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Association Between Toxoplasmosis Infection and Human Insulin In Pregnant and Aborted Women

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ABSTRACT: Background : People with compromised immune systems are more likely to get infected with the opportunistic parasite *Toxoplasma gondii* than healthy individuals. Diabetes and toxoplasmosis are two conditions that are rather prevalent in Iraq.

Objective : human insulin was the major objective of this study, along with determining the frequency of anti- *T. gondii* IgG antibodies in pregnant and abortion. In addition to this, establish a connection of (RBS) and (HbA1c).

Methods : The investigation was in Al-Qadisiyah. The term for collecting was from the November 2022 to the end of February 2023. where specimens were collected from the Maternity and Children's Teaching Hospital, 350 serum samples that were collected from pregnant, and abortion women. The healthy samples that served as a control group50 samples,. Both the ELISA technique and the Toxo Rapid Diagnostic Test (Cassette) were used to analyze the samples in order to determine the levels of human insulin present in pregnant and abortion women. Also measures (HbA1c) and (RBS).

Results : the Results According Cassette the 350 samples, only 130 (43.3%) positive, whereas 170 (56.7%) negative. Using ELISA, anti-*T. gondii* IgG was present in 90 (69.2%) of the women from 170 positive cassette. ELISA was also used to assess of human insulin that was present in order to get a better understanding of the role that toxoplasmosis plays in the development of diabetes. Patients with diabetes had considerably higher levels of human insulin (22.83 ± 13.91) when compared to patients with toxoplasmosis alone and healthy controls

Conclusion : *T. gondii* is more prevalent among pregnant women and abortions. Diabetes and toxoplasmosis have been shown to have a close connection, according to research. Our hypothesis is that toxoplasmosis provides the way for diabetes, and diabetes paves the way for toxoplasmosis. Toxoplasmosis is more likely to develop in people with diabetes since the disease lowers the immune response.

Keywords: *Toxoplasma gondii*, Aborted, Pregnant, ELISA, Insulin, Diabetic

1. INTRODUCTION

Toxoplasma gondii is a protozoan parasite that lives within its host and is obligate intracellular. It may infect any animal that has a warm-blooded host. Human beings, birds, and rodents are all examples of creatures with a warm-blooded metabolic system. Additionally, both domesticated cats and wild cats may play an important role in the transmission of the disease [1].The vast majority of T. gondii infections are asymptomatic; nevertheless, some people who are infected might acquire clinical indicators of toxoplasmosis, such as lymphadenopathy, chorioretinitis, and meningoencephalitis [2,3]. Asymptomatic T. gondii infections make up the bulk of all T. gondii infections. The

majority of T. gondii infections do not produce any noticeable symptoms in the host. People who are immunocompromised and have a reactivation of a T. gondii infection have an increased chance of having a condition that affects the central nervous system and has the potential to be fatal [1,2]. T. gondii may result in issues in a variety of the host's organs when it has established an infection[4]. Infections of the pancreas caused by T. gondii have been shown to be possible in both humans and other species as well. In humans, a T.gondii infection may result in the development of pancreatitis as a complication [5].T.gondii is responsible for the development of tissue cysts, which are responsible for the development of pancreatitis and tissue necrosis in both koalas and cats[6]. according to the results of various research. The brain, lungs, heart, pancreas, and lymph nodes are the organs in a host where T. gondii is most likely to spread after an initial infection [7]. Pancreatitis is another prominent site of T. gondii dissemination. Toxoplasmosis may express itself in a number of ways, including pancreatic disease, and has been seen in humans as well as other animals. When pancreatitis strikes a person, it is probable that an infection had a part in the progression of their condition [8]. Toxoplasmosis is more prevalent in diabetics because their immune systems are less able to successfully fight off infections [9]. Ninety percent of immunocompetent individuals who have been infected with toxoplasmosis will not exhibit any symptoms at all [10]. The adaptive responses known as humoral immunity (H.I.) and cell-mediated immunity (C.M.I.) are triggered by an infection with T. gondii [11]. This is because T. gondii is an intracellular parasite that is capable of migrating through extracellular space in order to recruit new host cells. As a result, this circumstance has arisen . T. gondii has the potential to cause a severe illness with significant ramifications, such as chorioretinitis, in people whose immune systems are weakened, including pregnant women. This condition may also occur in people whose immune systems are healthy[12,13].According to Carter [14] T. gondii has also been linked to a variety of autoimmune diseases, such as inflammatory bowel disease, rheumatoid arthritis, thyroid illness, and systemic sclerosis. In addition, T. gondii has been related with a number of other conditions. In addition to this, T. gondii has been linked to a variety of other illnesses. There is currently no medication that is both safe and effective for treating congenital toxoplasmosis or chronic infections [15]. This is the case even though there is a known link between the two conditions. In pregnant women and women who have just gone through an abortion, the goal of this research is to assess the amounts of human insulin and toxoplasmosis infection that are present in their bodies. In addition to this, it demonstrates how these two clinical criteria are connected to one another when contrasted with those who have a good state of health.

2. Material and Methods

2.1 Collection of Sample

The study was carried out in Al-Qadisiyah province. The collection duration extended period from the beginning of November 2022 to the end of February 2023. where samples were collected from the Maternity and Children's Teaching Hospital, the Diwaniyah General Teaching Hospital and different private laboratories. 350 samples serum were collected from pregnant, non-pregnant, and abortion women, including 300 serum patient samples and 50 healthy samples as a control group in gel tube. We conducted in-person interviews with the subject in order to learn more about her, including her age, location of residence, and history of past abortions. Veins were punctured in pregnant women to get three milliliters of blood. After that, the blood was spun for five minutes at a speed of 3000 revolutions per minute to separate its various components. The sera were initially kept in plane tubes and then put in the refrigerator to preserve their temperature before going through any kind of serological testing. The samples were diagnosed using Toxo Rapid Diagnostic Test (Cassette) and the ELISA test to detect the IgG antibody and also to measure the level of Human Insulin assayed by ELISA technique using (BT-Lab kit ,China Show how they relate to other clinical measures, such as random blood sugar (RBS), glycated hemoglobin (HbA1c), following the company's instructions attached.

2.2 Serological Technique

An ELISA test was performed on the blood of female participants to determine the amount of human insulin in their systems. In addition to this, the participants' serum was analyzed to determine whether or not they had specific anti-Toxoplasma gondii IgG antibodies. The ELISA kit was given by a commercial company (BT-Lab kit, China), and the process was carried out in line with the instructions that were provided by that manufacturer.

2.3 Statistical Analysis

The data was collected, summarized, analyzed, and displayed using Microsoft Office Excel 2010 and the statistical package for social sciences (SPSS) version 26. The range of the numerical data as well as its mean, standard deviation, and range were shown. unbiased samples The t-test was used to assess the mean difference between any two groups, with the stipulation that the variable in question had to follow a normal distribution.

The chi-square test was used to investigate the possible associations between any two categories of data. P-values of 0.01 or less were considered to be very significant, while those of 0.05 or less were considered to be significant.

3. Results

3.1 Rapid Diagnosis of Toxoplasmosis

The present study enrolled 350 samples from pregnant and aborted women and healthy women to investigated toxoplasmosis by using rapid diagnostic test (cassette) and the results was show as in table (1). The present results show 130 (43.3%) of pregnant and aborted women have positive results of rapid diagnostic test and 170 (56.7%) was negative results. But the present results show all healthy women subjects 50 (100.0%) have negative results of rapid diagnostic test, and the difference was highly significant ($p < 0.001$).

Table 1. - Rapid diagnosis of Toxoplasmosis in pregnant and aborted women and healthy women

Characteristics	Pregnant and aborted women *n* = 300	Healthy women *n* = 50	*P value*
Rapid diagnostic test (cassette)			
Positive, *n* (%)	130 (43.3%)	0	< 0.001 ¥ HS
Negative, *n* (%)	170 (56.7%)	50 (100.0%)	

¥: Chi-square test; HS: Highly significant at $P \leq 0.001$

3.2. Seroprevalence of Toxoplasmosis

To confirm the seroprevalence of Toxoplasmosis, the suspected patients and healthy control subjects submitted to IgG detection by ELISA technique and the results demonstrated in table (2). Positive results were seen in 90 (69.2%) of suspected patients in compared with 40 (30.8%) was negative. while all healthy control subjects 50 (100.0 %) were have negative results of IgG, and the difference was highly significant ($p < 0.001$),

Table 2. - Prevalence *T. gondii* infection according to ELISA IgG test in studied groups

Characteristics	Suspected patients *n* = 130	Healthy control *n* = 50	*P value*
IgG results			
Positive, *n* (%)	90 (69.2%)	0	< 0.001 ¥ HS
Negative, *n* (%)	40 (30.8%)	50 (100.0%)	

¥: Chi-square test; HS: Highly significant at $P \leq 0.001$.

3.3. Demographic characteristics of patients and healthy control

In the ongoing study, participants for the ELISA diagnostic tests comprised 50 healthy control volunteers as well as 90 patients suffering from toxoplasmosis. The demographic information pertaining to both the patients and the controls is included in Table 3. Patients with toxoplasmosis had a mean age of 28.54 ± 5.76, and healthy control subjects had a mean age of 25.22 ± 6.51. There was no obvious difference in mean age between suspected patients and control participants ($P = 0.110$) between toxoplasmosis patients and healthy control individuals, figure (1). The frequency distribution of toxoplasmosis patients and control individuals according to age is also shown in table (3), which may be seen below. On the other hand, there was not a statistically significant difference seen in the frequency distribution of patients and controls according to age group ($P = 0.178$). According to the findings of Residency, the ill group included 61 cases from urban regions and 29 cases from rural areas (representing 67.8% and 32.2%, respectively), while the healthy control group included 41 cases from urban areas and 9 instances from rural areas (representing 82.0% and 18.0%, respectively). There was not a statistically significant difference identified between the frequency distribution of patients and healthy control ($P = 0.070$), according to the findings of residency, figure (2).

Table 3. - Demographic characteristics of patients and healthy control

Characteristic	Patients *n* = 90	Healthy control *n* = 50	
Age (years)			
< 20, *n* (%)	2 (2.2%)	2 (4.0 %)	0.178 ¥
20-29, *n* (%)	50 (55.6%)	35 (70.0 %)	

30-39, *n* (%)	32 (35.6%)	9 (18.0 %)	NS
≥ 40, *n* (%)	6 (6.6 %)	4 (8.0%)	
Mean ±SD	28.54 ± 5.76	25.22 ± 6.51	0.101 † NS
Range	18 – 42 years	18 – 41 years	
Residency			
Urban, *n* (%)	61 (67.8%)	41 (82.0 %)	0.070 ¥ NS
Rural, *n* (%)	29 (32.2 %)	9 (18.0%)	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; **¥**: Chi-square test; **NS**: not significant at $P > 0.05$

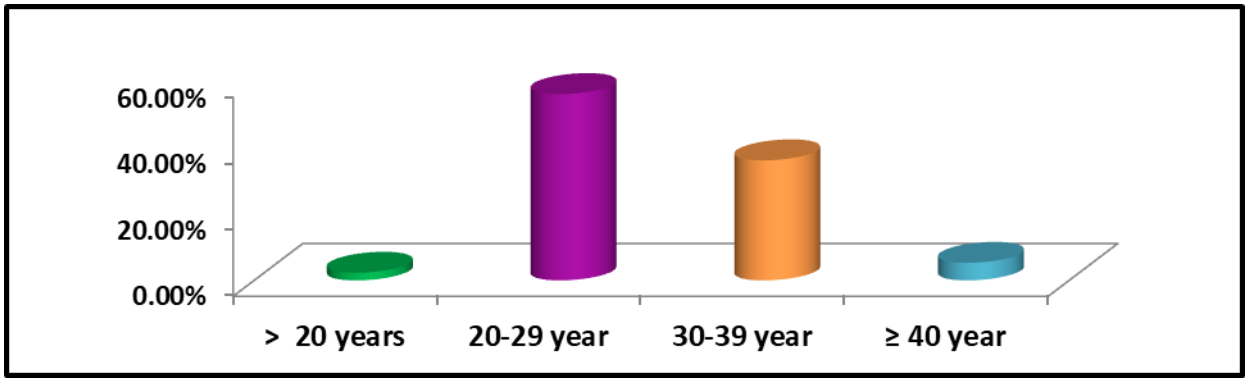

FIGURE 1. - Histogram showing the frequency distribution of women with toxoplasmosis according to age groups

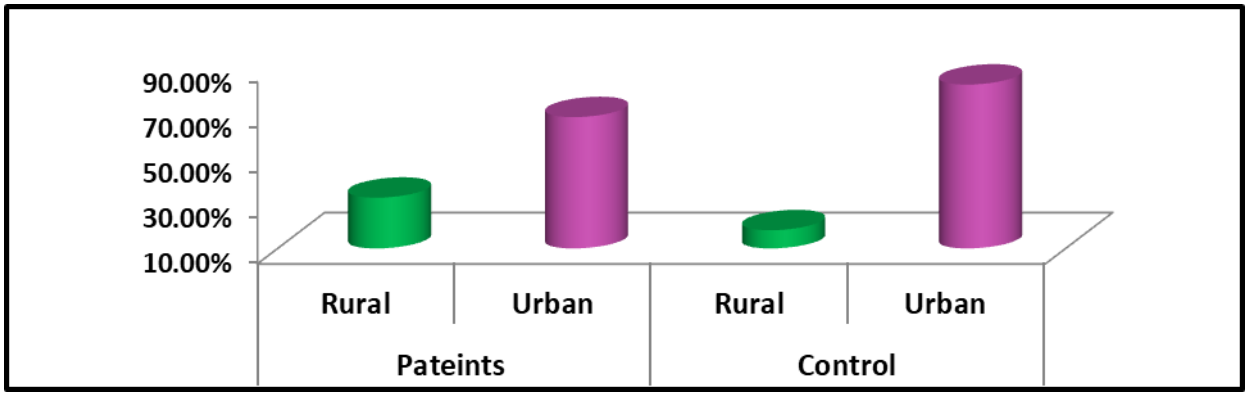

FIGURE 2. - frequency distribution of patients and healthy control according to residence

3.4. Diabetes mellitus (DM)

The frequency distribution of patients with toxoplasmosis and healthy control subjects according to diabetes mellitus was as following: 20 (22.2 %) of patients with toxoplasmosis have diabetes mellitus and 70 (77.8 %) of patients with toxoplasmosis don't have diabetes mellitus. While all healthy control subjects 50 (100.0 %) don't have diabetes mellitus and the differences was highly significant ($p < 0.001$), table (4).

Table 4. - Frequency distribution of patients and healthy control according to Diabetes mellitus

Characteristic	**Patients** ***n* = 90**	**Healthy control** ***n* = 50**	***P***
Diabetes mellitus			
Yes, *n* (%)	20 (22.2 %)	0	< 0.001 ¥ HS
No, *n* (%)	70 (77.8 %)	50 (100.0 %)	

n: number of cases; ¥: Chi-square test; **HS**: highly significant at $P \leq 0.05$

3.5. Levels of some biochemical markers (RBS, HbA1C) in patients and healthy control.

Table (5) compares numerous distinct biochemical markers discovered in the sick group to those found in the control group as the study's findings. Random blood sugar (RBS) readings for the toxoplasmosis patients with diabetes were 219.58 ± 62.55 mg/dL, for toxoplasmosis patients alone were 117.55± 22.38 mg/dL, and for healthy controls were 111.08± 14.59 mg/dL, respectively. This difference in RBS readings was statistically significant ($p < 0.001$). Hemoglobin A1C (HbA1C) mean values were also as follows: 7.61 ± 1.11 in patients with both toxoplasmosis and diabetes; 5.26± 0.78 in patients with toxoplasmosis alone; and 5.52± 0.441 in healthy controls, respectively. The diabetes patients' mean level differences were greater, which explained the extremely significant difference ($p < 0.001$) between these three groups of adults.

Table 5. - Levels of some biochemical (RBS, HbA1C) in patients and healthy control

	Cases–control comparison			
	Toxo. with diabetic ***n* = 20**	***Toxo. patients*** ***n* = 70**	**Healthy control *n* = 50**	***Total*** ***P value***
Random blood sugar (RBS) mg/dL				
Mean± SD	**219.58 ± 62.55^{A}**	**117.55± 22.38 ^{B}**	**111.08± 14.59^{B}**	**< 0.001 † HS**
Range	96.0- 305.0	82.0 – 180.0	88.0 – 127.0	
Different latters denote to the significant differences at p< 0.05				
Hemoglobin A1C (HbA1C) %				
Mean± SD	**7.61 ± 1.11 ^{A}**	**5.26± 0.78^{B}**	**5.52± 0.441^{B}**	**< 0.001 † HS**
Range	5.50- 9.40	4.00 – 6.70	4.80 – 6.0	
Different latters denote to the significant differences at p< 0.05				

SD: standard deviation; †: one way ANOVA; ¥: Chi-square test; HS: Highly significant at $P \leq 0.001$.

3.6. Serum Human Insulin level in patients and healthy control.

The table (6) method was used to figure out how much human insulin was in the blood of each person. The average amount of human insulin in the blood of people with toxoplasmosis was 17.43± 13.38, while the average amount of insulin in the blood of people who were healthy was 7.46± 6.73 The amount of human insulin in the blood of people who had both toxoplasmosis and diabetes was 22.83 ± 13.91 Compared to healthy control groups, these mean values were higher in toxoplasmosis patients with diabetes and in toxoplasmosis patients only, and the difference was highly significant ($p < 0.001$),

Table 6. - Levels of Human Insulin in patients and healthy control

	Cases–control comparison			
	Toxo. with diabetic ***n* = 20**	***Toxo. patients*** ***n* = 70**	**Healthy control *n* = 50**	***Total*** ***P value***
Human Insulin				
Mean± SD	**22.83 ± 13.91^{A}**	**17.43± 13.38 ^{A}**	**7.46± 6.73^{B}**	**< 0.001 † HS**
Range	0.69- 58.14	0.02 – 45.48	0.02 – 33.47	
Different latters denote to the significant differences at p< 0.05				

n: number of cases; **SD**: standard deviation; †: one way ANOVA; ¥: Chi-square test; HS: Highly significant at $P \leq 0.001$;

4. Discussion

A lateral flow chromatographic immunoassay, also known as a quick diagnostic test or caddy, is used in order to simultaneously detect and discriminate between IgG and IgM Abs that are present in human blood or plasma. This is accomplished by using a rapid diagnostic test. When it comes to determining whether or not an individual has T. gondii infection, this test kit may function both as a diagnostic tool and a screening test [16]. Cassettes are a great alternative for regular serological testing because they have a high degree of specificity, they are rapid, they have a cheap cost, and they are simple to use [17]. In addition, cassettes are very easy to use. Ali [18], whose research included the participation of 250 women from Kurdistan, Iraq, discovered that there was a frequency of 44.8% seropositive individuals in the area. The results of his investigation came to the same conclusions. The findings of the present research are in agreement with those discovered by Mohammed and Al-Janabi [19], which is another point of interest. According to the findings of that study, the researchers found that the prevalence of seropositivity among the subjects, which consisted of 75 women from the province of Babylon in Iraq, was 42.6%. The results of the current investigation are in line with the conclusions that Mohammed and Al-Janabi [19]found. The findings of the current investigation are, however, superior to those obtained by Mahmood et al., [20], who discovered that 136 patients (34%) from the national blood transfusion center were infected with T. gondii, while 246 patients (66%) exhibited a negative response to the latex agglutination test. The results of the present study are superior to those obtained by Mahmood et al., [20]. The results of the present investigation are, despite this, more impressive than those obtained by Mahmood et al., [20]. Using the ELISA method, the IgG levels of both the participants who were suspected of having toxoplasmosis and the healthy control participants in the study were assessed. This allowed the researchers to determine the seroprevalence of toxoplasmosis. The findings are consistent with the findings of an investigation that Hamad [21] conducted in Baghdad, Iraq, in the year 2017.

The age ranges of both the sick and the healthy persons in the control group made it clear that toxoplasmosis may affect people of any age. According to the findings of the present research, the seroprevalence rate increased among pregnant women in the age range of 20–29 years (55.6%), then again in the age group of 30-39 years (35.6%), but it decreased at age 40 and above (6.6%). This group had a higher risk of contracting toxoplasmosis, which is similar with the findings of previous studies carried out in a variety of countries [22]. According to the findings of these studies, the prevalence of T. gondii IgG antibodies was greatest in pregnant women who were between the ages of 10 and 20 years old, while the prevalence was lowest in pregnant women who were between the ages of 41 and 50 years old (25.0%). This might be due to the fact that younger pregnant women have different dietary and behavioral patterns than older pregnant women, and as a consequence, they could be more exposed to some of the risk factors for toxoplasmosis in pregnant women [23]. The outcomes of the research indicated that the percentage of pregnant and women who had an abortion was significantly higher among participants who came from urban areas as compared to those who came from rural areas. This is true for Iraqis who have had cats in their homes in the past and who, for the most part, do not exercise preventive measures or cat veterinary care [24]. The presence of cats in households is a key factor in the development of the parasite Toxoplasma, and this is true for Iraqis who have raised cats in their homes in the past. The greater frequency of toxoplasmosis among women in urban areas may be a consequence of their lifestyles, dietary habits, and environmental situations, all of which render them more sensitive to factors connected to toxoplasmosis [25].

According to the findings of that research, the seroprevalence level of *T. gondii* infection was 77.8% in a group of 180 diabetic people. In addition, the findings of the present study coincide with the data obtained by Molan and Ismail [26] in Diyala. These researchers looked at the same population. They carried out a research project with a control group consisting of 300 individuals who did not have diabetes and used their sera as samples. They identified a seropositive percentage of anti-*T. gondii* IgG antibody in diabetes patients at a rate of 66.6% and 33.4%, respectively, in diabetic patients. It is possible that toxoplasmosis comes before diabetes since it causes necrotic lesions in the pancreas of experimental animals that are infected with *T. gondii*. This is as a result of the fact that involvement of the pancreas may block insulin production, which in turn can result in the development of diabetes [26,27]. It's possible that diabetes and toxoplasmosis both arrive initially. Previous research has shown that when experimental mice are infected with *T. gondii*, a variety of organs, including the pancreas, stomach, lymph nodes, and intestine, develop necrotic lesions and inflammation [27]. This has been seen in a number of different organs. In addition to that, tachyzoites were shown to be present inside these tumors.

According to the information that is currently available, (22.2%) of the patients who were diagnosed with toxoplasmosis also suffered from diabetes mellitus. The concept that an infection with *T. gondii* may be connected to an increased risk of diabetes in adults is given more credence by these findings. In addition to this, it has been shown that the presence of *T. gondii* in the pancreas has a direct and deleterious effect on the pancreatic cells. Damaged cells would have an impact on insulin levels, and it's conceivable that damage induced by *T. gondi*i would upset the neurological system and influence pancreatic cells, which would increase the risk of developing diabetes [28]. In

addition, damaged cells would have an effect on insulin levels. According to the findings of the current research, diabetic patients who also had toxoplasmosis had much higher levels of FBS in their blood than patients who fell into any of the other categories. This was the case regardless of which group the patients belonged to. These findings are comparable to those discovered by Hilal [29], who found that the mean FBS levels in type 2 diabetes patients with toxoplasmosis, the group with toxoplasmosis alone, and healthy controls were, respectively247.24 ± 15.80, 98.83 ± 1.50 and 93.40 ± 0.93 with highly significant differences ($P \leq 0.01$), between each group. The findings presented here are comparable to those discovered by Hilal [29]. Kankova *et al*., [30], in their study, discovered that latently infected pregnant women had much higher levels of glucose in their blood than healthy pregnant women did. In addition, the results that have been reported here indicate that patients who were diagnosed with diabetes had mean HbA1C values that were significantly greater than those of patients who just had toxoplasmosis and healthy controls. These findings are in accordance with those that were discovered by Mohamed *et al.*, [31], who discovered that diabetes patients who had anti-Toxoplasma IgG antibodies had higher HbA1c levels than patients in other groups (8.163±0.9043) On the other hand, the findings of the current investigation run counter to the conclusions reached by Asgari *et al.*, (32), who found that all *T. gondii* patients had normal levels of HbA1c in their blood. The HbA1c test is the conventional method that is used for the purpose of identifying the average amount of glucose that has been present in the blood over the course of the most recent two to three months. When compared to healthy control groups, patients with toxoplasmosis who also had diabetes and patients with toxoplasmosis by themselves had considerably higher mean blood insulin levels. According to the findings, this difference was statistically significant ($P < 0.001$). in comparison to others. According to the results of the research conducted by Khalaf and AL-Jubouri [33], the majority of people who are diagnosed with type 2 diabetes have insulin resistance rather than an actual decline in insulin levels. Despite the fact that this causes a compensatory rise in insulin production, this assertion is only true in the earlier stages of the condition. As the condition progresses, people will have less insulin in their bodies than they had at the beginning of the disease. It is believed that this is caused by the destruction of pancreatic cells by inflammatory processes. This, in turn, leads to a reduction in cell mass as well as the buildup of amylin, a fibril-like amyloid in beta cells, which, in turn, ultimately increases the risk of both acute and chronic pancreatitis as well as diabetes [28].

In their research, Al-Qadi and Zangana [34], shown that high glucose levels cause beta cells to produce more insulin. As a consequence, blood glucose and insulin levels were found to be higher in toxoplasmosis patients with diabetes in comparison to the group that served as the control. The statistics that are given here are consistent with their results and support the inferences that they drew from those findings.

5. Conclusions

According to the findings of the study, *T. gondii* is more prevalent among pregnant women and women who have recently had abortions. Diabetes and toxoplasmosis have been shown to have a close connection, according to research. Our hypothesis is that toxoplasmosis provides the way for diabetes, and diabetes paves the way for toxoplasmosis; nevertheless, the order in which these conditions appear in a person's life is important to consider. Toxoplasmosis is more likely to develop in people with diabetes since the disease lowers the immune response.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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