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# Investigation of *bcr*1 Gene Expression in *Candida albicans* Isolates by RT-PCR Technique and its Impact on Biofilm Formation

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**Background:** Adhesion and biofilm formation are two important steps in *Candida* pathogenesis. The aim of the current study was to investigate the presence of *bcr*1 gene in *Candida albicans* (*C. albicans*) isolates from women with vaginal candidiasis and its impact on biofilm formation.
**Methods:** We used 50 clinical isolates which confirmed *C. albicans* by PCR-RFLP. Then total RNA was extracted from *C. albicans* isolates by glass bead and lysis buffer, and cDNA was synthesized using reverse transcriptase enzyme. RT-PCR (Reverse Transcriptase PCR) was used to evaluate the expression of *bcr*1 gene. Biofilm formation was evaluated in 96-well microplate and then tetrazolium reduction was assayed. All data were analyzed using t-test by SPSS software.
**Results:** Fifty clinical isolates out of 150 were confirmed as *C. albicans* by using PCR-RFLP method. All the isolates were resistant to fluconazole, 47/50(94%) isolates had *bcr1* gene by using PCR, and 45(95.7%) out of 47 isolates, showed BCR1 expression by the RT-PCR. Isolates which harbored *bcr*1 gene was succeed to form a dense biofilm on microplate. Comparison of the results of the tetrazolium reduction assay on the two isolates that had BCR1expression and two isolates that had no BCR1 expression showed significant differences (p=0.014).
**Conclusion:** According to our result, all of the isolates that had *bcr*1 gene expression according to RT-PCR, were also resistant to fluconazole in disk diffusion test and additionally, their adherence was higher compared to the control group. These results indicate that there is a positive relation between expression of *bcr*1 gene and biofilm formation.

*Keywords: Candida albicans*, *bcr*1 gene expression, RT-PCR

## 1. Background

*Candida albicans (C. albicans)* is an opportunistic pathogen that has pathogenic ability in both immunocompromised and immunocompetent individuals (1). The ability of pathogenic of *C. albicans* refers to the virulence factors, including the ability to adhere and biofilm formation on the living and non-living surfaces such as medical devices (2, 3). Biofilm formation starts with adherence of yeast-form cells on the surface (4). Many *Candida* infections arise from the formation of biofilms on implanted devices such as intravascular catheters (5-7). Adherence of *C. albicans* to surface is seen as critical stage of the infection, and thus the identification of genes involved in adherence is important. Obviously wide range of different genes and factors are involved in adherence of *C. albicans*. The *bcr*1 gene of *C. albicans* as a main transcription factor plays pivotal roles in biofilm formation (8, 9). The *bcr*1 gene (Biofilm and cell wall regulator 1) encoded a C2H2 zinc finger protein that has a significant role in biofilm formation (8, 10). On the other hand, the transcription factor BCR1 regulated biofilm formation occurs on mucosal surfaces or implanted medical devices (9, 11). BCR1 as a transcription factor has several target genes that govern cell- surface proteins (10, 12). These are expressed preferentially during hyphal differentiation; however, presence of BCR1 is not required for hyphal morphogenesis and it acts as a regulator of hyphal-specific adhesions. Some major target genes of BCR1 are: HYR1, ECE1, RBT5, HWP1, ALS3 and ALS1.

## 2. Objectives

In this study, we investigated the presence of the *bcr*1 gene of *C. albicans* in clinical samples and its role in the ability to adhere to non-living surface.

## 3. Materials and Methods

### *3.1. Patients*

A total of 150 women with yulvovaginitis were chosen randomly. The symptoms included white-patchy leucorrhea, itching and vulvar edema. Wet vaginal swabs were cultured on Sabouraud dextrose agar (Dipco, USA). All of the isolates were examined by PCR-RFLP and were confirmed as *C. albicans*. The age range of the studied population were 18-55 years and we had the history of the use of antibiotics and antifungal as well as underlying conditions such as diabetes.

### *3.2. Molecular identification*

All isolates were examined by RT-PCR method to evaluate the expression of *bcr*1 gene as following steps. DNA extraction: Total genomic DNA was extracted by glass bead and lysis solution according to previously described method (13). The cDNA synthesis: After DNase treatment with Vivantis kit, RNA was converted to cDNA, using cDNA synthesis kit (Fermentase K1621). The RT-PCR reaction: RT-PCR was carried out according to the manufacture's protocol (Table 1). The RT-PCR protocol was run as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation (94°C for 1 min), annealing (58°C for 1 min),





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and extension (72°C for 1 min), followed by a final extension step at 72°C for 3 min. Appropriate negative and positive controls were included in each test, viewed and blotted for band verification by hybridization. The remaining 25 µl of the PCR reaction could then be used, if positives were forth-coming to clone and sequence the band of interest. The *act*1 was used as a normalized gene (house-keeping gene) in all the molecular tests. We used *act*1 gene for positive control and confirmation test of PCR process.

**Table 1.** The BCR1 and ACT1 specific primers for RT-PCR

| Primer name | Sequence(5'→3') | PCR product size (bp) |
|---|---|---|
| ITS1-F | TCCGTAGGTGAACCTGCGG | 535 |
| ITS2-R | TCCTCCGCTTATTGA | |
| ACT1-F | CCAGCTTTCTACGTTTCC | 200 |
| ACT1-R | CTGTAACCACGTTCAGAC | |
| BCR1-F | CTTCAGCAGCTTCATTAACACCTA | 100 |
| BCR1-R | TCTTGGATCAGGTGTACTTTTCAA | |

### *3.3. Preparation of standard fungal cell suspension*

Susceptible *C. albicans* (ATCC 10231) and resistant isolates of *C. albicans* were used for forming biofilms. At first, two strains were grown on Sabouraud dextrose agar medium (SDA Merck, Germany) at 37°C for 18h. Then fresh colonies were inoculated into yeast nitrogen base medium (YNB medium; Himedia Co.) containing 100 mM glucose and incubated at 37°C for 24 hours. After that, a few colonies of the yeast were transferred into a test tube containing sterilized PBS with pH: 7.2 (Difco). Then the mixture was centrifuged (10000g, 10 min). Turbidity of suspension of cell was compared to 0.5 McFarland standard to estimate cell density and finally yeast cells were counted and adjusted at $1\times10^6$ cell/ml by Neubauer slide. ATCC 132 strain was used as the standard strain.

### *3.4. Biofilm formation and Tetrazolium reduction assay (MTT)*

Biofilm formation was assayed. The tetrazolium salt 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, Germany) was used in order to study living cells. MTT formazan formation was measured at 540 nm by using ELISA reader (star sate) (5). All data were analyzed using t-test with SPSS software.

## 4. Results

During sample collection from 2011 to 2012, total 150 samples were obtained from the Tehran clinic, direct examination showed that 110 (73%) were yeast. The results of *Candida* spp. identification showed that by a combination of the phenotypic and molecular method, of the 110 sample, we identified *C. albicans* (87.2%) and *C. galabrata* (10%).

Fifty candida isolates were detected by molecular identification using ITS1 and ITS4 primer pairs which led to PCR products of 535,871bp for *C. albicans* and *C. galabrata*. Two Segments produced for *C. albicans* and *C. galabrata* are shown Figure 1. All of the 50 isolates were resistant to fluconazole. Detection of *bcr*1 gene expression after PCR analysis showed that *bcr*1 gene was present in 47 out of 50 *C. albicans* isolates (94%). RT-PCR analysis was performed 3 µg of total RNA extract of isolates of 47 isolates which showed expression of *bcr*1 gene in 45 out of 47 isolates (95.7%). The results of the tetrazolium reduction test assay on the two isolates which had expression of BCR1 gene and two isolates without BCR1 gene expression showed significant difference with p-value =0.014 ($p<0.05$).

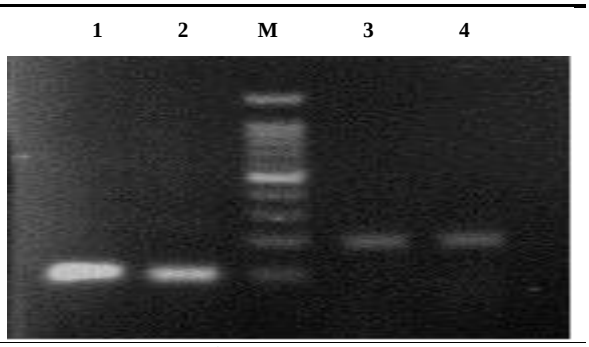


**Figure 1.** PCR product of *C. albicans* isolates. Lane 1 and 2 *bcr*1 gene expression; lane 3 and 4 *act*1 gene. M: 100 bp DNA size marker.

## 5. Discussion

The aim of the present study was to determine the expression of *bcr*1 genes in *C. albicans* isolated from women who were referred to health center (Tehran, Iran). The *bcr* gene expression can be detected by RT-PCR as a useful techn-ique for evaluation of gene expression.

Vaginitis is a world problem and approximately three-fourth of women during their life experience once vaginal candidiasis. The most prevalent etiological agent is *C. albicans* in over 80% of cases (14). Adherence of *C. albicans* to surface is a critical step for the formation of biofilm and initiation of infection. One of the important adherence genes is *bcr1*.

The importance of Bcr1 protein as a transcription factor is obvious thus assessment of *bcr1* gene expression is very necessary (15). However many environmental changes and disruption of anatomic integrity lead to colonization of candida and promotion of infection. On the other hand, biofilm formation contributes to drug resistance, thus expression of *bcr1* gene may be responsible for fluconazole resistance in *C. albicans* isolates. Due to this fact, all of isolates that expressed *bcr*1 were resistant to fluconazole. However, other genes such as *als* and *hwp*1 are involved in drug resistance (16, 17).

Previous studies have shown that the mutant of *bcr1*/*bcr1* is unable to form biofilm and this phenomenon is defective and also the ALS3 overexpression increased the *bcr1*/*bcr1* biofilm defect in vivo. Indeed, overexpression of Bcr1 targets such as ALS3, ECE1and HWP1 strongly restores biofilm formation of *bcr1*/*bcr1* mutant. These results show the pivotal role of BCR1 in the control of biofilm formation and expression of cell wall protein genes that mediate adherence of *C. albicans* on surface (18).

In this present study, RT-PCR was used as a reliable method to evaluate expression of *bcr1* gene in *C. albicans* isolates and also their capacity for adherence and attachment was assessed by using MTT test.

According to our finding, of 50 *C. albicans* isolates about 45 (95.7%) expressed *bcr* gene and only 5 isolates did not show any expression in RT-PCR method which suggests that this gene is conserved in *C. albicans* and contributes to fluconazole resistance, because all of isolates that were resis-tant to fluconazole had *bcr*1 gene.

The result of MTT assay in statistical analysis showed that there is a significant difference in attachment between the isolates of candida that expressed *bcr*1 gene and the control group that did not have any expression because of their ability to adhere to well was higher compared to the control group (p-value=0.014).



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## 6. Conclusion

In this research, our data support the role of *bcr1*gene as a transcriptional factor for the adhesion by increasing the ability of isolates in the attachment.

## Conflict of Interests

The authors declare they have no conflict of interests.


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## Authors' Contributions

All of authors contribute to this study.


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