Superficial Fungal Infections in 102 Renal Transplant Recipients: A Case-Control Study

102 RENAL TRANSPLANT ALICISINDA YÜZEYEL MANTAR ENFEKSİYONLARI: KONTROL GRUPLU ÇALIŞMA

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Summary

Background: Renal transplant recipients (RTRs) are predisposed to superficial fungal infections due to immunosuppressive therapy.

Objective: The aim of this study was to determine the prevalence rates and clinical and mycological features of superficial fungal infections in RTRs in our center.

Patients and Methods: One hundred and two consecutive RTRs (34 females and 68 males) and 88 healthy age- and sex-matched controls (30 females and 58 males) were screened for the presence of superficial fungal infection. Skin scrapings and swabs were taken from the dorsum of the tongue, upper back, toe webs, and any suspicious lesions. Nail clippings were also collected. All samples were examined by direct microscopy, and cultured in Sabouraud dextrose agar, mycobiotic agar, and dermatophyte test medium.

Results: Of the 102 RTRs, 65 (63.7%) had cutaneous-oral candidiasis, dermatophytosis, or pityriasis versicolor (PV), whereas only 27 (30.7%) controls showed fungal infection. PV was the most common fungal infection in the patient group (36.3%), followed by cutaneous-oral candidiasis (25.5%), onychomycosis (12.7%), and tinea pedis (11.8%). PV and oral candidiasis were significantly more common in the RTRs, whereas the frequency of dermatophytosis in patients and controls was similar. Candida albicans was the main agent responsible for oral candidiasis, and Trichophyton rubrum was the most common dermatophyte isolated.

Conclusions: Opportunistic infections with P. ovale and C. albicans are increased in RTRs probably due to the immunosuppressive state in this patient population. However, RTRs are not at increased risk for dermatophytosis.

Key Words: Candidiasis, dermatomycoses, Renal transplantation, Tinea versicolor

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Özet

Renal transplantasyon alıcıları (RTA) immünosüpresif tedavi nedeniyle yüzeyel mantar enfeksiyonlarına yatkındırlar.

Amaç: Bu çalışmanın amacı merkezimizdeki RTA'ndaki yüzeyel mantar enfeksiyonlarının prevalansını saptamak, klinik ve mikolojik özelliklerini değerlendirmektir.

Hastalar ve Yöntem: 102 RTA (34 kadın ve 68 erkek) ve 88 yaş ve cinsiyetleri kontrol grubuyla uyumlu sağlıklı birey (30 kadın ve 58 erkek) yüzeyel mantar enfeksiyonları açısından tarandı. Dil üstünden, sırt üst kısımdan, ayak parmak aralarından ve mantar şüphesi olan her lezyondan deri kazıntı ve sürüntüleri alındı. Tırnak örnekleri kesilerek toplandı. Tüm alınan örnekler direkt mikroskopi ile incelendi ve Sabouraud dektroz agar, mikobiyotik agar ve dermatofit test medyuma ekildi.

Bulgular: 102 RTA'sının 65'de (%63.7) kütanöz-oral kandidiyazis, dermatofitozis, ya da pityriazis versicolor (PV) saptanmışken, kontrol grubunun sadace 27'sinde (%30.7) mantar enfeksiyonuna rastlanmıştır. Hasta grubunda en sık görülen mantar enfeksiyonu PV olup (%36.3), bunu kütanöz-oral kandidiyazis (%25.5), onikomikozis (%12.7) ve tinea pedis izlemektedir. PV ve oral kandidiyazis RTA'larında anlamlı olarak daha fazla iken, hasta ve kontrol grubundaki dermatofitozis sıklığı benzer orandadır. Oral kandidiyazisde ana sorumlu etken Candida albicans iken en sık isole edilen dermatofit türü Trichophyton rubrum olmuştur.

Sonuç: RTA'nda P. ovale ve C. albicansın etken olduğu fırsatçı enfeksiyonların artması bu hastaların immünosupresif durumlarına bağlı olabilir. Öte yandan RTA dermatofitozlar açısından daha fazla bir riske sahip değildir.

Anahtar Kelimeler: Kandidiyazis, Dermatomikozis, Renal transplantasyon, Tinea versicolor

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Renal transplant recipients (RTRs) on chronic graft-preserving immunosuppressive therapy are predisposed to a variety of cutaneous complications (1-4). These include infections that tend to be

widespread and feature unusual clinical presentations, and thus present therapeutic challenges (5). A higher-than-normal frequency of superficial fungal infections is expected in these patients, mainly due to impaired cell-mediated immunity (1,6,7). Significantly reduced numbers of epidermal antigen-presenting Langerhans' cells (1,3,8), and corticosteroid-induced thickening and delayed desquamation of the stratum corneum due to treatment (9) are also suggested to contribute to the development of fungal infections in this group. Only a few studies have examined the prevalence and clinical features of superficial fungal infections in RTRs (1,5,6). Previous reports have noted frequencies of 7% to 75%, and most have described extensive lesions and mixed infections caused by different fungal species (1,2,5,6,8,10-12). Only one of the earlier investigations (6) of superficial fungal infections in RTRs included a control group.

This case-control study was undertaken to determine the prevalence rates and clinical and mycological features of superficial fungal infections in RTRs at our hospital.

Patients and Methods

The study included 102 consecutive RTRs (34 females and 68 males) who attended the Renal Transplantation Unit at Başkent University, Ankara, Turkey for routine check-up examinations between March and October 2001. The mean age in the group was 31.9±10.3 years (range, 18 to 61 years), and the mean time since transplantation was 53.6±54.6 months (range, 3 months to 24.9 years). Since the prevalence of fungal infections can be influenced by duration of immunosuppression, we divided the patients into three groups: group A (n=26) had undergone transplantation within the year; group B (n=45) was at 1-5 years of graft survival; and group C (n=31) was at more than 5 years of graft survival. Thirty-nine (38.2%) patients were taking prednisolone (P), cyclosporine (CyA), and mycophenolate mofetil; 21 (20.6%) were taking P and CyA; 20 (19.6%) were taking P, CyA, and azathioprine (Aza); 12 (11.8%) were taking P, mycophenolate mofetil, and tacrolimus; and 10 (9.8%) were taking other combinations of these four drugs. In the immunosuppression protocol, prednisolone was given at a dose of 1 mg/kg/day, and was slowly tapered to a maintenance dose of 10 mg/day by the end of the third month. The following conventional doses of the other immunosuppressive drugs were used: CyA 5 mg/kg/day; Aza 1-3 mg/kg/day; mycophenolate mofetil 1-2 g/day, and tacrolimus 0.1 mg/kg/day. Acute rejection episodes were treated by intravenous injection of methylprednisolone (0.25-1 g) every 24 hours, to a maximum total dose of 6-8 g. Steroid-resistant cases were treated with OKT3 (5 mg/day) for 10-14 days.

Eighty-eight age and sex-matched immunocompetent individuals (30 females and 58 males) randomly selected from patients in the dermatology department and from hospital personnel served as controls. The mean age in this group was 32.5±11.2 years (range, 18 to 64 years). Individuals were excluded from the control group if they presented with complaints related to mycological disease, or were immunosuppressed by drugs or diseases such as malignancy or diabetes mellitus. None of the patients or controls had used topical or systemic anti-fungal agents for at least 3 months prior to the dermatologic examination.

The same dermatologist investigated each patient according to a set protocol. Both the patient and control groups were screened for the presence of superficial fungal infections, namely, cutaneous-oral candidiasis, dermatophytosis and pityriasis versicolor (PV). The oral mucosa and the entire body surface were fully examined. Wood's lamp examination was performed when necessary. Clinical signs (erythema, scaling, maceration, desquamation, hypopigmentation, hyperpigmentation) and symptoms (itching, burning) were recorded. In cases of PV, the extent of the lesions was scored from 0 to 3, with 0 = no lesions; 1 = mild involvement; 2 = moderate involvement; and 3 = severe involvement. Skin scrapings were taken from the upper back, the toe webs, and any clinically suspicious lesions, and mucosal swabs were taken from the dorsum of the tongue. Toenail and fingernail clippings were collected from all subjects, regardless of whether the nails appeared dystrophic or normal.

All samples were examined by direct microscopy after clearing in 10-30% potassium hydroxide with glycerol, and were prepared with calcoflour white fluorescent staining to enhance the visibility of the fungal elements. In patients with nail involvement, the type of onychomycosis was also noted. Oral mucosa samples were cultured in Sabouraud dextrose agar (SDA) and incubated at 37 ºC for 72 hours. Each of the other samples (skin scrapings and nail clippings) was inoculated in two tubes of SDA, one tube of mycobiotic agar, and one tube of dermatophyte test medium. One of the SDA tubes was incubated at 37 ºC and the other was kept at 30ºC. All cultures were checked weekly for at least 4 weeks before they were discarded.

Candida species were identified by wet preparation of a colony for microscopic examination, and by germ-tube testing. The growth characteristics in Sabouraud dextrose broth, morphologic characteristics on cornmeal agar with Tween 80, and the ability of the fungi to grow in the presence of cycloheximide were evaluated. Urease activity testing, and carbohydrate assimilation and fermentation tests were also performed as required.

Dermatophytes were identified according to colonial and microscopic morphological features. Potato dextrose agar was used to stimulate the production of conidia. Physiological evaluations, including the *in vitro* hair perforation test, urease activity, and temperature tolerance testing, were performed as needed to identify dermatophyte species. Nutritional testing was used to classify *Trichophyton* species.

The presence of short septate filaments and observation of oval or round budding yeast cells on direct microscopic examination were accepted as typical diagnostic findings that identified *Pityrosporum ovale*.

Statistical anlysis were performed with Student's t test, and $p<0.05$ was considered significant. Pearson's correlation analysis was used to assess the effects of the following variables on the frequency of superficial fungal infections in RTRs: age, sex, duration of immunosuppression, and historical or current use of CyA and/or Aza.

Results

Sixty-five (63.7%) of the 102 RTRs were affected by cutaneous-oral candidiasis, dermatophytosis, or PV, whereas only 27 (30.7%) controls showed fungal infection ($p<0.001$). Cutaneous-oral candidiasis was detected in 26 RTRs (25.5%) and 13 controls (14.8%), a difference that was not statistically significant ($p>0.05$). There was also no significant difference between the RTRs and controls regarding the prevalence of dermatophytosis (n=20; 19.6% and n=18; 20.5%, respectively; $p>0.05$).

1. Mycological findings in suspicious lesions

In one patient and one control, scaling erythematous lesions in the inguinal region were positive on microscopic examination but cultures were negative. Another control subject had similar clinical lesions on his abdomen. Direct microscopy was positive in the sample from this area, and culturing revealed *Trichophyton rubrum*.

2. Mycology of the oral mucosa

Although there was no significant difference between the two groups regarding the frequency of cutaneous-oral candidiasis, the prevalence of candidal colonization and oral candidiasis in the RTRs was significantly higher than the rate in the controls (n=26; 25.5% and n=11; 12.5%, respectively; $p=0.028$). Tongue lesions with whitish or reddish coating and a burning sensation were noted in all patients diagnosed with oral candidiasis, but the buccal mucosa was normal in all patients and controls. *C. albicans* was the most frequent species identified in the two groups (Table 1).

3. Toe web mycology

The frequency of tinea pedis and candidal intertrigo combined was higher in the control group (n=13; 14.7%) than the RTRs (n=12; 11.8%), but this difference was not statistically significant ($p>0.05$). There was no significant difference between the two groups regarding the clinical presentation of these lesions. Concerning tinea pedis, *T. mentagrophytes* was most common isolate in the patient group, and *T. rubrum* predominated in the controls. For candidal intertrigo of the toe web, *C.*

Table 1. The frequency of oral candidiasis and oral candidal colonization in the renal transplant patients and controls

	Patients (n=102)	Controls (n=88)
Candida albicans	21 (20.6%)	8 (9.1%)
Candida parapsilosis	2 (1.9%)	1 (1.1%)
Candida krusei	2 (1.9%)	1(1.1%)
Candida tropicalis	1 (0.9%)	1(1.1%)
Total	26 (25.5%)	11 (12.5%)

Table 2. Microscopic and culture findings in the toe webs of the renal transplant patients and controls

	Patients (n=102)	Controls (n=88)
T. mentagrophytes	3 (2.9%)	3 (3.4%)
T. rubrum	2 (1.9%)	6 (6.8%)
T. tonsurans	1 (0.9%)	0 (0%)
Candida albicans	2 (1.9%)	1 (1.1%)
Microscopy alone (+)	4 (3.9%)	3 (3.4%)
Totals	12 (11.8%)	13 (14.7%)

Table 3. Microscopic and culture findings in the nails of the renal transplant patients and controls

	Patients (n=102)	Controls (n=88)
T. rubrum	7 (6.9%)	4 (4.5%)
Candida albicans	3 (2.9%)	0 (0%)
Microscopy alone (+)	3 (2.9%)	6 (6.8%)
Total	13 (12.7%)	10 (11.4%)

albicans was the only agent detected in both groups. The details of the microscopic and culture findings are given in Table 2.

4. Nail mycology

There was no significant difference between the RTRs (n=13; 12.7%) and controls (n=10; 11.4%) regarding prevalence of fungal nail infection ($p>0.05$). Also, the extent and severity of nail involvement were similar in the two groups. Of the 13 cases in the RTR group, 3 were proximal subungual onchyomycosis and 10 were distal subungual onchyomycosis. *T. rubrum* was the only dermatophyte identified as a causative agent in tinea unguium in both patients and controls. All the candidal onychomycoses were caused by *C. albicans*. The nail mycology results are shown in Table 3.

5. Mycological findings on the back

Direct microscopy revealed *P. ovale* in more RTRs (n=37; 36.3%) than controls (n=1; 1.1%), and this difference was statistically significant ($p<0.001$). The upper trunk was the site most commonly involved. Of these 37 cases, the extent of the lesions was mild in 7, moderate in 16, and severe in 14 patients. None of the samples from upper back sites that appeared normal were positive on direct microscopy.

Correlation analysis showed no significant effect of age, sex, and duration of immunosuppression on the prevalence rates of superficial fungal infections in this group of RTRs. CyA treatment and Aza therapy were identified as independent risk factors that affected the frequency of superficial fungal infections in this patient group (Table 4).

Table 4. The effects of different clinical variables on the prevalence of superficial fungal infections in RTRs

	RTRs with fungal infection	RTRs without fungal nfection	P
Age (yrs, mean±SD)	31.1±9.6	32.4±10.7	NS
Sex (F/M)	24/39	10/29	NS
Duration of Immunosuppression (months, mean±SD)	56.3±45.5	49.2±67.1	NS
CyA use (+)	62	31	p=0.002
CyA use (-)	1	8	
Aza use (+)	46	16	p=0.002
Aza use (-)	17	23	

RTRs, renal transplant recipients; CyA, cyclosporine; Aza, azathioprine

Discussion

Superficial fungal infections are expected to be more prevalent in RTRs because these patients take immunosuppressive agents to enhance graft survival (1,5,6). Previous studies have investigated a wide spectrum of cutaneous disorders in these patients (2,8,10-12); however, only a few have focused specifically on the cutaneous and laboratory features of superficial fungal infections in RTRs (1,5,6). Moreover, just one of these investigations included a control group (6). Earlier reports have documented wide variation in the frequency of superficial fungal infections, from 7% to 75% (1,2,5,6,8,10-12). Our case-control study of 102 RTRs revealed a higher prevalence of fungal infection in the patients (63.7%) than in immunocompetent controls (30.7%). The only other case-control study (6) in the literature reported a significant difference between 72 RTRs and 72 controls regarding the number of body sites affected (15% versus 9%, respectively).

We found that PV was the most common fungal infection (36.3%) in the group of RTRs we studied. Next in order of frequency were oral candidiasis (25.5%), onychomycosis (12.7%), and fungal toe-web infection (11.8%). These results confirm the findings of earlier studies, which have identified PV as the most frequent fungal infection (18-48%) in RTRs (1,6,13,14). Other authors have indicated that there is a high rate of *Pityrosporum spp.* colonization in RTRs. However, our findings provide no evidence that *P. ovale* colonization of the upper back without clinical evidence of PV is common in this patient group (1,6). Also in contrast to other reports, our data indicate that mucosal candidiasis is not uncommon in RTRs (1,6). We observed oral candidiasis significantly more frequently in RTRs than in controls, despite the fact that there was no difference between the two groups regarding overall rate of cutaneous-oral candidiasis. Our analysis identified *C. albicans* as the main agent responsible for cutaneous-oral candidiasis.

The results of this study show that the frequencies of onychomycosis and fungal toe-web infection are similar to the rates in immunocompetent hosts, which matches the findings of Shuttleworth et al (6). We found *T. rubrum* to be the most common dermatophyte in both patient and control samples, similar to what has been documented in several previous reports (5,15-17). However, some investigations of RTRs have noted *T. mentagrophytes* as a major isolate (1,6).

None of our transplant recipients exhibited cutaneous superficial fungal infections that were atypical, or more severe or widespread in comparison to findings in controls. Furthermore, none of the RTRs had mixed infections at any site.

In conclusion, the conditions for opportunistic infection with *P. ovale* and *C. albicans*, which are both normal inhabitants of the skin and oral mucosa (18,19) may be better in RTRs due to their immunosuppressive state. Our findings indicate that these patients do not differ markedly from immunocompetent individuals in terms of prevalence of dermatophytosis; thus, they are not at increased risk for this form of fungal infection. We suggest that not only administration of immunosuppressive agents, but also environmental exposure to pathogenic fungi is needed to facilitate dermatophytosis in RTRs.

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