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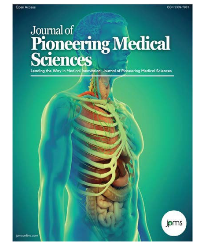

Association of Toxoplasma gondii Seropositivity, Obesity, and Type 2 Diabetes mellitus with Spontaneous Abortion among Women in Makkah: A Case-Control Study

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Abstract: **Background:** Spontaneous abortion is a common adverse pregnancy outcome caused by a number of infectious and metabolic risk factors. Toxoplasma gondii infection has been associated with loss of pregnancy and obesity and type 2 Diabetes mellitus (T2DM) are becoming increasingly important determinants of reproductive health in women. However, there is limited information on the combined effect of these factors on women in Saudi Arabia. **Methods:** In this study, the association between Toxoplasma gondii infection, obesity, T2DM, and spontaneous abortion was investigated in women in Makkah, Saudi Arabia. We employed a case-control study among 194 women of reproductive age who were in Makkah hospital and pregnant. The participants were classified into cases (women with a history of spontaneous abortion) and controls (women who did not have a history of spontaneous abortion). Sociodemographic, clinical, and obstetric records were collected using a questionnaire. Body mass index (BMI) was measured for obesity status and T2DM status was retrieved from medical records. Serum samples were examined for anti-Toxoplasma gondii IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA). The association between study variables and spontaneous abortion was tested by Chi-square tests and multivariable logistic regression. We calculated adjusted odds ratios and 95% confidence intervals (CIs) for these studies. **Results:** For 189 participants, the Toxoplasma gondii seropositivity was higher in women with a history of spontaneous abortion than in controls. The multivariable logistic regression of T. gondii infection revealed that T. gondii infection was associated with a higher likelihood of spontaneous abortion independently, and obesity and T2DM were also associated with pregnancy loss independent of any other potential factors. Additionally, toxoplasmosis and metabolic disorders were correlated to a higher chance of spontaneous abortion than without either exposure. T. gondii infection, obesity, and type 2 diabetes mellitus were associated with spontaneous abortion in women in Makkah. **Conclusion:** In conclusion, this study demonstrates that Toxoplasma gondii infection, obesity, and type 2 diabetes mellitus are independently associated with spontaneous abortion among women in Makkah, Saudi Arabia. Furthermore, evidence suggests a possible synergistic interaction between infectious and metabolic factors. These findings underscore the importance of integrated reproductive health strategies that address both infectious disease prevention and metabolic risk management to reduce the burden of spontaneous abortion.

Key Words: Toxoplasma Gondii, Toxoplasmosis, Spontaneous Abortion, Miscarriage, Obesity, Type 2 Diabetes Mellitus, Pregnancy Loss, Saudi Arabia, Makkah, Women's Health

INTRODUCTION

Spontaneous abortion remains one of the most common adverse pregnancy outcomes worldwide and a major public health concern due to its medical, psychological, and socioeconomic implications [1-2]. The procedure is generally said to occur before the fetus is viable and occurs in about 10–20% of clinically recognized pregnancies. Although chromosomal abnormalities account for large numbers of early pregnancy losses, a number of infectious, metabolic, immunological, and environmental factors are known to contribute to spontaneous abortion [3]. Modifiable risk factors are therefore essential for preventive strategies and maternal and fetal health outcomes [4]. T. gondii is an obligatory intracellular protozoan parasite that can infect almost all warm-blooded animals, including humans. The parasite lives a complex life cycle in felids as the definitive hosts and humans as intermediate hosts. Human infection is primarily due to eating tissue cysts in undercooked meat, eating food or water that is infected with oocysts, or the transmission of the parasite from mother to fetus during pregnancy [5-7]. Toxoplasmosis is one of the most prevalent parasitic infections worldwide, and a study showed that approximately one-third of the global population has been infected by the parasite. Infection in immunocompetent individuals is rarely detected, but toxoplasmosis in pregnancy is potentially life-threatening for the mother and fetus [8-10]. The primary maternal infection may lead to the transplacental transmission of the parasite and result in congenital toxoplasmosis, fetal growth restriction, neurological problems, visual impairment, stillbirth, and spontaneous abortion [11-12]. Fetal complications are highly diverse in different parts and vary in terms of the age at which infection occurred, the infection burden, maternal immune status, and diagnosis and treatment time [13]. Thus, toxoplasmosis remains a significant problem in reproductive and maternal health programs worldwide [14]. There are several epidemiological studies that have examined the relationship between T. gondii infection and spontaneous abortion [15]. The most recent evidence from a systematic review and meta-analysis showed that women with spontaneous abortion had significantly higher odds of T. gondii exposure compared to women with no history of abortion [15]. Also, recent infection was associated with an increased risk of pregnancy loss, which means that the active or newly acquired toxoplasmosis might be directly affecting pregnancy outcomes [16]. These results support the biological rationale that toxoplasmosis may play a major role in fetal loss through placental infection, inflammatory responses, and disruption of normal fetal development. The burden of toxoplasmosis is particularly relevant in the Middle East, where climate, diet, and environmental conditions may promote transmission [17]. The seroprevalence rates in many countries in the region have been reported in the past to be relatively high. Toxoplasmosis is still an important public health issue among women of reproductive age in Saudi Arabia [18]. A systematic review and meta-analysis of 13,000 Saudi women from 1994 to 2023 estimated a seroprevalence of 27.8%, indicating that approximately one in four women had previous exposure to T. gondii. Age, environmental exposure, and behavioral factors were associated with infection [19]. More recently, a comprehensive meta-analysis of studies performed in Saudi Arabia from 1994 to 2023 found an overall IgG seroprevalence of 27.5% and an IgM seroprevalence of 2.2% [20]. In pregnant women, the pooled IgG seroprevalence was 28%, and the parasite remained circulating in women in their reproductive years [20]. Importantly, the work included in this study also found a high prevalence of toxoplasmosis in patients with previous abnormal pregnancy outcomes such as spontaneous abortion [20]. The findings of this study underscore the necessity for further investigation into the role of toxoplasmosis in reproductive health in Saudi Arabia [20].

Metabolic disorders have been identified as major contributors to adverse reproductive outcomes along with infectious diseases [21]. Obesity is a worldwide epidemic and is very prevalent among women of childbearing age. Obesity is associated with chronic low-grade inflammation, insulin resistance, endocrine dysfunction, oxidative stress, and changes in reproductive physiology. Obesity has been shown to increase the risk of infertility, recurrent pregnancy loss, gestational complications, and adverse neonatal outcomes [22-25]. The connection between obesity and spontaneous abortion is thought to be mediated by impaired endometrial receptivity, abnormal placentation, altered embryo development, and inflammation [26]. Type 2 diabetes mellitus (T2DM) is also an increasing public health concern in the world and is more prevalent among women of reproductive age in the present era [27, 28]. Poor glycemic control before and during pregnancy has been linked to miscarriage, congenital anomalies, fetal development problems, and maternal complications. Hyperglycemia can induce oxidative stress, endothelial dysfunction, and inflammatory activities that can adversely affect implantation and embryonic development [29]. Diabetes-related vascular changes can also interfere with placental function and cause pregnancy loss. So obesity and T2DM are both significant risk factors for adverse reproductive outcomes [30].

New evidence suggests that metabolic disorders may also play a role in susceptibility to infectious diseases and in modifying host immune responses [31]. Obesity is characterized by immune dysfunction and chronic inflammation and impaired cellular immunity, which may impair the host's ability to control parasites [32]. Diabetes mellitus is associated with poor innate and adaptive immune responses that make it more susceptible to various infectious agents [33]. These observations suggest that obesity and T2DM might interact with T. gondii infection and potentially amplify its negative impact on pregnancy outcomes. However, the nature of this interaction remains insufficiently understood. The main aim of the article was to clarify the relationship between obesity, T2DM, T. gondii infection and the abortion.

METHODS

Study Design and Setting

In Makkah, Saudi Arabia, a case-control study to investigate Toxoplasma gondii infection, obesity, type 2 Diabetes mellitus (T2DM), and spontaneous abortion among women of reproductive age was conducted. The study was conducted in hospitals and laboratories in Makkah between June 2024 and April 2025.

Study Population

189 women aged 18-49 years were enrolled in the study. Women were divided into two groups based on their reproductive history:

- **Cases:** Women with a documented history of one or more spontaneous abortions

- **Controls:** Women with no history of spontaneous abortion and at least one successful pregnancy outcome

Women with induced abortions, ectopic pregnancies, known chromosomal abnormalities, autoimmune disorders, malignancies, or incomplete medical records were excluded from the study.

Sample Size

The study included 189 participants recruited through a consecutive sampling technique from eligible women attending participating healthcare facilities during the study period.

Data Collection

Data were collected using a structured questionnaire administered by trained research personnel. Information obtained included:

- Potential risk factors for toxoplasmosis include cat ownership or exposure, eating undercooked meat, source of drinking water, and contact with soil
- Anthropometric measurements were obtained according to standard procedures. Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Participants were classified according to World Health Organization criteria. Obesity was defined as BMI ≥30 kg/m^2
- Potential risk factors for toxoplasmosis, including cat ownership or exposure, consumption of undercooked meat, source of drinking water, and contact with soil

Laboratory Assessment

Venous blood samples were collected from all participants under aseptic conditions and stored at -20°C until analysis. Anti-Toxoplasma gondii IgG and IgM antibodies were detected using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's directions. Participants were classified as seropositive or seronegative according to cut-off values provided by the manufacturer. Type 2 diabetes mellitus was diagnosed based on a diagnosis from a doctor, documented diagnosis by the patient, or medical records indicating a confirmed diagnosis.

Outcome Variable

The primary outcome variable was a history of spontaneous abortion (yes/no).

Exposure Variables

The primary exposure variables included:

- Toxoplasma gondii seropositivity (IgG and/or IgM)
- Obesity (BMI ≥30 kg/m^2)
- Type 2 diabetes mellitus

Ethical Considerations

The study protocol was approved by the Institutional Review Board of PHHI (Approval No 1435). Written informed consent was obtained from all participants before enrollment. Confidentiality and anonymity were maintained throughout the study in accordance with the Declaration of Helsinki.

RESULTS

Sociodemographic and Clinical Characteristics of Participants

A total of 189 women were included in the analysis. Participants were classified into cases (history of spontaneous abortion, n = 102) and controls (no history of spontaneous abortion, n = 87). The mean age of the participants was 31.2±6.0 years. The majority of the participants had a graduate-level education (34.9%). Obesity (BMI ≥30 kg/m^2) was identified in 84.1% of the participants, while type 2 diabetes mellitus (T2DM) was present in 51.9%. Toxoplasma gondii IgG antibodies were detected in 35.4% of the study population, indicating previous exposure to the parasite, whereas IgM antibodies were detected in only 1.1%, suggesting that a very small proportion of participants had a relatively recent infection. Table 1 summarizes the baseline characteristics of the study population according to abortion status.

Association Between Study Variables and Spontaneous Abortion

In the univariate analysis, Toxoplasma gondii IgG seropositivity was significantly associated with spontaneous abortion (crude OR = 0.29, 95% CI: 0.15–0.54, P <0.001). Women aged 28–45 years had significantly higher odds of spontaneous abortion than those aged 18–27 years (OR = 2.45, 95% CI: 1.30–4.63, P = 0.009). Type 2 diabetes mellitus (T2DM) was also significantly associated with spontaneous abortion (OR = 0.39, 95% CI: 0.22–0.71, P = 0.002), whereas obesity was not significantly associated with abortion risk (OR = 0.75, 95% CI: 0.34–1.68, P = 0.601). No significant association was observed for Toxoplasma gondii IgM seropositivity (P = 0.409). Other variables, including parity and exposure to cats, were also evaluated and should be reported according to the results of the univariate logistic regression analysis.

Multivariable Logistic Regression Analysis

A multivariable logistic regression model was constructed to identify independent predictors of spontaneous abortion. The overall model was statistically significant (Omnibus χ^2 = 54.55, P <0.001), explained 33.5% of the variance in spontaneous abortion (Nagelkerke R^2 = 0.335), and demonstrated acceptable calibration according to the Hosmer–Lemeshow goodness-of-fit test (χ^2 = 8.92, df = 8, P = 0.349). The model correctly classified 72.0% of participants.

After adjustment for potential confounders, T. gondii IgG seropositivity remained a strong independent predictor

Table 1: Baseline Characteristics of Participants According to Abortion Status

Variable	Abortion Group (No. =102)	No abortion Group (No. = 78)	Total (No. = 189)	P-Value
Age-group				
18-27 Years	22 (21.6%)	35 (40.2%)	57 (30.2%)	0.009
28-45 Years	80 (78.4%)	52 (59.8%)	132 (69.8%)	
Education				
Educated	6 (5.9%)	8 (9.2%)	14 (7.4%)	0.556
Uneducated	96 (94.1%)	79 (90.8%)	175 (92.6%)	
BMI ≥30 kg/m²				
Yes	84 (82.4%)	75 (86.2%)	159 (84.1%)	0.601
No	18 (17.6%)	12 (13.8%)	30 (15.9%)	
Type 2 Diabetes mellitus				
Yes	42 (41.2%)	56 (64.4%)	98 (51.9%)	0.002
No	60 (58.8%)	31 (35.6%)	91 (48.1%)	
Toxo-IgG				
Positive	23 (22.5%)	44 (50.6%)	67 (35.4%)	
Negative	79 (77.5%)	43 (49.4%)	122 (64.6%)	0.001

Table 2: Multivariable Logistic Regression Analysis of Factors Associated with Spontaneous Abortion

Variable	Adjusted Odd Ratio (AOR)	95% CI	P-value
Race			
Arab	-	-	0.573
Asia	1.34	0.24–7.53	0.739
Africa	2.52	0.32–20.03	0.382
Education			
Graduate	-	-	0.062
Illiterate	5.07	1.18–21.82	0.029
Primary	3.85	1.17-12.65	0.027
Intermediate	4.25	1.22-14.88	0.024
Secondary	1.72	0.65-4.51	0.272
Duration of marriage			
>5 years	-	-	0.178
<1 year	3.85	0.89-16.66	0.072
1–5 years	1.19	0.48-2.97	0.709
Occupation			
Student	-	-	0.517
Housewife	0.94	0.12-7.26	0.949
Employee	1.84	0.21-15.91	0.578
Obesity (BMI ≥30 kg/m^2)			
No	-	-	-
Yes	1.17	0.43-3.20	0.766
T. gondii IgG			
Negative	5.38	2.50-11.58	<0.001
Positive	-	-	-
Type 2 diabetes mellitus			
Negative	-	-	-
Positive	3.71	1.77-7.75	<0.001

of spontaneous abortion (AOR = 5.38, 95% CI: 2.50–11.58, p<0.001). Likewise, women with type 2 diabetes mellitus had significantly greater odds of spontaneous abortion than non-diabetic women (AOR = 3.71, 95% CI: 1.77–7.75, P <0.001). Educational attainment was also independently associated with spontaneous abortion, with illiterate, primary, and intermediate educational levels showing significantly higher odds than graduate-level education. In contrast, age group, obesity, race, duration of marriage, and occupation were not independently associated with spontaneous abortion after adjustment for other variables Table 1-2.

DISCUSSION

This case–control study investigated the association between T. gondii infection and spontaneous abortion among women in Saudi Arabia while considering demographic and metabolic risk factors. The findings demonstrate that T. gondii IgG seropositivity and type 2 diabetes mellitus (T2DM) were independently associated with spontaneous abortion after adjustment for potential confounders. Although obesity and older age were associated with abortion in the univariate analysis, these associations were no longer significant after multivariable adjustment, indicating that their effects were largely explained by other covariates included in the model.

The overall seroprevalence of T. gondii IgG antibodies was 35.4%, indicating that more than one-third of the study participants had previous exposure to the parasite. In contrast, IgM seropositivity was rare (1.1%), suggesting that acute or recently acquired infection was uncommon in this population. These findings are consistent with the biological behavior of T. gondii, where IgG antibodies persist for life following infection, whereas IgM antibodies usually decline within months after primary infection [34- 35]. The relatively high IgG prevalence observed in the present study is comparable to reports from several Middle Eastern countries, where climatic conditions, dietary habits, and close contact with domestic animals contribute to sustained exposure to the parasite.

In the univariate analysis, women with T. gondii IgG seropositivity demonstrated a significant association with spontaneous abortion. More importantly, after controlling for age, race, education, duration of marriage, occupation, obesity, and diabetes, IgG seropositivity remained the strongest independent predictor of spontaneous abortion (AOR = 5.38, 95% CI: 2.50–11.58, $P < 0.001$). This finding suggests that previous exposure to T. gondii is strongly associated with pregnancy loss in the study population. Several biological mechanisms may explain this association. Although latent infection is generally considered clinically silent in immunocompetent individuals, parasite reactivation or chronic inflammatory responses during pregnancy may adversely affect placental function and fetal development [36]. In addition, women with chronic infection may experience altered immune regulation, increasing susceptibility to placental damage and fetal loss [37].

Type 2 diabetes mellitus also emerged as an independent predictor of spontaneous abortion, with diabetic women having approximately 3.7-fold higher odds of abortion than non-diabetic women. This observation is biologically plausible because maternal diabetes is associated with chronic systemic inflammation, endothelial dysfunction, oxidative stress, impaired placental vascularization, and poor glycemic control, all of which increase the likelihood of adverse pregnancy outcomes [38]. Furthermore, hyperglycemia has been reported to impair innate and adaptive immune responses, potentially increasing susceptibility to opportunistic infections, including T. gondii. The coexistence of diabetes and chronic toxoplasmosis may therefore contribute to a cumulative increase in reproductive risk [39].

Although obesity appeared to be associated with spontaneous abortion in the crude analysis, it was no longer statistically significant after adjustment for other variables (AOR = 1.17, 95% CI: 0.43–3.20, $P = 0.766$). This finding suggests that obesity itself may not independently increase abortion risk in this study population but may instead exert its influence indirectly through metabolic abnormalities, particularly diabetes. Previous epidemiological studies have similarly demonstrated that the adverse reproductive effects attributed to obesity are often attenuated after adjustment for glycemic status and other metabolic disorders [40-41].

Women aged 28–45 years showed higher crude odds of spontaneous abortion compared with younger women, however, age was not retained as an independent predictor in the adjusted model. This finding suggests that the increased risk observed among older women may be explained by the greater prevalence of comorbid conditions such as diabetes or previous reproductive complications rather than chronological age itself. Similar observations have been reported in reproductive epidemiology studies, where maternal age frequently loses statistical significance after adjustment for clinical risk factors [42].

Educational level demonstrated a significant association with spontaneous abortion. Women with illiterate, primary, or intermediate educational attainment had significantly higher odds of spontaneous abortion than women with graduate-level education. Educational attainment often reflects health literacy, socioeconomic status, access to healthcare services, nutritional practices, and awareness of preventive measures against infectious diseases. Consequently, lower educational status may indirectly increase exposure to preventable reproductive risk factors, including inadequate food hygiene and limited awareness of toxoplasmosis prevention.

No significant associations were observed for race, occupation, or duration of marriage after multivariable adjustment. These findings suggest that these demographic variables contributed relatively little to spontaneous abortion risk once biological and metabolic factors were considered. However, the wide confidence intervals observed for some categories indicate limited statistical precision due to relatively small subgroup sizes.

The multivariable logistic regression model demonstrated satisfactory performance. The model was statistically significant, explained approximately one-third of the variation in spontaneous abortion (Nagelkerke $R^2 = 0.335$), correctly classified 72.0% of participants, and showed good calibration according to the Hosmer–Lemeshow goodness-of-fit test. These findings indicate that the selected predictors provide a reasonable explanation of spontaneous abortion risk in this study, although additional biological, environmental, genetic, and obstetric factors not included in the model are likely to contribute to pregnancy loss.

The present study has several strengths. It represents one of the few investigations evaluating both infectious and metabolic determinants of spontaneous abortion among women in Saudi Arabia using multivariable logistic regression analysis. The inclusion of several demographic and clinical covariates allowed adjustment for important confounding factors, thereby strengthening the validity of the observed associations.

Nevertheless, several limitations should be acknowledged. First, the case–control design precludes establishing temporal or causal relationships. Second, exposure to T. gondii was assessed serologically, and molecular confirmation using polymerase chain reaction (PCR) was not performed. Third, information regarding dietary habits, consumption of undercooked meat, contact with cats, socioeconomic status, and glycemic control during pregnancy was not comprehensively evaluated. Finally, the study was conducted at a single center, which may limit the generalizability of the findings to other populations within Saudi Arabia.

Overall, this study demonstrates that T. gondii IgG seropositivity and type 2 diabetes mellitus are independent predictors of spontaneous abortion among women in Saudi Arabia. These findings support the importance of strengthening preconception and antenatal screening programs for toxoplasmosis, particularly among women with metabolic disorders. Public health interventions aimed at improving awareness of toxoplasmosis prevention, promoting food hygiene, encouraging appropriate glycemic control, and identifying women at increased reproductive risk may contribute to reducing spontaneous abortion and improving maternal and fetal outcomes.

Strengths and Limitations

This study has several strengths, including its relatively adequate sample size, the use of serological testing for T. gondii infection, and the simultaneous evaluation of multiple metabolic risk factors. The inclusion of women from Makkah enhances the relevance of the findings to a population with unique environmental and demographic characteristics. However, several limitations should be acknowledged. First, the case-control design limits the ability to establish causality. Second, recall bias may have affected self-reported data on exposure variables such as dietary habits and cat contact. Third, residual confounding cannot be excluded despite multivariable adjustment. Fourth, the use of serological markers alone may not distinguish between acute and chronic infection in all cases, particularly in IgG-positive individuals without IgM confirmation.

Recommendations For Future Research

Future prospective cohort studies are needed to confirm the temporal relationship between Toxoplasma gondii infection and spontaneous abortion. Additionally, studies incorporating molecular diagnostic methods, inflammatory biomarkers, and detailed metabolic profiling could provide deeper insight into the underlying mechanisms. Research exploring gene–environment and infection–metabolism interactions in reproductive outcomes are also recommended.

CONCLUSION

In conclusion, this study demonstrates that Toxoplasma gondii infection, obesity, and type 2 diabetes mellitus are independently associated with spontaneous abortion among women in Makkah, Saudi Arabia. Furthermore, evidence suggests a possible synergistic interaction between infectious and metabolic factors. These findings underscore the importance of integrated reproductive health strategies that address both infectious disease prevention and metabolic risk management to reduce the burden of spontaneous abortion.

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