Susceptibility to antifungal drugs of *Candida albicans* isolated from upper respiratory tract of patients with chronic hepatitis C

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INTRODUCTION

Hepatitis C infection is an infection of the liver caused by the hepatitis C virus (HCV). HCV can cause acute or chronic hepatitis and is a health problem worldwide. It is one of the leading causes of cirrhosis and hepatocellular carcinoma and is a common indication for liver transplantation [1, 2, 3, 4, 5, 6]. Therapy is based on markers that predict sustained virologic response, and the goal of therapy is to slow or to halt progression of fibrosis and prevent the development of cirrhosis. In future, multidrug regimens in combination with current therapies may be developed. A combination of pegylated interferon (peginterferon) alpha weekly and ribavirin daily represents the standard for the treatment of pegylated interferon (peginterferon) alpha weekly and ribavirin. None of the isolates resistant to ketoconazole (6.67%) or itraconazole (10%) were found in group I, while in group II amounting 9.68 – 48.39%, depending on the antifungal drugs. The isolates resistant to ketoconazole (6.67%) or itraconazole (10%) were found in group I, while resistant to miconazole (9.68%), ketoconazole (19.35%), itraconazole (22.58%) or fluconazole (3.22%) in group II.

MATERIALS AND METHOD

Swabs from the oral cavity and mucous membrane of throat and nose were obtained from 100 patients aged 30 – 65 years with chronic hepatitis C, belonging to 2 groups: group I – 46 patients without antiviral therapy and group II – 54 patients treated with peginterferon and ribavirin. None of...
the patients had lesions in the oral cavity. Samples were taken with a sterile cotton swabs and immediately streaked onto Sabouraud agar with chloramphenicol. The 30 isolates of \textit{C. albicans} from group I and 31 from group II were identified by standard methods – biochemical microtest API 20 C AUX (bioMérieux) on the basis of assimilation of various substrates.

The drug sensitivity was estimated by the Fungitest method (Sanofi Diagnostics Pasteur). This is a microplate-based procedure for the breakpoint testing of 6 antifungal drugs (amphotericin B, fluconysine, fluconazole, itraconazole, ketoconazole and miconazole). Each 16-well microplate contains 2 negative control wells, 2 positive growth control wells, and 12 drug-containing wells. Each antifungal agent was tested at 2 concentrations, selected to distinguish resistant isolates from susceptible ones. The drug concentrations were as follows: amphotericin B – 2 and 8 μg/ml; fluconysine – 2 and 32 μg/ml; fluconazole – 8 and 64 μg/ml; itraconazole and ketoconazole – 0.5 and 4 μg/ml; and miconazole – 0.5 and 8 μg/ml. Cell suspensions were prepared in sterile distilled water and were adjusted to a turbidity corresponding to a 1.0 McFarland standard; 100 μl of this suspension was added to 1.9 ml of sterile distilled water, and this was further diluted by adding 20 μl to 3 ml of pre-prepared RPMI 1640 suspension medium. This gave a final inoculum concentration of 10^6 CFU (Colony Forming Units)/ml. The microplates were inoculated by placing 100 μl of the appropriate cell suspension into each well. The plates were incubated at 37 °C for 48 h before reading. Two reference strains, \textit{C. albicans} ATCC 2091, \textit{C. albicans} ATCC 10231 and \textit{C. albicans} ATCC 22019, were included in each batch of broth microdilution tests to ensure quality control.

RESULTS

The prevalence of \textit{Candida} spp. in throat or/and oral cavity was found in 20 (43.48%) and 24 (44.44%) patients with HCV from group I and II, respectively. The predominant species was \textit{Candida albicans} identified in 20 (43.48%) patients of group I and 22 (40.74%) patients of group II.

On analysing the sensitivity of \textit{C. albicans} isolates from the group I of patients (Tab. 1), it was found that the isolates were sensitive in 100% to fluconysine and polyeve antibiotic – amphotericin B, but had decreased sensitivity, so-called sensitivity dose-dependent, to azole derivates – miconazole (16.67%), ketoconazole (10%), itraconazole (56.67%) or fluconazole (3.33%). The isolates resistant to azole derivates were found to be resistant to ketoconazole (6.67%) or itraconazole (10%). Moreover, 1 (3.33%) isolate possessed decreased sensitivity or resistance to all tested azoles.

Analysis of the sensitivity of \textit{C. albicans} isolates from group II of patients (Tab. 2), 100% sensitivity to fluconysine and polyeve antibiotic – amphotericin B was also found. A decreased sensitivity to miconazole, ketoconazole, itraconazole and fluconazole was observed, amounting 19.35%, 19.35%, 48.39%, 9.68% of the isolates, respectively. In turn, the isolates were observed to be resistant to miconazole (9.68%), ketoconazole (19.35%), itraconazole (22.58%) or fluconazole (3.22%). In addition, 3 (9.68%) isolates showed decreased sensitivity or resistance to all tested azoles.

### Table 1. Sensitivity to antifungal drugs of \textit{Candida albicans} isolated from the upper respiratory tract of patients with chronic hepatitis C from group I without antiviral therapy

<table>
<thead>
<tr>
<th>Antifungal drug</th>
<th>No. (percentage) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>Fluconysine</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Miconazole</td>
<td>2 (6.67)</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>7 (22.58)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

### Table 2. Sensitivity to antifungal drugs of \textit{Candida albicans} isolated from the upper respiratory tract of patients with chronic hepatitis C from group II with antiviral therapy

<table>
<thead>
<tr>
<th>Antifungal drug</th>
<th>No. (percentage) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>Fluconysine</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Miconazole</td>
<td>3 (9.68)</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>6 (19.35)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>7 (22.58)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1 (3.22)</td>
</tr>
</tbody>
</table>

DISCUSSION

Fungal infections are an important medical problem in patients from risk groups, among them in patients with chronic hepatitis C. Increased frequency of fungal infections in recent years is associated with inappropriate use of antifungal drugs. This is a major cause for the emergence of resistant or multi-drug resistant strains, which may lead to many therapeutic failures [1, 8, 12].

The prevalence of \textit{Candida} spp. in the upper respiratory tract of patients with chronic hepatitis C from both groups – without or with the standard antiviral therapy (peginterferon and ribavirin), ranging from 43.48 – 44.44%, was similar to that found in healthy people [7, 13, 14]. Similarly, other authors [15] showed that cultures of \textit{Candida} sp. from the tongue surfaces were positive in 50% patients with HCV infection at least once during therapy with interferon. The incidence of \textit{Candida} spp. in these patients during interferon treatment did not increase, compared to that before treatment.

Data presented in this study showed that all of the \textit{C. albicans} strains colonizing upper respiratory tract of patients with chronic hepatitis C from both groups were sensitive in 100% to amphotericin B and fluorcytosine, which is in agreement with data obtained by other authors [7, 12, 15, 17]. The high sensitivities to both the antifungal drugs were observed, by Oberoi J. K. et al. [5], amounting 89.6% and 90.9% of the studied isolates, respectively, and by Krajewska-Kulak E. et al. [16] amounting 84.2 – 91.5% and 67.7 – 93.6-% of the isolates studied, respectively.

Recently, the decreased susceptibility of \textit{Candida} spp., including \textit{C. albicans} to azole derivatives, has been increasing [5, 12, 17, 19]. The data in the presented study are in agreement...
with this tendency, showing the decreased susceptibility to azole derivatives, in the case of itraconazole reaching even 56.67% and 48.39% of C. albicans isolates. Similar results have been obtained by other authors for C. albicans isolated from the oral cavity in patients with HIV/AIDS, from the upper respiratory tract of patients with lung cancer, from blood specimens or from neoplasmatic patients [12, 15, 17, 18]. According to other literature data [5, 19], the decreased sensitivity to fluconazole was found with a frequency amount of 6 – 31.2%, while to itraconazole with a frequency of 14 – 45.7%.

The results from group I in the presented study confirm that isolates were resistant to ketoconazole and itraconazole in 6.67% and 10%, respectively. In turn, in group II there appeared resistance to fluconazole (3.22%), miconazole (9.68%), and significantly often, resistance to ketoconazole (19.35%) and itraconazole (22.58%). Data presented in this paper and those from literature indicate that the successful treatment of candidiasis by azoles has been also been impaired by the emergence of drug-resistant strains. Mulu A. et al. [18] also found that Candida spp. isolated from oropharyngeal candidiasis from patients with HIV/AIDS were resistant to fluconazole (12.2%), ketoconazole (7.7%) and itraconazole (4.7%). The results of studies by Zomorodian K. et al. [17] demonstrated that among the clinical isolates of C. albicans the resistance to ketoconazole was found to be 3.2%. Jin-Sol L. et al. [19] assessed that resistance to fluconazole and itraconazole was found in 2% and 4% of the bloodstream Candida isolates, respectively. In turn, Pfaffer at al. [6] showed that among the clinical isolates of Candida spp., 3.96% were resistant to fluconazole and up to 71% of those were resistant to itraconazole.

Data presented in this study and those from the literature strongly indicate that the increasing rate of resistance to azoles among Candida spp., including C. albicans isolates, has become a major problem, especially among immunocompromised patients, in which these drugs, mainly fluconazole, are usually used in prophylaxis of fungal infections [4, 7, 9, 12, 19].

CONCLUSIONS

Actual knowledge about the drug sensitivity of Candida spp. including C. albicans isolated from the upper respiratory tract, allow its proposal as successful prophylaxis and treatment of potential respiratory tract infections caused by pathogenic yeasts in a given population, including patients with HCV.

REFERENCES


