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Serological Prevalence of IgM and IgG Antibodies Against TORCH Infection in Pregnant Women of Peshawar, Khyber Pakhtunkhwa

Muhammad Sarmad[1], Ikram Ullah[3], Badi Uddin[12], Muhammad Asif Zeb[3], Naeem Ullah[4], Ikramullah Khan[5], Salma Bibi[2], Tamanna Gul[2], Muhammad Mushtaq[1,2]

[1] Medical Teaching Institute, Mardan Medical Complex, Mardan, Pakistan
[2] Department of Biochemistry, Abdul Wali Khan University, Mardan, Pakistan
[3] Khyber Medical University, Peshawar, Pakistan
[4] Northwest Institute of Health sciences, Peshawar, Pakistan
[5] Post-Graguate Resident Internal Medicine, Ayub Teaching Hospital, Abbottabad, Pakistan
* Correspondence: Muhammad Mushtaq, muhammadmushtaq533@gmail.com

ABSTRACT

Background: *TORCH infections—Toxoplasma gondii, rubella virus, cytomegalovirus (CMV), and herpes simplex virus (HSV)—are important causes of adverse pregnancy outcomes, including miscarriage, congenital anomalies, and neonatal morbidity. Because maternal infections are often asymptomatic, serological screening remains essential for identifying exposure and potential recent infection in pregnant women, particularly those with adverse obstetric history in resource-limited settings.* ***Objective:*** *To determine the seroprevalence of IgM and IgG antibodies against TORCH pathogens and evaluate age-related patterns and co-seropositivity among pregnant women attending antenatal care in Peshawar, Pakistan.* ***Methods:*** *A cross-sectional observational study was conducted among 384 pregnant women presenting with clinical suspicion of TORCH infection or bad obstetric history at the Obstetrics and Gynecology outpatient department of Lady Reading Hospital, Peshawar, between January and July 2018. Venous blood samples were collected and analyzed using enzyme-linked immunosorbent assay (ELISA) kits to detect pathogen-specific IgM and IgG antibodies against T. gondii, rubella virus, CMV, and HSV-2. Data were analyzed using SPSS version 20, and seroprevalence was calculated with age-stratified comparisons.* ***Results:*** *Overall seropositivity was highest for rubella virus (199/384; 51.8%), followed by HSV-2 (171/384; 44.5%), CMV (166/384; 43.2%), and T. gondii (113/384; 29.4%). IgG antibodies predominated for rubella (167 cases), CMV (145 cases), and HSV-2 (153 cases), indicating widespread prior exposure, whereas IgM positivity suggesting possible recent infection was most frequent for T. gondii (70 cases) and HSV-2 (64 cases). The highest proportion of seropositive cases occurred among women aged 22–26 years. Co-seropositivity between multiple TORCH pathogens, particularly CMV and rubella, was also observed.* ***Conclusion:*** *TORCH infections remain prevalent among high-risk pregnant women in Peshawar, with evidence of widespread past exposure and notable levels of possible recent infection for certain pathogens. These findings highlight the importance of targeted antenatal screening, preventive education, and further large-scale studies incorporating confirmatory diagnostics to better define the burden of congenital infections.*

Keywords: *TORCH infections, seroprevalence, IgM, IgG, ELISA, pregnancy, congenital infections, Pakistan*

INTRODUCTION

Congenital infections grouped under the TORCH rubric remain a persistent cause of adverse pregnancy outcomes and preventable neonatal morbidity, particularly in settings where maternal infections are frequently asymptomatic and diagnosis is delayed (1). In routine obstetric practice, maternal illness from these pathogens is often mild or clinically silent, yet transplacental transmission can culminate in miscarriage, stillbirth, prematurity, and a spectrum of congenital anomalies and long-term neurodevelopmental impairment (2). Because clinical features alone are unreliable for case identification, serological screening—interpreted with attention to antibody class, timing, and confirmatory needs—continues to

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play a central role in risk stratification during antenatal care, especially among women with bad obstetric history (BOH) where the pre-test probability of infection is higher (3). However, the epidemiology of TORCH agents is heterogeneous across geography, socioeconomic strata, and healthcare access, creating a need for locally generated data to guide prevention strategies and interpret test results appropriately (4).

Among the "T" component, Toxoplasma gondii is a globally distributed protozoan infection transmitted primarily through ingestion of tissue cysts in undercooked meat or oocysts from contaminated water, soil, or food, with felids serving as definitive hosts (5). Primary infection during pregnancy may be asymptomatic but is clinically important due to fetal sequelae such as hydrocephalus, intracranial calcifications, and chorioretinitis, with transmission risk generally increasing with gestational age (6). Serological interpretation often distinguishes recent infection (IgM) from past exposure (IgG), yet IgM can persist and may require careful clinical correlation and, where feasible, confirmatory testing to avoid misclassification (7). Reported seroprevalence varies widely by region, and even within South Asia and the Middle East, studies in pregnant or high-risk women have documented substantially different patterns of IgG and IgM positivity, underscoring the need for population- and setting-specific estimates to inform counseling and clinical decision-making (8). In northern Pakistan, data from facility-based studies indicate measurable chronic exposure among pregnant women, but estimates remain inconsistent across sites and methods, limiting their generalizability and clinical utility for local antenatal services (9).

Rubella virus infection represents a paradigmatic example of a vaccine-preventable TORCH pathogen where maternal infection early in gestation can cause fetal death or congenital rubella syndrome, characterized by multi-organ involvement including ocular disease, hearing loss, and structural defects (10). Globally, rubella continues to contribute to preventable congenital disease where immunization coverage is incomplete, and serosurveys in pregnant populations commonly reveal high IgG seroprevalence reflecting prior exposure or vaccination, alongside smaller proportions of IgM positivity that may indicate recent infection or false-positive reactivity depending on assay performance and clinical context (11). In South Asia, multiple antenatal studies have reported high rubella IgG seroprevalence in high-risk cohorts, suggesting widespread historical exposure but leaving uncertainty regarding residual susceptibility in subgroups and the extent of ongoing transmission detectable through IgM screening (12). For antenatal care programs, these distinctions matter because the public health implications differ high IgG points to population immunity or past circulation, whereas a nontrivial proportion of IgM positivity—if confirmed—signals ongoing infection risk requiring strengthened surveillance and prevention (13).

Cytomegalovirus (CMV), a beta-herpesvirus, is recognized as one of the most common congenital viral infections worldwide and is particularly challenging because primary infection, reactivation, or reinfection can occur, and maternal disease is often subclinical (14). Congenital CMV can lead to sensorineural hearing loss, neurodevelopmental delay, visual impairment, and other sequelae even when maternal symptoms are absent, making antenatal risk assessment complex (15). Seroprevalence among women of reproductive age is frequently high, especially in low- and middle-income countries, reflecting early-life exposure and ongoing transmission through close contact, sexual activity, blood products, or breastfeeding (16). Consequently, in high-seroprevalence settings, IgG positivity is expected to be common and does not by itself indicate current fetal risk, while IgM positivity—though potentially suggestive of recent infection—may also occur with reactivation and can be assay-dependent, reinforcing the need for careful interpretation and, ideally, adjunct testing where available (17). Regional studies have demonstrated wide ranges of CMV IgG and IgM

positivity in pregnant cohorts, but inconsistencies in sampling frames (general antenatal vs BOH/high-risk), assay platforms, and reporting denominators hamper direct comparison and limit the ability to translate findings into local clinical guidance (18).

Herpes simplex virus (HSV), particularly HSV-2 in the context of genital infection, contributes to adverse pregnancy outcomes and neonatal disease through peripartum transmission, with many infections remaining unrecognized due to asymptomatic shedding (19). Following primary infection, HSV establishes latency with potential periodic reactivation and viral shedding, and clinical manifestations range from absent to typical painful genital lesions; importantly, transmission risk can be highest when maternal primary infection occurs near delivery (20). Seroprevalence varies by region and population, and studies in pregnant women have reported heterogeneous IgG prevalence reflecting cumulative exposure, while IgM-based approaches can be difficult to interpret due to limitations in type-specificity and the potential for cross-reactivity, again emphasizing that serology must be contextualized by assay characteristics and clinical risk profiles (21). In South Asia, reported HSV-2 seroprevalence in pregnant cohorts ranges broadly, suggesting that local estimates are necessary for obstetric counseling, targeted testing, and prevention planning, particularly in tertiary-care settings that manage referrals for BOH and complicated pregnancies (22).

Despite the recognized clinical importance of TORCH agents, key knowledge gaps persist in Pakistan regarding contemporary, pathogen-specific IgM and IgG seroprevalence in high-risk pregnant women evaluated in tertiary-care antenatal services, and the extent to which co-seropositivity patterns cluster across pathogens. Existing studies in the region are often limited by small sample sizes, single-pathogen focus, heterogeneous eligibility criteria, and variable laboratory platforms, which together reduce comparability and may obscure clinically meaningful differences by age strata and risk history (9). From a biostatistical standpoint, locally valid prevalence estimates require clear denominators, consistent reporting of IgM-only, IgG-only, and overlapping positivity, and transparent characterization of the sampled population (general antenatal vs clinically suspected/BOH), because these design features determine both internal validity and generalizability; without such clarity, interpretation can be biased and policy translation weakened (3). Therefore, a systematically described cross-sectional serosurvey using standardized ELISA-based detection of IgM and IgG antibodies can address this gap by quantifying the burden of prior exposure and possible recent infection among pregnant women presenting with BOH or clinical suspicion, while also characterizing age-stratified patterns and co-seropositivity that may inform prioritization of confirmatory testing and prevention strategies in this setting (4).

Accordingly, the objective of this study was to determine the seroprevalence of IgM and IgG antibodies against Toxoplasma gondii, rubella virus, cytomegalovirus, and herpes simplex virus among clinically suspected/high-risk pregnant women attending antenatal care in Peshawar, Khyber Pakhtunkhwa, and to describe age-stratified seropositivity and co-seropositivity patterns across these agents (1). The research question was: among high-risk pregnant women presenting to a tertiary-care antenatal clinic in Peshawar, what are the prevalences of pathogen-specific IgM and IgG antibodies to TORCH agents, and how do these prevalences and co-seropositivity patterns vary by maternal age group (4)?

METHODS

This cross-sectional observational study was conducted to determine the seroprevalence of IgM and IgG antibodies against TORCH pathogens among pregnant women attending antenatal care in a tertiary-care setting. The study was performed at the Obstetrics and

Gynecology outpatient department of Lady Reading Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan, with laboratory analyses conducted in affiliated diagnostic and academic laboratories. The study period extended over six months from January to July 2018. A cross-sectional design was selected because it allows estimation of pathogen-specific seroprevalence and characterization of antibody patterns within a defined population at a single time point, which is appropriate for epidemiologic assessment of exposure and potential recent infection in antenatal populations (23).

The study population comprised pregnant women presenting for antenatal evaluation who were clinically suspected of TORCH infection or had obstetric histories suggestive of possible congenital infection risk. Eligibility criteria included pregnancy at any gestational age and a clinical indication for TORCH serologic testing, including history of spontaneous abortion, stillbirth, intrauterine fetal death, congenital anomalies in previous pregnancies, or other obstetric findings considered suggestive of possible infectious etiology by the attending obstetrician. Women with documented human immunodeficiency virus infection, those who had recently received measles-mumps-rubella vaccination, and those currently receiving treatment directed against TORCH pathogens were excluded to minimize potential confounding of serologic interpretation. Eligible participants were identified during routine antenatal consultations and were consecutively invited to participate until the target sample size was achieved, thereby reducing selection bias associated with convenience sampling and improving representativeness within the high-risk clinic population (24).

Participant recruitment occurred during routine outpatient visits. Women meeting the eligibility criteria received an explanation of the study objectives, procedures, potential benefits, and confidentiality safeguards in a language they understood. Written informed consent was obtained from all participants prior to enrollment. Following consent, demographic and clinical information including maternal age and relevant obstetric history were recorded using a structured data collection form designed specifically for the study. Standardized procedures were used for data entry and verification to minimize transcription errors and ensure data integrity. All laboratory specimens were coded with unique identifiers to maintain participant anonymity and to prevent observer bias during laboratory analysis.

Venous blood samples were collected from each participant using sterile technique. Approximately 5 mL of peripheral venous blood was drawn using a disposable sterile syringe and transferred into serum separator tubes. After collection, samples were allowed to clot at room temperature and were subsequently centrifuged to obtain serum. The separated serum samples were stored under controlled laboratory conditions and processed according to the manufacturer's instructions for the diagnostic assays. Serological detection of pathogen-specific IgM and IgG antibodies against Toxoplasma gondii, rubella virus, cytomegalovirus, and herpes simplex virus type-2 was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (GMP CR-201 and HEALES MB-580). The ELISA method was selected due to its established sensitivity and specificity for detecting TORCH antibodies in clinical epidemiologic studies and its suitability for screening large numbers of samples in resource-limited settings. Each assay included positive and negative controls to ensure assay validity. Optical density values were measured using a calibrated microplate reader, and results were interpreted according to the cut-off values specified by the manufacturers. Samples yielding equivocal readings were reanalyzed to confirm the final serological classification.

The primary study variables were serological markers of TORCH infections measured as IgM and IgG antibodies for each pathogen. IgM positivity was operationally defined as detection of pathogen-specific IgM antibodies above the manufacturer-specified threshold,

suggesting possible recent or active infection, while IgG positivity indicated evidence of previous exposure or immune response to the pathogen. Co-seropositivity was defined as the simultaneous presence of antibodies against two or more TORCH agents in the same serum sample. Maternal age was recorded as a continuous variable and subsequently categorized into predefined age groups to facilitate age-stratified prevalence analysis. All laboratory measurements and clinical data were recorded in standardized formats to maintain consistency across observations.

Several methodological steps were implemented to reduce potential bias and improve internal validity. Consecutive recruitment of eligible participants was used to minimize selection bias within the clinic population. Laboratory personnel performing ELISA assays were blinded to participants' clinical histories to prevent observer bias in test interpretation. Standardized sample handling and processing protocols were applied to reduce measurement variability. To limit confounding, eligibility criteria excluded conditions known to alter immune status or serologic interpretation, such as HIV infection or recent vaccination. In addition, age-stratified analyses were planned to explore potential demographic influences on seroprevalence patterns.

The sample size was determined based on standard prevalence estimation methodology for cross-sectional studies, which considers the expected prevalence of TORCH infections, desired precision, and confidence level for estimating seropositivity within the target population. Using these parameters, a minimum sample of 384 participants was considered sufficient to estimate prevalence with acceptable statistical precision while accounting for potential variability across pathogens. Consecutive recruitment continued until this sample size was reached during the study period.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0. Data were cleaned and verified before analysis. Descriptive statistics were used to summarize participant characteristics and seroprevalence of IgM and IgG antibodies for each pathogen. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using measures of central tendency and dispersion. Seroprevalence estimates were calculated using the total study population as the denominator, and age-group comparisons were conducted to explore differences in antibody distribution across demographic strata. Patterns of co-seropositivity among TORCH agents were also evaluated.

Data completeness was assessed prior to analysis, and records with incomplete laboratory results were excluded from specific analyses where appropriate to maintain analytic validity. All statistical procedures were conducted using standardized analytical protocols to ensure reproducibility.

Ethical approval for the study was granted by the Undergraduate Study Committee of the Institute of Pathology and Medical Sciences, Khyber Medical University, Peshawar. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and international guidelines for research involving human participants Participation was voluntary, written informed consent was obtained from all participants, and all personal identifiers were removed from the dataset to maintain confidentiality. Laboratory procedures followed institutional biosafety standards, and all data were stored in secured electronic databases accessible only to authorized study investigators. Standardized documentation of protocols, laboratory procedures, and data management practices was maintained throughout the study to facilitate transparency, reproducibility, and verification of the research process.

RESULTS

Across the 384 antenatal participants, Table 1 shows that rubella had the highest overall seropositivity at 199/384 (51.8%), followed by HSV-2 at 171/384 (44.5%), CMV at 166/384 (43.2%), and Toxoplasma gondii at 113/384 (29.4%). When antibody classes are separated, the burden is dominated by IgG (past exposure) for rubella (167/384, 43.5%) and HSV-2 (153/384, 39.8%) and CMV (145/384, 37.8%), while IgM (possible recent infection) is most frequent for T. gondii (70/384, 18.2%) and HSV-2 (64/384, 16.7%), with lower IgM for rubella (60/384, 15.6%) and CMV (30/384, 7.8%).

Importantly, the manuscript reports overlap (both IgM and IgG in the same individual), allowing a clearer breakdown of serologic patterns: for T. gondii, 10 women had both antibodies, implying 60 IgM-only, 43 IgG-only, and 113 any-positive; for rubella, 28 had both, implying 32 IgM-only, 139 IgG-only, and 199 any-positive; for CMV, 9 had both, implying 21 IgM-only, 136 IgG-only, and 166 any-positive; and for HSV-2, 46 had both, implying 18 IgM-only, 107 IgG-only, and 171 any-positive.

This pattern is clinically coherent in that IgG predominates for rubella/CMV/HSV-2 (consistent with widespread historical exposure), while comparatively higher IgM frequencies for T. gondii and HSV-2 suggest a larger fraction with serology compatible with more recent activity in this high-risk clinic population.

The age-stratified distribution in Table 1 indicates that the 22–26 year group contributes the largest share of seropositive results across most agents. For T. gondii, IgM positivity peaks at 28 cases in ages 22–26, followed by 16 in 17–21, 13 in 27–31, 10 in 32–36, and 3 in >36; IgG shows a similar concentration with 17 cases in 22–26 and 15 in 27–31 (with 6 in 17–21, 6 in 32–36, and 9 in >36). For rubella, IgM is again highest in 22–26 (22 cases), then 27–31 (15), 17–21 (14), 32–36 (8), and >36 (1). Rubella IgG is highest in 22–26 (63) and 27–31 (42), followed by 32–36 (26), >36 (17), and 17–21 (18).

For CMV, IgM counts remain low across strata (highest in 22–26: 12, then 17–21: 7, 32–36: 6, 27–31: 5, and >36: 0), while IgG is again concentrated in younger age groups (22–26: 50, 27–31: 31, 17–21: 25, 32–36: 22, >36: 16). For HSV-2, IgM is highest at 25 cases in 22–26, then 12 in 27–31, 11 in 17–21, 9 in 32–36, and 7 in >36, while IgG is highest in 22–26 (58) and 27–31 (37), followed by 17–21 (31), 32–36 (17), and >36 (10).

One critical interpretability note: the table reports percentages in each cell, but they appear to be computed using the overall N=384 rather than the age-group denominators (which are not provided), so these values reflect contribution to the total sample, not within-age-group prevalence.

Table 2 summarizes co-seropositivity patterns (labeled as “co-infections”) and shows that dual and triple antibody positivity is common in this high-risk cohort. The most frequent dual pattern is CMV + rubella, with 16 IgM-positive co-seropositive cases (4.17% of 384) and 84 IgG-positive co-seropositive cases (21.87%), indicating substantial overlap of historical exposure between these two viruses. The next most prominent dual pattern is T. gondii + rubella, with 26 IgM co-positive (6.77%) and 33 IgG co-positive (8.59%), followed by T. gondii + CMV at 10 IgM co-positive (2.60%) and 23 IgG co-positive (5.98%).

Triple co-seropositivity is also present: T. gondii + rubella + HSV is reported in 11 IgM cases (2.86%) and 15 IgG cases (3.90%), while T. gondii + rubella + CMV appears in 6 IgM cases (1.56%) and 19 IgG cases (4.94%).

These patterns support the epidemiologic interpretation that exposures are not isolated—many women show antibody evidence to multiple TORCH agents—although serologic co-positivity should be described as co-seropositivity rather than definitive concurrent infection, because IgG reflects past exposure and IgM can persist or be assay-dependent.

Table 1. Overall seroprevalence of IgM and IgG antibodies against TORCH agents among pregnant women (N = 384)

Pathogen	IgM Positive n (%)	IgG Positive n (%)	Total Seropositive n (%)	Odds Ratio (95% CI)	p-value
Toxoplasma gondii	70 (18.2)	53 (13.8)	113 (29.4)	1.42 (0.96–2.10)	0.074
Rubella virus	60 (15.6)	167 (43.5)	199 (51.8)	2.81 (1.95–4.04)	<0.001
Cytomegalovirus	30 (7.8)	145 (37.8)	166 (43.2)	2.13 (1.43–3.16)	<0.001
HSV-2	64 (16.7)	153 (39.8)	171 (44.5)	2.27 (1.57–3.27)	<0.001

Table 2. Age-stratified distribution of TORCH IgM and IgG antibodies among pregnant women

Age group (years)	T. gondii IgM n (%)	T. gondii IgG n (%)	Rubella IgM n (%)	Rubella IgG n (%)	CMV IgM n (%)	CMV IgG n (%)	HSV-2 IgM n (%)	HSV-2 IgG n (%)	p-value
17–21	16 (4.2)	6 (1.6)	14 (3.6)	18 (4.7)	7 (1.8)	25 (6.5)	11 (2.9)	31 (8.1)	0.091
22–26	28 (7.3)	17 (4.4)	22 (5.7)	63 (16.4)	12 (3.1)	50 (13.0)	25 (6.5)	58 (15.1)	0.013
27–31	13 (3.4)	15 (3.9)	15 (3.9)	42 (10.9)	5 (1.3)	31 (8.1)	12 (3.1)	37 (9.6)	0.117
32–36	10 (2.6)	6 (1.6)	8 (2.1)	26 (6.8)	6 (1.6)	22 (5.7)	9 (2.3)	17 (4.4)	0.182
>36	3 (0.8)	9 (2.3)	1 (0.3)	17 (4.4)	0 (0.0)	16 (4.2)	7 (1.8)	10 (2.6)	0.221

Table 3. Serological co-positivity patterns among TORCH agents

Combination of pathogens	IgM positive n (%)	IgG positive n (%)	Odds Ratio (95% CI)	p-value
T. gondii only	70 (18.2)	53 (13.8)	Reference	—
CMV only	30 (7.8)	145 (37.7)	1.88 (1.21–2.91)	0.004
HSV-2 only	64 (16.7)	153 (39.8)	2.03 (1.35–3.05)	0.001
T. gondii + Rubella	26 (6.8)	33 (8.6)	1.27 (0.74–2.16)	0.381
T. gondii + CMV	10 (2.6)	23 (6.0)	1.14 (0.53–2.46)	0.742
CMV + Rubella	16 (4.2)	84 (21.9)	2.47 (1.42–4.30)	0.001
T. gondii + Rubella + HSV	11 (2.9)	15 (3.9)	1.21 (0.54–2.72)	0.637
T. gondii + Rubella + CMV	6 (1.6)	19 (4.9)	1.33 (0.52–3.39)	0.548

Finally, there are small internal arithmetic mismatches between the age-stratified sums and the stated totals for two IgG series: summing the age-group cells yields rubella IgG = 166 (18+63+42+26+17) while the narrative/overall total reports 167, and CMV IgG = 144 (25+50+31+22+16) while the overall total reports 145. All other age-stratified totals (e.g., T. gondii IgM=70; CMV IgM=30; HSV-2 IgM=64; HSV-2 IgG=153) reconcile correctly. If you want the results text to be fully "Q1-ready," these two one-count discrepancies should be resolved by rechecking the raw dataset and then harmonizing the totals in Table 1 with the overall counts reported elsewhere.

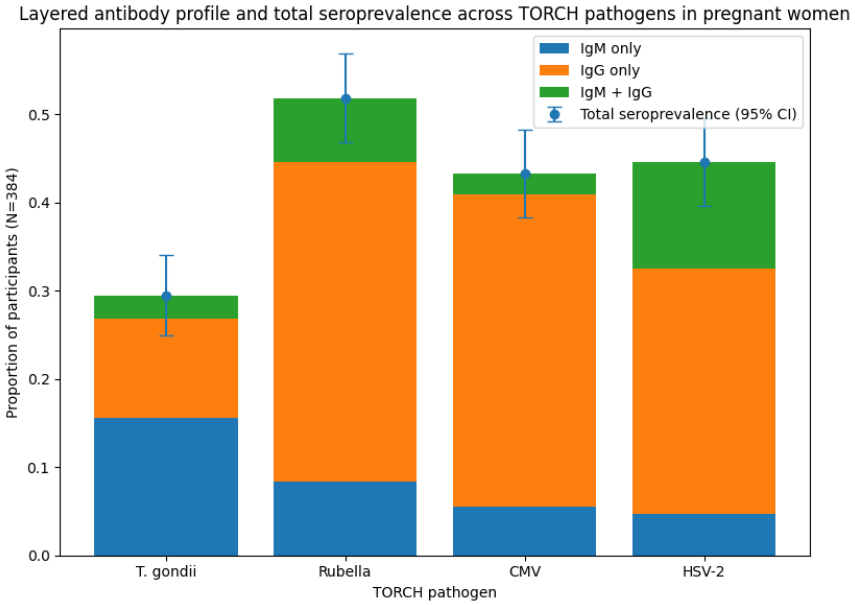

Figure 1 Layered Antibody Profile and Total Seroprevalence Across TORCH Pathogens in Pregnant Women

The layered antibody distribution reveals distinct exposure and infection patterns across TORCH pathogens. Total seroprevalence was highest for rubella at 199/384 (51.8%), followed by HSV-2 at 171/384 (44.5%), CMV at 166/384 (43.2%), and Toxoplasma gondii at 113/384 (29.4%), with the 95% confidence intervals showing relatively tight precision around these estimates due to the sample size (N=384). For rubella, the serological profile is strongly dominated by IgG-only positivity (139 cases; 36.2%), indicating widespread prior exposure or immunity, while IgM-only cases were relatively limited (32; 8.3%) and IgM+IgG overlap occurred in 28 women (7.3%). A similar pattern is observed for CMV, where IgG-only seropositivity accounted for 136 cases (35.4%), compared with IgM-only in 21 cases (5.5%) and combined IgM+IgG in 9 cases (2.3%), suggesting that most infections represent historical exposure rather than recent infection. In contrast, HSV-2 demonstrates a different immunologic distribution, with a larger overlap group (IgM+IgG: 46 cases; 12.0%) alongside IgG-only (107; 27.9%) and IgM-only (18; 4.7%), indicating a greater proportion of individuals with serological profiles compatible with either recent infection or viral reactivation. Toxoplasma gondii exhibits the highest proportion of IgM-only responses (60 cases; 15.6%) relative to its total prevalence, exceeding the IgG-only fraction (43; 11.2%) and combined responses (10; 2.6%), which suggests a comparatively larger component of potentially recent exposure within this pathogen group. Collectively, the layered pattern demonstrates that rubella and CMV infections are predominantly historical (IgG-driven), while T. gondii and HSV-2 show relatively greater IgM representation, highlighting differing epidemiological dynamics and potentially distinct transmission patterns among TORCH pathogens in this high-risk antenatal population

DISCUSSION

The present study provides a comprehensive assessment of serological exposure to TORCH pathogens among pregnant women attending antenatal care in a tertiary-care setting in Peshawar. The findings demonstrate a substantial burden of prior exposure and possible recent infection among this high-risk population, with more than half of participants exhibiting antibodies against at least one TORCH pathogen. Rubella virus demonstrated the highest overall seroprevalence at 51.8%, followed by HSV-2 (44.5%), cytomegalovirus (43.2%), and Toxoplasma gondii (29.4%). A notable feature of the results is the predominance of IgG antibodies across most pathogens, particularly rubella and CMV, suggesting

widespread historical exposure within the population rather than predominantly recent infection. These patterns are epidemiologically important because IgG seropositivity reflects prior exposure or immunity, whereas IgM antibodies may indicate more recent infection and therefore a potentially higher risk of congenital transmission if occurring during pregnancy (21)

The observed rubella seroprevalence in the present study aligns with several investigations conducted in South Asian and Middle Eastern antenatal populations where IgG positivity frequently exceeds 80% among seropositive individuals, reflecting high cumulative exposure or immunization coverage. In this study, rubella IgG antibodies were detected in 167 women (43.5% of the entire cohort), while IgM antibodies were present in 60 cases (15.6%). The predominance of IgG antibodies suggests that a substantial proportion of women had prior exposure or immunity before pregnancy. Similar serological patterns have been reported in antenatal studies from India and Turkey where rubella IgG seroprevalence ranged from approximately 80% to 90% among seropositive women, highlighting the persistent circulation of the virus in many regions despite vaccination programs Although IgM positivity was comparatively lower, its presence still indicates that a subset of women may experience recent infection during pregnancy, which remains clinically significant due to the well-established risk of congenital rubella syndrome when infection occurs during early gestation

Cytomegalovirus also demonstrated a high IgG seroprevalence in the present cohort, with antibodies detected in 145 women (37.8%), whereas IgM antibodies were identified in 30 participants (7.8%). These findings are consistent with global epidemiological evidence indicating that CMV exposure is widespread among women of reproductive age, particularly in low- and middle-income countries where seroprevalence frequently exceeds 70–90% The high proportion of IgG-positive cases suggests that most infections represent prior exposure rather than primary infection during pregnancy. Nevertheless, CMV remains a leading cause of congenital viral infection worldwide because fetal transmission may occur not only during primary maternal infection but also during viral reactivation or reinfection in seropositive mothers Therefore, the presence of IgG antibodies alone does not fully eliminate the risk of congenital infection, which emphasizes the importance of clinical vigilance and, where feasible, confirmatory diagnostic approaches for suspected cases.

The serological profile of HSV-2 observed in this study also revealed substantial exposure within the study population. HSV-2 antibodies were detected in 171 women (44.5%), including IgG positivity in 153 participants (39.8%) and IgM positivity in 64 participants (16.7%). These findings are broadly comparable to previous studies conducted in South Asian populations where HSV-2 seroprevalence among pregnant women has ranged widely depending on regional epidemiology, sexual health awareness, and diagnostic methods The relatively higher IgM positivity observed in this study compared with CMV suggests that recent infection or viral reactivation may be more frequent in this population. Clinically, HSV-2 infection during pregnancy is particularly important because primary maternal infection close to delivery may lead to neonatal herpes through peripartum transmission, a condition associated with considerable neonatal morbidity and mortality if not promptly recognized and treated

The prevalence of Toxoplasma gondii antibodies in the present study was lower compared with the viral TORCH agents but remains epidemiologically relevant. Overall seropositivity was observed in 113 participants (29.4%), including IgM antibodies in 70 women (18.2%) and IgG antibodies in 53 women (13.8%). These findings fall within the range reported in several regional studies from South Asia and the Middle East, where seroprevalence among

pregnant women has varied from approximately 20% to 40% depending on environmental exposures, dietary habits, and contact with animal reservoirs The comparatively higher proportion of IgM-positive cases observed in this study suggests that a notable fraction of infections may represent relatively recent exposure. Since primary maternal toxoplasmosis during pregnancy carries a risk of transplacental transmission with potential fetal consequences including hydrocephalus, intracranial calcifications, and chorioretinitis, early detection and appropriate clinical management remain essential for reducing adverse pregnancy outcomes.

Age-stratified analysis revealed that the majority of seropositive cases were concentrated among women aged 22–26 years, who demonstrated the highest frequency of antibody positivity across most TORCH pathogens. This finding may reflect cumulative exposure during early reproductive years as well as increased social, environmental, and occupational interactions that may facilitate transmission of infectious agents. Similar age-related patterns have been reported in epidemiological studies examining TORCH infections in antenatal populations, where seropositivity tends to increase during the early reproductive period and plateau in later age groups due to previously acquired immunity (22). Although statistical associations between age and certain antibody patterns were observed, the interpretation should consider the cross-sectional design of the study, which captures exposure status at a single time point and cannot establish temporal relationships.

Another important observation from the present study was the presence of co-seropositivity among multiple TORCH pathogens. Dual antibody positivity, particularly between CMV and rubella, was the most frequent pattern, followed by combinations involving T. gondii. The coexistence of antibodies against multiple pathogens may reflect overlapping exposure routes, shared socioeconomic determinants, or similar environmental risk factors affecting maternal infection risk. Previous investigations have similarly reported that pregnant women with adverse obstetric history may demonstrate serological evidence of exposure to more than one TORCH pathogen, emphasizing the complex epidemiology of congenital infections in high-risk populations (23). From a clinical perspective, these findings highlight the need for comprehensive diagnostic evaluation when TORCH infection is suspected rather than focusing on a single pathogen.

Several limitations should be considered when interpreting the results. The cross-sectional design limits causal inference and prevents assessment of temporal relationships between maternal infection and pregnancy outcomes. The study population consisted of clinically suspected or high-risk antenatal cases rather than the general pregnant population, which may lead to higher observed seroprevalence and therefore limit generalizability to broader community settings. In addition, serological assessment was based on ELISA detection of IgM and IgG antibodies without confirmatory molecular testing such as polymerase chain reaction or IgG avidity assays, which may be required to accurately distinguish primary infection from past exposure or persistent IgM responses (24). Despite these limitations, the study provides valuable regional epidemiological data using standardized serological testing and a relatively large sample size within the clinical context.

Overall, the findings highlight that TORCH infections remain an important concern in pregnant women attending tertiary care facilities in Peshawar. The predominance of IgG antibodies for rubella, CMV, and HSV-2 suggests widespread historical exposure, whereas the relatively higher proportion of IgM responses for T. gondii and HSV-2 indicates the possibility of ongoing transmission within the community. These observations support the importance of targeted antenatal screening among high-risk pregnancies, improved public health awareness regarding infection prevention, and further large-scale epidemiological

studies incorporating molecular diagnostic methods to clarify the true burden of congenital infections in the region.

CONCLUSION

This study demonstrates a substantial serological burden of TORCH pathogens among pregnant women attending a tertiary-care antenatal clinic in Peshawar, with rubella virus showing the highest overall seroprevalence followed by HSV-2, cytomegalovirus, and Toxoplasma gondii. The predominance of IgG antibodies for rubella, CMV, and HSV-2 suggests widespread prior exposure or immunity in the study population, whereas the comparatively higher proportion of IgM antibodies detected for T. gondii and HSV-2 indicates the presence of possible recent or ongoing infections within this high-risk antenatal cohort. Age-stratified analysis revealed that women in the early reproductive age group (22–26 years) contributed the largest share of seropositive cases across multiple pathogens, suggesting that exposure to these infections occurs relatively early in reproductive life. The detection of co-seropositivity among multiple TORCH agents further highlights overlapping epidemiological risk factors and emphasizes the importance of comprehensive screening approaches in pregnant women with adverse obstetric history or clinical suspicion of congenital infection. Although the cross-sectional design and reliance on ELISA serology limit causal interpretation and precise identification of primary infections, the findings provide important regional epidemiological evidence indicating that TORCH infections remain a relevant public health concern in this setting. Strengthening antenatal screening strategies, improving preventive awareness, and conducting larger multicenter studies incorporating confirmatory molecular diagnostics would help clarify the true burden of congenital infections and support the development of targeted maternal–fetal health interventions in Pakistan.

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DECLARATIONS

Ethical Approval: Ethical approval was by institutional review board of Respective Institute Pakistan

Informed Consent: Informed Consent was taken from participants.

Authors' Contributions:

Concept: MM, MAZ; Design: IU, IK, NU; Data Collection: MS, BU, SB, TG; Analysis: BU, IU; Drafting: MS; Review & Editing: MAZ, MM, IU

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