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Prevalence of *Toxoplasma gondii* and Herpes Simplex Virus Utilising Real-Time PCR in Pregnant and Aborted Woman

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Abstract

Toxoplasmosis and Herpes simplex virus (HSV) infections may have severe effects especially in pregnancy resulting in a greater risk of unfavorable pregnancy outcomes. The purpose of this study was to research the prevalence of T. gondii and HSV mixed infection in pregnant women and aborted women. The sampled population consisted of 250 patients; 100 pregnant, 100 abortions, and 50 healthy controls. Rapid diagnostic tests of Toxoplasma IgG antibodies and real-time PCR of the T. gondii and HSV DNA were conducted. Positive Toxoplasma IgG showed in 23% of pregnant, 21% of abortion and 0% in healthy women. It was found by real-time PCR that 19% of pregnant women and 16% of aborted women were positive of T. gondii, and 7% of pregnant and 17% of abortion women were positive of HSV. The difference was significant in the prevalence of T. gondii and HSV infection in study groups ($p < 0.05$).

Keywords: Herpes simplex virus, *Toxoplasma gondii*, and Real-time PCR.

1. Introduction

One of the main concerns of public health for people worldwide is Toxoplasmosis, which is a parasitic infection brought by a protozoan organism called *T. gondii* [1, 2]. Most healthy individuals do not show any symptoms of the infection, and it may lead to serious complications in immunocompromised patients and pregnant women [3].

The infection of *T. gondii* during pregnancy may cause congenital toxoplasmosis, which may become manifested in different ways, such as miscarriage, stillbirth, and neurological or ocular problems in the fetus [4]. On the

contrary, HSV infections also pose a significant health problem especially among pregnant women and neonates. During pregnancy, delivery, or postpartum, HSV may be passed to the child and cause severe neonatal infections, which may be life-threatening [5]. Having a mixed infection with *T. gondii* and HSV may lead to a synergetic effect, which may result in the risk of having an adverse outcome during pregnancy [6].

Historically, the diagnosis of toxoplasmosis has been carried out through serological tests, e.g. enzyme-linked immunosorbent assay (ELISA). These tests however have weaknesses in sensitivity and specificity particularly in acute infection or reactivation [7]. In more recent times, the molecular methods, including real-time-PCR, have become useful either in the direct detection of *T. gondii* and HSV DNA in a wide variety of biological samples, including blood, amniotic fluid, and cerebrospinal fluid [8]. Some of studies have examined the prevalence of *T. gondii* and HSV mixed infection among various groups of people such as pregnant women and immunocompromised [9].

The proposed investigation is focused on thoroughly examining the *T. gondii* and HSV mixed infections prevalence in pregnant women, women who undergo an abortion, and healthy controls using real-time PCR and rapid diagnostic tests. Results of the present study could be of great clinical value in the prevention, early detection, and management of these mixed infections.

2. Materials and Methods

2. 1 Sample Collection and Serological Screening for *T. gondii*

Venous blood samples (5 mL) were collected from 250 participant (100 pregnant, 100 abortion, and 50 healthy women as a control) under aseptic conditions. A rapid immunochromatographic test (Toxoplasma IgG Rapid Test Card) was used for the detection of *T. gondii* - specific IgG antibodies in serum, following the manufacturer's instructions.

2. 2 Molecular Detection of *T. gondii* and HSV by Real-Time PCR

2. 2. 1 Genomic DNA Extraction

DNA was extracted from 200 µL of whole blood using the gSYAN DNA Extraction Kit (Geneaid, Taiwan) according to the manufacturer's protocol. DNA concentration and purity were assessed using a Nanodrop spectrophotometer.

2. 2. 2 Primers and Probes

Sequence-specific primers and TaqMan probes were used for the detection of *T. gondii* (B1 gene) and HSV (DNA polymerase gene). All oligonucleotides were synthesized by Macrogen (Korea) as listed in table 1.

Table1: Primers and probes for RealTime-PCR detection of T. gondii and HSV.

Target	Primer/Probe	Sequence (5'→3')	Product Size
T. gondii	Forward	TCCCCTCTGCTGGCGAAAAGT	113 bp
	Reverse	AGCGTTCGTGGTCAACTATCGATTG	
	Probe	FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA	
HSV	Forward	CATCACCGACCCGGAGAGGGAC	92 bp
	Reverse	GGGCCAGGCGCTTGTTGGTGTA	
	Probe	FAM-CCGCCGAACTGAGCAGACACCCGCGC-TAMRA	

2. 2. 3 Real-Time PCR Assay

Each reaction was performed in a final volume of 20 µL using the RealMOD™ Probe 2X qPCR Mix (INtRON, Korea) on a MiniOpticon Real-Time PCR System (Bio-Rad, USA) as listed in table 2.

Table 2: Real-Time PCR master mix composition.

Component	Volume (µL)
qPCR Master Mix (2X)	10.0
Forward Primer(10pmol/µL)	1.0
Reverse Primer (10pmol/µL)	1.0
Probe (20pmol/µL)	1.0
DNA Template	5.0
Nuclease-Free Water	2.0
Total Volume	**20.0**

3. Results

3. 1 Serological Evidence of Past *Toxoplasma gondii* Exposure

The first diagnostic screening was used to determine the seroprevalence of previous exposure to *T. gondii* by using rapid diagnostic test of Toxoplasma - IgG antibodies tables 3, and 4. This test gives an account of lifetime exposure to the parasite. Their findings, which are fully reported in table 5, showed that the different groups had different serological patterns. The difference was significant at P = 0.001.

Table 3: Rapid diagnosis of Toxoplasmosis in pregnant, abortion and healthy women.

Antibody (IgG)	Pregnant women *n* = 100	Aborted women *n* = 100	Healthy control *n* = 50
Positive, *n* (%)	23 (23.0%)	21 (21.0%)	0
Negative, n (%)	77 (77.0%)	79 (79.0%)	50 (100.0%)
P value	0.001 S		

Among the 100 pregnant women, IgG of *T. gondii* was positive in 23 out of 100. This shows that close to a quarter of the pregnant participants had been infected with the parasite at some time before the study. This tendency was also present in the aborted women group in which 21 out of 100 participants were seropositive. These statistics imply that the prevalence of toxoplasmosis in the female population of reproductive age in study is significant background rate. Nevertheless, it should be

noted that a positive test only indicates prior exposure, which does not distinguish between a remote, latent infection, and an acute, recent, or reactivated infection, which may be clinically pertinent to the outcome of pregnancy.

3. 2 Molecular Detection of Active Infection (Real-Time PCR Findings)

A TaqMan probe-based RT-qPCR assay was used to replicate pathogens. This is a very sensitive and specific molecular method that targeted a conserved B1 gene of *T. gondii* and the DNA polymerase gene of HSV and enabled the direct identification of a pathogen DNA in whole blood samples.

3. 2. 1 Prevalence of *T. Gondii* and HSV Infection

The molecular method Real-Time PCR was used to detection the DNA of *T. gondii* and HSV in blood samples. The result was show 19% of pregnant women were positive for *T. gondii* and 7% were positive for HSV infection while aborted women show 16% were positive for *T. gondii* infection and 17% were positive for HSV infection as listed in tables 6, and 7 where differences were significant.

Table 4: Real Time PCR diagnosis of *T. gondii* among the study groups.

Real time-PCR of *T. gondii*	Pregnant women *n* = 100	Aborted women *n* = 100	Healthy control *n* = 50
Positive, *n* (%)	19 (19.0%)	16 (16.0%)	0
Negative, n (%)	81 (81.0%)	84 (84.0%)	50 (100.0%)
P value	0.005 S		

Table 5: Real Time PCR diagnosis of HSV among the study groups.

Real time-PCR of HSV	Pregnant women *n* = 100	Aborted women *n* = 100	Healthy control *n*= 50
Positive, *n* (%)	7 (7.0%)	17 (17.0%)	0
Negative, n (%)	93 (93.0%)	83 (83.0%)	50 (100.0%)
P value	0.002 S		

The comparatively high prevalence of active HSV infection in the group of aborted women is a relevant finding of the given study, which should be followed up with the studies on viral load, the time of infection in comparison with pregnancy loss, and whether it interacts with other pathogenic agents, such as *T. gondii*.

3. 3 Analysis of Risk Factors

Determination of the possible risk factors related to active infection, the cases which were positive in qPCR were compared to demographic characteristics.

3. 3. 1 Prevalence of Toxoplasmosis and HSV according to the Age

The positivity rate among pregnant women with *T. gondii* seemed to rise with age, where it reached 10% in women under

20, 19% in women between the age of 20-29, and 27.3% in women aged 30 or older. A similar (but less) pronounced trend was seen in aborted women.

However, these observed differences in *T. gondii* prevalence across age categories were not statistically significant. For HSV, the pattern was different. Among pregnant women, all seven positive cases were in the 20-29 and ≥30 age groups. Among aborted women, the highest positivity rate was surprisingly in the youngest cohort (<20 years) at 26.9%. Again, statistical analysis found no significant association between age category and HSV PCR positivity. This collective analysis suggests that within this adult female population, chronological age was not a primary independent predictor for the presence of active *T. gondii* or HSV DNA in the blood.

Table 6: Prevalence of *T. gondii* and HSV infection according to age.

Age group	*Total*	*T. gondii + ve*	*Herpes + ve*	X^2	*P value*
Pregnant women					
< 20 years	20	2 (10.0%)	0	NA	NA
20-29 years	58	11 (19.0%)	4 (6.9%)	3.752	0.053
≥ 30 years	22	6 (27.3%)	3 (13.6%)	1.257	0.262
X^2		2.031	02.995		
P value		0.362	0.224		
Aborted women					
< 20 years	26	3 (11.5%)	7 (26.9%)	1.981	0.159
20-29 years	52	11 (21.2%)	8 (15.4%)	0.580	0.446
≥ 30 years	22	2 (9.1%)	2 (9.1%)	NA	NA
X^2		2.194	2.886		
P value		0.334	0.236		

3. 3. 2 Prevalence of Toxoplasmosis and HSV according to the Residency

A larger percentage of PCR-positive cases were found in rural regions than in urban regions in both the pregnant and the aborted group of women. An example is that of pregnant women, the proportion of rural residents who were positive was 26.1% compared to 13% urban residents. This observation was not statistically significant in the group-wise comparison of pregnant women, but this observation showed significant result in the analysis of the data of the rural pregnant group. The rate *T. gondii* positivity rate was significantly higher than that of HSV positivity.

This indicates there exists a specific epidemiological profile in rural environments where *T. gondii* turns out to be more prevalent than HSV in pregnant women. In case of HSV infection, there was a more equal distribution of the urban and rural populations between the two groups of patients, and no statistically significant associations with residency identified. These data are in line with known epidemiology of toxoplasmosis under which rural living is frequently linked with the greater exposure to *T. gondii* oocysts in soil (e.g., through gardening, contact with contaminated water

or food) or the close contact with feline definitive hosts.

Table 7: Prevalence of *T. gondii* and HSV infection according to the Residency.

Residency	*Total*	*T. gondii + ve*	*Herpes + ve*	X^2	*P value*
Pregnant women					
Urban	54	7 (13.0%)	3 (5.6%)	1.763	0.184
Rural	46	12 (26.1%)	4 (8.7%)	6.046	0.014
X^2		2.780	0.376		
P value		0.095	0.540		
Aborted women					
Urban	56	6 (10.7%)	7 (12.5%)	0.087	0.768
Rural	44	10 (22.7%)	10 (22.7%)	NA	NA
X^2		2.646	1.827		
P value		0.104	0.177		

4. Discussion

The current paper gives a significant revealing the epidemiology and clinical importance of *T. gondii* and HSV infection in women of reproductive age with specific reference to pregnancy and poor outcomes. These results demonstrate that there is a significant carrying capacity of infections in the examined population both active and past with significant variations among pregnant women, women who had miscarriage previously, and healthy controls. The seroprevalence of T. *gondii* IgG antibodies in this cohort of 23% and 21% of pregnant women and aborted women, respectively, are in line with the global and regional epidemiological outcomes.

Seroprevalence estimates in the Middle East tend to be similar, with estimates being 20-40% [10]. Nevertheless, the presence of IgG antibodies just shows past exposure and cannot distinguish between latent and active infection, the latter with direct clinical consequences, particularly in pregnancy [11]. Such a drawback highlights the importance of molecular diagnostics. Real-time PCR data showed active *T. gondii* parasitemia in 19% of pregnant women and 16% of aborted women. This is a rather active infection rate compared to certain past reports in comparable environments which tend to only use serology and may potentially underestimate the weight of parasitemia [12].

The fact that found one out of every five women during pregnancy had parasitemia that was detectable, demonstrates a possible problem that has not been adequately realized in the community and hence needs to be systematically screened by prenatal screening in endemic areas. Outcomes were also very persuasive in the case of HSV. Although HSV is a prevalent viral infection worldwide, its presence in the blood through PCR implies active systemic replication, which in the case of a female of reproductive age may cause serious complications neonatal herpes, premature birth, and spontaneous abortion [13]. In current research, HSV DNA was found in 7% of the pregnant women, which is in line with the

reports that demonstrate active HSV infection in pregnant women of 5-15% [14].

Nevertheless, it increased more than twice to 17% in women who had a history of miscarriages. This is an indication of disparity that may indicate an etiological link between active HSV infection and pregnancy loss. Moreover, physiological and immunological stress is known to affect HSV reactivation, which can be increased after a miscarriage and this forms bidirectional relationship [15].

Finding that age has no significant relationship with active infection in either pathogen would indicate that during the reproductive age window, behavioral, environmental and immunological variables could play a more important role in determining the infection status compared to the chronological age. This is consistent with modern conceptions about both toxoplasmosis as well as herpes epidemiology, in which exposure risk (e.g., dietary habits, exposure to cats in the case of *T. gondii*, sexual behavior in the case of HSV) and host immunity are more important than age perse [16, 17].

This tendency toward increasing *T. gondii* PCR positivity in rural residents, especially in pregnant women, resonates with established risk factors of toxoplasmosis. Rural conditions mean more direct contact with soil, unfiltered water, and domestic or stray cats, all of which are the main sources of *T. gondii* oocysts [18]. The high disparity that was found in the rural pregnant subgroup with *T. gondii* positivity being higher than HSV positivity highlights the requirement for targeted preventive education in such communities [19].

Methodologically, this research highlights the superiority of real-time PCR to that of serological testing to detect active infection. This is in line with the present guidelines provided by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) that suggests PCR as the gold standard in the diagnosis of active toxoplasmosis in immunocompromised patients and pregnant women suspected of being infected [20].

In the case of HSV, PCR has been known to be the most sensitive technique of identity any viral DNA in the blood and other clinical specimens, particularly when undergoing asymptomatic or sub-clinical reactivation [21]. Thus suggest the inclusion of *T. gondii* and HSV PCR testing in the standard workup of pregnant women in endemic regions especially when they have a history of miscarriage or other risk factors.

5. Conclusion

The prevalence of *T. gondii* and HSV infections especially among pregnant women is very high and thus necessitates extensive screening and diagnostic strategies during routine antenatal care. Furthermore, the comparison of *T. gondii* and HSV mixed infections in pregnant women and aborted women can possibly indicate the possible synergistic outcome of these two pathogens and their relationship with a negative pregnancy outcome, including miscarriage. Such knowledge can assist in the creation of more effective preventive and management strategies for these mixed infections among high-risk groups.

Besides, the prevalence of *T. gondii* and HSV mixed infections in healthy controls may be assessed to gain a better idea about the background prevalence of such infections in the general population that is of paramount importance in the interpretation of the results in the high-risk groups. The findings advocate for a paradigm shift in prenatal and post-miscarriage care, moving beyond serology to embrace molecular diagnostics for accurate identification of active infections. Healthcare providers can implement targeted interventions that may reduce the risk of congenital disease, improve pregnancy outcomes, and ultimately enhance maternal and child health. Public health initiatives should also focus on education and preventive measures, especially in rural communities, to mitigate exposure to these consequential pathogens.

6. References

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