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Article

Placentitis and Determination of Alpha-fetoprotein in the Se-rum of a Rats Infected with Experimental Toxoplasmosis

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Annotation: *Toxoplasma gondii* is the cause of toxoplasmosis, which can be congenital or acquired. The parasite is an obligate intracellular protozoan It represents one of the most common parasites among population groups. It is thought because vertical transmission of a *T. gondii* infection from a woman having systemic infection to the fetus causes congenital toxoplasmosis (CT). According to age-adjusted statistics from a recent French study, approximately 31% of pregnant women have antibodies against *T. gondii*. The current study included the isolation of the *T. gondii* parasite from placenta samples of aborted women infected with Toxoplasma who visited Bint Al-Huda teaching hospital in Thi-Qar province of southern Iraq. After confirming the presence of the parasite stages in those samples, 0.3 ml of the parasite suspension was injected into the peritoneum of female white rats (*Rattus norvegicus*). The animals were divided into two groups, an uninfected group as a control group that was given Normal Slaine solution, and a second group infected with the toxoplasma parasite, rats were placed for mating, and after confirming pregnancy and determining the first day of pregnancy, they were killed by ether and explained in the last trimester of pregnancy for gross and histological examinations. In contrast with pregnant rats given via saline, toxoplasmosis led to Elevated alpha-fetoprotein in mother serum levels and placental inflammatory, high levels of alpha-fetoprotein (AFP) were linked to adverse pregnancy results, that resulted in malformed and sometimes congenital fetuses. High (AFP) is associated with the occurrence of weak and deformed births. It was noted in our current study that placental inflammation resulting from toxoplasmosis may have led to high levels of alpha-fetoprotein in the mother's serum. Our current study may be a model for other future studies to shed more light on the damage resulting from infection with the toxoplasmosis.

Keywords: toxoplasmosis, alpha-fetoprotein (AFP), placentitis

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1. Introduction

Among these infectious pathogens is *T. gondii*, which can result in early embryonic difficulties such abortion, stillbirth, mortality, and mummification [1]. As the parasite's ultimate hosts, cats emit oocysts in their feces, which then infect intermediate animals includes ruminants [2]. Toxoplasmosis-related reproductive issues result in decreased lambing intervals and the death of offspring [3]. The three primary routes of transmission for *T. gondii* are ingestion of oocysts, ingestion of infected tissues, and congenital infection. Less than 1% of cattle and humans get infected with *T. gondii* through the placenta. It is unknown what percentage of people get infections from eating meat, food, or water contaminated with oocyst-contaminated *T. gondii*, and there are presently no tests to differentiate between infections acquired through meat and those acquired through oocysts [4]. It is considered because vertical transmission of a *T. gondii* infection from a woman with systemic infection to the fetus causes congenital toxoplasmosis (CT).

According to age-adjusted statistics from a recent French study, approximately 31% of pregnant women have antibodies against *T. gondii* [5]. Alpha-fetoprotein (AFP) was discovered for the first time as an immigrant postalbumin migration in the embryo serum by Bergstr and Czar in 1956 using the electro-deportation technique, an album-like protein that results from the yolk sac bag early during pregnancy and is then produced from the fetus liver during an advanced period of pregnancy and is also produced from the placenta [6]. It is a blood sugar protein containing up to 35% carbohydrates with a molecular weight of 68-73 kVA [7]. APOs, or adverse pregnancy outcomes, contain pre-eclampsia, stillbirth, preterm birth, and fetal growth restriction. If the mother's level of serum is elevated during her pregnancy and her fetus does not have an open neural tube defect but is known to be at an increased risk, alpha-fetoprotein has been used as a marker. of these outcomes in their later gestation. It is sometimes referred to in the literature as "inexplicably high MS-AFP" because of the unidentified cause [8]. Still, it is unknown what system connects high MS-AFP with APO. Fetal growth restriction and stillbirth are linked to placental inflammation resulting from either causes, infectious or noninfectious [9]. According to a recent morphological investigation, the two leading diseases in the placentas of preterm birth were malperfusion and inflammation/infection [10]. So, the most common dangerous change in nearly all cases of APO is placental inflammation. Regarding the potential mechanism behind the relationship between MS-AFP and APO, some evidence from placental ultrasonography and histology also suggested that it originated in the placenta [11]. Consequently, we suggested that a possible cause of the association between high MS-AFP and APO could be placental inflammation.

2. Materials and Methods

2.1. Animals

The study was conducted on female white rats of the type (*Rattus norvegicus*) in the Animal House Department-College of Education for Pure Science/The Qar University. They were given access to water and a stable habitat with a 12-hour light/dark cycle, 23–25°C ambient temperature, and 50–60% humidity.

2.2. Expermental infection

Animal grouping twenty-eight rats were divided into two groups: the control group (treated with normal saline, n = 14) and the infected groups (the Toxoplasma parasite, n = 14). The parasite was isolated from placental specimens of who visited Bint Al-Huda teaching hospital in Thi-Qar province of southern Iraq. Those who have been serologically proven to be infected with the parasite. After confirming the presence of the parasite stages in those samples, 0.3 ml of the parasite suspension was injected into the peritoneum of female rats.

The parasite infection was diagnosed ten days after the injection using the ELISA technique. Two milliliters blood from the inferior vena cava was collected from the mother. To separate the serum for the enzyme-linked immunosorbent test, blood samples were centrifuged at 1000 g for 15 minutes after being allowed to clot for two hours at room temperature. (ELISA). The animals were placed for mating, Vaginal swabs were taken. The ladies were kept overnight with. the following morning. The male fertility is found in a 2:1 ratio. The sperm's existence Verify pregnancy with vaginal cleansing, and it was noted as "Conception Day" on that day.

2.3. Blood samples

The blood samples were allowed to clot at room temperature for half an hour and centrifuged at 1000 g for 15 minutes to isolate the serum to measure alpha-fetoprotein (AFP) with a device Electrochemiluminescence technology (Cobas 411 E).

2.4. Tissue preparation and histology

Tissue preparation was made according to Bancroft and Gamble (2008) [12], a small piece of the targeting organ (placenta) of the rates was kept in 10% formaline till tissue preparation for histological study.

2.5. Statistical analysis

GraphPad Prism 8 For all statistical tests, $P < 0.05$ was considered significant. Significant differences in figures were noted by asterisks: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***). Parametric data were presented as mean SEM and were analyzed using two-tailed unpaired t-test.

3. Results

3.1. Toxoplasma infection resulted in elevated MS – AFP

The results of the current study showed an increase in alpha-fetoprotein in the mother's serum in the group infected with the Toxoplasma conidia parasite (Figure 1) compared to the group treated with normal saline solution. This could be related to the occurrence of infections in the placenta and the decrease in the number of fetuses, as well as the occurrence of congenital malformations, and this is what we found in the fetuses when they were born.

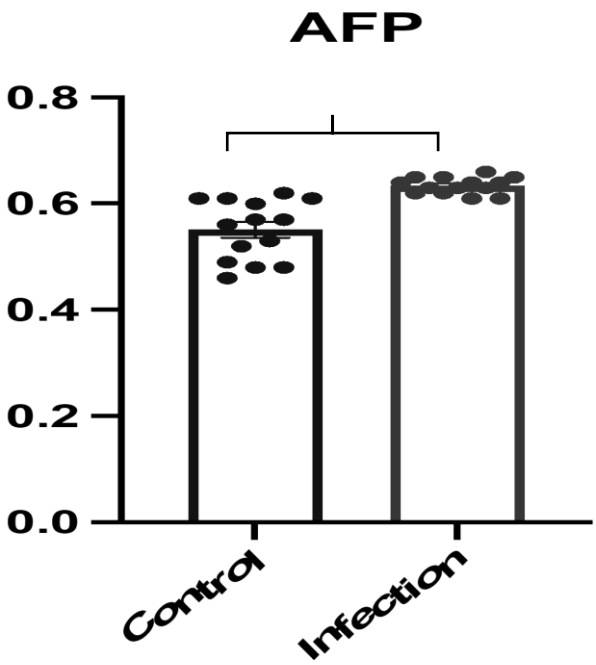

Figure 1. Maternal serum alpha-fetoprotein (MS-AFP) Significant differences at $P \leq 0.001$

3.2. Toxoplasma infection led to an inflammatory response Placentitis and low fetal weight

Infection with Toxoplasma gondii led to pathological histopathological changes in the placenta, such as tissue necrosis and inflammatory infiltration in the tissue, compared with the uninfected group treated with normal saline solution (Figure 2 (A)). Under a microscope, inflammatory cell infiltration and various forms of tissue necrosis could be seen in contrast to the typical placental histology (Figure 2 (B)).

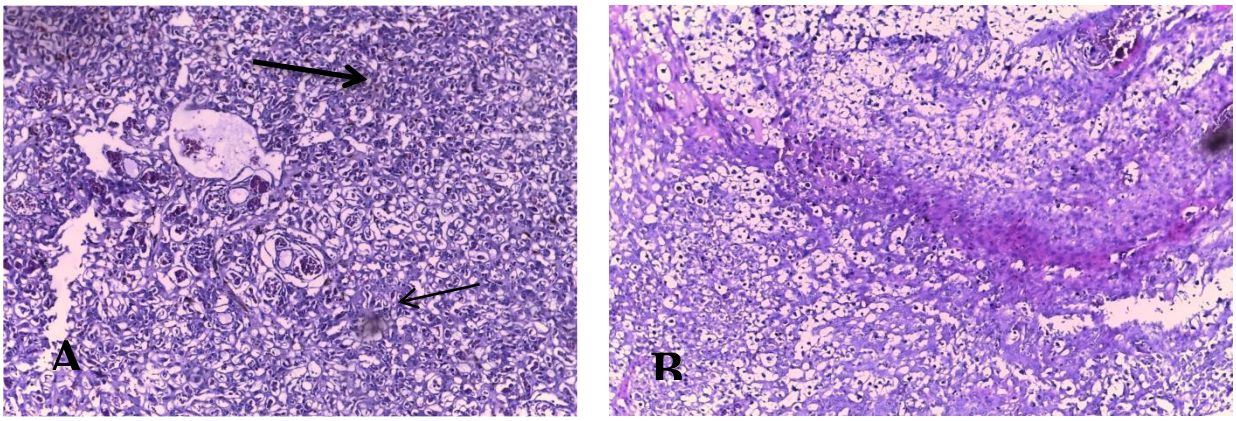

Figure 2. Photomicrograph of placenta, (A) Placental inflammation: necrosis of the tissue and infiltration of inflammatory cells, (B) Normal placental histology (hematoxylin-eosin stain, magnification×100)

The pathological changes varied in severity, from weak to moderate and more severe, which later affected the growth of the embryos, as the embryos were weak and had varying weights between the affected and healthy groups (Figure 3).

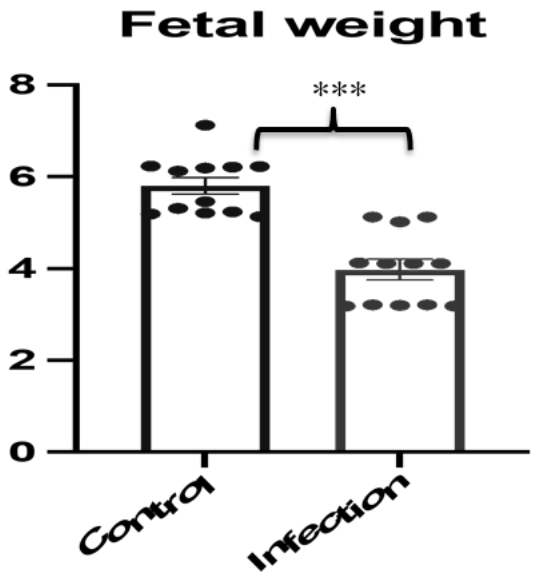

Figure 3. Fetal weight significant differences at $P \leq 0.001$

4. Discussion

The current study's finding was that the group with toxoplasmosis had higher levels of alpha-fetoprotein. Since placental inflammation may have helped to this increase, an experiment by Hu et al. (2019) [13] established a connection between placental infections and elevated alpha-fetoprotein levels. It was carried out with the use of sugary, fatty substances like lipopolysaccharide, which increases the level of protein in both the mother's and the fetus's serum and can have detrimental effects on pregnancy, including placentitis, preeclampsia, and premature births. An increase in this protein is considered an indicator of fetal malformations during pregnancy through the mother's serological tests in the prenatal period, as the test is considered an indicator of chromosomal imbalances and neural tube abnormalities for care during pregnancy [8]. We hypothesized that elevated MS-AFP may be the outcome of increased fetal-maternal AFP transfer led on by the placental barrier's reduced integrity as a result of inflammation brought on by toxoplasmosis. This is a result of the significant AFP concentration gradient between the fetal and mother circulations. during physiological conditions causes the trans-placental fetalmaternal AFP transfer to occur through the placental barrier. The placenta is recognized to have significant roles in the pathophysiology of certain adverse pregnancy outcomes. Pregnancy-related systemic infections, including toxoplasmosis, can result in placental infection and have a significant impact on the mother-child bond as well as the success of the pregnancy. One of the key factors influencing the likelihood of parasite transmission to the fetus is placental permeability to *Toxoplasma gondii* [14]. *T. gondii* types I, II, and III were compared in a study by Robbins et al., (2012) [15] and were found to be prevalent inside cells. They can cross the placenta and induce spontaneous abortion, premature birth, or significant sickness in the newborn that survives. The ability of type II parasites to reproduce within the placental tissue may differ, although research on the cellular and tissue components of the placental barrier is still in its early stages.

The researcher did not find a significant difference in the ability to invade cells. When a fetus becomes infected in utero as a result of vertical transmission of Toxoplasma from the mother, congenital toxoplasmosis (CT) usually occurs. In this instance, we found Toxoplasma organisms in the placenta of a baby who passed suddenly four days after birth from multiple organ failure together with extensive villitis, which might suggest delayed-onset severe neonatal CT Al-Hamod et al., (2010) [16] revealed severe chronic granulomatous villitis with multinucleated giant cells and localized remains of Toxoplasma organisms by histological investigation of the placenta [17]. Found that Stensvold et al. (2022) [17] the placenta of a kid who passed away four days after birth had focal remnants of Toxoplasma and a severe case of granulomatous chronic villitis, as verified by immunohistochemical and DNA-based techniques. At delivery and ten months later, the immunocompetent mother's toxoplasma test results were negative. Without a systemic infection in the mother, placental infection can occur. The results showed lower birth weights of the fetuses in the group affected by placentitis, which led to negative effects on the fetuses [13]. Hurt (2022) showed that there is a direct relationship between infection with toxoplasmosis and low birth weight of fetuses [18].

5. Conclusion

High (AFP) is associated with the occurrence of weak and deformed births. It was noted in our current study that placental inflammation resulting from toxoplasmosis may have led to high levels of alpha-fetoprotein in the mother's serum. Our current study may be a model for other future studies to shed more light on the damage resulting from infection with the toxoplasmosis.

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