Original Article

Molecular Detection of *Candida albicans* Virulence Genes That Isolated from Periodontitis Patients in Al-Hillah City

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Abstract

Background: An inflammatory condition known as periodontitis causes the tooth's supporting tissues to be destroyed over time. One of the current dental problems is its high prevalence and detrimental consequences on quality of life. An oral candidiasis lesion often develops as a result of the development of a complex biofilm containing *Candida albicans* and other bacteria. **Objectives:** The objective of this study was to detect *C. albicans* virulence gene agglutinin-like sequence 1 and hyphal wall protein 1 (ALS1 and HWP1) by molecular method from clinical oral infection sample. **Materials and Methods:** One hundred fifty oral swabs were obtained from periodontitis patients who attended to a specific dental health center and outpatient clinics of dentistry in Al-Hillah city, Iraq during the period from April 2022 to September 2022. Patient age ranges from 5 to 72 years. The sample was taken by disposable cotton swabs. This swab was cultured on various culture media including CHROM agar and Sabouraud's dextrose agar for isolation of *C. albicans*. After that genomic DNA was extracted from confirmed colony. Virulence genes (ALS1 and HWP1) were detected by polymerase chain reaction technique. **Results:** The result showed that out of 150 samples 25 (16.66%) belong to *C. albicans* isolated; also the result showed that ALS1 gene detected in a rate 15 (22.05%) while HWP1 detected in a rate 19 (27.94%). **Conclusion:** The Als1 and HWP1 virulence genes were expressed in most isolates of *C. albicans* which suggests that the *ALS1 and HWP1* protein play an important role in the pathogenesis of infection.

Keywords: ALS1 gene, *Candida albicans*, Hwp1 gene, PCR, periodontist

Introduction

Candida albicans is the most common species of *Candida*; it is associated with both pathological and healthy oral conditions.[1] This opportunistic microbe does, in fact, belong to a healthy human digestive tract's commensal microbiota, but it has the potential to turn pathogenic when exposed to favorable local or general environmental conditions. Periodontitis is an oral disease that affects people all over the world and has a highly convoluted etiopathogenesis, involving dysbiosis, and host immunological responses that maintain the conditions for the development of periodontal disease in susceptible people.[2,3] Throughout the world, tooth decay is a contagious illness that causes tooth discomfort from dental cavities, which eventually ends in tooth loss.[4] Bisphosphonates (BPs) are medications employed widely in the management of metabolic bone diseases. Dental implants are a new therapy for the replacement of missing teeth depending on the osseointegration process. There is a considerable debate on the effect of oral BPs on the osseointegration process and subsequently on the success rate of dental implants and development of BRNOJ.[5]

Periodontitis is characterized clinically by attachment loss around teeth, forming periodontal pockets, and bone destruction.[6] The mouth cavity is a reflection of overall health and a target organ for a variety of negative drug-related responses. Numerous researches had connected

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oral carcinoma use. Some people hypothesized that periodontal attachment loss is likely to happen in women who use contraceptives due to increased gingival inflammation.[7] Numerous studies highlighted how the microbiota's variability and periodontal disease are related. Actually, the onset and progression of periodontitis have been linked to a variety of microorganism species, including Gram-negative anaerobic bacteria arranged in a complex biofilm.[8] Various virulence factors support *C. albicans* to infect such complex host niches.[9] Virulence factors are found in many attributes like adhesive and invasive expression on the cell surface, biofilm production, secretion of hydrolyses enzymes, and phenotypical switching.[10] Furthermore, *C. albicans* supports virulence factors, colonizes, and avoids host defense acts through oral tissue adherence.[11] Numerous putative virulence factors found in *Candida* species, such as phospholipases, secreted aspartyl proteinases, the *ALS* gene, hyphal wall protein (HWP), and cell wall glycoproteins (adhesions), helped *C. albicans* cells adhere to a variety of targets, such as cells of other microorganisms, abiotic surfaces, and various host cells.[12,13] Expression of numbers genes, including *ALS1*, *ALS3*, *ECE1*, and *HWP1*, is required for systemic candidiasis.[14] In particular, it is known that the gene hyphal wall protein 1 (*HWP1*) encodes a major *C. albicans* protein involved in several mechanisms, such as assembly of cell wall, intracellular signaling, and hyphal growth, through cross-linking to the glucans of the cell wall.[15] Additionally, *HWP1* increases *Candida* binding to epithelial cells, which is the first stage in colonization and it increases virulence in systemic candidiasis.[16] *Candida albicans* ALS1 gene product is a cell surface protein that improves the adherence to endothelial and epithelial cells and the investigation of the research on biofilm production mechanism shows that the expression level of the ALS1 gene is increased. The research revealed that the ALS1 gene is crucial for the production of biofilms.[17,18] Similar to the ALS proteins, HWP1 is a glycosylphosphatidylinositol-linked mannoprotein that is upregulated throughout the biofilm formation process and helps *C. albicans* adhere covalently to several surfaces and host cells.[18,19] This study was conducted to detect *C. albicans* virulence gene (ALS1 and HWP1) by molecular method from clinical oral infection samples.

Materials and Methods

Sample collection

One hundred and fifty cotton swabs from oral patients and the suspected plaque samples were transported to the laboratory of the microbiology department of medicine college after being deposited in 2 mL of sterile phosphate-buffered saline as a transport medium. All samples obtained from an individual were attended to a specific dental health center and outpatient clinics of dentistry in Al-Hillah city, Iraq, during the period from April 2022 to September 2022, after taking permission from the patients for examination and sampling.

Detection of *yeast*

Colony morphology and microscopic examination

Colony was diagnosed after growth on culture media (CHROM agar medium and Sabouraud's dextrose agar with chloramphenicol for selective isolation and culture of yeasts) based on its morphological characteristics (colony form, size, color, borders, and texture) according to Nas *et al.*[19]

DNA extraction

DNA was extracted according to Geneaid kit. In accordance with the manufacturer's recommendations, 4–6 yeast colonies were suspended in 1.5 mL of sterile 0.9% NaCl. The microtubes were centrifuged at 1,216,000*g* for 2 min; the supernatant was collected and was washed once with 2-mM EDTA (pH 8). The microtubes were then centrifuged for 10 min at 5000*g*; the supernatant was discarded and the pellet was stored. Nanodrop spectrophotometer was used for checking the concentration and purity of DNA that

Table 1: Primer and condition for amplification *Candida albicans virulence genes*

C albicans Genes		Sequence of Primer (5' -------- 3')	PCR amplicon size	Condition	References
ALS1	F	GACTAGTGAACCAACAAATACCAGA	318	**Initial Denaturation** (94C° for 5 min No.of cycle 35)	[21]
	R	CCAGAAGAAACAGCAGGTGA		**Denaturation** (94C° for 1 min)	
				Annealing (55C°for 30sec)	
				Extension (72C°for 1min)	
				Final extinction (72C°for 5min)	
HWP1	F	ATGACTCCAGCTGGTTC	503	**Initial Denaturation** (94C° for 5 min No.of cycle 35)	[22]
	R	TAGATCAAGAATGCAGC		**Denaturation** (94C° for 30 sec)	
				Annealing (55C°for 40sec)	
				Extension (72C°for 1min)	
				Final extinction (72C°for 5min	

were extracted, checked, and measured by reading the absorbance at 260/280 nm.

Primer and uniplex polymerase chain reaction

Molecular detection was conducted by uniplex polymerase chain reaction (PCR) with a primer as shown in Table 1.

Polymerase chain reaction ***mixture***

The reaction of PCR for detection of *C. albicans* virulence gene was done in a final volume, as shown in Table 2.

*Polymerase chain reaction****thermocycler program***

PCR conditions were used to detect virulence genes of *C. albicans*, as shown in Table 1. After that, agarose gel electrophoresis confirmed the PCR amplicon in the concentration of 1.5% agarose.[20]

Statistical analysis

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS) software program. The statistical difference between groups was ascertained using the Pearson chi-square test.

Table 2: PCR reaction volume

Content	Volume (ml)
Master Mix	7.5
DNA template	5
Forward Primer	2.5
Reverse Primer	2.5
Nuclease Free Water	2.5
Total	20

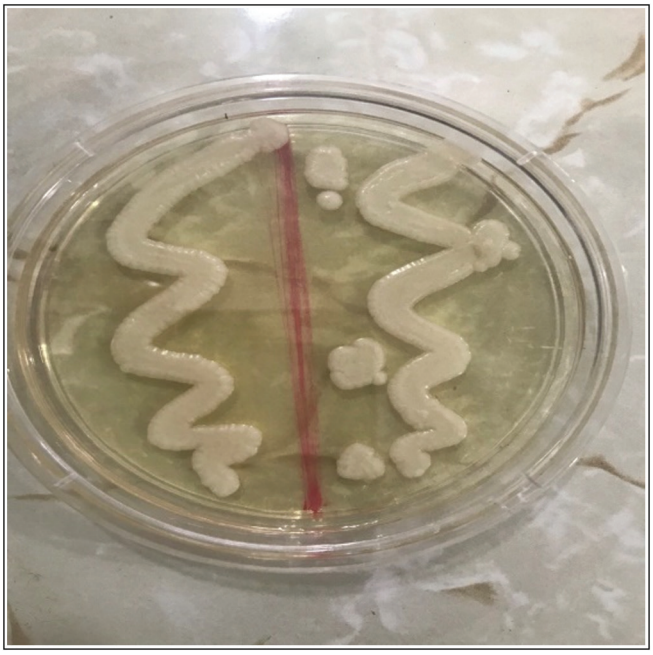

Figure 1: Colony morphology of *C. albicans* on Sabouraud chloramphenicol medium

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 1234 (including the number and the date on March 1, 2021) to get this approval.

Results

Cultural and molecular detection of isolates

This study was conducted on 150 oral cotton swabs, during the period from April 2022 to September 2022. The results indicated that clinical specimens were distributed into 25 (16.6%) specimens as *C. albicans* and 125 (83.3%) specimens negative for *C. albicans*; all these specimens were cultured on different media. After the recording of patient information, the obtained samples were cultured on a Sabouraud chloramphenicol medium and Chroma agar media for the isolation of yeasts [Figure 1]. Pure colonies of yeast were identified by culturing on CHROM agar as shown in Figure 2.

The result of this study shows that the agglutinin-like sequence 1 (ALS1) gene detection by using PCR was 13 (52%) and was positive for ALS1 gene with amplicon 318 bp, as shown in Figure 3 and Table 3.

This study detected other *C. albicans* virulence gene (*HWP1*) in which 17 (68%) isolates were found out of 25 isolates of *C. albicans* with amplicon 503 bp, as shown in Figure 4 and Table 3.

Figure 2: Colony morphology of *C. albicans* on CHROM agar

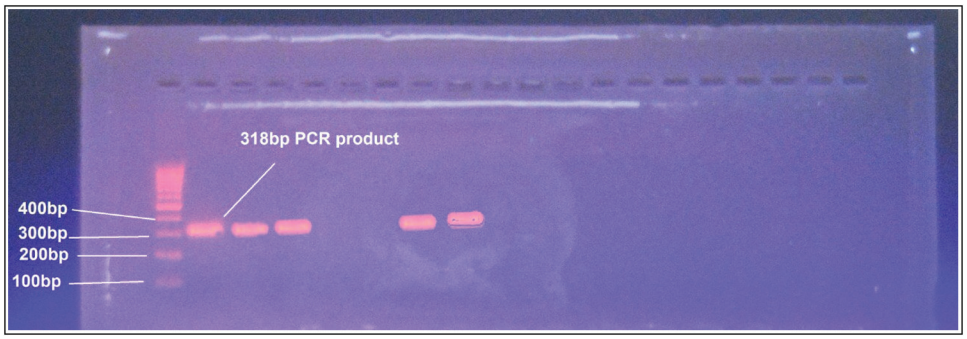

Figure 3: Gel electrophoresis of PCR amplicon (ALS1 genes (318 pb) with in *C. albicans*) on 1.5% agarose gel at 7 V/cm for 30 min. Lane 1: 1000 bp DNA ladder

Table 3: PCR detection percentage of *C albicans* virulence gene

Gene	Positive Number	Negative Number	Total
C. albicans ALS1	13(52%)	12(48%)	25(100%)
C. albicans HWP1	17(68%)	8(32%)	25(100%)

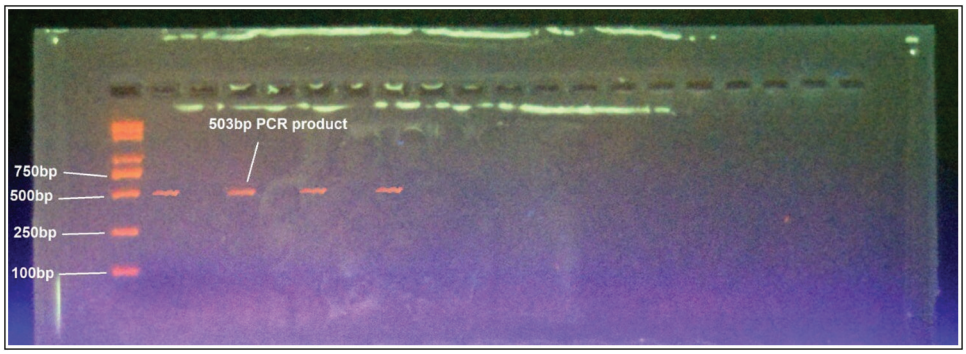

Figure 4: Gel electrophoresis of PCR amplicon (HWP1 genes (503 pb) in *C. albicans*) on 1.5% agarose gel at 7 V/cm for 30 min. Lane 1: 1000 bp DNA ladder

Discussion

Periodontitis is a complicated inflammatory condition that affects the tooth's supporting components and is linked to other inflammatory diseases of the system.[23] The identification of *C. albicans* from other *Candida* spp. was based on morphological characteristics (colony form, size, color, borders, and texture).[24,25] *Colony of C. albicans on the Sabouraud chloramphenicol medium is smooth and creamy white*.[26] The result *showed C. albicans isolated were higher* than the rates of results conducted in Iraq by Sardi *et al.*[27] who found that the rates of isolated *C. albicans* from oral infection were 8 (7.4%) from 108 of the total sample number.

Molecular detection of ALS1 gene was similar to a study conducted by Ali *et al.*[28] who isolated the ALS1 gene in 12 from 25 isolates. The result of the present study disagrees with the study conducted in Iraq by Mohammed *et al.*[29] where they found that 100% of *C. albicans* isolates from oral and vaginal infection are ALS1 positive. This difference may be due to the quantity of samples examined, the pathogenicity of the strains investigated, and the methods used, or the microorganism may contain other genes responsible for the same virulence factors.

The Als1–Als9 genes produce the ALS proteins, which are crucial extracellular elements for colonization and adherence.[30,31] Additionally, it has been shown that the virulence factors SAP4 and HWP1 are crucial for the development of biofilms, host tissue damage, and hyphae production, respectively.[20-22,24-32]

It has been reported that the HWP1 gene allowed *C. albicans* to bind covalently to epithelial cells, and previous studies have demonstrated that the association between the HWP1 surface protein and ALS1/3 is required for the start and growth of biofilm formation. ALS1, ALS3, and HWP1 genes were proposed to play complementary roles in biofilm formation.[33] Additionally, it was found in numerous studies that a number of genes, including HWP1 and HWP2, were primarily responsible for the production of biofilms, but HWP1 was the crucial component.[20-22,24-34] Previous research suggested that HWP1 is a crucial substrate that may help *C. albicans* to adhere covalently to host cells.[34] This finding supports the

idea that the HWP1 gene is crucial for the development and progression of *Candida* infections at different tissue sites.[35,36]

Conclusions

The Als1 and HWP1 virulence genes were expressed in most isolates of *C. albicans* which suggests that the *ALS1 and HWP1* protein play an important role in the pathogenesis of infection.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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