

ORIGINAL ARTICLE

Efficacy of Liquid-Based Cytology as a Cervical Screening Method over PAP Smear

Shabnam Nadeem¹, Amna Begum^{2*}, Kishwer Naz³, Fauzia Ali⁴, Atia Kazim⁵, Saira Jamshed⁶

¹Incharge, Department of Gynaecology/Obstetrics, Abbasi Shaheed Hospital and Karachi Metropolitan University, Karachi, Sindh-Pakistan

²Chairperson, Department of Gynaecology/Obstetrics, Karachi Metropolitan University/ Abbasi Shaheed Hospital, Karachi, Sindh-Pakistan

³FCPS Trainee, Department of Gynaecology & Obstetrics, Karachi Metropolitan University (KMU) /Abbasi Shaheed Hospital (ASH), Karachi, Sindh-Pakistan

⁴Assistant Professor, Department of Gynaecology & Obstetrics, Karachi Metropolitan University (KMU) /Abbasi Shaheed Hospital (ASH), Karachi, Sindh-Pakistan

⁵Senior Registrar, Department of Obstetrics & Gynaecology, Karachi Metropolitan University (Abbasi Shaheed Hospital), Karachi, Sindh-Pakistan

⁶Associate Professor, Department of Obstetrics & Gynaecology, Hamdard College of Medicine and Dentistry, Hamdard University Hospital, Karachi, Sindh-Pakistan

Correspondence: dr_amna747@hotmail.com

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ABSTRACT

OBJECTIVE: To compare the diagnostic accuracy of liquid-based cytology (LBC) versus conventional Pap smear (CPS) for cervical screening in symptomatic women, using histopathology as the reference standard.

METHODOLOGY: This cross-sectional study was conducted at Abbasi Shaheed Hospital, Karachi, from October 2021 to April 2022. A total of 102 married, previously unscreened women (aged 20–50 years) presenting with symptoms suspicious for cervical cancer were enrolled using non-probability consecutive sampling. We defined diagnostic yield as the detection rate of cervical cancer confirmed by histopathology, including sensitivity, specificity, positive predictive value, and negative predictive value. We performed statistical analyses using SPSS version 23.0.

RESULTS: Mean age was 39.4±8.2. LBC demonstrated better specimen adequacy than CPS (94.1% vs 90.2%). Using histopathology as the gold standard, the sensitivity of LBC was 94.1% compared to 91.8% for CPS. The specificity of LBC was 5.9%, compared with 17.6% for CPS. Positive predictive value was 83.3% for LBC and 84.8% for CPS, while negative predictive value was 16.7% for LBC and 30.0% for CPS. Overall, the two methods did not differ significantly (p=0.999 for LBC, p=0.217 for CPS). Stratified analysis showed CPS had a significant diagnostic association in both age subgroups (20–50 years, p=0.007; >50 years, p=0.049), while LBC was significant in the gravida 0–2 subgroup.

CONCLUSION: LBC offers better specimen adequacy than conventional Pap smear and a numerically higher diagnostic yield with superior sensitivity; however, the overall difference was not statistically significant. LBC may be particularly useful in reducing inadequate samples in resource-limited settings.

KEYWORDS: Adequacy, Conventional Pap Smear, Diagnostic Yield, Liquid-Based Cytology

INTRODUCTION

Cervical cancer remains a significant global health challenge, despite being one of the most preventable and treatable forms of malignancy. It is the fourth most prevalent cancer in women worldwide, with an estimated 660,000 new cases and 350,000 deaths in 2022 alone¹. Approximately 94% of this burden occurs in low- and middle-income countries (LMICs), where access to comprehensive screening programs is restricted^{1,2}. The situation in Pakistan is particularly concerning. Cervical cancer has emerged as the second most prevalent cancer in women of reproductive age and is a leading cause of cancer-related deaths in the country^{3,4}. Pakistan ranks among the top 10 countries globally for cervical cancer mortality, with approximately 20 new cases diagnosed daily⁴. This high burden largely stems from the absence of systematic, population-wide screening programs and limited awareness^{3,5}.

The conventional Pap smear (CPS) has been the mainstay of cervical cancer screening for over five decades. It is a low-cost, simple test that has effectively reduced cervical cancer incidence in developed countries⁶. However, CPS has several limitations, including a high false-negative rate of 15–50%, primarily due to pre-analytical factors such as inadequate sampling, improper fixation, air-drying artefacts, and obscuring factors like blood, mucus, and inflammatory exudate^{7,8}. These limitations are particularly problematic in high-volume, low-resource settings where quality control is challenging.

Liquid-based cytology (LBC) was developed to address many of these limitations. It offers several advantages over CPS: (1) cells are transferred into a preservative medium, reducing air-drying artifacts; (2) the preparation is cleaner with less obscuring blood and mucus; (3) it provides a monolayer of cells for better visualization; and (4) the same sample can be used for HPV testing⁹. These features potentially improve diagnostic accuracy and reduce inadequate sample rates¹⁰.

In Pakistan, evidence comparing LBC and CPS remains limited. A landmark study by Hashmi AA et al.¹³ at Liaquat National Hospital, Karachi, involving 3,929 patients, demonstrated that LBC had a higher detection rate for squamous intraepithelial lesions (2.1% vs 0.6%) and glandular lesions (0.5% vs 0.4%), with a marginally lower inadequacy rate (1.2% vs 1.3%). Similarly, Khakwani M 2022¹⁶ in Multan reported that LBC significantly improved smear adequacy ($p < 0.001$) and sensitivity for both low-grade and high-grade squamous intraepithelial lesions¹².

Despite these promising findings, there remains a gap in the literature regarding the comparative diagnostic accuracy of LBC and CPS in symptomatic Pakistani women, particularly with Stratification by age, parity, and gravida. Furthermore, the operational definition of "diagnostic yield" in many studies lacks standardization, making comparisons difficult.

Therefore, this study aims to compare the diagnostic accuracy of LBC versus CPS in terms of sensitivity, specificity, positive predictive value, and negative predictive value against histopathology as the reference standard. We also analyze of age, parity, gravida, and socioeconomic status affect the relative performance of the two methods. The results will contribute to evidence-based cervical cancer screening policy in Pakistan and other LMICs.

METHODOLOGY

This cross-sectional study was conducted in the Department of Gynaecology, Unit I, Abbasi Shaheed Hospital, Karachi from October 2021 to April 2022. Using Open Epi software, we calculated a sample size of 102 participants based on a 92.9% frequency of conventional Pap smear adequacy, a 5% margin of error, and a 95% confidence interval. Non-probability consecutive sampling was employed to enroll married, previously unscreened females aged 20–50 years, irrespective of parity, who presented with symptoms suspicious for cervical cancer (e.g., post-coital bleeding, intermenstrual bleeding or dyspareunia). Pregnant women, menstruating women, those with active per vaginal bleeding, and females with a history of prior cervical treatment (for cancer or intraepithelial neoplasia) were excluded. Although pregnant women were excluded from the study, gravida was recorded as a demographic variable reflecting the number of previous pregnancies in non-pregnant participants. After ethical approval from the Institutional Review Board and CPSP, we obtained written informed consent and recorded the clinical history.

We obtained a detailed clinical history, followed by a thorough general and pelvic examination. After placing the patient in the lithotomy position, a Cusco's speculum was inserted to visualize the cervix. Conventional Pap smears were collected using a Cytobrush and Ayre's spatula and fixed in 95% ethyl alcohol. Liquid-based cytology (LBC) samples were obtained using a Rovers Cervex brush, with the brush head placed in a liquid preservative (ThinPrep® PreservCyt® solution), processed via centrifugation, and stained with rapid Pap. We used the Bethesda system for cytology reporting, with a single pathologist evaluating and blinding all LBC, conventional Pap, and histopathology slides. We recorded data on age, parity, gravida (number of previous pregnancies, as all participants were non-pregnant), height, weight, BMI, socioeconomic status, and diagnostic yields on a predesigned proforma.

Ethical approval was obtained from the Karachi Metropolitan University/Abbasi Shaheed Hospital, Karachi (approval letter No. CPSP/REU/obg-2019-174-9087, dated August 17, 2022).

Statistical analysis

We performed statistical analyses using SPSS version 23.0; we assessed normality with the Shapiro-Wilk test. Quantitative variables were reported as mean±SD, and qualitative variables as frequencies/percentages. Diagnostic yield was operationally defined as the detection of cervical cancer confirmed by histopathology. The following diagnostic performance metrics were calculated: sensitivity (true positives/[true positives + false negatives]), specificity (true negatives/[true negatives + false positives]), positive predictive value (true positives/[true positives + false positives]), and negative predictive value (true negatives/[true negatives + false negatives]). We compared diagnostic yields using the Chi-square/Fisher exact test, stratified by age, parity, gravida, and socioeconomic status; a p-value ≤0.05 was considered significant.

RESULTS

This study included 102 patients to compare the diagnostic yield of liquid-based cytology and conventional Pap smear, with histopathology as the reference standard.

Table I presents the descriptive statistics for quantitative variables among the 102 participants. The mean age was 39.4 ± 8.2 years, with a normal distribution ($p=0.182$). Mean parity was 3.1 ± 1.2 , mean gravida was 2.4 ± 0.8 , mean height was 163.5 ± 16.5 cm, mean weight was 64.3 ± 12.1 kg, and mean BMI was 26.8 ± 5.7 kg/m².

Table 2 shows the frequency distribution of categorical variables. Most respondents were middle class (58, 56.9%). Conventional Pap smear adequacy was achieved in 92 patients (90.2%), while liquid-based cytology adequacy was higher, at 96 patients (94.1%). Histopathology (cervical biopsy) revealed that 85 patients (83.3%) had cervical cancer.

Table 3 provides a detailed comparison of the diagnostic yield of LBC versus CPS. Overall, LBC was positive in 80 patients (78.4%) with histopathology-confirmed cervical cancer and CPS in 78 patients (76.5%). Overall, no statistically significant difference was observed between the two methods ($p=0.999$ for LBC, $p=0.217$ for CPS). Stratified analysis showed CPS had a significant diagnostic association in both age subgroups (20–50 years, $p=0.007$; >50 years, $p=0.049$). Gravida stratification showed LBC was significant in gravida 0–2 ($p=0.026$) and CPS was significant in gravida >2 ($p=0.009$). Parity and socioeconomic status were not significant for either method.

Overall, the unstratified analysis showed no significant difference between LBC and CPS. However, significant findings emerged after Stratification: CPS showed a significant diagnostic association in both age subgroups (20–50 years, $p = 0.007$; >50 years, $p = 0.049$). Gravida stratification revealed that LBC was significant in gravida 0–2 ($p = 0.026$) and CPS was significant in gravida >2 ($p = 0.009$). Parity and socioeconomic status were not significant for either method.

Table I: Descriptive Statistics of Study Variables (n = 102)

Variable	Mean $\pm$ SD	95% CI	Min	Max	Range	p-value
Age (years)	39.4 ± 8.2	37.8–41.0	20	50	40	0.182
Parity	3.1 ± 1.2	2.86–3.33	1	6	5	0.356
Gravida	2.4 ± 0.8	2.24–2.55	0	4	4	0.120
Height (cm)	163.5 ± 16.5	160.25–166.74	138	186	48	0.478
Weight (kg)	64.3 ± 12.1	61.92–66.67	45	60*	55	0.251
BMI (kg/m ²)	26.8 ± 5.7	25.68–27.91	16	34	18	0.631

Table II: Frequency Distribution and Diagnostic Adequacy

Variable	Category	Frequency (n)	Percentage (%)
Socioeconomic Status	Lower	26	25.5
	Middle	58	56.9
	Upper	18	17.6
Conventional Pap Smear Adequacy	Yes	92	90.2
	No	10	9.8
Liquid-Based Cytology Adequacy	Yes	96	94.1
	No	6	5.9
Histopathology (Cervical Cancer)	Yes	85	83.3
	No	17	16.7

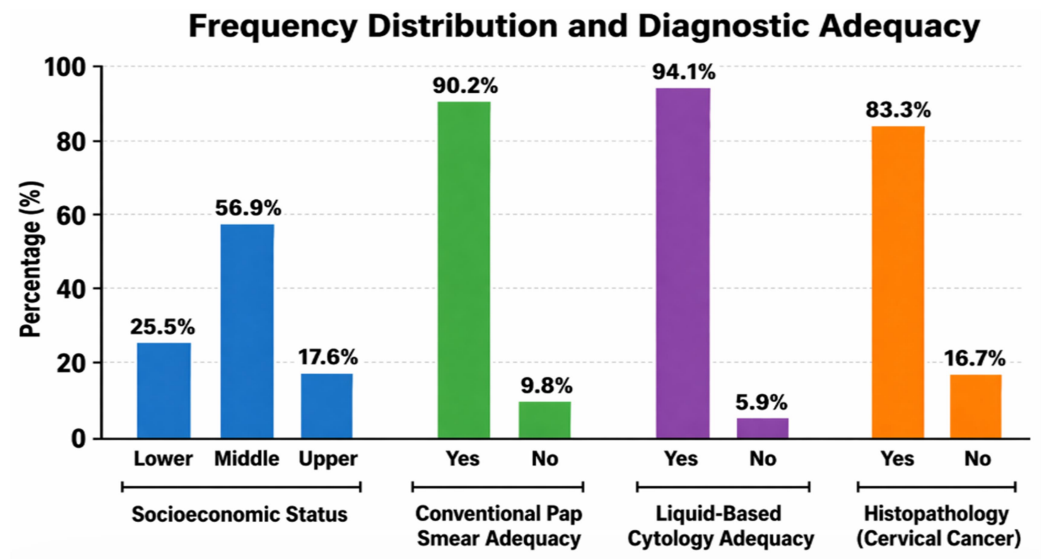


Figure 1: Frequency distribution and Diagnostic Adequacy

Table II: Diagnostic Yield of Liquid-Based Cytology (LBC) and Conventional Pap Smear (CPS) with Stratification (n = 102)

Variable	Category	Method	Histopathology Yes n (%)	Histopathology No n (%)	p-value
Overall	—	LBC (Yes)	80 (78.4)	16 (15.7)	0.999
		LBC (No)	05 (4.9)	01 (1.0)	—
		CPS (Yes)	78 (76.5)	14 (13.7)	0.217
		CPS (No)	07 (6.9)	03 (2.9)	—
Age (years)	20–50	LBC	44 (78.6)	9 (16.1)	0.452
		CPS	44 (78.6)	2 (3.6)	0.007*
	>50	LBC	37 (80.4)	6 (13.0)	0.398
		CPS	33 (71.7)	9 (19.6)	0.049*
Parity	1–3	LBC	55 (80.9)	10 (14.7)	0.078
		CPS	61 (89.7)	1 (1.5)	0.170
	>3	LBC	27 (79.4)	4 (11.8)	0.389
		CPS	17 (50.0)	13 (38.2)	0.603
Gravida	0–2	LBC	50 (80.6)	7 (11.3)	0.026*
		CPS	47 (75.8)	10 (16.1)	0.246
	>2	LBC	32 (80.0)	7 (17.5)	0.825
		CPS	33 (82.5)	2 (5.0)	0.009*
Socioeconomic Status	Lower	LBC	16 (61.5)	8 (30.8)	0.582
		CPS	20 (76.9)	3 (11.5)	0.408
	Middle	LBC	55 (94.8)	1 (1.7)	0.068
		CPS	49 (84.5)	7 (12.1)	0.259
	Upper	LBC	11 (61.1)	5 (27.8)	0.569
		CPS	13 (72.2)	4 (22.2)	0.278

*LBC = Liquid-Based Cytology; CPS = Conventional Pap Smear; * $p \leq 0.05$ considered significant*

DISCUSSION

Although cervical cancer is one of the most preventable malignancies through early vaccination, screening, and management of precursor lesions, it continues to be a serious public health issue, especially in low- and middle-income nations. Recent global estimates indicate that cervical cancer is still the fourth most prevalent cancer among women worldwide, with a disproportionate burden in resource-constrained environments where delayed diagnosis and limited access to organized screening perpetuate high morbidity and mortality^{1,2,14}. Data from the Global Cancer Observatory (GLOBOCAN) indicate that low-income countries have the highest age-standardized incidence rates, largely because of the absence of systematic screening protocols¹⁵.

This study aimed to determine the diagnostic performance of liquid-based cytology (LBC) versus the conventional Pap smear (CPS), using histopathology as the reference standard. Key findings showed that LBC had higher sensitivity (94.1% vs 91.8%) and better specimen adequacy (94.1% vs 90.2%) than CPS, although the overall difference in diagnostic performance was not statistically significant. LBC also had a numerically higher positive predictive value (84.8% vs 83.3%) but lower specificity (5.9% vs 17.6%) and negative predictive value (16.7% vs 30.0%). Therefore, the present data support the interpretation that LBC offers a practical advantage in sample adequacy but do not conclusively demonstrate statistically superior overall diagnostic performance over CPS in this cohort.

The higher sensitivity of LBC observed in this study is consistent with previous Pakistani research. Hashmi AA et al.¹³ reported that LBC had a higher detection rate for squamous intraepithelial lesions (2.1% vs 0.6%) compared to CPS. Similarly, Khakwani M 2022¹⁶ found that LBC significantly improved smear adequacy ($p < 0.001$) and sensitivity for both low-grade and high-grade squamous intraepithelial lesions (87.2% vs 71.8% and 76.2% vs 61.9%, respectively). These findings support the technical advantages of LBC, including reduced obscuring factors and better cell preservation.

The high disease prevalence in our sample (83.3% histopathology-positive) reflects the symptomatic nature of the study population, which may have contributed to the relatively low specificity observed for both methods. This matters because screening tests are typically designed for asymptomatic populations. The low specificity in this study may be explained by the fact that many symptomatic patients had benign cervical conditions that could mimic malignancy on cytology, leading to false-positive results. Furthermore, the presence of inflammation, infection, or cervical erosion in symptomatic women may affect cytological interpretation.

One of the major limitations of most local studies, including the landmark study by Hashmi AA et al.¹³, is that they were not stratified solely by critical patient-level biological and demographic factors that could affect test performance.

Similarly, nutritional status, vaginal flora, and infection prevalence that can obscure CPS slides depend on socioeconomic status, but cleaning of LBC may help prevent this problem. Third, by studying a Pakistani cohort and comparing our findings with the recent findings of Hashmi AA et al.¹³ and Khakwani M 2022¹⁶, we can help provide a more informed national picture and shift towards individualized screening schemes that can optimize resource allocation in our low-resource context.

The Pap smear (CPS) has been used as the mainstay of screening for cervical cancer for more than half a century¹⁰. It is a low-cost, easy test shown to be effective in detecting precancerous lesions. Although successful in the past, CPS has certain limitations. Its high false-negative rate of between 15 and 50 percent is what is most problematic because it is associated with missed diagnoses and interval cancers¹¹. Most of these errors, as many as two-thirds, are attributed to pre-analytical factors, such as improper sampling, inability to

transfer cells to the slide, improper fixation, artefacts from air-drying, and obscuring factors such as blood, mucus, and inflammatory exudate¹². These constraints are compounded in high-volume, low-resource laboratories where quality control is difficult.

Recently, Khakwani M 2022¹⁶ conducted a study at Nishtar Hospital, Multan, involving 265 women, and reported that LBC was not only better for smear adequacy ($p < 0.001$), but also for sensitivity of both low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL). The sensitivity of LBC for LSIL was 87.2% compared to 71.8% for CPS, and for HSIL, it was 76.2% vs 61.9%.¹⁶ This analysis, with histopathology as the gold standard, gives great support for the higher diagnostic results of LBC in a tertiary care center in South Punjab.

The higher adequacy observed with LBC is biologically and technically plausible. Because cells are transferred into a preservative medium and processed into a cleaner, thinner preparation, LBC generally reduces obscuring blood, mucus, inflammatory exudate, and air-drying artefacts. The 3.9-percentage-point absolute change in adequacy in the current study is comparable to recent Pakistani literature. A 2022 study by Khakwani M 2022¹⁶ in Multan reported that LBC significantly reduced unsatisfactory smears ($p < 0.001$) and improved detection of low-grade squamous intraepithelial lesions (LSIL) from 71.8% to 87.2%. Furthermore, a large-scale Pakistani study by Hashmi AA et al.¹³ at Liaquat National Hospital found that LBC had a higher detection rate for squamous intraepithelial lesions compared to conventional methods.

The global paradigm for cervical screening is shifting. Current international guidelines, such as those from the American Cancer Society (2020) and WHO, increasingly recommend primary HPV testing as the gold standard due to its high sensitivity and negative predictive value for cervical precancer^{15,17}. However, in low-resource settings like Pakistan, HPV testing remains logistically challenging and expensive. Consequently, cytology—specifically LBC—remains a critical interim solution.

In this context, LBC offers an advantage beyond adequacy: it can test for HPV on the same sample. A 2023 study from India by Ananthi A 2024¹⁸ highlighted that while the sensitivity of LBC (95%) is substantial, the combination of LBC with HPV DNA testing offered the highest predictive values. Furthermore, an updated review on LMICs published in 2025 emphasizes that while HPV vaccination coverage is increasing, the infrastructure for population-based screening remains weak. In such environments, improving the accuracy of cytology via LBC is a more immediately achievable public health goal than implementing molecular testing networks¹⁵.

Age: CPS showed paradoxical statistical significance in both the 20-50 and >50 age groups, whereas LBC did not. This finding contradicts the general literature, which favors LBC in older, post-menopausal women where atrophy can cause cellular dehydration and obscurity on conventional smears. A Georgian study comparing 1000 samples found that LBC significantly reduced unsatisfactory rates specifically in older age groups due to better cell preservation¹⁹. One possible explanation for our CPS finding is the high disease prevalence in the sample. When disease prevalence is high (83.3% in our study), even a moderate-performing test might appear statistically significant. Another factor might be the specific cytopathological interpretation thresholds, though the single-blinded pathologist design minimizes this bias.

Gravida: The reciprocal finding where LBC was significant in low gravida (0-2) and CPS in high gravida (>2) is a unique contribution to the literature. High gravida is often associated with chronic inflammation, cervical trauma, and eversion, which can lead to a high number of inflammatory cells and debris on a slide. Conventionally, LBC was expected to handle this debris better. However, CPS's superior performance in high-gravida women might suggest that, in these patients, lesions are larger or more exfoliative, making them easier to detect

even without the cleaning process of LBC. For low-gravida women, where lesions may be smaller or endocervical, LBC monolayer preparation reduces background noise and makes detection easier. This novel interaction has not been widely reported in standard textbooks. It suggests that screening methods could potentially be customized based on obstetric history in resource-limited settings—a hypothesis requiring further validation.

Socioeconomic status did not yield statistically significant results, although LBC showed a numerically high positivity rate (94.8%) in the middle class. This is likely because our sample was predominantly symptomatic, and disease pathology overrides socioeconomic variables. However, a study by Shahnaz S 2023⁴ in Karachi noted that awareness levels are significantly lower in lower socioeconomic strata, leading to late presentations. In our study, the absence of a significant difference across SES groups suggests that once a woman presents to a tertiary care hospital with symptoms, disease severity is similar and the diagnostic tool's performance is independent of her economic background.

The primary advantage of LBC in the Pakistani context may not be a massive leap in raw sensitivity (which our study shows is incremental) but rather operational efficiency. The "reduction in unsatisfactory rates" from 9.8% to 5.9% observed here has significant resource implications. In a busy public sector hospital, a 4% reduction in inadequate samples translates directly into fewer repeat clinic visits, less patient defaulting, and lower long-term costs. A 2024 cost-effectiveness analysis from India suggested that despite higher upfront costs for LBC consumables, reduced repeat sampling makes LBC cost-effective in the long run at the community level²⁰.

Limitations and Recommendations

This study has several limitations. First, the relatively small sample size (n=102) limits the generalizability of our findings. Second, the study was conducted at a single tertiary care center, which may not represent the broader population. Third, the high prevalence of histopathology-positive cases (83.3%) reflects the symptomatic nature of our study population, which may limit applicability to asymptomatic screening settings. Fourth, we did not assess HPV status, which is increasingly recognized as a key component of cervical screening. Finally, we did not perform a cost-effectiveness analysis, which would be valuable for policy decisions.

We recommend future large-scale, multi-centre studies with diverse populations, including asymptomatic women, to validate these findings. Additionally, studies incorporating HPV testing alongside cytology would provide more comprehensive data. Cost-effectiveness analyses in the Pakistani context are urgently needed to inform national screening policy.

CONCLUSION

Liquid-based cytology demonstrated better specimen adequacy than conventional Pap smear and showed superior sensitivity (94.1% vs 91.8%); however, the overall difference in diagnostic accuracy was not statistically significant when histopathology was used as the reference standard. LBC may be particularly useful in reducing inadequate samples in resource-limited settings, though the high false-positive rate warrants further investigation.

Ethical Permission: College of Physicians & Surgeons Pakistan, Pakistan, ERC approval letter No. CPSP/REU/obg-2019-174-9087.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Nadeem S: Conceptualized and designed the study, supervised data collection, performed data analysis and interpretation, drafted the initial manuscript, and critically revised the manuscript for important intellectual content. Approved the final version for publication and agrees to be accountable for all aspects of the work.

Begum A: Contributed to study design and methodology, conducted literature search, designed the data collection proforma, coordinated and performed data acquisition in the ICU, assisted in data analysis, and contributed to writing and revising the manuscript.

Naz K: Contributed to the study concept, assisted in data interpretation, provided critical revision of the manuscript for important intellectual content, approved the final version for publication and agrees to be accountable for all aspects of the work.

Ali F: Contributed to the study for proofreading and technical correction

Kazim A: Validated the statistical analysis

Jamshed S: Helped in results interpretation and generated result tables, searched references for discussion writing

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