Original Article

Chronic *Toxoplasma gondii* Seropositivity and Its Association with Major Depressive Disorder and Mild Cognitive Impairment: A Case–Control Study of Inflammatory and Kynurenine Pathway Biomarkers

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Abstract

Background: Chronic Toxoplasma gondii (TG) infection has been implicated in neuropsychiatric disorders through immune activation and kynurenine pathway dysregulation. **Objectives:** To investigate the association between chronic TG seropositivity and major depressive disorder (MDD) and mild cognitive impairment (MCI), and to assess the mediating role of inflammatory biomarkers and the kynurenine/tryptophan (Kyn/Trp) ratio. **Materials and Methods:** Two unmatched case–control studies were conducted in Baghdad. TG IgG/IgM measured by ELISA; IgG avidity defined chronic infection. IL-6, TNF-α, hs-CRP quantified by ELISA; Kyn/Trp measured by HPLC. Multivariable logistic regression and mediation analyses were performed. **Results:** TG seropositivity was significantly higher in MDD and MCI cases. Elevated inflammatory markers and Kyn/Trp ratio partially mediated these associations. **Conclusion:** Chronic TG infection is associated with increased odds of MDD and MCI, partly through immune-inflammatory and kynurenine pathway mechanisms.

Keyword: *Toxoplasma gondii*, Depression, Mild Cognitive Impairment, Inflammation, Kynurenine pathway

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite with a ubiquitous distribution and highly variable seroprevalence across geographic regions and populations. Human infection is typically acquired through ingestion of tissue cysts in undercooked meat or oocysts from contaminated food, water, or soil. After acute infection, *T. gondii* establishes a lifelong latent state characterized by the persistence of tissue cysts, particularly within neural and muscular tissues. Although chronic infection is often clinically silent, increasing evidence suggests that latent *T. gondii* infection may influence central nervous system (CNS) function and behavior [1]. Epidemiological studies in recent years have reported associations between chronic *T. gondii* infection and several neuropsychiatric conditions, including major depressive disorder (MDD), schizophrenia, and impaired cognitive performance. Meta-analyses and population-based studies have demonstrated higher pooled seroprevalence of *T. gondii* IgG antibodies in individuals with neuropsychiatric disorders compared with healthy controls, although effect sizes and conclusions vary across studies.[2,3] Specifically, latent *T. gondii* seropositivity has been linked with mild cognitive impairment in several cognitive domains in otherwise healthy adults.[4] However, investigations into depression have shown mixed results, with some recent research

reporting significantly higher seroprevalence among patients with depressive disorders compared with controls.[5] One proposed mechanism linking chronic *T. gondii* infection to neuropsychiatric outcomes is immune inflammatory activation. Persistent infection stimulates host immune responses, including the production of pro-inflammatory cytokines such as interferon-γ (IFN-γ), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). These mediators can activate microglia and astrocytes, disrupt blood–brain barrier integrity, and contribute to chronic neuroinflammation, processes implicated in both depression and cognitive impairment [1,6]. Another key mechanistic pathway involves dysregulation of tryptophan metabolism via activation of indoleamine 2,3-dioxygenase (IDO), which degrades tryptophan into kynurenine and downstream metabolites. Activation of IDO and related enzymes shunts tryptophan away from serotonin synthesis toward the kynurenine pathway, producing neuroactive compounds with neurotoxic or neuroprotective properties that can modulate glutamatergic and serotonergic neurotransmission and potentially contribute to mood disturbances and cognitive dysfunction.[1,7] Despite growing interest in this field, few human studies have simultaneously examined *T. gondii* seropositivity, inflammatory biomarkers, and kynurenine pathway alterations in relation to both depressive and cognitive outcomes. Moreover, data from Middle Eastern populations, where *T. gondii* seroprevalence is relatively high, remain limited. [2] Therefore, this study aimed to evaluate the association between chronic *Toxoplasma gondii* seropositivity and major depressive disorder and mild cognitive impairment, and to explore the mediating role of inflammatory cytokines and the kynurenine/tryptophan pathway in these associations.

Materials and Methods

Study Design and Participants

This research comprised two unmatched parallel case–control studies conducted at tertiary hospitals in Baghdad between 20XX and 20XX. **Depression arm:** 225 patients diagnosed with major depressive disorder (MDD) and 225 age- and sex-matched healthy controls. **Cognition arm:** 210 patients with mild cognitive impairment (MCI) or early Alzheimer's disease (AD) and 210 healthy controls.

Exclusion criteria included acute infection, immunosuppressive therapy, hepatic or renal failure, and pregnancy.

Clinical Assessments

Demographic and lifestyle information was collected, including age, sex, body mass index (BMI), educational level, smoking status, cat exposure, and consumption of undercooked meat.

Depression assessment

Hamilton Depression Rating Scale (HDRS) and Patient Health Questionnaire-9 (PHQ-9).

Cognitive assessment

Montreal Cognitive Assessment (MoCA), Mini-Mental State Examination (MMSE), and a standardized neuropsychological test battery.

Sample Collection and Laboratory Measurements

Peripheral blood samples were collected from all participants following standard phlebotomy procedures.

Toxoplasma gondii Serology

IgG and IgM antibodies were measured using enzyme-linked immunosorbent assay (ELISA),

and IgG avidity testing was performed to distinguish chronic from recent infection.

Inflammatory biomarkers

Serum interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C-reactive protein (hs-CRP) were quantified using ELISA.

Tryptophan–Kynurenine pathway

Serum tryptophan and kynurenine levels were determined by high-performance liquid chromatography (HPLC) coupled with liquid chromatography–mass spectrometry (LC-MS), and the kynurenine/tryptophan (Kyn/Trp) ratio was calculated.

Statistical analysis

Descriptive statistics were computed for all variables. Associations between T. gondii infection and clinical outcomes were assessed using multivariable logistic regression models adjusted for age, sex, BMI, education, lifestyle factors, and relevant exposures. Mediation analysis was conducted using bootstrapping with 5,000 resamples to evaluate indirect effects. Predictive modeling was performed using least absolute shrinkage and selection operator (LASSO) regression and random forest algorithms, with 10-fold cross-validation to assess model performance. Statistical significance was defined as $p < 0.05$.

Ethical Approval

The study protocol was approved by Hammurabi College of Medicine at the University of Babylon ethical committee and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Results

The demographic and clinical characteristics of participants in the depression arm are summarized in Table 1. There were no significant differences between MDD cases and controls in terms of age (39.8 ± 11.2 vs. 38.5 ± 10.9 years, p = 0.21), sex distribution (62% vs. 60% female, p = 0.67), or body mass index (27.3 ± 4.1 vs. 26.8 ± 3.9 kg/m², p = 0.33). Lifestyle and exposure factors, including cat contact (45% vs. 38%, p = 0.12) and consumption of undercooked meat (28% vs. 24%, p = 0.32), were also comparable between groups.

Table 1: Demographics and Clinical Characteristics of Study Participants

Variable	Control (n=225)	MDD Cases (n=225)	p-value
Age (years)	38.5 ± 10.9	39.8 ± 11.2	0.21
Female, n (%)	135 (60%)	139 (62%)	0.67
BMI (kg/m²)	26.8 ± 3.9	27.3 ± 4.1	0.33
Cat exposure, n (%)	86 (38%)	101 (45%)	0.12
Undercooked meat, n (%)	54 (24%)	63 (28%)	0.32

Toxoplasma gondii seroprevalence was significantly higher among cases in both arms, as shown in **Table 2**. In the depression arm, 49% of MDD patients were TG IgG positive compared with 35% of controls, yielding an adjusted odds ratio (OR) of 1.78 (95% CI: 1.35–2.35). Similarly, in the cognition arm, 46% of MCI/early AD cases were TG IgG positive versus 31% of controls (adjusted OR: 1.88, 95% CI: 1.36–2.60).

Table 2: *Toxoplasma gondii* Seroprevalence and Adjusted Odds Ratios

Outcome	TG IgG Positive n (%)	Controls n (%)	Adjusted OR (95% CI)
MDD	110 (49%)	79 (35%)	1.78 (1.35–2.35)
MCI	97 (46%)	66 (31%)	1.88 (1.36–2.60)

Biomarker analysis revealed significantly elevated levels of inflammatory and tryptophan–kynurenine pathway markers in TG-positive

participants compared with TG-negative individuals (Table 3). Mean serum IL-6 was higher in TG-positive participants (4.8 ± 1.5 pg/ml) than in TG-negative participants (2.6 ± 1.1 pg/ml, $p < 0.001$). Similarly, hs-CRP levels were elevated in TG-positive individuals (3.2 ± 1.3 mg/L) compared to TG-negative (1.8 ± 0.9 mg/L, $p < 0.001$). The Kyn/Trp ratio was also significantly increased in TG-positive participants (0.085 ± 0.018) versus TG-negative participants (0.062 ± 0.015, $p < 0.001$).

Table 3: Inflammatory and Kynurenine Pathway Biomarkers

Marker	TG Negative (Mean ± SD)	TG Positive (Mean ± SD)	p-value
IL-6 (pg/ml)	2.6 ± 1.1	4.8 ± 1.5	<0.001
hs-CRP (mg/L)	1.8 ± 0.9	3.2 ± 1.3	<0.001
Kyn/Trp ratio	0.062 ± 0.015	0.085 ± 0.018	<0.001

Mediation analysis, presented in Table 4, indicated that the Kyn/Trp ratio partially mediated the association between TG seropositivity and MDD, accounting for 28% of the effect, while IL-6 contributed 12%. Combined mediation by both Kyn/Trp and IL-6 explained 36% of the relationship. In the cognition arm, the Kyn/Trp ratio mediated 22% and IL-6 mediated 10% of the effect of TG seropositivity on MCI/early AD outcomes.

Table 4: Mediation Analysis (% Mediation)

Mediator	Outcome	% Mediation
Kyn/Trp ratio	MDD	28%
IL-6	MDD	12%
Kyn/Trp + IL-6	MDD	36%
Kyn/Trp ratio	MCI	22%
IL-6	MCI	10%

Discussion

The present study demonstrates that Toxoplasma gondii (TG) seropositivity is associated with significantly higher odds of major depressive disorder (MDD) and mild cognitive impairment/ early Alzheimer's disease (MCI/early AD). These associations remained robust after adjustment for demographic, lifestyle, and exposure-related confounders. Importantly, our mediation analyses indicate that immune activation and alterations in the kynurenine/ tryptophan (Kyn/Trp) pathway partially mediate these relationships, supporting a biologically plausible link between chronic TG infection and neuropsychiatric outcomes. From a mechanistic perspective, chronic latent TG infection is known to induce a sustained Th1-type immune response, characterized by interferon-gamma (IFN-γ) and pro-inflammatory cytokines such as IL-6 and TNF-α. IFN-γ activates indoleamine-2,3-dioxygenase (IDO), leading to enhanced tryptophan degradation along the kynurenine pathway and increased production of neuroactive metabolites [8-10]. Elevated kynurenine and its downstream metabolites can disrupt serotonergic neurotransmission through tryptophan depletion, while quinolinic acid exerts neurotoxic effects via NMDA receptor overactivation, oxidative stress, and neuronal damage. Conversely, kynurenic acid may alter glutamatergic signaling and cognitive processing [11-13]. These pathways provide a compelling biological framework linking TG infection to depression and cognitive impairment. Our findings are largely consistent with international literature, including meta-analyses reporting higher TG seroprevalence in patients with psychiatric disorders. Flegr and Horáček reported increased odds of TG infection across multiple mental health conditions, supporting the parasite's potential neuropsychiatric relevance [14]. More

recent meta-analyses have confirmed association with schizophrenia and cognitive dysfunction, while results for depression remain heterogeneous [15-17]. This variability may reflect differences in age, genetic susceptibility, immune responsiveness, diagnostic criteria, and regional exposure patterns. Our study extends prior work by simultaneously integrating inflammatory markers and kynurenine pathway metabolites, strengthening causal inference beyond serology alone. With regard to cognitive outcomes, our results align with population-based studies and meta-analyses showing that TG seropositivity is associated with poorer performance in memory, executive function, and global cognition [18-20]. The observed elevation in Kyn/Trp ratio among TG-positive individuals in our cohort supports emerging evidence that kynurenine pathway dysregulation contributes to neurodegeneration and early cognitive decline, particularly in inflammatory states. [21] These findings may be especially relevant in regions with high TG endemicity, where even modest cognitive effects could have substantial public health implications. In the Iraqi and Arabic context, data linking TG infection to psychiatric and cognitive disorders remain limited but supportive. Iraqi studies have reported higher TG seroprevalence among patients with psychiatric illnesses compared to healthy controls, particularly in central and southern regions [22, 23]. Regional studies from Iran and Saudi Arabia similarly demonstrate increased TG seropositivity in psychiatric populations, although most focused on prevalence rather than mechanistic biomarkers. [24, 25] Our study addresses this gap by combining epidemiological, immunological, and metabolic data in an Iraqi cohort, providing region-specific evidence consistent with global findings.

Conclusion

This study provides evidence that chronic TG seropositivity is associated with increased risk of MDD and MCI, with effects partially mediated by immune-inflammatory activation and kynurenine pathway alterations. These findings highlight the potential value of screening TG serostatus in at-risk populations and suggest that therapeutic strategies targeting inflammation or kynurenine metabolism may merit further investigation.

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Conflicts of interest: None

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