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# Evaluation of the Sensitivity of Three Immunological Diagnostic Techniques for the Diagnosis of Toxoplasmosis in Aborted Women in Kirkuk/ IRAQ

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## ABSTRACT

**Background:** Toxoplasmosis is widely recognized as a highly widespread parasitic infection in humans, capable of vertical transmission from mother to fetus. Clinical problems associated with toxoplasmosis during pregnancy include spontaneous miscarriage, premature labor, stillbirth, and fetal abnormalities.

**Objective**: to evaluate the sensitivity of three immunological methods for the diagnosis of *Toxoplasma. gondii* in serum and placental tissue of aborted women.

**Materials and Methods:**80 blood and placental Specimens were obtained from women who underwent abortion and were admitted to the Maternity ward and Child hospital in Kirkuk City. Blood samples were analyzed for anti-*Toxoplasma* antibodies IgM and IgG using ELISA and Combo Rapid test and placental samples were tested for *T. gondii* antigen in the placental tissue using the Immunohistochemistry technique.

**Results:** The presence of anti- *T. gondii* antibodies in women who have undergone abortions was 30% (IgG) when the Combo Rapid test was used and 16.3% (IgG) when ELISA was used. In all cases, neither the combo rapid test nor the ELISA demonstrated seropositivity of IgM. IHC examination revealed that 60% of tissues had toxoplasmosis infection, whereas 40% of tissues were negative.

**Conclusion:** the finding of this study indicates that IHC is a more sensitive immunological technique than a serological assay for the direct diagnosis of *T. Gondii* in the placental tissue of aborted women.



*Keywords: Toxoplasmosis, Enzyme-Linked immunosorbent Assay, Immunohistochemistry, Abortion.*

## 1. Introduction

The genus *Toxoplasma* only contains one species, *T. Gondii*. Most mammals, including humans, dogs, and cattle, are susceptible to infection by the protozoan parasite *T. gondii*. Because of the serious illnesses it causes, it is an important issue in public health worldwide[1]. An estimated one-third of the world's population is thought to have been infected with *T.Gondii* and may have a long-lasting infection[2].

The two primary modes of transmission to humans are vertical transmission from the mother to embryo through the placenta in a pregnant woman and horizontal transmission such as accidental consumption of food or water contaminated with infectious oocysts or the utilization of uncooked meats that contain bradyzoite tissue cysts[3].

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Currently, there exists no officially approved vaccine for human toxoplasmosis due to the presence of several virulence factors at each stage of the life cycle, which contribute to immune system suppression and the establishment of a persistent infection[4]. Since *T. gondii* is an intracellular pathogen, infection triggers all immune defense lines including innate and adaptive responses which are necessary for the pathogen's movement through extracellular spaces in search of a new host cell[5].

Infection with *T. gondii* is a key contributor to the emergence of illnesses affecting the central nervous system and the eye in both immunocompetent and those having an impaired immune system. Fetuses may be exposed to this infection after their mothers contract it during pregnancy. Infants who were infected during the first trimester, when the immune system of the fetus is compromised, exhibit the most severe clinical signs in the brain and eye[6]. Although most individuals with chronic infection do not display symptoms, fetal toxoplasmosis can result in abortion, stillbirth, or profound cognitive impairment. Infections acquired during the later stages of pregnancy may not initially present symptoms, but can later lead to retinal or nervous system damage[7].

The manifestation of clinical signs in cases of toxoplasmosis infection is infrequent, making them an unreliable basis for diagnosis. As a result, laboratory diagnosis serves as the principal method of detection. Numerous laboratory diagnostic procedures have been employed throughout scientific inquiry and investigation[8].

In the majority of instances, the diagnostic process involves the utilization of immunological testing and molecular methods, either independently or in conjunction, to establish a diagnosis[9].

The diagnostic process for *T. gondii* infection frequently entails a histological examination that employs immunohistochemistry (IHC) techniques[10]. Immunohistochemistry (IHC) is a commonly utilized technique in the field of diagnostic pathology for the detection and characterization of pathological agents[11]. The current study aims to evaluate the sensitivity of three immunological methods for the detection of Toxoplasmosis in aborted women in Kirkuk City – northern Iraq.

## 2. Methods and Materials

The investigation was conducted from January to October 2023. were 80 blood and placental samples collected from aborted women admitted to the Maternity and Child Hospital in Kirkuk City. Samples of blood tested for anti-*toxoplasma* IgM and IgG using an Enzyme-Linked immunosorbent assay and Combo Rapid test and placental samples were examined for *T. gondii* antigen in the placental tissue using the Immunohistochemistry technique. Donors were invited to take part in the study following a concise description of the project's objectives, and verbal agreement was acquired from all patients.

### 2.1 Blood Sample Collection

4 ml of blood was obtained from each patient in a gel and clot vacuum tube to separate the serum to detect anti-Toxoplasma IgM and IgG using the Combo Rapid Cassette Test and ELISA respectively.

### 2.2 Placental Sample Collection

Fresh placental tissue was collected from aborted women in a clean plastic cup containing **10%** neutral buffered formalin for fixation and sent to the library for the diagnosis of *T. gondii* antigen using an immunohistochemistry test.

### 2.3 Serological testing

Serum samples screened for anti-T. *gondii* IgG and IgM using commercially available (CTK BIOTECH onsite Toxo IgG/IgM Combo Rapid Test, CA 92064, USA) and ELISA (CAMP media group, No.29 Stanei Street Bucharest, Romania). The guidelines provided by the manufacturer carried out both ELIZA and Combo Rapid tests. In the ELISA test, negative specimens (A/C.O < 1) are those whose absorbance is less than the cut-off value. Positive specimens are those with absorbances that are equal to or higher than the cut-off value (A/C.O ≥ 1).

### 2.4 Immunohistochemistry

The immunohistochemistry was conducted utilizing the Dako EnVision detection immunohistochemistry kit (Envision FLEX, Dako, K8000, Denmark) following the instructions provided by the manufacturer.

**Statistical analysis**

The data was analyzed using the $X^2$ test with a 95% confidence interval (CIs) in SPSS 26.0 (SPSS Inc., Chicago, IL, USA) to identify statistically significant differences (P-value <0.05).

## 3. Results

### 3.1 ELISA and Rapid Test

No Seropositivity of anti-Toxoplasma IgM antibodies showed in all cases in both rapid test and ELISA. As demonstrated in table (1) Anti-Toxoplasma IgG antibodies percentage was 30% and 16.3% in both rapid test and ELISA respectively.

**Table 1:** A comparison of anti-Toxoplasma IgG antibodies seropositivity using Rapid test and (ELISA) tests.

| Infection Status | Rapid Test | | ELIZA Test | | P. Value | Odds Ratio |
|---|---|---|---|---|---|---|
| | Number | Percentage % | Number | Percentage % | | |
| Negative | 56 | 70.0% | 67 | 83.8% | 0.039 | 0.45 |
| Positive | 24 | 30.0% | 13 | 16.3% | | |
| Total | 80 | 100.0% | 80 | 100.0% | | |

*. The association is significant at the 0.05 level.

### 3.2 Immunohistochemistry

placental tissue (n=80) were subjected to immunohistochemistry for the detection of T.gondii antigen in the examined tissues as shown in figures (1,2). to prove that the method of immunohistochemistry is more specialized. The method was applied to one of the samples of tissue sections of an infected placenta, but without the use of an antibody, so the sections showed negative immunological expression as shown in igure (3). IHC analysis revealed that 60% of tissues had toxoplasmosis infection, whereas 40% of tissues were negative. as shown in table (2).

A, B, C&D/ Positive expression of anti-Toxoplasma gondii antibody (arrow) was observed in spaces between placental villi. Also, Positive expression of anti-Toxoplasma gondii antibody (arrowhead) in the lumen of villous capillaries. DAB & Hematoxylin. A&C: 100x and B&D: 400x.

A, B, C&D/ Massive expression of anti-Toxoplasma gondii antibody (arrow) that separated between stratum basalis and decidual cells indicating that Toxoplasma was invaded the placental tissue. DAB & Hematoxylin. A&B: 100x and C&D: 400x.

**Table 2:** Distribution of T.Gondii antigen in the placental tissue using IHC.

| Infection status | Number of samples | (%) |
|---|---|---|
| Positive | 48 | 60% |
| Negative | 32 | 40% |
| Total | 80 | 100 |

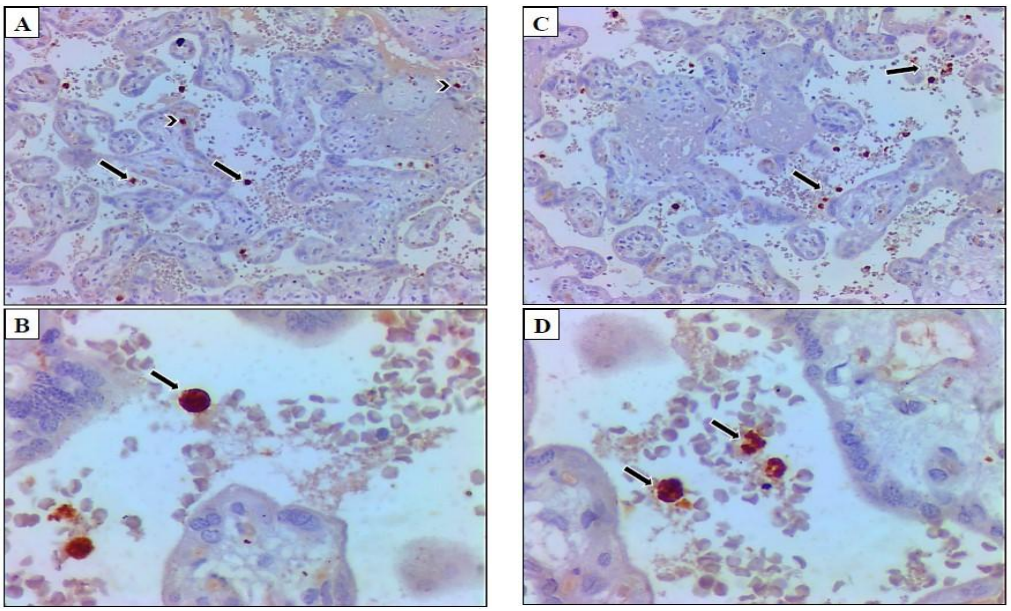


**Figure 2:** Photomicrograph of placenta infected with Toxoplasma gondii.

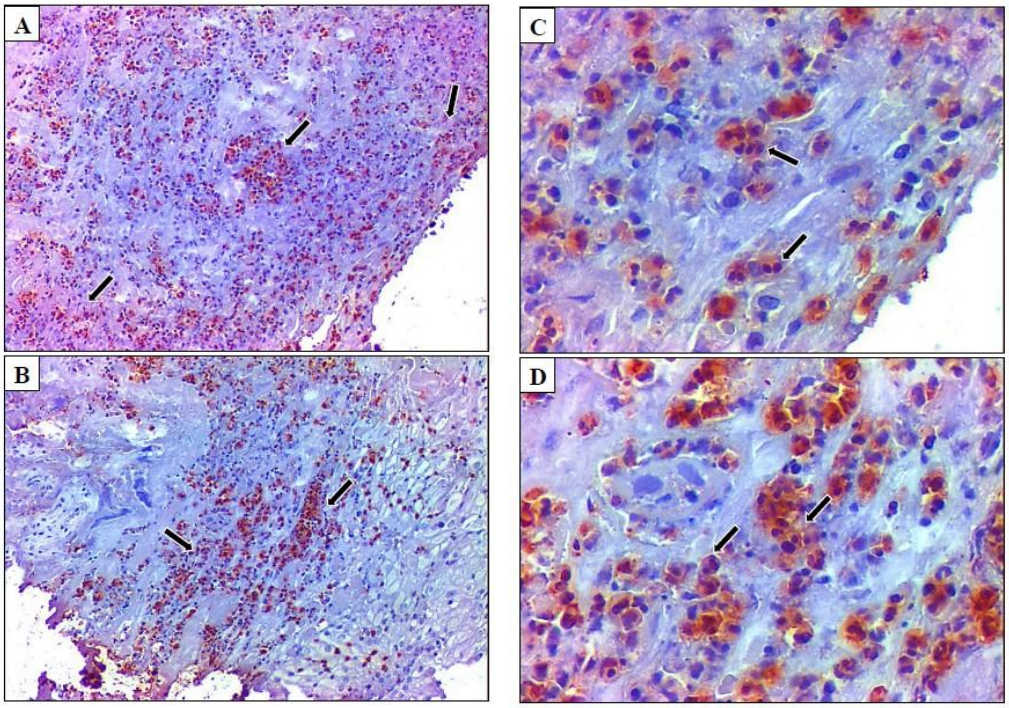


**Figure 1:** Photomicrograph of placenta infected with Toxoplasma gondii.

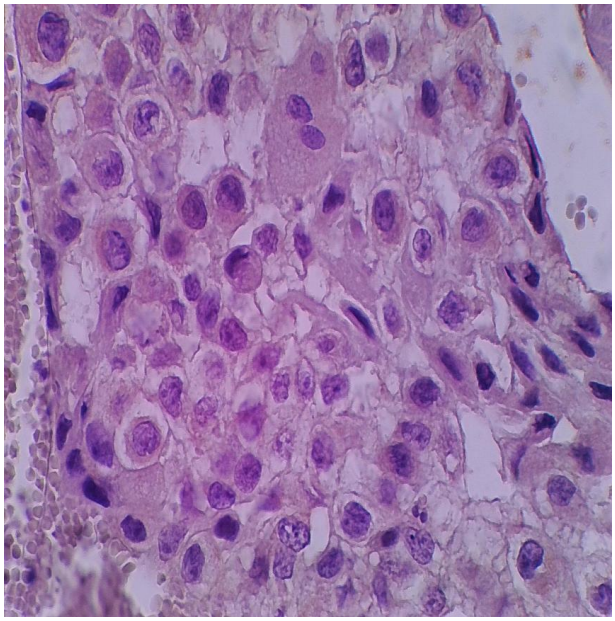

**Figure 3:** Photomicrograph of T. gondii infected placenta. Control negative of T. gondii primary antibody showed no expression. Hematoxylin & DAB. 40x.

## 4. Discussions

Intracellular parasite T. gondii infiltrates mammalian cells through an actin-dependent mechanism, resulting in the creation of a parasitophorous vacuole (PV) that is predominantly altered by the parasite. The parasite can impact several cell types seen in warm-blooded vertebrates[12]. The predominant techniques employed in contemporary research and investigations to validate the presence of toxoplasmosis encompass direct identification of parasites in bodily tissues or fluids, cultivation and isolation of protozoa in mice or tissue culture, and the detection of antibodies targeting IgM, IgG, and IgA in serum or other bodily fluids[13]. The selection of ELISA for the identification of IgM and IgG anti-Toxoplasma antibodies was motivated by the fact that the diagnosis of acute toxoplasmosis typically relies on the detection of IgM antibodies. In the context of acute infections, it is typically observed that the levels of IgM antibodies exhibit an increase within a time frame of around one to two weeks after the onset of illness[14]. Due to its simplicity, low price, and speed of results, the rapid test cassette was widely used for diagnosing Toxoplasmosis infection[15]. The technique of immunohistochemistry (IHC) involves the utilization of an antigen-antibody reaction to visually assess the distribution and quantity of a certain molecule within different tissue samples. The procedure is conducted while preserving the histologic structure of tissues, so enabling the observation of the molecule's expression pattern within the microenvironment[16].

This study found that among 80 samples examined, the overall seroprevalence of anti-T. gondii antibodies (IgG) in females who had abortions was 30% in the combo rapid test and 16.3% in the ELISA test. The difference was significant between the Rapid test and ELISA for IgG ($P<0.05$). None of the 80 samples tested positive for IgM seropositivity in either combo rapid test or ELISA. the result of this study is in agreement with previous studies conducted on pregnant women in Kirkuk city by [17] they reported a seropositivity rate of 36.67% using LAT and 16.92% using ELISA additionally[18], found that the overall T. gondii antibody infection rate was 9.05% for IgG among female students at Kirkuk University, using the ELISA assay as well as[19] they recorded 31.2% T.gondii antibodies in aborted women using VIDAS technique. The obtained seroprevalence data from this study are less than the previous studies conducted in various regions in Iraq: in Babylon 42.6% when used LAT while 4% and 22.6% when ELISA is used for the detection of anti-T. gondii IgM and IgG respectively[20], in Baghdad the anti-T. gondii antibodies seroprevalence was 59% in women who had abortion[21], in Erbil 27.03% of aborted individuals had an inactive infection (IgG)[22]. The obtained data in the present study were also less than the seroprevalence already found in different neighboring countries in and/or close to the Middle East, including Iran 75.7%, Yemen 45.4%[23-24] and Ethiopia 85.4%[25]. The variations in the seropositivity rates may be due to several factors, for example; sample size, local nutritional habits, climate, personal hygiene manners, socio-economic status, and geographic location [26]. Regarding the results of the IHC test, the rate of infection with T.gondii in 80 aborted women was 60% (48) cases while 40% were negative. Many local studies were conducted to deal with toxoplasmosis in women who had abortions using immunohistochemical detection. the rate of toxoplasmosis in the current study when the IHC test was used was higher than in the study conducted by [27] they confirmed T.Gondii Ags in the placentae of 25.2% (34/135) spontaneously aborted women when the IHC technique was used and the study carried out by[28] they confirmed (26) infections of a total 120 women with spontaneous abortion and 6 women with induced abortion by IHC method. IHC results in this study agree with that conducted by[29] they concluded that IHC is considered a confirmatory test to classify aborted women into T. gondii-infected women and non-T. gondii-infected women.

## Conclusion

Serological diagnoses such as Combo rapid test and ELISA are most commonly used for the diagnosis of anti-*Toxoplasma gondii* IgM and IgG antibodies in the patient's serum, but serological diagnosis has some limitations for example anti-*T. Gondii* IgG detection may represent the past infection. In this study, IHC was employed to validate toxoplasmosis in women who experienced abortion. The results revealed notable disparities between serological and immunohistochemical examinations, indicating substantial differences between the two testing methods.

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## Conflict of interests

None

## Ethical approved

This study was approved by the Kirkuk Health Department with No. 796 on 15/11/2023 to the Maternity and Child Hospital in Kirkuk.

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