

Exploring the Antimicrobial Effects of Cervical Mucus in Pregnants and Its Relationship with the Course of Pregnancy

Gebelerde Servikal Mukusun Antimikrobiyal Etkilerini ve Gebelik Seyri ile Olan İlişisini Araştırmak

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ABSTRACT Objective: To investigate whether the cervical mucus in pregnant has antimicrobial activity in vitro and to determine its relationship with prognosis of pregnancy. **Material and Methods:** Cervical mucus samples were collected from 50 women with single pregnancy during the first trimester. Sterilized Wartman papers were soaked into mucus samples and dried under sterile conditions. Culturing was performed by disk diffusion method which uses Miller Hinton medium and Mac-Farland 0.5 solution prepared with *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Candida albicans*. Positive or negative zones were determined by measuring the diameters of inhibition zones occurred following 18 hours incubation period. Prognosis of pregnant with or without antimicrobial effect of cervical mucus were compared. **Results:** In 28 cases, it was demonstrated that cervical mucus had antimicrobial effects. During the follow-up period of 27 pregnant, 22 (81.5%) gave viable birth in term, 3 (11.1%) had abortus, and 1 (3.7%) had early membrane rupture at 36th week of gestation. In 22 cases, cervical mucus showed no antimicrobial effect. 20 of these followed-up and 14 (70.0%) participants gave viable birth at term, 3 (15.0%) had abortus, and 2 (10.0%) had preterm delivery. There was no statistically significant difference between the two groups in terms of pregnancy prognosis. **Conclusion:** Cervical mucus prevents infections from vagina to upper genital organs not only mechanically but also by functioning as a chemical barrier. In our study, it is established that presence or absence antimicrobial effect of mucus does not have a significant impact on the prognosis of pregnancy. We think that studies with greater number of participants may give different results.

Key Words: Cervix mucus; pregnancy outcome; anti-infective agents; secretory leukocyte peptidase inhibitor

ÖZET Amaç: İn vitro olarak gebelerdeki servikal mukusun antimikrobiyal aktiviteye sahip olup, olmadığını araştırmak ve gebelik prognozu ile olan ilişkisini saptamak. **Gereç ve Yöntemler:** Elli adet tekiz ilk trimestir gebeden servikal mucus alındı. Mukuslar sterilize Wartman kağıtlarından hazırlanmış disklerle emdirildi ve steril şartlarda kurutuldu. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* ve *Candida albicans*'la hazırlanmış Mac-Farland 0.5 solusyonları, Miller Hinton besi yerleri kullanılarak disk diffüzyon yöntemi ile ekim yapıldı. On sekiz saatlik inkübasyon periyodundan sonra oluşan inhibisyon zonların çapları ölçülerek pozitif ve negatif olanlar belirlendi. Servikal mukusları antimikrobiyal etkiye sahip olan ve olmayan gebelerin prognozları karşılaştırıldı. **Bulgular:** Yirmi sekiz olguda servikal mukusun antimikrobiyal etkiye sahip olduğu saptandı. Bunlardan takibi yapılabilen 27 gebenin 22'sinde (%81.5) termde canlı doğum, birinde (%3.7) ise 36. haftada erken membran rüptürü geliştiği saptandı. Yirmi iki olguda servikal mukusun antimikrobiyal etkiye sahip olmadığı saptanmadı. Bu olgulardan takibi yapılabilen 20 gebenin 14'ünde (%70.0) termde canlı doğum, ikisinde (%10.0) preterm doğum, oldu. İki grup arasında gebelik prognozu bakımından istatistiksel olarak fark görülmedi. **Sonuç:** Servikal mucus sadece mekanik değil aynı zamanda kimyasal bir bariyer olarak vajenden yukarı genital organlara gelecek enfeksiyonları önlemektedir. Servikal mukusun antimikrobiyal etkiye sahip olup, olmamasının gebelik prognozu üzerine anlamlı bir etkiye sahip olmadığı çalışmamızda saptandı. Daha geniş serilerle yapılacak çalışmalarda farklı sonuçların alınabileceğini düşünmekteyiz.

Anahtar Kelimeler: Servikal mucus; gebeliğin seyri; anti enfeksiyon ajanları; sekretuar lökosit peptidaz inhibitör

Cervix regulates sperm transport for fertilization and prevents female upper genital system from invasion of microorganisms. There are many temporary and/or permanent colonisations of microorganisms at cervical external ostium and vagina, whereas uterine cavity generally remains sterile.¹ It is known that cervical mucus has a multifactorial filter system. However, our knowledge on its antimicrobial activity is limited.²⁻⁴

The natural defense function of cervix, that has an important barrier effect against ascending infections, is also important for preventing sexually-transmitted diseases that is an important factor in women morbidity since they lead to infertility, and affect negatively the results of pregnancy.³ The lower-pole of fetal membrane-decidua junction is associated with entrance of cervical canal that opens anatomically to the vagina. Cervical mucus plug forms a functional barrier in cervical canal during pregnancy, and additionally, it has antimicrobial properties. With these physiological characteristics, cervical mucus forms an obstacle which prevents microorganisms from reaching intrauterine tissue, fetal membrane and fetus.⁴⁻⁶

There are various microorganisms in the vaginal fluid of women. In theory, microorganisms in vagina or endocervical canal may reach neighboring decidua and lower pole of fetal membrane via ascending way. However, fetal membranes and amniotic fluid are considerably resistant to invasion of many microorganisms. One of the leading factors that provide the resistance is decidual leukocytes which show antimicrobial properties.^{3,4}

It is suggested that many microorganisms were demonstrated to be associated with disorders that cause preterm delivery. Nevertheless, a certain relationship between preterm delivery and a specific microorganism of vaginal or endocervical fluid has not been demonstrated. Many researchers suggested that the infection responsible for the preterm labor might originate from vagina or cervix.⁵⁻⁷

In this study, we aimed to investigate antimicrobial properties of cervical mucus and to determine its effects on the prognosis of pregnancy.

MATERIAL AND METHODS

Fifty women with single pregnancy with the ages of 18-38 years who admitted to Obstetrics and Gynaecology Department of Yüzüncü Yıl University Faculty of Medicine during their first trimester, were included in the study. The presence of an intrauterine pregnancy and the gestational ages were determined by ultrasonography. After obtaining informed consent, patients were examined using a sterile speculum in gynaecologic position. Mucus was aspirated from cervical external ostium using a 20 cc sterile syringe, and they were sent to the microbiology laboratory within five minutes. Samples were stored at +4 °C whenever needed. Sent mucus samples were diluted with 1 cc physiological serum in order to make them more fluent as they were thick. Disks prepared from sterilized Wartman papers were soaked in cervical mucus samples and the disks were dried under sterile conditions. Then, microorganisms were cultured by disk diffusion method which used Miller Hinton medium and Mac-Farland 0.5 solution prepared with *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*. Disks saturated with cervical mucus placed in the middle of these media. After one night of incubation (18 hours) at 37 °C, diameters of inhibition zones were assessed. Disks with an inhibition zone were referred as positive whereas disks without an inhibition zone were recorded as negative (Figure 1). All participating women were invited for the routine visits at least once a month. Pregnancy results were recorded. The relationship between pregnancy results and the antimicrobial effects of cervical mucus samples that were collected during the first trimester of pregnancy was analysed. Data were analysed by one-way analysis of variance (ANOVA). A p value less than 0.05 was accepted as statistically significant.

RESULTS

Of the 50 pregnant women included in the study, 28 participants (66.0%) showed cervical mucus with antimicrobial effects against at least one of tested microorganisms. At the end, 22 of 27 cases (81.5%) completed pregnancy in term and gave live birth, three cases (11.1%) had *in utero mort fe-*

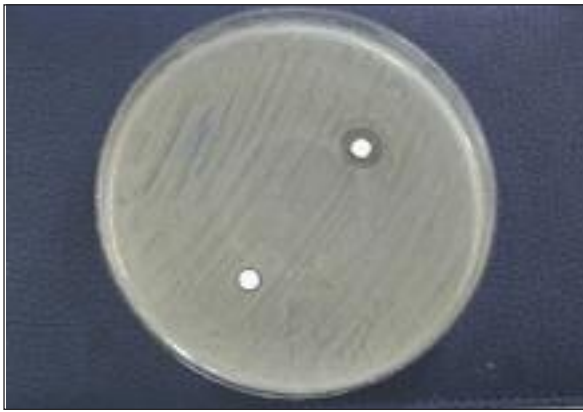


FIGURE 1: The upper disk has a halo zone (is antimicrobial activity positive cervical mucus) The lower disk has no halo zone (is antimicrobial activity negative cervical mucus).

tal at 14th, 16th and 18th weeks of gestation respectively, one case (3.7%) had termination of pregnancy due to fetal abnormality at 32nd week, and one case (3.7%) had early membrane rupture at 36th week.

It is determined that in 22 cases (44.0%), cervical mucus failed to show any antimicrobial effect against tested microorganisms. Of these, 20 patients had been followed up and 14 (70.0%) patients had term delivery with live birth, three (15.0%) patients had in utero mort fetal at 12nd, 14th, and 15th weeks of gestation respectively, two (10.0%) patients had preterm labor, and one patient (5.0%) had consented to terminate pregnancy by dilatation & curattage.

We failed to find any statistically significant relationship between the results of pregnancy and antimicrobial effects of cervical mucus against *S. aureus*, *E. coli*, *K. pneumonia*, and *C. albicans*. P values were P: 0.31, P: 0.46, P: 0.47, and P: 0.25 respectively.

There was no statistically significant difference between the results of pregnancy when group with antimicrobial cervical mucus and the group without antimicrobial cervical mucus were considered (P: 0.84) (Figure 2).

DISCUSSION

Cervix with its specific anatomic structure, cervical mucus production, and antimicrobial factors wit-

hin the cervical mucus are considered as a barrier of female upper genital system against the ascending infections stemming from lower genital system.^{2,4,6} In our study, we found that 28 (66.0%) of tested cervical mucus samples showed antimicrobial effects at different levels against one or more of the tested microorganisms. Antimicrobial efficacy rates in our study were in accordance with previous published reports.

Cervical glands that produce thick secretion of cervical canal during non-pregnant periods, show hypertrophy and hyperplasia during pregnancy. Furthermore, increase in vascularity and edema occur in cervix during pregnancy.⁵ Immediately after conception, a quite thick mucus plug closes the cervical canal. The so-called “mucus plug” is thrown out at the beginning and/or just before the beginning of labor.^{5,7} The antimicrobial effect of cervical mucus is not clearly known. The two possible reasons of this are the difficulties in obtaining that viscous hydrogel and rheologic properties of the mucus. Cervical mucus can be obtained in minute amounts and be affected by many different factors.^{6,8,9} There was not any case that we failed to obtain or to assess cervical mucus. However, we think that rheologic properties of cervical mucus is also important for the differences in antimicrobial activity.

In some studies investigated the relationship of endocrinologic status and antimicrobial activity, estrogen was shown to decrease viscosity of cervical mucus by inducing microcapillary permeability changes and thus, it caused lower enzyme concentrations.^{7,8} Although there are some studies in the

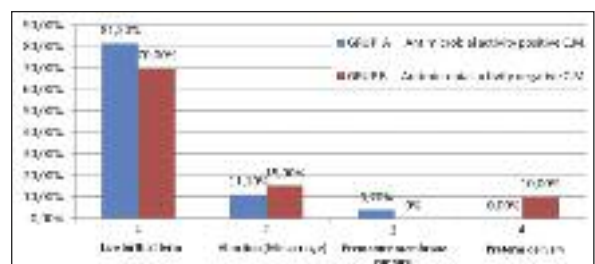


FIGURE 2: Live birth, abortion, premature membrane rupture and preterm delivery ratios in pregnancies in cervical mucus with or without antimicrobial activity. (C.M: Cervical mucus).

literature that report decrease in immunoglobulin (Ig) levels at midcycle phase,^{9,10} there are also studies that report no change in the levels of Ig.^{4,10,12} Aside from systemic immunoglobulins, proteins originating from serum transudation, local immune system, and especially IgA and enzymatic factors play major role on antimicrobial properties of cervical mucus. There are a few studies focusing on the enzymatic properties of human cervical mucus.^{2,6,10} For instance, lysosime is an important factor for antimicrobial effect of many bodily fluids in humans. This enzyme leads to bacteriolysis as a component of non-specific defense system against microorganisms in synergy with local immunoglobulins and factors of complement cascade.^{6,11,12} Genital secretions contain enzymes other than lysosime for antimicrobial activity such as lactoferrin.^{9,11} In our study, we did not have any opportunity to investigate which of the above factors contributed to antimicrobial activity in the positive samples.

In a study with 133 mucus samples, it has been shown that all samples inhibited bacterial growth in agar plates at measureable levels.² In our study, antibacterial effect rate of cervical mucus samples was 66% which is not comparable with the results of the afore mentioned study. We think this difference may originate from population differences, the studied microorganisms, study methods or rheologic properties of cervical mucus.

It has been demonstrated that antimicrobial effect was more potent in women under long-term estrogen treatment when compared to women with a spontaneous cycle.^{9,13} High estrogen levels of pregnant may be an explanatory factor for high antimicrobial activity in the present study.

In a study examining cervical mucus samples collected during delivery in 56 healthy pregnant, it was determined that a substance with low-molecular weight protected fetus against ascending infections by its antimicrobial action.¹⁴ In different studies performed by collecting cervical mucus from healthy pregnant during delivery, there were sufficient neutrophil defense, lactoferrin, lysozime, secretory leukoprotease inhibitor (SLPI) and

high molecular group nucleosomal-binding area (HMG N2) for antimicrobial action and it was reported that the cervical mucus functioned not only as a mechanical barrier but also as a chemical barrier for ascending infections of vagina.^{6,12,15} We think that antimicrobial action of cervical mucus in 28 (66.0%) participants of our study may be due to above mentioned factors. However, we failed to find any statistically significant difference between prognosis of pregnancy in women with or without cervical mucus with antimicrobial action.

In studies performed immediately after membrane rupture, it was shown that bacterial infection may have a role in the pathogenesis of membrane rupture evidenced by isolation of pathologic microorganisms from vaginal flora.^{11,15,16} In our study, cervical mucus showed positive antibacterial activity in 28 (66.0%) subjects and negative antibacterial activity in 22 (44.0%) subjects. One patient (3.7%) in positive group had early membrane rupture whereas none of the patients in negative group had early membrane ruptures. This suggests that the antibacterial action of cervical mucus may not be an effective factor in the early membrane rupture. Nevertheless, with increasing number of patients, the results may also be different.

It has been known that hundreds of maternal or fetal factors may determine the prognosis of pregnancy. We consider antibacterial activity of cervical mucus is effective to some extent amongst those hundreds of factors. We think that larger studies should be performed to elucidate the effects of antimicrobial activity of cervical mucus on the prognosis of pregnancy and to reduce controversial results.

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