Does alternation of *Candida albicans TUP1* gene expression affect the progress of symptomatic recurrent vulvovaginal candidiasis?

Mona Ghazanfari¹, ², Azam Fattahi⁴, Mehraban Falahati¹*, Majid Bakhshizadeh⁵, Maryam Roudbar⁴, Faramarz Masjedian Jazi⁶, Mohsen Keykhoosravi⁴, Ensieh Lotfali⁷

¹ Department of Medical Mycology, Faculty of Medical Sciences, Iran University of Medical Sciences, Tehran, Iran  
² Invasive Fungi Research Centre, Communicable Diseases Institute / Department of Medical Mycology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran  
³ Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran  
⁴ Center for Research and Training in Skin Diseases and Leprasy, Tehran University of Medical Sciences, Tehran, Iran  
⁵ Department of Community Medicine, Faculty of Medical Sciences, Iran University of Medical Sciences, Tehran, Iran  
⁶ Department of Microbiology, Faculty of Medical Sciences, Iran University of Medical Sciences, Tehran, Iran  
⁷ Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

**Article Info**

**Article type:** Original article

**Article History:**
Received: 11 October 2019  
Revised: 01 January 2020  
Accepted: 12 February 2020

**Corresponding author:** Mehraban Falahati
Department of Medical Mycology, Faculty of Medical Sciences, Iran University of Medical Sciences, Tehran, Iran.
Email: falahati_mehraban@yahoo.com

---

**ABSTRACT**

**Background and Purpose:** Recurrent vulvovaginal candidiasis (RVVC) is one of the most common gynecological conditions in healthy and diabetic women, as well as antibiotic users. The present study was conducted to determine the relationship between *TUP1* gene expression patterns and symptomatic recurrent *C. albicans* infections.

**Materials and Methods:** This research was performed on *C. albicans* samples isolated from the vaginal specimens obtained from 31 individuals with RVVC in 2016. The reference strain *C. albicans* ATCC 10231, 10 *C. albicans* strains isolated from minimally symptomatic patients, and 10 isolates from asymptomatic patients were also used as control strains. The relative mRNA expression of the *TUP1* gene was quantified using quantitative real-time polymerase chain reaction (QRT-PCR).

**Results:** The QRT-PCR results revealed that *TUP1* mRNA expression was significantly decreased (0.001-0.930 fold) in the *C. albicans* isolates obtained from RVVC patients (*P* < 0.001). However, no *TUP1* expression was detectable in the isolates collected from asymptomatic patients. The results also indicated a significant correlation between *TUP1* mRNA expression level and the severity of itching and discharge (*P* < 0.001).

**Conclusion:** The present results were suggestive of the probable contribution of *TUP1*, as part of the transcriptional repressor, to the severity of the symptoms related to *C. albicans* infections in the vagina. Regarding this, it is required to perform more in vivo studies using a larger sample size to characterize the regulatory or stimulatory function of *TUP1* in the severity of RVVC symptoms. Furthermore, the study and identification of the genes involved in the severity of the symptomatic manifestations of *C. albicans*, especially in resistant strains, may lead to the recognition of an alternative antifungal target to enable the development of an effective agent.

**Keywords:** Candida albicans, Expression, Filamentous growth, TUP1 gene, Vulvovaginal candidiasis

---

**How to cite this paper**
Ghazanfari M, Fattahi A, Falahati M, Bakhshizadeh M, Roudbar M, Masjedian Jazi F, Keykhoosravi M, Lotfali E. Does alternation of *Candida albicans TUP1* gene expression affect the progress of symptomatic recurrent vulvovaginal candidiasis? Curr Med Mycol. 2020; 6(2): 7-10. DOI: 10.18502/CMM.6.2.2694

---

**Introduction**

Recurrent vulvovaginal candidiasis (RVVC) is one of the most common gynecological conditions in healthy [1-3] and diabetic women, as well as antibiotic users [4, 5]. Currently, 5-8% of women worldwide suffer from RVVC, with a minimum recurrence rate of 4 episodes per year [5, 6]. Although *Candida albicans* has been considered the primary cause of RVVC, emerging evidence increasingly points to the causal role of non-*Candida albicans* Candida (NCAC) species, particularly *C. glabrata* [7, 8].

The frequent occurrence of RVVC in different populations without identifiable predisposing factors highlights the role of unknown possible genetic

---

**License**

Copyright © 2020. Published by Mazandaran University of Medical Sciences on behalf of Iranian Society of Medical Mycology and Invasive Fungi Research Center. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY) License (http://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution and reproduction in any medium, provided appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.
deficiencies in the host, as well as the pivotal virulence factors of the pathogenic fungi [3]. Several characteristics of C. albicans are directly involved in the pathogenesis of this species [9-13]. For instance, the transition between yeast and filamentous growth is one of the most outstanding virulence factors [14, 15]. There is a hypothesis suggesting that the filamentous form is more invasive than the yeast form [16] because it can penetrate the tissue and escape from the immune system of the host [17].

It seems that this morphogenesis reflects the integrity/interaction of multiple genetic and environmental factors responsible for full virulence. The morphogenesis also emphasizes the necessity of the recognition of the genes involved in morphogenesis and accounting for pathogenesis during symptomatic recurrent infections, especially in patients with unknown underlying diseases. It is believed that hyphal formation is controlled by a panel of transcriptional activators (e.g., EFG1 and CPH1) [18, 19] and co-repressor complexes (e.g., TUP1, NRG1, and RFG1) [10].

In addition, several signaling pathways, such as mitogen-activated protein kinase (MAPK or MAP kinase), Ras/cyclic AMP signaling, calcium/calmodulin-dependent pathways, and some environmental conditions are also involved [20]. According to the literature, the deletion of TUP1, EFG1, and CPH1 genes could induce constitutive filamentous growth [21-23]. Moreover, the mutation of TUP1 reportedly contributed to the increase of the expression levels of several genes, such as ALS and SAP, promoting C. albicans and increasing virulence [24]. However, the activation of TUP1 transcription repressor complexes results in the repression of filament-specific gene expression [24].

The exact mechanism of the functional role of TUP1 in morphogenic switching is controversial. There is a bulk of evidence indicating that TUP1 directly represses filamentation or encodes a panel of repressor genes to induce filamentous formation [23]. However, the exact relevant factors, which are responsible for the incidence of symptomatic and recurrent C. albicans infections, are not known yet.

Experimental evidence regarding hyphal formation suggests that TUP1 is involved in morphogenesis via various signaling pathways and encodes the genes promoting mucosal pathogenesis [23]. Hence, a hypothesis arose from the fact that TUP1 expression might have a stimulatory effect on symptomatic pathology. With this background in mind, the present study was conducted as the first attempt to determine the correlation between TUP1 gene expression patterns and symptomatic recurrent vulvovaginal candidiasis caused by C. albicans using the QRT-PCR.

Materials and Methods

Study design and participants

The present experimental study was performed on 31 C. albicans samples isolated from vaginal specimens obtained from 31 individuals with RVVC (with the presence of filament in the direct examination of vaginal discharge) in 2016 [3]. In addition, C. albicans ATCC 10231, 10 C. albicans strains isolated from minimally symptomatic patients, and 10 isolates from asymptomatic patients were used as control strains.

The ability of all 31 strains to develop filaments were confirmed on the yeast extract peptone dextrose (YE PD) broth without any pretreatment (Merck, Germany) at 39°C for 1 h [25, 26]. The symptoms of RVVC patients, including itching and discharge, were monitored by physician visits and recorded precisely during the sample collection. In addition, the identification of the clinical control isolates was performed according to a previous study performed by Ghazanfari et al. [3].

Primer design

The PCR primers were designed using online Primer3 software (version 0.4.0) (http://primer3.ut.ee/) and synthesized by the Bioneer Company (Korea; Table1).

RNA Extraction

To ensure the relationship of mRNA expression with the infection and promote hyphal growth, total RNA (for clinical isolates and ATCC strain) was extracted in the early stage of mycelia growth (log-phase) on the YE PD broth without any pretreatment (Merck, Germany) at 39°C (25, 26), using the RNASE Plus solution (Cinnagen, Iran). The qualities and concentrations of the extracted RNA were checked with agarose gel electrophoresis and a spectrophotometer (ND-1000, Thermo Scientific Fisher, US), respectively. In order to remove any DNA contamination, the RNA was treated by DNase1 (Fermentas, USA) according to the manufacturer’s instructions.

Complementary DNA Synthesis

Complementary DNA (cDNA) was synthesized using 3 μg RNA, 20 pmol/μL random hexamer (Fermentas, Burlington, Canada), and 20 pmol/μL Oligo-dT (Fermentas, Burlington, Canada) [27]. Subsequently, it was incubated at 65°C for 5 min and then added with 10 μL Hyerscript RT Master Mix (GeneAll, Korea). In the next stage, the sample was kept at 25°C for 5 min, followed by incubation at 42°C for 60 min and finally warmed up to 85°C for 5 min.

The integrity of the cDNA was checked using the housekeeping gene ACTIN primers. The PCR denaturation process was carried out for 5 min at 96°C, 45 sec at 94°C, 45 sec at 60°C, and 1 min at 72°C for

| Table 1. Primer sequences used in the study |
|--------------------------------------------|
| **Gene name** | **Primer** | **Nucleotide sequence (5’→3’)** |
|----------------|-----------|---------------------------------|
| **TUP**       | **TUPF**  | TCAAGGAATCCCACTTCATTC           |
| **TUP**       | **TUPR**  | AAATCTCAGCGACGAACAAAC           |
| **ACT**       | **ACTF**  | GCCGTTTCCCATCCTGTGT             |
| **ACT**       | **ACTR**  | GCTCGGTCAACAAAATGCGGG           |

Materials and Methods

Study design and participants

The present experimental study was performed on 31 C. albicans samples isolated from vaginal
an extension of 30 cycles. Finally, it was heated up to 72°C for 10 min. In the next stage, all appropriate cDNAs were stored at -20°C.

Quantitative real-time polymerase chain reaction

The QRT-PCR was conducted in duplicate with 20 μL volumes using the Q-Master Mix SYBR Green I (2X) (Ampliqon, Denmark) and RG-3000 (Corbett, Australia). Subsequently, 1 μL single-stranded cDNA was added to a microtube, containing 10 μL Q-Master Mix SYBR Green I (2 X), 0.8 μL of each of the forward and reverse TUP1 primers (10 pmole/μL), and H₂O of up to 20 μL. The thermal protocol was performed by activation at 95°C for 15 min, followed by amplification at 95°C for 35 sec and 55°C for 40 sec for 35 cycles. The ACT gene was used as a house-keeping agent to normalize the data. In order to ensure the accuracy of the examination, the average TUP1 mRNA expression level of the control strains (i.e., reference strain and clinical strains isolated from patients with minimally symptomatic RVVC) was measured and used as the baseline.

The results were analyzed using the comparative Ct method (ΔΔCt) by REST© software (2009, version 2.0.13). Study approval was obtained from the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (NO. 26990).

Statistical analysis

Data analysis was conducted in SPSS software, version 16 (SPSS, Chicago, IL, USA) using Fisher’s exact and Mann-Whitney U tests. A P-value less than 0.5 was considered statistically significant.

Results

The itching and discharge were reported in 17 (54.83%) and 8 (25.80%) RVVC patients, respectively. Furthermore, 6 (19.35%) cases developed both itching and discharge simultaneously. The QRT-PCR results revealed a significant decrease (0.001-0.930 fold) in the TUP1 mRNA expression in all 31 C. albicans isolates (P<0.001) in comparison to that in the control group (Figure 1). In addition, a significant correlation was observed between TUP1 mRNA expression level and the severity of itching and discharge (P<0.001).

Discussion

The results of the present study demonstrated a significant downregulation in TUP1 mRNA expression levels in the isolates with filamentous growth in the microscopic specimen of vaginal discharge after 1 h of growth in the YEPD broth at 39°C, compared to that in the control specimens. This finding, along with moderate to severe clinical manifestations, suggests a direct correlation between the downregulation of TUP1 mRNA expression with hypha formation and the severity of symptomatic recurrent vaginal infections. In other words, it seems that highly symptomatic infections are associated with a higher down regulation level of TUP1 mRNA and vice versa.

Moreover, the importance of TUP1 level in C. albicans-induced infection severity has been shown in corneal infections. The RBT4 gene expression level and/or downstream of TUP1 refer to the present results. There was a relationship between the higher expression level of TUP1 mRNA and the presence of asymptomatic recurrent infections. These findings were obtained from the comparison of the results obtained from symptomatic patients with asymptomatic controls with no expression of TUP1 mRNA and confirm our hypothesis (i.e., a relationship between the higher expression level of TUP1 mRNA and the presence of asymptomatic recurrent infections).

Two explanatory hypotheses were raised from the recent imaging of TUP1 gene expression. Firstly, EFG1, as a key activator of filamentation, could interfere with TUP1 expression via the inhibition of NRG1 expression. Secondly, possible genetic changes in the inhibitory complexes (e.g., NRG1, RFG1, and TUP1) can inhibit TUP1 gene expression.

Conclusion

The present results were suggestive of the contribution of TUP1, as a part of the transcriptional repressor, to the severity of symptoms related to C. albicans infections in the vagina. However, it is required to perform more in vivo studies on a larger sample size to characterize the regulatory or stimulatory function of TUP1 in the severity of RVVC symptoms. Furthermore, the study and identification of the genes involved in the severity of the symptomatic manifestations of C. albicans, especially those of resistant strains, may lead to the recognition of an alternative antifungal target to facilitate the development of an effective agent.

Acknowledgments

This research was financially supported by the Iran University of Medical Sciences (grant No: 26990).

Author’s contribution

M. G. and M. F. designed the study, M. G. and A.
References

1. Adib SM, Bared EE, Fanous R, Kyriacos S. Practices of Lebanese gynecologists regarding treatment of recurrent vulvovaginal candidiasis. N Am J Med Sci. 2011; 3(9):406-10.

2. Lema VM. Recurrent vulvovaginal candidiasis: diagnostic and management challenges in a developing country context. Obstet Gynecol Int J. 2017; 7(5):260.

3. Ghazanfari M, Falahati M, Fattahi A, Bazrafshan F, Nami S, Hosseinzadeh M, et al. Is MBL serum concentration a reliable predictor for recurrent vulvovaginal candidiasis? Mycoses. 2017; 62(2):106-11.

4. Bernstein JA, Seidu L. Chronic vulvovaginal Candida hypersensitivity: an underrecognized and undertreated disorder by allergists. Allergy Rhinol. 2015; 6(1):44-9.

5. Rosentul DC, Delsing CE, Jaeger M, Plantinga TS, Oosting M, Costantini I, et al. Gene polymorphisms in pattern recognition receptors and susceptibility to idiopathic recurrent vulvovaginal candidiasis. Front Microbiol. 2014; 5:483.

6. Aguin TJ, Sobel JD. Vulvovaginal candidiasis in pregnancy. Curr Infect Dis Rep. 2015; 17(6):462.

7. Gonçalves B, Ferreira C, Alves CT, Henriques M, Azeredo J, Silva S. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. Curr Rev Microbiol. 2016; 42(6):903-27.

8. Whaley SG, Berekow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species. Front Microbiol. 2017; 12:2173.

9. Gunther LS, Martins HP, Gimenes F, Abreu AL, Consolaro ME, Svidzinska T. Prevalence of Candida albicans and non-albicans isolates from vaginal secretions: comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. Sao Paulo Med J. 2014; 132(2):116-20.

10. Kadosh D, Johnson AD. Induction of the Candida albicans filamentous growth program by relief of transcriptional repression: a genome-wide analysis. Mole Boil Cell. 2005; 16(6):2903-12.

11. Saville SP, Lazzell AL, Monteagudo C, Lopez-Ribot JL. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of Candida albicans during infection. Eukaryot Cell. 2003; 2(5):1053-60.

12. Brown AJ. Morphogenetic signaling pathways in Candida albicans. In: Calderone R, editor. Candida and candidiasis. Washington, DC: ASM Press; 2002. P. 95-106.

13. Kadosh D, Gow NA. Host recognition by Candida species. In: Calderone R, editor. Candida and candidiasis. Washington, DC: ASM Press; 2002. P. 67-86.

14. Romani L, Bistoni F, Puccetti P. Adaptation of Candida albicans to the host environment: the role of morphogenesis in virulence and survival in mammalian hosts. Curr Opin Microbiol. 2003; 6(4):338-43.

15. Achkar JM, Fenes BC. Candida infections of the genitourinary tract. Clin Microbiol Rev. 2010; 23(2):253-73.

16. Thompson DS, Carlisle PL, Kadosh D. Coevolution of morphology and virulence in Candida species. Eukaryot Cell. 2011; 10(9):1173-82.

17. Sudbery PE. Growth of Candida albicans hyphae. Nat Rev Microbiol. 2011; 9(10):737-48.

18. Lane S, Biese C, Zhou S, Matson R, Liu H. DNA array studies demonstrate convergent regulation of virulence factors by Cph1, Cph2, and Efg1 in Candida albicans. J Biol Chem. 2001; 276(52):48988-96.

19. Felk A, Kretschmar M, Albrecht A, Schaller M, Beinhaus S, Richterlein T, et al. Candida albicans hyphal formation and the expression of the Efg1-regulated proteinases Sap4 to Sap6 are required for the invasion of parenchymal organs. Infect Immune. 2002; 70(7):3689-700.

20. Braun BR, Johnson AD. TUP1, CPH1 and EFG1 make independent contributions to filamentation in Candida albicans. Genet. 2000; 155(1):57-67.

21. Brand A. Hyphal growth in human fungal pathogens and its role in virulence. Int J Microbiol. 2011; 2012:517529.

22. Jackson BE, Mitchell BM, Wilhelmus KR. Corneal virulence of Candida albicans and characterization of TUP1, CPH1 and EFG1 in Candida albicans. Eukaryot Cell. 2008; 7(6):980-9.

23. Braun BR, Head WS, Wang MX, Johnson AD. Identification and characterization of TUP1-regulated genes in Candida albicans. Genet. 2000; 156(1):31-44.

24. Kebara BW, Langford ML, Navarathna DH, Dumitríu R, Nickerson KW, Atkin AL. Corneal virulence and susceptibility to Candida albicans infection by allergists. Allergy Rhinol. 2015; 6(1):44-9.

25. Gunther LS, Martins HP, Gimenes F, Abreu AL, Consolaro ME, Svidzinska T. Prevalence of Candida albicans and non-albicans isolates from vaginal secretions: comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. Sao Paulo Med J. 2014; 132(2):116-20.

26. Fließmann J, Rocha MA. Decrease in ribosomal RNA in Candida albicans induced by serum exposure. PLoS One. 2015; 10(5):e012430.

27. Kim D, Shin WS, Lee KH, Young Park J, Koh CM. Rapid differentiation of Candida albicans from other Candida species using its unique germ tube formation at 39 C. Yeast. 2002; 19(11):957-62.

28. Fattahi A, Zaini F, Kordbacheh P, Rezae S, Safara M, Fateh R, et al. Evaluation of mRNA expression levels of cyp51a and mdr1, candidate genes for voriconazole resistance in Aspergillus flavus. Jundishapur J Microbiol. 2015; 8(12):e26990.