Results of Real-time Multiplex Polymerase Chain Reaction Assay in Renal Transplant Recipients With Sterile Pyuria

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ABSTRACT

Urinary tract infections are a major cause of morbidity and hospitalization after renal transplantation. Patients treated with immunosuppressive drugs suffer not only from common uropathogens but also from opportunistic infections caused by unusual uropathogens. Sterile pyuria is associated with numerous infectious agents including viruses, fungi, and atypical or fastidious organisms. The objective of this study was to investigate the pathogens using real-time multiplex polymerase chain reaction (rtMPCR) assay in sterile pyuria of renal transplant recipients. In this prospective controlled study, pathogen detection was performed with rtMPCR assay on October 2016 in 60 patients with sterile pyuria who had undergone kidney transplantation. A total of 40 renal transplant patients were determined as the control group. Male-to-female ratio was same. The mean age of the subjects with sterile pyuria was 45.7 ± 12.1 (25–74). The mean duration after transplantation was 28.8 ± 3.97 (3–102) months. Pathogens were detected with rtMPCR in 61.7% of sterile pyuria group. This rate was significantly higher compared with the control group ($P < .001$). Two or more different pathogens were found in 13 (21.7%) patients in sterile pyuria group. The pathogens found included cytomegalovirus in 10 patients (19%), *Gardnerella vaginalis* and obligate anaerobes in 20 patients (38%), Ureaplasma spp in 17 patients (33%), Candida spp in 2 patients (4%), *Mycoplasma hominis* in one patient (2%), herpes simplex virus-2 in one patient (2%), and *Trichomonas vaginalis* in one patient (2%). Sterile pyuria may indicate the presence of genitourinary pathogens that cannot be detected with conventional urine culture method in renal transplantation patients. rtMPCR is an accurate and convenient method for detection of multiple potential pathogens of sterile pyuria in renal transplant patients.

KIDNEY transplantation is the best options for treatment of patients with end-stage renal disease. However, the kidney recipient may present complications including graft rejection, infections, and neoplasms [1]. Urinary tract infections are the major cause of hospitalization and morbidity following kidney transplantation [2] and seriously threaten success of the operation [3]. The incidence of bacterial infections in kidney transplantation patients is directly associated with the net immunosuppressive effect achieved and the period of time this treatment is administered. Patients treated with immunosuppressive drugs suffer not only from common uropathogens but also from opportunistic infections caused by unusual uropathogens [4]. Sterile pyuria is the presence of elevated number of white cells in the urine in absence of bacteria as determined by means of standard aerobic laboratory techniques [5]. Sterile pyuria is associated with numerous infectious

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agents including viruses, fungi, and atypical or fastidious organisms such as *Chlamydia trachomatis*, Ureaplasmas, and Mycoplasmas [6]. Although these pathogens are correlated with several diseases of the genitourinary tract, they are usually not determined by routine microbiological diagnosis [7]. Molecular genetic assays such as polymerase chain reaction (PCR) are useful tools for the detection of microorganisms that are difficult to cultivate and those that grow slowly [8]. Because multiplex technique includes the concurrent identification of multiple pathogens in a single clinical sample, it has an additional superiority in screening [9]. Quantitative real-time PCR, which evaluates bacterial loads in specimens, can provide useful information to understand the pathogenic role of opportunistic pathogens in the urogenital tract. The objective of this study was to evaluate results of quantitative real-time multiplex PCR test (rtMPCR) in organ transplantation patients.

MATERIALS AND METHODS

This prospective controlled analysis included a total of 60 consecutive patients (30 male and 30 female patients) who had undergone renal transplantation in our hospital in the past and presented for routine outpatient visits in October 2016. Patients with growth in urine culture and those receiving prophylactic antiviral therapy for cytomegalovirus (CMV) infections were excluded from the study. Sterile pyuria was defined as the presence of 3 or more white cells per high-power field of unspun urine and a urinary dipstick test positive for leukocyte esterase (Combur-Test, Roche, Switzerland) with a negative conventional urine culture test [5]. A total of 40 consecutive patients (20 male and 20 female patients) who had undergone renal transplantation in the past in our hospital and who presented for routine outpatient visits at the same dates with the presence of 2 or fewer white cells per high-power field of unspun urine and a urinary dipstick test negative for leukocyte esterase were assigned to the control group. Genitourinary pathogens were studied in all patients with rtMPCR assay. First voiding urine samples and urethral swabs were collected from male and female patients. The study was approved by the local ethics committee, and written informed consent forms were received from all participants.

Microbial detection of the samples was carried out with rtMPCR technique. This is a molecular-based method that identifies DNA and RNA of micro-organisms. In our study, real-time PCR kits manufactured by DNA Technology LLC (Moscow, Russia) were used. The tests were studied with Elite Prime real-time PCR, which is manufactured and programmed by the same company. This test kit determines the total microbial load of bacteria, viruses, parasites, and fungi in terms of DNA. In addition, it gives the number of potential pathogen micro-organisms and its ratio to total microbial load. Furthermore, pathogen micro-organisms are also detected. Besides total microbial load; the presence of *Gardnerella vaginalis* and obligate anaerobes (*Prevotella bivia* + Porphyromonas spp), Candida spp, *M hominis*, and Ureaplasma spp that are among the potential pathogens, and their ratios to total microbial load were detected using this test. In addition, the presence of *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *C trachomatis* among pathogen microorganisms and, herpes simplex virus (HSV)-1, HSV-2, and CMV among viruses is demonstrated. The presence of *G vaginalis* and obligate anaerobes, Candida spp, *M hominis*, and Ureaplasma spp with a microbial load $\geq 10^4$ was considered positive as recommended by the manufacturer.

Statistical analysis was performed using SPSS statistical software (SPSS for Windows, version 22.0; SPSS, Inc., Chicago, IL, USA). Normality of the variables was studied using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed parameters are expressed as mean ± standard deviation, and non-normally distributed parameters are given as median and standard error of mean (SEM). Differences between age and month variables were evaluated with Student *t* test and Mann-Whitney *U* tests. Differences between the groups were compared using χ^2 and Fisher tests. *P* values less than .05 were considered statistically significant.

RESULTS

The mean age was 45.7 ± 12.1 (25–74) in the 60 patients with sterile pyuria and 48.1 ± 11.7 (30–67) in the control group. The mean duration after transplantation was 28.8 ± 3.97 (SEM) (95% confidence interval [CI] 20.86–36.72) (3–102) months in sterile pyuria group and 27 ± 6.28 (SEM) (95% CI 13.87–40.16) (3–94) months in the controls. No significant difference was found between the 2 groups in terms of age ($P = .203$), gender ($P = .99$), and duration after transplantation ($P = .820$). A total of 52 pathogens were detected with rtMPCR in 37 (61.7%) of 60 patients, whereas only 4 pathogens were detected with rtMPCR in 4 (10%) patients in the control group. The presence of pathogens was significantly higher in sterile pyuria group than in the controls ($P < .001$). The presence of pathogens was significantly higher in separately evaluated female and male patients with sterile pyuria than in the control group (63% and 60%, $P = .003$ and $P = .006$, respectively), whereas no significant difference was found between the samples from female and male patients with sterile pyuria in terms of the presence of pathogens ($P = .822$). Two or more different pathogens were found in 13 (21.7%) patients in sterile pyuria group. Two different pathogens were detected in 11 patients and 3 different pathogens in 2 patients. None of the patients from the control group showed polymicrobial status. Pathogens detected with rtMPCR included CMV in 10 patients (19%), *G vaginalis*, and obligate anaerobes in 20 patients (38%), Ureaplasma spp in 17 patients (33%), Candida spp in 2 patients (4%), *M hominis* in one patient (2%), HSV-2 in one patient (2%), and *T vaginalis* in one patient (2%) (Fig 1). *C trachomatis*, *N gonorrhoeae*, and *M genitalium* that are included in rtMPCR kit were not detected in any case with sterile pyuria, whereas CMV was found in 2 patients and Ureaplasma spp in 2 patients from the control group. When both groups were compared in terms of the presence of *G vaginalis* and obligate anaerobes and CMV, which are not reported in the literature among the infectious causes of sterile pyuria, the presence of *G vaginalis* and obligate anaerobes was significantly higher in sterile pyuria group than the controls ($P = .004$), and no statistically significant difference was found between the 2 groups in terms of CMV ($P = .117$).

DISCUSSION

Sterile pyuria is a highly common condition in patients both with and without transplantation. Unfortunately, studies in

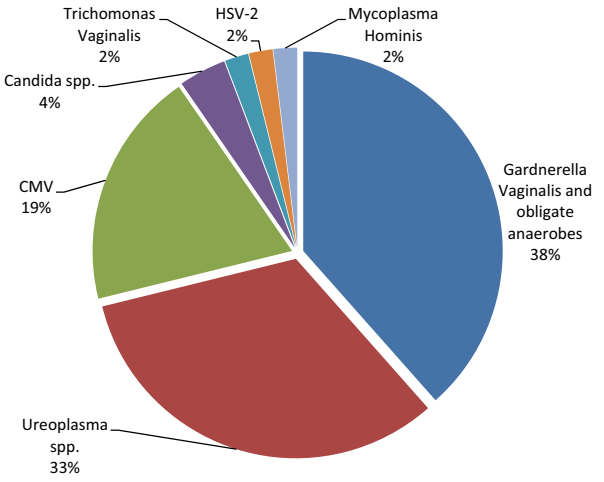

Fig 1. Distribution of pathogens detected in sterile pyuria of renal transplant recipients.

the literature evaluating sterile pyuria in organ transplant patients are scarce. Sterile pyuria is related to both infectious and noninfectious causes, as well as localized and systemic diseases. Among the infectious causes of sterile pyuria, *C trachomatis*, Ureaplasma spp, *N gonorrhoeae*, Mycoplasma spp, *T vaginalis*, HSV I–II, human papillomavirus, human immunodeficiency virus, *Genitourinary tuberculosis*, Schistosomiasis, and Candida spp are reported in the literature [5]. Noninfectious causes of sterile pyuria include rejection of a renal transplant, acute appendicitis, urinary tract stones, presence or recent use of a urinary catheter, recent cystoscopy or urologic endoscopy, foreign body such as surgical mesh in the urethra or a retained stent, pelvic irradiation, urinary fistula, polycystic kidney, renal vein thrombosis, interstitial nephritis or analgesic nephropathy, papillary necrosis, interstitial cystitis, urinary tract neoplasm, and inflammatory disease such as Kawasaki disease or systemic lupus erythematosus [5,10,11]. Another reason for sterile pyuria has been reported as the use of several drugs including indinavir, olsalazine, and nitrofurantoin [12,13]. In addition, tubulointerstitial diseases such as renal transplant rejection can cause sterile pyuria [12].

The present study defines the assessment of an rtMPCR assay in sterile pyuria in renal transplant recipients for the simultaneous identification in clinical specimens of 11 pathogens including those that are difficult to detect by other methods. These micro-organisms are related to various diseases of genitourinary tract. This is the first study to utilize rtMPCR assay for identification of these organisms in sterile pyuria specimens in renal transplantation patients. The result of this study indicated that rtMPCR testing of sterile pyuria could detect a considerable number of causative micro-organisms and should show to the clinicians the advantage of detection of the fastidious micro-organisms in urine from the renal transplantation patients when standard cultures fail to identify microbial infection, because identification of those micro-organisms should constitute an essential part of diagnosis and management in these patients.

Ureaplasma was discovered in 1954 as a causative pathogen of nongonococcal urethritis in men [14]. This bacterium is commonly isolated in humans as a part of the normal flora [15]. It has been demonstrated to play a causal role in up to 30% of persons with nongonococcal urethritis and cystitis [16]. In a study by Ekiel et al, prevalence of Ureaplasma was reported to be higher in the samples from renal transplant recipients (40%) compared with controls (27.5%), because of immunosuppressive therapy [17]. Studies from the literature report that Ureaplasma caused serious problems in kidney transplant patients. There was one case report of ureaplasma meningitis in a renal transplant patient [18]. Another report described a renal abscess in a transplanted patient due to ureaplasma infection [19]. And the other case of severe disseminated Ureaplasma infection has been reported in a kidney transplanted patient [20]. In our study, Ureaplasma spp were found in 28% of patients with sterile pyuria.

M hominis is a part of the urogenital commensal flora as ureaplasma, and infections of *M hominis* are less frequently seen compared with Ureaplasma species [21]. *M hominis* has been the cause of several genital and extragenital infections after organ transplantation [22]. *M hominis* infections have resulted in graft loss and death in the setting of kidney transplants [23]. *M hominis* infections primarily occur in severely immunosuppressed patients [24]. Because *M hominis* cannot be demonstrated by Gram stain, its isolation is difficult and it grows slowly, requiring specific media that may delay the diagnosis of *M hominis* infection. PCR test is a good option for an easy, fast, and accurate detection of this pathogen.

M genitalium is a sexually transmitted pathogen associated with nongonococcal urethritis in men and several inflammatory reproductive tract syndromes including pelvic inflammatory disease, cervicitis, and infertility in women [25]. In our study, *M genitalium* was not detected in any patient with sterile pyuria and in the control group.

G vaginalis is a commensal in the vaginal microflora and grows excessively in bacterial vaginosis as a predominant bacterial species [26]. There is strong evidence suggesting that this pathogen is among the causal factors of nongonococcal urethritis in men [27]. Although involvement of *G vaginalis* out of the genitourinary system is rare, hip joint infection due to *G vaginalis* following renal transplantation has been reported in the literature [28]. In addition, perinephric abscess has been reported in a patient who had undergone kidney transplantation [29]. Using real-time PCR assay, quantification of *G vaginalis* presented a sensitivity of 100% with a specificity of 93% for bacterial vaginosis [30]. In our study, *G vaginalis* and other obligate anaerobes were detected with $\geq 10^4$ microbial load in 20 patients, which was significantly higher compared with the control group. The literature shows that *G vaginalis* and obligate anaerobes (*P bivia* + Porphyromonas spp) are not shown among the infectious causes of sterile pyuria

[5,10,11]. However, these results from our study indicate that *G vaginalis* and obligate anaerobes may also be involved among the infectious causes of sterile pyuria in patients with renal transplantation. We believe that further randomized prospective studies with larger series are needed to demonstrate that especially *G vaginalis* may cause sterile pyuria.

Trichomoniasis is a sexually transmitted disease resulting from infection with the protozoan parasite *T vaginalis* as the causative agent. It is recognized as the most common nonviral sexually transmitted disease worldwide [31]. Trichomoniasis has been associated with vaginitis, urethritis, cervicitis, adverse birth outcomes, and pelvic inflammatory disease [32]. The number of publications about *T vaginalis* infection in kidney transplantation patients is quite limited. In our study *T vaginalis*, which is among infectious causes of sterile pyuria, was detected in one woman.

Although normally *Candida albicans* lives as a harmless commensal, it is the most common human yeast pathogen and can also lead to invasive fungal diseases and mucosal infections [33]. *C albicans* is commonly found in mucosal surfaces of the genitourinary and gastrointestinal tracts [34]. However, excess growth of *C albicans* causes superficial and mucosal diseases such as oral candidiasis and vulvovaginal candidiasis. Early detection of this pathogen is required to guide prevention and treatment. The identification of fungal DNA through PCR has been reported as an important tool in the early diagnosis of fungal infection [35]. In our study Candida spp was detected in 2 patients with sterile pyuria.

HSV, which belongs to the herpes virus family, has the ability to establish latency and become reactivated later [36]. The most common clinical manifestation of HSV disease among kidney transplant patients is oral or genital mucocutaneous disease. In severely immunosuppressed patients, the lesions may have an atypical appearance and become large or confluent. Ultimately, infrequent but potentially fatal forms of HSV disease in the patients undergoing kidney transplantation can occur in the form of pneumonitis, hepatitis, and disseminated disease [37–39]. HSV, which is one infectious cause of sterile pyuria, was found positive in one patient.

CMV infections are frequent during the first months following renal transplantation and may result in severe disease, multiorgan involvement, and death [40]. There are several methods for diagnosis of CMV infections including pp65 in blood (antigenemia), CMV isolation in urine (viruria) and DNA detection by PCR in blood [41]. Today there is no current consensus on the most efficient technique for detection of CMV following kidney transplantation [42–44]. When the literature was assessed in terms of sterile pyuria, although it was reported that CMV may cause hemorrhagic cystitis in immunocompromised patients, this pathogen was not shown among the viral causes of sterile pyuria [5,10,12]. In our study, CMV accounted for 19% of the pathogens found in sterile pyuria group. However, no significant difference was found compared with the controls. Thus we concluded that CMV can not be included among infectious causes of sterile pyuria. However, we think that further studies with larger series are needed on this topic.

CONCLUSION

Sterile pyuria may indicate the presence of genitourinary pathogens that cannot be detected with conventional urine culture method in renal transplantation patients. These pathogens may lead to serious problems in patients with organ transplantation. rtMPCR is an easy, effective, and successful technique for detection of these pathogens. Especially *G vaginalis* and obligate anaerobes should be considered as one of the infectious causes of sterile pyuria in patients who had undergone kidney transplantation.

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