Concentration of lactoferrin and interleukin-6 in cervical mucus from patients being treated for infertility

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Background: The concentrations of the iron-binding protein lactoferrin (LF) and interleukin-6 (IL-6) were measured in the cervical mucus of patients being treated for infertility throughout the menstrual cycle.

Methods: A total of 251 cervical mucus samples were obtained from the patients throughout the menstrual cycle. One hundred and fifty samples were from primary infertility patients with unexplained infertility and 101 samples were from secondary infertility patients as a control. The concentrations of LF and IL-6 were measured by enzyme immunoassays. The standard curve of LF concentrations ranged from 1.6 to 50 ng/mL.

Results: The mean LF and IL-6 concentrations in the cervical mucus of primary infertility patients were higher than that of the control patients (P = 0.04, P = 0.032, respectively). The LF and IL-6 concentrations were highly correlated (P < 0.0001).

Conclusion: Elevated levels of IL-6 and LF in the cervical mucus were obtained from primary infertility patients. We speculate that LF might also be one of the causes of infertility and might play an important role in reproductive processes in the cervix. (Reprod Med Biol 2006; 5: 105–109)

Key words: human cervical mucus, IL-6, lactoferrin.

INTRODUCTION

CERVICAL CANAL MUCUS is a gel-like material that is secreted from the epithelial membrane of the cervix. The mucus functions as a plug that prevents infectious agents from ascending to the uterus.1,2 Cervical mucus is both a mechanical and chemical barrier to infection. After conception, the appearance and physicochemical properties of cervical mucus change dramatically as a result of hormonal influences.3,4

In the follicular phase, the cervical mucus becomes thick, sticky, viscous, opaque and gelatinous, and forms a plug that obstructs the cervical canal.5 One day before ovulation, because there is the highest amount of cervical mucus, the greatest degree of spinnbarkeit, and the lowest degree of viscoelasticity, spermatozoa have their greatest ability to penetrate the cervical mucus.5 However, the opportunity for bacterial infection might also increase along with the penetration ability of the sperm. The defense mechanism of cervical mucus against infection is quite important. Interleukin-6 (IL-6) is an inflammatory cytokine that plays a central role in the body's defense against infection. Although the cellular origin of IL-6 is unclear, IL-6 has been localized to leukocytes, and glandular and surface epithelium in the cervix at term.6 Levels of IL-6 in infertility patients have been previously reported.7,8

Lactoferrin (LF) is a glycoprotein present in most secretions found at mucosal surfaces and in secondary granules of polymorphonuclear neutrophils.9,10 LF is regulated by estrogen, and its expression appears to be an early and sensitive bioassay for estrogen activity in the epithelium of the mouse uterus and vagina.11–13 The functions of LF in the immune system have been widely studied. It has been shown that LF inhibits the cytokine production of macrophages in vitro.14,15

It is very useful to evaluate the state of cervical mucus in patient being treated for. In the present study, IL-6 and LF concentrations in cervical mucus during the menstrual cycle were examined, and the levels of IL-6 and LF among primary infertility patients and the control group were compared.
MATERIALS AND METHODS

Materials

Samples of human cervical mucus

INFORMED CONSENT WAS obtained from all patients who participated in the present study. Cervical mucus was collected from 251 non-pregnant women who visited an outpatient clinic at Showa University Hospital, Tokyo, Japan. One hundred and fifty samples were from primary infertility patients diagnosed with unexplained infertility and 101 samples were from a control group of secondary infertility patients who had at least once spontaneous pregnancy. Although it was checked that there had been no sexual intercourse within the past 2 days, the possibility of recent semen exposure was not undeniable. A doctor collected all samples in order to avoid technical errors. To collect cervical mucus, a cotton swab was gently placed in the cervix and allowed to passively absorb mucus for approximately 20–30 s. At the same time, culture of the cervical mucus was also carried out in order to rule out vaginitis. The swab was then immersed in 1 mL of phosphate-buffered saline (PBS; 0.05% NaN₃ (Wako Pure Chemical Industries, Tokyo, Japan), 10% aprotinin (Sigma Aldrich, Tokyo, Japan), and NaCl (Wako Pure Chemical Industries, Tokyo Japan), pH 6.5. The specimens were separated by centrifuging at 15 000 r.p.m. for 10 min at 4°C. The extractions were stored at −80°C until they were assayed.

Methods

Measurement of LF and IL-6 concentrations

Lf concentration was determined by a peroxidase-based enzyme-linked immunosorbent assay procedure, as previously described. Briefly, the immunoglobulin fraction of goat antihuman lactoferrin (Nordic Immunological Laboratories, Tilburg, Netherlands) was diluted to 20 µg/mL in 0.2 mol sodium phosphate buffer (Wako Pure Chemical Industries, Tokyo, Japan), pH 6.5. The 96-well microtiter plate (Nalge Nunc International, Tokyo, Japan) was coated with 0.1 mL/well of antibody for 2 h at room temperature. Each well was washed three times with 0.3 mL PBS (DAKO cytoma- tion, Kyoto, Japan)/Tween20 (Wako Pure Chemical Industries). Washings were carried out with NUNC Immuno Wash (Nalge Nunc International). Each well received 0.3 mL of block buffer (Snow Brand Milk Products, Tokyo, Japan) was incubated for at least 1 h at room temperature. Purified human milk LF (Sigma Aldrich) was diluted in Block Ace (Snow Brand Milk Products) and used as the standard with concentrations ranging from 1.6 to 50 ng/mL. The standards or unknowns were added to the plates (0.1 mL/well) and incubated for 2 h at room temperature. The plates were washed three times with PBS/Tween-20. Peroxidase-conjugated goat antihuman lactoferrin (Nordic Immunological Laboratories) was diluted to 5 µg/mL in Block Ace (Snow Bland Milk Products). Each well received 0.1 mL of diluted antibody and the plates were incubated for 1 h at room temperature. The plates were washed three times with PBS/Tween-20. ABTS color development solution (BIORAD Laboratories, Tokyo, Japan) and 30% H₂O₂ (Wako Pure Chemical Industries) were added to the wells. The plates were then incubated for 45 min at room temperature with shaking. The reaction was stopped with 2% oxalic acid (Wako Pure Chemical Industries) and the plates were immediately read on a NUNC micro plate reader (Nalge Nunc International) at 415 and 450 nm. The intra-assay and interassay coefficients of variance were both <10%.

The IL-6 concentrations in the cervical mucus samples were measured using ELISA kits (Peprotech EC, London, United Kingdom).

Statistical analysis

All data was analyzed using the Mann–Whitney U-test. P-values less than 0.05 were considered statistically significant.

RESULTS

CONCENTRATIONS OF LF and IL-6 did not vary significantly during the menstrual cycle. The LF mean concentrations at follicular and luteal phase were 6.076 ± 0.976 µg/mL and 7.045 ± 8.718 µg/mL respectively. For IL-6, the mean concentrations at follicular and luteal phase were 0.1 ± 0.141 pg/mL and 0.118 ± 0.165 pg/mL respectively (Fig. 1). The mean LF concentrations in the cervical mucus of the primary infertility group (7.12 ± 12.7 µg/mL) was significantly (P = 0.04) higher than that in the control group (4.66 ± 6.87 µg/mL). The mean IL-6 concentration in the cervical mucus of the primary infertility group (0.12 ± 0.17 ng/mL) was significantly (P = 0.032) higher than that of the control group (0.08 ± 0.08 ng/mL, Figure 2).

The IL-6 and LF concentrations in cervical mucus were highly correlated (P < 0.0001, Figure 3). There

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