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Research Article

Alternation of *HWP1* and *PLB1* mRNA Expression Level in Progression of *Candida albicans* in Different Anatomical Sites

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Abstract

Background: *Candida albicans* can cause oral, vaginal, and cutaneous infections, as well as systemic candidiasis. It has been recently documented that molecular factors play significant roles in the pathogenesis of *Candida albicans* in various anatomical sites of the host.
Objectives: The present study was designed to answer the hypothesis of whether *PLB1* and *HWP1* mRNA expression patterns are related to the progression of infection in different anatomical sites of the body.
Methods: The experimental study was performed on 120 clinical isolates of *C. albicans* obtained from various sites of non-immune-compromised and immune-compromised patients. Initially, all samples were cultured on Sabouraud-dextrose agar and then CHROM agar *Candida* medium to isolate and obtain a pure colony of yeasts. Quantitative real-time PCR was carried out for the quantitative evaluation of *HWP1* and *PLB1* mRNA expression in all clinical samples. The frequency of the *PLB1* and *HWP1* genes among *C. albicans* strains isolated from four clinical sites was analyzed using Fisher's exact test with a significance threshold of $P < 0.05$. Finally, data obtained from real-time PCR was interpreted using the comparative Ct method ($\Delta\Delta$Ct) by REST© software.
Results: The *HWP1* gene was detected at a higher frequency than the *PLB1* gene in *C. albicans* strains. The *HWP1* mRNA expression level of clinical samples was upregulated by 70, 83.3, 43.3, and 33.3% in four sites (oral, vaginal, BAL, and cutaneous sites), respectively. The *PLB1* mRNA expression level of all samples was upregulated by 46.7, 53.3, 40, and 3.3% ($P < 0.001$) in four sites compared to the control group.
Conclusions: The *PLB1* and *HWP1* genes were expressed predominantly in mucosal (oral, vaginal, and BAL) specimens. This clearly shows that the expression pattern of these candidate genes depends on the organ localization. Furthermore, the presence of samples with no expression of *HWP1* and *PLB1* genes mRNA confirmed the recent hypothesis that there is a meaningful relationship between the higher expression level of candidate genes mRNA and the presence of infections in a specific site of the body. However, more studies are required on larger samples to characterize the exact molecular mechanism of candidate genes involved in the severity of symptoms, as well as their contribution to the site of infection.

Keywords: *Candida albicans*, Gene Expression, *HWP1* Protein, *PLB1* Protein

1. Background

Candida albicans can cause a variety of infections, ranging from superficial mucocutaneous candidiasis to systemic infections that involve major organs including the lungs, kidneys, liver, spleen, heart, and other organs (1). Recently, with the increasing number of immune-compromised populations, the incidence of candidiasis has also remarkably increased (2).

It has recently documented that molecular factors play significant roles in the pathogenesis of *Candida albicans* in various anatomical sites of the host (3). A set of virulence factors are involved in *C. albicans* infection including hyphae formation, phenotype switching, biological substrates' adhesion, and extracellular production of hydrolytic enzymes (4).

Previous research found that alternation in the mRNA expression level of specific Secreted Aspartyl Proteinase (SAP) genes was related to the active form of the disease and the anatomical site of infection (5). The first step of

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Candida infection is the formation of hyphae and adhesion to host cells. It seems that the filamentous form is more invasive than the yeast form to the progression of *Candida* infection (6). Candidal cell adherence to host tissues is a complex multifactorial phenomenon. A 34-kDa adhesin molecule named *HWP1*, which is found in the hyphal wall protein, is a gene required for virulence in mucocutaneous candidiasis because it encodes an outer surface mannoprotein (7). Hypha-specific expression of *HWP1* results in the increased fungal growth in the host in candidiasis in the murine stomach (8). The contribution of *HWP1* to mucosal symptomatic and asymptomatic infections has been reported (1).

The most important hydrolytic enzymes produced by *C. albicans* include Phospholipases (PLBs) and Secreted Aspartyl Proteinases (SAPs) (9). Phospholipase A, B, C, and D have been recognized in *C. albicans*. Phospholipases have the properties of both lysophospholipase and hydrolase (7). The *PLB1* and *PLB2* gene products have been detected extracellularly. An 84-kDa *PLB1* glycoprotein isolated from the hyphal tip in the course of tissue invasion has shown to be required for virulence in a murine model of candidiasis (5, 7).

2. Objectives

The present study was designed to answer the hypothesis of whether *PLB1* and *HWP1* mRNA expression patterns are related to the progression of infection in different anatomical sites of the body.

3. Methods

3.1. Sample Collection and Identification of C. albicans Isolates

An experimental study was performed on 120 clinical isolates of *C. albicans* obtained from various sites of immune-compromised and non-immune-compromised patients who were admitted to teaching hospitals of Shahid Beheshti University of Medical Sciences, Tehran, Iran from 2016 to 2019 (Table 1).

Table 1. Distribution of Clinical Isolates of *Candida albicans*

Source	Number of Samples
BAL	30
Oral	30
Vaginal	30
Cutaneous (nail, axillary, groin)	30

Abbreviation: BAL, Bronchoalveolar lavage.

The specimens were submitted to the Medical Mycology Laboratory of the Medical School. Initially, all samples were cultured on Sabouraud-dextrose agar (Merck, Germany) at 32°C for 48 hours. Then, a single colony was subcultured on CHROMagar *Candida* medium (CHROMagar, France), followed by incubation at 35°C for 48 hours to obtain a pure colony of yeasts. A single colony of all isolates was subjected to *HWP1* gene amplification to discriminate against the *C. albicans* complex.

3.2. Amplification of HWP1 Gene

The *HWP1* gene was amplified to differentiate the *C. albicans* complex comprising *C. albicans*, *C. Africana*, and *C. dubliniensis*. The PCR with paired primers *HWP1*-F (5′-GCTACCACTTCAGAATCATCATC-3′) and *HWP1*-R (5′-GCACCTTCAGTCGTAGAGACG- 3′) was done in the following condition: 95°C for 15 minutes; 35 cycles of 30 seconds at 95°C, annealing for 30 seconds at 60°C, one minute at 72°C, and a final extension of 5 minutes at 72°C. The data were interpreted according to bands sizes of ~900 bp, ~700 bp, and ~ 560 bp for *C. albicans*, *C. Africana*, and *C. dubliniensis*, respectively. Five microliters of PCR products were electrophoresed on a 1.5% agarose gel in TBE buffer (Merck, Germany); then, they were observed and photographed under ultraviolet irradiation.

3.3. RNA Extraction

All *C. albicans* strains were cultured on Sabouraud Dextrose Agar (Merck, Germany) and incubated for two days at 35°C. Total RNA was extracted from an exponential phase using the commercial kit (Fermentas, EU) (10, 11). The total RNA was treated using the DNase1 (Fermentas, USA) to improve the reliability of the quantitative method.

3.4. cDNA Synthesis

Single-strand complementary DNA (ScDNA) was synthesized using 2 μg of total RNA in a 10-μL reaction mixture using the Prime Script RT reaction kit (Takara, Japan) according to the manufacturer's instruction (12). The accuracy of ScDNA was checked with actin (*ACT1*) gene primers as the housekeeping gene. The PCR condition was as follows: initial denaturation for 5 minutes at 94°C, 30 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 60°C, extention for 45 seconds at 72°C, with a final extension for one minute at 72°C.

3.5. Quantitative Real-Time PCR Assay

The set of primers used the Oligo Explorer (version 15) software. Each primer pair was aligned with sequences from the NCBI GenBank database using BLAST

(http://www.ncbi.nlm.nih.gov/BLAST) to ensure the specificity of the sequences. The primers used in this study were synthesized by Bioneer Company (Korea) as listed in Table 2. Quantitative real-time PCR (QRT) was performed with Applied Biosystems 7300 Real-Time PCR System (Singapore). SYBR Premix Ex Taq II (Amplicon, Korea) was used as a reagent specifically designed for intercalator-based real-time PCR. The PCR mixture contained 2 μL of the first-strand cDNA, 10 μL of SYBR green, 0.8 μL of each primer, 0.4 μL of Rox reference dye (50x), and 6 μL of dH_2O to make a final volume of 20 μL. The PCR was performed with a preliminary hold at 95°C for 30 seconds as the initial denaturation step, followed by 40 cycles of PCR consisting of 95°C for 5 seconds and 60°C for 31 seconds. Final holding was performed at 72°C for 60 seconds.

The *ACT1* gene was used as a housekeeping gene to normalize the data. For the accuracy of the examination, we measured the *PLB1* and *HWP1* mRNA expression levels of control strains (including reference strain and clinical strain isolated from patients with no invasive candidiasis). The average expression level of them was used as the baseline. All experiments were done in duplicate and each experiment was repeated twice on two different days to assess reproducibility (13).

3.6. Statistical Analysis

The frequency of the *PLB1* and *HWP1* genes among *C. albicans* strains isolated from four clinical sites was analyzed using the Fisher exact test with a significance threshold of $P < 0.05$. Finally, data obtained from real-time PCR were interpreted using the comparative Ct method ($\Delta\Delta$Ct) using REST© software (2009, V. 2.0.13).

4. Results

4.1. Interpretation of Demographic Information

We collected 120 samples from four clinical sites. Demographic information (age and sex) is shown in Table 3. The immune-compromised patients (n = 80) profile included solid-organ cancers (n = 25), bone marrow transplant recipients (n = 32), and intensive care unit (ICU) patients (n = 23). No patients were treated with antifungal agents at the time of sampling.

4.2. Result of Amplification of HWP1 Gene

The initially identified *C. albicans* complex on chromogenic media was discriminated using the amplification of the *HWP1* gene. Based on bands revealed on gel electrophoresis, all strains were known as *C. albicans* (Figure 1) (14).

4.3. Result of QRT PCR

The *PLB1* and *HWP1* genes were detected in *C. albicans* strains. The *HWP1* gene was detected at a higher frequency than the *PLB1* gene in *C. albicans* strains. There were statistically significant differences in *PLB1* and *HWP1* gene expressions between *C. albicans* strains isolated from four clinical sites (P = 0.003 and 0.001, respectively) (Figures 2 and 3).

The *HWP1* mRNA expression levels of clinical samples were upregulated by 70%, 83.3%, 43.3%, and 33.3% in oral, vaginal, BAL, and cutaneous samples, respectively (Table 3). The *PLB1* mRNA expression level of all samples was upregulated by 46.7%, 53.3%, 40%, and 3.3% ($P < 0.001$) in oral, vaginal, BAL, and cutaneous samples, respectively, compared to the control group (Table 3).

5. Discussion

In this study, BAL samples from patient specimens had positive blood cultures. Given the significant expansion of *Candida* spp. infections, as well as differences in

Table 2. Primers Used in Quantitative Real-time PCR Analysis

Gene	Primer	Sequence
HWP1	Forward	5' GACCGTCTACCTGTGGGACAGT 3'
	Reverse	5' GCTCAACTTATTGCTATCGCTTATTACA 3'
PLB1	Forward	5' GGTGGAGAAGATGGCCAAAA 3'
	Reverse	5' AGCACTTACGTTACGATGCAACA 3'
ACT1	Forward	5'TGGAGCTTCGGTCAACAAAACTGG 3'
	Reverse	5' ACGGTATTGTTTCCAACTGGGACG 3'

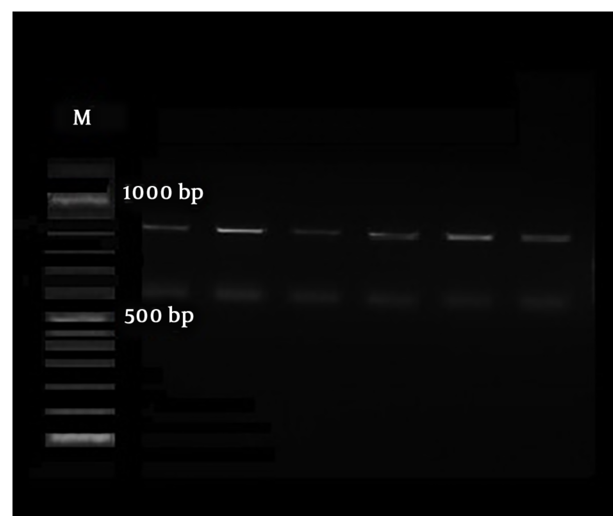

Figure 1. The gel electrophoresis of *HWP1* gene amplification of *Candida albicans* (900 bp)

Table 3. Frequency of *Candida albicans PLB1* and *HWP1* Genes and Their Expression (Up and Downregulation) in Oral, Vaginal, BAL, and Cutaneous Samples[a]

Specimens	Age	Sex		Expression of *PLB1*			Expression of *HWP1*		
		Male	Female	Up	Down	Up and Down	Up	Down	Up and Down
Oral (n = 30)	46 ± 8.72	16 (53.3)	14 (46.6)	14 (46.7)	5 (16.6)	19 (63.3)	21 (70)	9 (30)	30 (100)
Vaginal (n = 30)	41 ± 10.29		30 (100)	16 (53.3)	9 (30)	25 (83.3)	25 (83.3)	4 (13.3)	29 (96.6)
BAL (n = 30)	47 ± 8.38	17 (56.6)	13 (43.3)	12 (40)	8 (26.6)	20 (66.6)	13 (43.3)	10 (33.3)	23 (76.6)
Cutaneous (n = 30)	42 ± 11.68	8 (26.6)	22 (73.3)	1 (3.3)	15 (50)	16 (53.3)	9 (30)	10 (33.3)	19 (63.3)
Total	-	-		43 (35.8)	37 (30.8)	80 (66.6)	68 (56.6)	33 (27.5)	101 (84.1)
P value (between groups)	-	-		-	-	0.003	-	-	0.001

[a]Values are expressed as mean ± SD or No. (%).

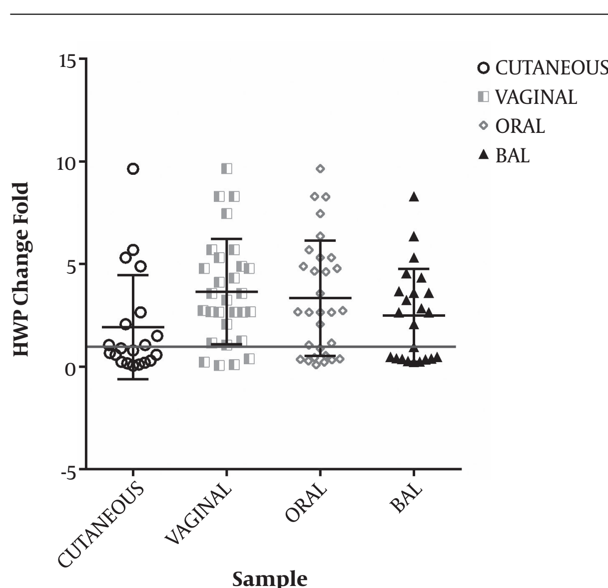

Figure 2. Comparison *HWP1* mRNA expression level in 120 clinical samples compared to the control group in four anatomical sites

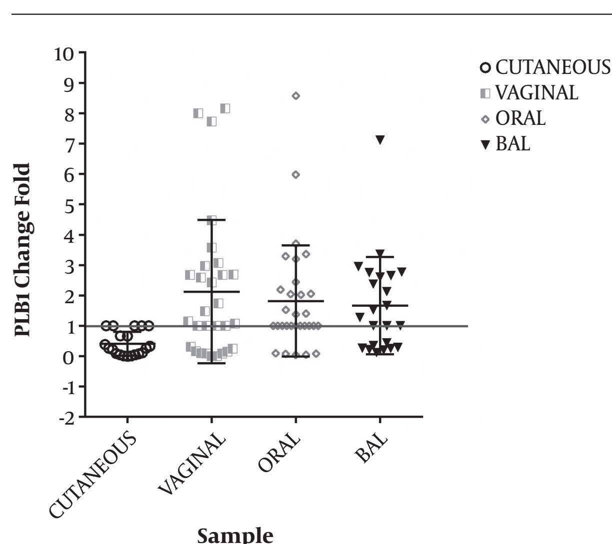

Figure 3. Comparison *PLB1* mRNA expression level in 120 clinical samples compared to the control group in four anatomical sites

pathogenicity, the rapid and accurate identification at the species level seems crucial for clinical management. This study analyzed the mRNA expression levels of the *HWP1* and *PLB1* genes in *C. albicans* isolated from 120 oral, vaginal, BAL, and cutaneous samples.

The results provided insights into the patterns of the two genes' mRNA expression levels in four anatomical sites. In our cases, the expression rates of *HWP1* and *PLB1* genes were significantly different (P = 0.001 and P = 0.003, respectively) at the four anatomical sites. The results showed 56.6% and 35.8% upregulation in the *HWP1* and *PLB1* genes, respectively.

The present findings suggest a meaningful correlation between the up-regulation of *HWP1* mRNA expression level with the pathogenicity of *C. albicans* strains and the sites of infection. The data showed that the *HWP1* gene could be expressed by *C. albicans* strains *in vitro* from the vagina (83.3%). The *HWP1* expression was significantly different between vaginal samples from oral and cutaneous samples ($P \leq 0.05$) but the difference was not significant between vaginal and BAL samples (P = 1).

The *HWP1* mRNA expression is the key player in the activation of vaginal candidiasis and hence, it may have an essential role in the clinical presentation of vaginitis. The *HWP1* gene expression was upregulated in mucosal samples compared to cutaneous samples (only 30%). The importance of the *HWP1* level in the severity of mucocutaneous candidiasis by *C. albicans* has been shown previously. The *HWP1* gene expression level has been found to affect the pathogenesis of candidiasis in vaginal and oral sites (5). A survey by Nas et al. showed that the expression of *HWP1* was

detected as 62% in *C. albicans* strains isolated from women with vulvovaginal candidiasis (10). Pakdel et al. reported that the presence of *HWP1* is an important factor for vaginal infection (11).

The results also showed that the *PLB1* expression in vaginal samples had a significant difference with the *PLB1* expression in cutaneous samples ($P \leq 0.05$) while there was no difference with the two other samples (BAL and oral samples). The role of phospholipases in *C. albicans* infections is also well established through the cloning of a phospholipase gene, *PLB1*, the deletion of which did not result in alterations in adherence, but generated cells with reduced invasion ability (12).

The phospholipase activity was detected in 35.8% of the *C. albicans* isolates in the present study. Previous studies have shown phospholipase activity in 30% to 100% of *Candida* isolates from various groups of patients and various sites (13, 15). The proportion may depend on the site; for example, the phospholipase activity was found in 55%, 50%, and 30% of *Candida* spp. isolated from blood, wound infection, and urine, respectively (13). The phospholipase gene expression has shown to be affected by growth conditions (15).

In a study by Hoover et al., a little phospholipase activity was detected in *C. albicans* strains isolated from the oral cavity of carriers (16). The *PLB1* expression may be regulated by factors that also regulate the expression of hyphal morphology.

Other possible factors affecting candidiasis should be addressed by evaluating virulence factors other than these genes. If the critical virulence genes were identified in the pathogenesis of candidiasis, it could be an important practical application in developing new diagnostic tests and therapeutic strategies for the treatment of candidiasis (10).

The presence of samples with no expression of *HWP1* and *PLB1* genes mRNA confirms the recent hypothesis that there is a meaningful relationship between higher expression levels of candidate genes mRNA and the presence of infections in a specific site of the body.

In conclusion, the present study is the only report addressing the expression of the *C. albicans HWP1* and *PLB1* genes in BAL and cutaneous samples, which demonstrated differences in expression during the course of the disease. Once more, *PLB1* and *HWP1* were expressed predominantly in mucosal specimens (oral, vaginal, and BAL). This clearly shows that the expression pattern of these candidate genes depends on the organ localization. However, more studies on larger samples are required to characterize the exact molecular mechanism of candidate genes involved in the severity of symptoms, as well as their contribution to the site of infection.

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Footnotes

Authors' Contribution: Study concept and design and technical supervision: Ensieh Lotfali; obtaining the samples from patients and interpretation: Fatemeh Noorbakhsh; technical support: Saham Ansari; acquisition of data and drafting of the manuscript: Reza Ghasemi and Mohammad Mahdi Rabiei; critical revision of the manuscript: Azam Fattahi.

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References

1. Naglik JR, Fostira F, Ruprai J, Staab JF, Challacombe SJ, Sundstrom P. Candida albicans HWP1 gene expression and host antibody responses in colonization and disease. *J Med Microbiol*. 2006;**55**(Pt 10):1323–7. doi: 10.1099/jmm.0.46737-0. [PubMed: 17005778]. [PubMed Central: PMC3244616].
2. Abastabar M, Hosseinpoor S, Hedayati MT, Shokohi T, Valadan R, Mirhendi H, et al. Hyphal wall protein 1 gene: A potential marker for the identification of different Candida species and phylogenetic analysis. *Curr Med Mycol*. 2016;**2**(4):1–8. doi: 10.18869/acadpub.cmm.2.4.1. [PubMed: 28959789]. [PubMed Central: PMC5611690].
3. Falkow S. Is persistent bacterial infection good for your health? *Cell*. 2006;**124**(4):699–702. doi: 10.1016/j.cell.2006.02.004. [PubMed: 16497581].
4. Monroy-Perez E, Paniagua-Contreras G, Vaca-Paniagua F, Negrete-Abascal E, Vaca S. SAP expression in Candida albicans strains isolated from Mexican patients with vaginal Candidosis. *Int J Clin Med*. 2013;**4**(1):25–31. doi: 10.4236/ijcm.2013.41006.
5. Naglik JR, Rodgers CA, Shirlaw PJ, Dobbie JL, Fernandes-Naglik LL, Greenspan D, et al. Differential expression of Candida albicans secreted aspartyl proteinase and phospholipase B genes in humans correlates with active oral and vaginal infections. *J Infect Dis*. 2003;**188**(3):469–79. doi: 10.1086/376536. [PubMed: 12870130].
6. Thompson DS, Carlisle PL, Kadosh D. Coevolution of morphology and virulence in Candida species. *Eukaryot Cell*. 2011;**10**(9):1173–82. doi: 10.1128/EC.05085-11. [PubMed: 21764907]. [PubMed Central: PMC3187052].

7. Khan MSA, Ahmad I, Aqil F, Owais M, Shahid M, Musarrat J. Virulence and pathogenicity of fungal pathogens with special reference to Candida albicans. *Combating fungal infections*. Springer; 2010. p. 21–45. doi: 10.1007/978-3-642-12173-9_2.
8. Sundstrom P, Balish E, Allen CM. Essential role of the Candida albicans transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. *J Infect Dis*. 2002;**185**(4):521–30. doi: 10.1086/338836. [PubMed: 11865405].
9. Tsang CS, Chu FC, Leung WK, Jin LJ, Samaranayake LP, Siu SC. Phospholipase, proteinase and haemolytic activities of Candida albicans isolated from oral cavities of patients with type 2 diabetes mellitus. *J Med Microbiol*. 2007;**56**(Pt 10):1393–8. doi: 10.1099/jmm.0.47303-0. [PubMed: 17893179].
10. Nas T, Kalkanci A, Fidan I, Hizel K, Bolat S, Yolbakan S, et al. Expression of ALS1, HWP1 and SAP4 genes in Candida albicans strains isolated from women with vaginitis. *Folia Microbiol (Praha)*. 2008;**53**(2):179–83. [PubMed: 18837169].
11. Pakdel M, Zarrabi M, Asgarani E, Mohammadi P. [Prediction of antigenic sites on ALS1 and HWP1 protein sequences in vaginal isolated C. albicans of using bioinformatics analysis]. *Iran J Med Microbiol*. 2015;**9**(1):29–34. Persian.
12. Leidich SD, Ibrahim AS, Fu Y, Koul A, Jessup C, Vitullo J, et al. Cloning and disruption of caPLB1, a phospholipase B gene involved in the pathogenicity of Candida albicans. *J Biol Chem*. 1998;**273**(40):26078–86. doi: 10.1074/jbc.273.40.26078. [PubMed: 9748287].
13. Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in Candida albicans. *Sabouraudia*. 1982;**20**(1):7–14. doi: 10.1080/00362178285380031. [PubMed: 7038928].
14. Yazdanparast SA, Khodavaisy S, Fakhim H, Shokohi T, Haghani I, Nabili M, et al. Molecular characterization of highly susceptible Candida africana from vulvovaginal candidiasis. *Mycopathologia*. 2015;**180**(5-6):317–23. doi: 10.1007/s11046-015-9924-z. [PubMed: 26183965].
15. Samaranayake YH, Dassanayake RS, Cheung BP, Jayatilake JA, Yeung KW, Yau JY, et al. Differential phospholipase gene expression by Candida albicans in artificial media and cultured human oral epithelium. *APMIS*. 2006;**114**(12):857–66. doi: 10.1111/j.1600-0463.2006.apm_-479.x. [PubMed: 17207086].
16. Hoover CI, Jantapour MJ, Newport G, Agabian N, Fisher SJ. Cloning and regulated expression of the Candida albicans phospholipase B (PLB1) gene. *FEMS Microbiol Lett*. 1998;**167**(2):163–9. doi: 10.1111/j.1574-6968.1998.tb13223.x. [PubMed: 9809417].