Vaginal fluid pH and buffer capacity for predicting false preterm labor in Japanese women

Takotoshi Noguchi, Toshiyuki Sado, Katsuhiko Naruse, Hiroshi Kobayashi *

Department of Obstetrics and Gynecology, Nara Medical University, Nara, Japan

1. Introduction

The most common obstetric complication experienced during pregnancy is preterm delivery; it is currently the leading cause of perinatal morbidity and mortality worldwide [1]. It is difficult to discriminate between patients who will actually experience preterm delivery and those who experience preterm uterine contractions but do not undergo delivery until at least full term, with more than half of patients thought to be at risk of preterm delivery ultimately experiencing a full-term delivery [2]. The multifactorial etiology of preterm labor [2,3] explains this difficulty in identifying specific biomarkers for preterm delivery.

Recent attempts to accurately predict preterm delivery have included the use of ultrasonographic measurements of the cervix [4,5] and measuring (cervico)vaginal fluid properties [6], including fetal fibronectin (fFN) [7] and phosphorylated insulin-like growth factor-binding protein 1 [8]. Analytical tests using fFN have demonstrated some accuracy in predicting spontaneous preterm delivery among patients experiencing preterm labor [9–13] and a quantitative phosphorylated insulin-like growth factor-binding protein 1 test has demonstrated accuracy in predicting preterm delivery among patients experiencing preterm labor [14] and in patients during the first trimester of pregnancy [15]. Additionally, considerable interest has been shown in developing safe, effective, simple, and inexpensive biomarker assays for predicting preterm delivery [16,17].

The novel idea explored in the present study originated from the concept that saliva provides protection against dental erosion and caries [18]. The healthy oral microbiota performs a protective role against pathogenic bacteria. Significant correlations have been demonstrated between an increased risk of dental caries and both saliva Streptococcus mutans counts and buffer capacity [19]. In comparison with healthy controls, patients with dental erosion have demonstrated larger decreases in pH following citric acid rinses or drinking orange juice, with the pH of patients’ saliva remaining decreased. Low saliva buffer capacity has been found to be a risk factor for the development of dental caries [18,20]. It was hypothesized that, similarly, reductions in vaginal buffer capacity could result in a decrease in vaginal pH, and that this could, in turn, influence the likelihood of preterm labor and delivery.

Consequently, the aim of the present study was to evaluate the pH and buffer capacity of vaginal secretions of patients who were pregnant to identify any associations between these values and preterm labor. To the best of our knowledge, no previous studies have investigated the association between preterm labor and the buffer capacity of vaginal secretions.
The present study included data from two prospective cohorts enrolled at Nara Medical University Hospital, Japan, between January 1, 2009 and March 31, 2012. The first cohort study (cohort 1) examined the pH of patients vaginal secretions only and enrolled patients attending the study hospital owing to symptoms of preterm labor (P = 0.001), between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001), and between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001).

2. Materials and methods

The present study included data from two prospective cohorts enrolled at Nara Medical University Hospital, Japan, between January 1, 2009 and March 31, 2012. The first cohort study (cohort 1) examined the pH of patients vaginal secretions only and enrolled patients attending the study hospital owing to symptoms of preterm labor and a control group between January 1, 2009 and December 31, 2009. Following this, the second prospective cohort (cohort 2) examined the pH and buffer capacity of vaginal secretions, enrolling further patients experiencing preterm labor and a control group between January 1, 2010 and March 31, 2012. Both cohorts were enrolled according to the same criteria; the preterm-labor groups comprised patients at 22–36 weeks of pregnancy attending the study hospital owing to increasingly symptomatic uterine contractions at shorter than 10-min intervals, who had cervical dilation up to 3 cm or had premature effacement of the cervix. The control groups enrolled patients at 22–36 weeks of pregnancy who were experiencing no pregnancy complications, had no systemic diseases, and were not regularly taking any medications. Patients were recruited to the control groups when attending routine prenatal checkups. The exclusion criteria for all potential study participants included preterm rupture of membranes, cervical dilatation greater than 3 cm, multiple pregnancies, non-reassuring fetal testing, chronic hypertension, pre-eclampsia, pre-existing diabetes, gestational diabetes mellitus, lupus erythematosus, abruptio placentae, intrauterine growth restriction, fetal anomalies, placenta previa, clinical signs of

Table 1
Patient characteristics among all study participants (cohorts 1 and 2).a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control patients (n = 189)</th>
<th>Patients exhibiting symptoms of preterm labor (n = 48)</th>
<th>Patients who delivered at term after demonstrating symptoms of preterm labor (n = 30)</th>
<th>Patients who delivered preterm after demonstrating symptoms of preterm labor (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of vaginal mucus samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.112</td>
</tr>
<tr>
<td>Age, y</td>
<td>29.5 ± 5.54 (16–43)</td>
<td>30.8 ± 4.41 (22–39)</td>
<td>31.2 ± 4.60 (22–39)</td>
<td>30.1 ± 3.99 (23–37)</td>
<td>0.255</td>
</tr>
<tr>
<td>Duration of pregnancy at delivery, wk</td>
<td>27.2 ± 3.94</td>
<td>31.2 ± 2.87</td>
<td>31.4 ± 3.18</td>
<td>30.9 ± 2.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vaginal secretion pH</td>
<td>4.05 ± 0.34</td>
<td>4.38 ± 0.54</td>
<td>4.35 ± 0.536</td>
<td>4.44 ± 0.52</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2
Patient characteristics among study participants in cohort 2. a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control patients (n = 96)</th>
<th>Patients exhibiting symptoms of preterm labor (n = 27)</th>
<th>Patients who delivered at term after demonstrating symptoms of preterm labor (n = 16)</th>
<th>Patients who delivered preterm after demonstrating symptoms of preterm labor (n = 11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of vaginal mucus samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.118</td>
</tr>
<tr>
<td>Age, y</td>
<td>30.2 ± 5.19 (18–40)</td>
<td>30.9 ± 4.32 (22–39)</td>
<td>31.1 ± 4.68 (22–39)</td>
<td>30.5 ± 3.70 (23–35)</td>
<td>0.550</td>
</tr>
<tr>
<td>Neonate weight at delivery, g</td>
<td>2955.9 ± 358.3</td>
<td>2638.9 ± 565.0</td>
<td>2809.5 ± 415.8</td>
<td>2390.7 ± 654.3</td>
<td>0.011</td>
</tr>
<tr>
<td>Duration of pregnancy at recruitment, wk</td>
<td>28.1 ± 3.84</td>
<td>31.0 ± 3.00</td>
<td>31.4 ± 3.30</td>
<td>30.5 ± 2.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of pregnancy at delivery, wk</td>
<td>38.7 ± 1.30</td>
<td>36.7 ± 2.08</td>
<td>37.8 ± 0.95</td>
<td>35.2 ± 2.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Buffer capacity</td>
<td>0.743 ± 0.372</td>
<td>0.668 ± 0.457</td>
<td>0.747 ± 0.639</td>
<td>0.671 ± 0.30</td>
<td>0.014</td>
</tr>
</tbody>
</table>

a Values are given as number, number (percentage), mean ± SD (range), or mean ± SD, unless indicated otherwise.
b Significant differences were observed between the control group and patients exhibiting symptoms of preterm labor (P = 0.001), between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001), and between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001).
c Significant differences were observed between the control group and patients exhibiting symptoms of preterm labor (P = 0.001), between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001), and between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001).
d Significant differences were observed between the control group and patients exhibiting symptoms of preterm labor (P = 0.001), between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001), and between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001).
e Significant differences were observed between the control group and patients exhibiting symptoms of preterm labor (P = 0.001), between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001), and between the control group and patients who delivered preterm after demonstrating symptoms of preterm labor (P = 0.001).
infection at the time of recruitment, positive screening test results for Candida spp. or Trichomonas vaginalis, symptoms suggesting bacterial vaginosis, and recent (within 24 h) sexual intercourse. Additionally, any patients who had recently undergone digital vaginal examination, transvaginal ultrasonography, or any additional treatment were also excluded. The study was approved by the Research and Ethics Committee of Nara Medical University and written informed consent was provided by all participants.

Patients presenting with symptoms of preterm labor were assessed for cervical length, effacement, and dilatation. At the discretion of the attending physician, tocolytic therapy, including intravenous magnesium sulfate, or intravenous or oral beta-mimetic therapy, could be administered.

To assess the pH and buffer capacity of vaginal secretions, samples from the posterior vaginal fornical mucus were obtained using a polyethylene terephthalate swab (Becton, Dickinson and Co, Franklin Lakes, NJ, USA) during an examination with a sterile speculum prior to any digital vaginal examination. A sterile swab was rotated across the posterior fornix of the vagina for 5 s to absorb secretions to be assayed directly. Any samples with blood visible on the swab were excluded. Samples were collected prior to the administration of any medications following hospital admission. The pH and buffering capacity of all samples were tested immediately using a micro pH electrode and pH meter (pH/mV meters CI-9D02 and TN208-031; Unique Medical Co LTD, Tokyo, Japan) (Supplementary material S1). A pilot study had previously determined that the intra-assay and inter-assay coefficients of variation of vaginal-secretion pH were lower than 5% (unpublished data).

A second vaginal-secretion sample was collected with a separate swab and was used for the vaginal-secretion buffer capacity assay. The buffer capacity of vaginal secretions was defined as the capacity of the mucus to maintain a consistent pH (i.e. the titratable acidity). pH values were tested before and after adding phosphate buffered saline (PBS; Sigma-Aldrich Co, St. Louis, MO, USA) to vaginal mucus, providing precise estimations of the change in pH (ΔpH, calculated by subtracting the initial pH from the pH after PBS had been added). The sample swab was placed in 0.1 mL of 10 mmol/L PBS that was then shaken for 5 s before being allowed to stand for 10 s; following this, the final pH was measured with a manual pH meter. If a patient demonstrated a higher buffer capacity, the vaginal fluid ΔpH value would be low. ΔpH appeared to be an adequate parameter for calculating the buffer capacity. In the present study, the buffer capacity was expressed as ΔpH.

Data were analyzed using SPSS version 21.0 (IBM Corp, Armonk, NY, USA). The Student’s t test and Mann-Whitney U test were used to assess differences in the pH and buffer capacity, respectively, between the patient groups. A receiver operating characteristic curve (ROC) analysis was used to determine the optimum threshold pH value and buffer capacity values for predicting pregnancy outcomes, specifically the values that maximized the sum of specificity and sensitivity. Pregnancy outcomes were defined as either preterm delivery (<37 weeks) or term delivery (≥37 weeks). The statistical tests performed were two sided and P < 0.05 was considered statistically significant.

3. Results

In total, 237 patients were recruited across the entire study; 114 patients were recruited to cohort 1, including 21 (18.4%) patients experiencing preterm labor. Among the patients in cohort 1 experiencing preterm labor, 7 (33.3%) experienced preterm delivery. Cohort 2 enrolled 123 patients, including 27 (22.0%) who were experiencing preterm labor; of these 27 patients, 11 (40.7%) experienced preterm delivery. All patients recruited to the control group underwent delivery at term. The characteristics of cohorts 1 and 2 combined are summarized in Table 1. Table 2 summarizes the characteristics of cohort 2 only, including vaginal-secretion buffer capacity. The neonatal weight at delivery was significantly lower among patients who experienced preterm labor compared with the control group in both cohort 2 alone and in the combined complete study population. The duration of pregnancy at recruitment was shorter in the control group compared with the patients who attended the study institution owing to preterm labor in both the complete study population and among patients in cohort 2. However, the duration of pregnancy at delivery was lower in the patients who experienced preterm labor than in the control group.

Some patients had samples collected multiple times throughout the study period; predictive modelling was performed including all patient samples and using only the first sample collected from each patient. In total, 619 individual samples from 237 patients were included. The pH value. Whiskers indicate the 10th and 90th percentiles, respectively. Outlier values are indicated by crosses.
of vaginal mucus samples are presented in Fig. 1. In the control group, the vaginal pH was not affected by the duration of pregnancy (Fig. 1).

When all the vaginal-mucus samples were included, elevated pH levels were recorded in patients experiencing symptoms of preterm labor compared with the control group (Tables 1, 2, Fig. 2). When the vaginal-mucus pH of patients experiencing preterm labor who experienced preterm delivery was compared to that of symptomatic patients who did not experience preterm delivery, no significant difference was observed (Table 1). The ROC curve analysis using all patient samples demonstrated an optimal cutoff value of pH 4.18, resulting in a sensitivity and specificity of 62.7% and 71.7%, respectively, and a positive predictive value (PPV) and negative predictive value (NPV) of 34.3% and 89.1%, respectively, for predicting if patients were currently experiencing symptoms of preterm labor (Table 2). Among the control group, the buffering capacity of patients’ vaginal mucus was not altered significantly by the duration of the index pregnancy (Fig. 4).

When including all patient samples, the mean buffer capacity was higher in the control group than among patients experiencing symptoms of preterm labor (Table 4). Lower buffer capacities were also demonstrated in both the patients demonstrating symptoms of preterm labor who experienced preterm delivery and the patients experiencing preterm labor who did not, when each was compared with the control group; however, no significant difference was observed when comparing the patients experiencing symptoms of preterm labor who went on to experience preterm delivery with the patients displaying symptoms of preterm labor who underwent delivery at term (Table 2, Fig. 5). The ROC curve analysis that included all patient samples from cohort 2 demonstrated that the optimum buffer-capacity cutoff value was 0.578, corresponding to a sensitivity, specificity, PPV, and NPV of 69.5%, 57.0%, 0.717, and 0.627. Abbreviation: ROC, receiver operating curve.
34.7%, and 85.0%, respectively, for predicting if patients were currently experiencing symptoms of preterm labor (Fig. 6 and Table 4).

4. Discussion

The present study demonstrated increased pH and lower buffering capacity in the vaginal mucus of patients who were experiencing the symptoms of preterm labor. These findings suggest that both tests could be useful markers for identifying an increased risk of preterm labor for patients. For both markers, the NPV recorded was higher than the PPV, demonstrating that both markers were more effective in identifying healthy individuals than patients experiencing preterm labor at that time. Unfortunately, both markers were unable to discriminate between patients experiencing symptoms of preterm labor who would go on to experience preterm delivery and those who were experiencing similar symptoms but would undergo delivery at term.

To our knowledge, this is the first clinical study investigating the utility of vaginal buffer capacity in predicting preterm labor. Furthermore, the present study can provide evidence regarding the cost-effectiveness of using pH and buffer capacity for diagnostic purposes and an ongoing retrospective survey is currently being conducted to estimate the direct and indirect costs of preterm labor-associated outpatient visits and hospitalizations.

Prediction of spontaneous preterm delivery remains a challenge in obstetric medicine. There has been limited success in attempts to accurately predict whether preterm labor will result in preterm delivery or whether the pregnancy will continue to term. In previous studies, cervical-length measurements using transvaginal ultrasonography and the presence of fFN in the cervicovaginal fluid have been described as reliable markers for predicting preterm delivery [21,22]. Tanvir et al. [21] reported that cervical-length measurements, made at patients’ mid-trimester prenatal scan, are a safe, accurate, and reproducible method for identifying the risk of preterm delivery, demonstrating high specificity and NPV for this method in predicting preterm delivery. Furthermore, fFN has been found to be increased in patients at risk of experiencing spontaneous preterm delivery [22]. fFN demonstrated high NPV in predicting spontaneous preterm delivery, but not in predicting preterm labor. Consequently, a negative result with an fFN

Table 4
Predictive value of vaginal mucus sample buffer capacity in diagnosing preterm labor or preterm birth.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Patient samples included</th>
<th>Buffer capacitya</th>
<th>P value</th>
<th>Optimal cut-off value from ROC curve</th>
<th>AUC (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control patients</td>
<td>All samples</td>
<td>0.743 ± 0.372 (0.617)</td>
<td>0.014</td>
<td>0.578</td>
<td>0.608 (0.528–0.688)</td>
<td>0.695</td>
<td>0.57</td>
<td>0.347</td>
<td>0.85</td>
</tr>
<tr>
<td>Control patients</td>
<td>All samples</td>
<td>0.668 ± 0.457 (0.552)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients exhibiting symptoms of preterm labor</td>
<td>Initial sample only</td>
<td>0.731 ± 0.360 (0.604)</td>
<td>0.436</td>
<td>0.578</td>
<td>0.549 (0.435–0.664)</td>
<td>0.704</td>
<td>0.542</td>
<td>0.311</td>
<td>0.839</td>
</tr>
<tr>
<td>Patients exhibiting symptoms of preterm labor</td>
<td>Initial sample only</td>
<td>0.716 ± 0.529 (0.555)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients who delivered preterm after demonstrating symptoms of preterm labor</td>
<td>All samples</td>
<td>0.667 ± 0.452 (0.555)</td>
<td>0.62</td>
<td>0.495</td>
<td>0.536 (0.38–0.693)</td>
<td>0.4</td>
<td>0.824</td>
<td>0.625</td>
<td>0.651</td>
</tr>
<tr>
<td>Patients who delivered at term after demonstrating symptoms of preterm labor</td>
<td>All samples</td>
<td>0.669 ± 0.461 (0.551)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients who delivered preterm after demonstrating symptoms of preterm labor</td>
<td>Initial sample only</td>
<td>0.671 ± 0.301 (0.571)</td>
<td>0.3</td>
<td>0.613</td>
<td>0.378 (0.155–0.601)</td>
<td>0.818</td>
<td>0.25</td>
<td>0.429</td>
<td>0.667</td>
</tr>
<tr>
<td>Patients who delivered at term after demonstrating symptoms of preterm labor</td>
<td>Initial sample only</td>
<td>0.747 ± 0.638 (0.542)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

* Values given as mean ± SD (median).

Fig. 5. Box and whisker plot of vaginal buffer capacity from all cohort-2 samples collected from the control group, patients displaying symptoms of preterm labor who experienced term delivery, and patients symptomatic for preterm labor who experienced preterm delivery. Boxes represent the interquartile range and the line within each box represents the median value. Whiskers indicate the 10th and 90th percentiles, respectively. Outlier values are indicated by crosses.

Fig. 6. ROC curve of the use of vaginal buffer capacity for predicting if patients were currently experiencing symptoms of preterm labor. The optimal buffer capacity cutoff was 0.578, the specificity was 0.581, and the sensitivity was 0.644. Abbreviation: ROC, receiver operating curve.
test could help in reducing the use of unnecessary interventions and prophylaxis for patients who do not require them [22]. However, despite diagnostic advances, no ideal marker has been found for predicting preterm labor or delivery.

There are several limitations that should be considered when evaluating the results of the present study. First, the results of the present study are based on study samples from only one region. Additionally, the significant shortcomings of the present study include that the relatively small sample size prevents the evaluation of outcomes such as earlier preterm delivery among patients experiencing preterm labor. Whereas differences between the study groups at recruitment were observed, no changes in vaginal-mucus pH were detected as normal pregnancies in the control group progressed. Considerable work is needed to evaluate whether the use of pH or buffer capacity could assist in identifying which patients experiencing preterm labor will go on to undergo preterm delivery and in identifying patients who are asymptomatic but are at increased risk of spontaneous preterm delivery.

Second, it is possible that both markers could have been influenced by common resident vaginal microflora. Infection is a leading cause of preterm delivery and the presence of several anaerobic or facultative microorganisms in the vagina could impact on the risk of preterm delivery, possibly through patients’ immune responses [23]. In the present study, vaginal-swab specimens were not tested to examine vaginal microflora. Microflora alterations could explain differences in susceptibility to preterm delivery between individual patients [24]. Evaluating the effectiveness of the combined or sequential use of pH and buffer-capacity tests, in addition to analyses of patient microflora, warrants further research.

Finally, vaginal-swab specimens were not tested for the presence of fFN, which has been demonstrated to have a sensitivity, specificity, PPV, and NPV of 66.7%, 87.9%, 36.4%, and 96.2%, respectively, in predicting preterm delivery within 7 days of testing [25]. Additionally, the present study did not include cervical-length measurements. Future studies should examine whether the addition of vaginal pH and buffer capacity could improve on existing techniques for predicting which patients are at risk of spontaneous preterm delivery.

In conclusion, the present preliminary study could provide the first evidence that vaginal pH and buffer capacity measurements have predictive value for preterm labor. More studies are necessary to develop practical criteria for predicting preterm delivery specifically and not preterm labor, which may or may not proceed to preterm delivery; this could be useful in recognizing susceptible individuals and preventing preterm delivery.

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Conflict of interest

The authors have no conflicts of interest.

References