Prevalence of Sexually Transmitted Diseases in Asymptomatic Renal Transplant Recipients

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Abstract

Objectives: **Sexually transmitted diseases, which may be asymptomatic, have the potential to cause serious health problems in renal transplant recipients. The aim of this study was to determine the prevalence of sexually transmitted diseases in sexually active asymptomatic renal transplant patients by using real-time multiplex polymerase chain reaction assays.**

Materials and Methods: **This prospective controlled study was conducted between November 2016 and January 2017 in our hospital. Our study group included 80 consecutive, sexually active asymptomatic patients (40 men and 40 women) who had undergone renal transplant in our hospital and who presented to our outpatient clinic for routine follow-up. We also included a control group of 80 consecutive, sexually active nontransplant patients (40 men and 40 women). All patient samples were tested for *Gardnerella vaginalis* and obligate anaerobes (*Prevotella bivia, Porphyromonas* species), *Candida* species, *Mycoplasma hominis*, *Mycoplasma genitalium, Ureaplasma* species, *Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis,* herpes simplex virus 1 and 2, and *Cytomegalovirus* by real-time multiplex polymerase chain reaction.**

Results: **The prevalences of infection with *Gardnerella vaginalis* and obligate anaerobes (*P* = .043), *Ureaplasma* species (*P* = .02), and *Cytomegalovirus* (*P* = .016) were found to be significantly higher in the study group versus the control group. However, there was no difference between the 2 groups regarding the prevalence of *Mycoplasma* infection (*P* = .70).**

Conclusions: **Sexually transmitted diseases may occur more frequently in sexually active asymptomatic renal transplant recipients than in nontransplanted individuals. Real-time multiplex polymerase chain reaction analysis may be a suitable method for determining these pathogens.**

Key words: *Infection, Kidney transplantation, Polymerase chain reaction*

Introduction

Renal transplant is the best therapeutic option for patients with end-stage renal disease.[1] Renal transplant not only improves renal function but also quality of life and sexual function. Several studies have reported improvements in libido and sexual function of patients after renal transplant.[2] Transplant recipients often receive numerous immunosuppressants to prevent graft loss, which is associated with an increased risk of infection.[3]

Sexually transmitted diseases (STDs) are particularly problematic in transplant recipients with improved sexual functions, since these patients may not be able to generate sufficient inflammatory response due to immunosuppressant medications.[4] Most STDs are asymptomatic. In such cases, the patients continue to spread the infection through sexual transmission. Therefore, it is highly important to screen asymptomatic patients for STDs.[5]

Polymerase chain reaction (PCR) assays have been considered to be a highly sensitive method for detecting STD pathogens.[6] The aim of this study was to determine the prevalence of STDs in sexually active asymptomatic renal transplant patients by using real-time multiplex PCR assays.

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Materials and Methods

This prospective controlled study was conducted between November 2016 and January 2017 in our hospital. The study group consisted of 80 consecutive, sexually active patients (40 men and 40 women) who had undergone renal transplant in our hospital and presented to our nephrology outpatient clinic for routine follow-up, who had no medical history suggestive of STDs, and whose gynecologic or urologic physical examinations were normal. The control group consisted of 80 consecutive sexually active patients (40 men and 40 women) who presented to the urology or gynecology outpatient clinic for reasons other than infection, who had no medical history suggestive of STDs, and whose gynecologic or urologic physical examinations were normal.

Patients who received any antimicrobial or antiviral treatment within the last 4 weeks were excluded from the study. All renal transplant patients received an immunosuppressive treatment regimen consisting of mycophenolate mofetil, prednisolone, and tacrolimus/cyclosporine.

The study was approved by the local ethics committee, and written informed consent was received from all participants. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration.

According to patient sex, vaginal or urethral samples were collected from all patients in the study and control groups using a cotton-tipped swab. In addition, first void urine samples (15 mL) were collected from all patients in the study and control groups. All samples were stored at -80°C before analysis.

We used the PREP-NA PLUS and PREP-GS PLUS extraction kits manufactured by DNA Technology (Moscow, Russia) for our study. The tests were analyzed by Elite Prime Real-Time PCR, which is manufactured and programmed by the same company. All samples were tested for *Gardnerella vaginalis, Candida* species, *Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma* species, *Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis*, herpes simplex virus 1, herpes simplex virus 2, and *Cytomegalovirus* (CMV) using real-time multiplex PCR with STD kits (DNA Technology). These test kits quantified the amount of microbial DNA (bacteria, viruses, parasites, and fungi) in clinical specimens. The number of potentially pathogenic microorganisms and its ratio to total microbial load were determined. The presence of *Gardnerella vaginalis, Candida* species, *Mycoplasma hominis,* and *Ureaplasma* species with a microbial load $\geq 10^4$ was considered positive, as recommended by the manufacturer.

Statistical analyses

All statistical analyses were performed using SPSS statistical software (SPSS for Windows, version 22.0; SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as means and standard deviation. Differences between groups (STDs, sex, and polymicrobial infections) were evaluated using *t* tests. All other data were analyzed using one-way analysis of variance. $P < .05$ was considered statistically significant.

Results

In the study group, the mean duration between renal transplant and STD testing was 22.8 months (range, 8-64 mo). The mean age was 45 ± 11.4 years (range, 23-63 y) in the study group and 40 ± 10.8 years (range, 20-60 y) in the control group. Male-to-female ratios were the same between groups. The prevalence of patients infected with at least one STD pathogen was found to be significantly higher in the study group (51.3%) than in the control group (32.5%) ($P = .016$). The prevalence of STDs among female patients was significantly higher in the study group (55%) than in the control group (35%) ($P = .004$). Similarly, the prevalence of STDs among male patients was significantly higher in the study group (47.5%) than in the control group (27.5%) ($P = .002$). In addition, polymicrobial infection rates were also found to be significantly higher in the study group (15%) than in the control group (2.5%) ($P = .002$). The infection rates of the control and study groups are shown in Table 1.

The prevalences of pathogens isolated from the study and control groups are presented in Table 2. The prevalences of *Gardnerella vaginalis* ($P = .043$), *Ureaplasma species* ($P = .02$), and CMV ($P = .016$) were found to be significantly higher in the study group than in the control group. However, there was no difference between the 2 groups regarding the prevalence of *Mycoplasma species* ($P = .70$) (Table 2). With real-time multiplex PCR, none of the specimens gave positive results for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or herpes simplex virus 1.

Table 1. Distribution of Data Between the Study and Control Groups According to Age, Sex, and Polymicrobial Infections

	Study Group	Control Group
Age, y (range)	45 ± 11.4 (23-63)	40 ± 10.8 (20-60)
Patients with STDs	51.3% (41/80)	32.5% (25/80) (*P* = .016)
Male patients with STDs	47.5% (19/40)	27.5% (11/40) (*P* = .002)
Female patients with STDs	55% (22/40)	35% (14/40) (*P* = .004)
Patients with polymicrobial infections	15% (12/80)	2.5% (2/80) (*P* = .022)

STDS, sexually transmitted diseases

Table 2. Prevalences of Detected Pathogens in Study Group and Control Group

Pathogen	Study Group (n = 80)	Control Group (n = 80)
Gardnerella vaginalis and obligate anaerobes	20 (25%)	13 (13.75%) (*P* = .043)
Ureaplasma species	19 (23.75%)	7 (8.7%) (*P* = .02)
CMV	9 (11.25%)	1 (1.25%) (*P* = .016)
Mycoplasma species	3 (3.75%)	4 (%5) (*P* = .70)
Mycoplasma hominis	2 (2.5%)	2 (2.5%)
Mycoplasma genitalium	1 (1.25%)	2 (2.5%)
Candida species	2 (2.5%)	3 (3.75%)
HSV-2	1 (1.25%)	
Trichomonas vaginalis	1 (1.25%)	
Chlamydia trachomatis		2 (2.5%)

Abbreviations: CMV, cytomegalovirus; HSV, herpes simplex virus

For pathogens other than CMV, patients with positive PCR results were started on antimicrobial treatment. In patients with positive results for CMV, the quantification of CMV DNA level in the blood was used to determine the need for antiviral therapy. In patients with negative CMV DNA results, antiviral treatment was not deemed necessary.

Discussion

Sexually transmitted diseases constitute a public health problem throughout the world. In addition, STDs are often asymptomatic or cause nonspecific symptoms, which increase the risk of transmission to sexual partners. There are numerous studies in the literature reporting the prevalence of STD pathogens in sexually asymptomatic individuals. However, the prevalence of STDs in sexually active asymptomatic renal transplant recipients is not well-established. Sexually transmitted diseases may require long-term treatment and may lead to chronic rejection in renal transplant patients, increasing morbidity and mortality.[7] To our knowledge, this study is the first in the literature to assess STDs in sexually active asymptomatic renal transplant recipients.

In transplant recipients, the risk of infection is determined by the intensity of exposure to potential pathogens and the combined effect of all factors that contribute to a patient's susceptibility to infection.[8] The incidence of infection in renal transplant recipients is directly related to the immunosuppressive drugs used and the duration of the immunosuppressive therapy.[9] Several reports have indicated that immunosuppressants such as sirolimus or everolimus may be associated with an increased rate of bacterial infections, including infections by opportunistic organisms.[10,11] In addition to affecting the immune system, immunosuppressants also have negative effects on the microbiota of the mucosal flora of transplant recipients.[12] These mechanisms explain why opportunistic infections are more common in these patients. In a study evaluating the effects of immunosuppressant agents on the flora, prednisone was found to have the most significant effect on bacterial diversity and on the colonization of potentially opportunistic pathogens, whereas mycophenolate mofetil had a more limited effect on the bacterial flora but was associated with increased colonization by non-*albicans Candida* species.[12]

As expected, in our study, STDs with opportunistic microorganisms were found to be more frequent in the study group than in the control group. The data on STDs in renal transplant recipients are limited. Asymptomatic individuals do not seek medical attention; therefore, there may be a large pool of undiagnosed cases. Sexually transmitted diseases must be diagnosed and treated correctly to prevent graft loss and/or other problems that may arise in transplant patients.

Mycoplasma hominis, *Ureaplasma* species, and *Gardnerella vaginalis* may be found in the urogenital system as a part of the commensal flora, especially in sexually active individuals. It is known that these pathogens may cause serious extra-urogenital complications in renal transplant patients who are under immunosuppressive treatment.[13-15] The quantification of the microbial load in clinical specimens may provide useful information for differentiating between infection and commensalism. In this study, the microbial loads of the above pathogens were calculated using quantitative PCR analysis, which is helpful in predicting patient prognosis by determining the severity of the infection.

The reported incidence of CMV and/or CMV-related infections during the first months after renal

transplant ranges from 20% to 60%. These infections may result in multiorgan involvement and death.[16,17] As expected, CMV viruria is more common in renal transplant patients. *Cytomegalovirus* may be found in the body fluids and in the urine.[18] In our opinion, sexually active asymptomatic renal transplant patients have a higher risk of acquiring CMV through sexual transmission. There are numerous studies in the literature reporting on the sexual transmission of CMV.[19] In our study, the prevalence of CMV was higher in the study group than in the control group. This result may be due to past CMV infections. Here, the most important parameter is the viral load. Viral load quantification for CMV was not performed in this study. Further studies are needed to explain about the sexual transmission of CMV in renal transplant patients.

Ureaplasma species (*Ureaplasma urealyticum* and *Ureaplasma parvum*) are commonly isolated from patients as part of the normal flora.[20] They are also regarded as important opportunistic pathogens. *Ureaplasma* species have been demonstrated to play a causal role in up to 30% of patients with nongonococcal urethritis and cystitis.[21] In a study by Ekiel and associates, the prevalence of *Ureaplasma* was reported to be higher in samples from female renal transplant recipients than in controls because of immunosuppressive therapy.[22] Likewise, in our study, the prevalence of *Ureaplasma* species was found to be much higher in the transplanted group. More analytical research is needed on *Ureaplasma* species infections in sexually active renal transplant recipients.

Similar to *Ureaplasma* species, *Mycoplasma* hominis is also part of the urogenital commensal flora. However, *Mycoplasma hominis* infections are less common than *Ureaplasma* infections.[23] Likewise, in this study, *Mycoplasma* infections were found to be less common than *Ureaplasma* infections. In a previous study, Meyer and associates have reported that *Mycoplasma hominis* infections occur mainly in severely immunosuppressed patients.[24] In the present study, although the prevalence of *Ureaplasma* was significantly higher in renal transplant patients, no increase was observed in the prevalence of *Mycoplasma*. Similarly, Ekiel and associates found no difference between transplanted and nontransplanted patients in terms of *Mycoplasma* species prevalence.[22] We believe that larger studies on patients with solid-organ transplants will be more enlightening in terms of determining the true prevalence of *Mycoplasma* species.

Our study had some limitations. First, although STDs are dependent on the sexual behavior patterns of individuals, a complete sexual history (sexual partners, marital status, sexual behaviors) was not obtained from patients. Second, the single time point sampling used for the study may have affected the results. Third, the content of the multiplex PCR kit includes CMV, obligate anaerobes, and *Candida* species, which are not necessarily acquired through sexual intercourse like human immunodeficiency virus and syphilis. Despite these limitations, this study highlights the importance of taking into consideration asymptomatic STDs, which may cause significant morbidity and mortality.

The growing health burden of STDs and their high costs have led to a need for rapid and reliable laboratory techniques to identify the causative pathogens. Most pathogens and commensal micro-organisms causing STDs are difficult to cultivate by routine microbiologic methods.[25] Molecular genetic assays such as PCR have been found to be highly sensitive in detecting these STD pathogens.[6] A multiplex assay has an additional advantage in screening because it involves the simultaneous detection of multiple pathogens in a single sample.[26] Multiplex PCR assays are reported to have an overall sensitivity of 96% and specificity of 100% compared with uniplex PCR assays.[27] Quantitative real-time PCR, which measures the bacterial load in specimens, may provide useful information to understand the pathogenic role of opportunistic pathogens in the urogenital tract.

Conclusions

Sexually transmitted diseases may occur more frequently in sexually active asymptomatic renal transplant recipients than in nontransplanted individuals. These pathogens may cause significant morbidity in renal transplant patients. Real-time multiplex PCR analysis may be a suitable method for the diagnosis and management of these pathogens.

References

1. Castaneda DA, Leon K, Martin R, Lopez L, Perez H, Lozano E. Urinary tract infection and kidney transplantation: a review of diagnosis, causes, and current clinical approach. *Transplant Proc.* 2013;45(4):1590-1592.

2. Toorians AW, Janssen E, Laan E, et al. Chronic renal failure and sexual functioning: clinical status versus objectively assessed sexual response. *Nephrol Dial Transplant*. 1997;12(12):2654-2663.
3. Schmaldienst S, Dittrich E, Horl WH. Urinary tract infections after renal transplantation. *Curr Opin Urol*. 2002;12(2):125-130.
4. Ashoor I, Aviles D, Pasternak R, Vehaskari VM. Sexually transmitted infections in pediatric renal transplant recipients: Time to take notice! *Pediatr Transplant*. 2015;19(6):584-587.
5. Shafer CW. STD prevention: why limit ourselves to just an ounce? *S D Med*. 2015;Spec No:53-57.
6. Chen MY, Donovan B. Changes in testing methods for genital Chlamydia trachomatis in New South Wales, Australia, 1999 to 2002. *Sex Health*. 2005;2(4):251-253.
7. Yacoub R, Akl NK. Urinary tract infections and asymptomatic bacteriuria in renal transplant recipients. *J Glob Infect Dis*. 2011;3(4): 383-389.
8. Fishman JA. Infection in solid-organ transplant recipients. *N Engl J Med*. 2007;357(25):2601-2614.
9. Domann E, Hong G, Imirzalioglu C, et al. Culture-independent identification of pathogenic bacteria and polymicrobial infections in the genitourinary tract of renal transplant recipients. *J Clin Microbiol*. 2003;41(12):5500-5510.
10. Ibanez JP, Monteverde ML, Goldberg J, Diaz MA, Turconi A. Sirolimus in pediatric renal transplantation. *Transplant Proc*. 2005;37(2):682-684.
11. Hymes LC, Warshaw BL. Sirolimus in pediatric patients: results in the first 6 months post-renal transplant. *Pediatr Transplant*. 2005;9(4):520-522.
12. Diaz PI, Hong BY, Frias-Lopez J, et al. Transplantation-associated long-term immunosuppression promotes oral colonization by potentially opportunistic pathogens without impacting other members of the salivary bacteriome. *Clin Vaccine Immunol*. 2013;20(6):920-930.
13. Geissdorfer W, Sandner G, John S, Gessner A, Schoerner C, Schroppel K. Ureaplasma urealyticum meningitis in an adult patient. *J Clin Microbiol*. 2008;46(3):1141-1143.
14. Pastural M, Audard V, Bralet MP, et al. Mycoplasma hominis infection in renal transplantation. *Nephrol Dial Transplant*. 2002;17(3):495-496.
15. Finkelhor RS, Wolinsky E, Kim CH, Tchou P, Frengley JD. Gardnerella vaginalis perinephric abscess in a transplanted kidney. *N Engl J Med*. 1981;304(14):846.
16. Sola R, Diaz JM, Guirado L, et al. Significance of cytomegalovirus infection in renal transplantation. *Transplant Proc*. 2003;35(5):1753-1755.
17. Umesh L, Mahesh E, Kumar A, Punith K, Lalitha K, Suman G. Infections in renal transplant recipients. *JIACM*. 2007;8(4):316-323.
18. Sola R, Rabella N, Guirado LL, Diaz JM, Facundo C, Garcia R. Relation between pp65 antigenemia, RT-PCR and viruria for cytomegalovirus detection in kidney transplant recipients. *Transplant Proc*. 2005;37(9):3768-3769.
19. Handsfield HH, Chandler SH, Caine VA, et al. Cytomegalovirus infection in sex partners: evidence for sexual transmission. *J Infect Dis*. 1985;151(2):344-348.
20. Pinna GS, Skevaki CL, Kafetzis DA. The significance of Ureaplasma urealyticum as a pathogenic agent in the paediatric population. *Curr Opin Infect Dis*. 2006;19(3):283-289.
21. O'Leary WM. Ureaplasmas and human disease. *Crit Rev Microbiol*. 1990;17(3):161-168.
22. Ekiel AM, Pietrzak B, Kaminski P, Dolezych H, Jozwiak J, Martirosian G. Prevalence of urogenital mycoplasmas and ureaplasmas in women after kidney transplantation. *Transplantation*. 2009;87(6): 848-851.
23. Nulens E, Van Praet J, Selleslag D, et al. A disseminated Mycoplasma hominis infection in a patient with an underlying defect in humoral immunity. *Infection*. 2016;44(3):379-381.
24. Meyer RD, Clough W. Extragenital Mycoplasma hominis infections in adults: emphasis on immunosuppression. *Clin Infect Dis*. 1993;17 Suppl 1:S243-249.
25. Piemont Y, Jaulhac B. [Value of molecular biology methods for diagnosis in bacteriology]. *Ann Dermatol Venereol*. 1995;122(4): 206-212.
26. Kong F, Gilbert GL. Multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB)--a practical epidemiological and diagnostic tool. *Nat Protoc*. 2006;1(6):2668-2680.
27. Horii T, Ohtsuka H, Osaki M, Ohkuni H. Use of a dual priming oligonucleotide system to detect multiple sexually transmitted pathogens in clinical specimens. *Lett Appl Microbiol*. 2009;49(1):46-52.