In Search of Dermatophytes – Frequency and Etiology of Fungal Infections in Patients with and without Diabetes Mellitus

Gergana Z. Vazheva1, Liliya G. Zisova1, Elena A. Becheva2, Veselin R. Chonov3, Valentin D. Dichev4, Tsonka M. Miteva-Katrandzhieva5, Zoya V. Rachkovska6, Maria M. Orberzova7,8, Veselin T. Belovezhdov9

1 Department of Dermatology and Venereology, Medical University of Plovdiv, Plovdiv, Bulgaria
2 Department of Endocrinology and Metabolic Diseases, Medical University of Plovdiv, Plovdiv, Bulgaria
3 Department of General and Clinical Pathology, Medical University of Plovdiv, Plovdiv, Bulgaria
4 Department of Medical Biology, Medical University of Plovdiv, Plovdiv, Bulgaria
5 Department of Medical Informatics, Biostatistics and E-learning, Medical University of Plovdiv, Plovdiv, Bulgaria
6 Department of Microbiology and Immunology, Medical University of Plovdiv, Plovdiv, Bulgaria
7 Clinic of Endocrinology and Metabolic Diseases, St George University Hospital, Plovdiv, Bulgaria
8 Department of Endocrinology, Medical University of Plovdiv, Plovdiv, Bulgaria
9 Department of General and Clinical Pathology, Medical University of Plovdiv, Plovdiv, Bulgaria

Corresponding author: Gergana Z. Vazheva, Department of Dermatology and Venereology, Medical University of Plovdiv, 15A Vassil Aprilov Blvd., Plovdiv, Bulgaria; Email: g.vazheva@gmail.com; Tel.: +359 886 962 602

Received: 21 Apr 2022 ♦ Accepted: 12 July 2022 ♦ Published: 31 Dec 2022

Citation: Vazheva GZ, Zisova LG, Becheva EA, Chonov VR, Dichev VD, Miteva-Katrandzhieva TM, Rachkovska ZV, Orberzova MM, Belovezhdov VT. In search of dermatophytes – frequency and etiology of fungal infections in patients with and without diabetes mellitus. Folia Med (Plovdiv) 2022;64(6):922-931. doi: 10.3897/folmed.64.e85486.

Abstract

Introduction: Onychomycosis is a frequent nail disorder, accounting for up to 50% of all nail problems. Treatment of onychomycosis is expensive and requires a long time of antifungal medications. Consequently, a proper and faster diagnosis is necessary. Especially for those patients with diabetes mellitus, where onychomycosis is among the most significant predictors of foot ulcer and possible severe complications.

Aim: To compare the sensitivity, specificity, and turnaround time between direct microscopy, culture, histology, and real-time PCR. In addition, to compare the frequency and etiology of onychomycosis in patients with and without DM.

Materials and methods: This study included 102 patients, divided into two groups. One group consisted of patients with diabetes mellitus and the other – without diabetes. Nail samples were collected and examined by direct KOH microscopic examination, culture, histology, and real-time PCR.

Results: From the 102 patients with clinical onychomycosis, positive KOH was found in 38 (37.3%). Culture – 82 out of 102 samples (80.4%) were positive for dermatophytes, yeasts, and/or NDM. Positive histology samples were 32 (41.6%). The PCR was positive in 57 (55.9%) out of the 102. We discovered that there is no significant statistical difference in the etiology of the fungal infections between the two groups.

Conclusions: All mycological investigations have their place in the diagnosis of onychomycosis. Direct microscopy, culture, and histology are useful methods for clinicians to diagnose and follow up the post-treatment period. The advantages of RT-PCR include obtaining results faster and accurately identifying fungi, thus becoming more valued in the diagnosis of OM.

Keywords
dermatophytosis, histology, onychomycosis, real-time PCR, Trichophyton rubrum
Onychomycosis (OM) is the most common cause of nail infections, representing up to 90% of toenail and at least 50% of fingernail infections. It is present in 2-13% of the general population increasing up to 48% at 70 years of age. OM is caused by three groups of fungi – dermatophytes, yeasts, and non-dermatophyte molds (NDM). The term “dermatophytosis” describes infections by members of the genera Trichophyton, Microsporum, and Epidermophyton. It is considered that over 90% of onychomycoses are caused by two dermatophytes – Trichophyton rubrum and Trichophyton mentagrophytes. Yeasts and NDM are the pathogens in about 7% of fungal nail infections. The yeasts causing OM are namely Candida spp. with its most frequent representative C. albicans, but also C. krusei, C. glabrata, C. parapsilosis, and C. tropicalis. As far as non-dermatophyte molds are concerned – Alternaria spp., Aspergillus spp., Acremonium spp. are among the most common species.

Fungal nail infections are frequently recurrent and evolve into chronic conditions. This not only leads to nail thickening, discoloration, and onycholysis, but also causes discomfort, embarrassment, and in some cases, physical pain. For some patients with concomitant diseases, such as diabetes mellitus, mycotic infection of the nails is a risk factor that could lead to severe possible complications. It is well known that diabetic patients often have problems with their feet, generally due to neuropathy and arterial insufficiency. Traumatic ulcerations, fissures, and following secondary infection lead to an increased risk of toe or lower leg amputation. Infections are a common problem among diabetic patients. Fungal infections are estimated to exceed 50% of all infections in diabetic patients. They are twice as likely to suffer from onychomycosis compared to diabetes-free individuals.

Diabetes mellitus has emerged as one of the most serious and common chronic diseases of our times, causing life-threatening, disabling, costly complications, and reducing life expectancy. The global prevalence of diabetes had reached pandemic proportions with the 10th edition of the IDF reporting a prevalence of 337 million adults (20-79 years) living with diabetes. This number is predicted to rise to 643 million by 2030 and 783 million by 2045.

The aim of the study was to compare the sensitivity, specificity, and turnaround time between four different diagnostic methods – direct microscopy, culture, histology, and real-time PCR. Also, to compare the frequency and etiology of onychomycosis in patients with and without diabetes mellitus, using these four methods.
blade) and clippings (taken with small clippers) were collected at the Mycology Laboratory, Department of Dermatology and Venereology in the Medical University of Plovdiv.

The collected specimens were divided into four portions for most of the patients. For some of them, it was only possible to collect enough material for three portions, and histology for those was not possible. The first portion of the specimens was examined microscopically after incubation in 10% KOH. The second portion was cultured on two sets of media: Sabouraud Dextrose Agar (SDA) containing chloramphenicol with and without cycloheximide. The third portion was used to perform real-time PCR and the fourth one was used to perform histology using PAS stain technique.

Direct microscopic examination – the sample to be examined was placed on a clean glass slide. A drop of 10% KOH reagent was added, mixed with the sample, and incubated for 1 hour. The softened nail material was examined under both low (10×) and high (40×) power fields of the microscope for the presence of fungal elements – hyphae, spores, and pseudo-hyphae.

Cultures were carried out on all nail specimens obtained from patients from both diabetic and non-diabetic groups. Samples were inoculated on two sets of media – SDA with chloramphenicol with and without cycloheximide and incubated at 28°C - 36°C and examined regularly for 4 weeks, for any growth. If there was no growth after 4 weeks, the result was reported as negative. Identification of obtained growth was done by macroscopic examination of the culture – observing morphological characteristics of the colony including size, shape, consistency, margins, color both in recto and verso sides, type of the growth whether fluffy, cottony or creamy, and the presence or absence of diffusible pigments (Figs 1, 2). Microscopic examination of the cultures was also performed by tease mount which is the most common technique used for rapid mounting of fungi. In the cases where Candida spp culture was obtained, an additional Chrom agar test was performed to differentiate the most common species of Candida – C. albicans, C. glabrata, C. krusei, and C. tropicalis. The other species were indicated as C. non-albicans.

Histological examination – nail clippings were sent for histopathologic examination in a 10% buffered formalin container. The stain chosen in this study was the Periodic Acid-Schiff (PAS). Then each material was observed under a microscope in low (10×) and high (40×) power fields for the presence of fungal elements in a magenta-red color – hyphae, spores, pseudo-hyphae (Figs 3, 4).

Real-time PCR (RT-PCR) was performed using DermaGenius® 2.0 Complete multiplex real-time PCR kit (PathoNostics B.V., The Netherlands) which can detect 12 pathogens, including Candida albicans, Trichophyton rubrum, Trichophyton interdigitale, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton soudanense, Trichophyton violaceum, Trichophyton benhamiae, Trichophyton verrucosum, Microsporum canis, Microsporum audouinii, and Epidermophyton floccosum. RT-PCR was performed according to the manufacturer’s instructions. First, the DNA extraction was carried out and then 5 µl of DNA extract was added both to the PCR mix 1 and PCR mix 2, and a D'Tprime Real-time Detection Thermal Cycler was used for amplification and melting curve analysis.

**Statistical analysis**

Descriptive and nonparametric (Fisher’s test and McNemar) analyses were performed (SPSS Program, version 19). Some of the results are presented as frequency tables. The sensitivity, specificity, and efficacy of each test are presented and these diagnostic parameters were compared using the
In Search of Dermatophytes

McNemar nonparametric test. Values of $p<0.05$ were considered to be significant.

**Onychomycosis**

To define a true case of onychomycosis in this study, we have taken into account the combination of clinical symptoms of nail disorder with positive histology, and/or a positive culture for a true pathogen (dermatophyte or *Candida spp.*), and/or positive RT-PCR result.

**RESULTS**

One hundred and two patients were included in this study. Fifty-five of them were women (53.9%) and the rest 47 were men (46.1%). The youngest patient was 20 years of age, whereas the oldest patient was 89 years old. The age mode of patients was 55 with a SD of 16 years. Most of the patients had nail changes in their toenails – 98 (96.1%), and only 4 of them (3.9%) had problems with their fingernails.

The severity of the onychomycosis was divided into categories, depending on the number of nails affected – mild (4 or fewer nails affected), moderate (5 to 8 nails affected), severe ($\geq$ 9 nails involved)\(^{[10]}\), and the results were respectively 70 (68.6%), 4 (3.9%), and 28 (27.5%) (Table 1).

Most commonly, both big toes of the patients were affected – 55 cases (53.9%), secondly, all of the toenails – 24 cases (23.5%). According to the clinical presentation of the nail changes, the most frequent type of onychomycosis was the distal subungual, representing 93 (91.2%) of patients. Second in frequency was the TDO with 6 cases (5.9%), and then 2 cases for DLSO, and 1 case of SWO.

The duration of the nail changes varied from 3 months to 30 years. The following scale was introduced to categorize them – less than 1 year, 1 to 5 years, 6 to 10 years, and more than 10 years. The majority of patients had started having their consultation, whereas 32 (31.4%) used either local or systemic treatment. Out of those 32, 26 had local treatment (81.25%), and the rest 6 patients (18.75%) had systemic treatment. As previously established, patients were advised to stop applying local medication on their nails at least 3

---

**Table 1. Level of severity of nail involvement**

<table>
<thead>
<tr>
<th>Level of severity of nail involvement</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>70</td>
<td>68.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>3.9</td>
</tr>
<tr>
<td>Severe</td>
<td>28</td>
<td>27.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>102</td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

**Table 2. Duration of nail changes**

<table>
<thead>
<tr>
<th>Duration of nail changes</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq$ 1 year</td>
<td>19</td>
<td>18.6</td>
</tr>
<tr>
<td>1-5 years</td>
<td>50</td>
<td>49.0</td>
</tr>
<tr>
<td>6-10 years</td>
<td>25</td>
<td>24.5</td>
</tr>
<tr>
<td>$\geq$ 10 years</td>
<td>8</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>102</td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
days before the sample taking, and none of them had been taking systemic treatment for at least a month before it. The group of patients with diabetes mellitus included 51 patients with controlled DM. Their disease was established 1 to 5 years ago in 18 cases (35.29%), 6-10 years ago in 18 (35.29%), and over 10 years ago in 15 patients (29.41%) (Table 3).

Trichophyton rubrum was the most frequently detected species with 20 (60.6%), followed by T. mentagrophytes with 10 (30.3%). Twenty-four (29.3%) mixed cultures were obtained including a dermatophyte in 11 (13.4%) cases. The sensitivity of this method was 73.1%, and the specificity was 100% (p<0.001, McNemar test).

Table 3. Duration of nail changes and duration of DM

<table>
<thead>
<tr>
<th>Duration of nail changes (years)</th>
<th>&lt;1</th>
<th>1-5</th>
<th>6-10</th>
<th>&gt;10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5 N</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>% duration of DM</td>
<td>16.7%</td>
<td>55.6%</td>
<td>22.2%</td>
<td>5.6%</td>
<td>100.0%</td>
</tr>
<tr>
<td>6-10 N</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>% duration of DM</td>
<td>22.2%</td>
<td>44.4%</td>
<td>22.2%</td>
<td>11.1%</td>
<td>100.0%</td>
</tr>
<tr>
<td>&gt;10 N</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>% duration of DM</td>
<td>0.0%</td>
<td>46.7%</td>
<td>40.0%</td>
<td>13.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total N</td>
<td>7</td>
<td>25</td>
<td>14</td>
<td>5</td>
<td>51</td>
</tr>
<tr>
<td>% duration of DM</td>
<td>13.7%</td>
<td>49.0%</td>
<td>27.5%</td>
<td>9.8%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Out of the total of 18 patients with DM from 1 to 5 years, 3 (16.7%) have had nail changes for less than one year. For the group of patients with a duration of DM of 6 to 10 years, results were as follows – 18 cases in total, 12 of which (66.7%) with nail changes for less than a year, or between 1 and 5 years. For the group of patients with diabetes mellitus longer than 10 years – 15 patients, 13 (86.7%) of them have experienced nail changes for less than one year, between 1 and 5 years, and between 6 and 10 years.

Direct microscopy

Of the 102 patients with clinical onychomycosis, positive KOH was found in 38 (37.3%), where 5 (4.9%) with positive KOH alone, and the rest 33 (32.4%) positive for both KOH and culture. The sensitivity of the direct microscopy was 47.4%, and the specificity – 95.8% (p<0.001, McNemar test).

Culture

In total, 82 out of 102 samples (80.4%) were positive for dermatophytes, yeasts, and/or NDM. Among them, 33 were dermatophytes, 29 were yeasts including 5 C. albicans, and 45 (20 of which found in mixed cultures with yeasts and/or dermatophytes) were positive for non-dermatophyte molds including Alternaria, Scopulariopsis brevicaulis, Aspergillus spp., Penicillium spp., Rhodotorula spp., and Cladosporium spp.

Trichophyton rubrum was the most frequently detected species with 20 (60.6%), followed by T. mentagrophytes with 10 (30.3%). Twenty-four (29.3%) mixed cultures were obtained including a dermatophyte in 11 (13.4%) cases. The sensitivity of this method was 73.1%, and the specificity was 100% (p<0.001, McNemar test).

Histology

Histological examination was possible in 77 out of 102 patients (75.5%). The positive samples were 32 (41.6%). The estimated sensitivity was 55.2%, and specificity was 100% (p<0.001, McNemar test). Negative results were observed in 45 samples among which 18 samples had a positive culture – 12 Candida albicans and non-albicans, and 6 dermatophytes, 26.7%, and 13.3%, respectively. A total of 13 out of 45 histology negative samples (28.9%) were associated with real-time PCR positive results.

RT-PCR

The PCR was positive in 57 (55.9%) out of the 102 patients included in the study, among which 28 (49.1%) had positive histologic results. The kit detected a majority of dermatophytes – 55 (96.5%), and 3 C. albicans (5.3%). There were 3 mixed infections detected among the 57 positive results that were associated with double signals – 2 samples – T. rubrum and T. interdigitale, and 1 – T. rubrum and C. albicans. The most frequent dermatophyte was T. rubrum with 37 cases (64.9%), followed by T. interdigitale with 20 cases (35.1%). The PCR was positive in spite of the presence of molds (19 cases) or yeasts other than C. albicans (8 cases), which could have been a reason for PCR inhibition. PCR was positive in 13 out of 28 (46.4%) samples with negative culture for a dermatophyte or C. albicans but with positive
histologic result, showing its ability to identify non-growing fungal agents. In total, RT-PCR was positive in 57 out of 78 cases of onychomycosis (73.1%). In addition, the test could detect the presence of non-growing dermatophytes in 11 cases (19.2%) and 2 cases of C. albicans where histologic result was negative. Real-time PCR had a sensitivity of 73.1%, specificity of 100%, and efficacy of 79.4% (p<0.001, McNemar test) (Table 4).

<table>
<thead>
<tr>
<th>Type of examination</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Efficacy (%)</th>
<th>McNemar test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct KOH microscopy</td>
<td>N=37 (47.4%)**</td>
<td>N=23 (95.8%)</td>
<td>N=60 (58.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Culture</td>
<td>N=57 (73.1%)**</td>
<td>N=24 (100.0%)</td>
<td>N=81 (79.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>N=57 (73.1%)**</td>
<td>N=24 (100.0%)</td>
<td>N=81 (79.4%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* True positive cases of OM (78), true negative cases of OM (24) – according to the set standard; ** Positive cases of direct microscopy and culture after the standard for a true OM is considered.

Eventually, according to the set definition of a true case of OM, the number of the positive cases in our study was 78 and the number of the negative ones was 24.

**RT-PCR discrepancies with culture results**

In two cases, real-time PCR missed the detection of the dermatophyte M. ferrugineum because it was not included in the original kit. In four cases, PCR missed the detection of dermatophytes included in the kit that were detected by culture. In three cases, culture was positive for T. rubrum, whereas PCR showed T. interdigitale. Discordance with culture results regarding T. mentagrophytes (on culture) and T. interdigitale (on real-time PCR) was observed in eight cases, but these results could be positively confirmed in favor of PCR by ITS sequencing.

**Combination of tests**

The combination of histologic examination and real-time PCR had a better sensitivity, specificity, and efficacy than both tests separately with 77.6%, 100%, and 83.1%, respectively. (p<0.001, McNemar test).

**Onychomycosis – diabetic and non-diabetic group**

As far as the frequency of onychomycosis among the diabetic and the non-diabetic patients is concerned, and the etiology of the pathogens, we have found that there was no significant statistical difference between the two groups from RT-PCR (p>0.05, Fisher’s exact test). The real-time PCR showed negative results in 21 patients of the diabetic group (46.7%) and 24 patients from the non-diabetic group (53.3%) from a total of 45 negative results from both groups.

According to the culture investigation, our data show a slight difference in the etiology but still there was no significant statistical difference in the results between the two groups (p>0.05, Fisher’s exact test). Out of a total of 20 negative results, 13 were in the diabetic group (65.0%) and 7 in the non-diabetic group (35.0%). Out of 3 C. albicans infections, one was in the diabetic group and the other 2 in the non-diabetic group. Whereas, the total number of C. non-albicans pathogens was 21, 11 for the diabetic group (52.4%) and 10 for the non-diabetic group (47.6%). For the dermatophytes – T. mentagrophytes was isolated in a total of 8 cases, 2 of them in the diabetic group and 6 in the non-diabetic group, 25% and 75%, respectively. There was only one T. interdigitale infection isolated in the non-diabetic group. All cases of T. mentagrophytes grown with culture were later confirmed by RT-PCR to be in fact T. interdigitale, hence the difference in the results. Due to their macroscopic and microscopic resemblance, and the fact that T. interdigitale was confirmed to be a variation of T. mentagrophytes[11], it is always a challenge to identify the two with cultural examination. T. rubrum was again the most frequent dermatophyte isolated with a total of 17 cases, with 7 (41.2%) and 10 (58.8%) for the diabetic and non-diabetic groups, respectively. A total of 5 mixed infections were detected – 2 consisting of T. mentagrophytes and Candida, 1 of T. rubrum and Candida in the diabetic group, and 2 cases of T. rubrum and Candida in the non-diabetic group.

**DISCUSSION**

Onychomycosis is a frequent nail infection caused by filamentous fungi. The fungal infections diagnosis based on traditional methods has its advantages but there are also
disadvantages that require a more accurate and fast method to facilitate the diagnostic process. In this study, we evaluated the use of four methods for fungal detection in 102 samples of fingernails (4 samples) and toenails (98 samples). As a positive result for onychomycosis, we have taken into account the combination of clinical symptoms of a nail disorder with positive histology, and/or a positive culture for a true pathogen (dermatophyte or \textit{Candida spp.}), and/or positive RT-PCT result.

Other Candida species have been included in the positive results for culture because multiple studies have reported that they could also cause onychomycosis (\textit{C. parapsilosis, C. krusei, C. glabrata, C. guilliermondii, C. zeylanides, and C. tropicalis}), especially in patients with immunosuppression and concomitant diseases such as diabetes mellitus, which is the other aspect of our study.\textsuperscript{[12-15]} NDM were not included as positive results for culture, since it is difficult to determine whether they are concomitants or the real pathogen causing onychomycosis. To determine that, the culture should be repeated several times with the same non-dermatophyte mold as a result.\textsuperscript{[16]} There are, though, some species of NMD, such as \textit{Scopulariopsis brevicaulis} and \textit{Fusarium spp.}, which can be pathogenic in some cases.\textsuperscript{[17]}

The combination of direct microscopy and culture, despite the long turnaround time (TAT) of the culture, is still the most used one in the clinical practice to diagnose onychomycosis.\textsuperscript{[18]} Direct microscopy is indeed the easiest, cheapest, and fastest method for the detection of OM showing hyphae, spores, and arthrospores that a skilled technician can spot in a matter of minutes. The downsides of this method are that it does not determine the etiology of the pathogens and has a low sensitivity and specificity, plus false negative or false positive results are possible. Our study evaluated that the sensitivity of this method was 47.4%, and specificity was 95.8%, which correlates with the results for the same method that Gupta et al. observed in their study – 48%.\textsuperscript{[18]}

Table 5. Distribution of etiological agents, according to real-time PCR

<table>
<thead>
<tr>
<th>Etiological agent, according to real-time PCR</th>
<th>Diabetes Mellitus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Negative</td>
<td>N</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>46.7%</td>
</tr>
<tr>
<td>\textit{Candida albicans}</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>50.0%</td>
</tr>
<tr>
<td>\textit{T. interdigitale}</td>
<td>N</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>50.0%</td>
</tr>
<tr>
<td>\textit{T. interdigitale + Tr. rubrum}</td>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>50.0%</td>
</tr>
<tr>
<td>\textit{T. rubrum}</td>
<td>N</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>55.9%</td>
</tr>
<tr>
<td>\textit{T. rubrum + Candida}</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>50.0%</td>
</tr>
</tbody>
</table>

Although the PAS stain does not allow differentiation of dermatophyte from non-dermatophyte fungi, histologic examination allows the visualization of spores and hyphae, and can also determine the degree of invasiveness of the nail plate. It can also be used to differentiate fungal infections from psoriasis, lichen planus, or yellow nail syndrome.\textsuperscript{[19]} This method also requires well-trained medical specialists. The specificity of PAS stain, however, is low because the morphology of hyphae and spores detected inside the tissue does not offer any indication as to the fungal genus or species.\textsuperscript{[20]} The sensitivity of the PAS stain obtained in our study is 55.2%, which correlates with the one from a study carried out by Alkhayat et al. (60.9%).\textsuperscript{[21]} For some studies, histologic investigation is a method that is often used and thus, a greater experience is gathered in its performance. The highest sensitivity of this method had been evaluated by a recent meta-analysis that reported a sensitivity of 98% obtained by Shenoy et al.\textsuperscript{[20,22]}

Culture examination, still considered the gold standard, has its limitations that include long TAT and the microorganisms being alive. For studies that compare different diagnostic methods, the samples are divided into portions for each investigation. In our study, we divided the materials into four portions, which can additionally hinder the diagnostic process since unequal distribution of fungal elements in clinical specimens is quite likely. Thus, in some cases, false-negative results could happen. Moreover, the isolation of various NDM such as \textit{Fusarium spp., Asper-
CONCLUSIONS

In conclusion, the progress of mycological investigations and the statistical data obtained from the molecular techniques encourage considering new algorithms in the diagnosis of fungal infections, including real-time PCR. While it has still not been converted into the gold standard for fungal detection, it has demonstrated its advantages. It is also important to emphasize the fact that the combination of histologic examination and real-time PCR had a better sensitivity, specificity, and efficacy than both tests separately. Nevertheless, real-time PCR is a distinguished method to embrace, especially in cases with negative culture or NDM growth when a distinct clinical picture is observed.

Acknowledgments

This study was possible with the funding of Medical University of Plovdiv, Plovdiv, Bulgaria.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

В поисках дерматофитов – частота и этиология грибковых инфекций у больных с сахарным диабетом и без него

Гергана З. Важева1, Лилия Г. Зисова1, Елена А. Бечева2, Веселин Р. Чонов3, Валентин Д. Дичев4, Цонка М. Митеева-Кантранджиева5, Зоя В. Рачковска6, Мария М. Орбецова7,8, Веселин Т. Беловеждов9

1 Кафедра дерматологии и венерологии, Медицинский университет – Пловдив, Пловдив, Болгария
2 Кафедра эндокринологии и болезней обмена веществ, Медицинский университет – Пловдив, Пловдив, Болгария
3 Кафедра общей и клинической патологии, Медицинский университет – Пловдив, Пловдив, Болгария
4 Кафедра медицинской биологии, Медицинский университет – Пловдив, Пловдив, Болгария
5 Кафедра медицинской биомедицины и биостатистики, Медицинский университет – Пловдив, Пловдив, Болгария
6 Кафедра микробиологии и иммунологии, Медицинский университет – Пловдив, Пловдив, Болгария
7 Клиника эндокринологии и болезней обмена веществ, УМБАЛ “Св. Георги”, Пловдив, Болгария
8 Кафедра эндокринологии, Медицинский университет – Пловдив, Пловдив, Болгария
9 Кафедра общей и клинической патологии, Медицинский университет – Пловдив, Пловдив, Болгария

Адрес для корреспонденции: Гергана З. Важева, Кафедра дерматологии и венерологии, Медицинский университет – Пловдив, бул. „Васил Априлов” № 15А, Пловдив, Болгария; Email: g.vazheva@gmail.com; Тел.: +359 886 962 602

Дата получения: 21 апреля 2022 ♦ Дата приемки: 12 июля 2022 ♦ Дата публикации: 31 декабря 2022


Резюме

Введение: Онихомикоз является частым заболеванием ногтей, на которое приходится до 50% всех проблем с ногтями. Лечение онихомикоза дорого и требует длительного приема противогрибковых препаратов. Следовательно, необходима правильная и более быстрая диагностика. Особенно для тех больных сахарным диабетом, у которых онихомикоз является одним из наиболее значимых предикторов язвы стопы и возможных тяжелых осложнений.

Цель: Сравнить чувствительность, специфичность и время выполнения прямой микроскопии, посева, гистологии и ПЦР в реальном времени. Кроме того, сравнить частоту и этиологию онихомикоза у больных с СД и без него.

Материалы и методы: В исследование включено 102 пациента, разделённых на две группы. Одну группу составили пациенты с сахарным диабетом, другую – без диабета. Образцы ногтей собирали и исследовали с помощью прямого микроскопического исследования KOH, посева, гистологии и ПЦР в реальном времени.

Результаты: Из 102 пациентов с клиническим онихомикозом положительный КОН был обнаружен у 38 (37.3%). Культура – 82 из 102 образцов (80.4%) были положительными на дерматофиты, дрожжи и/или НДП (недерматофитные плесени). Положительные гистологические образцы были 32 (41.6%). RT-PCR была положительной у 57 (55.9%) из 102. Мы обнаружили, что нет существенной статистической разницы в этиологии грибковых инфекций между двумя группами.

Заключение: Все микологические исследования имеют своё место в диагностике онихомикоза. Прямая микроскопия, посев и гистология являются полезными методами для клиницистов для диагностики и наблюдения за периодом после лечения. К преимуществам RT-PCR можно отнести более быстрое получение результатов и точную идентификацию грибов, что делает её более ценной при диагностике ОМ.

Ключевые слова
derматофития, гистология, онихомикоз, ПЦР в реальном времени, Trichophyton rubrum