BIOCHEMICAL PHENOTYPES OF CANDIDA ALBICANS
ISOLATED FROM THE UPPER RESPIRATORY TRACT
OF PATIENTS WITH CHRONIC HEPATITIS C

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Abstract

The aim of this paper was to analyse the biochemical phenotypes of Candida albicans colonising the upper respiratory tract in 100 patients with chronic hepatitis C from group I (without antiviral therapy) and from group II (treated with peginterferon and ribavirin). The ability of the assimilation of carbon from various substrates (assimilation phenotypes) or activity of hydrolytic enzymes (biotypes) of 61 C. albicans isolates were estimated using API 20 C AUX and API ZYM microtests, respectively. Among 30 isolates of C. albicans from the group I, seven assimilation phenotypes and six biotypes were determined, while among 31 isolates from the group II – eleven assimilation phenotypes and five biotypes. The most frequently isolated assimilation phenotype in both groups of patients with API numerical profile of 2576174 comprised about 50%-70% of all phenotypes. The predominant biotype E belonging to the classification of Williamson comprised about 39%-50% of all biotypes. Our results and those from the literature suggest that C. albicans biotypes but not assimilation phenotypes may be related with some diseases. However, this requires further detailed studies.

Key words: human, chronic hepatitis C, Candida albicans, upper respiratory tract, biochemical phenotypes.

The outcome of hepatitis C virus (HCV) infection is determined by the interaction between the virus and the host immune system (18). The dominant cause of viral persistence during chronic HCV infection may be the development of a weak antiviral immune response to the viral antigens, with corresponding inability to eradicate infected cells. Some patients with the advanced cirrhosis of the liver have immunity alterations leading to immunosuppression (8). Over 130 million people worldwide suffer from chronic hepatitis C, defined by the presence of HCV RNA for more than six months (5).

Yeasts belonging to Candida sp., including Candida albicans, are a part of the normal human microflora of mucocutaneous areas, mainly within upper airways, gastrointestinal tract, and vagina. C. albicans is still the major aetiological agent of both nosocomial and community-acquired candidiasis. Such infections are usually endogenous in origin (16, 19, 21). According to the literature (1, 3, 9, 10, 13, 23), several factors e.g. state of health, hospitalisation, occurrence in different part of human body, drug treatment, or geographical regions may be related with colonisation of human body by different biochemical phenotypes of C. albicans.

The aim of this paper was to analyse the biochemical phenotypes of C. albicans colonising upper respiratory tract in patients with chronic hepatitis C, with or without standard antiviral therapy, including peginterferon and ribavirin. Biochemical diversity of C. albicans isolates was assessed on the basis of assimilation phenotypes representing the ability of carbon assimilation from various substrates or biotypes differing in the activity of hydrolytic enzymes.

Material and Methods

Swabs from the oral cavity and mucous membrane of the throat and nose were obtained from 100 patients aged 30–65 years with chronic hepatitis C. The patients were divided into two groups: group I – 46 patients without antiviral therapy and group II – 54 patients treated with peginterferon and ribavirin. None of the patients had cirrhosis of the liver.

The samples were taken up with a sterile cotton swabs and immediately streaked onto Sabouraud agar with chloramphenicol. The isolates were identified by biochemical microtest API 20 C AUX (bioMérieux) on the basis of assimilation of D-glucose, glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylene, adonitol, xylitol, D-galactose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, N-acetyl-glucosamine, D-cellobiose,
D-lactose, D-maltose, D-saccharose, D-trehalose, D-melezitose, and D-rafinose. The obtained results were also used for the evaluation of the diversity of assimilation phenotypes between isolates identified as C. albicans on the basis of API numerical profiles. The ability of the isolated strains to produce hyphae, pseudohyphae, or chlamydomspores was also assessed.

The activity of 19 hydrolytic enzymes: alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase was estimated by using a API ZYM microtest (bioMérieux). The activity of some enzymes, e.g. esterase (C 4), valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and N-acetyl-β-glucosaminidase is the base of biotyping of Candida sp. (25). The biotyping was performed according to the classification of Williamson (25) – biotypes A-H, Kurnatowska and Kurnatowski (14) – biotypes I-N, Krajewska-Kula et al. (12) – biotypes O-R, Batura-Gabryel et al. (1) – biotype S, Brajer et al. (3) – biotypes T-W, and Mnichowska et al. (17) – biotype Z.

Results

As shown in Fig. 1, in 20 (43.48%) and 24 (44.44%) patients with chronic hepatitis C from groups I and II, respectively, the occurrence of Candida sp. was found in the throat or/and oral cavity. No Candida sp. was isolated from nasal specimens in the both groups. The predominant species of Candida albicans was isolated from 20 (43.48%) patients of the group I and 22 (40.74%) patients of the group II. Other Candida species were found in four (8.70%) patients of the group I (C. glabrata, C. tropicalis, C. lusitaniae, and C. parapsilosis) and in four (7.41%) patients of the group II (C. glabrata, C. tropicalis).

Sixty-one isolates of C. albicans were classified on the basis of assimilation phenotypes and biotypes. As shown in Table 1, among 30 isolates of C. albicans from the group I, seven assimilation phenotypes were determined, while among 31 isolates from the group II – eleven assimilation phenotypes. The most frequently isolated assimilation phenotype with numerical profile of 2576174 comprised 21 (70%) and 16 (51.61%) isolates, in groups I and II, respectively. The occurrence of specific phenotypes for the group I or II was also observed.

According to Fig. 2, 30 isolates of C. albicans isolated from the group I belonged to six biotypes: C, D, E, F, and H classified by Williamson (93.33%) and K – by Krajewska-Kula et al. (6.67%), while 31 isolates from group II to – 5 biotypes: C, E, F, H classified by Williamson (87.1%) and K – by Krajewska-Kula et al. (12.9%). The most frequently isolated was biotype E, comprising 15 (50%) and 12 (38.71%) isolates in groups I and II, respectively.

Fig. 1. The prevalence of Candida sp. in the upper respiratory tract of patients with chronic hepatitis C from group I (without antiviral therapy) and from group II (treated with peginterferon and ribavirin).
Fig. 2. The prevalence of biotypes of *C. albicans* in the upper respiratory tract of patients with chronic hepatitis C from group I (without antiviral therapy) and from group II (treated with peginterferon and ribavirin).

**Table 1**

<table>
<thead>
<tr>
<th>Assimilation phenotype (API numeral profile)</th>
<th>Group I (n = 30)</th>
<th>Group II (n = 31)</th>
<th>All (n = 61)</th>
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<tr>
<td>2576174</td>
<td>21 (70.0%)</td>
<td>16 (51.61%)</td>
<td>37 (60.66%)</td>
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<tr>
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<td>1 (3.33%)</td>
<td>3 (9.68%)</td>
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<td>1 (3.22%)</td>
<td>2 (3.28%)</td>
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<td>6172134</td>
<td>3 (10%)</td>
<td>1 (3.22%)</td>
<td>4 (6.56%)</td>
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<td>1 (1.64%)</td>
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<td>0 (0%)</td>
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</tbody>
</table>

**Discussion**

The local and systemic candidiases are preceded by the colonisation of human body by *Candida* sp. (20, 24). The prevalence of *Candida* sp. in the upper respiratory tract of patients with chronic hepatitis C from both groups – without or with the standard antiviral therapy (peginterferon and ribavirin), was similar to that found in healthy people (5, 20, 24). However, it should be noted that candidiases were diagnosed statistically more frequently in patients with alteration in the immunity than in healthy population (2, 3, 20, 23). It was also found that the standard antiviral therapy used in the treatment of chronic hepatitis C had no effect on the occurrence of *Candida* sp. The predominant yeast species isolated from both studied groups of the patients was *C. albicans*, similarly as in healthy people (5, 17, 19, 20, 24).

It is well-known that *C. albicans* strains differ in the biochemical properties, e.g. assimilation of carbon from various substrates (assimilation phenotypes) or activity of hydrolytic enzymes (biotypes). This is the basis of their phenotypic differentiation. A detailed analysis of assimilation phenotypes of *C. albicans* isolates from the upper respiratory tract of patients with chronic hepatitis C revealed that despite the observed significant diversity, one assimilation phenotype with API numerical profile of 2576174 predominated in both group of patients (about 60% in the whole population studied). Similar assimilation phenotype was found the most frequently by other authors in both healthy people and those suffering from candidiases (2, 6, 7, 11, 15, 22). It is most probable that it may be the predominant assimilation phenotype of *C. albicans* colonising human body, irrespective of other factors.

It was found on the basis of detailed analysis of *C. albicans* biotypes from the upper respiratory tract of
patients with chronic hepatitis C that biotype E belonging to the classification of Williamson was the most frequently isolated (25). According to our unpublished data in healthy people from the same geographical region – the Lublin region, biotype P, belonging to classification of Krajewska-Kułak et al. (12) and biotype K – to classification of Kurnatowska and Kurnatowski (14) were the most frequently isolated. Data of Brajer et al. (4), showed that biotype E also predominated in patients with lung cancer (36.36%), while in healthy people from the same geographical region – Wielkopolska, biotype F was the most frequently isolated (69.23%), classified by Williamson (3).

Our results and those from the literature suggest that C. albicans biotypes colonising human body may be related with some diseases, e.g. chronic hepatitis C, irrespective of geographical region, but this requires further detailed studies. The presented results also suggest that the immunomodulation therapy used in the standard treatment of chronic hepatitis C with peginterferon and ribavirin had no effect on the occurrence of C. albicans biotypes.

References