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Seroprevalence of TORCH Infections and Associated Influential Factors among Pregnant Women in Khost Province, Afghanistan: A Cross-Sectional Study

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ABSTRACT

Background: TORCH infections (*Toxoplasma gondii*, Rubella virus, Cytomegalovirus, and Herpes simplex virus) are major causes of adverse outcomes in pregnancy. Data on seroprevalence and associated risk factors are limited in Afghanistan. We aimed to assess the seroprevalence of TORCH infections (IgG/IgM) and associated risk factors among pregnant women in Khost Province, Afghanistan.

Methods: This cross-sectional study was performed on 369 pregnant women attending antenatal services in Khost Province, Afghanistan, from 01 January 2024 to 30 December 2024. All participants answered a questionnaire on demographic, behavioral, and obstetric data. Sera were tested for IgG and IgM antibodies for current or past TORCH infections. Significant associations between risk factors and seropositivity were tested with Chi-square and Mann-Whitney U tests, and predictors of IgM seropositivity were identified by binary logistic regression.

Results: Overall, IgG seropositivity was 62.6%, IgM positivity 49.6%. *Toxoplasma* was the most prevalent (IgG 38.5%; IgM 22.2%), followed by CMV (IgG 19.0%; IgM 19.2%) and rubella (IgG 5.1%; IgM 6.5%). Significant factors for IgG seropositivity: cat presence, poor hygiene, raw meat consumption (all $P<0.05$). For IgM positivity, significant associations were cat presence and husband's occupation ($P<0.05$). Logistic regression identified cat ownership (OR=0.64, 95%CI [0.42–0.98], $P=0.039$) and raw meat consumption (OR=0.62, 95%CI [0.40–0.95], $P=0.029$) as independent predictors of lower likelihood of recent IgM positivity. There was a correlation with age and parity with regard to history of abortion, but none with TORCH serology.

Conclusion: TORCH infections (especially toxoplasmosis and CMV) were common among the pregnant women in Khost, driven by environment, feeding habits, and living standards. Maternal age and parity mattered, but abortion history did not. Combating these infections in southeastern Afghanistan calls for hygiene/food safety education, improved living conditions, and better antenatal screening.

Keywords: TORCH infections, Pregnancy, Seroprevalence, Afghanistan

Introduction

TORCH infections that consist of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV) and Herpes simplex viruses (HSV) are common factors for untoward outcome of pregnancy such as fetal loss, congenital defects, intrauterine growth restriction and neonatal morbidity (1–3).

In pregnant women, TORCH infections can be asymptomatic but it has grave outcomes to the fetus when the pregnant women acquired primary infection during pregnancy (4). These infections represent about 2-3% of all congenital abnormalities occurring world-wide (5). In low resource country, antenatal screening is minimal, due to lack of awareness of infection prevention, personal hygiene, environmental and dietary habits (2,6). TORCH infections have also been identified as an important cause of morbidity and mortality in the third world country with variation according to geographical distribution (7).

There have been, as far as we are aware, no published seroprevalence studies of rubella, CMV or HSV among the pregnant women in Afghanistan. Although some localized or organism-specific studies in pregnant women have been undertaken (e.g., toxoplasmosis) (8), there have been no systematic analyses of the status of TORCH infection in pregnant women in Afghanistan.

Currently, Afghanistan has no national TORCH screening program for pregnant women. TORCH screening is not part of the standard antenatal care package, which focuses on blood pressure measurement, weight monitoring, blood and urine testing, nutritional advice, and tetanus toxoid vaccination. Rubella-containing vaccine has not yet been introduced into the national immunization program, leaving most women of childbearing age susceptible to rubella infection. Laboratory capacity for TORCH serology is limited and largely concentrated in urban areas, making routine screening inaccessible to most rural women. These contextual factors underscore the importance of our study in providing baseline seroprevalence data to inform future policy decisions.

To formulate a targeted strategy of prevention, an understanding of seroprevalence (IgG and IgM) and any related demographic and behavioral risks is crucial. In this study we sought to: Establish seroprevalence of TORCH infections (IgG and IgM) in pregnant women in Khost, Determine the demographic and behavioral factors associated with TORCH infection seropositivity, Identify the factors associated with previous abortion history and, Establish the predictive factors of acute infection (positive IgM antibody titer) using logistic regression.

Materials and Methods

Study design and setting

This cross-sectional study was conducted among pregnant women attending antenatal services in Khost Province, Afghanistan between 01 January 2024 and 30 December 2024.

Study population and data collection

A total of 369 pregnant women were enrolled. All participants had complete demographic, behavioral, clinical, and laboratory data. A structured questionnaire was designed containing data on demographic variables (age, education, residence, occupation, husband's occupation, income), obstetric history (gestational age, number of offspring, abortion history), behavioral factors (raw meat consumption, milk boiling, presence of cats at home), hygiene practices, type of sanitation facility, and antenatal check-up regularity.

Laboratory analysis

Blood samples were collected and examined for TORCH infections using serological assays: IgG antibodies (indicating past exposure), and

IgM antibodies (indicating recent/acute infection).

All samples yielded definitive positive or negative results; no equivocal (borderline) results were observed in this study. In accordance with manufacturer guidelines, any equivocal samples would have been retested in duplicate and excluded from final analysis if they remained indeterminate

Statistical analysis

All collected data were first entered and organized using Microsoft Excel. The finalized dataset was then exported to SPSS software Version 27 (IBM Corp., Armonk, NY, USA) or comprehensive statistical analysis.

Descriptive statistics were calculated to summarize the demographic, clinical, and laboratory characteristics of the study population. Categorical variables were presented as frequencies and percentages. Continuous variables were assessed for distributional normality using appropriate methods (e.g., visual inspection and normality tests).

The chi-square (χ^2) test was applied to examine associations between categorical variables, including the relationship between seropositivity and obstetric or demographic factors. When expected cell counts were small, appropriate corrections were considered.

The Mann–Whitney U test was used to compare non-normally distributed continuous variables between the abortion groups, as it is a non-parametric alternative to the independent samples *t*-test.

To identify independent predictors of IgM seropositivity, binary logistic regression analysis was performed. Variables that demonstrated potential association in bivariate analysis were entered into the regression model to estimate adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Model fitness and multicollinearity assumptions were evaluated prior to final interpretation. A *P*-value of ≤ 0.05 was considered statistically significant for all analyses.

Results

Participant characteristics

Of the 369 subjects, 85.4% were house wives; 74.3% were rural inhabitants; 53.1% were illiterate; 55.3% consumed raw meat; 57.7% kept cats at home; 41.7% do not boil milk; and 45% had irregular antenatal check-up.

The largest proportion of participants (47.4%, n=175) were aged 31–40 years, followed by 21–30 years (40.1%, n=148), 15–20 years (8.9%, n=33), and >40 years (3.5%, n=13).

18.4% of the subjects had a past history of abortion, and 15.4% had past history of TORCH infection (Table 1).

Table 1: Socio-demographic and behavioral characteristics of pregnant women in Khost Province, Afghanistan

Characteristic	***Category***	***Frequency (n)***	***Percentage (%)***
Age group(yr)	15–20	33	8.9
	21–30	148	40.1
	31–40	175	47.4
	>40	13	3.5
Occupation	Housewife	315	85.4
	Other	54	14.6
Education	Not educated	196	53.1
	Primary school	119	32.2
	High school	18	4.9

	University	36	9.8
Husband's education	Not educated	229	62.1
	Primary school	43	11.7
	High school	19	5.1
	University	78	21.1
Husband's occupation	Farmer	83	22.5
	Shopkeeper	23	6.2
	Passenger/Driver	85	23.0
	Medical professional	32	8.7
	Other	146	39.6
Residence	Rural	274	74.3
	Urban	95	25.7
Current residence	Khost	357	96.7
	Other	12	3.3
Personal hygiene	Poor	84	22.8
	Good	285	77.2
Presence of cats at home	Yes	213	57.7
	No	156	42.3
Raw meat consumption	Yes	204	55.3
	No	165	44.7
Boiling milk before consumption	Yes	215	58.3
	No	154	41.7
Type of bathroom/sanitation	Simple (Traditional)	186	50.4
	WC (Modern/Flush)	183	49.6
Antenatal check-ups	Regular	203	55.0
	Irregular	166	45.0
Previous abortion	Yes	68	18.4
	No	301	81.6
Self-reported past history of TORCH	Yes	57	15.4
	No	312	84.6

Seroprevalence of TORCH infections and factors associated with IgG positivity

Totally, 231 out of 369 participants (62.6%) were seropositive for IgG antibodies against at least one TORCH pathogen, indicating widespread past exposure.

Among the seropositive cases, *Toxoplasma* infection was the most prevalent (38.5%, n=142), followed by CMV (19.0%, n=70) and rubella (5.1%, n=19) (Table 2).

Several demographic and behavioral factors were significantly associated with IgG seropositivity. Presence of cats at home was strongly associated ($P < 0.001$); women living in households with cats showed higher *Toxoplasma* IgG positivity (45.5%) compared to those without cats (28.8%). Conversely, CMV IgG was less frequent among cat owners (9.9% vs. 31.4%). Poor personal hygiene was also significantly associated with IgG seropositivity ($P = 0.003$), with a higher proportion of *Toxoplasma* IgG (52.4% vs. 34.4%) and CMV IgG (22.6% vs. 17.9%) among women reporting poor hygiene practices.

Raw meat consumption showed a strong association with IgG results ($P < 0.001$). Notably, women who did not consume raw meat had higher *Toxoplasma* IgG seropositivity (51.5%)

compared to those who consumed raw meat (27.9%), a finding that may reflect complex exposure patterns or recall bias. Boiling milk was also significantly associated (P = 0.006); women who did not boil milk had higher CMV IgG (24.0% vs. 15.3%) but lower *Toxoplasma* IgG (29.2% vs. 45.1%) compared to those who boiled milk.

Irregular medical checkups during pregnancy were significantly associated with IgG seropositivity (P = 0.010), with higher *Toxoplasma* IgG rates among women with irregular visits (45.8% vs. 32.5%). In contrast, no significant associations were found for participant's job, husband's job, current residence, education level, husband's education, or type of bathroom (P > 0.05) (Table 1).

Table 2: Seroprevalence of TORCH infections (IgG and IgM) among pregnant women in Khost Province, Afghanistan

Infection	***No. (%) IgG***	***No. (%) IgM***
Negative for all TORCH pathogens	138 (37.4)	186 (50.4)
Positive for any TORCH pathogens	231 (62.6)	183 (49.6)
Toxoplasma gondii	142 (38.5)	82 (22.2)
Cytomegalovirus (CMV)	70 (19.0)	71 (19.2)
Rubella	19 (5.1)	24 (6.5)
Herpes simplex virus (HSV)	-	2 (0.5)
Other unspecified TORCH pathogens	-	4 (1.1)

Seroprevalence of TORCH infections and factors associated with IgM positivity

Overall, 183 participants (49.6%) tested positive for IgM antibodies, suggesting a high burden of recent or acute infections during pregnancy. Among the seropositive cases, *Toxoplasma* infection demonstrated the highest prevalence (22.2%), followed by CMV (19.2%) and rubella (6.5%) (Table 2).

For HSV, none of the 369 participants tested positive for HSV-specific IgG antibodies, indicating an absence of prior exposure to HSV-1 or HSV-2 in this cohort. However, 2 participants (0.5%) tested positive for HSV IgM, suggesting a possible recent primary infection. Among these two IgM-positive cases, neither was co-infected with other TORCH pathogens (i.e., no concurrent *Toxoplasma*, Rubella, or CMV IgM positivity was detected).

Analysis of IgM seropositivity revealed different patterns. A significant association was found between the presence of cats in the household and recent infection (P = 0.017). Husband's occupation was also significantly associated (P = 0.031), with the highest rate of recent CMV infection observed among women whose husbands were medical professional workers (40.6%). Furthermore, the type of bathroom showed a significant association (P = 0.015); women using a simple latrine had higher rates of recent *Toxoplasma* infection (28.5%) compared to those with a WC (16.9%) (Table 3).

In contrast, no statistically significant associations were identified between infection status and educational level, place of residence, or occupation. Dietary-related factors such as raw meat consumption (P = 0.173) and boiling milk (P = 0.748) were not significantly associated with recent IgM positivity (Table 3).

Notably, women who reported having cats in their household exhibited significantly higher *Toxoplasma* IgG positivity compared to those without cats, indicating increased prior exposure among this group.

Table 3: Factors associated with IgG and IgM seropositivity among pregnant women in Khost Province, Afghanistan

Variable	*Category*	*NO. (%) IgG positive*	*P-value*	*NO. (%) IgM positive*	*P-value*
Presence of cats at home	Yes	125/213 (58.7)	<0.001	113/213 (53.1)	0.017
	No	106/156 (67.9)		67/156 (42.9)	
Personal hygiene	Poor	65/84 (77.4)	0.003	37/84 (44.0)	0.805
	Good	166/285 (58.2)		143/285 (50.2)	
Raw meat consumption	Yes	117/204 (57.4)	<0.001	110/204 (53.9)	0.173
	No	114/165 (69.1)		70/165 (42.4)	
Boiling milk	Yes	143/215 (66.5)	0.006	101/215 (47.0)	0.748
	No	88/154 (57.1)		79/154 (51.3)	
Antenatal check-ups	Regular	114/203 (56.2)	0.010	109/203 (53.7)	0.217
	Irregular	117/166 (70.5)		71/166 (42.8)	
Type of bathroom	Simple	—	—	93/186 (50.0)	0.015
	WC	—		87/183 (47.5)	
Husband's occupation	Farmer	—	—	48/83 (57.8)	0.031
	Shopkeeper	—		8/23 (34.8)	
	Passenger/Driver	—		41/85 (48.2)	
	Medical Professional	—		19/32 (59.4)	
	Other	—		64/146 (43.8)	
Occupation	Housewife	198/315 (62.9)	0.673	163/315 (51.7)	0.918
	Other	33/54 (61.1)		26/54 (48.1)	
Education	Not Educated	125/196 (63.8)	0.600	94/196 (48.0)	0.310
	Primary	71/119 (59.7)		60/119 (50.4)	
	High School	14/18 (77.8)		6/18 (33.3)	
	University	21/36 (58.3)		20/36 (55.6)	
Residence	Rural	169/274 (61.7)	0.796	—	—
	Urban	62/95 (65.3)		—	

Logistic regression analysis

Binary logistic regression analysis was performed to identify independent predictors of IgM seropositivity. The overall model was marginally statistically significant ($\chi^2 = 9.392$, $P = 0.052$), indicating limited but borderline evidence that the included variables collectively contributed to predicting recent infection status. Among the examined variables, the presence of cats was identified as a statistically significant predictor of lower odds of IgM seropositivity (OR = 0.64, $P = 0.039$). Similarly, consumption of raw meat was significantly associated with

reduced odds of a recent infection (OR = 0.62, $P = 0.029$) (Table 4).

The explanatory power of the model was low, as indicated by a Nagelkerke R^2 value of 0.034, meaning that only about 3.4% of the variability in IgM positivity was explained by the variables. The model's overall predictive accuracy was 58.0%, which is only marginally better than chance.

Table 4: Logistic regression analysis for predictors of IgM seropositivity among pregnant women in Khost province, Afghanistan

Variable	*B*	*S.E.*	*Wald*	*df*	*P-value*	*Odds Ratio (OR)*	*95% Confidence Interval for OR*	
Presence of Cats at Home	-0.445	0.216	4.241	1	0.039	0.641	0.420	0.979
Raw Meat Consumption	-0.484	0.222	4.768	1	0.029	0.617	0.399	0.952
Type of Bathroom (Simple vs. WC)	-0.051	0.214	0.057	1	0.812	0.950	0.624	1.446
Boiling Milk	0.063	0.224	0.080	1	0.777	1.066	0.687	1.652
Constant	1.269	0.652	3.791	1	0.052	3.557	-	-

Factors associated with abortion history

Factors associated with abortion history were analyzed using the Mann–Whitney U test. The analysis revealed significant differences for age ($P < 0.001$) and the number of offspring ($P = 0.045$). No significant association was observed between abortion history and either TORCH IgG or IgM serological results (Table 5).

Table 5: Association between TORCH seroprevalence, demographic factors, and history of abortion

Variable	*Test Used*	*Test Statistic*	*P-value*
Age(yr)	Mann-Whitney U	Z = -4.071	<0.001
Number of Offspring (Parity)	Mann-Whitney U	Z = -2.001	0.045
IgG Seropositivity	Mann-Whitney U	Z = 0.295	0.768
IgM Seropositivity	Mann-Whitney U	Z = -0.096	0.923
Weight	Mann-Whitney U	Z = -1.412	0.158
Gestational Age	Mann-Whitney U	Z = -1.110	0.267
Personal hygiene	Mann-Whitney U	Z = -0.473	0.636
Education	Mann-Whitney U	Z = 0.117	0.907
Family size	Mann-Whitney U	Z = 0.064	0.949
Monthly income	Mann-Whitney U	Z = -0.777	0.437
Antenatal check-ups	Mann-Whitney U	Z = 0.429	0.668
Husband's education	Mann-Whitney U	Z = 1.056	0.291

Discussion

To the best of our knowledge, this is the first report to determine TORCH seroprevalence in the pregnant women in Khost Province, Afghanistan. Overall IgG seroprevalence reveals a large burden of history of infection with at least one TORCH agent, while the IgM seroprevalence demonstrates that a large proportion of these are recent/acute infections, which carry risk of transplacental passage to the fetus with

potential miscarriages, birth defects and neonatal morbidity (1,2).

Among the infections that we studied, toxoplasmosis was found to be the most common infection, with IgG and IgM positivity rates of 38.5% and 22.8% respectively, comparable to the prevalence figures found by a study in Kabul, Afghanistan (IgG: 48.3%) (8). The strong association between having a cat in the house and being positive for IgG confirms the worldwide established association between ownership of the domestic cat and the risk of being infected with toxoplasmosis during pregnancy (4,9). Another risk factor we identified for having the IgG seropositivity for toxoplasmosis, was eating raw meat. 55.3% of our subjects reported eating raw meat and this was found to be a significant factor in predicting IgG positivity, which confirms the findings of several previous studies indicating that the ingestion of undercooked meat is one of the main transmission routes of *T. gondii* (10–12).

In this study, CMV was the second most frequently observed infection. The high percentage of IgM suggests an infection acquisition during the current pregnancy which might be alarming given the possible implications of primary CMV infection to the fetus causing the potential for congenital infection which may present with sensorineural hearing loss and neurodevelopmental delay (13). The relationship found between poor hygiene practice and CMV seropositivity in this study is consistent with the known mechanism of transmission which is through bodily fluids (14–16), which will be exacerbated by crowded households. On the other hand, the finding of a positive association between inconsistent antenatal visit and CMV seropositivity can mean that women with less healthcare contact are likely exposed more or less likely to be aware of protective measures (17).

The prevalence of rubella antibodies was very low, which seems unusual because rubella is usually vaccine-preventable, and not widespread in the Afghan population, with most childbearing women unprotected by the vaccine (18). The presence of IgM antibodies in 6.5% reflects recent infection, a particularly dangerous period to acquire during the first trimester of pregnancy as congenital rubella syndrome (CRS) risks are high (19). This evidence highlights the need for a rubella immunization campaign in Afghanistan.

Only 2 cases of HSV IgM were detected in our population, but as an established contributor to TORCH infections, linked to neonatal herpes encephalitis and disseminated infection (20), HSV serology needs to be included in the TORCH panel for future studies conducted in this population.

Cat ownership and raw meat consumption were found to be statistically significant independent variables associated with reduced odds of current IgM positivity via binary logistic regression. The unexpectedly inverse relationship can perhaps be attributed to the notion that chronically IgG-positive (previously infected) women are protected against current re-infection, especially in toxoplasmosis (21,22). Conversely, behavioral shifts within the pregnancy period including decrease in cat handling or meat consumption and better cooking may influence exposure patterns differently. In other seroprevalence surveys there were paradoxical associations as well (23). Even so, the bivariate analysis indicated a strong positive correlation between having cats and higher *Toxoplasma* IgG, suggesting greater prior exposure.

Husband's occupation showed strong correlation with IgM positive in bivariate analysis with highest percentage among women whose husbands were health professionals. This might be related to possible increase in occupational exposure to agents of infection among health professionals (24). However, the results should be interpreted with reservation owing to the small sample size of subgroups.

Lack of personal hygiene and low attendance to antenatal care visits showed significant correla-

tion with overall seropositivity thus implicating health awareness and access to healthcare. Promoting proper handwashing and food hygiene as well as consistent antenatal visits can decrease TORCH infections prevalence (2,25).

We found no correlation between history of abortion and TORCH seropositivity in contrast to a number of studies, where acute toxoplasmosis or CMV have been reported as having associations with miscarriage. We also did observe that both age and parity were associated with the history of abortion and this has been consistently described as associated with risk of miscarriage (2,26). It may be the case that in this population, other causes of miscarriage overrode TORCH mediated fetal loss (i.e., Chromosomal abnormalities or bacterial infections or social/ economic reasons), or that our study design was cross-sectional in nature, and could not discern temporal events.

Policy implications

The elevated seroprevalence of TORCH infections in Khost Province suggests a number of policy interventions. The Ministry of Public Health should consider making screening of pregnant women for TORCH infections a standard part of antenatal care, at a minimum for subsets of women at higher risk of exposure, such as women who own cats or women who consume raw meat or practice poor hygiene. Focused educational programs are essential to inform women of measures to prevent exposure to raw meat, to wash hands after handling cats, to boil milk, and to have regular antenatal visits. Third, as few seronegative women of childbearing age are susceptible to rubella, Rubella vaccination for such women should be recommended. Postpartum vaccination of seronegative women would be most effective. Last, improvements in antenatal care accessibility in rural settings would allow for timely diagnosis and counseling of acute TORCH infections. The limited investment required for these recommendations would dramatically decrease congenital TORCH related morbidity in southeastern Afghanistan.

The implementation of routine TORCH screening for pregnant women in rural Afghanistan faces a complex web of interconnected barriers spanning infrastructure, finance, sociocultural factors, and the broader political context.

Policy recommendations to address these barriers are: 1) Integrate TORCH screening into existing ANC platforms rather than creating parallel programs, to minimize additional resource requirements; 2) Prioritize rubella vaccination for women of childbearing age, as this is a highly cost-effective intervention that does not require laboratory infrastructure; 3) Strengthen community-based health services through midwives and community health workers who can provide health education and basic screening; 4) Target high-risk groups (e.g., cat owners, raw meat consumers) for screening rather than attempting universal screening, given resource constraints; and 5) Advocate for sustained international support for Afghanistan's health system, recognizing that maternal and child health cannot be addressed without basic functional infrastructure.

Limitations

The limitations of this study need to be highlighted when interpreting the findings: a cross sectional design that can only show association between risk factors and TORCH infections, not causation; only serological tests were conducted without the confirmation by PCR which could affect accuracy; Behavioral data was self-reported thus is susceptible to recall bias; Age variable was categorical in its classification, and hence its statistical associations to the dependent variables might not have been adequately assessed; a very low explanatory capacity of the logistic regression model indicates that variables entered only represented a fraction of the variation for IgM seropositivity.

Molecular confirmation via PCR was not performed in this study due to logistical and resource constraints inherent to conducting research in a low-resource setting. PCR is significantly more expensive and requires specialized laboratory infrastructure, reliable electricity, cold chain storage, and trained personnel that were unavailable in Khost Province at the time of the study. Additionally, our cross-sectional seroprevalence study aimed to estimate population-level exposure (IgG and IgM) rather than confirm active infections, making serological assays the appropriate and most cost-effective choice for this study objective

The reliance on self-reported behavioral data introduces the potential for recall bias, particularly regarding dietary habits and hygiene practices. Participants may have underreported socially undesirable behaviors or modified their practices during pregnancy, which could have influenced the observed associations. Future prospective cohort studies with repeated behavioral assessments are needed to confirm these findings.

Conclusion

TORCH infections, specifically toxoplasmosis and CMV were prevalent in Khost Province, especially amongst pregnant women. Positive predictors of infection were having cats at home, consuming undercooked meat, low level of hygiene, and inadequate number of antenatal visits. Abortion history was significantly correlated to age and parity but not with TORCH serology status.

Health intervention programs should be focused on this region of Afghanistan to alleviate the burden of this infection. Educational interventions aimed at improving food hygiene, importance of boiling milk and enhancing personal hygiene and strengthening the antenatal screening programs are important. General screening may be particularly beneficial for high-risk groups in the region.

Ethics approval

The study protocol was reviewed and approved by the Biomedical Ethics Committee of Ghalib University, Kabul, Afghanistan (Approval No. AF.GUK.REC.1402.008). Written informed consent was obtained from all participants; for illiterate participants, oral informed consent was obtained. Consent covered participation in the laboratory examination and completion of questionnaires. To ensure privacy and confidentiality, all data were anonymized using unique participant codes and handled confidentially throughout the study.

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Conflicts of interest

The authors declare no competing interests.

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