Presence of interleukin-8 and the IL-1 receptor antagonist in the cervical mucus of fertile and infertile women

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Background. Cytokines are involved in almost every aspect of reproduction, and recent studies suggested a relationship between cytokines and male/female infertility. In the present study, the levels of interleukin-8 (IL-8) and interleukin-1 receptor antagonist (IL-1Ra) were determined in the cervical mucus of fertile and infertile women. Methods. Groups of patients were formed according to the results of the standard procedure for infertility investigation, including postcoital test and the presence of antispermatozoid antibodies in the sera of both partners and in seminal plasma, by mixed antiglobulin reaction (MAR) test and Kibrick agglutination test. IL-8 and IL-1Ra levels were determined in solubilized (ultrasonographic sonicaton) cervical mucus sampled in the midcycle by commercial ELISA kits and expressed as pg/mg proteins. Results. The groups were designated as fertile (n=20) and infertile (n=20). The latter was divided into two subgroups, one consisting of infertile women with positive postcoital test and without antispermatozoid antibodies (n=10), and the other designated as infertile women with negative postcoital test (n=10). This subgroup was composed of women with negative postcoital test and without antibodies (n=10) and the women with negative postcoital test with antibodies (n=10). Similar levels of IL-8 and IL-1Ra were noted in the cervical mucus of fertile women and women with positive postcoital test and without antispermatozoid antibodies. A tendency of decrease (p=0.052) and significant decrease in IL-8 levels (p<0.05) was noted in negative postcoital test group and negative postcoital test group without antibodies, respectively, compared to the levels in the fertile examinees. A significant rise in IL-1Ra levels (p<0.05) was detected in the mucus of negative postcoital test group with further increase in negative postcoital test group with antibodies (p<0.02). Conclusion. Changes in IL-8 and IL-1Ra levels in the cervical mucus of infertile patients with negative postcoital test suggested the existence of the relationship between cervical cytokines and infertility in these women.

Key words: infertility, female; cervix mucus; interleukin-8; receptors; interleukin-1.

Introduction

The survival and function of the gametes, protection, and proliferation of the embryo during its migration through the uterus, as well as its nidation and subsequent development, depend on local regulatory mechanisms in which the hormones and the growth factors play an important role. Recent investigations pointed out the importance of the small soluble polypeptide mediators, cytokines, in various aspects of reproduction (1–3). The role of cytokines in the reproductive process has been insufficiently examined, and the available data are mostly based on the detection of cytokines in the compartments of reproductive system and during various phases of reproductive cycle.

Cytokines are present in a large number in human reproductive tract fluids, including cytokines that participate in the inflammatory and immune response: interleukins IL-1 and IL-6, chemokines, tumor necrosis factor alpha (TNF-α), hematopoietic cytokines — stem cell factor (SCF), and leukemia inhibitory factor (LIF), as well as regulatory cytokines, such as transforming growth factor β (TGF-β) (4). In the female genital tract, cytokines are present in the oviduct, amnion, and follicular liquors, as well as in the endometrium. The occurrence of inflammatory cytokines is consi-
dered important for the protection from infections, while hematopoietic cytokines seem to play a role in oogenesis and embryonic development (4, 5). Some cytokines, such as chemokines, are involved in the regulation of ovarian function, including the follicle development and atresia, ovulation, steroidogenesis, as well as in the formation, development, and regression of the yellow body (6, 7). One of the clinical signs of ovulation is based on the effect of the increased IL-1 levels in plasma on thermo regulatory hypothalamic centres during the luteal phase (8, 9). The presence of cytokines in the endometrium is related to the secretory and/or proliferative activity of this compartment, as shown for chemokines IL-8 and monocytic chemokinetic protein (MCP-1) (10), and for IL-6 (5, 11) in earlier studies. Previous research on humans and animals stressed the importance of the IL-1 system, which consists of cytokines (IL-1α, IL-1β), receptors (IL-1RI, IL-1RII), and receptor antagonist (IL-1Ra) in the process of implantation (12, 13). Cytokines also play an important role in mother-fetus relation during pregnancy, and are considered to be the effector mechanisms of endocrine activity in pregnancy (14). Normal pregnancy is characterized by the absence of a strong immune cell response to the fetus and a dominant humoral response, owing to the beneficial effect of Th2 cytokines on the conceptus (15). The presence of IL-8 in the human cervix in pregnancy and its increased levels in women with vaginal delivery suggests the importance of this cytokine both in the cervix maturation and in labour (16). The occurrence of cytokines in the cervical mucus of healthy women is considered an important factor of the local immunity in the cervix, as well as in the development of vaccines against sexually transmissible diseases (17). In addition, a large number of cytokines detected in seminal plasma and the high levels of the immunoregulatory cytokine TGF-β probably prevent spermatozoids to be rejected in the lower region of the female genital tract.

Some cytokines exert effects that might be detrimental to reproductive process. Thus, it was shown that TNF-α, IFN-α, and IFN-γ might be cytotoxic to spermatozoids and to the preimplanted embryo in vitro (18–20).

Moreover, some cytokines might have harmful effects on the gonad functions (TNF-α) (21), prevent implantation (IL-1Ra) (12, 13) or postimplantational survival of conceptus (21), while the shift towards the Th1 cytokines (TNF-α and IFN-γ) is related to the repeated abortions (22, 23). The increased levels of IL-6 and IL-8 in the seminal plasma of infertile in comparison to fertile males imply their possible role in male infertility (24, 25). Raised levels of TNF-α, IFN-γ, IL-6, and IL-8 in the genital secretion of infertile females (26, 27) also points to their significance in female infertility.

 Bearing in mind the importance of cytokines present in the lower part of the genital tract for the normal function of this region, as well as the described detrimental effects that pointed to a possible relation between cytokines and infertility, the present study aimed to support the hypothesis that the levels of IL-1 receptor antagonist and IL-8 in the cervical mucus in fertile women differed from those in infertile women, the difference being especially obvious in the group of women with the negative postcoital test. Negative result of this test implies the hostility of lower genital tract microenvironment to spermatozoids, and the changes in the cytokine content in this region might imply a possible association between these mediators and the test results.

Methods

The study included infertile (n=48) and fertile (n=20) married couples, aged 22–38. Along with the examinations for the presence of morphological anomalies, the infertile couples were subjected to the standard procedure for the estimation of infertility (in accordance with the American Society for Reproductive Medicine, ASRM), which included sperm analysis, ovulation rating, hysterosalpingogram, laparoscopy (if indicated) and postcoital test. Hormonal status (serum progesterone, estrogen, LH and FSH level), and the time of ovulation (evaluated by body temperature and ultrasonographic folliculometry) were also normal. There was no Chlamydia or Mycoplasma in the cervical swab. Urological findings were normal, sperm grains and sperm cultures showed normosperma, no Ureaplasma was detected, and patients had no history of previous infections.

Postcoital test was done from 11th to 14th of the cycle. The test was considered negative when only dead spermatozoids were found in the vaginal swab and cervical aspirate, slightly positive when a few unprogressively mobile spermatozoids were found, and positive when progressively mobile spermatozoids where found in the cervical swab.

Fertile women tested had two children each, their deliveries being in the interval of not less than a year and not more than two years; they had normal bacteriological results. At the time of the sample collection none of the tested women were under any therapy. The fertile group did not have contraceptive protection.

Antispermatozoid antibodies were determined in the serum of both partners by the method of mixed antiglobulin reaction (MAR) and in the seminal liquor by the Kibrick agglutination reaction at the Institute of Immunology and Virology, Torlak. Sample collection for the determination of antispermatozoid antibodies was not related to the day of the menstrual cycle.

Cervical mucus was collected from day 8 to the midst of ovarian phase as described by Naz, et al (26). The mucus was aspirated from the endocervical chanulas with sterile canules for intravenous use, diluted with 0.4 ml of sterile saline, and frozen at −20 °C. The samples were sonicated (4×10s on ice) at 30% of maximum intensity amplitude on MSE sonicator (USA) and then centrifuged; the supernatant was kept at −20 °C. Protein con-
centration in solubilized cervical mucus was determined by the Lowry method (29).

Cytokines in the samples of cervical mucus were determined with the commercial ELISA tests (Quantikine R&D Systems, McKinley USA). The kits included the “sandwich” ELISA with monoclonal antibodies to IL-8 or IL-1Ra bound to the microtitre plate. Cytokine values were calculated from the standard curve drawn on the basis of cytokine standard concentrations and expressed in pg/mg proteins.

The results were expressed as mean values ± SEM. Statistical analysis was done with the Mann-Whitney test, and the differences were considered significant at \( p<0.05 \).

Table 1

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<th>Cytokine Interleukin-8 (IL-8) and Interleukin-1 Receptor antagonist (IL-1Ra) in patient's cervical mucus</th>
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\(^*\) mean ± S.E.M; \(^1\)median; \(^2\)range: min-max; \(^3\)p < 0.05 vs. _fertile_; \(^4\)p = 0.052 vs. _fertile_.

Results

It is well known that the negative postcoital test is one of the standard criteria for the determination of infertility (28), and the low result of this test is usually associated with the antispermatozoid antibodies in males (30). Hence, in the present study the groups were formed on the basis of the results of postcoital test and the presence of antispermatozoid antibodies.

Eighteen women had a negative postcoital test. All women in whom antispermatozoid antibodies were detected (n=3) had the positive postcoital test, and so had the women (n=5) whose partners had antispermatozoid antibodies. In 10 women with the negative postcoital test antispermatozoid antibodies were not found. On the basis of these findings women were put into one of 3 groups for the determination of the presence of cytokines in the cervical mucus. The control group was marked as fertile (n=20). The total number of infertile women amounte 48 (n=48), and these women were divided into two subgroups. One was composed of women who had the positive postcoital test and in whom antispermatozoid antibodies were not detected, nor were they detected in their partners. This group was designated as the group with positive postcoital test and without antispermatozoid antibodies (n=30). Another subgroup was composed of women with the negative postcoital test (n=18), and was designated as such. In the latter subgroup, 3 women had antispermatozoid antibodies in the serum and the partners of 5 of them had antispermatozoid antibodies in the serum and in seminal fluid. These women (n=8) were marked as the group with negative postcoital test with antispermatozoid antibodies. The group designated as the one with negative postcoital test without antispermatozoid antibodies included women (n=10) with the negative result of postcoital test who were negative, or whose partners were negative for antispermatozoid antibodies. The results of the assay for IL-8 and IL-1Ra presence in the cervical mucus are presented in Table 1. The cervical mucus of fertile women had detectable IL-8, and similar concentrations were found in the mucus of women belonging to the group with positive postcoital test, and without antispermatozoid antibodies. In the whole group with negative results of postcoital test, IL-8 levels tended to decrease (p=0.052) in comparison to IL-8 levels in fertile women. However, in the group with negative postcoital test, without antibodies, significantly lower IL-8 levels were observed (p<0.05) in respect to fertile women. It was also shown that there was no difference in the IL-1Ra levels between the fertile women and those from the group with positive postcoital test and without antispermatozoid antibodies. Significantly increased IL-1Ra level (p<0.05) was detected in the cervical mucus of women with negative postcoital test. Higher increase (p<0.02) was detected in women and/or their partners with negative postcoital test and with antispermatozoid antibodies.

Discussion

The results of determining the presence of cytokines in the cervical mucus of fertile and infertile women are presented in this paper. The occurrence of IL-8 and IL-1Ra in
the cervical mucus of fertile women supported the findings on the role of cytokines in the local immunity of the cervix (31, 17). IL-8 levels detected in fertile women in the present study (914±317 pg/mg) were similar to those obtained in the study on fertile women from USA (630±30 pg/mg proteins) (27). The American study established the increased IL-8 level in the cervical mucus of women with unexplained infertility (without immunological signs), as well as in those with immunologic findings (with antispermatozoid antibodies) compared to fertile women. In contrast, in the present study no differences in IL-8 levels were found between these two groups. Moreover, our results demonstrated that in the group with negative postcoital test IL-8 level tended to decrease, while in the group with negative postcoital test and without immunological findings (without antispermatozoid antibodies) it was significantly decreased. It is not yet clear what might be the cause of the differences between IL-8 levels in the cervical mucus found in these two studies. It seems that they stem from the different criteria applied for group forming, as well as from demographic differences. The biological importance of the decrease in IL-8 level may be discussed on the basis of the known role that this cytokine plays in local homeostasis/immunity of the female genital tract (6). In the microenvironment of the cervix, IL-8 may affect the leukocyte migration, and hence the maintenance of the cervix homeostasis and local immunity. Since there is the evidence on the increased accumulation of leukocytes, mostly neutrophils, in the cervical mucus after insemination (32), the change in IL-8 level might affect the migration of leukocytes under such conditions. Analogous to these observations, it was assumed that IL-8 might influence the accumulation of sperm after coitus (27). Thus, the decrease of IL-8 in women with negative postcoital test might result in the modulation of leukocyte migration and in the expression of their functions.

No literature data are yet available concerning on the presence and the quantitative changes of IL-1Ra in cervical mucus of infertile women. The increase of IL-1Ra level found in the group of women with the negative postcoital test pointed to the possible role of this cytokine in the microenvironment of the lower part of the reproductive tract. The significant increase in IL-1Ra level in women with negative postcoital test who, or whose partners had, antispermatozoid antibodies in the serum suggested a possible relation between the presence of antibodies and the increased IL-1Ra levels. Other findings on the relation between antibodies and IL-1Ra production by monocytes (33) supported our observations. In the cervix of normal women, IL-1Ra might act as a competitive inhibitor of IL-1. According to some authors, this activity might lower the toxicity of high local concentrations of IL-1 (31). Although there are no data on infertility and IL-1 in the cervical environment, it is necessary to monitor changes of both mediators in order to understand the biologic significance of the IL-1Ra increase. IL-1Ra may exert some specific activities affecting spermatozoids in the local environment of the lower genital tract. In this respect, the anti-implantation effect of this mediator in experimental mice is indicative. It is based on the decreased expression of α4, α5, and β1 integrins, adhesion molecules relevant to the endometrium receptiveness for the embryo in the process of implantation (12, 13). Conceding the above mentioned facts, IL-1Ra might influence the surface molecules of spermatozoids, thus changing their characteristics and behavior in the cervix microenvironment. In that respect, there are data which show that the cytokine IL-6, also present in the cervical mucus, changes the fertilization capacity of spermatozoids by increasing the capacitation and the acrosomal reaction (34). Also, since it has been demonstrated that mechanical changes of the skin epithelium stimulate the production of IL-1Ra (35), the raised IL-1Ra levels in a contractile environment of the uterus during the process of fertilization might be relevant to the change of characteristics and/or behavior of spermatozoids. Future investigations on the presence of IL-1Ra activity in the lower part of female genital tract will provide further insight into the significance of IL-1Ra detection in the cervical mucus of infertile women with negative postcoital test.

The mechanisms underlying cytokines increase/decrease in the cervical mucus are not clear. Changes in cytokine levels in the cervical mucus may result from the changes in local environment (17, 31), particularly in hormonal activity (36), as shown in fertile women. They may be also associated with the exudation from the serum. Comparison of serum levels of cervical cytokines investigated so far (IFN-γ, TNF-α, IL-6, IL-8) with those in the cervical mucus of infertile women indicated that a local production in the cervix might be a possible mechanism of their increase (26, 27). The activity of resident cells and of leukocytes in the cervical microenvironment may contribute to the change of cytokine levels in this region of the reproductive tract.

In conclusion, the results obtained in this study showed that there are no differences in IL-8 and IL-1Ra levels in the cervical mucus between fertile and infertile women, but that there were differences in the levels of these mediators between the infertile women with the negative postcoital test and the fertile ones. Differential changes (decrease in IL-8 and increase in IL-1Ra) require further investigation related to the changes in the presence of these mediators in the cervix microenvironment in order to elucidate the possible underlying mechanisms of these changes.


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A p s t r a k t


PRISUSTVO INTERLEUKIN-8 I ANTAGONISTA RECEPTORA ZA IL-1 U CERVIKALNOJ SLUZI PLODNIH I NEPLODNIH ŽENA

Uvod. Citokini su uključeni u gotovo svaki aspekt reprodukcije, a novija istraživanja ukazuju na vezu između citokina i neplodnosti kod muškaraca i žena. U ovom radu su prikazani rezultati određivanja interleukina-8 (IL-8) i antagonista receptora za IL-1 (IL-1Ra) u cervikalnoj sluzi plodnih i neplodnih žena. Metode. Grupe ispitanica su formirane prema rezultatima standardne procedure ispitivanja neplodnosti oba partnera, uključujući postkoitusni test, kao i prema prisustvu antitela na spermatozoide. Antispermatozoidna antitela su određivana u serumu oba partnera i ejakulatu mešanom antiglobulinom reakcijom (MAR) i Kribk testom aglutinacije. IL-8 i IL-1Ra su određivani u solubilizovanoj (sonifikacija ultrazvukom) cervikalnoj sluzi uzetoj 11–14. dana ciklusa pomoću komercijalnih ELISA kompleta. Rezultati su izraženi kao pg/ml proteina. Rezultati. Grupe su označene kao fertiline (n=20), infertiline (n=48). Infertiline su podeljene u dve podgrupe – jednu sa neobjašnjenom neplodnošću (pozitivni postkoitusni test i bez anti-spermatozoidnih antitela) (n=30), i drugu sa negativnim postkoitusnim testom (n=18). Poslednja grupa je podeljena na grupu sa negativnim postkoitusnim testom bez antitela (n=10) i negativnim postkoitusnim testom sa antitelima (n=8). Zapaženi su slični nivoi IL-8 i IL-1Ra u cervikalnoj sluzi neplodnih žena i žena sa pozitivnim postkoitusnim testom, a bez anti-spermatozoidnih antitela. U grupi sa negativnim postkoitusnim testom zapažena je tendencija sniženja nivoa IL-8 (p=0,052), a u grupi sa negativnim postkoitusnim testom bez antitela i značajno niži nivoi IL-8 (p<0,017) u poređenju sa nivoima kod fertilnih ispitanica. Značajno povećanje nivoa IL-1Ra (p<0,05) detektovano je u cervikalnoj sluzi ispitanica koje su imale negativan postkoitusni test, kao i dalje poštenje (p<0,02) u grupi ispitanica sa negativnim postkoitusnim testom i antitelima.

Zaključak. Promene zastupljenosti IL-8 i IL-1Ra u cervikalnoj sluzi neplodnih ispitanica sa negativnim postkoitusnim testom, ukazuju na postojanje veze između citokina u cervikusu i neplodnosti kod ovih žena.

K l j u č e n e r e ć i: neplodnost, žena; grlić materice, sluz; interleukin-8; receptori, interleukin-1.