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Prevalence of Chlamydia trachomatis, associated reproductive factors, and comparison of molecular specimen types among pregnant women attending a tertiary hospital in Malaysia

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Abstract

Objectives

Genital *Chlamydia trachomatis* infection during pregnancy is frequently asymptomatic and is associated with adverse maternal and perinatal outcomes. *Chlamydia trachomatis* screening—defined here as nucleic acid amplification test (NAAT)–based testing of pregnant women to identify largely asymptomatic infection—is essential for preventing adverse maternal and perinatal outcomes. In Malaysia, data on the burden of infection, associated risk factors, and optimal diagnostic specimen types in pregnancy remain limited.

Methods

This cross-sectional study evaluated the prevalence, risk factors, and diagnostic performance of different specimen types for *C. trachomatis* detection among pregnant women in Malaysia. A total of 100 pregnant women were recruited at a tertiary hospital, and endocervical, vaginal, and first-void urine samples were analyzed using nested polymerase chain reaction (PCR) targeting the major outer membrane protein (MOMP) gene. In the absence of a single definitive diagnostic test, a composite molecular reference conclusion was used to classify infection status.

Results

Thirty-six participants were classified as infected, yielding a prevalence of 36%. A history of pregnancy loss was significantly associated with current infection (OR 2.72, p = 0.03). Using a composite reference conclusion as the gold standard, endocervical samples demonstrated the highest sensitivity (61.1%), followed by vaginal (47.2%) and urine specimens (41.7%). Pairwise agreement between specimen types was fair (κ = 0.27–0.33), with no statistically significant directional differences.

Conclusion

This study demonstrates a substantial burden of undiagnosed *C. trachomatis* infection among pregnant women attending a tertiary care setting in Malaysia and identifies prior pregnancy loss as a potential risk marker for infection. Endocervical sampling showed the highest diagnostic sensitivity, although vaginal sampling represents a feasible alternative where self-collection may improve screening uptake. Larger, multicenter studies are needed to confirm these findings and to inform antenatal screening strategies in similar settings.

Background

Chlamydia trachomatis (CT) is the most frequently reported bacterial sexually transmitted infection (STI) worldwide and remains a major public health concern, particularly among women of reproductive age. The World Health Organization (WHO) estimates that more than 128 million new chlamydial infections occur annually, with the majority affecting young sexually active adults[1]. Because approximately 70–90% of infections in women are asymptomatic, many cases remain undiagnosed and untreated, allowing persistent infection, continued transmission, and preventable reproductive complications[2,3].

During pregnancy, untreated CT infection has been associated with several adverse maternal and neonatal outcomes, including miscarriage, premature rupture of membranes, preterm birth, postpartum endometritis, low birth weight, neonatal conjunctivitis, and neonatal pneumonia[3,4]. Although the strength of these associations varies across studies, systematic reviews consistently support early diagnosis and timely treatment to reduce maternal and neonatal morbidity[3,4].

Nucleic acid amplification tests (NAATs) are currently regarded as the diagnostic method of choice because of their excellent analytical sensitivity and specificity[2,5]. These assays can be performed using endocervical swabs, vaginal swabs, or first-void urine specimens. Increasing evidence suggests that self-collected vaginal swabs provide diagnostic performance comparable to clinician-collected specimens while improving patient acceptability and screening uptake[2]. Current recommendations from the United States Centers for Disease Control and Prevention (CDC) advise CT screening for all pregnant women younger than 25 years and for pregnant women aged 25 years or older with identifiable risk factors, with repeat testing during the third trimester for women at continued risk[5].

Despite the global burden of CT infection, epidemiological data from many low- and middle-income countries, including Malaysia, remain limited. Routine antenatal screening for CT is not currently incorporated into Malaysian national antenatal care, and published data describing infection prevalence, associated reproductive risk factors, and the relative performance of different clinical specimen types are scarce. Consequently, clinicians have limited local evidence to guide decisions regarding targeted antenatal screening or specimen selection for molecular diagnosis.

Generating locally relevant evidence is important because disease prevalence, healthcare access, behavioural risk factors, and healthcare delivery differ substantially between countries[1,4]. Hospital-based studies can provide valuable preliminary data to identify women who may benefit most from future screening programmes and to inform the design of larger multicentre investigations.

Therefore, this study aimed to determine the prevalence of *Chlamydia trachomatis* infection among pregnant women attending a tertiary referral hospital in Malaysia, examine selected demographic and reproductive factors associated with infection, and compare the diagnostic performance of endocervical, vaginal, and first-void urine specimens using nested polymerase chain reaction. We hypothesised that CT infection would be relatively common within this hospital-based cohort and that endocervical specimens would demonstrate the highest diagnostic sensitivity compared with alternative specimen types.

Methods

Study design and setting

This cross-sectional observational study was conducted at the Department of Obstetrics and Gynaecology, Hospital Sungai Buloh, Selangor, Malaysia, over a 12-month period. The study aimed to determine the prevalence of Chlamydia trachomatis (CT) infection among pregnant women, examine selected reproductive risk factors associated with infection, and compare the diagnostic performance of endocervical, vaginal, and first-void urine specimens using a molecular assay.

Ethical approval was obtained from the Malaysian Medical Research and Ethics Committee (MREC) (Approval No. 24-02000-FEE). We obtained written informed consent from all participants before enrolment.

Study participants

Pregnant women aged 18 years or older attending the obstetric ward during the study period were invited to participate. Women from all trimesters of pregnancy were eligible.

Participants were recruited consecutively after providing written informed consent. We excluded women who declined participation or could not provide all three clinical specimens from the analysis.

Because recruitment occurred in a tertiary referral hospital, the study population may not represent the general antenatal population. Therefore, the findings should be interpreted as representative of this hospital-based cohort rather than the wider Malaysian pregnant population.

Sample size

A total of 100 pregnant women were recruited.

This exploratory study was designed to generate preliminary epidemiological and diagnostic data regarding CT infection in pregnancy in Malaysia, where published data remain limited. Because reliable local prevalence estimates were unavailable at the time of study planning, no formal sample size calculation was performed. Consequently, the study should be considered hypothesis-generating rather than definitive.

Data collection

Demographic characteristics, obstetric history, reproductive history, and behavioural variables were collected using a standardised data collection form completed during participant enrolment.

Variables collected included maternal age, ethnicity, educational level, occupation, household income, obstetric history, previous pregnancy loss, history of sexually transmitted infections, condom use, and number of previous sexual partners.

Clinical information was verified against the participants' medical records where appropriate.

Specimen collection

Three specimens were collected from each participant during the same clinical visit:

- endocervical swab
- vaginal swab
- first-void urine specimen

Endocervical and vaginal swabs were collected using the BD Universal Transport System (Becton Dickinson, USA).

Approximately 20 mL of first-void urine was collected in a sterile container before any pelvic examination.

All specimens were transported to the laboratory under standard biosafety procedures and processed within the recommended time according to the manufacturer's protocol.

DNA extraction

DNA extraction was performed using the Bioteke DNA Extraction Kit (Bioteke Corporation, China) according to the manufacturer's instructions.

For swab specimens, transport medium was vortexed and centrifuged at 800 × g for 10 minutes before DNA extraction.

Urine samples underwent identical centrifugation before DNA extraction.

We stored extracted DNA at −80°C until molecular analysis.

Nested PCR assay

Detection of *C. trachomatis* DNA was performed using nested polymerase chain reaction targeting the major outer membrane protein (ompA/MOMP) gene as previously described by Yeow et al., with minor modifications[6].

We used outer primers for the first amplification, followed by a second amplification with inner primers.

We separated PCR products on 1.5% agarose gels stained with RedSafe nucleic acid stain and visualised them under ultraviolet illumination.

The human β-globin gene was amplified in all samples as an internal control to confirm specimen adequacy and successful DNA extraction.

We included positive and negative controls in every PCR run to monitor contamination and assay performance.

Reference standard

Because no universally accepted reference standard exists for comparing multiple specimen types using the same molecular assay, we adopted a composite molecular reference standard.

Participants were classified as CT-positive when at least two of the three specimen types tested positive by nested PCR. Participants who did not meet this criterion were classified as CT-negative.

This composite reference standard has been used in diagnostic accuracy studies where no single specimen can be regarded as an independent gold standard, and it reduces the likelihood of misclassification from isolated false-positive results. Nevertheless, this approach may introduce incorporation bias because the evaluated specimen types contribute to the reference classification. This limitation is considered in the interpretation of the findings.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA).

Continuous variables were summarised as means and standard deviations or medians and interquartile ranges where appropriate. Categorical variables were summarised as frequencies and percentages.

The prevalence of CT infection was calculated with corresponding 95% confidence intervals.

Associations between CT infection and selected reproductive and behavioural factors were initially examined using Pearson's Chi-square test or Fisher's Exact test, as appropriate. Crude prevalence odds ratios (PORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of association.

Variables demonstrating evidence of association in the bivariate analysis ($p < 0.25$) and variables considered clinically relevant based on prior literature were entered into a multivariable binary logistic regression model to identify factors independently associated with CT infection. CT infection status (infected/not infected) was specified as the dependent variable. Adjusted odds ratios (AORs) with corresponding 95% confidence intervals were estimated. Statistical significance was defined as a two-sided *p-value* < 0.05.

Diagnostic performance of each specimen type was evaluated by calculating sensitivity relative to the composite molecular reference standard, together with 95% confidence intervals calculated using the Wilson method.

Agreement between specimen types was assessed using Cohen's kappa coefficient and interpreted according to the Landis and Koch classification.

McNemar's test was used to evaluate paired differences in detection rates between specimen types.

All statistical tests were two-sided, and p-values <0.05 were considered statistically significant.

Results

Participant characteristics

As seen in Table 1, participants were predominantly young (mean age 29.9 years), married (95%), and mostly Malay (88%), with nearly half holding a tertiary education (48%). More than half reported low household income (< RM 2000). These figures describe a predominantly young, married, lower- to middle-income cohort.

Table 1
– Sociodemographic characteristics of respondents (N = 100)

Variable		Frequency (n, %)
Age	Mean (SD)	29.9 (5.43)
Marital status	Married	95 (95.0%)
	Single	5 (5.0%)
Race	Malay	88 (88.0%)
	Non-Malay	12 (12.0%)
Level of education	Primary	9 (9.0%)
	Secondary	43 (43.0%)
	Tertiary	48 (48.0%)
Occupational status	Working	54 (54.0%)
	Not working	46 (46.0%)
Monthly household income (RM)	< 2000	53 (53.0%)
	2000–5000	39 (39.0%)
	5000–10000	6 (6.0%)
	≥ 10000	2 (2.0%)

Burden of CT infection

The study found that among the 100 participants screened for CT infection, 36 were classified as infected and 64 as not infected, yielding a prevalence of 36% (95% CI: 26.6%-45.4%). This prevalence

reflects infection classification within a hospital-based sample and is not intended to represent population-level antenatal prevalence.

Nevertheless, it indicates a substantial burden of CT infection within the pregnant population studied and aligns with global evidence that CT is a highly prevalent, frequently asymptomatic sexually transmitted infection.

Association of CT infection status with adverse reproductive health variables

A significant association was observed between a history of pregnancy loss and CT infection (POR = 2.72, 95% CI: 1.06–6.97; p = 0.03) (Table 2). Women with a previous pregnancy loss had approximately 2.7 times higher odds of testing positive for CT infection than those without a history of pregnancy loss. This finding is consistent with previous evidence suggesting that untreated CT infection may contribute to adverse reproductive outcomes, including miscarriage, ectopic pregnancy, and infertility.

No statistically significant associations were observed between CT infection and a history of preterm delivery, use of protection during sexual intercourse, number of previous sexual partners, or previous STI diagnosis ($p > 0.05$). Although some directional trends were observed, these findings should be interpreted cautiously given the relatively small sample size and limited statistical power.

Table 2
– Association between adverse reproductive health variables and *Chlamydia trachomatis* infection among study participants

Variable		Infected	Not infected	OR	P - value	95% CI
Previous loss of pregnancies	Yes	13 (54.2%)	11(45.8%)	2.72	**0.03***	1.06–6.97
	No	23 (30.3%)	53 (69.7%)			
Pre-term deliveries	Yes	9 (47.4%)	10 (52.6%)	1.80	0.25	0.65–5.02
	No	27 (33.3%)	54 (66.7%)			
Use of protection during sex	Yes	2 (20.0%)	8 (80%)	0.41	0.32†	0.08–2.05
	No	34 (37.8%)	56 (62.2%)			
Number of previous sexual partners	Nil	22 (42.3%)	30 (57.7%)	Ref		
	1	12 (28.6%)	30 (71.4%)	0.55	0.18	0.23–1.32
	> 1	2 (33.3%)	4 (66.7%)	0.68	0.67	0.11–4.04
Previous diagnosis of STI in self/ partner	Yes	0 (0.0%)	1(100.0%)	Not estimable‡	1.00 †	
	No	36 (36.4%)	63 (63.6%)			

* *Statistically significant at p < 0.05*

† *Fisher's Exact Test was used because of sparse data*

‡OR could not be estimated due to zero events in the exposed group

In the bivariate analysis, a history of pregnancy loss was significantly associated with CT infection (OR = 2.72, 95% CI: 1.06–6.97; *p* = 0.03). Pre-term delivery, use of protection during sexual intercourse, number of previous sexual partners, and previous STI diagnosis were not significantly associated with CT infection.

Given the relatively small number of CT-positive cases (n = 36), only variables meeting the predefined inclusion criterion were retained in the final multivariable model to reduce the risk of model overfitting.

Variables meeting the pre-specified inclusion criterion ($p < 0.25$) and variables considered clinically relevant were subsequently entered into a multivariable binary logistic regression model. After adjustment, none of the variables remained significantly associated with CT infection. Women with a history of pregnancy loss had higher odds of CT infection (AOR = 2.22, 95% CI: 0.83–5.98), although this association was not statistically significant (p = 0.11). Likewise, no significant associations were observed for preterm delivery (AOR = 1.60, 95% CI: 0.54–4.73; p = 0.39), use of protection during sexual activity (AOR = 0.36, 95% CI: 0.07–1.89; p = 0.23), or number of previous sexual partners (overall p = 0.48) as seen in Table 3 below.

Table 3
Multivariable binary logistic regression analysis of factors associated with CT infection

Variable	AOR	P - value	95% CI
Previous pregnancy loss	2.22	0.11	0.83–5.98
Pre-term delivery	1.60	0.39	0.54– 4.73
Use of protection during sex	0.36	0.23	0.07–1.89
Previous sexual partners (overall)			0.48
1 partner vs Nil	1.16	0.87	0.18–7.40
>1 partners vs Nil	0.66	0.67	0.10–4.40

Detection performance and agreement between sample types

Relative to the composite molecular reference standard, endocervical specimens demonstrated the highest sensitivity for CT detection (61.1%, 95% CI: 44.9–75.2%), followed by vaginal specimens (47.2%, 95% CI: 32.0–63.0%) and urine specimens (41.7%, 95% CI: 27.1–57.8%) seen in Table 4. Although endocervical samples yielded the highest detection rate, substantial overlap between confidence intervals suggests that these differences should be interpreted cautiously.

Table 4
Sensitivity of different sample types for Chlamydia detection relative to the composite molecular reference standard

Sample type	True positives	False Negatives	Sensitivity (%)*	95% CI
Endocervical	22	14	61.1%	44.9–75.2%
Vaginal	17	19	47.2%	32.0–63.0%
Urine	15	21	41.7%	27.1–57.8%

**Sensitivity was calculated using the composite molecular reference standard as the reference classification*,

whereby participants were considered infected when at least two of the three specimen types tested positive.

As seen in Table 5, pairwise agreement between specimen types was fair, with Cohen's κ values ranging from 0.27 to 0.33. The highest agreement was observed between vaginal and urine specimens (κ = 0.33, 95% CI: 0.11–0.55), while agreement between endocervical and vaginal specimens was slightly lower (κ = 0.27, 95% CI: 0.05–0.49). Although observed agreement ranged from 77.0% to 82.0%, the κ values indicate only fair concordance beyond chance. McNemar's tests showed no significant differences in positivity rates between specimen pairs (all p > 0.05), suggesting balanced discordance between sampling methods despite differences in observed sensitivity.

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Table 5
– Agreement and paired comparison of endocervical, vaginal, and urine samples for CT detection using the MOMP assay

Sample comparison	Observed agreement (%)	Cohen's κ (95% CI)	Agreement level	McNemar p-value
Endocervical and Vaginal	77.0	0.27 (0.05–0.49)	Fair	0.41
Vaginal and Urine	82.0	0.33 (0.11 - 0.55)	Fair	0.82
Urine and Endocervical	79.0	0.31 (0.09–0.53)	Fair	0.19

κ was interpreted according to the Landis and Koch classification.

McNemar's test was used to assess paired differences in positivity rates between specimen types.

Taken together, these findings indicate that while non-cervical samples show reduced sensitivity relative to endocervical specimens, no alternative sampling site performed statistically better in paired comparisons.

These findings highlight the high CT prevalence estimate in the study population. This study has contributed to the identification of a targetable high-risk group, e.g., women with a history of pregnancy loss. Evidence-based, optimal specimen type for detection of CT infection remains endocervical sampling; however, for self-sampling, vaginal sampling can be recommended.

Discussion

This study provides contemporary data on the burden of CT infection among pregnant women attending a tertiary referral hospital in Malaysia and evaluates the diagnostic performance of different specimen types using a molecular assay. The findings indicate a substantial prevalence of CT infection within this hospital-based cohort and demonstrate variation in detection across specimen types. In addition, previous pregnancy loss emerged as a potential marker of infection in the bivariate analysis, although this association did not remain statistically significant after adjustment for other factors

Burden of CT infection in pregnancy

The prevalence of CT infection observed in this study (36.0%, 95% CI: 26.6%-45.4%) was considerably higher than estimates reported in most international studies of pregnant women[1,3,4]. Nevertheless, caution is required when interpreting this finding. Participants were recruited from a single tertiary referral centre, and infection status was determined using a composite molecular reference standard rather than a routinely implemented commercial NAAT platform. These factors may have contributed to the elevated prevalence estimate and limit generalisability to the broader Malaysian antenatal population. The relatively small, hospital-based sample may overrepresent women with pregnancy complications or unmeasured risk factors. Consequently, the prevalence estimate cannot be generalised to the broader antenatal population.

Despite these limitations, the findings suggest that a substantial burden of asymptomatic CT infection may remain undetected within antenatal settings where routine screening is not performed. Given the well-established association between untreated CT infection and adverse maternal and neonatal outcomes, including miscarriage, preterm birth, neonatal conjunctivitis, and neonatal pneumonia, improved case detection may represent an important public health priority[1,3,11]. The cohort's demographic characteristics—predominantly young, married women from lower- to middle-income households—highlight a critical public health gap. Previous studies have demonstrated that perceived low behavioural risk, including marital status, often leads to under-screening for STIs, despite substantial background prevalence[2,8]. This underscores the importance of reframing CT infection as a population-level antenatal health concern rather than a behaviour-defined condition.

Given that 85–90% of chlamydial infections are asymptomatic[5], reliance on symptom-based detection is likely to underestimate CT burden and may allow infection to persist undetected, contributing to ongoing transmission with implications for maternal morbidity and adverse pregnancy outcomes [1,2]. These findings therefore reinforce WHO and CDC recommendations to use NAATs for screening and diagnosis in pregnant women, particularly in contexts with an uncertain baseline CT prevalence, to enable timely treatment and prevent complications [1,6].

Reproductive and sexual behavioural factors associated with CT infection

In the bivariate analysis, previous pregnancy loss was significantly associated with CT infection. Women reporting a history of pregnancy loss had approximately 2.7-fold higher odds of infection than women without previous pregnancy loss. However, after adjustment for preterm delivery, condom use, and number of previous sexual partners, the association no longer reached statistical significance. The attenuation of the association following adjustment suggests that pregnancy loss may represent a clinical marker associated with prevalent CT infection rather than an independently predictive factor. Nevertheless, the adjusted odds ratio remained elevated, indicating that a potential relationship cannot be excluded. Given the relatively small sample size and limited number of infected participants, the study may have been underpowered to detect moderate associations. Larger prospective studies are required to determine whether previous pregnancy loss represents a clinically useful indicator for targeted antenatal screening.

No statistically significant associations were observed for preterm delivery, use of protection during sexual activity, number of previous sexual partners, or previous STI diagnosis. The absence of significant findings should not be interpreted as evidence of no association. Wide confidence intervals around several estimates indicate limited precision and suggest that larger studies are required before definitive conclusions can be drawn.

Although the cross-sectional nature of our study limits causal inference, this finding is biologically plausible, linking untreated CT infection to endometrial inflammation, placental dysfunction, miscarriage, and infertility [3,4,11]. Previous pregnancy loss may represent a clinical marker associated with prevalent CT infection for identifying women who may benefit from targeted antenatal screening in settings where it has not yet been implemented.

In settings where universal screening is not yet incorporated into antenatal care, a risk-based approach incorporating obstetric history may offer an interim way to improve detection. This aligns with previous reports recommending risk-stratified screening to improve detection efficiency in resource-constrained settings [5,6,7].

Diagnostic performance and specimen agreement

Consistent with prior studies, endocervical specimens demonstrated the highest sensitivity for CT detection relative to the composite reference conclusion, followed by vaginal and urine samples[1,5,6]. These findings align with the biological localisation of CT infection within the endocervical epithelium and support continued use of endocervical sampling as a clinically useful specimen for detection. However, the modest sensitivity observed, even with endocervical samples, highlights the challenges of detecting low organism loads, particularly in asymptomatic pregnant populations, even with nucleic acid amplification tests (NAATs)[2,6]. Additionally, substantial overlap in the confidence intervals for sensitivity estimates suggests that these differences should be interpreted cautiously. Furthermore, because the evaluated specimen types contributed to the composite reference classification, the reported sensitivities should be regarded as relative rather than absolute measures of diagnostic performance.

Agreement analyses revealed only fair concordance between specimen types, with no statistically significant directional differences observed in paired comparisons. This suggests that no specimen type consistently outperformed another despite the numerically higher sensitivity observed for endocervical samples. Similar variability in detection across anatomical sites has been reported previously and is likely attributable to heterogeneous bacterial distribution rather than NAAT methodological limitations [15,6]. From a clinical perspective, these findings support continued use of endocervical sampling as the preferred diagnostic specimen while recognising vaginal self-sampling as a feasible and acceptable alternative, particularly for improving screening uptake[15].

Public health implications

Routine antenatal CT screening is not currently incorporated into standard antenatal care in Malaysia. The findings suggest that asymptomatic infection may be more common than previously appreciated in some clinical settings. Although the present data are insufficient to support universal screening recommendations, they provide preliminary evidence that may help design larger multicentre studies and future cost-effectiveness evaluations.

Strengths and limitations

Key strengths include its multi-specimen molecular diagnostic approach, assessment of both detection performance and inter-specimen agreement, and exploration of reproductive risk factors in a setting with limited existing data. However, several limitations remain. Single-centre recruitment limits generalisability and may overstate prevalence. Most reproductive and behavioural variables did not show statistically significant associations with CT infection, likely reflecting limited statistical power due to small subgroup sizes.

The cross-sectional design precludes causal inference, and the reference conclusion, while representing the best available determination of infection status, may introduce classification uncertainty. Although this approach has been employed in diagnostic accuracy studies where no single specimen can serve as a definitive reference, incorporation bias cannot be excluded. Therefore, the findings should be interpreted as preliminary and require validation in larger multicentre cohorts.

Future directions and conclusion

Future research should involve larger, multicenter cohorts with longitudinal follow-up to assess pregnancy and neonatal outcomes following diagnosis and treatment. Evaluating the effectiveness of digital health interventions in improving screening uptake, treatment adherence, and partner therapy would further strengthen the evidence base for integrated antenatal screening models.

In conclusion, this study reveals a substantial burden of CT infection among pregnant women attending a tertiary care health institution in Malaysia, identifies prior pregnancy loss as a potential risk marker, and underscores key diagnostic considerations for antenatal screening strategies. From a public-health perspective, the high prevalence of asymptomatic CT infection has significant implications for maternal,

neonatal, and population health. Undiagnosed infection during pregnancy contributes to preventable adverse outcomes, ongoing community transmission, and long-term reproductive morbidity. The findings support the urgent need for enhanced antenatal screening policies tailored to local epidemiology and linkage to optimised antenatal and perinatal care and wellbeing. Future evaluation of targeted screening strategies may inform SDG-3-aligned maternal health interventions.

Declarations

Ethics approval and Consent to Participate

Ethics approval for this study was obtained from the Ministry of Health, Medical Research Ethics Committee (MREC) (24-02000-FEE). Malaysia. Written informed consent was obtained from all participants prior to participation in the study.

Consent for Publication

Not applicable

Competing Interests

The authors declare no competing interests.

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Availability of data and materials

Clinical data supporting the findings of this study are available from the corresponding author upon reasonable request. Access is restricted to protect patient privacy in accordance with institutional and ethical guidelines.

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