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Detection of drug resistance gene expression in *Candida albicans* isolated from oral thrush of children via real-time PCR technique

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Abstract

The present study was intended to check the candida drug resistance (CDR) gene expression in isolates of *Candida albicans* (*C. albicans*) of oral thrush, a disease condition that affects inner lining of mouth of immunosuppressed people especially children. From Al-Diwaniya hospital/Al-Diwaniyah City/Iraq, 30 isolates were obtained as 3 groups of tens. Depending on the age of the children, the isolates were categorized into under one year of age (T1), older than one year of age (T2), and nonpathogenic isolates (C). Using quantitative real-time PCR (qPCR), T1 showed significant (p<0.05) expression of the CDR1 and CDR2 genes and higher than T2 and C groups. On the other hand, T2 did not reveal any differences (p>0.005) when compared to C group for both genes. The results indicate that the isolates belong to the children of less than 1 year of old activated resistance against some known and used anti-thrush drugs. This study alarms medical officials and encourages future studies of how to overcome this risky problem.

Keywords: *Candida albicans*, children, CDR gene, qPCR

INTRODUCTION

Oral thrush caused by *C. albicans* is considered as an important disease condition that affects human beings of different ages but mostly infants, toddlers, elderly, and immunocompromised people [1, 2, 3]. *C. albicans* is localized normally on the body mucosa in low numbers, however these numbers get increased when the yeast causes infection [4] . It takes additional factors for the *C. albicans* to initiate infection. Some of these factors are ranged from smoking, immunosuppression, pregnancy, age, and diabetes mellitus [5,6,7]. The seriousness of the disease is limited, but it influences the lifestyle of sick people due to some reasons such as the bad odor of an affected mouth [8]. Early-age infections occur due to incomplete immune system which leaves babies and toddlers prone to *C. albicans* [9,10]. The treatment of this disease is considered as a challenge and this is mainly because the drug resistance that is developed by *C. albicans* [11]. Moreover, biofilm produced by *C. albicans* could protect the yeast from anti-fungal drugs and also increase the time needed to eliminate the infection [12]. The candida drug resistance (CDR) genes are well-known genetic components for developing resistance against some anti-yeast drugs [13]. Some of the famous used anti-fungal drugs are fluconazole, ketaconazole, voriconazole, and Nystatin [14]. Increasing anti-fungal drug resistance cases led the current study to investigate CDR1 and CDR2 gene expression in isolates of *C. albicans* provided by a local hospital in Al-Diwaniyah City, Iraq. The detection of this expression was performed using qPCR technique, and the results revealed interesting information about the presence of antifungal drug resistance in these isolates of *C. albicans*. The results point out some of the major problems of treatment failure in fungal diseases in different ages and more specifically in children.

MATERIALS AND METHODS

Sampling

In Al-Diwaniyah City/Iraq, Al-Diwaniya hospital had provided the isolates of *C. albicans* that were used in this study. The samples were obtained as 3 groups of tens. Depending on the age of the children, the isolates were categorized into under one year of age (T1), older than one year of age (T2), and nonpathogenic isolates (C).

Total RNA extraction

Using Accuzol® reagent kit (Bioneer, South Korea) and depending on the manufacturer protocol, total RNA was extracted from *C. albicans* isolates. Briefly, the isolates were pre-incubated on broth for 4 hrs, and then sterile 1.5ml eppendrof tubes were used to place 200µl of the broth growth in each tube. Then the extraction processes were initiated. DNase I enzyme kit (Promega Company, USA) was used to eliminate any remaining DNA in the extracted solutions. Finally, the extracted RNA was stored at -20 ℃ for later analyses. NanoDrop (THERMO, USA) was employed to measure the quality and quantity of the extracted RNA.

CDNA synthesis

AccuPower® RocktScript RT PreMix kit (Bioneer Company, South Korea) was utilized to synthetize cDNA from the RNA extracts and the manufacturer protocol was followed. Then the pre-RT mix was generated using 10µl RNA (100ng/ul), 1µl random hexamer primer (10pmol), and 9µl DEPC water. The mix was added the kit tubes that contain reverse transcriptase followed by vortexing and spinning down. The thermocycler conditions used to synthetize cDNA are 1 hour at 50 °C of cDNA synthesis (RT step) and 5 min at 95 °C of heat inactivation.

Quantitative real-time PCR (qPCR)

The CDR1 and CDR2 gene expression of the *C. albicans* isolates was evaluated using qPCR technique and following 2-ΔΔCT-Livak method [15]. A real-time PCR system (BioRad, USA) was employed to perform the qPCR process. To detect the amplification of the target genes plus actin which worked as a housekeeping gene for normalization purposes, SYBER Green dye qPCR mastermix was utilized. The Primer 3 Plus was used to design the primers for the qPCR process. These primers are F: AGGTGCTGCCATGTTCTTTG and R: TCGACAATTGGTCTGGCTTC to amplify a 90bp-sized piece of the CDR1 gene, F: ATGTGATTCCCGGGTTTTGG and R: GGTGCACAAGTGACTTTTGC to amplify a 109bp-sized piece of the CDR2 gene, and F: ATGGACGGTGAAGAAGTTGC and R: TGGTCTACCAACAAGAGATGGG to amplify a 117bp-sized piece of the actin gene. These primers were deposited in the GeneBank Database under the numbers XM_718116.2, XM_718076.2, and XM_019475182.1 respectively. AccuPowerTM 2XGreen Star qPCR master mix kit (Bioneer, South Korea) was used to prepare the mastermix for the qPCR assay, and the company protocol was followed. The mastermix was then placed in Miniopticon Real-Time PCR system (BioRad, USA). Here, the conditions of the thermocycler used are 1 cycle

of initial denaturation for 1 hr at 50 °C, 40 cycles of denaturation and annealing/extension (detection scan) for 20 sec at 95 °C and 30 sec at 60 °C respectively, and finally 1 cycle of melting for 0.5 sec at 60-95°C.

Statistical analysis

One-way ANOVA was used to analyze and interpret the results of the current study. Data showed elsewhere are represented by Mean ± Standard Error (Mean±SE) unless otherwise mentioned. When the resulted probability was $p<0.05$, rejection of the null hypothesis was decided. Graphs were drawn using Microsoft Office Excel Worksheet 2007.

RESULTS

The present study results have shown the expression of CDR1 and CDR2 in the isolates of the *C. albicans* that was provided by the healthcare provider in the city of Al-Diwaniyah. Interestingly, T1 showed significant ($p=0.000$) expression of the CDR1 gene and higher than T2 and C groups. Moreover, T1 declared significant ($p=0.002$) and ($p=0.000$) expression of the CDR2 gene when compared to T2 and C groups respectively. On the other hand, T2 did not reveal any differences ($p>0.005$) when compared to C group for both genes. For the illustration of the purposes, figure 1 and figure 2 show the appreciated results of the fold changes regarding the gene expression of the CDR1 and CDR2 genes respectively.

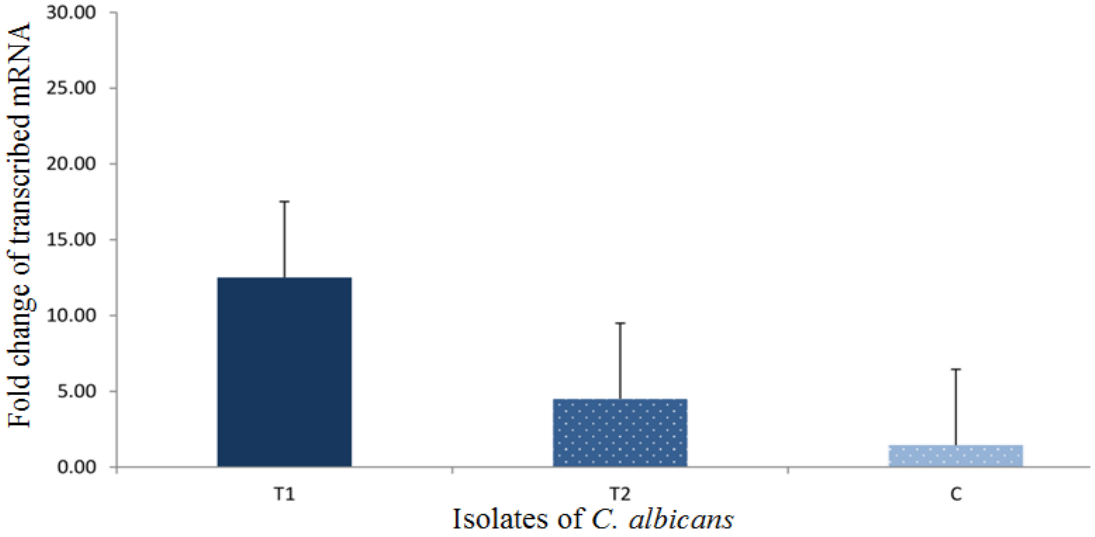

Fig. 1: Relative gene expression of the CDR1 gene in the *C. albicans* isolates. T1 showed significant ($p=0.000$) expression of the CDR1 gene and higher than T2 and C groups. T2 did not reveal any differences ($p>0.005$) when compared to C group.

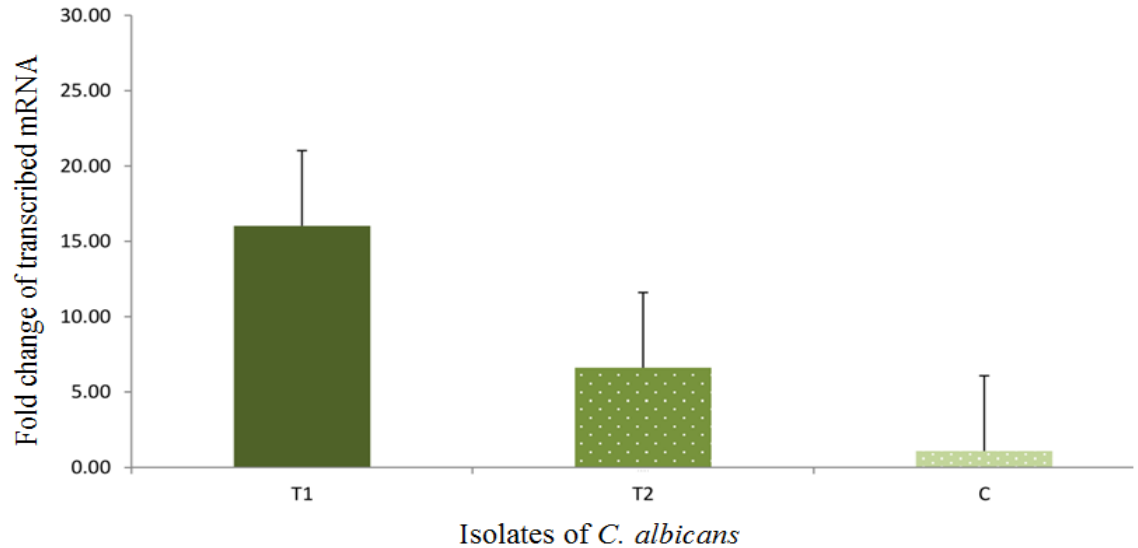

Fig. 2: Relative gene expression of the CDR2 gene in the *C. albicans* isolates. T1 declared significant ($p=0.002$) and ($p=0.000$) expressions of the CDR2 gene when compared to T2 and C groups respectively. T2 did not reveal any differences ($p>0.005$) when compared to C group.

DISCUSSION

The issue of antifungal drug resistance is an escalating problem in many diseases and more specifically in fungal and yeasted infections [16]. Especially in immunecompromised people and children, yeast infections in presence of anti-fungal drug resistance may worsen the infection situation and lead to endanger the life of patients [17]. The current study was aimed to explore some of the antifungal drug resistance reasons that are represented, here, by expression of genes responsible for this problem. CDR1 and CDR2 play important roles in developing this dilemma in *C. albicans* [18]. The results appeared in the present study assure that the emerging resistance by *C. albicans* against antifungal drugs in the city children was as a result to the activation of the CDR1 and CDR2 genes. Amazingly, children of under or more than 1 year of old showed significant expression of these genes in their *C. albicans* isolates. In recent published articles, [19] have inferred that these genes might place valuable impact on the development of the antifungal drug resistance especially against fluconazole. The authors, [20], have realized that antifungal drug resistant cases of *C. glabrata* were mainly produced by CDR1 and CDR2 genes, and this agrees with the current study results of the expression of these genes in the *C. albicans* of children. The CDR1 gene was found to be the main reason of developing drug resistance in *C. albicans* and thus complies with the present study findings [21]. Frighteningly, the isolates of the *C. albicans* from the children of less than one year of old gave expression of the CDR1 and CDR2 genes higher than those from children of older than one year of age and that is when compared to the gene expression of the nonpathogenic isolates of the investigated yeast. These variations could be reasoned to some factors but mainly to low immunity that infants characterized by, biofilm formation, and virulence of the yeast strains [22]. In conclusion, the current study results are important and provide deep knowledge about the problem of *Candida albicans* infections in mouths of children. This also alarms medical officials and researchers to take actions to fight this condition.

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