**Original Article**

**Distribution of Candida albican genotype and Candida species is associated with the severity of vulvovaginal candidiasis**

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**Abstract:** Objective To investigate the distribution of pathogenic *C.albicans* genotype and *Candida* species in association with the severity of vulvovaginal candidiasis (VVC). Methods Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) of the internal transcribed spacer analysis was employed to identify the *Candida* species isolated from the vaginal secretions of 198 patients with acute VVC. SSCP and GeneScan analyses of microsatellite locus I polymorphism were employed to determine the genotypes of the clinical isolates of *C. albican* associated with VVC. All the patients were scored for clinical signs and symptoms to evaluate the severity of VVC. Results A total of 198 *Candida* strains were isolated from VVC patients, including 140 (70.7%) *C. albicans* strains and 58 (29.3%) non-*albicans* strains. In the 95 patients with severe VVC and 103 with mild-moderate VVC, *C. albicans* was detected in 62.1% and 76.6% of the patients, respectively ($P=0.011$). Thirty-eight microsatellite locus I genotypes were detected in 140 unrelated *C. albicans* strains, among which the dominant genotypes 30-45 (44 strains, 31.43%) and 32-46 (23 strains, 16.43%) were the most common, followed by genotypes 30-46 (4 strains, 2.86%) and 32-47 (9 strains, 6.42%). The overall frequencies of the 4 genotypes were significantly higher in severe VVC than in mild-moderate VVC cases (77.9% vs 42.0%, $P<0.001$). Conclusion *C. albicans* remains the most common pathogenic *Candida* species in patients with VVC, but the non-*albicans* species seem more likely to cause severe VVC. The dominant genotypes of *C. albicans* with a tropism for the vagina are correlated to the severity of VVC.

*Key words:* vulvovaginal candidiasis; *Candida albicans*; microsatellite locus I genotype; single-strand conformation polymorphism; GeneScan

**INTRODUCTION**

As one of the most common vaginitis caused by *Candida* species, vulvovaginal candidiasis (VVC) affects 70%-75% of child-bearing women at least once during their life time [3]. On the basis of the severity of symptoms, frequency, causative agents and host factors, VVC is usually classified into uncomplicated and complicated VVC, and the latter included recurrent VVC, severe VVC, non-*albicans* VVC, and VVC with immunodeficiency disorders. Approximately 10%-20% of women have complicated VVC and 5%-8% of the adult women experience recurrent VVC [2]. Severe VVC was defined by the presence of severe symptoms, damages of the vulva skin or vaginal mucosa, and a VVC symptom score over 7. Although *Candida albicans* remains the most common causative agent for vaginitis in approximately 85%-95% of the cases [3, 4], the spectrum of the pathogenic strains isolated from the vagina of VVC patients tends to undergo alterations [3, 4]. Currently the relationship between the severity of VVC and the pathogenic strains of *Candida* species is poorly understood. In this study, we explored the association of the pathogenic *Candida* species and the genotype distribution of *C. albicans* with the severity of VVC using polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and GeneScan to analyze microsatellite locus I polymorphism of *C. albicans* strains, and attempt to identify the spectrum of the pathogenic *Candida* species responsible for VVC.

**MATERIALS AND METHODS**

**Patients**

This study was conducted among 198 child-bearing women with VVC receiving treatment in the Department of Gynecology, Zhujiang Hospital, Southern Medical University during the period from September 2009 to...
October 2010. The patients aged between 18 and 50 years (average 32.5 years) and were matched for working environment, living habits, diet and previous medication. Informed consent was obtained from the patient before entry. The diagnosis of VVC was established by the presence of vulva itching, vaginal discharge, microscopic detection of blastoconidia or pseudohyphae in a wet vaginal smear treated with 10% potassium hydroxide, and a positive result of Candida culture. Patients with bacterial vaginosis, trichomoniasis vaginosis or with only a positive Candida potassium hydroxide, and a positive result of Candida pseudohyphae in a wet vaginal smear treated with 10% medication. Informed consent was obtained from the patient before entry. The diagnosis of VVC was established by the presence of vulva itching, vaginal discharge, microscopic detection of blastoconidia or pseudohyphae in a wet vaginal smear treated with 10% potassium hydroxide, and a positive result of Candida culture. Patients with bacterial vaginosis, trichomoniasis vaginosis or with only a positive Candida culture without typical signs or symptoms were excluded. Pregnant women or patients with diabetes, immune deficiency or long-term use of antibiotics were not included in this study.

The severity of VVC was scored by evaluating the symptoms and vulvo-vaginal signs (e.g., itching, burning, erythema, discharge and fissure). Each sign or symptom was scored individually according to a four-score rating scale (Tab. 1) and their total score was used for rating the severity of VVC. Mild to moderate VVC was defined by a total score below 7, and severe VVC by a higher score. The rating was carried out in line with the diagnostic criteria of VVC postulated by the Obstetrics and Gynecology Branch of the Chinese Medical Association Infectious Diseases Collaborative Group.

### Tab. 1 Scoring criteria of VVC

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>0 point</th>
<th>1 point</th>
<th>2 point</th>
<th>3 point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inching</td>
<td>Absent</td>
<td>Occasional</td>
<td>Obvious</td>
<td>Persistence</td>
</tr>
<tr>
<td>Pain</td>
<td>Absent</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Congestion or edema</td>
<td>Absent</td>
<td>&lt;1/3 vaginal wall</td>
<td>1/3-2/3 vaginal wall</td>
<td>&gt;2/3 vagina wall</td>
</tr>
<tr>
<td>Vaginal secretion</td>
<td>Normal</td>
<td>Mildly increased</td>
<td>No vaginal discharge</td>
<td>Large volume of vaginal discharge</td>
</tr>
</tbody>
</table>

**Culture and yeast identification**

Vaginal secretions were collected from the patients using sterile swabs and cultured in enriched YPD medium (containing 2% yeast extract, 2% glucose, 1% peptone, and 18% glycerin) until the medium became turbid, followed by inoculation of the medium on YPD agar plates containing 100 mg/ml chloramphenicol at 35°C for 24 to 48 h. One colony was picked from the plate, suspended in 5 ml medium and oscillated for 24 h at 28°C. Nuclear DNA extraction and PCR amplification of the microsatellite internal transcribed spacer (ITS) of Candida strains were performed following the protocols as described [5]. The sequences of the amplified fragments were compared against those in GenBank, and the *C. albicans* strains were identified while non-albicans *Candida* discarded.

**SSCP and GeneScan analyses**

PCR amplification of the microsatellite CAI was performed following the protocols described previously [5]. The polymorphisms of the microsatellite locus I of *C. albicans* strains were detected by single-strand conformation polymorphism (SSCP) and GeneScan analyses. The fragment sizes were determined automatically by GeneScan analysis software (version 3.7). The CAI genotypes of *C. albicans* were designated according to the number of trinucleotide repeat units in both alleles of the microsatellite locus based on the diploid nature of the strains [5].

**Statistical analysis**

All statistical analyses were conducted using SPSS 13.0 statistical software. Chi-square test was used to determine the differences between different groups, and *t* test was used to analyze the measurement data between *C. albicans* and non-albicans groups and between the dominant and minor genotypes of *C. albicans*. A *P* value less than 0.05 was considered to indicate a significant difference.

**RESULTS**

**Distribution of Candida species and VVC scores of the patients**

A total of 198 *Candida* strains were isolated from the vagina of the VVC patients, including 140 *C. albicans* strains (70.7%) and 58 non-albicans strains (29.3%); the non-albicans strains included *Candida glabrata* (52 cases, 26.2%), *Candida parapsilosis* (2 cases, 1.0%), *Candida tropicalis* (1 case, 0.5%), and other yeasts (1.6%). Among the 198 patients, 95 were found to have severe VVC and 103 had mild to moderate VVC.

**Candida species and severity of VVC**

Among the 95 patients with severe VVC, *C. albicans* were isolated from 59 cases (62.1%), *C. glabrata* from 31 cases (32.6%), *C. parapsilosis* from 1 case (1.0%), *C. tropicalis* from 1 case (1.0%), and other yeasts from 3 cases (3.1%). In the 103 mild-moderate VVC patients, *C. albicans* was isolated from 81 cases (78.6%), *C. glabrata* from 21 cases (20.4%), and *C. parapsilosis* from 1 case (1.0%). The VVC scores of...
patients with *C. albican* and non-albicans infections were 6.26 ± 2.147 and 5.64 ± 1.507, respectively, showing a significant difference between them (*P*=0.001, Tab.2).

**CAI genotype distribution of *C. albicans* and severity of VVC**

From the 140 isolated *C. albican* strains associated with VVC, 38 distinct CAI genotypes were identified using microsatellite locus I polymorphism analysis. The dominant genotypes 30-45 (44 strains, 31.43%) and 32-46 (23 strains, 16.43%) were the most common, followed by the genotypes 30-46 (4 strains, 2.86%) and 32-47 (9 strains, 6.42%). The overall frequencies of the 4 genotypes accounted for 57.1% (80 strains) of the total strains isolated. The remaining 34 less common genotypes were detected in 1 to 3 strains each.

Among the 59 strains isolated from severe VVC patients, 17 genotypes were identified, including 4 dominant genotypes 30-45 (49.2%), 32-46 (13.6%), 32-47 (10.2%) and 30-46 (5.1%), and 15 minor genotypes found in 1-2 strains each. In the 81 strains isolated from mild-moderate VVC patients, 31 genotypes were identified with the 4 dominant genotypes of 30-45 (18.5%), 32-46 (18.5%), 30-46 (1.2%) and 32-47 (3.7%) (Tab.3). The VVC scores of the patients infected by *C. albican* of the dominant genotypes and minor genotypes were 6.81±2.081 and 5.52±2.021, respectively (Tab.4).

**DISCUSSION**

PCR-SSCP and GeneScan analysis of the microsatellite CAI is a powerful approach for strain typing of *C. albican*. The polymorphisms of microsatellite locus of strains show a species-specific pattern with a low mutation rate and a discriminatory power of 0.97±0.6. SSCP technique for CAI microsatellite analysis allows rapid strain typing with a discriminatory power of 0.99, and the result can be identified by GeneScan analysis.

We found in this study that *C. albican* alone remains the most common pathogenic strain for VVC, accounting for 70.7% (140/198) of the total strains isolated. Of the non-albicans species identified, *C. glabrata* was the most common (26.2%). According to a report, the prevalence of *C. albican* ranged from 85% to 95% in VVC patients, a rate higher than our finding, possibly due to the differences in the samples, climate and geographic conditions. Our study suggests a correlation between infection by the *Candida* strains and the severity of VVC. Patients with severe VVC had a lower infection rate by *C. albican* than those with mild-moderated VVC (62.1% vs 76.6%, *P*=0.011).
Sobel [16] commented that vaginitis induced by non-
albicans species was clinically indistinguishable from
that caused by C albicans; moreover, non-albicans
species are often more resistant to treatment. The
relationship between non-albicans Candida species and
the severity of VVC had not been reported in China
before this present study.

The vaginopathic C. albicans strains with a tropism
for the vagina were first reported in 2008 based on
findings by genotype analysis [10-11]. The dominant CAI
genotypes of these strains isolated from the vagina of
Chinese patients were rarely found in the strains from
other sources (e.g. oral, skin, and rectum) [10-11], and this
genotype distribution was not reported in other countries.
Using SSCP analysis, we identified 4 dominant genotypes
of C. albican strains from the vagina of VVC patients,
including 3 previously described genotypes A (30-45),
B(32-46) and C (30-46) [11] and another dominant
 genotype 32-47, instead of the genotype D (30-47)
reported previously. This discrepancy in the dominant
genotypes may arise from the evolution of the original
genotypes 30-45 and 32-46 over time, as indicated by
the results of cluster analysis [10].

We also found that the genotype distribution of C.
albicans was related to the severity of VVC. The
dominant genotype was found more frequently in
strains isolated from patients with severe VVC than in
strains from patients with mild-moderate VVC (77.9% vs
42.0% , P<0.001). Patients with VVC caused by C.
albicans strains of the dominant genotypes had a mean
symptom score of 6.81 ± 2.08, significantly higher than
that in patients with infection by C.albican strains of
the minor genotypes (5.52 ± 2.021, P<0.001), suggesting a
relationship between the symptom scores of the patients
and the genotypes of the pathogenic Candida strains.

Our results suggest that the distribution of C.
albicans genotype and the Candida species is associated
with the severity of VVC. The virulence is enhanced by
proteolytic enzymes, phospholipase elaborated, toxins,
high-frequency heritable switching of the Candida
species, and these virulence factors all affect the gene
expression profiles of the strains [12-13]. The virulence factors of C.albicans strains of the dominant genotypes
may importantly contribute to the severity of VVC.
Studying the correlation between the virulence factors of
the strains of the dominant genotypes and the severity of
VVC may yield important insights into the pathogenic
characteristics of these strains.

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References

  1961-71.
  clinical diagnosis of lower genital tract infection in women[J]. Am J
  (2000-2004) epidemiological survey of Candida and non-Candida
  yeast species causing vulvovaginal candidiasis in Graz, Austria [J].
  incidence and treatment of non-Candida albicans infection. Curr
[7] Li J, Bai FY. Single-strand conformation polymorphism of
  microsatellite for rapid strain typing of Candida albicans [J]. Med
  microsatellite for identification of Candida albicans strains [J]. J Clin
  PCR for Candida albicans strain typing reveals microevolutionary
  albicans strains associated with vulvovaginal candidosis and
  candidal balanoposthitis in China [J]. Clin Infect Dis, 2008, 47(9):
  1119-25.
  albicans strains associated with different conditions of vulvovaginal
  candidiasis, as revealed by microsatellite typing [J]. Sex Transm
  Infect, 2008, 84(2): 103-6
  profile and proteinase production of yeasts causing vaginovaginitis
  Candida albicans agglutinin-like sequence gene expression patterns
  in human clinical specimens and models of vaginal candidiasis [J].
  aspartyl proteinases in virulence and pathogenesis [J]. Microbiol Mol
  B gene PLB5 in wild-type Candida albicans reduces cell-associated
  phospholipase A2 activity and attenuates virulence [J]. Int J Med
  Candida albicans secreted aspartyl proteinase gene family in human
  oral and vaginal candidiasis [J]. Microbiology. 2008, 154(Pt 11):
  3266-80.
  secreted aspartyl proteinase in human vulvovaginal candidiasis[J].
摘要：探讨致病假丝酵母菌菌群分布以及假丝酵母菌基因型与外阴阴道假丝酵母菌病症状严重程度的关系。方法：我们对2009年9月~2010年10月在我院就诊的，以及生活习惯、饮食、既往用药及工作环境相似的急性VVC患者，利用聚合酶链反应-单链构象多态性分析技术(PCR-SSCP)对其阴道来源的假丝酵母菌进行分子水平的菌种鉴定，结合SSCP和基因扫描(GeneScan)对白假丝酵母菌CAI区进行多态性分析确定其基因型，对VVC的严重程度进行临床症状体征的评分。结果：从获得的198份标本中分离白假丝酵母菌140株(70.7%);58株非白假丝酵母菌(29.3%)。198名患者中重度VVC95人，轻中度VVC103人。白假丝酵母菌在重度VVC和轻中度VVC患者中所占比列为62.1%和76.6%(P=0.011)。140株C.albican中共检出38种CAI基因型且集中分布于少数几种，其中基因型30-45(44株，31.43%)和32-46(23株，16.43%)最常见，其次为基因型30-46(4株，2.86%)和32-47(9株，6.42%)。4种优势基因型菌株在重度VVC和轻中度VVC患者中的分布差异有统计学意义(77.9% vs 42.0%，P<0.001)。结论：白假丝酵母菌仍是VVC的主要致病菌，但非白假丝酵母菌与白假丝酵母菌相比更容易引起重度VVC，白假丝酵母菌的基因型与VVC严重程度有关。

关键词：外阴阴道假丝酵母菌病;白假丝酵母菌;CAI基因型;单链构象多态性分析;基因扫描