Changes in cervical neutrophil elastase levels during the menstrual cycle

Fumiko Tawara · Naoaki Tamura ·
Nobuhiko Suganuma · Naohiro Kanayama

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Abstract

Purpose Cervical mucus plays an important role in host defense mechanisms and is involved in the ascendance of sperm. To determine the change of neutrophil mediators contained in cervical mucus, we measured total neutrophil elastase (NE) levels throughout the menstrual cycle.

Methods Cervical NE concentrations in 31 healthy women having regular ovulatory cycles were measured during the follicular, ovulatory and luteal phases.

Results The cervical NE levels during the follicular, ovulatory and luteal phases were 0.73 ± 0.57 (mean $\pm$ SD), 0.29 ± 0.33 and 1.32 ± 1.24 $\mu\text{g/ml}$, respectively. The NE concentrations during the ovulatory phase were significantly lower than the other phases.

Conclusions The decrease in total cervical NE level during the ovulatory phase suggests that neutrophil activity in cervical mucus is depressed around ovulation. The decreased cervical NE levels and the depressed neutrophil activity in cervical mucus at ovulation may be suitable for sperm passage through the cervical canal.

Keywords Cervical mucus · Enzyme immunoassay · Menstrual cycle · Neutrophil elastase · Ovulation

Introduction

Cervical mucus faces outward, and possesses various self-protective substances such as lactoferrin, lysosome, and immunoglobulin (Ig) A to prevent microorganisms from ascending into the intrauterine cavity [1–4]. Cervical mucus also contains leukocytes and chemical mediators derived from leukocytes [5]. Although changes in lactoferrin [2] and immunoglobulin [3, 4] during the menstrual cycle have been reported, there are no studies available that analyze the alteration and regulation of the mediators derived from leukocytes in cervical mucus.

Neutrophil elastase (NE) is a neutral protease with a molecular weight of about 30,000 Da. It is released from neutrophils and accumulates at sites of inflammation when leukocytes are activated by bacterial infection. NE degrades not only extracellular matrix components such as fibrin, fibronectin, and collagen [6–8] but also the bacterial wall [9]. Clinically, NE is used as a sensitive serum marker for inflammation [10]. To elucidate NE action in the cervical canal of women during the ovulatory cycle, we measured the cervical total NE levels consisting of both free elastase and elastase-alpha 1 protease inhibitor complexes at various phases of the menstrual cycle.

Materials and methods

Subjects

Thirty-one non-pregnant women who had spontaneous ovulations confirmed by basal body temperature were enrolled in this study. The samples of cervical mucus were collected after obtaining informed consent under ethical consideration at Tawara Fumiko IVF clinic. The mean age

F. Tawara
Tawara Fumiko IVF Clinic, Shizuoka, Japan

N. Tamura (✉) · N. Kanayama
Department of Obstetrics and Gynaecology,
Hamamatsu University School of Medicine,
1-20-1 Handayama,
Higashiku, Hamamatsu 431-3192, Japan
e-mail: ntamura@hama-med.ac.jp

N. Suganuma
Human Health Sciences, Kyoto University Graduate
School of Medicine, Kyoto, Japan

of the subjects was 32.4 ± 3.1 years (mean $\pm$ SD, range 27–39). Fifteen women were nullipara, and 16 were multipara (Table 1).

Cervical mucus was collected during the follicular phase (cycle day 5–9), the ovulatory phase (the day of ovulation or 1 day before or after the ovulation day) and the luteal phase (5–9 days after ovulation) after careful observation of the properties. In order to avoid the influence of the presence of sperm in the cervical mucus, the women abstained from sexual intercourses for at least 2 days before the sample collection. Ovulation was determined with basal body temperature, vaginal ultrasound imaging, and/or the level of urinary luteinizing hormone. Women with cervical disease such as cervical polyps or cervicitis were excluded from the study. Women having a history of cervical laceration, diabetes mellitus, hyperthyroidism, cardiac disease, or renal disease were also excluded.

Measurement of cervical NE

The vagina and ectocervix were cleansed with warm saline. A cotton swab was inserted into the cervical canal and gently rolled around twice so that 10 μ l (range 9.5–10.5) of the cervical fluid penetrated the swab. The samples of cervical fluid were mixed and diluted with 1 ml of phosphate buffered saline. Centrifugation was carried out at 1,000 rpm for 10 min, and the supernatants were collected. All the samples were kept at -80°C until use.

In the present study, we used an enzyme immunoassay (EIA) kit for NE (Sanwa Kagaku Co., Nagoya, Japan) that has been developed specifically for the measurement of both free elastase and elastase- α 1 protease inhibitor complexes [11]. In brief, the first antibody to NE (Ela 1) was coated on a 96-well plastic plate. After blocking with 2% bovine serum albumin, the cervical fluid sample was added, and the plates were incubated for 30 min at room

Fig. 1 Cervical neutrophil elastase levels during the normal menstrual cycle ($n = 31$). Significant differences in cervical neutrophil elastase values between the ovulatory phase and the follicular phase or the luteal phase ($P < 0.05$)

temperature. After washing three times with bicarbonate buffer, pH 9.6, peroxidase-conjugated second antibody (Ela 2) was added and incubated for 30 min at room temperature. Following a further three washes, *o*-ortho-phenyl-diamine and 0.5% hydrogen peroxide were added for color reaction. The absorbance at 492 nm was read by the EIA reader. The use of human subjects in this study was approved by the Ethics Committee at Tawara Fumiko IVF clinic.

Statistical analysis

Statistical analysis was performed with the Student's *t* test. A *P* value of less than 0.05 was considered statistically significant.

Results

Changes in cervical total NE levels during the menstrual cycle are shown in Fig. 1. Cervical NE concentration showed minimal values during the ovulatory phase. The cervical NE levels during the follicular, ovulatory and luteal phases were 0.73 ± 0.57 , 0.29 ± 0.33 and 1.32 ± 1.24 $\mu\text{g}/\text{ml}$, respectively. There were significant differences in the cervical NE values between the ovulatory phase and the follicular phase ($P = 0.024$) or the luteal phase ($P = 0.049$). The cervical NE levels during the follicular, ovulatory and luteal phases in the nullipara women ($n = 15$) and the multipara women ($n = 16$) were 0.73 ± 0.52 , 0.26 ± 0.23 , 1.25 ± 0.89 $\mu\text{g}/\text{ml}$ and 0.73 ± 0.42 , 0.29 ± 0.26 , 1.35 ± 0.95 $\mu\text{g}/\text{ml}$, respectively. There were no significant differences in cervical NE levels between the nullipara and multipara women.

Table 1 Characteristics of enrolled cases

No. of subjects	31
Age (years)	32.4 ± 3.1
Body mass index (kg/m^2)	22.5 ± 2.4
Menstrual cycle length (days)	28.4 ± 5.5
Gravida	1.0 ± 1.24
Parity	0.9 ± 0.65
Nulliparous (%)	15 (48.4)
Multiparous (%)	16 (51.6)
Complications	
Endometriosis (%)	3 (9.6)
Uterine myoma (%)	1 (3.2)
Recurrent pregnancy loss (%)	3 (9.6)
Hyperprolactinemia (%)	3 (9.6)

Discussion

Measurement of cervical NE with EIA has been established in the field of obstetrics [5]; however, the conventional NE assay can only detect elastase-alpha 1 protease inhibitor complexes [12]. Moriyama et al. [13] previously demonstrated no significant difference of elastase concentrations in cervical mucus during the menstrual cycle by using the conventional NE assay kit. In contrast, the kit that we used in the present study (ELASPEC; Sanwa Kagaku) is able to measure both free elastase and elastase-alpha 1 protease inhibitor complexes [11]. Based on the fact that cervical mucus is known to contain free elastase that is biologically active, this NE assay kit is highly sensitive for measuring cervical NE concentration. We evaluated the coefficients of variation (CV) and measurable range of this assay. Two control samples, with NE concentrations of 1.51 and 5.18 µg/ml, were measured 5 times resulting in mean values (µg/ml)/standard deviations/CV (%) of 1.56, 0.03, 2.12 and 5.01, 0.02, 0.50, respectively. Furthermore, NE had a measurable range of 0.05 to 10 µg/ml. These data suggest that the present NE assay has the potential to sufficiently represent total NE levels in cervical mucus.

The properties of cervical mucus change during the ovulatory period [14]. We also observed typical qualities of cervical mucus particularly during the ovulatory phase in which the mucus presented profuse, more watery and tenacious, so-called 'Spinnbarkeit'. This finding suggests our cases represented the cervical physiological changes in the menstrual cycle. As a result, the present study clearly showed a marked decrease in cervical NE concentration during the ovulatory phase compared to the other phases. The NE activity accurately reflects neutrophil activity. The decrease of cervical NE concentration during the ovulatory phase indicates that neutrophil activity can be depressed around ovulation [1–4]. We speculate that the decrease of cervical NE concentration locally at periovulation correlates with these phenomena.

Neutrophil mediators such as elastase and free radicals are thought to injure sperm. The decrease of cervical NE concentration around ovulation may facilitate the smooth ascendance of sperm through the cervical canal. Similarly, the concentrations of IgA, lactoferrin, and lysosomes in cervical mucus are low around ovulation. The concentration of various biologically active substances may be altered at ovulation for the passage of sperm into the uterine cavity.

Although the mechanism responsible for a decrease in cervical NE at ovulation is unknown, hormonal changes may be involved. The observation that estrogen augments ferning of cervical mucus suggests that estrogen regulates neutrophil activity. We have previously analyzed cervical

NE levels of patients ($n = 8$) treated with clomiphene citrate (anti-estrogen drug) in which the mean value of NE was 0.55 ± 0.25 µg/ml during the ovulatory phase; this level was higher than during the natural menstrual cycle. Clomiphene citrate has also been reported to depress the amount of cervical mucus [15]. According to our data, clomiphene citrate affects not only the amount but also the quality of cervical mucus.

Objective evaluation of cervical mucus is important in the diagnosis of infertility. Although there are various causes of infertility, there are few basic and clinical evaluations on cervical factors [16]. The post-coital test is used for assessment of cervical mucus and sperm, and ferning of cervical mucus is also determined as ovulation; however, these methods are based on a morphological, not a quantitative assay. In this regard, cervical NE measurement has the potential to become a useful tool for clarifying the cervical factors involved in infertility. The cervical NE kit we used in the present study is commercially available in Japan and is covered by national health insurance for preterm delivery (ELASPEC, Sanwa Kagaku). Further investigations are necessary to examine cervical total NE concentrations in infertile women.

We conclude that the cervical total NE level is decreased during the ovulatory phase suggesting that neutrophil activity in cervical mucus is depressed around ovulation. This alternation in the properties of cervical mucus may be suitable for sperm passage through the cervical canal. The present non-invasive and quantitative measurement of cervical total NE will contribute to clinical practice in the field of reproduction.

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