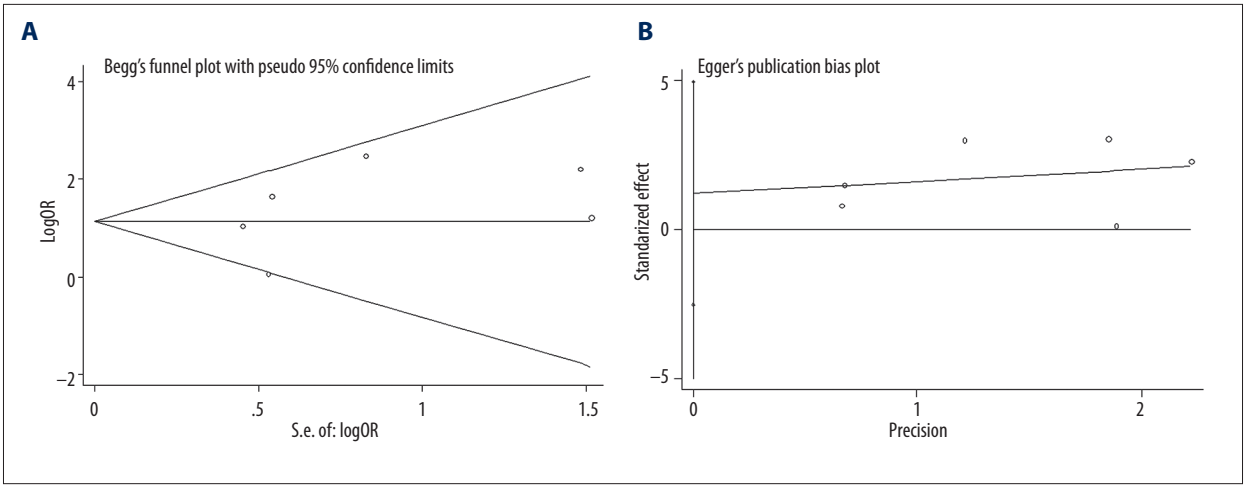

Figure 3. Publication bias test for the overall data (case *vs.* control) (**A**) Funnel plot; (**B**) Egger's linear regression test.

infection as a risk factor for malignancy may be of interest and value for research in this field. To the best of our knowledge, the present meta-analysis is the first to address the possible relationship between *T. gondii* infection and leukemia risk, and it provides new insights into research on cancer etiology.

The mechanisms by which *T. gondii* initiates tumorigenesis are unclear. Reports showed that *T. gondii* can export miRNAs into its host cell, which might regulate the hosts' gene expression, and thus cause cancer onset [37]. By modifying host miRNA expression, *T. gondii* infection has been reported to initiate and develop brain carcinoma [38]. Since the genes involved in apoptosis or anti-apoptosis were both targeted by the differentially-expressed miRNAs, the change in balance of power between the miRNAs targeting host apoptosis genes and those modulating host anti-apoptosis genes leads to the fate of the host apoptosis process [39]. The above evidence might be helpful in elucidating the roles of *T. gondii* in the genesis of leukemia. However, the evidence addressing the mechanisms is rather sparse. Future studies are needed to provide more precise evidence.

Considering that hospital-based controls were patients with other benign disorders that might have an association with *T. gondii* infection, we conducted subgroup analysis stratified by source of controls. Nevertheless, the results of the 2 subgroups were consistent with the overall data, indicating that source of controls probably did not affect the results. Moreover, *Toxoplasma* DNA was used as a target for detection in some studies, while in other studies *Toxoplasma* Ig G was used. Similarly, subgroup analysis regarding detection target also indicates little influence of this factor on the overall results.

Several limitations must be considered in the present meta-analysis when interpreting the results. First, only studies written in Chinese and Turkish were included because papers in English did not meet the inclusion criteria. Most included studies were conducted in China, leading to possible selection bias. This might be because *T. gondii* infection mainly affects people in low- or middle-income countries [6], such as China and Turkey. Second, the types of leukemia were not specified in all included studies. Since the biological characteristics and clinical features are different between acute leukemia and chronic leukemia, and between lymphocytic leukemia and myeloid leukemia, it will be necessary to increase the sample sizes and divide the cases according to the clinical type in future primary studies. Third, the controls in most included studies were not well-matched to the cases, and this might have affected the accuracy of the estimates. Furthermore, as shown in our previous paper [4], genetic factors might exert an influence on the susceptibility to leukemia. Whether *T. gondii* infection interacts with genetic factors and contributes to leukemia risk remains uncertain. This subject could not be assessed in the present meta-analysis because this interesting point has not been evaluated in the primary literature. Since *T. gondii* might influence host gene expression, ethnic variation may play a role in the pathogenesis of leukemia. Therefore, studies in different geographic areas and involving different ethnicities are needed to obtain a more reliable estimate.

Conclusions

The results of the present meta-analysis indicate that *T. gondii* infection might have an association with increased leukemia risk. These interesting results may be helpful in research on leukemia etiology. Studies in different regions and ethnicities, with large sample sizes, together with basic laboratory studies, are warranted to confirm this association and explore the potential molecular mechanisms.

Conflict of interest

None declared.

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