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Research Article

Effects of experimentally induced toxoplasmosis on blood glucose in albino mice Balb/c

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Abstract
Toxoplasmosis is one of the most important and prevalent disease affecting a wide range of hosts. It is caused by the parasite called *T.gondii*. The present study aimed to evaluate the blood glucose in serum in albino mice Balb/c induced toxoplasmosis serum. Ten placenta samples were collected from aborted women from Al-Batoul and Al-Salam Hospital in Mosul city from October 2023 to December 2024 . Eighty mice were infected experimentally with toxoplasmosis (20 male and 20 female) by injecting tissue cysts isolated from the placenta into the peritoneal cavity of laboratory mice and five mice were dissected after each after 1, 2, 3, and 6 weeks post infection (p.i)).A serum was collected to determine the glucose and insulin levels, and the titer of anti-portant toxoplasma antibodies was measured using an ELISA kit. The number of tissue cysts in the impressions brain was numerate. Results showed an increase in glucose level concentration in all groups. The highest rate of glucose was recorded in infected males after the sixth week p.i (167.40±9.46)mg/dl. The highest increase in insulin levels was recorded in all infected groups (male and female) at all periods compared to control. Furthermore, There were significant differences in the rate of IgG antibody titer, as the highest rate of antibody concentration was recorded at the first-week p.i in female (0.54±0.06) titer .Also, the results showed significant differences in the number of cysts in all groups at the sixth week post–infected.

Keywords: Diabetes mellitus, ELISA , Insulin level ,*Toxoplasma gondii*, Toxoplasmosis

INTRODUCTION

Toxoplasmosis is one of the most important widespread zoonosis diseases that affects about a third of the world's population (Graham *et al.,* 2021) .These diseases are considered one of the most important food-borne pathogens (Kuruca *et al.,* 2023). It is caused by the obligatory intracellular parasite, an opportunistic called *Toxoplasma gondii* (Robert Gangneux *et al.,* 2022). This is one of the most successful pathogens (Maus *et al.,* 2024).. The parasite is transmitted horizontally and vertically as infection occurs through raw meat, affecting the tissue cysts. Infection may occur through eating sporulation oocysts shed by cats (Almeria and Dubey, 2021). It is then transmitted vertically from the infected mother to the fetus through the placenta (Imam *et al.,* 2021). The parasite invades any cells that have nuclei reticuloendothelial tissue cells and many other organs such as the liver, spleen, pancreas, and the brain (Beshay *et al.,* 2018; Pazoki *et al.,* 2020).

Many studies have indicated a correlation between *T. gondii* and various diseases, including primary neurological disorders (Asgari *et al.,* 2021) It induces immune disturbances in patients with liver fibrosis (Robert -Gangneux *et al.,* 2012).Acute and chronic may be linked to pathological changes in pancreatic tissues, including enlargement, reduced number of islets of Langerhans, and programmed cell death (apoptosis) in the acute phase and a significant decrease in the size and number of islets of Langerhans in the chronic phase, potentially impacting insulin-secreting beta cells which leads to the development of diabetes(El-Kady *et al.,* 2022).

The relationship between *T. gondii* infection and diabetes is still controversial.There are many studies with conflicting results have shown that *T. gondii* infection may significantly affect diabetes (Soltani *et al.,* 2021).This may be due to decreased immunity, which leads to increased susceptibility to infection with the parasite (Janssen *et al.,* 2021). Because the parasite can cause infection in most organs, such as the brain, liver and pancreas, diabetes may be increased according to this damage to describe this relationship (Catchpole *et al.,* 2023). The present study aimed to follow up on the effect of experimentally toxoplasmosis infection on induced diabetes in mice.

MATERIALS AND METHODS

Experiment design

The experiment was done by taking 80 albino mice Balb/c, (20 for each male and female) and 20 mice in each control group for male and female, which were experimentally infected with *T. gondii*. The mice were dissected after 1, 2, 3, and 6 weeks (3 mice were dissected for each male and female) of experimental infection.,

The glucose and insulin concentrations were measured in blood serum.The severity of *T. gondii* infection was measured using ELISA kit.Tissue cysts were counted in brain impressions.

Sample collection and source

The placenta samples were collected from aborted women from Al-Batoul and Al-Salam Hospital in Mosul city from October 2023 to December 2024.The samples were kept in clean sterile containers containing phosphate buffer saline (PBS), until the samples arrived at the laboratory to isolate parasites.

Parasite isolation and microscopic examination

The parasites were isolated from placental samples as per (Dubey (1998).The microscopic examination was conducted to confirm the presence of the parasites in the samples.

Preparation of the injection dose

The injection dose was prepared by counting the tissue cysts of the parasite in suspension respectively, by taking a 10-microliter drop of the suspension, placing it on a glass slide and staining it with Giemsa dye (Liesenfeld, 2002).

Experimental animals

In this experimental albino mice *Balbs/c*, aged 25-30 days old and weighing 15 to 30 grams. The mice were used an intraperitoneal injection (IP) with a 0.1 ml dose containing approximately 100 tissue cysts (Al- Hayali, 2002).

Collecting blood samples

Blood samples were collected from infected mice by drawing blood from the posterior cavity of the eye using a capillary tube (Parasuraman *et al.,* 2015) and placed in yellow gel tubes containing a gelatin layer to prevent the mixing of serum with blood cells. The samples were left for 20-25 minutes and then centrifuged for 3000 rpm \15 minutes and, collected serum in Eppendorf tubes and kept at -20 °C until used for laboratory tests15(Soares *et al.,* 2022; Ulvi *et al.,* 2002).

Serological tests

Determination of glucose level

The glucose level in the blood serum was measured by the enzymatic process (glucose - oxidase - peroxidase) using a measuring kit (Biolab Reagents - France - REF: 87409). The method is based on the Trindnder reaction (Trinder, 1969).

Determination of insulin level

The insulin levels in the blood serum of mice were measured using an ELISA Kit (Sunlong Biotec) Biotechnology Co., Ltd.

Measuring the concentration of *T. gondii* antibodies using ELISA KIT

The severity of *T. gondii* infection was measured using the Mouse Toxo IgG ELISA Kit prepared by USCN China. The color change was considered from blue to yellow, and the color intensity was measured at 450 nm using a Spectrophotometer WTW-Germany.....)

Counting parasite cysts in brain impressions

The tissue cysts of the parasite were counted in the brain impressions. The brain was crushed and homogenised with phosphate buffer saline solution added to it, and the mixture was centrifuged. A 0.1 drop of the sediment was taken and stained with Giemsa stain, and then a diagnostic was done at 100X (Gatkowska *et al.,* 2012).

Statistical analysis

The statistical program SPSS was used to analyse the results by Tow- way Analysis of Variance at a significant level of $P < 0.01$.

Ethical approval

This study was conducted in accordance with the ethical standards and guidelines set forth by the University of Mosul, College of Education for Girls, Department of Biology Ethical Review Committee. Ethical approval was obtained prior to the commencement of the research(Um.edu.girl.2023/9/7) ,ensuring all procedures involving animals were carried out in compliance with institutional and international ethical standards regarding animal care and use.

RESULTS AND DISCUSSION

Glucose level in blood serum

The results of measuring the glucose concentration in the serum of mice infected with *T. gondii* (males, females) after 1,2,3 and 6 weeks p.i. showed significant differences in the glucose level according to gender, infection, and time, It was found that the highest concentration of glucose level in the serum of infected males was in the sixth week p.i. 167.40±9.46 mg/dl and the highest significant increase (167.40±9.46 mg/dl) recorded as compared to the period, as shown in Table 1.

It may be due to the parasites stimulation of autoimmunity against pancreatic beta cells, thus causing type 1 diabetes and high concentrations of glucose by damaging beta cells and reducing insulin secretion (Prandota., 2013). Furthermore, parasite may leading to activation of autoimmunity pathways, as well as induce the inflammation islets of Langerhans (insulitis) and ultimately developing diabetes (Oz., 2014). Volpatti *et al.* (2019) examined the immunological responses in murine models infected with *T. gondii*. They noticed that glucose may have a synergistic activity that enhances the reproduction of *T. gondii*, as it affects cell division and reproduction with long-term effects. Another study also indicated the corroborated *T. gondii* modulation of a few diabetes-related indicators, such as glucose, insulin, and HOMA-IR ,also glucose levels increased significantly in patients who had positive parasite antibodies(Al-Halbousi and Al- Warid.,2024).

Insulin level in blood serum

The results showed that the insulin level Pmol/L in Table 2 indicates significant differences between gender, infection, and period. The highest increase in insulin levels was recorded in the group of males and females infected in all weeks compared to the control. This may be due to insulin resistance,one of the most prominent metabolic disorders, as the action of insulin in muscle tissues is impaired (Beale, 2013) and caused by a defect in primary insulin signalling or by the presence of mutations and defects in the insulin-like growth factor gene (INSR1), as a result of increase programmed cell death, (Melvin *et al.,* 2018). It is characterized by high blood serum insulin concentration and increased glucose response to endogenous and exogenous insulin.
Severe insulin resistance may present with abnormal glucose homeostasis, requiring large amounts of exogenous insulin to maintain blood sugar (Semple *et al.,* 2011). Furthermore, T2DM development has been increasingly more correlated with dysfunction and/or necrosis of cells with the aid of reactive oxygen species, nitric oxide, and some pro-inflammatory cytokines, main to feasible insulin resistance in animals (Li *et al.,* 2018). A study indicates that insulin has the function of enhancing glucose absorption and stimulating the proliferation of parasite cells (Volpatti *et al*.,2019). The results of this study are consistent with the results of Moudgil *et al*. (2019) who showed that high levels of HomA-IR are associated with the incidence of toxoplasmosis.

Table 1. Average glucose level in mg/dL in blood serum in mice experimentally infected with *T. gondii* (males, females) after 1,2,3 and 6 weeks post infection

Times	Groups			
	Control Male 20	**Infected Male 20**	**Control Female 20**	**Infected Female 20**
1 week	109 .31± 4.67 C	125.60±16.36 C	111.56 ± 4.53 c	134.03±11.88 Bc
2 week	114.50 ±10.30 C	158.43±44.14 Ab	105.90±4.73 c	138.63±21.20 Abc
3 week	112.56 ± 9.75 C	131.73±109.12 Ab	106.20 ± 5.86 c	130.86±19.84 Bc
6 week	115.60± 9.65 Bc	167.40±9.46 A	114.76±27.24 c	131.66±16.46 Bc

Similar letters indicate the absence of a significant difference, and different letters indicate the presence of a significant difference; Values represent the mean ± standard deviation for each of 3 mice in the group at a significant level of P<0.01.

Table 2. Average insulin concentration Pmol/ L in mice experimentally infected with *T. gondii* (males, females) after 1,2,3 and 6 weeks post infection

Times	Groups			
	Control Male 20	**Infected Male 20**	**Control Female 20**	**Infected Female 20**
1 week	4.02±0.73 B	11.80 ±1.48 A	4.18 ± 0.50 b	10.87±1.11 a
2 week	3.86±0.34 B	11.08 ± 1.67 A	4.25 ± 0.26 b	10.97±2.16 a
3 week	3.93±0.52 B	10.96±1.51 A	3.81±0.56 b	11.10±1.80 a
6 week	3.67± 0.31 B	9.94±2.12 A	3.81±0.36 b	12.12±1.14 a

Similar letters indicate that there is no significant difference in gender, infection, or time period between the group of infected males and females, and different letters indicate the presence of a significant difference.
Values represent the mean ± standard deviation for each of 3 mice in the group at a significant level of P<0.01.

Table 3. The average concentration of IgG titer antibodies in the blood serum of mice experimentally infected with *T. gondii* (males, females) after 1,2,3 and 6 weeks post *infection*

Times	Groups			
	Control Male 20	**Infected Male 20**	**Control Female 20**	**Infected Female 20**
1 week	0.41 ± 0.01 cdef	0.52±0.04 Ab	0.40±0.04 cdef	0.54±0.06 a
2 week	0.34±0.03 fgh	0.48±0.03 Abc	0.36±0.04 efgh	0.48±0.07 abc
3 week	0.29±0.04 h	0.38±0.07 Defg	0.34±0.04 fgh	0.48±0.01 abc
6 week	0.29±0.06 gh	0.44±0.06 Bcde	0.31±0.04 gh	0.45±0.02 abcd

Similar letters indicate that there is no significant difference in the concentration of antibodies between control and infected males and females, and different letters indicate the presence of a significant difference.
Values represent the mean ± standard deviation for each of 3 mice in the group at a significant level of P<0.01.

Concentration of IgG antibodies to *Toxoplasma gondii* in blood serum : The results of concentration rate of IgG antibodies in the blood serum in mice experimentally infected with *T. gondii* (males, females) after 1,2,3 and 6 weeks p.i. showed significant differences in the concentration rate of IgG antibodies in mice experimentally infected with *T. gondii*. The highest average of antibodies (0.54±0.06) was recorded in the first week for infected females compared with the control group, as shown in Table 3.

This may be because the presence directs the immune machinery towards the production of autoantibodies, including IgG, against beta cells in the islets of Langerhans, Furthermore, the infection of white blood cells also makes it probable for the parasite to spread throughout the body's organs, such as the pancreas (Prandota, 2013). In another study conducted by AL-Khafajii *et al.* (2021) to evaluate the incidence of *T. gondii* infection and its relationship to diabetes, the results showed that normal diabetic patients and overweight diabetic patients had the highest positive rate of IgG against *T. gondii*. This result is consistent with Al-shakir'*et al*. (2020), who detected *T. gondii* infection using the ELISA technique in addition to measuring the fasting blood sugar level. The sugar level in most diabetic patients was between 150-200. Also, out of 98 patients, 32 patients were positive for Igg, meaning that diabetes may increase the susceptibility to *T. gondii* infection. This result is also consistent with a study conducted by Shakiba *et al*. (2020) on the relationship between toxoplasmosis and diabetes. The study results showed that seropositivity for Igg antibodies in the case group was 33% in the patient group and 15.6% in the control group, indicating an important statistical relationship between diabetes and toxoplasmosis.

Table 4. Showing the average number of tissue cysts in mice experimentally infected with *Toxoplasma gondii* (males, females) after 1,2,3 and 6 weeks post infection

Times	Groups	
	Infected Male 20	**Infected Female 20**
1 week	7.33±3.05 D	14.00±2.00 cd
2 week	38.00±15.87 Cd	51.00±16.52 c
3 week	105.66±31.87 B	118.66±32.02 b
6 week	226.66±16.07 A	257.33±26.76 a

Similar letters indicate that there is no significant difference between infected males and females, and different letters indicate that there is a significant difference.
Values represent the mean ± standard deviation for each of 3 mice in the group at a significant level of P<0.01.

Tissue cysts in brain impression

By counting tissue cysts in brain impressions, it was observed that there were significant differences in the average of cysts. The highest increase in the number of cysts was recorded in the sixth – week post-infection (p.i) of males and females, as 226.66±16.07 and 257.33±26.76 cysts per brain, respectively as shown in Table 4. This is due to diabetes eventually inducing production of reactive oxygen and nitric oxide, which are the most stimulant-initiating opportunist intracellular pathogens associated with benign or severe encephalitis course due to inflammatory reaction of migrated tachyzontes (Wulf *et al.,* 2005). This result is consistent with El Saftawy *et al*. (2023), who reported that the toxoplasmosis, when challenged with diabetes, showed massive pathological features and higher parasite load in the cerebral tissues. This may be due to the successful transformation of the parasite into the bradyzoite stage. Many studies have also confirmed the importance of rapid diagnosis of parasite infection in diabetic patients (Abdelhamid *et al*.,2023).

Conclusion

The present study concluded that there was relationship between diabetes and toxoplasmosis. The higher glucose concentration in the blood serum was accompanied by an increase in the severity of toxoplasmosis and an increase in brain mice's tissue cysts. *Toxoplasma gondii* seropositive can be correlated with changes in some diabetic-related biochemical parameters may be involved, such as insulin levels and higher concentration of glucose. Further studies need to measure the effect of toxoplasmosis on blood sugar in humans, especially pregnant women.

Conflict of interest

The authors declare that they have no conflict of interest.

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