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## Role of nitric oxide in detection toxoplasmosis patients in Al-Najaf Province

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**Abstract**---This study was aimed to estimate the prevalence of *Toxoplasma gondii* infection and associated risk factors among patients in Al-Najaf Province and to investigate whether there is a relationship between this infection and one of oxidative stress marker level (nitric oxide (NO)). A total of 117 blood samples were collected from Iraqi women with toxoplasmosis at Al-Zahra Teaching Hospital for Maternity and Children in AL-Najaf province. The PCR test was carried out in the Advanced Research Laboratory of the Department of Laboratory Investigations / Faculty of Science – University of Kufa. The estimation of NO marker level in patients and 30 healthy women (control group) was conducted in Al-Ameen Center for Research and Advanced Biotechnology/ Imam Ali Holy Shrine. The results of the PCR (to confirm the toxoplasmosis infection) had shown that 53(45.30%) patients were found positive and 64 (54.70%) were negative. The results of biochemical study revealed that the concentrations of NO level were increased in toxoplasmosis patients compared with the controls. The results of this study highlight on the wide spread of Toxoplasmosis in Iraq, especially in Najaf Governorate. The use of molecular methods to diagnose toxoplasmosis remains the most accurate technique for diagnosing infection. The results of the study documented that there is a relationship between high levels of oxidative stress markers (NO) and infection with *T. gondii*.

**Keywords**---Genotyping, *Toxoplasma gondii*, B1 gene, Relation, Nitric Oxide.

## Introduction

*Toxoplasma gondii*, an obligate intracellular protozoan parasite with noteworthy zoonotic importance, causes toxoplasmosis in humans and warm-blooded animals (Ybañez *et al.*, 2020). Consumption of raw or uncooked meat and fish containing bradyzoites (water, milk, and vegetables contaminated with oocysts) and transfusion and transplantation of blood and organs, respectively, harboring tachyzoites from patients infected with *T. gondii* are the major sources of *T. gondii* infection in humans (Kolören and Dubey, 2020). As a final host, cats play an important role in spreading *T. gondii* infection (Dubey, 2009). Therefore, poor hygienic management of farms, climate, presence of cats in farms, consuming raw or uncooked meat and vegetables, and inter-current diseases may act as potential risk factors influencing this disease (Ferra *et al.*, 2020). The sexual life cycle of *T. gondii* is restricted to cats; however, the asexual cycle depends on humans and other intermediate hosts (Dubey *et al.*, 2020). The prevalence range of toxoplasmosis has remarkably increased in humans and animals (Asgari *et al.*, 2006; Nasiru *et al.*, 2020). The highest risk of toxoplasmosis is due to congenital infection of the fetus during pregnancy, patients with an immunity disorder such as AIDS, recipients of organ transplants, and immunosuppressive drug users, in which the parasite spreads into the organs and destroys their tissues, leading to severe complications (Montoya and Liesenfeld, 2004; fonso, *et al.*, 2009). Molecular studies have shown that *T. gondii* is subdivided into three main types named G1, G2, and G3. A variety of genotypic distributions have been observed in different geographical areas and among different hosts. The severity of *T. gondii* pathogenicity in the host is dependent on the parasite type so that type I is more pathogenic than types II and III (Mammari *et al.*, 2019; tiu *et al.*, 2019; Fernandez-Escobar *et al.*, 2020; M'ev'elec *et al.*, 2020). Although G2 is more common in human infections in terms of frequency, G1 and atypical isolates seem to be more likely to cause severe complications, such as retinochoroiditis (Ayo *et al.*, 2016) respectively. Although serological methods are conventional in the diagnosis of toxoplasmosis, molecular methods have recently received more attention due to their higher sensitivity and specificity. Moreover, Polymerase chain reaction (PCR)-based methods, such as PCR-RFLP, are widely used to determine the strains of the parasite (Liu *et al.*, 2015). PCR amplification of different genes to detect the *T. gondii* from aborted samples is a valuable and reliable technique (Abu-Dalbouh *et al.*, 2012). Nitric oxide (NO) is produced by many cell types, such as dendritic, mast, natural killer, and phagocytic cells, in response to cytokine stimulation and plays important roles in immunologically mediated protection against many types of pathogens *in vitro* and in animal models, including protozoans (Christian, 2001). During infection by protozoans such as *Plasmodium* spp. and *Toxoplasma gondii*, increased NO production mediates host protection by either killing parasites directly or limiting parasite growth (Brunet LR., 2001). Previous studies have reported higher NO production during primary *E. tenella* infection, such that NO acts as an anticoccidial effector (Lillehoj, 1998).

## Materials and Methods

### Sampling of cases

A) Study group: A total of 117 blood samples were collected from Iraqi women with toxoplasmosis at Al-Zahra Teaching Hospital for Maternity and Children in AL-Najaf province. They previously detected as *Toxoplasma* positive cases by using the Vitek Immuno-Diagnostic Assay System (VIDAS) for detection of immunoglobulins toxoplasma antibodies. The PCR test was carried out in the Advanced Research Laboratory of the Department of Laboratory Investigations / Faculty of Science – University of Kufa. The estimation of oxidative stress markers (NO) level was conducted in Al-Ameen Center for Research and Advanced Biotechnology / Imam Ali Holy Shrine.

B) Control group: It consists of 30 healthy women; all were without any inflammatory disorders or clinical manifestation of any disease. The tests were carried out on 5 mL of venous blood drawn from each subject (Al-Labban, 2017). 2 milliliters were gathered in anticoagulant EDTA vials and utilized in the testing of PCR to confirm the *T. gondii* infection. 3 ml was collected in gel tubes and centrifuged for 5 minutes at 3000 rpm. The serum was placed into a 1.5milliliter microcentrifuge tube and kept at  $-80^{\circ}\text{C}$  for subsequent utilize in estimation of oxidative stress markers (NO) level. This marker was measured for only the PCR positive samples and control (Medhat and Aljanabay, 2022; Hadi and Aljanaby, 2022; Aljanaby et al., 2022; Alhasnawi and Aljanaby, 2022).

### Results and Discussion

From present study results in Table 1, it can be noted that the NO level in patients is increased compared with the control this may be retrain to a defensive mechanism to protect the patient against the harmful effects of the parasite. Also it was suggested that it can play a role in the pathogenesis of acute toxoplasmosis. NO is the product of arginine metabolism and one of the most effective O<sub>2</sub>-free toxins. There are also previous studies reporting an increase in the NO level in parasitic diseases (Kang KM, *et al.*, 2004). It was concluded that an increase in the NO level can be associated with the stimulation of the cell mediated immune system.

Table 1  
The NO concentrations in control and toxoplasmosis patients

Parameters	Groups		P-value
	control N (30) Mean± SD	Patients N (53) Mean± SD	
NO (μmole/ml)	0.030±0.009	0.146±0.052	0.0001*

\*means significant difference at ( $P \leq 0.05$ )

## Conclusion

The results of the study documented that there is a relationship between high levels of oxidative stress markers (NO) and infection with *T. gondii*.

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