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Isolation of *Toxoplasma gondii* from Placental Tissue and its Effect on Malondialdehyde Levels in Pregnant Women in Al-Anbar Province of Iraq

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Abstract

Background: Toxoplasmosis is recognized as among the prevalent ailments globally affecting mammalian creatures. Around a third of the human population is affected with the condition. Miscarriage, also known as spontaneous abortion or pregnancy loss, pertains to an unviable intrauterine pregnancy that occurs within 20 weeks of gestation. Early pregnancy loss, which occurs throughout the first trimester, is the most common miscarriage type.

Methods: Alternative techniques that do not involve enzymes have been employed to separate the *Toxoplasma gondii* parasite from placentas that have been aborted or not in women with toxoplasmosis in Al-Anbar Province. The purpose of such techniques is to preserve the parasite's viability while it rapidly multiplies inside placental tissue. Moreover, the current investigation measures the amounts of malondialdehyde (MDA) to determine how the parasite affects lipid peroxidation.

Results: Upon examining the pellet and suspension obtained from the placental solution, the microscopic analysis revealed the existence of bradyzoite in a majority of the identified specimens, along with the appearance of the swiftly reproducing tachyzoite phase in some. Lipid Peroxidation measurements showed that the infected samples' MAD levels had significantly increased relative to control samples.

Conclusion: Non-enzymatic methods of isolating the toxoplasma parasite are better at maintaining the vitality of the different phases of the parasite. Moreover, the parasite's effect is evident in raising lipid peroxidation levels and increasing risk factors.



Introduction

T. gondii can be defined as a protozoan that belongs to the apicomplexan group that is intracellular and obligate. It can be found worldwide [1]. In North Africa, at the Pasteur Institute in Tunis, *T. gondii* was discovered. Manceaux and Nicolle have discovered an arc-shaped protozoan in North African rodent tissues known as gundis (i.e., *Ctenodactylus gundi*) while performing a study on the leishmaniosis [2].

The toxoplasmosis-causing agent, *T. gondii*, can impact various warm-blooded creatures, like birds and mammals [3]. This illness is globally prevalent, with about 500 million cases worldwide [4]. Toxoplasmosis prevalence varies considerably across the globe and is highly affected by some factors, such as food production and harvesting practices, water sources, environmental conditions, and exposure to sand or soil [5]. Geographical factors, such as the climate (which includes temperature and rainfall), are highly important in the prevalence of this disease. For example, high degrees of temperature and humidity are believed to play a role in oocysts' survival in the soil, thus maintaining a high prevalence of toxoplasmosis. In addition to that, differences in dietary habits could contribute to prevalence variations as well [6]. In some parts of the globe, up to 95% of the population can be infected with *T. gondii*, with the infections happening most often in Africa, South America, and Europe [7].

T. gondii infection prevalence in pregnant women varies a lot amongst nations, with the estimates ranging from 9% to 67% in Europe and 92.50% in Ghana, based on epidemiological research that has been conducted worldwide. Also, some American nations have significant rates of *T. gondii* infection. In contrast, the incidence is quite low in East Asian nations, particularly Japan and Korea [8]. There were reports of *T. gondii* seroprevalences in 43% and 28.3%, respectively, in European nations, including Italy and France [9]. Barbosa and associates found a 66.3% prevalence rate among expectant mothers in Brazil [4]. The term "abortion" describes the process of ending a pregnancy when a fetus is lost before it can leave the uterus following nine months. A natural abortion is referred to as a miscarriage, while intentional abortions are known as induced abortions [10]. A newborn with infectious toxoplasmosis may experience neurocognitive deficiencies, a neuronal abnormality, and chorioretinitis. This particular form of toxoplasmosis is transmitted by the placenta and is linked to fetal death and abortion. The most typical kind of pregnancy loss is early pregnancy loss, which happens in the first trimester. The non-specific symptoms regarding uterine cramps as well as vaginal bleeding linked to miscarriages, could arise in ectopic, molar, and normal pregnancies, which can

be confusing for medical professionals and frustrating for patients [11].

Malondialdehyde (MDA) present in human cells and tissues is specified as a marker of oxidative stress caused by lipid peroxidation [8]. In addition, lipid peroxidation is a weak unsaturated fatty acid derivative enzyme that arises from depositing a complex set of components [9].

Preventing lipid peroxidation is crucial for all aerobic organisms since it produces various oxidation products, such as malondialdehyde (MDA), which can damage DNA [12].

This study aims to isolate the *Toxoplasma gondii* parasite by non-enzymatic methods from the placenta treated by the parasite in addition to studying the extent of the parasite's effect in increasing risk factors represented by measuring the level of lipid peroxidation in the studied samples.

Methods

Blood samples

Blood specimens were obtained from untreated individuals during the initial pregnancy trimester. Pertinent data regarding each patient, such as age, residential locality, primary occupation, offspring count, feline exposure, toxoplasma infection history, and any adverse effects on prior children, were documented. Two parts of the serum were isolated and split. For the ensuing malondialdehyde analysis, the first segment was kept at -20°C. The second portion was utilized immediately for antibody detection.

Placenta Samples

The Ramadi Teaching Hospital's obstetrics units provided the specimens. A total of 8 non-infected specimens were used as a control group. Also, eight specimens had miscarriages caused by *T. gondii*, and eight specimens had unaffected pregnancies caused by *T. gondii*. The specimens were then stored in sterile vessels holding regular saline (NaCl) and antibiotics like streptomycin and Penicillin to prevent contamination.

Detect IgG and IgM antibodies by using the ELISA technique.

ELISA assay was employed in the current research to detect and differentiate IgM and IgG immunoglobulins. The German enterprise (Human) employed various assays. The antigens coated onto the kit's wells and the antibodies in the patient's serum interacted to form the basis of the testing mechanism. This assay was designed to verify samples that were already *T. gondii*-infected.

Measuring Malondialdehyde (MDA)

Malondialdehyde (MDA) levels were assessed in human serum using the Malondialdehyde (MDA) Assay Kit sourced from the Cayman Chemical Company, USA. The process involved a Microtiter Plate Enzyme immunoassay for accurate quantitative analysis.

Isolation *Toxoplasma gondii* from placenta

The samples were then transported to the Anbar University's College of Science Laboratories. The specimens were ground up and mixed with a specific volume of normal saline solution using a ceramic mortar. The resulting mixture was then strained through sterile gauze to eliminate large fragments. Next, it was centrifuged at 3000 rpm for 10 minutes at a temperature of 37°C. The first adhered tube was retained for examination, while the residue was suspended in a normal saline solution, and the process was repeated thrice. To prevent contamination, 100 grams of streptomycin and 1000 units of Penicillin have been added to the output. To examine the material under a microscope and confirm the presence of parasitic stages, a small quantity was put on a glass slide and colored with Giemsa dye [3].



Figure 1: The hemolysis and centrifugation of samples from placentas with and without abortion.

Statistical Analysis

The data is shown as average \pm deviation. The data has been statistically analyzed using the Statistica 8.0 program (Stat Soft Ltd., Bedford, UK), which comprised descriptive statistics and linear regression. A paired t-test was employed to investigate the differences between the approaches and the samples. Significant differences were considered when the two-tailed p-value was less than 0.

Results

Percentage of *T. gondii* infection

Using an ELISA kit, serum samples from 85 women have been tested for certain IgM and IgG antibodies to confirm the presence of toxoplasmosis in those women. Table 1 displays the results, which indicate

that 35 out of 85 women (41.2%) have antibodies against *T. gondii*.

ELISA test				
Number of samples	Number of positive samples	Positive %	Number of negative samples	Negative %
85	35	41.2%	50	58.8%

Table 1: Percentage of *T. gondii* infection using the ELISA method.

The results of anti-*Toxoplasma* antibodies showed that 18 (21.18 %) women had IgM antibodies alone and 7 (8.24%) women had IgG antibodies alone, while 10 (11.77 %) women had both antibodies (IgM and IgG). Upon isolating the parasite from the placenta, it was observed through microscopic examination that most of the samples contained various parasite stages; this confirmed the occurrence of the parasite in placentas that were preserved without the use of enzymes to maintain the vitality of the parasite's reproductive stages.

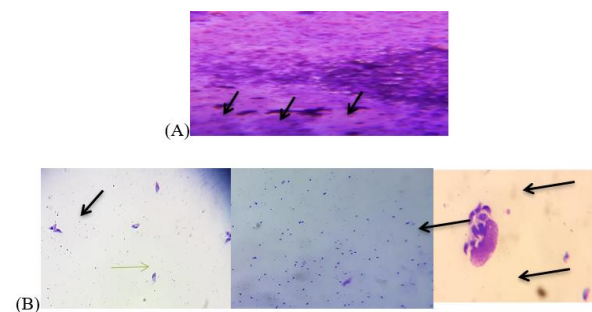


Figure 1: The histological analysis of tissue revealed the separation of placenta, displaying various parasitic stages after centrifugation and application of Giemsa stain. (A) bradyzoite. (B) trachyzoite.

As seen in the table and figure below, the research's findings demonstrated a significantly higher amount of lipid peroxidation in *Toxoplasma gondii*-infected samples in comparison with control samples.

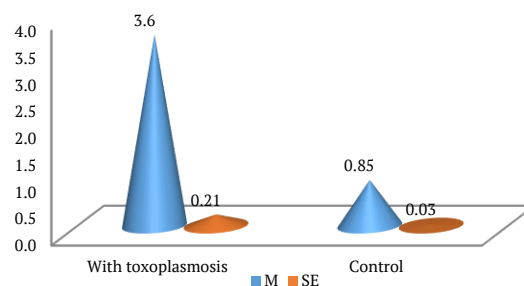


Figure 2: Mean \pm SE of malondialdehyde levels

Discussion

Toxoplasmosis is a widespread zoonotic ailment discovered globally. Contracting *T. gondii* while

pregnant could result in severe consequences for the fetus. In healthy individuals, toxoplasmosis produces mild indications, but in patients with weakened immune systems, it is considered fatal. The presence of IgM and IgG in the blood serum indicates the type and stage of *T. gondii* infection. It was demonstrated that IgM antibodies manifest earlier and decline quicker than IgG antibodies, often the first antibodies detected following primary infection [14].

The infection rate in this investigation was almost comparable to specific investigations conducted in [15], where he documented an infection rate of 49%. However, it was less than the investigations conducted in Baghdad by Qazaz [16], where they recorded the infection in 78.33% and 80.6% of samples, respectively.

In 1974, Remington and Desmontsin developed an isolation process that involved dispensing with tissue digestion [17]. They used acid pepsin or trypsin solution to isolate placental tissue. However, Jacobs and his team attempted to isolate *T. gondii* parasite from placental tissue using an acid basin in 1960. They were unsuccessful, and Jacobs noted that the acid pepsin solution killed the rapidly multiplying phase in the tissue, which may have contributed to their failure. In this study, the researchers did not use tissue digestion of placental tissue to isolate the parasite, which may be one reason why Jacobs and others were unsuccessful. The difficulty in identifying the parasite could have arisen from using an acid pepsin solution in tissue digestion, which reduced the quantity of tissue bags, including placental tissue, compared to the fast proliferating phase.

The numerical balance between antioxidants and oxidants [18] maintains a healthy pregnancy, and any disturbance of such balance could lead to endometrial malfunction. Free radicals have been linked to the development of diverse illnesses. Therefore, the excessive buildup of lipids in the placenta in multiple conditions, such as toxoplasma infestation, must be averted by generating antioxidants [19].

The current research shows that toxoplasma infestation resulted in a notable rise in lipid peroxidation injury, as evidenced by the serum concentration of malondialdehyde; this could be due to the generation of free radicals and oxidants caused by the parasitic infectious disease [20]. These free radicals have the potential to intermingle with DNA, leading to mutation or cytotoxicity. Additionally, they can attach to membrane lipids that are abundant in polyunsaturated fatty acid, causing cellular damage and membrane destruction [21].

Elevated levels of lipid peroxidation and reduced antioxidant defense mechanisms can result in the formation of byproducts that may react spontaneously with nucleophilic centers within the cell, leading to

covalent binding with DNA, RNA, and proteins [22]. The damage caused by such reactions may vary from cytotoxicity to allergy, mutagenicity, and carcinogenicity, depending on the properties of the specific byproduct involved [23]. Malondialdehyde, recognized to make up around 20% of end products produced by oxidative damage regarding lipids in vitro, is a frequently utilized marker for determining the amount of lipid peroxidation byproducts in blood [24].

Author Contributions

All the authors contributed significantly to completing this work, as Mr. Saif and Mr. Adeeb collected different samples from different governorate areas. Mrs. Ridhab and Mrs. Fatin completed the practical part and wrote and directed the research as required.

Author Contributions

All the authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Competing Interests

The authors declared that there were no conflicts of interest.

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