

Results of Multiplex Polymerase Chain Reaction Assay to Identify Urethritis Pathogens

Üretrit Patojenlerinin Saptanmasında Multipleks Polimeraz Zincir Reaksiyonu Yöntemi

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What's known on the subject? and What does the study add?

We assume that using a multiplex polymerase chain reaction test, enabling results in a rapid and reliable way and performing the procedure with only one swab sample from the patient, provides the clinician with a big advantage as to identifying the pathogens within the ethiology of urethritis, which is a common sexually transmitted disease in males.

Abstract

Objective: The purpose of this study was to evaluate the results of multiplex polymerase chain reaction (PCR) test applied to identify the pathogens in male patients who attended our urology clinic with a pre-diagnosis of urethritis related with sexual intercourse.

Materials and Methods: In this study, we included a total of 91 male patients, who sought medical advice in our clinic between August 2015 and October 2016 due to complaints of urethral discharge, dysuria and urethral itching, having a visible urethral discharge during the physical examination or a positive leukocyte esterase test (Combur-Test®-Roche) in the first urine sample. In the urethral swab samples of these patients, urethritis pathogens were searched with a multiplex PCR test. The multiplex PCR kit, which is able to identify nine pathogens and produced by PathoFinder® (Holland), was used in the process. The pathogens that could be detected by the kit were *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Treponema pallidum*, and *Candida albicans*.

Results: The average age of the subjects was 35.1 (19-57) years. Sixty one out of 91 patients (67%) were found to have a pathogen in the urethral swab sample. In 45 patients (49.4%), only one pathogen, in 12 (13.1%) - two different pathogens and in 4 (4.3%) patients, 3 different pathogens were detected. The pathogens found were as follows: *Ureaplasma urealyticum* in 22 patients (27.1%), *Gardnerella vaginalis* in 15 (18.6%), *Neisseria gonorrhoeae* in 13 (16.1%), *Mycoplasma genitalium* (10 patients; 12.3%), *Mycoplasma hominis* (8 patients; 9.9%), *Chlamydia trachomatis* (8 patients; 9.9%), *Trichomonas vaginalis* (3 patients; 3.8%), and *Candida albicans* (2 patients; 2.4%). None of the patients were identified with *Treponema pallidum*. None of the pathogens were identified in 30 patients (32.9%) whose samples were examined by PCR method.

Conclusion: Sexually transmitted pathogens that are quite difficult to identify and that cause urethritis are possibly defined with only one swab sample in a short time using multiplex PCR method providing new possibilities and scopes for the diagnosis.

Keywords: Polymerase chain reaction, urethritis, mycoplasma, ureaplasma, *Gardnerella vaginalis*, sexual transmitted disease

Öz

Amaç: Bu çalışmanın amacı; cinsel ilişkiye bağlı üretrit ön tanısı ile kliniğimize başvuran erkek hastalarda, patojenin tespiti için uygulanan multipleks polimeraz zincir reaksiyon (PCR) testinin sonuçlarının değerlendirilmesidir.

Gereç ve Yöntem: Kliniğimize Ağustos 2015-Ekim 2016 tarihleri arasında cinsel ilişki sonrası üretral akıntı, disüri, üretral kaşıntı şikayetleri ile başvuran, fizik muayenesinde görünür üretral akıntısı olan veya ilk idrar örneğinde lökosit esteraz testi (Combur-Test®-Roche) pozitif olan 91 erkek

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hasta çalışmaya dahil edildi. Bu hastaların üretral swap örneğinde multipleks PCR testi ile üretrit patojenleri araştırıldı. Bu amaçla PathoFinder® (Hollanda) firmasının 9 patojeni tespit eden multipleks PCR kiti kullanıldı. Kitin saptadığı etkenler; *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Treponema pallidum* ve *Candida albicans*'tı.

Bulgular: İnceleme yapılan 91 hastanın ortalama yaşı 35,1 idi (19-57). Doksan bir hastanın 61'inde (%67) üretral sürüntü örneğinde patojen saptandı. Bunlardan 45 (%49,4) hastada tek patojen, 12'sinde (%13,1) 2 farklı patojen ve 4'ünde (%4,3) ise 3 farklı patojen saptandı. Hastalarda saptanan patojenler sırasıyla, 22'si (%27,1) *Ureaplasma urealyticum*, 15'i (%18,6) *Gardnerella vaginalis*, 13'ü (%16,1) *Neisseria gonorrhoeae*, 10'u (%12,3) *Mycoplasma genitalium*, 8'i (%9,9) *Mycoplasma hominis*, 8'i (%9,9) *Chlamydia trachomatis*, 2'si (%2,4) *Candida albicans* ve 3'ü (%3,8) de *Trichomonas vaginalis*'ti. Hastaların hiçbirinde *Treponema pallidum* tespit edilmedi. Örnek alınan hastaların 30'unda (%32,9) PCR yöntemi ile hiçbir patojen saptanmadı.

Sonuç: Multipleks PCR yöntemi ile, saptanması oldukça zor olan cinsel yolla geçen ve üretrit nedeni patojenlerin tek bir üretral sürüntü örneğiyle, kısa sürede saptanır hale gelmesi, tanıya yeni olanaklar ve fırsatlar sağladığı görülmektedir.

Anahtar Kelimeler: Polimeraz zincir reaksiyonu, üretrit, mikoplazma, üreaplasma, *Gardnerella vaginalis*, cinsel yolla bulaşan hastalıklar

Introduction

Sexually transmitted diseases (STDs) are significant cause of morbidity in sexually active individuals and still remain to be a major medical, social and economic burden all over the world. Urethritis, characterized by dysuria and urethral discharge, is the most common clinical picture and, globally annual incidence is estimated to be ~150 million cases (1). As a part of STDs, urethritis is widely classified as non-gonococcal urethritis (NGU) and gonococcal (2). STDs are highly varied and often involve more than one pathogen (3). The growing burden of STDs and ever-increasing costs have created a need for rapid and reliable laboratory techniques in order to detect the cause pathogens. There are continuing researches investigating methods that may detect more than one pathogen simultaneously in a single clinical patient. Polymerase chain reaction (PCR) assay has been found to be highly sensitive method detecting these STD pathogens (4). A multiplex assay has an additional advantage in screening since it involves simultaneous detection of multiple pathogens (5). The purpose of this study was to evaluate the results of multiplex PCR (MPCR) test performed to identify the pathogens in patients having complaints such as urethral discharge and dysuria after sexual intercourse.

Materials and Methods

We included 91 male patients who were admitted to our clinic between August 2015 and October 2016 due to complaints of urethral discharge, dysuria, and urethral itching, having a visible urethral discharge during the physical examination and those with a positive leukocyte esterase test (Combur-Test®-Roche) in the first urine sample studied on urethritis pathogens using MPCR. The study was approved by Medical Park Hospital Ethics Committee (approval number: 189). Written informed consent was obtained from all patients before MPCR test and the study was performed in accordance with the principles of the Helsinki Declaration. To identify the pathogens in the swab samples, a MPCR kit which is able to identify nine pathogens and produced

by PathoFinder® (Holland) was used. The pathogens that could be detected by the kit were *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Treponema pallidum* and *Candida albicans*. The first nucleic acids (DNA and RNA) were extracted in accordance with the kit protocol. Afterwards, amplification, detection and data analysis processes were carried out using Rotor-Gene Real Time PCR System® (Qiagen, Germany) in compliance with the manufacturer's instructions. The results were examined retrospectively.

Results

The average age of the patients was 35.1 (19-57) years. A total of 81 pathogens were identified in the urethral swab samples of 61 out of 91 patients (67%). Only one pathogen in 45 samples (49.4%), two different pathogens in 12 (13.1%), and 3 different pathogens in 4 (4.3%) samples were detected. The pathogens found were respectively as follows: *Ureaplasma urealyticum* in 22 patients (27.1%), *Gardnerella vaginalis* in 15 (18.6%), *Neisseria gonorrhoeae* in 13 (16.1%), *Mycoplasma genitalium* in 10 patients (12.3%), *Mycoplasma hominis* in 8 (9.9%), *Chlamydia trachomatis* in 8 (9.9%), *Trichomonas vaginalis* in 3 patients (3.8%) and *Candida albicans* in 2 (2.4%) (Figure 1). None of the patients were identified with *Treponema pallidum*. No pathogen was detected in 30 patients (32.9%) whose samples were examined using the PCR method.

Discussion

Urethritis or inflammation of the urethra is a multifactorial condition which is sexually acquired in the majority cases. It is characterized by discharge, dysuria and/or urethral discomfort but may also be asymptomatic. The diagnosis of urethritis is confirmed by demonstrating an excess of polymorphonuclear leukocytes (PMNLs) in the anterior urethra. This is generally assessed by using a urethral swab. Urethritis is defined as

gonococcal when *Neisseria gonorrhoeae* is detected and as NGU when it is not detected. There exist many uncertainties in the event of NGU. Especially in cases having a low-grade inflammation, there are significant intra- and inter-observer mistakes in applying and reading the urethral slides and also in counting the PMNL (6). In many men with urethritis, a known pathogen is not detected (7). The high sensitivity levels of nucleic acid amplification tests like PCR enable the use of less invasive sampling, use of initial flow urine samples or swabs taken by one's own, which are not suitable for less sensitive tests like culture and antigen tests (8). MPCR, uses the advantage of the amplification of various target series under one single reaction condition set of more than one primer sets (9). Samples taken from patients having STDs (including symptomatic and asymptomatic cases) may frequently contain more than one pathogens (10). Therefore, in order to detect low levels of multiple (nine different) pathogens in one analysis, tests like MPCR having sufficient sensitivity and high specificity, have a significant potential in diagnosing STDs rapidly and reliably. MPCR also does not exhibit cross reaction with other relevant target species or many other common urogenital organisms.

Today, for patients diagnosed with urethritis, starting an antibiotic treatment is recommended before the results of culture tests (2). The results of culture tests take 3-7 days. During this period, it is within the bounds of possibility that the patient could contaminate others through new sexual intercourses. However, only the PCR method allows obtaining a result in less than 24 hours. This gives rise to the thought that it allows starting the correct treatment without delay. Identifying the pathogen in the early period will also make the sexual partner available for a correct treatment. Thus, the control of the infection will be easier. Here comes the cost as a significant issue. The cost of a PCR kit to the hospital is 280 TL (77\$). Though this appears to be a high amount for a single test, being able to identify 9

different pathogens in one single sample shows that the test could be considered in terms of cost and profit. Apart from this, in resistive cases in which the patient has not recovered after empirical antibiotic treatment, or in urethritis cases where the pathogen cannot be identified through conventional methods, MPCR analysis could be considered.

Neisseria are gram-negative cocci that require nutritional supplement to be cultured in laboratory environment and their reproduction in culture is not easy. It is estimated that gonococcal urethritis accounts for approximately 5-20% of cases (11). 10% of males infected by gonorrhoeae are asymptomatic (12). *Chlamydia trachomatis* is generally defined as a required intracellular pathogen in the literature and it is responsible for 30-40% of NGU (13). PCR analysis has a high sensitivity and specificity in the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

Studies of the role of ureaplasma in the pathogenesis of urethritis have revealed inconsistent results. Ureaplasmas can be isolated from culture in 30-40% of asymptomatic males (14). In the literature, the prevalence of *Ureaplasma urealyticum* has been reported to be between 5% and 26% (2). *Ureaplasma* and *Mycoplasma species*, due to having no cell walls, are required intracellular parasites (15). Among the 9 pathogens detected by MPCR assay within this study, the most frequently identified ones are *Ureaplasma* and *Mycoplasma hominis* and *genitalium* (with a rate of 46.9 % within all urethritis pathogens) which indicates that intracellular parasitery infections occur much more frequently in the ethiopathogenesis of urethritis. It is rather difficult to isolate and identify these pathogens using conventional methods (16). MPCR assay is remarkably efficient in terms of making these pathogens detectable.

There is a lack of evidence for sexual transmission of genitourinary *Candida* infection. Transmission of *Candida* infection between partners in heterosexual intercourse is seen and genital *Candida albicans* isolates were alleged to transmit sexually in the studies where genotypes were used (17). However in the literature there is a limited number of publications about incidence of *Candida* infection in urethritis ethiology. In a study carried out by Obisesan et al., (18) the incidence of candida species in males was found to be 4.9% (18). In our study, however, the incidence of candida species was 2.4%.

There is increasing evidence that bacteria associated with bacterial vaginosis may cause NGU (19). However, there is no enough data on the association between *Gardnerella vaginalis*, and NGU in males. In a study including 80 heterosexual males and 79 controls carried out by Iser et al. (20), based on the microscobic criteria, the prevalence of *Gardnerella vaginalis* was found to be 14% (20) among heterosexual men with non-gonococcal urethral symptoms. In our study, the prevalence of *Gardnerella*

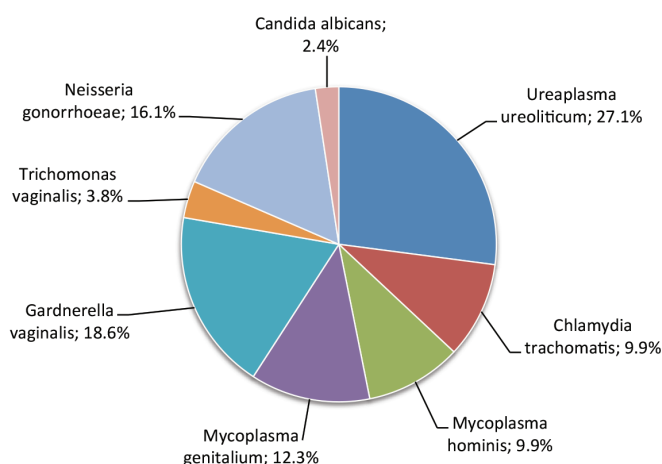


Figure 1. The distribution of urethritis pathogens

vaginalis was found to be 18.6%. *Gardnerella vaginalis* can be seen as commensal organisms in the genitourinary system. Since MPCR kits have been used in this study, a quantitative assessment could not be done for *Gardnerella vaginalis* as a microbial load. This could pose a handicap as to false positive result for such bacteria as *Gardnerella vaginalis* that can be commensal. With the method of quantitative PCR, we assume that the position of *Gardnerella vaginalis* in urethritis will be much more clearly understood.

In the literature, it has been reported that the pathogen associated with urethritis could not be identified in 20-30% of males (21). In a recent study by Wetmore et al. (7), the rate of patients in whom the pathogen could not be identified and who were considered as having idiopathic urethritis was 45.8% among all patients with urethritis. Whereas in our study, the rate of patients, so called idiopathic urethritis group in whom no pathogen could be detected, was found as 32.9%. The condition of idiopathic urethritis may not have an infectious etiology, or different from the ones infected by traditional STD pathogens, but this condition might result from unidentified infection agents in circulation in subgroups of population.

As explained before, identification of most pathogens causing STDs is not easy using routine microbiological diagnose methods. However, PCR assay is useful for identification of microorganisms that are difficult to cultivate and those grow slowly (22). In MPCR, more than one target sequence can be amplified by including more than one pair of primers in the reaction. MPCR has the potential of producing considerable savings in time and effort in the laboratory without compromising test utility. Furthermore, when the amount of the clinical sample is limited, multiplexing allows more targets to be analysed by using a single aliquot of sample material (23). When compared to uniplex PCR test, MPCR test has a general sensitivity of 96% and a specificity of 100% (24).

Conclusion

Identifying pathogens causing sexually transmitted infections and urethritis in males and, are quite difficult to detect with one urethral swab sample in a short time using MPCR method shows that this method can provide new possibilities for the diagnosis.

Ethics

Ethics Committee Approval: The study was approved by Medical Park Hospital Local Ethics Committee (Approval number: 189), Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Mehmet Sarier, Concept: Mehmet Sarier, Design: Erdal Kukul, Data Collection or Processing: Şafak Gökteş, Analysis or Interpretation: Meltem Demir, Literature Search: İbrahim Duman, Writing: Mehmet Sarier.

Conflict of Interest: No conflict of interest was declared by the authors.

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