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Screening for *Chlamydia trachomatis* Infection Using Direct Fluorescein Antibody Method in Female Sex Workers Registered in Ankara, Turkey: Pros and Cons

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Aim: This study aimed to screen for *Chlamydia trachomatis* infection by direct fluorescein antibody (DFA) method in a high-risk population, registered female sex workers (FSWs) in Ankara, Turkey, to investigate the advantages and disadvantages of using this method in a routine screening program of the FSW population.

Materials and Methods: All registered FSWs (n = 152) in Ankara were enrolled in the study. None of them were showing any genital signs or symptoms of sexually transmitted infections (STIs). Endocervical swabs were collected and examined for *C. trachomatis* by DFA method (Fluorotect Chlamydia, Omega Diagnostics, UK). Cellular adequacy of the specimens was examined for the presence of at least five columnar epithelial cells per X400 field. A positive diagnosis was made when fixed and stained specimens showed at least 10 chlamydial bodies under the fluorescein microscope. A positive control slide was also stained and examined on each study day.

Results: Of the 152 specimens, only 112 (73.7%) were adequate; it was not possible to obtain adequate specimen from the remainder of the women because the cervical os was too tight to insert the swab. Of the 112 adequate specimens, 3.6% (4/112) were positive for *C. trachomatis* serotype-specific major outer membrane proteins (MOMP), based on DFA method.

Conclusions: *C. trachomatis* infection rate of 3.6% was relatively low in this group of women compared to a priori expectations, yet still remarkable. A significant level of "inadequacy" in obtaining endocervical specimens (in 26.3% of FSWs) could have limited the external validity of the prevalence rate estimation in this population and the practicality of DFA use for *C. trachomatis* screening. *C. trachomatis* screening should be integrated in the STI control program for sex workers in Turkey. However, further research is warranted to optimize the method to be used in screening and/or diagnosis in such high-risk groups.

Key Words: *Chlamydia trachomatis*, direct fluorescein antibody method, DFA, sex workers

Ankara İlinde Kayıtlı Kadın Seks Çalışanlarında *Chlamydia trachomatis* Enfeksiyonu Taramasında Direkt Floresan Antikor Yönteminin Uygulanabilirliği: Yarar ve Kısıtlılıkları

Amaç: Bu çalışmada, Ankara ilinde, yüksek risk grubu olan kayıtlı kadın seks çalışanları (KSÇ)'nda *Chlamydia trachomatis* enfeksiyonu taramasında, direkt floresan antikor (DFA) yönteminin uygulanabilirliğinin araştırılması ve rutin sürveyans programlarında uygulanabilirliği açısından yarar ve kısıtlılıklarının incelenmesi amaçlanmıştır.

Yöntem ve Gereç: Ankara ilinde kayıtlı KSÇ'ndan (n = 152) endoservikal sürüntü örnekleri toplanmıştır. Seks çalışanlarının hiçbirinde cinsel yolla bulaşan enfeksiyon (CYBE) varlığını gösteren genital belirti ve bulgu saptanmamıştır. Bu örneklerde *C. trachomatis* enfeksiyonunun saptanması için DFA (Fluorotect Chlamydia,Omega Diagnostics,UK) yöntemi kullanılmıştır. Örneklerin yeterliliği X400 büyütmede, her alanda en az beş kolumnar epitel hücresinin varlığına göre değerlendirilmiştir. Tespit edilmiş ve boyanmış örnekler floresan mikroskobu altında incelendiğinde, en az 10 klamidyal form varlığı tespit edildiğinde pozitif olarak sonuçlandırılmıştır. Her çalışma gününde pozitif kontrol örneği boyanarak incelenmiştir.

Bulgular: Yüzelliiki KSÇ'ndan alınan 152 örneğin 112'si (% 73.7) değerlendirme için yeterli bulunmuştur. Geri kalan KSÇ'ndan endoservikal kanala girilemediği için örnek alınamamıştır. Yeterli bulunan 112 örneğin 4 tanesi (% 3.6) DFA yöntemi ile *C. trachomatis* serotipe özgü dış membran proteinleri açısından pozitif bulunmuştur.

Sonuç: Bu grup kadında tespit edilen *C. trachomatis* enfeksiyon hızı (%3.6) daha önceki çalışmalar ışığında beklenene kıyasla daha düşük olmakla birlikte yine de dikkat çekici boyuttadır. Alınan endoservikal örneklerin % 26.3'ünün yetersiz örnek olması, *C. trachomatis* varlığı tespitinin geçerliliğini ve DFA yönteminin tarama testi olarak kullanılabilirliğini kısıtlar görünmektedir. Türkiye'de seks çalışanlarında CYBE'ın kontrolü programı kapsamında *C. trachomatis* varlığının da taranması kuşkusuz önem taşımaktadır. Ancak, yüksek riskli davranışı olan bu gruplarda tarama ve tanı yöntemlerinin optimasyonu için ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Sözcükler: *Chlamydia trachomatis*, direkt floresan antikor yöntemi, DFA, seks çalışanları

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Introduction

Sex workers are high-risk populations for which comprehensive strategies, including screening for sexually transmitted infections (STIs), have to be implemented. In Turkey, registered female sex workers (FSWs) are routinely screened for *Neisseria gonorrhoeae* twice a week by Gram staining of the endocervical swab specimens and for human immunodeficiency virus (HIV), hepatitis B and syphilis every three months, by specific serological tests. However, little is known about the prevalence of *Chlamydia trachomatis* infection in FSWs in Turkey because *C. trachomatis* is not included in the routine screening program of FSWs, although it is one of the important curable STIs.

In an effort to prevent the spread of *C. trachomatis* infections, increased attention has to be given to early diagnosis and treatment (1). The introduction of sensitive and specific nucleic acid amplification tests for detection of this infection has made the use of noninvasive testing feasible in women, but the cost of the tests has unfortunately restricted their usage in developing countries. Direct fluorescein antibody (DFA) method is another nonculture test method with high sensitivity (78%) and specificity (99%). However, it requires a fluorescent microscope, an experienced observer and a pelvic examination to obtain an endocervical specimen, which restrict its widespread use in all settings and all population subgroups (2).

The aim of this study was to screen for *C. trachomatis* infection by DFA method in a high-risk population, registered FSWs in Ankara, Turkey, to investigate the advantages and disadvantages of using this method in a routine screening program of the FSW population.

Materials and Methods

All registered FSWs (n = 152) in Ankara were enrolled in the study. None of them presented any signs or symptoms of genital STIs. Endocervical swabs were collected and examined for *C. trachomatis* by DFA method according to the instructions given in the package insert of the assay (Fluorotect Chlamydia, Omega Diagnostics, UK). In addition, Gram staining was performed for evaluation of the existence of polymorphonuclear leukocytes (PMNLs) and intracellular Gram-negative diplococci. Cellular adequacy of the specimens was examined for the presence of at least five columnar epithelial cells per ×400 field. A positive diagnosis was made when fixed and stained specimens showed at least 10 chlamydial bodies under fluorescein microscope. A positive control slide was also stained and examined on each study day.

Results

Of the 152 specimens from FSWs aged 26-60 years old, 112 (73.7%) were adequate. In the remainder of the FSWs screened, it was not possible to obtain an adequate specimen because the cervical os was too tight to insert the swab. Thus, 26.3% of these women could not be screened for *C. trachomatis* using the DFA method.

Of the 112 adequate specimens, 3.6% (4/112) were positive for *C. trachomatis* serotype-specific major outer membrane proteins (MOMP), according to the DFA method. Among the positives, 2 had >25 PMNLs in the Gram-stained slides, 1 had >15, and 1 did not contain any leukocytes. Other than these, 2 different specimens were positive for intracellular Gram-negative diplococci with >25 PMNLs in the Gram-stained slides. However, the number of leukocytes were >25 in 38 and >15 in 33 collected specimens, without presenting any bacterial morphology.

Discussion

C. trachomatis is the most prevalent sexually transmitted bacterial infection worldwide. A large proportion of those, particularly women, are asymptomatic, and these individuals serve as a major reservoir of infection. Asymptomatic infections are of particular concern also for sex workers because of the potential to spread the infection to several others. Thus, FSWs, as a high-risk population, have to be given special attention in comprehensive prevention strategies, including screening strategies for STIs.

There have been limited studies in different groups about the prevalence of *C. trachomatis* infection in Turkey. In one of the studies performed in Istanbul in 1996 in an urban population of married Turkish women, the infection was diagnosed according to clinical algorithms and reported in 4.9% of the women (3). In another study, 251 registered prostitutes working in a brothel in İzmir were screened for *C. trachomatis* using DFA method. After excluding 27 (10.7%) of the inadequate specimens, 57 (25.4%) were found to be positive (4).

Among all other methods that can be used for *C. trachomatis* screening, DFA has a high yield of usage in high-risk populations, is relatively cheaper, has fast (in 30 minutes) turnaround for results, and can be processed simply. Yet, its validity is dependent on the observer's experience and it requires a fluorescein microscope, which is not readily available in all settings.

This study investigated the practicality of using DFA method in *C. trachomatis* screening in FSWs and to evaluate its advantages and disadvantages. The financial cost of the kit (354 YTL for 50 tests) made it the best choice for use in this study. On the other hand, a significant level of "inadequacy" in obtaining endocervical specimens limited the yield and practicality of DFA for *C. trachomatis* screening. The main limitation we faced, when considering DFA for future use, was the difficulty in obtaining "adequate" endocervical specimens from all FSWs. The cervical os was too tight to insert the swab, even though the collection process was repeated twice; thus, about one-third of the females could not be screened. Inadequate samples were also examined for chlamydial bodies, but they were all negative and were not included in the study. Since the possibility of *Chlamydia* infection could not be ruled out in these women, they were advised to be screened further using another method.

We concluded that endocervical specimen would not be the best option for *C. trachomatis* detection in a routine screening program of FSWs. The increased number of PMNLs (>15) in the Gram-stained slides was not very helpful in the presumptive diagnosis of *C. trachomatis* infection. While 76 of the specimens had an increased number of PMNLs, only six of them had the specific pathogen.

In our study, *C. trachomatis* infection rate (3.6%) was lower compared to the previous studies performed both in Turkey and in some other countries, which reported rates varying between 6.9 to 38.3% (5-10). In the registered group, the prevalence was reported as 12.4% in three northern Mexican cities (11); however, the prevalence was higher among street workers, such as 22.9% in the Czech and Slovak Republics (12). One of the reasons for the lower *C. trachomatis* infection rate in FSWs in this study might have been due to the habit of using antichlamydial drugs without consulting a physician. This situation is highly common in this group of women and also an important problem for the control of emerging antibiotic resistance. The other reasons for the lower infection rate in FSWs in this study might be due to: 1) regular application of successful screening programs, which possibly increased awareness among FSWs of the need for condom usage, and/or 2) the limited yield of the screening method (namely DFA) and its recommended specimen type (endocervical specimens) used for detection of *Chlamydia* infection in the study group.

In conclusion, we believe that *C. trachomatis* screening should be integrated in the STIs control program for sex workers in Turkey. However, further research is clearly warranted to optimize the method to be used in screening and/or diagnosis in such high-risk groups.

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