Ref: Ro J Med Pract. 2024;19(3)
DOI: 10.37897/RJMP.2024.3.1

Seroconversion Toxoplasmosis as a predictor for gestational diabetes mellitus among pregnant ladies in Iraq

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ABSTRACT

Background. *Toxoplasma gondii (T.g.) can reach many host organs including the pancreas and pancreatic tissue may be directly attacked and compromised by the organisms.*

Aim. *This study aimed to evaluate the risk of toxoplasmosis infestation as a predictor of gestational diabetes mellitus (GDM) among pregnant ladies in Thi-Qar, Southern Iraq.*

Methodology. *A case-control study was conducted on 151 pregnant ladies classified (61) with GDM and (89) healthy pregnant ladies attending a tertiary Endocrine Center. All patient data were gathered from direct interviews and the digital records of the tertiary center, which used an internal network system and Microsoft Access program. Each pregnant woman was diagnosed with new GDM according to the American Diabetes Association (ADA) definition criteria.*

Results. *The mean ages of the whole participants were 28 ±6 years old and more than half of them were in the second decade of life, their mean weight was 75.38±13.29) kg and their body mass index was 29.82 ± 5.23 (Kg/m^2). Family history of DM, history of toxoplasmosis infestation, and positive serological test of IgG for toxoplasmosis was significantly predominant among women with GDM rather than those without (p-value= 0.007, 0.002, 0.004 respectively).*

Conclusions. *The second decade of age is the independent risk factor for developing new GDM, while toxoplasmosis infestation and positive IGG were additive-dependent risk factors for developing new GDM in collaboration with other patients' profiles. Pregnant women with GDM were significantly older, heavier in body weight, and obese than those without GDM making them at higher risk factors for getting hyperglycemia.*

Keywords: Anti-Toxoplasma antibodies, gestational diabetes mellitus, incidence, risk factor, seroconversion, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii (T.g.) is an endemic zoonotic disease that affects all warm-blooded animals, including humans who serve as intermediate hosts [1,2]. The incidence of human toxoplasmosis varies throughout Iraq; from 12.4% in Basra to 62.9% in Al-Najaf [3,4], in addition to other countries. In immunocompetent individuals, 90% of toxoplasmosis infections are asymptomatic [5], Pregnancy-related T.g. infection has been associated with fetal loss and birth defects among human and animal fetuses. T.g. can infect a variety of host organs, including the pancreas [6,7]. When T.g. infects nucleated cells, such as pancreatic cells, it can directly target and compromise pancreatic tissue. By damaging β cells and insulin secretion, they may increase the risk of diabetes and both acute and chronic pancreatitis [8].

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Article History:
Received: 3 August 2024
Accepted: 28 August 2024

Opportunistic infections can be brought on by diabetes, much as HIV infections and other immune system problems can. People with diabetes are more susceptible to several specific diseases, such as toxoplasmosis, according to several studies [2,8]. The World Health Organization (WHO) states that diabetes is a chronic illness that arises from the body's inability to produce enough insulin or use it appropriately. Gestational diabetes mellitus (GDM) is a form of diabetes mellitus (DM) characterized by pregnancy-onset glucose intolerance [2]. Globally, GDM affects more than 14% of pregnancies, or 18 million infants annually, based on the most recent data published by the International Diabetes Federation (IDF) in 2017[9]. Before 2009, research found that the prevalence of GDM was 10.6%, but studies conducted in or after 2010 found that the prevalence was 14.0% [10]. Since there is insufficient data about the evidence linking toxoplasmosis and GDM, our objective aimed to investigate how toxoplasmosis infestation affects the development of new GDM among pregnant women from Thi-Qar, Southern Iraq.

MATERIALS AND METHODS

Study Setting

From September 2023 to February 2024, pregnant women undergoing treatment at the tertiary Thi-Qar Specialized Diabetes Endocrine and Metabolism Center (TDEMC) in Thi-Qar, Southern Iraq, were the subjects of this case-control study.

Patients arrived from Souq Al-Shuyukh General Hospital, Hay Al-Askary Health Center, the city center, and all districts and sub-districts. Any pregnant ladies with new onset GDM between 20 and 40 weeks of gestation who are eligible and willing to take part were included in this study. Women with Type-1 DM, Type-2 DM, drugs-induced diabetes, transient hyperglycemia, and non-pregnant women were excluded from enrollment.

A baseline demographic information of each pregnant woman was gathered by direct interview of studied variables including age, residency, education attained, family history of DM, number of children, number of dead births, previous GDM, history of the polycystic ovarian syndrome (PCOS), previous abortion, stillbirth with congenital malformation, previous babies born with weight more than 4 kg, previous toxoplasmosis infection, and contact with an indoor cat.

Together digital records from the data system of the TDEMC center by a Microsoft Access Program (MSAP) and an internal network to store all patients' data.

The sample size was calculated by using the following equation [11]:

$$\text{Sample size} = (r+1)(P^*)(1- P^*)(Z_\beta + Z_{\alpha/2})^2/r(P1 - P2)^2$$

r = Ratio of control to cases,
p^* = Average proportion exposed = [proportion of exposed cases + proportion of control exposed]/2,
Z_β = Standard normal variate for power 80% which is 0.84.
$Z_{\alpha/2}$ = Standard normal variate for the level of significance which is 1.96,
p1 – p2 = Effect size or difference in proportion expected based on previous studies,
p1 = the proportion in cases and p2 is the proportion in control.

So the prevalence of GDM (10.6%) [10], and the prevalence of toxoplasmosis (30%), the minimum number of the sample is 147, but we enrolled 151 participants in this study for more satisfaction. Pregnant women were measured anthropometrically for height (in meters) and weight (in kg) while wearing loose clothing and no shoes. The woman's height was measured while she was standing straight, looking straight ahead, and touching her heels, hips, and occiput on a straight measurement wall. The nearest 0.1 kg flat-surface weighing equipment were then used to record the weight. The majority of computerized medical records compute the patient's Body Mass Index (BMI) automatically by dividing their weight in kilograms by the square of their height on average (kg/m^2). The categories of obesity are class I (30–34.9), class II (35-39.9), class III (>40 kg/m^2), underweight (less than 18.5 kg/m^2), normal (18.5-24.9) kg/m^2, overweight (25-29.9) kg/m^2, and class III obesity [12].

Biochemical Measurements

Each pregnant woman was diagnosed with GDM according to ADA definition criteria [13] as fasting blood glucose of more than 92 mg/dL (5.1 mmol/L), one-hour glucose tolerance test ≥180 mg/dL (10.0 mmol/L), two-hour glucose tolerance ≥153 mg/dL (8.5 mmol/L). Some pregnant ladies were confirmed as having new GDM by getting 6.5% or more glycated hemoglobin (HbA1c) level which was measured by using a well-qualified method (Bio-Rad Variant II Turbo HbA1c Kit – 2.0 Quick Guide 270-2455EX). Duration of GDM was specified as the period (in the nearest month) between the visit date and the patient's diagnosis date.

14 Blood samples were collected from each pregnant lady and tested for detection of anti-Toxoplasma IgM and IgG using the Enzyme-linked immunosorbent assays (ELISA) the sensitivity ranged from 93-100% and the specificity ranged from 78-99% [14]. Let the specimen, controls, and/or test apparatus come to room temperature (15-30°C) before beginning any testing. The test gadget should then be used immediately after being removed from its sealed bag. When doing a

whole blood test, make sure the test apparatus is labeled with the specimen's ID number and place it on a spotless, flat surface. When doing a serum or plasma test, fill the plastic dropper with the specimen, hold it vertically, and carefully inject one drop (about 30-45 µL) into the sample well, making sure to get rid of any air bubbles in the process. Then, immediately add 1 drop (35-50 µL) of sample diluent. The result ought to appear in fifteen minutes, according to the stopwatch. Add one drop of whole blood to the sample well (around 40–50 µL). In barely one minute, positive results were evident. Refrain from reading the findings after fifteen minutes. To avoid confusion, throw away the test instrument once the results have been evaluated [14] .

Statistical Analysis

The one-sample Kolmogorov-Smirnoff test was used to determine if the parametric variables were normally distributed. The results were shown with the mean and standard deviation (SD) displayed. Statistical Statistics Package of Socio Science version 23 (SPSS) was used to apply the Chi-Square test to assess the data for independent qualitative variables.

Independent student t-tests and analysis of variance (ANOVA) were then employed to investigate the variable's independence for continuous variables, followed by binary logistic regression analysis. A more thorough examination was considered required when the p-value was less than 0.05.

RESULTS

The baseline characteristics of pregnant ladies. One hundred and fifty-one pregnant ladies were enrolled in this study. They were classified as (62 cases with new GDM) and (89 women without GDM were considered as control). The mean ages of the whole participants were 28 ±6 years old and more than half of them were in the second decade of life, their mean weight was 75.38±13.29 kg and their BMI was 29.82±5.23 Kg/m^2. For education level, those women were distributed as illiterate, primary school, intermediate, and university level at 9.9%, 37.7%, 22.5%, and 29.8% respectively (Table 3-1).

One hundred and thirty-seven (90.7%) women were living in urban districts, and 14(9.3%) women were from rural populations. Around 68 (45.5%) pregnant ladies had a strong family history of 645 (29.8%) pregnant ladies with seropositive Toxoplasmosis infestation. The mean glycemic parameters of the participants were as RBS 126.63±57.21 mg/dl, FBS 110.61±26.48 mg/dl, and HbA1c 7.07±1.52% (Table 1).

Table 2 compare means of continuous variables between pregnant ladies with or without GDM. Pregnant ladies with GDM (32 ± 6) years were significantly older

TABLE 1. *Baseline characteristics of pregnant ladies*

Variable		Frequency	Relative frequency	Mean ± SD	Range
Age (years)	<20	11	7.3	28.52 ± 6.53	(17-46)
	20-29	82	54.3		
	30-39	44	29.1		
	≥40	14	9.3		
Weight (Kg)		---	---	75.38 ± 13.29	(42-120)
BMI (Kg/m^2)	<18.5	1	0.7%	29.82 ± 5.23	(16.6-44.8)
	18.5-24.9	25	16.6%		
	25-29.9	54	35.8%		
	30-34.9	47	31.1%		
	35-39.9	18	11.9%		
	>40	6	4.0%		
RBS (mg\dl)		---	---	126.63 ± 57.21	(50-350)
FBS (mg\dl)		---	---	110.61± 26.48	(80-166)
HBA1C (%)		---	---	7.07 ± 1.52	(4.7-10.2)
Residence	Urban	137	90.7	---	---
	Rural	14	9.3		
Education	Illiterate	15	9.9	---	---
	Primary	57	37.7		
	Intermediate	34	22.5		
	University	45	29.8		
Positive family history of DM		68	45.0	---	---
Positive history of PCOS		21	13.9	---	---
Positive Toxoplasmosis infestation		45	29	---	---

than those without GDM (26 ± 5) years (p-value <0.001). They had higher weight 82.74 ± 13.258 kg and BMI 32.8 ± 5.3 kg\m^2 than those without GDM (weight 70.25 ± 10.71 kg, BMI=27.753 ± 4.09 kg\m^2) (<0.001, <0.001 respectively). Women with GDM had higher blood glucose 177.84 ± 64.166 mg\dl than the other group 94.98 ± 11.83 mg\dl (p-value <0.001) while HbA1c was not significantly different between both groups (p-value=0.125) (Table 2).

Table 3 shows the distribution of the categorical variables among pregnant ladies with or without GDM. Interestingly, family history of DM, history of toxoplasmosis infestation, and positive serological test of IGG were significantly predominant among women with GDM rather than those without (p-value = 0.007, 0.002, 0.004 respectively). While residency, education levels, history of PCOS, history of macrosomia, and contact with in-door cats were not significantly different among both groups (p-value= 0.886, 0.347, 0.265, 0.64, 0.454 respectively).

Cross-tabulation of categorical characteristics between pregnant women with and without toxoplasmosis revealed that women with positive toxoplasmosis had a substantially greater history and number of abortions than those without (p-value <0.001, <0.001). Additionally, among pregnant women with positive toxoplasmosis, the history of congenital deformity and new GDM was substantially greater than in the other group (p-value = 0.044, 0.002, respectively). Other factors, however, did not significantly differ between pregnant women who had toxoplasmosis and those who did not Table 4.

TABLE 2. *Compare the mean of continuous variables between pregnant ladies with or without GDM.*

Variables		New GDM	No GDM	5% Confidence Interval for Mean		p-value
				Upper bound	Lower bound	
Age (years)	Mean ± SD	32.03 ± 6.58	26.07 ± 5.28	27.47	29.57	<0.001
	<20	0.0%	12.4%			
	20-29	38.7%	65.2%			
	30-39	41.9%	20.2%			
	≥40	19.4%	2.2%			
Weight (Kg)		82.74 ± 13.26	70.25 ± 10.71	73.24	77.52	<0.001
BMI (Kg\m^2)	Mean ± SD	32.8 ± 5.3	27.75 ± 4.09	28.98	30.66	<0.001
	<18	0.0%	1.1%			
	18.5-24.9	4.8%	24.7%			
	25-29.9	27.4%	41.6%			
	30-34.4	33.9%	29.2%			
	35-39.9	24.2%	3.4%			
	>40	9.7%	0.0%			
RBS (mg\dl)		178 ± 64	95 ± 12	117	136	<0.001
HBA1C (%)		7.12 ± 1.5.48	4.90	6.60	7.53	0.152
Total		62	89	---		

TABLE 3. *Cross tab of categorical variable among pregnant ladies with or without gestational diabetes*

Variable		New GDM	No GDM	Total	p-value
Resident	Urban	56 (90.3%)	81 (91.0%)	137 (90.7%)	0.886
	Rural	6 (9.7%)	8 (9.0%)	14 (9.3%)	
Education					
	Illiterate	8 (12.9 %)	7 (7.9%)	15 (9.9%)	0.347
	Primary	24 (38.7%)	33 (37.1%)	57 (37.7%)	
	Intermediate	16 (25.8 %)	18 (20.2%)	34 (22.5%)	
	University	14 (22.6 %)	31 (34.8%)	45 (29.8%)	
Family history of DM		36 (58.1 %)	32 (36.0%)	68 (45.0%)	0.007
History of PCOS		11(17.7 %)	10 (11.2%)	21 (13.9%)	0.265
History of macrosomia		9 (14.5 %)	5 (5.6%)	14 (9.3%)	0.64
Contact with indoor cats		31 (50.0%)	39 (43.8%)	70 (46.4%)	0.454
History of Toxoplasma Infestation		27 (43.5%)	18 (20.2%)	45 (29.8%)	0.002
Positive Serology of IGG		26 (41.9%)	18 (20.2%)	44 (29.1%)	0.004
Negative Serology of IGM		62 (100.0%)	89(100.0%)	151(100.0%)	
Total		62	89	151	

TABLE 4. *Cross tab of categorical variables among pregnant women with or without toxoplasmosis*

Variable		Positive Toxoplasmosis	Negative Toxoplasmosis	Total	p-value
Residence	Urban	38 (84.4%)	99 (93.4%)	137 (90.3%)	0.083
	Rural	7 (15.6%)	7 (6.6%)	14 (9.7%)	
Education	Illiterate	6 (13.3%)	9 (8.5%)	15 (9.9%)	0.508
	Primary	19 (42.2%)	38 (35.8%)	57 (37.7%)	
	Intermediate	10 (22.2%)	24 (22.6%)	34 (22.5%)	
	University	10 (22.2%)	35 (33.0%)	45 (29.8%)	
History of PCOS		7 (15.6%)	14 (13.2 %)	21 (13.9%)	0.703
Family history of DM		24 (53.3%0	44 (41.5 %)	68 (45.0%)	0.182
History of abortion		32(71.1%)	25 (23.6%)	57 (37.7%)	<0.001
Number of abortions	0	14 (31.1%)	79 (74.5%)	93(61.6%)	<0.001
	1	17 (37.8%)	19 (17.9%)	36 (23.8%)	
	≥ 2	14 (31%)	8 (7.6%)	22 (14.5%)	
Dead Birth	0	36 (81.8%)	99 (93.4%)	135 (90%)	0.056
	≥ 1	7(15.9%)	8 (8.9%)	15 (10%)	
Contact with indoor cats		26 (57.8%)	44 (41.5%)	81 (53.6%)	0.067
History of. Macrosomia		6 (13.3%)	8 ((7.5%)	14 (9.3%)	0.262
History of congenital malformation		4 (8.9%)	2 (1.9%)	6 (4.0%)	0.044
GDM	New GDM	27 (60.0%)	35 (33.0%)	62 (41.1%)	0.002
	No GDM	18 (40.0%)	71 (67.0%)	89 (58.9%)	
Duration GDM (months)	1	5 (20.0%)	6 (17.6%)	11 (18.6%)	0.721
	2	8 (32.0%)	12(35.3%)	20 (33.9%)	
	3	7 (28.0%)	12(35.3%)	19 (32.2%)	
	4	2 (8.0%)	3 (8.8%)	5 (8.5%)	
	5	3 (12.0%)	1(2.9%)	4 (6.8%)	

TABLE 5. *Binary logistic regression analysis to study the independence of the variables*

Variables in the equation		B	S.E.	Wald	Df	Sig.	Exp(B)
Step 1[a]	Family history of DM	-.290	.740	.154	1	.695	.748
	History of Toxoplasma	-20.055	41380.004	.000	1	1.000	.000
	IGG2(1)	20.960	41380.004	.000	1	1.000	1267195753.747
	Age. Groups			5.719	3	.126	
	Age. Groups(1)	-20.972	11237.371	.000	1	.999	.000
	Age. Groups(2)	-2.589	1.146	5.109	1	.024	.075
	Age. Groups(3)	-1.471	1.203	1.495	1	.221	.230
	BMI.25			1.671	5	.893	
	BMI.25(1)	-20.106	43666.471	.000	1	1.000	.000
	BMI.25(2)	-20.222	18944.809	.000	1	.999	.000
	BMI.25(3)	-.870	17067.099	.000	1	1.000	.419
	BMI.25(4)	-1.705	17067.099	.000	1	1.000	.182
	BMI.25(5)	-.221	17067.099	.000	1	1.000	.802
	RBS.153(1)	-21.349	5487.320	.000	1	.997	.000
	FBS.92(1)	-20.881	8589.138	.000	1	.998	.000
	HbA1c.6.5			.000	2	1.000	
	HbA1c.6.5(1)	-21.179	6393.040	.000	1	.997	.000
	HbA1c.6.5(2)	.835	13566.725	.000	1	1.000	2.306
	Constant	64.285	19993.165	.000	1	.997	82913644234868450 00000000000.000

a. Variable(s) entered on step 1: Family. Hx.DM, Hx.Toxoplasma2, IGG2, Age.Groups, BMI.25, RBS.153, FBS.92, HbA1c.6.5

DISCUSSION

The association between GDM and toxoplasmosis infestation has limited data to be reviewed so this study aims to explain this issue among pregnant ladies in Thi-Qar province [15]. The mean age of the pregnant ladies was 28 ±6-years-old and more than half of them were in the second decade of life and more than 80% of them were in the second and third decades of age. This illustrates that most of the enrolled ladies were getting their pregnancies during the maximum fertile period between 20-40 years. However, pregnant women with GDM in this study were significantly older in age than those without GDM and they were heavier in body weight, and more obese making them at multiple risk factors for getting hyperglycemia ($p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). These findings were highly logical and acceptable as the onset of gestational diabetes is significantly influenced by age and pregnant women between the ages of 25 and 40 have a gradually higher incidence of GDM as they mature [10,16,17].

As pregnant ladies with GDM had a higher body weight and higher BMI than those without GDM, these findings were statistically significant (p-value<0.001%), and it was similar to the studies in the Middle East and North Africa [10,18]. A higher risk of GDM has been linked to maternal overweight/obesity and increased prenatal weight gain, according to meta-analyses of research mostly in European and North American populations [19]. Also, prior research has indicated that a higher BMI in pregnant women is a risk factor for GDM and the increase in body weight is due to increased insulin resistance which enhances the development of GDM [20].

As a common clue, the pregnant women with GDM had a statistically significantly higher blood sugar level than those without GDM as same as in a case-control study [18], but HbA1c was statistically not significant among both groups ($p=0.152$). This was in contrast to the study of Shandong, China [21]. There is strong evidence that HbA1c is an inaccurate test for assessing glycemic levels during pregnancy and that anemia and kidney illness may have an impact on it [22]. Pregnant women often have anemia, especially in the later stages of their pregnancy [23].

Most of the pregnant ladies in both groups were living in urban districts without significant differences between women with or without GDM and the degree of education levels was comparable between them where no significant difference in the level of education was observed and it was in agreement with the Iranian study[18]. In general, the rural population is less likely to develop GDM due to the nature of the rural lifestyle, which requires doing work with great physical effort. In addition, the modernization habits among the urban population by excess fast food and sedentary lifestyle behaviors.

A strong family history of DM was found in more than half (58%) of ladies with GDM as compared with one-third (35%) of ladies without GDM; this may predispose those women to a higher additive risk for developing new GDM and it was as higher as than the HAPO study cooperative research group [24] and in agreement with studies in Shandong, China, and Vienna [21,25]. The occurrence of GDM is significantly influenced by a family history of the disease, and genetics may be connected to susceptibility genes and an intrauterine high glucose environment [26].

Nonetheless, PCOS is a prevalent endocrine and metabolic condition in women and is thought to be a significant risk factor for pregnant women's increased prevalence of GDM [27]. In this study, pregnant ladies with GDM had a higher rate of PCOS (17%) than those without (11%), it was numerically not significant ($p = 0.265$). These data were in contrast to the evidence in Greece and Shandong, China [21,28], while some evidence demonstrates the incidence of GDM complicated by the increasing PCOS[29]. Although a history of PCOS played an important risk factor for the development of T2D as documented in a local study by dysregulated insulin secretory function and glucose intolerance [30], there is debate and a paucity of data on the association between PCOS and GDM [31].

The American College of Obstetricians and Gynecologists defines macrosomia as a birth weight greater than 4,000 grams, and it affects approximately 9% of births globally. It has been shown that a higher prevalence of macrosomia was linked to both pre-GDM and GDM. This is probably going to raise the chance of maternal T2DM in the future [32]. Despite, the high rate of macrosomia among ladies with GDM (14.5%) than those without (5.6%), there was no significant difference between both groups. This agreed with the study in Tanzania [33]. Further studies are required to judge this issue in the future to allocate this unpredicted truth.

In this study, contact with indoor cats was observed among half of pregnant ladies with GDM and 43.8% of pregnant ladies without GDM. These findings were statistically no significant association between contact with domestic animals and GDM.

Toxoplasmosis infestation depending on positive serology of IGG was significantly higher among ladies with GDM (43.5%) than those without (20.2%) in this case-control study ($p = 0.002$). this was also seen in a cross-sectional study in Prague [34], and in Kurdistan, west of Iran [35]. According to these results, people who have toxoplasmosis may be more susceptible to developing new GDM with an enigmatic etiology [8]. Previous studies show a plausible correlation between

toxoplasmosis and DM would have noteworthy clinical implications, providing insight into the intricate pathophysiology of the disease. Overall, the current theory indicates that toxoplasmosis raises the risk of developing diabetes, but it also makes diabetic individuals more susceptible to opportunistic infections like toxoplasmosis [2]. Various empirical evidence has been evaluated and suggested as plausible pathophysiological mechanisms to explain this association. These include (1) enhanced migratory feature of infected white blood cells, which facilitates the spread of Toxoplasma in body organs, such as the pancreas; (2) the possibility that *T. gondii* infection could initiate a clinically noticeable autoimmune process, driving immune machinery toward the production of autoantibodies, such as those against Langerhans islets cells [36]. Pancreatic tissue may be directly attacked and compromised by organisms. They may also raise the risk of both acute and chronic pancreatitis, as well as diabetes, by destroying β cells and insulin secretion [37]. Toxoplasmosis infestation was not statistically significantly associated with the type of residency of the pregnant ladies. The most seropositive of toxoplasmosis infestation were living in urban areas (84.4%) than those in rural regions. Their results were in agreement with a study in Kirkuk [38]. The reason behind this could be that women's immunity is weakened by the high population density and air pollution in the city center as compared to rural areas. Apart from unhealthful dietary habits like consuming fast food, they also have the possibility of acquiring toxoplasmosis [39].

The current study showed no statistically significant association between the education level of pregnant ladies and the seroconversion of toxoplasmosis. It was more predominant seropositive toxoplasmosis among primary schools than intermediate and university level and less frequent among illiterates. This could be aberrantly unlogic especially when some studies showed a low level of education was associated with a higher rate of toxoplasmosis making our finding in contrast to the study of Sanandaj, west of Iran [35]. The risk of infection during pregnancy can be increased by low socioeconomic status and a lack of valuable knowledge about the disease's route of transmission [40]. However, both the history of PCOS and the family history of DM were not statistically significant in the incidence of toxoplasmosis infestation among pregnant ladies.

In this study, Toxoplasmosis infestation has a significant effect on the fate of pregnancy as an increase in the risk and frequency of abortion is correlated positively with positive toxoplasmosis (71.1% vs 23.6%, p-value<0.001). This was as same as the study in Yemen [41], But it was inconsistent with a study in Sanandaj, west of Iran [42,43]. The whole risk for abortion is approximately 0.5% among pregnant individuals who had positive seroconversion and in pregnancies with documented fetal infection, the risk of fetal demise seems to be 1.3 to 1.6 %[44]. Although there was a higher frequency of dead births in the seropositive toxoplasmosis than negative group, there was no significant association between these parameters (p=0.056) as also observed in a cross-sectional study in Mexico [45]. In this study, contact with indoor cats was more frequent among ladies with positive toxoplasmosis (57.8%) than (41.5%) with negative toxoplasmosis, but this result cleared no significant statistical difference (p-value =0.067). this finding was in agreement with the study in Sanandaj, west of Iran[42]. Pregnant women's history of cat contact is frequently cited as a risk factor for T. gondii infection. Moreover, the type of cat in a given country may have an impact on the parasite's prevalence among domestic cats [42].

The current study did not find any significant statistical difference between the history of macrosomia with toxoplasmosis (p=0.262). despite the results showing a higher rate of macrosomia in positive toxoplasmosis (13.3%) than (7.5%) with negative toxoplasmosis. Furthermore, the incidence of congenital malformation was significantly higher among seropositive toxoplasmosis (9%) than those negative (2%) (p=0.004). The low rate of vertical transmission supported this, as did a research conducted in Saudi Arabia [46], which was further supported by a thorough assessment of the literature. The point in pregnancy at which a mother contracts toxoplasmosis has a major impact on the likelihood of transmission and the intensity of fetal illness. By the end of the pregnancy, transmission has increased to about 80% from a very low (<20%) rate in the first trimester [47]. Despite the fact that transmission rates are highest in the final trimester, the majority of cases are subclinical, leading to recurrent chorioretinitis or silent infections that persist into early adulthood and can threaten eyesight and even cause blindness. The infection causes spontaneous miscarriage, hydrocephaly, and mental disability in early-gestation instances, which are severe [48].

As mentioned earlier, the proportion of new GDM among seropositive toxoplasmosis pregnant ladies was significantly higher than those with negative toxoplasmosis (60% vs 33%, p=0.002). This study agrees with a cross-sectional study in Prague [34]. Li et al. demonstrated T. gondii infection is associated with different types of DM in eastern China and patients with DM had higher frequencies of antibodies against *T. gondii* as compared to control subjects [49]. Regarding the duration of GDM, it had no effect during this study which also was disagreeable with that documented in Southwest Iran [50]. After doing a logistic regression analysis, the second decade of age is the independent risk factor for developing new GDM, while other parameters like

toxoplasmosis infestation and positive IGG were dependent risk factors for developing new GDM in collaboration with other patients profiles.

Limitations

This study has certain limitations. Firstly, the small sample size may have an impact on the results. Secondly, polymerase chain reaction is not available for a conclusive toxoplasmosis diagnosis instead of serological testing. Lastly, the gold standard method for detecting GDM in pregnant women is lacking the two- or three-step glucose tolerance testing. Therefore, more research is needed to evaluate these problems in the future.

CONCLUSION

The second decade of age is the independent risk factor for developing new GDM, while toxoplasmosis infestation and positive IGG were additive-dependent risk factors for developing new GDM in collaboration with other patients' profiles. Pregnant women with GDM were significantly older, heavier in body weight, and obese than those without GDM making them at higher risk factors for getting hyperglycemia.

Conflict of interest: none declared

Financial support: none declared

Ethical considerations:
Informed consent was taken from each enrolled pregnant lady and ethical approval was cleared from the ethical committee of the targeted institutions according to Helsinki rules by the number (143/2023 on 6 July 2023)

Author's contribution:
Conceptualization, Zainab A. Abd Alredha, Dawood S. Mahdi; methodology Zainab A. Abd Alredha; software, Dawood S. Mahdi; validation, Zainab A. Abd Alredha, formal analysis, Zainab A. Abd Alredha; investigation, Mahmood Th. Altemimi; resources, Dawood S. Mahdi; data curation, Mahmood Th. Altemimi; writing–original draft preparation, Mahmood Th. Altemimi; writing–review and editing, Dawood S. Mahdi; visualization Zainab A. Abd Alredha; supervision, Mahmood Th. Altemimi; project administration, Zainab A. Abd Alredha; funding acquisition, Dawood S. Mahdi. All authors have read and agreed to the published version of the manuscript.

Acknowledgment: Authors would like to thank the participants for their acceptance and cooperation in this study.

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