Gynecology; *and Gynecologial Oncology*

The Detection of Genital Actinomyces by Cytological and Microbiological Methods

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OBJECTIVE: To detect the genital Actinomyces in women by both cytological and microbiological methods.

STUDY DESIGN: Cervico-vaginal samples were obtained from 200 patients attending the Gynecology and Obstetrics Clinics of Hacettepe University. All these samples were screened in view of Actinomyces-like organisms (ALOs) cytologically and were examined microbiologically by the BBL Crystal Identification System with regard to the presence of Actinomyces.

RESULTS: ALOs was detected in 6 (3%) women by cytological method and Actinomyces positivity was found in 7 (3.5%) samples by the BBL Crystal ID System. Of 6 samples that gave positive results by Pap-staining, only one was positive by the BBL Crystal ID system. We found a lack of agreement statistically between these methods (p>0.05) for the detection of Actinomyces.

CONCLUSION: The detection of genital Actinomyces both in smears and cultures might be more sensitive to prevent the misdiagnosed and undiagnosed cases and for early diagnosis and treatment.

Key Words: Actinomyces, Cervicovaginal smear, Papanicolaou staining, The BBL Crystal ID System

Gynecol Obstet Reprod Med;15:3 (157 - 161)

Introduction

Actinomyces species are frequent inhabitants of mucosal surfaces of human urogenital tracts. However, they are also involved in pelvic infections as oppurtinistic pathogens, especially in women using Intrauterine Contraceptive Devices (IUCDs).[1-3] The identification and differentiation of these anaerobic bacteria in clinical specimens is very diffucult because they are fastidious, oxygen-sensitive and slow-growing organisms.[1,4]

Several studies have been reported the detection rates of Actinomyces in IUCD wearers and non-IUCD wearers using various techniques.[5-11] In some of these studies, there is a disagreement between the methods which are used to detect Actinomyces. Nayar et al. reported that Actinomyces was observed in 7 of 193 (3.63 %) Papanicolaou-stained cervicovaginal smears, but none of these were confirmed by culture.[8] In Hager's study, 40 study patients had Actinomyces on cytological smears, but only one positive result was confirmed by culture.[9] Valicenti et al. also reported that 9 of 13 patients, whose smears had Actinomyces were confirmed by immunoflourescence, but not by culture.[10] In addition to these studies, Cleghorn and his colleagues compared culture, immunoflorescence and Pap staining in view of detecting Actinomyces and suggested that at least two methods were required for the detection of genital Actinomyces.[11]

In literature, there are several opinions of which method is helpful in identifiying Actinomyces. However, there is not still a reliable, accessible and consistent method for the identification of this genus. Thus, the aim of the present study was to confirm the diagnosis of Actinomyces-like organisms (ALOs) on Pap smears by the BBL Crystal ID System and to be able to increase the sensitivity of Pap smears which are rapid and cost-effective for the preliminary identification of genital Actinomyces in routine cytology laboratories.

Material and Method

Study Population

This study comprised 200 patients attending the Gynecology and Obstetrics Clinics of Hacettepe University, Ankara, Turkey for routine gynecological examination during a six-month period. The age group of these patients varied from 21 to 68 years. The questionnaire containing information about age, menstruation date, gravity and clinical symptoms were completed.

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Submitted for Publication: *14.07.2009*
Accepted for Publication: *14.08.2009*

Papanicolaou staining

Cervico-vaginal samples were taken with a cytobrush for cytologic examination, these samples were fixed with absolute alcohol without air drying and then were stained with routine Papanicolaou technique and screened in detail for the detection of ALOs.

Isolation of Actinomyces from cervico-vaginal specimens

The microbiological examination for isolating and identifying Actinomyces involved culture, Gram staining, biochemical testing and the BBL Crystal Identification System in this study. The specimen for anaerobic culturing was obtained with a sterile swab and it was transferred into a plastic tube consisting of the combination of medium and anaerobic atmosphere. This combination prolongs the survival of microorganisms for transportation (Anaerobic Culturette Collection and Transport System). Then, specimens were transported to the Microbiology Laboratory and cultured as quickly as possible. In the laboratory, the specimen-containing swab removed from the transport container and inoculated on Schadler Agar in plates and Thioglycolate broth in tubes. After inoculation, the swab was smeared on glass slides and stained with Gram staining method to asses cell morphology. The media that had been inoculated with the swab incubated anaerobically in GasPak jars (BioMerieux sa, Maray-l'Etoile, France). The incubation lasted 3 weeks at 37 ˚C. After a three-week incubation period, rough and dry colonies on Schaedler Agar and colonies at the bottom of tubes in Thioglycolate broth were considered to be positive for Actinomyces. These isolates were subcultured on Schadler Agar medium again and incubated 48 hours anaerobically to prepare pure cultures of isolates. These pure cultures were further examined with The BBL Crystal Identification System (BBL Crystal Identification Systems, Shannon County Clare, Ireland). This test was performed according to the manufacturers' instructions. The BBL Crystal ID System also requires catalase and indole results for each isolate tested. For this purpose, production of catalase was tested with 15% hydrogen peroxide and those of indole was tested with a spot test with 1% p-dimethylaminocinnamaldehyde. In addition to these tests, the results of nitrat reduction, esculin hydrolysis and carbohydrate fermentation were used to differentiate Actinomyces from other genera which are similar to Actinomyces morphologically.

Statistical analysis

The statistical analysis were performed by using the SPSS package programme version 11.5 (Chicago, Illinois, U.S.A). The Fischer's exact test was used to test the difference between the two methods in respect of diagnosing Actinomyces. A p-value < 0.05 was considered statistically significant.

Results

Cervico-vaginal samples obtained from 200 patients were tested by cytology and by the BBL Crystal ID System with regard to the presence of Actinomyces. Table 1 lists the detection of Actinomyces by the BBL Crystal ID system or cytological screening of the specimens from the patients. As it is seen in this table, detection rates for each technique alone were: cytology 3% and the BBL Crystal ID System 3.5 %. Only one patient has a positive smear and culture and there is a lack of agreement between these two methods statistically ($p>0.05$). The results of these two methods were also given in detail below.

Table 1: Comparison of cytological and microbiological methods for diagnosing Actinomyces

Methods	Act(+)	Act(-)	Total	P value
Cytological method	6 (3 %)	194(97%)	200(100%)	> 0.05
Microbiological method	7(3.5 %)	193(96.5%)	200(100%)	

Light microscopic examination of Pap-stained cervico-vaginal smears

The Papanicolaou-stained smears obtained from 200 patients were examined promptly by an experienced cytologist. During this examination, ALOs was defined as the presence of dense and basophilic aggregations in the center which is surrounded by radially oriented filament-like structures (Figure 1) and ALOs were detected in 6 (3%) of 200 smears.

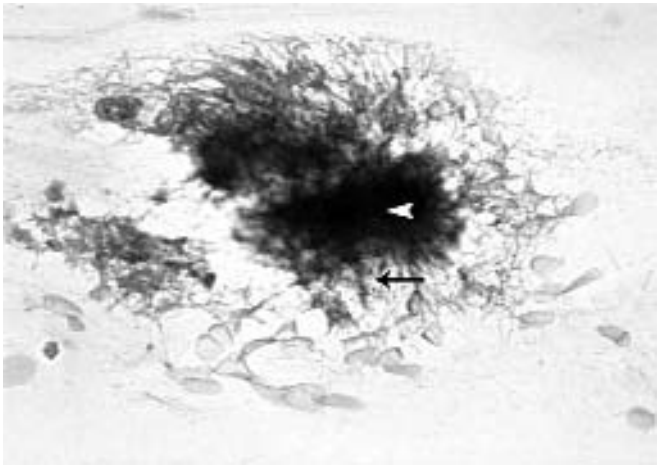

Figure 1: Pap-smear revealing ALOs in the apperance of a dense and basophilic central core (white arrowhead) surrounded by radially oriented filament like structures (black arrow) (Papanicolaou, x400)

Microbiological investigation of cervico-vaginal specimens

The findings of Gram-stained direct smears and anaerobic culturing results were evaluated together before the identification with the BBL Crystal ID System. Gram-positive branching bacilli (Figure 2) seen in Gram-stained smears and dry-rough colonies on Schaedler Agar plates (Figure 3) were sup-

posed to be Actinomyces. In 19 of 200 (9.5 %) Gram-stained smears demonstrated Gram-positive pleomorphic rods consistent with Actinomyces. In 5 of these 19 patients' media (26.3 %), dry and rough colonies were also detected. But only one of them were diagnosed as Actinomyces with the BBL Crystal ID System. Other 4 patients were diagnosed as Propionibacterium[2] and Lactobacilli.[2] Fourteen patients, whose Gram-stained smears have not Gram-positive branching rods but cultures have rough colonies, were also examined with the BBL Crystal ID System and 6 of them were diagnosed as Actinomyces. The other 8 "suspicious" colonies- that is, colony morphologically similar to an actinomycetes- were identified as Propionibacterium,[4] Mobiluncus,[2] Bifidobacterium[1] and Lactobacilli.[1] The details of these results were given in Table 2.

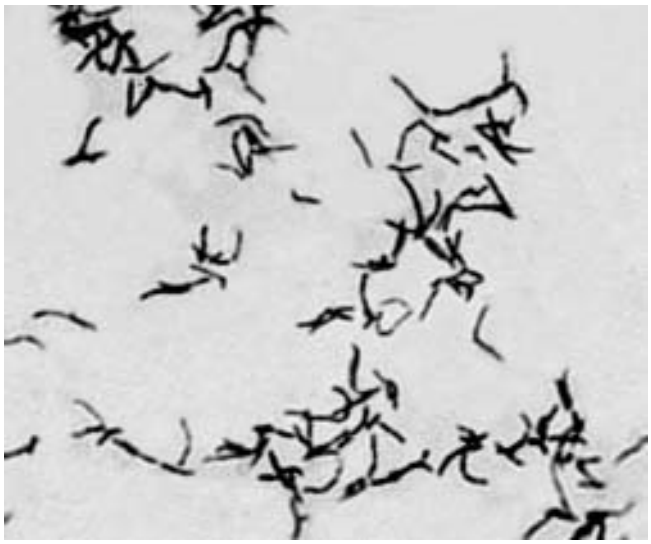

Figure 2: Gram-stained smear reveals Gram-positive, branching, filamentous rods (Gram stain, x1000)

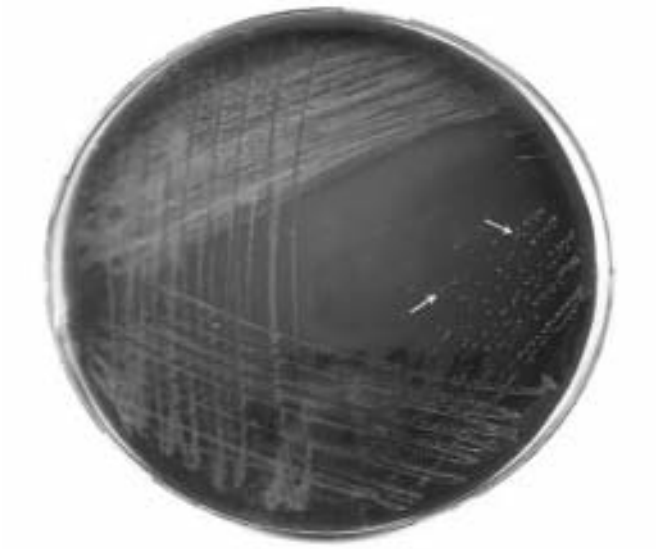

Figure 3: It is seen dry and rough colonies (white arrow) consistent with Actinomyces on Schaedler Agar

Table 2: The details of microbiological examination of 200 samples

1. The examination of Gram-stained direct smears	2. Anaerobic cultivation	3. the BBL Crystal Identification Sysytem
Smears with Gram-positive bacilli (n=19)	Media with dry and rough colonies (n=5)	2 Lactobacillus 2 Propionibacterium 1 Actinomyces
	Media without dry and rough colonies (n=14)	-
	Media with dry and rough colonies (n=14)	6 Actinomyces 4 Propionibacterium 2 Mobiluncus 1 Bifidobacterium 1 Lactobacillus
Smears without Gram-positive bacilli (n=181)	Media without dry and rough colonies (n=167)	

Discussion

Pap staining is the widely used, economical and practical method for the early detection of the genital infectious agents such as Actinomyces. However, Pap smears do not justify the definitive identification for Actinomyces species and it is prefered to use the term Actinomyces-like organisms.[12] Thus, we wanted to confirm our results microbiologically by the BBL Crystal ID System and to increase the performance of Pap smears for the early detection of genital Actinomyces. It is well established that this system is the most succesfull, reliable and acceptable system for the rapid identification of classical Actinomyces spp without the need for anaerobic incubation. It is also found that this system is more discriminatory with respect to identfying different Actinomyces species [13,14] In this study, we found a lack of agreement between these two methods for the identification of Actinomyces ($p>0.05$). There can be several reasons of this disagreement, but we thougt that the main reason is using the different cervico-vaginal specimens for each methods. The cytological samples were collected using a cyto-brush whereas the specimens for microbiological examination was obtained with a swab of anaerobic transport system. In other words, the samples (which were) tested were different. The other reason may be the biological features of Actinomyces. Because these bacteria are fastidious, oxygen-sensitive and slow-growing organisms and it is diffucult to select them from other faster-growing anaerobes.

As a result of our microbiological investigation, 7 patients were diagnosed as Actinomyces by the BBL Crystal ID

System. Among these 7 patients, 4 Actinomyces viscosus, 2 Actinomyces israelii and 1 Actinomyces naeslundii were detected. In literature it is reported that Actinomyces israelii is the most common agent in pelvic actinomycosis, whereas Actinomyces viscosus and Actinomyces naeslundii were generally related to oral infections.[1,4,12,15,16] In contrast to these studies, in our study Actinomyces viscosus and Actinomyces naeslundii were identified as an etiological agents of pelvic actinomycosis. Moreover, Actinomyces viscosus was detected the most common Actinomyces spp. in our patient group. It is considered that these species, whose principal flora is the oral cavity, may be isolated from the genital tract of healty women.

Some colonies suspected to be Actinomyces on Gram-staining smears were identified as Propionibacterium, Eubacterium, Bifidobacterium and Mobiluncus by the BBL Crystal ID System in microbiological examination of our study. These microorganisms are Gram-positive, non-spore forming and highly pleomorphic organisms and they morphologically very similar to Actinomyces. They can be differentiated from each other by some biochemical tests as well.[17] Because nitrat reduction, esculin hydrolysis and carbohydrate fermentation are important tests for discrimination of these genera from each other, these tests were applied to suspicious colonies and the results of these tests were given in Table 2. As is seen in this table, it was detected that 6 patients had Propionibacterium, 2 Bifidobacterium, 1 Mobiluncus, 1 Eubacterium, although they were suspected to be Actinomyces. Despite of the fact that Gram-stained smears are generally rapid, simple and provide early detection, accurate diagnosis must be given by the identification kits and/or by biochemical tests. Also, for correct treatment identification kits provides the definitive means of identifying Actinomyces as the causative agent.[14]

In this study, the incidence of Actinomyces was found low by the two methods. The major reason might be the high socioeconomic level of patients, the regular routine controls and the application of treatment in advised way and time by patients. In addition to these reasons, the overgrowth of concomitant bacteria and possible supressive effect of prior antimicrobial therapy might be the other reasons of low incidence.

Diagnosis of Actinomyces infections may present problems because of the abundance of other filamentous and anaerobic bacteria in the female vagina. It is reported that Pap smear did not depend on viable organisms so it is considered to be more sensitive. It is also inexpensive, simple and direct method for detecting Actinomyces. However, it is impossible to determine which Actinomyces species is present. If a Pap smear includes Actinomyces, culture must be obtained under anaerobic conditions for an accurate antibiotic therapy. In conclusion, detection of Actinomyces both in smears and cultures from the genital tract together might be more sensitive for genital actinomycosis to prevent the misdiagnosed and undiagnosed cases and for early diagnosis and treatment.

Genital Actinomyces'in Sitolojik ve Mikrobiyolojik Yöntemlerle Araştırılması

AMAÇ: Bu çalışmada, genital Actinomyces'in hem sitolojik hem de mikrobiyolojik olarak teşhis edilmesi amaçlanmıştır.

GEREÇ VE YÖNTEMLER: Serviko-vajinal örnekler Hacettepe Üniversitesi Kadın Hastalıkları ve Doğum Anabilim Dalı'na başvuran 200 hastadan alınmıştır. Alınan bu örnekler Papanicolaou boyama yöntemine göre boyanarak Actinomyces-benzeri organizma (ABO) varlığı açısından sitolojik olarak değerlendirilmiştir. Mikrobiyolojik inceleme için de aynı hastalardan alınan örneklere BBL Crystal Tanımlama Sistemi uygulanmıştır.

BULGULAR: Sitolojik yöntemle 6 hastaya (% 3) ABO tanısı konmuştur. BBL Crystal tanımlama sistemiyle ise 7 hastada (3.5 %) Actinomyces tespit edilmiştir. Sitolojik olarak ABO tanısı konan 6 hastadan sadece birine BBL Crystal tanımlama sistemi ile de Actinomyces tanısı konabilmiştir. Genital Actinomyces tanısı açısından bu iki yöntem arasında istatistiksel açıdan anlamlı bir ilişki olmadığı bulunmuştur ($p>0.05$).

SONUÇ: Genital aktinomikoz tanısında sitolojik ve mikrobiyolojik yöntemlerin birlikte kullanılması gerektiğini, böylece tanının erken ve doğru konabileceğini ve yanlış tanıların da önlenebileceğini düşünülmüştür.

Anahtar Kelimeler: Actinomyces, Serviko-vajinal yayma, Papanicolaou boyama yöntemi, BBL Crystal tanımlama sistemi

References

1. Westhoff C. IUDs and colonization or infection with Actinomyces. Contraception 2007;75: 48-50.
2. Merki-Field GS, Rosselli M, Imthurn B. Comparison of two procedures for routine IUD exchange in women with positive Pap smears for Actinomyces-like organisms. Contraception 2008;77: 177-80.
3. Feiter PW, Soeters PB. Gastrointestinal actinomycosis: an unusual presentation with obstructive uropathy. Dis Colon Rectum 2001; 44: 1521-25.
4. Tamer A, Gündüz Y, Karabay O, İka H, Aksel F. Abdominal Actinomycosis: a case report mimicking colon tumor. Türkiye Klinikleri J Med Sci 2006;26:330-33.
5. Demirezen Ş, Kaya D, Beksaç MS. Cytologic findings in Pap smears with Actinomyces-like organisms. Acta Cytol 2005; 49(3):257-61.
6. Ocak S, Çetin M, Hakverdi S, Dolapçıoğlu K, Güngören A, Hakverdi AU. Effects of intrauterine device and oral contraceptive on vaginal flora and epithelium. Saudi Med J 2007;28(5): 727-31.

7. Nawroth F, Foth D, Schmidt T, Römer T. Differential diagnosis and non-surgical treatment of pelvic actinomycosis. Acta Obstet Gynecol Scand 2000;79:1024-25.

8. Nayar M, Chandra M, Chitraratha K, Das SK, Chowdhary GR. Incidence of Actinomycetes infection women using intrauterine contraceptive device. Acta Cytol 1985; 29(2): 111-16.

9. Hager WD, Douglas B, Majmudar B et al. Pelvic colonization with Actinomyces in women using intrauterine contraceptive devices. Am J Obstet Gynecol 1979; 135: 680-684.

10. Valicenti JF, Pappas AA, Graber CD, Williamson HO, Wills NF. Detection prevalence of IUD-associated Actinomyces colonization and related morbidity. JAMA 1982; 247: 577.

11. Cleghorn AG, Wilkinson RG. The IUCD-associated incidence of Actinomyces israelii in the female genital tract. Aust NZ J Obstet Gynecol 1989; 29(4): 445-449.

12. Merki-Field GS, Lebeda E, Hogg B, Keller PJ. The incidence of Actinomyces-like organisms in Papanicolaou-stained smears of copper- and levonorgestrel-releasing intrauterine devices. Contraception 2000; 61: 365-68.

13. Cavallaro JJ, Wiggs LS, Miller JM. Evaluation of the BBL Crystal Anaerobe Identification System. J Clin Microbiol 1997; 35(12): 3186-91.

14. Santala AM, Sarkonen N, Hall V, Carlson P, Jousimies H, Könönen E. Evaluation of four commercial test systems for identification of Actinomyces and some closely related species. J Clin Microbiol 2004; 42(1): 418-20.

15. Scribner DR, Baldwin J, Johnson GA. Actinomycosis mimicking a pelvic malignancy: a case report. J Reprod Med 2000; 45: 515-518.

16. Sandin RL, Greene JN, Sarzier JS et al. Pelvicoabdominal actinomycosis associated with an intrauterine contraceptive device: a case of liver dissemination mimicking metastatic ovarian cancer. Ann Clin Lab Sci 1993; 23(6): 448-455.

17. Hillier S, Moncla B. Anaerobic Gram-Positive nonsporing Bacilli and Rods. In: Balows A, ed. Manual of Clinical Microbiology, 5th edition.Washington: ACM, 1991: 1700-1701.