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Evaluation of cervical maturity by cervical collagen measurement using light-induced fluorescence (LIF) during pregnancy

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Abstract

Objective: The study aimed to evaluate cervical ripening by measuring cervical collagen levels in non-pregnant women, women with a normal pregnancy, and postpartum women by light-induced fluorescence (LIF).

Methods: Cervical collagen content in normal pregnant women (n = 165) at various times of gestation was measured by LIF with a collascope, which is specifically designed to measure fluorescence of collagen. Cervical LIF in non-pregnant women (n = 12) and postpartum women (n = 14) was also detected. The demographic characteristics of women at various times were recorded. The Bishop score at 40 to 41 gestational weeks (n = 37) before the onset of labor was analyzed.

Results: Cervical LIF values progressively declined from the non-pregnant state to late gestation ($R = -0.836$) and reached their lowest levels during parturition and then increased at postpartum. LIF values and the Bishop score were significantly negatively correlated ($R = -0.83$). In patients with a Bishop score ≥ 6, the first stage of labor was shortened with a decrease in LIF values ($R = 0.718$).

Conclusions: Cervical collagen levels as measured by LIF could be a useful method for evaluating cervical maturity.

Keywords

Cervical maturity, collagen, light-induced fluorescence, Bishop score, labor, pregnancy

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Introduction

In preparation for labor and delivery, the cervix softens and becomes more distensible, then it dilates for passage of the developed fetus.[1–5] The sequence of structural and functional processes that occur during pregnancy and parturition is referred to as cervical ripening.[6,7] Cervical ripening is a process, which undergoes three steps, including softening, effacement, and dilation consecutively and irreversibly.[6–8] Cervical ripening is regulated in part by steroid hormones, including progesterone, and is independent of uterine contractions.[6,7,9–12]

The Bishop score is widely used for evaluating cervical maturity and it is determined by digital examination. This score is calculated on the basis of the station of the presenting part and cervical dilatation, effacement, consistency, and position. The major problems of this method are imprecision and subjectivity. Currently, no objective measurements are clinically used to assess cervical function during pregnancy, or to accurately predict term or preterm labor. Investigation of cervical function has focused on two basic areas of quantification of tissue deformability and the presence, orientation, and/or concentration of microstructural components (e.g., collagen).[13] Various experimental methods have been used to assess cervical collagen, including light-induced fluorescence (LIF) using a collascope.[11,14–17]

Cervical LIF measurements in pregnant humans and animals have shown that the cervix gradually softens during pregnancy.[14–16,18–21] The advantage of LIF is its noninvasive characteristic, it can measure cervical collagen rapidly, and it can be used *in vivo* repeatedly in the same patient. Cervical LIF can be used as an assisted diagnostic tool during pregnancy, and therefore, it may play a potential role in monitoring of patients. However, clinical measurements in studies have only been performed in a limited number of women and gestational ages, and the association of LIF values with the Bishop score has not been investigated yet.[15,18,22]

This study aimed to: 1) assess and compare the density of cervical collagen in non-pregnant women, women with a normal pregnancy, and postpartum women by LIF, 2) to analyze the correlation of LIF values with the Bishop score at term pregnancy; and 3) to develop a new method to evaluate cervical ripening.

Method and materials

Patients

Each participant who was enrolled was informed about the detailed procedure of measurement and related risks, and signed an informed consent form at Guangzhou Women and Children's Medical Centre, Guangzhou, China. This study was approved by the Institutional Review Board of the Medical Center (protocol number: 2017041001).

A total of 165 normal nulliparous pregnant women were recruited in this study, including early pregnancy ($< 14+0$ weeks of gestation, $n=25$), mid-trimester (from $14+0$–$27+6$ weeks of gestation, $n=58$), and late pregnancy ($>28+0$ weeks of gestation, $n=82$), as well as non-pregnant ($n=12$) and postpartum ($n=14$) women.

LIF measurements of non-pregnant women were obtained from those without a history of pregnancy with a non-menstrual status. For pregnant women, the first day of the last menstrual period was used to estimate the completed gestational date and then confirmed by an ultrasound scan.

The inclusion criteria of the participants were an age from 18 to 35 years and there was no gynecological history of cervical dysplasia, conization, lacerations, genital herpes, or other complications. Women with any medical complication, such as

diabetes, or an obstetric complication, such as preeclampsia, were excluded from the study. Maternal demographic characteristics, including maternal age, body weight, body height, body mass index, and gestational age, were recorded.

Collascope and LIF measurements

The collascope used in this study was made by Reproductive Research Technologies, LLP; Houston, TX, USA) and purchased from funds provided by the National Institutes of Health. The collascope consisted of an excitation light source from a non-ozone xenon lamp (250 W) and a selective filter system. This filter excluded all light wavelengths, except for those centered at 339±3 nm (wavelength at which insoluble collagen fluoresces). The excitation light was carried by an optical fiber to the tip of a probe, which was placed on the surface of the exocervix. The probe was a stainless-steel rod with optical fiber bundles. The excitation fiber bundle was in the probe surrounded by fluorescence emission fibers. The probe was inserted into a stainless sterile sheath with a sapphire window at the end to protect the optical fibers from direct contact with the tissue. The fluorescence emitted from the cervix (wavelength of 390 nm) was collected by the probe and carried by optical fibers into a charge coupled device spectrum analysis system, which was connected to an on-line computer. The spectrum of the analyzed tissue was displayed and the photons were measured for each examined cervix. The exposure time for excitation was approximately 0.1 s.

The collascope equipment was used by well-trained professional physicians to avoid errors. The patients underwent measurement using the collascope in the clinic or in the labor and delivery ward in our medical center. First, the women were told to adopt the lithotomy position and the vulva was disinfected. A speculum was used to open and distend the vagina to fully expose the cervix. The cervix was then disinfected with povidone iodine solution, and blood and/or discharge were subsequently removed from the cervical surface with a gauze pad. At the same time, the collascope was allowed to become warm for approximately 10 minutes and an operator inserted the probe into the vagina and lightly touched the cervical surface. Minimum pressure from the collascope probe on the cervix was used to perform LIF measurements. Usually five to six LIF measurements were made at different positions on the cervix from each patient and measurements are expressed as mean photons. LIF measurement started from the 12 o'clock point of the cervical surface, and was then moved clockwise at the other four to five points of the cervix randomly in each patient. The coefficient of variation of LIF was 4% to 10%. We then averaged the LIF value of these measurements for data analysis.

Bishop score and induction of labor

Women without complications of pregnancy were admitted for induction of labor when they were at a gestational age of 40 to 41 weeks. The Bishop score was evaluated immediately upon admission. A modified Bishop scoring system was commonly used to assess the cervix for preparation of delivery. The score for this study was calculated on the basis of the station of the presenting part, cervical dilatation, effacement, consistency, and position. In this study, two experienced obstetricians were assigned for cervical assessment to minimize inter-observer variation of the Bishop score. Women with a Bishop score $\geq$6 were selected. All of these patients were sent to the delivery room at 07:00 hours the next morning and underwent induction of labor with oxytocin after artificial rupture of the membranes. These women received epidural anesthesia and were transferred to the labor room after a cervical dilation of 2 cm.

Statistical analysis

Statistical analysis was performed with IBM SPSS version 24.0 software (IBM Corp., Armonk, NY, USA). The normality of the data was visually checked by a histogram and further examined by Q-Q plots. For data with a skewed distribution, logarithmic transformation was performed before further analysis. All data are expressed as mean + standard deviation or median (interquartile range) on the basis of the distribution. One-way ANOVA was used for multiple mean comparisons and the Bonferroni test was used for post-hoc comparisons. A scatter plot and Pearson correlation analysis were used to indicate the associations of cervical LIF with the duration of labor and interval to delivery. A P value ≤ 0.05 indicates a significant difference. Based on our pilot experiment, a minimal sample size of 10 cases in each group was required to reach a power of 0.80 and an alpha value of 0.05.

Results

Demographics of patients

The demographic data of pregnant, non-pregnant, and postpartum women in this study are shown in Table 1. There were no significant differences in maternal age and body mass index among the groups.

LIF values in non-pregnant women, pregnant women at different gestational ages, and postpartum women that delivered at term

Figure 1 shows LIF values in non-pregnant women, pregnant women at various gestational ages, and postpartum women. LIF values in non-pregnant women were significantly higher than those in pregnant women ($P < 0.001$). LIF values gradually decreased during pregnancy, fell to the lowest levels before birth (approximately 300–500 photons, $R = -0.836$), and increased postpartum. Cervical LIF values were negatively correlated with gestational age. (Pearson correlation $R = -0.914$, $P < 0.001$). In postpartum women (6 weeks after delivery), LIF values were significantly elevated compared with those obtained in late pregnancy ($P < 0.05$).

Relationship between LIF values and the Bishop score at term pregnancy

There was a significantly negative correlation between LIF values and the Bishop

Table 1. Demographic data of pregnant (n=165), non-pregnant (n=12), and postpartum(n=14) women.

Demographic data	n	Range	Mean±SD
Non-pregnant women	12		
Age (years)		22–34	29.67±0.93
Body mass index (kg/m^2)		16.94–22.86	19.88±0.45
Pregnant women	165		
Age (years)		19–35	28.73±3.72
Body mass index (kg/m^2)		16.65–33.10	23.76±3.37
Postpartum women	14		
Age (years)		20–33	28.38±0.56
Body mass index (kg/m^2)		17.78–24.12	20.57±0.81
Total number	191		

SD, standard deviation.

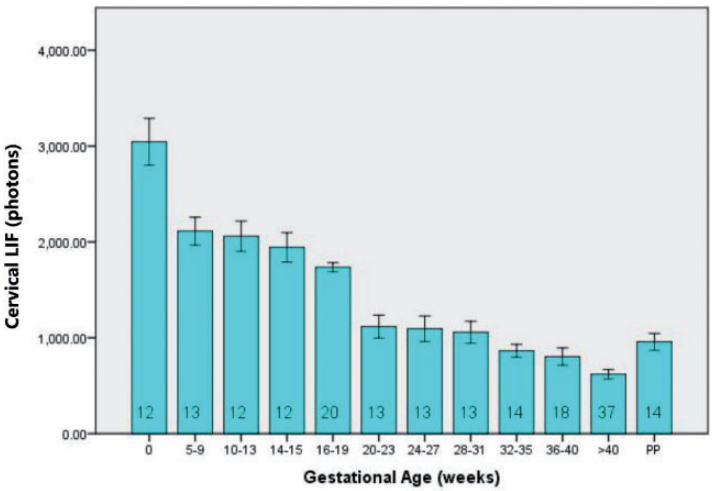

Figure 1. Relationship between cervical LIF in non-pregnant women, pregnant women at various gestational ages, and postpartum women. There was a negative correlation between gestational age and LIF counts (n=165, R=−0.914, P<0.001). LIF values in non-pregnant women (n=12) were significantly higher than those in pregnant women (P<0.001). In postpartum women (6 weeks after delivery), LIF counts were significantly higher compared with those obtained in late pregnancy (P<0.05).
LIF, light-induced fluorescence; PP, postpartum.

score at term ($n = 37$) ($R = -0.83$, $P < 0.001$) (Figure 2).

Relationship between LIF values and the first stage of labor at term pregnancy in women with a Bishop score ≥6

Figure 3 shows the relationship between cervical LIF values and the duration of the first stage of labor. When the Bishop score was ≥6 ($n = 23$), the first stage of labor shortened with a decrease in LIF values ($R = 0.718$, $P < 0.001$).

Discussion

In this study, we indirectly measured the collagen content of the cervix by a collascope in non-pregnant women, pregnant women at various times of gestation, and postpartum women. We found the following. 1) Cervical light-induced fluorescence values changed with pregnancy. 2) LIF values in non-pregnant women were higher than those in pregnant women. LIF values gradually decreased during pregnancy, reached lowest levels before delivery, and increased postpartum. 3) LIF values were significantly positively correlated with the length of the first stage of labor.

The human uterine cervix plays an important role in protecting the intrauterine milieu from the outside environment. The uterine cervix mainly contains the extracellular matrix and its major component is collagen (70% for type I and 30% for type III). During gestation and parturition, the cervix undergoes a process named cervical ripening and the cervix becomes softened, effaced, and eventually dilated.[23]

For most cases during pregnancy, the cervix does not undergo ripening successfully and it may be rigid and even closed to protect products of conception. However, the cervix greatly dilates during parturition and enters a cervical destructive period. Changes in collagen levels over the course of pregnancy and labor determine the

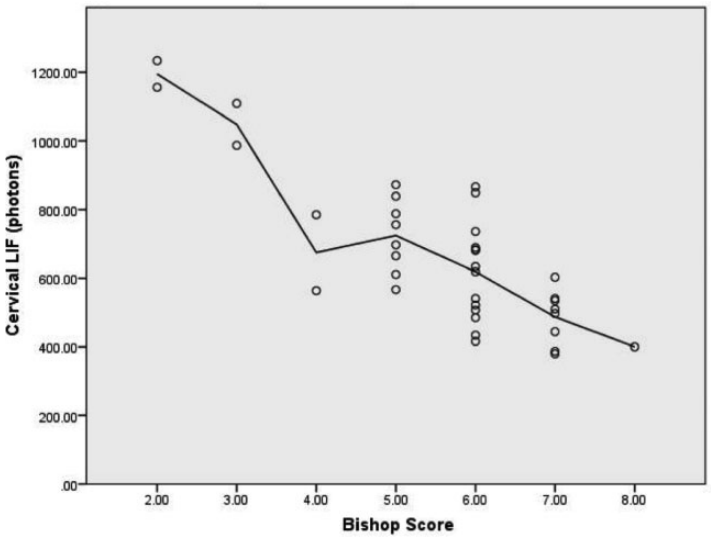

Figure 2. Relationship between the Bishop score and cervical LIF. The Bishop score and cervical LIF showed a negative correlation (n=37, R=−0.83, P<0.001).
LIF, light-induced fluorescence.

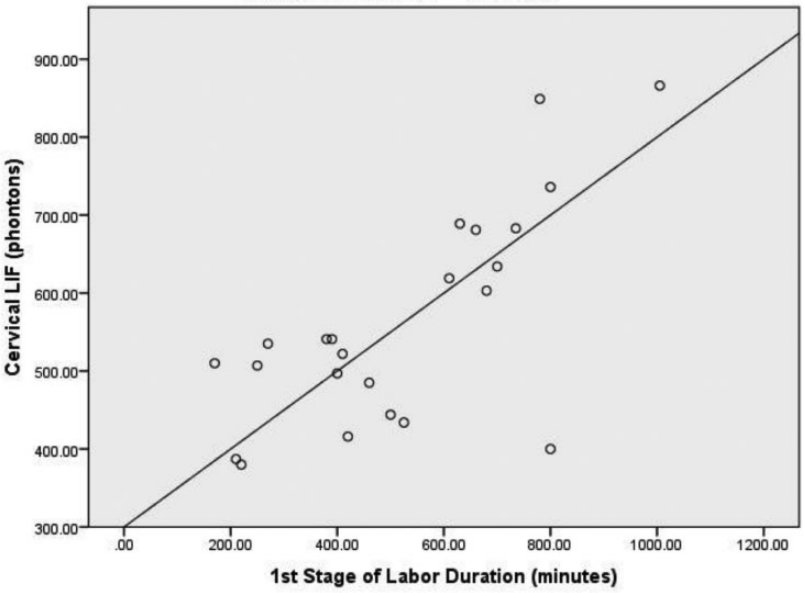

Figure 3. Relationship between cervical LIF and the duration of the first stage of labor in patients with a Bishop score ≥6. Cervical LIF was positively correlated with the duration of the first stage of labor in women with a Bishop score ≥6 (n=23) (R=0.718, P<0.001).
LIF, light-induced fluorescence.

flexibility and mechanical strength of the cervix.

A collascope was used to measure cervical collagen fluorescence to reflect cervical collagen concentrations in our study. Our study is consistent with previous studies in pregnant humans and animals, which reported that the cervix softens gradually during pregnancy.[14–16,18–20] Maul et al. found that LIF markedly decreased as gestational age increased, which is consistent with our study.[22] To the best of our knowledge, this is the first study to compare cervical LIF content among the non-pregnant state, across pregnancy, and at postpartum. We found a dynamic change in cervical collagen levels during pregnancy. Our results suggest that cervical LIF measurements could be useful in evaluating cervical maturation.

There are various other methods to evaluate cervical maturation in the clinic, of which the most commonly used is the Bishop score first described by Dr. Edward Bishop in 1964.[24] The Bishop scoring system evaluates the position of the cervix in relation to the vagina, cervical consistency, dilation, effacement, and station of the presenting part. Bishop scores for the cervix indicate the successful probability of induction of labor. A higher Bishop score indicates a higher rate of successful induction of labor, while a score < 5 indicates an unfavorable cervix for induction.[24] Our study showed that LIF values were consistent with the Bishop score, where a lower LIF value indicated a higher rate of successful induction. Furthermore, we found that cervical LIF values were more useful than the Bishop score for predicting the length of the first stage of labor. In this study, we evaluated the relationship between the duration of labor and cervical LIF in women with a Bishop score of ≥ 6 who were treated with oxytocin for induction of labor. Women with higher LIF values had a longer first stage of labor.

Clinically, the Bishop score is widely used to assess cervical maturation before induction of labor, but it is imprecise and subjective, and an experienced, professional obstetrician is required. Women with a Bishop score of ≥ 6 had different durations of the first stage of labor in our study. A novel method to test cervical ripening is required. Changes in cervical LIF during pregnancy correspond to cervical softening in animal and human studies.[14–16,18–21,23,25] In view of the precision of cervical LIF values for evaluating cervical maturation in predicting the length of the first stage of labor, performing cervical LIF measurements before induction of labor is reasonable. However, there are several other factors that may affect the duration of labor besides cervical LIF. These factors include uterine and abdominal muscle contractions, fetal characteristics (fetal position, weight, and head circumference), dimensions of the pelvis, analgesia, and psychological influences.[26,27] Interestingly, LIF values did not return to pre-pregnant levels in our study. This may help explain why the first stage of labor is shorter in multiparous women than in nulliparous women.

Our study has some limitations as follows. First, this was a cross-sectional study. Longitudinal measurements of cervical LIF in the same women might be more accurate. Additionally, postpartum women should be followed up for a longer interval for measuring cervical LIF to determine if LIF can increase to levels in the non-pregnant state. Finally, the LIF value is only an indirect measurement of collagen cross-link and it requires further verification in a future study.

In conclusion, cervical collagen concentrations detected by cervical LIF significantly decrease as gestational age increases. This method may be a useful tool for evaluating cervical ripening and

predicting the timing of the first stage of labor.

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Declaration of conflicting interest

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