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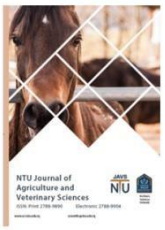

Influence of Induced Diabetes on the Severity of Experimental Toxoplasmosis in Mice

1st Samah Fathy Ramadan[1], 2nd Reeda Nathem Hamoo[2], 3rd Baydaa Ghanim Mohammed[3]
1,2,3 Department of Biology, College of Education for Girls, University of Mosul, Mosul, Iraq.

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Corresponding author:
Samah fathy Ramadan
University of Mosul, College of Education for Girls Email:
samahfathy717@gmail.com

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A B S T R A C T

This study aimed to evaluate the effect of induced diabetes on the severity of experimental toxoplasmosis in mice. 10 placenta samples were collected from Al-Salam Hospital in the city of Mosul. The parasite isolated and identified. Eighty mice were experimental diabetes were induced with Alloxan, and divided into three groups; two groups 40 mice of both sexes (20 males and 20 females) infected in Toxoplasma gondii (*T. gondii*), and 20 mice consider as a control group. Mice were sacrificed at one, two, three and six weeks post infection (p.i.), blood serum was collected and evaluated for the severity of T. gondii infection using an ELISA kit, and glucose and insulin levels were measured. Tissue cysts in brain casts were also assessed. The results showed a significant difference at $P < 0.01$. in the glucose level in all groups, the highest glucose level was recorded at six week i.p in females which recorded 274.13±74.13. It was also observed that there is an increase in the rate of insulin level, the highest rate was showed in the second and sixth weeks p.i in male group which recorded16.15±4.14. furthermore, it was found that the highest concentration level forIGg was recorded at third week p.i females which recorded 0.52±0.06 , Furthermore, a statistical relationship was found between males and females according to number of cysts.

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Introduction

Toxoplasmosis is a prevalent disease affecting a wide range of hosts including approximately one-third of the human population. It is caused by the sporozoan parasite T.gondii. It causes a fatal encephalitis in immunosuppressed people (Hajj et al., 2021).The infection spreads both horizontally and vertically. It is spread through raw meat. The tissue cyst infection may occur through eating oocysts released by cats.(Almeria and Dubey, 2021 and Smith et al., 2021). There was indicated a correlation between *T. gondii* and various diseases, such as neurological, behavioral and psychological disorders also different types of cancer such as pancreatic cancer,several studies have been conducted to investigate the impact and correlation with liver diseases, including diabetes (Pazoki et al., 2022).

Diabetes is a common metabolic disorder in all world relatively, manifested by an increase in blood glucose levels. It is an important factor due to increases the susceptibility and risk of infection with *T. gondii* (Robert-Gangneux and Dardé 2012). Toxoplasmosis causes pathological changes in pancreatic tissues including enlargement, programmed cell death (apoptosis) in acute phase as well as a decrease in the size and number of islets of In the chronic phase, there is a decrease in the size and number of islets of Langerhans. This decrease potentially impacts insulin-secreting beta cells. This can lead to the development of diabetes.`(El-Kady et al., 2022).

Some studies suggest that a parasite called T. gondii may be a factor that increases the risk of developing type 1 or type 2 diabetes. However, other studies have found conflicting results, so the relationship between T. gondii and diabetes is still controversial.(Soltani et al., 2021), especially in uncontrolled cases (Ahmadikia et al., 2021). It is associated with immunosuppression, which increases the risk of *T. gondii* seroprevalence (Lewis et al., 2015). There is some evidence that this parasite may have an undiscovered role in causing autoimmune diseases, including diabetes. The presence of the parasite in the pancreas may leads to directly undermine the pancreatic cells (Shapira et al., 2012).

Due to unfit host immune condition, the latent bradyzoites have the fitness to transmutation with powerful opportunistic variability to revert acute blood phase through tachyzoites re-conversion, and order to immunological and physiological stressors or progression to intrinsic stimulus orchronic disease (Boothroyd, 2009). But, the actual stimulating dynamic of opportunistic toxoplasmosis is still vague (Tenter et al., 2000). Consequently, latent opportunistic human toxoplasmosis possibly consequence to diabetes (Buxton, 1990).

Additional research isrequired to determine whether changes in immune function due to type-1 diabetes increase the risk ofinfection with Toxoplasma gondii, infection with Toxoplasma gondii increases the risk of type-1 diabetes (Catchpole et al., 2023). A litle studies have been conducted on the prevalence of toxoplasmosis with diabetics. For this purpose, the aims of this work is to study the severity of toxoplasmosis among diabetics, by determin anti *T. gondii* immunoglobulin (Ig) G titers in the blood serum of mice with diabetic.

Materials and Methods

Experiment design

Eighty mice Balb /c (Mus musculus) aged 25-30 day weight ranged between 15 -30 gms. were used for induced diabetes experimentally with Alloxan which were divided into three groups; 40 mice of both sex (20 males and 20 females) infected in *T. gondii*, and 40 mice where considered as control group. The mice were sacrificed after one , two, three and six week ip, serum samples were separated to measuring glucose and insulin levels. In addition, tissue cysts in brain impressions were used to evaluate the severity of *T. gondii* infection using an ELISA kit (USCN China)

Preparing the injection dose with Alloxan

The dose of Alloxan was prepared according to body weight at a dose of 200 mg/kg. 200 milligrams of alloxan were weighed and dissolved in 12.5 ml of normal saline. The mice were injected subcutaneous, and blood glucose levels were determined (Yamagami et al., 1985 and Rahman et al., 2017).

Diabetes induced by alloxan

Mice fasted for 24 hours except for water (Brosius, 2019) injected s/c with a dose of 0.25 ml containing 4 mg of alloxan. 5% glucose solution was given to the mice immediately after the injection to avoid severe hypoglycemia (Shahid et al., 2019). A drop of blood was taken from the tail, glucose levels were measured by using a glucometer viva accu chek (Kennard et al., 2021).

Placenta samples with T.gondii collection and source

Samples of the placenta were collected from women who underwent abortions at Al-Salam Hospital in Mosul City. The samples were stored in a sterile environment.and sterile containers with phosphate buffer saline (PBS) to be used for parasite isolation.

Parasite isolation and microscopic examination

In order to isolate parasite, placenta was cut into small pieces and suspended with 5 ml of pepsin enzyme at a temperature of 37 °C. The suspension was incubated at 37 °C for10 minutes then filtered with gauze. The mixture was centrifuged at 3000 rpm for10 minutes, then the precipitate was suspended in a PBS solution (Dubey, 1998).

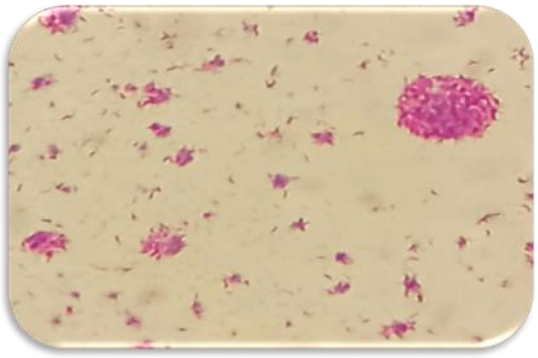

Fig 1. Show the tissue cyst of parasite 100X.

A 0.1-ml dose containing approximately 100 tissue cysts was administered intraperitoneally to the mice.(Al Hayali, 2022).

Collection of Blood Samples

Blood samples were obtained from infected mice by puncturing the posterior eye chamber with a fine needle and subsequently drawing blood into a capillary tube.(Parasuraman et al., 2015) Serum samples were collected in Eppendroff tubes and keep it at -20 C° until 15 minute (Ulvi et al., 2002 and Soares et al., 2022).

Serological tests

A special kit (from Biolab Reagents) was used to measure the glucose level in the blood. The kit had an enzyme called glucose oxidase-peroxidase. The test was based on a method invented by Trinder.(Trinder, 1969).

Insulin level serum:

Insulin level in the blood serum of mice was determined using an analysis kit prepared by the ELISA Kit (Sunlong Biotec) Biotechnology Co according to the manufacturer's instructions (Sunlong Biotec).

Measurement the concentration of *T. gondii* antibodies:

We used a kit made by USCN China to measure how bad the T. gondii infection was. The test changes color from blue to yellow, which is considered positive. The intensity of the color change was measured using a spectrophotometer at 450 nm.(Lunn et al., 2012).

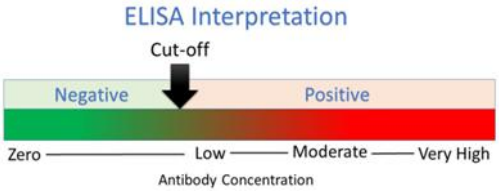

Fig 2. Show Antibody Concentration.

Quantifying tissue cysts in brain impressions:

Tissue cysts of the parasite were counted from brain impressions by using 100x lens as shown figure (3) (Gatkowska et al., 2012).

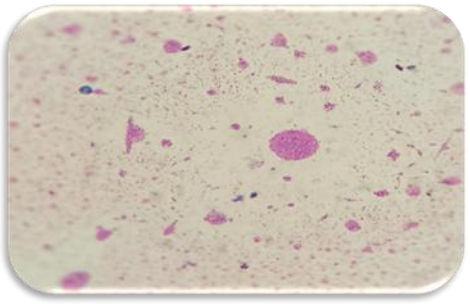

Fig 3. Show the tissue cyst of parasite 100X.

Statistical analysis

The statistical program SPSS was used to analyze the results of the study Two- way Analysis of Variance at a significant level of $P < 0.01$.

Results and discussion

Results of Glucose level in blood serum

The average level of glucose in the blood serum of mice experimentally infected with parasite p.i after 1, 2, 3 and 6 weeks were significant differences in glucose level. highest glucose level was recorded at sixth week post infected females which recorded 274.13±74.13while no significant differences were recorded between male and Female the time periods, as shown in Table 1.

Table 1. levels of glucose in mg/dL in blood serum in mice experimentally infected with *T. gondii*

Times	Groups			
	Control Male Number.20	Infected Male Number.20	Control Female Number.20	Infected Female Number.20
1 week	109.70±4.67 c	273±123.72 a	111.56±4.53 c	217.98±85.35 Abc
2 week	114.50±10.30 c	184.53±62.56 abc	132.94±12.44 bc	260.30±122.90 A
3 week	112.56±9.75 c	247.73±109.12 ab	106.20±5.69 c	123.31±14.42 C
6 week	1115.60±9.65 c	136.40±5.65 bc	141.76±27.24 bc	274.13±74.13 A

*Different letters indicate the presence of a significant difference at P<0.01.

*Values represent the mean ± standard deviation for each of 3 mice in the group.

Results of measuring the average insulin level in blood serum:

The results showed that insulin concentration in the blood serum in experimental mice infected with diabetes p.i, were a significant difference, the highest concentration level were recorded in the second week in infected females which recorded14.95±4.51 compared to the control group, as shown in Table 2.

Figures. All figures need to be in a .jpg or .png file format and inserted separately, and it must be referred to in the text. Figure legend should descriptive and written below the figure.

Table 2. concentration of insulin (pmol/L) in the blood serum of mice experimentally infected with *T. gondii* .

Times	Groups			
	Control Male Number.20	Infected Male Number.20	Control Female Number.20	Infected Female Number.20
1 week	0.73±4.02 D	12.75±0.80 bc	4.18±0.50 d	10.43±1.11 C
2 week	3.86±0.34 D	19.09±5.78 a	4.25±0.26 d	14.95±4.51 abc
3week	0.52±3.93 D	11.68±2.34 c	3.81±0.56 d	11.08±3.30 c
6 week	3.67±0.31 D	16.15±4.14 ab	3.81±0.36 d	13.83±1.59 bc

*Different letters indicate the presence of a significant difference at P<0.01.
*Values represent the mean ± standard deviation for 3 mice in the group.

Concentration of IgG antibodies to T. gondii in blood serum:

The results showed that there were big differences in the levels of certain antibodies. The highest levels of these antibodies were found in infected females during the third week. The second and sixth weeks had similar levels. There were no significant differences in the levels of these antibodies in infected males during any of the weeks (Table 3)..

Table 3. Concentration of IgG antibodies in the blood serum of mice experimentally infected with T. gondii .

Times	Groups			
	Control Male Number.20	infected Male Number.20	Control Female Number.20	Infected Female Number.20
1 week	0.29±0.04 cd	0.47±0.03 ab	0.02±0.22 d	0.38±0.12 bc
2 week	0.23±0.03 D	0.48±0.04 ab	0.29±0.02 cd	0.43±0.05 ab
3 week	0.25±0.03 D	0.49±0.03 ab	0.26±0.05 d	0.52±0.06 a
6 week	0.26±0.06 D	0.42±0.08 ab	0.26±0.09 d	0.44±0.04 ab

*Different letters indicate the presence of a significant difference at P<0.01.
*Values represent the mean ± standard deviation for each of 3 mice in the group

Results of counting tissue cysts in brain impressions:

By following up on the results of counting tissue cysts in the brain impressions, there were a significant differences between groups, the highest number of cysts were seen at six week p.i in females groups 329.00±18.24 . While the lowest rate of number of cysts was recorded in the first week for infected males and females, as shown in Table 4.

Table 4. Mean number of tissue cysts in brain mice experimentally infected with T. gondii .

Times	Infected Male Count.20	Infected Female Count.20
1week	10.66±4.50 a	23.33±7.63 a
2 week	51.33±10.26 b	64.33±4.04 b
3 week	149.66±15.01 c	203.33±15.27 c
6 week	268.66±17.95 f	329.00±18.24 a

*Different letters indicate the presence of a significant difference at P<0.01.
*Values represent the mean ± standard deviation for 3 mice in the group.

Toxoplasmosis is one of the highly prevalent zoonotic diseases worldwide caused by the parasite T. gondii (Doghish et al., 2023). This parasite infecting about 2 billion people. It can cause fatal encephalitis in people with weakened immune systems (Almeria and Dubey, 2021). Several studies have been conducted on the association of infectious and environmental pathogens with diabetes. Some studies instrument the relationship between chronic T. gondii infection and the development of Type 2 of diabetes mellitus in humans and animals (Nosaka et al., 2018). The highest glucose concentration was recorded in the sixth week in infected females. This result agree with the result that found through Nassief Beshay et al., (2018) in a murine model. Toxoplasmosis may increase susceptibility to developing type-1 diabetes due to the ability of Toxoplasma gondii to invade and replicate inside pancreatic cells, inhibit insulin production and increase glucose concentration. When beta cells are destroyed, insulin secretion will be affected, as it is possible that the pancreatic cells will be damage. In addition, T. gondii is considered a trigger for Type 1 of diabetes mellitus by damaging pancreatic cells to prevent insulin secretion (Shirbazou et al., 2013). T. gondii has a role in type 2 diabetes by causing chronic inflammatory reactions. On the other hand, infection with T. gondii can stimulate autoimmunity and thus reduce insulin secretion from pancreatic beta cells (Nosaka et al., 2016 and Li et al., 2018).

Furthermore, Nassief Beshay et al., (2018) found that all mice with chronic toxoplasmosis with inflammation of the islets of Langerhans.Through observation of the results, it was noted that there was an increase in the insulin concentration rate in the blood. The highest concentration rate was recorded in the second week of affected female group compared to the control group. This may be due to insulin resistance, a common metabolic disorder where the body can't properly use insulin.(Beale 2013). Also development has been increasingly more correlated with dysfunction or necrosis of cells with the aid of reactive oxygen species, nitric oxide, and some pro-inflammatory cytokines, main to insulin resistance in animals (Li et al., 2018).

On the other hand, the study revealed an increase in the concentration of IgG antibodies, as evidenced by the results. The highest concentrations were found in the male group affected in the third week, followed by the second and sixth weeks (Sharma et al., 2022). However, high increase T. gondii infection rates, This due to increase in neutrophil, monocyte, and T-cell dysfunction in type-1 diabetes, as both the innate and adaptive immune responses may be impaired in type-1 diabetes (Calvet and Yoshikawa, 2001). This result is compatible

with the results obtained by Li et al., (2018) in China confirmed an appreciably greater Toxoplasama seroprevalence in 120M sufferers (23.5%) than in the control team (11.75%), Recently, there is global necessity and curiosity over the previous two decades, direct and oblique associations between irritation and T2DM have been elucidated, suggesting that this kind of DM is a continual inflammatory ailment (Li et al., 2018). Furthermore, it appears that the activation of latent toxoplasmosis raise Ig levels, increase the proportion of seropositive individuals, or, as noted in the majority of publications, make persons diabetic (Elkholy et al., 2022).

It was noted that there were significant differences between the mean number of cysts in the sixth week for infected females. While the lowest rate of number of cysts was recorded in the first week for infected males and females more than %25 of patients with type-1 diabetes lack complement component 4 (C4), a protein found on the surface of cells that is crucial in the opsonization of pathogens (Shirbazou et al., 2013).

In addition to the decreased immune function associated with type-1 diabetes, another potential explanation for a possible positive association between parasite infection and type-1 diabetes is that T. gondii could induce Autoimmunity defensiveness against beta cells in the pancreas, thereby causing type-1 diabetes by damaging insulin secretion from (Moutschen et al., 1992). This result is consistent with the results reached by Hassanain et al., (2024) as he pointed out hyperglycemic factor was confirmed to opportunist higher T. gondii brain parasite load and elevate Immunoglobulin titer in latent infected and diabetes induced rat, possible match with T.gondiisero-positive diabetic patient , also normoglycemic level is very important factor for controlling extra brain. parasite load, and diminishes nervous signs in diabetic patients, where the fit normoglycemic diabetic will be successfully interacting with the outside escaping tachyzoits.

Conclusions

Toxoplasmosis may reveal to increase in susceptibility for diabetes. The higher level of glucose in the blood serum, number of parasite cysts , invasion of tachyzoites , plays a role in stimulating susceptibility to diabetes.

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