

DIAGNOSTIC UTILITY OF VAGINAL PLACENTAL PROTEIN 14 AS A NOVEL BIOMARKER FOR PRETERM PRELABOR RUPTURE OF MEMBRANES

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Preterm prelabor rupture of membranes (PPROM) is a major cause of neonatal morbidity and mortality. Placental Protein 14 (PP14), a glycodelin glycoprotein, has emerged as a potential biomarker for the diagnosis of PPRM. The study aimed to evaluate the diagnostic accuracy of PP14 in detecting fetal membrane rupture. This case-control study was conducted at Al Yarmouk Teaching Hospital from January to October 2023. The sample included 45 pregnant women diagnosed with PPRM who were compared to 45 pregnant women with intact membranes. Vaginal fluid samples collected from women in both groups were tested with nitrazine paper and then sent to the laboratory for PP14c measurement using immunoassay. PPRM cases had significantly higher vaginal PP14 levels than controls (mean 0.0108 ± 0.002 ng/mL vs. 0.0056 ± 0.003 ng/mL; $p < 0.001$). ROC analysis showed an AUC of 0.852 (95% CI: 0.762–0.941; $p < 0.001$). Using a cut-off of 0.0078 ng/mL, PP14 yielded a sensitivity of 97.8% and specificity of 77.8%. The odds of elevated PP14 (≥ 0.0078 ng/mL) were significantly higher in PPRM cases (OR = 154; 95% CI: 18.8–1261.4; $p < 0.001$). In conclusion, vaginal PP14 demonstrates strong diagnostic performance for PPRM, combining high sensitivity with good specificity. PP14 is a promising, low-cost potential adjunct to conventional bedside tests and could improve early, accurate detection of membrane rupture, facilitating timely clinical management.

Keywords: PROM, PP14, nitrazine

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INTRODUCTION

Prelabor rupture of membranes (PROM) occurs when the chorioamniotic membrane ruptures before labor onset. Preterm PROM (PPROM) before 37 weeks contributes significantly to preterm births and neonatal complications, including infection, respiratory distress, and long-term neurodevelopmental impairment (1,2).

Current bedside tests (speculum exam, fern test, nitrazine paper) are rapid and inexpensive (3). Still, they can yield false positives and false negatives because vaginal pH and visual findings may be altered by blood, semen, infection, or topical agents (4).

Novel biomarkers such as PAMG-1 and IGFBP-1 improve accuracy but may be costly, variably available, or affected by contamination (5). These limitations can delay correct diagnosis or lead to unnecessary interventions.

Diagnosis typically involves clinical examination and laboratory tests, including speculum exams, the fern test (6), and the nitrazine test, though these can have false positives and negatives (4).

Novel biomarkers, such as PAMG-1 and IGFBP-1, offer enhanced diagnostic accuracy for PROM (5), while ultrasound is vital for evaluating amniotic fluid volume and overall fetal well-being (7).

Biological rationale for Placental Protein 14

Placenta Protein 14 (PP14) is an important glycoprotein in the endometrial and decidual context of pregnancy, characterized by its 42,000 MW and 162 amino acids with potential immunosuppressive properties, relevant to pregnancy maintenance (8).

PP14's immunosuppressive role supports the establishment and maintenance of pregnancy by inducing apoptosis in T cells without affecting monocytes (9). Furthermore, because it modulates T-cell activity and cytokine responses, PP14 may be linked to preterm prelabor rupture of membranes (PPROM), thus presenting a potential biomarker for PPRM diagnostics (10).

The tissue specificity and presence of PP14 in amniotic fluid make it a biologically plausible biomarker for vaginal amniotic leakage (11). Although early studies indicate that PP14 concentrations vary significantly between women with intact versus ruptured membranes (11,12), the existing evidence remains limited and heterogeneous.

Gap in knowledge and justification for this study

A critical need exists for novel diagnostic biomarkers that combine high sensitivity with acceptable specificity and

affordability to prevent missed diagnoses of PPRM. Existing biomarker studies vary in population, assay method, and reported thresholds, and few have evaluated PP14 in a well-defined preterm population with standardized sample handling. Demonstrating reproducible diagnostic accuracy for PP14 would support its incorporation into clinical algorithms and could reduce diagnostic uncertainty, unnecessary interventions, and adverse outcomes. Therefore, this study aimed to evaluate the diagnostic accuracy of placental protein 14 (PP14) in detecting rupture of fetal membranes in a standardized clinical setting.

METHODS

A case-control study was conducted in the Obstetrics and Gynecology Department at Al Yarmouk Teaching Hospital from January to October 2023. The study enrolled 90 pregnant women via consecutive sampling based on eligibility criteria during the study period. The cohort comprised 45 pregnant women diagnosed with preterm PROM at gestational age ≥ 28 to < 37 weeks (cases), who were compared to 45 pregnant women at a similar gestational age with intact membranes (controls). The study used the available consecutive eligible patients during the study period.

Group allocation criteria

Cases (PPROM) were defined as pregnant women presenting with clinical evidence of amniotic fluid leakage on sterile speculum examination and a positive nitrazine test. Controls were defined as pregnant women with no observed leakage and a negative nitrazine test.

Exclusion criteria

Exclusion criteria for both groups included use of vaginal medications within 72 hours prior to the interview, recent antibiotic use (which can lead to alkaline urine), active genitourinary tract infection, sexual intercourse within the preceding 48 hours, presence of chronic diseases (e.g., hypertension and diabetes), history of trauma during the current pregnancy, and gross blood contamination in the vaginal fluid sample.

Specimen collection

Following initial history taking, each participant underwent a thorough clinical evaluation, including a sterile speculum examination. Vaginal fluid was collected from the posterior vaginal fornix using a sterile cotton swab or by direct aspiration when pooling was visible. One aliquot was used

immediately for the nitrazine test. A second aliquot was placed into a labeled sterile tube, stored at 2–8°C, and transported daily to the laboratory for PP14 measurement.

Nitrazine test (reference standard)

The nitrazine test was performed immediately at the bedside using sterile nitrazine paper strips (pH range 4.5–7.5). Following application of the vaginal fluid sample to the strip, the color shift was evaluated against the manufacturer-provided pH color chart. A pH ranging from 7.1 to 7.3 (indicated by a dark blue color shift) was considered positive for amniotic fluid leakage (13). Women with a positive nitrazine test and visible fluid pooling were assigned to the PPRM group, while women with a negative test and no pooling were assigned to the control group. Specimens with significant blood contamination were excluded from analysis to prevent diagnostic interference.

Placental Protein 14 (PP14) ELISA (Index Test)

Concentration of PP14 in vaginal fluid was determined using a commercially available enzyme-linked immunosorbent assay (Human Placental Protein 14 ELISA Kit, Catalog No. YLA2850HU; Shanghai YL Biotech Co., Shanghai, China) according to the manufacturer's instructions.

To remove cellular debris, vaginal fluid samples were centrifuged at 1000 x g for 10 minutes. All samples were processed within 48 hours of collection, and the supernatant was collected and stored at –20 °C until analysis. The kit uses a double-antibody sandwich ELISA method. Briefly, 50 microliters (μL) of the standard, controls, or diluted samples were added to designated wells pre-coated with an anti-PP14 monoclonal antibody.

Then, a biotin-conjugated detection antibody was added, followed by the addition of a streptavidin-horseradish peroxidase (HRP) conjugate. After the incubation period, tetramethylbenzidine (TMB) was added as a chromogenic substrate. After adding sulfuric acid to halt the reaction, the optical density was measured using a microplate reader set to 450 nm.

Concentrations were interpolated from a standard curve generated with known PP14 standards supplied in the kit. All samples were assayed in duplicate, and the mean value was used for analysis. The assay sensitivity was 0.001ng/mL, and the intra- and inter-assay coefficients of variation were <10%. Results were expressed as ng/mL of PP14 in vaginal fluid. Laboratory personnel were blinded to the participants' group assignment.

Rigid attention to detail during history-taking, examination, and vaginal fluid collection was maintained, as the nitrazine test can yield false-positive or false-negative results due to contamination with alkaline urine, semen, blood, meconium, vaginitis, cervicitis, or antibiotic use.

The questionnaire

Data were collected using a structured questionnaire that included maternal demographics, such as age and smoking history, as well as obstetric history. Obstetric data comprised gravidity, parity, number of abortions, date of the last menstrual period, gestational age, and estimated date of delivery. Information on current vaginal bleeding, previous premature rupture of membranes (PROM), and prior preterm deliveries was also recorded.

Ethical considerations

The study protocol was reviewed by the Ethics Committee of Al Yarmouk Teaching Hospital (Approval No. 758; Approval Date: November 2, 2022), and administrative and ethical approvals were obtained. All participant interviews for both the PPRM group (cases – group 1) and the intact membrane group (controls – group 2) were conducted within the premises of the Obstetrics and Gynecology Department. All procedures adhered to the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment. To maintain confidentiality, all personally identifiable data were anonymized, and the collected information was used exclusively for the purposes of this study.

Statistical methods

Continuous variables were reported as mean ± standard deviation (SD) or median with interquartile range (IQR) after normality assessment using the Shapiro–Wilk test and Q-Q plots. Categorical variables were presented as frequencies and percentages. Group comparisons used the independent t-test or Mann–Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables.

The diagnostic performance of PP14 was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with a 95% confidence interval (CI) was reported. The optimal cut-off was selected using the Youden index, and the corresponding sensitivity and specificity with 95% CIs were calculated. The unadjusted association between elevated PP14 (dichotomized at the optimal cut-off) and PPRM was assessed using the odds ratio.

RESULTS

The mean age of women in the PPROM group was 26.6 ± 5.2 years (range: 19–37 years), while the mean age in the control group was 27.9 ± 5.1 years (range: 19–37 years), as shown in Table 1. No significant differences were observed between the two groups with regard to age, gravidity, parity, history of miscarriages, gestational age, socioeconomic status (SES), smoking, education, or employment. The majority of women in the PPROM group, 41 (91.1%), were non-smokers, while 4 (8.9%) reported a history of smoking. In the control group, smoking was reported by only 2 (4.4%) participants.

Significant differences were observed concerning the previous history of PROM and preterm delivery. Approximately 14 (31.1%) women in the PPROM group had a history of previous PROM, and another 14 (31.1%) had a history of previous preterm delivery. In contrast, no history of previous PROM was reported in the control group, and a history of previous preterm delivery was confirmed in only 4 (8.9%) control participants.

The mean PP14 level in vaginal fluids in the PPROM group was 0.0108 ± 0.002 ng/ml. while the control group was 0.0056 ± 0.003 ng/ml.

Table 1. Baseline demographic and clinical characteristics of cases and controls

Variables	Cases (n = 45)	Control (n = 45)	p-value
Continuous Variables (mean $\pm$ SD)			
Age (in years)	26.6 ± 5.20	27.9 ± 5.18	0.27 *
Gravidity	3.80 ± 1.455	3.53 ± 1.05	0.323 *
Parity	2.56 ± 1.235	2.33 ± 1.04	0.36 *
Previous history of miscarriages	0.24 ± 0.48	0.13 ± 0.34	0.213 *
Gestational weeks	31.13 ± 2.17	31.33 ± 2.62	0.69 *
Categorical variables, n (%)			
Smoking history			
No	41 (91.1%)	43 (95.6%)	0.677 *
Yes	4 (8.9%)	2 (4.4%)	
Previous PROM			
No	31 (68.9%)	45 (100%)	<0.001*
Yes	14 (31.1%)	0	
Previous history of pre-term delivery			
No	31 (68.9%)	41 (91.1%)	0.008 †
Yes	14 (31.1%)	4 (8.9%)	
Total	45 (100%)	45 (100%)	

*Independent t test, † Chi square test, ‡ Fisher's exact test

There was a highly significant difference in PP14 levels (p-value <0.001) between the two groups. As shown in Figure 1, women in the PPROM group had a higher level of PP14 compared to women in the control group.

Analysis of PP14 concentrations between gestational weeks 24 and 36 demonstrated consistently higher levels in women with PPROM compared to controls. As shown in Figure 2, PP14 values in the PPROM group remained elevated across all time points, with a pronounced increase around week 34. In contrast, the control group exhibited lower and relatively stable concentrations throughout the same gestational period. These findings suggest a potential association between elevated PP14 levels and the pathophysiological processes underlying PPROM.

Receiver operating characteristic curve and odds ratio Receiver operating characteristic (ROC) curve analysis was performed to identify the optimal PP14 cut-off value for

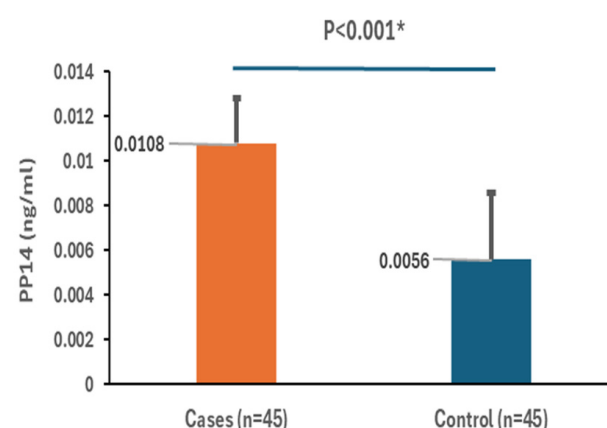


Figure 1. Distribution of controls and cases by the PP14 level. *Mann–Whitney U test

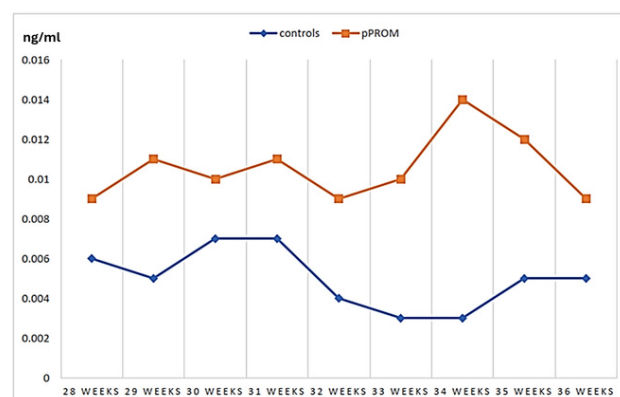


Figure 2. Distribution of PP14 levels across gestational weeks 24 to 36, demonstrating elevated concentrations in cases relative to controls.

diagnosing PPROM. As shown in Figure 3, the area under the curve (AUC) for PP14 was 0.852 (standard error = 0.046; $p < 0.001$; 95% CI: 0.762–0.941).

At the optimal cut-off threshold of 0.0078 ng/mL, PP14 demonstrated good diagnostic performance, with a sensitivity of 97.8% (95% CI: 88.2–99.9%) and a specificity of 77.8% (95% CI: 62.9–88.8%).

Table 2 depicts the distribution of cases and controls according to the vaginal fluid PP14 cut-off level. Using the optimal cut-off of ≥ 0.0078 ng/mL, 44 of 45 PPROM cases (97.8%) tested positive compared to 10 of 45 controls (22.2%).

Women with PPROM had 154 times higher odds of presenting with elevated PP14 (≥ 0.0078 ng/mL) levels than controls (odds ratio (OR) = 154.0, 95% confidence interval (CI): 18.8–1261.4; $p < 0.001$).

Assessment of potential circularity bias

We acknowledge that using a reference standard that includes the nitrazine test may theoretically introduce circularity bias, as PP14 could be correlated with vaginal pH. To assess this, we calculated the Spearman rank correlation between PP14 levels and nitrazine pH values (treated as a continuous variable based on color chart equivalents). The correlation was weak and not statistically

significant (Spearman's $\rho = 0.21$, $p = 0.09$), indicating that PP14 provides diagnostic information largely independent of the nitrazine result. Furthermore, PP14 measurement was performed blinded to group allocation, while group assignment was based on a composite reference standard (sterile speculum pooling plus nitrazine test), not on nitrazine alone. Therefore, the risk of overestimation of diagnostic accuracy is limited.

DISCUSSION

These results indicate that PP14 measured in vaginal fluid is a highly sensitive, reasonably specific, and low-cost biomarker that could substantially improve the diagnostic workup of PPROM, particularly in settings where conventional tests are ambiguous or where more expensive commercial immunoassays are not readily available.

Comparison with conventional diagnostic methods

Current bedside tools, including the nitrazine test, fern test, and sterile speculum examination, are rapid and inexpensive but suffer from well-documented limitations. The nitrazine test, which detects a pH shift (≥ 6.5 –7.1) due to amniotic fluid, has a reported sensitivity of 70–95% and specificity of 70–90%, and they both vary widely due to contamination with blood, semen, alkaline urine, or bacterial vaginosis (1,2,14). False negatives can occur when leakage is minimal or when the fluid is diluted, while false positives are common in the presence of cervicovaginal infections.

In our study, PP14 achieved a sensitivity of 97.8%, which is at the upper bound of what is achievable with nitrazine, and a specificity that, although not perfect, is sufficient to reduce the diagnostic uncertainty that frequently leads to unnecessary hospitalization or missed intervention.

The fern test, which relies on the crystallization of amniotic fluid on a slide, is similarly operator-dependent and yields a sensitivity of 50–90% (4,15). Both conventional tests also have poor reproducibility. By contrast, PP14 measurement is an objective, laboratory-based immunoassay that eliminates observer variability.

Thus, PP14 is best viewed not as a replacement for bedside tests but as a powerful adjunct, especially when the nitrazine or fern result is equivocal or when clinical suspicion remains high despite a negative bedside test.

Comparison with other emerging biomarkers

Several protein biomarkers have been developed for the diagnosis of PPROM, most notably insulin-like growth factor

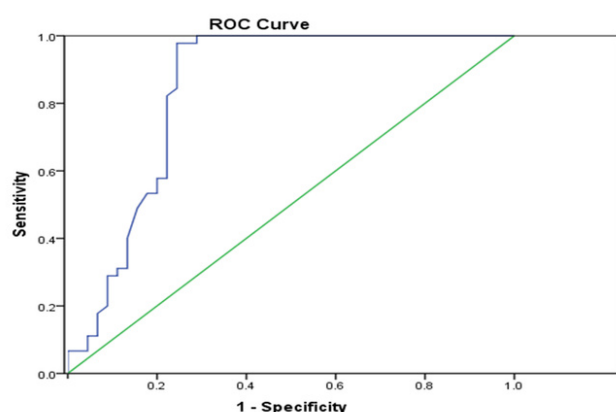


Figure 3. Roc analysis for PP14

Table 2. Distribution of cases and controls according to vaginal fluid PP14 cut-off Levels

PP14 level	Cases (n = 45)	Control (N + 45)	p-value
≥ 0.0078 ng/ml	44 (97.8%)	10 (22.2%)	$p\text{-value} < 0.001$
< 0.0078 ng/ml	1 (2.2%)	35 (77.7%)	

[†] Analyzed using Pearson's Chi-square test $\chi^2 = 53.519$, $df = 1$

binding protein-1 (IGFBP-1) (16) and placental alpha microglobulin-1 (PAMG-1) (5). Pooled sensitivity estimates for PAMG-1 range from 87% to 99%, while pooled specificity estimates range from 88% to 100%. In contrast, the diagnostic performance of IGFBP-1 appears to be more variable, with pooled sensitivity estimates ranging from approximately 90% to 94% and specificity estimates ranging from approximately 90% to 97% (5,7).

The results for PP14 observed in our study (sensitivity 97.8%, specificity 77.8%) are comparable to those reported for PAMG-1 in terms of sensitivity but demonstrate somewhat lower specificity than that reported in some studies (5,11). However, direct comparisons should be interpreted with caution because of differences in study populations, reference standards, and disease prevalence across studies.

Importantly, the cost and accessibility of these biomarkers are what differentiate them. The PAMG-1 and IGFBP-1 tests are often offered as point-of-care assays, which, despite their convenience, incur relatively high per-test costs, sometimes exceeding 10 to 20 US dollars(17).

In our specific environment, the ELISA-based PP14 assay costs roughly \$3.60 per sample and may be performed using standard laboratory equipment (18). Additionally, the requirement for a microplate reader, stable electricity, refrigeration, and trained technicians means that PP14 testing may not be immediately feasible in the most resource-constrained environments without additional investment. This is true even though the cost of each test is lower than that of some commercial point-of-care immunoassays. Furthermore, because PP14 is assessed from the same vaginal fluid sample that is acquired during a speculum examination, there is no need for any further invasive treatment to be performed.

Comparison with previous studies on PP14

Our findings are in close agreement with the initial research conducted by Wang et al. (2018), who were the first to investigate PP14 in vaginal fluid for the diagnosis of PPROM (11). The area under the curve (AUC) in that study was 0.855, the sensitivity was 90%, and the specificity was 84%, which is in striking similarity to the values that we obtained (AUC 0.852, sensitivity 97.8%, specificity 77.8%). It is possible that the modest variances can be attributed to variations in demographic characteristics (for example, the gestational age range or inclusion of term PROM in their cohort), sample handling, or the particular ELISA kit used. It is important to note that the reproducibility of PP14 as a diagnostic marker is strongly supported by the consistency

observed across independent cohorts.

Notably, our research focused on patients with PPROM between 28 and 37 weeks of gestation, as this timeframe presents the highest risk. The diagnostic performance was not affected by the inclusion of both preterm and term cases of premature rupture of membranes (PROM) in the study by Wang et al. (11). Although further stratified studies are needed to confirm this finding, the results suggest that the PP14 test performs consistently across a broad range of gestational ages.

Biological plausibility

The key physiological roles of PP14 (glycodelin) include immunosuppression, which involves triggering T-cell death to increase fetal tolerance, and modulation of endometrial receptivity (10). The fetal membranes act as a barrier in the course of a normal pregnancy, thereby preventing large quantities of PP14 from entering the vagina (19). The amniotic fluid, which contains PP14 at quantities that are significantly higher than those found in cervicovaginal secretions (20), seeps into the vaginal canal when the membranes tear. The significant diagnostic signal is supported by this physiologic gradient throughout the study. Previous research has shown that PP14 is relatively stable in the presence of blood and other contaminants, which is one of its advantages over IGFBP-1 or PAMG-1(8). Although our study did not include samples with substantial blood contamination, the ability of PP14 to maintain diagnostic accuracy despite minimal contamination may be advantageous in clinical obstetric settings where vaginal bleeding is present.

Future directions

The excellent diagnostic performance of PP14, characterized by very high sensitivity (97.8%), supports further investigation of its potential as a reliable biomarker for the diagnosis of PROM. A negative PP14 test (<0.0078 ng/ml) would be a strong indicator against the presence of PPROM in clinical practice. This could help physicians avoid unnecessary interventions, such as hospitalization, corticosteroid administration, or tocolysis, thereby reducing patient stress and healthcare costs.

On the other hand, a positive result, particularly when combined with a positive nitrazine or fern test, can confirm the diagnosis with a high degree of certainty, allowing for a timely initiation of appropriate management. This management may include antibiotic prophylaxis, antenatal corticosteroids, and transfer to a tertiary center if required.

In secondary-level hospitals that have at least basic

laboratory capabilities, it may be feasible to include PP14 in standard diagnostic procedures. This is because the sample is already collected during a speculum examination, which carries a relatively low cost.

Nevertheless, in order to achieve widespread use in primary or remote institutions, the assay would need to be simplified even further, or a point-of-care format would need to be developed initially. Whenever nitrazine or fern is unable to provide a definitive answer because of limited leakage or contamination, equivocal bedside tests are performed.

Populations at high risk include women who have a history of previous preterm birth or premature preterm delivery, for whom a prompt and precise diagnosis is of the utmost importance.

Settings with limited resources are those in which costly commercial point-of-care testing is not readily available.

Several other directions for future research are warranted. To confirm the proposed cut-off value and evaluate the test's predictive performance in everyday clinical practice, it is required to conduct prospective validation through a large-scale, multicenter study with consecutive recruitment. It would be helpful to explain the relative diagnostic accuracy and cost-effectiveness of PP14 and other established biomarkers, such as PAMG-1 and IGFBP-1, in the same population by comparing these biomarkers head-to-head. This would also help to determine the optimal clinical role for each biomarker.

Additionally, studies evaluating the stability of PP14 in vaginal fluid samples under various storage and transport conditions may provide valuable insights into its feasibility for use in remote or resource-limited settings, where rapid laboratory processing may not be readily available.

As of yet, the value of PP14 in identifying membrane rupture during the second trimester of pregnancy, the time when management decisions about fetal viability are particularly difficult, has not been well investigated and should be the subject of devoted research.

In conclusion, investigating the combined use of PP14 with other biomarkers, such as IGFBP-1, may enhance diagnostic accuracy compared with either marker alone, and thereby help determine whether a multi-marker strategy provides additional clinical value.

Limitations

While the case-control study design offers a high level of internal validity for diagnostic accuracy, it may overestimate test performance compared to a prospective cohort in which

the prevalence of PPRM is considerably lower. As this was a single-center study, the findings cannot be generalized to populations with different demographic features, ethnic backgrounds, or clinical practices. It is crucial to perform multicenter validation.

The sample size was not designed to support subgroup analyses, such as those based on gestational age, the presence of bacterial vaginosis, or the extent of membrane rupture, although it provided adequate statistical power for the primary analysis. Consequently, larger studies are required to investigate the test's performance across these subgroups.

Although samples containing coarse blood were not included in the study, the impact of microscopic blood or other vaginal fluids on the concentration of PP14 was not rigorously quantified. According to the findings of prior studies, PP14 appears to maintain stability even when blood is present (8).

A final point to consider is that the research did not include women in the second trimester of pregnancy or those who had term or preterm labor; hence, the diagnostic performance of PP14 in these populations is still unknown.

Placental protein 14 (PP14) measured in vaginal fluid demonstrates strong diagnostic performance for PPRM, characterized by very high sensitivity and excellent specificity. This biomarker has the potential to complement standard bedside tests, minimize diagnostic uncertainty, and improve rapid clinical care. Furthermore, it is a promising and inexpensive biomarker. With additional prospective validation, PP14 could become a useful, easily accessible diagnostic tool for PPRM, particularly in settings with limited resources.

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Author Contributions

Conceptualization: A.A.R. and M.A.H.A.; Methodology: A.A.R. and M.A.H.A.; Formal analysis: A.A.R. and M.A.H.A.; Investigation: A.A.R. and M.A.H.A.; Writing – Original draft: A.A.R. and M.A.H.A.; Writing – review & editing: A.A.R. and M.A.H.A. All authors have read and agreed to the published version of the manuscript.

Statement of Ethics

The study protocol was reviewed and approved by the Ethics Committee of Al Yarmouk Teaching Hospital (Approval No. 758; Date: November 2, 2022). All procedures adhered to the ethical principles of the Declaration of Helsinki, and written informed consent was obtained from all participants prior to enrolment. Participant data were fully anonymized to maintain strict confidentiality.

Statement of Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Data Availability

The statistical data supporting the findings of this study are included within the article. The raw datasets generated and analyzed are available from the corresponding author upon reasonable academic request.

Statement of Generative AI Use

No generative AI was used.

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