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Hwp1 gene Expression of *Candida albicans* and Study its role in adherence

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ABSTRACT

Candida albicans is an opportunistic fungal pathogen found as mycoflora in the human body surfaces. Sevral genes play a crucial rule in its virulence including Hwp1 (hyphal wall protein 1), BCR1 and ALS gene family. Hwp1 gene is a responsible for coding a cell surface protein, which mediates biofilm formation in *candida albicans*. Here we investigated the presence of the HWP1gene was characterised among *Candida albicans* isolates in women with recurrent vaginal canididasis and further we studies its role in cell adherence. We used 50 *Candida albicans* clinical isolates resistant to Fluconazole. RNA (of samples were extracted using glass bead and lysis buffer and cDNA was synthesized using reverse transcriptase enzyme. Expression of (HWP1) gene was analysed using reverse transcriptase-plolymerase chain reaction (RT-PCR). The ability of adherence of the isolates with or without the expression of HWP1 were characterized using Hela cells. Statistical analysis were performed using t-test and two-way ANOVA SPSS software. Our results showed that the HWP1 gene were present in 47 samples (94%) out of 50 isolates, 27 samples (57%) had expression of HWP1. The result of adherence assay revealed that the isolates with the expression of HWP1 gene and control isolates was statistically different ($p<0.05$). In conclusion, the isolates with the expression of HWP1 gene has the higher ability to adhere the epithelial mucosal cell surface. Our data support a positive correlation between the expression of HWP1 gene and the ability of adherence to epithelial cells.

1. Introduction

Candidiasis is a fungal infection caused by commensal fungi mainly *Candida albicans.* This fungus mainly causes mucosal and systemic diseases, and sometimes serious infections in immunocompromised patients (Calderone, 2002).

The underlying predisposing factors such as increased in PH, *Candida* spp. mainly cause a variety of mucosal diseases such as vulvovaginal candidiasis which is an important gynecology disorder in women world wide (Sobel et al., 1998). Clinical manifestations can be non–specific, but may including vaginal discharge, irritation. About three-quarters of all women suffer from vaginitis at least once during their life time (Sobel, 2007). Because of increasing antifungal resistance and

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creating recurrent candidiasis, identification and detection some genes responsible for adherence is very important in therapy (Mardh et al., 2003). There are many mechanisms involved in the development of adherence in yeasts such as Overexpression of some adhesion proteins, mutations on another target protein, including mannoproteins (Frinkel, 2012).

It has been established that genes family contribute to generate Adherence of microorganisms to host surface is a critical step in the development of biofilm and initiation of infection. Adhesion of *C.albicans* is required to some adhesion molecules, such as family, hyphal wall protein *(HWP)* (Dwivedi et al., 2011) and cell wall glycoproteins ALS (Agglutinin-Like Sequence). (Nobile and Mitchell, 2006; Chandra et al., 2001).

HWP1 encodes an adhesion receptor that operates through a transglutaminase-mediated mechanism which Plays a critical role in adhesion and biofilm formation. Hwp1p are predicted to be cross-linked to the 1,6-glucans of the cell wall of *candida albicans* (Staab et al., 2004).

Among them, it has been shown that genes have adhesive function in *C.albicans* and associated with pathogenesis of *C.albicans* (Fu et al., 2002).

Capable of forming biofilm, Because of the increasing prevalence of antibiotic resistance, it is necessary to find a sterategy for biofilm inhibition and therapy.

The purpose of the present study was to determine the presence of *Hwp1* gene among the isolates by specific primers in PCR methods. Also Expression of HWP1 and its correlation with cell adherence was evaluated.

2. Matherials and Methods

2.1. Sample collection and Identication of C.albicans

In this Study, About 50 Vaginal sampling that collected in pervious study and confirmed by PCR-RFLP technique by using *Msp*I restriction enzyme (Ferementas) were examined (Roudbary et al., 2013). Also all of isolates were resistant to fluconazol. We had informed history from all patients.

Samples cultured on Sabaouraud Dextros agar containing Chloramphenicol (Merck, Germany) and incubated for 48 h at 35°C. Microscopic observation of *Candida* pseudohypha or filamentous blastoconidia in direct preparations was done.

2.2. DNA and RNA Extraction

DNA extraction was performed using by glass bead and lysis solution as described previously by (Yamada et al., 2002).

For RNA extraction we used the methods described previously, in brief about 10^3 CFU (Colony forming unit) of a fresh colony was transferred to a 1.5 ml eppendorf tube and then 300 µl of lysis buffer (200 mM Tris-Hcl, pH7.5, 10 mM EDTA, 0.5 M NaCl), 300 µl of phenol- chloroform-Isoamillalchol (1:24:25) and 200 µl of glass beads, with a diameter of 1 mm, were added and the tube was vigorously shake for 60 minutes. Then, the sample was centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a clean tube and 400 µl of chloroform was added. After centrifuging as the previous conditions, the aqueous phase was transferred to a clean tube and then 1 volume of cold isopropanol and 5 of 3M sodium acetate (pH: 5.2) were added and was kept at -20°C for 10 minutes. After that, the sample was washed by 70% ethanol. Then 30 µl distilled water was added and the sample was kept at -20°C.

2.3. DNase treatment

RNA treatment was performed using a two-step process: First a sterile microtube without nucleaze 10µl RNA was added, 1µl 10X DNase 1 reaction buffer, 1µl RNase-free and DNase1 was added to the microtube which contain 1-2µl RNA, microtube for 30 min at 37°C were incubate. In continue 1µl EDTA 25 mM added to each microtube and microtube for 10 min at 65°C were incubate. RNA Concentration determined by absorbance in 260 nm.

2.4. cDNA synthesis and RT-PCR Assay

The cDNA was synthesized using 2-step RT-PCR kit (ViVantis, Malaysia) following manufacture's instruction and was stored at -20°C. 2 µl 10 X Viva Buffer A, 18.5µl Distilled water, 1-2µl cDNA, 0.35µl forward primer and 0.35 µl revers primer (Table 1). In continue The microtube put into thermocycling device: initial denaturation step at 94°C for 5 min followed by 35 cycles

consisting of denaturation (94°C for 1 min), annealing (58°C for 1 min), and extension (72°C for 1 min) were followed by a final extension step at 72°C for 3 min. Appropriate negative and positive controls were included in each test running. And final 25 µl of the final PCR product should be run out on a 1% agarose gel, viewed and blotted for band verification by hybridization. ACT1 was used as a normalized gene (house keeping gene) in all molecular tests. We used ACT1 gene for positive and confirmation of PCR process.

2.5. PCR with specific primer gene

The confirmed *C.albicans* isolates were used in PCR analysis for presence genes. *ACT1* was used as a control housekeeping gene (Table 1). PCR reactions were carried out in a reaction mixture with total volume of 25 µl containing 20 ng of genomic DNA, 20 µl sterile water, 2.5 µl 10x Taq polymerase buffer, 0.3 µl dNTPs (10 mmol l^{-1}), 1 U Taq DNA polymerase and25 pmol l^{-1} from each primer.PCR reactions were denatured for 5 min at 94°C and subjected to 40 cycles at 94°C (30 s), 58°C (30 s) and 72°C (30 s). A final 7 min extension at 72°C is completed the reaction.

2.6. Adherence assay

We used previously methodology according Robert et al., 1980. Brifly hella cells were cultured in RPMI 1640 with its supplement. Then 1×10^5 hella cells

Co–cultured with1×10^5 isolated which Hwp1 experesion and without experetion on microtiter plate. After 24 h incubation, supernatant of wells whose contain non-adherent *candida* was cultuered on SDA medium. Also adherence candida aspirated by tripsin and cultured on SDA CFU (Colony Forming Unit) were count after 24 h.

Table 1. Hwp1and *ACT1* specific primers for PCR and RT-PCR amplification

Primer name	Sequence (5'→3')	PCR Product
ACT1-F	CCAGCTTTCTACGTTTCC	200bp
ACT1-R	CTGTAACCACGTTCAGAC	
HWP1-F	GCTGGAACAGAAGATTCAGG	572bp
HWP1-R	GCTGGAACAGAAGATTCAGG	

3. Result

PCR analysis detected that HWP1 gene were present in 47 samples (94%) out of 50 isolates under study. The result of RNA extraction was shown in (Figure1).

RNA extract of isolates showed Among the 50 samples, 27 samples (57%) had expression of HWP1 by using RT-PCR technique. results of PCR and RT-PCR analysis are shown in (Figure 2 and 3).

The result of adherence assay exposed that the isolates with the expression of HWP1 gene and the isolated whitout the expression of HWP1 gene was statistically different ($p<0.05$) that is shown in (Table 2 and 3).

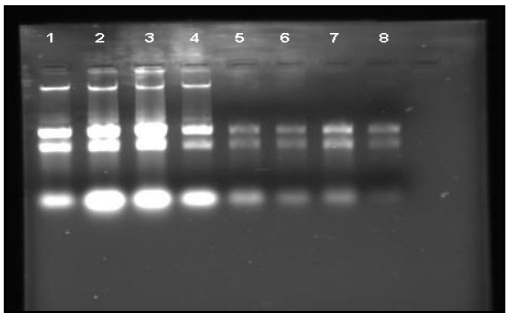

Figure 1. line1-4; RNA extraction from Clinical isolates of *Candida albicans.* line5-8; Traetment of DNA with DNase and elimination of DNA.

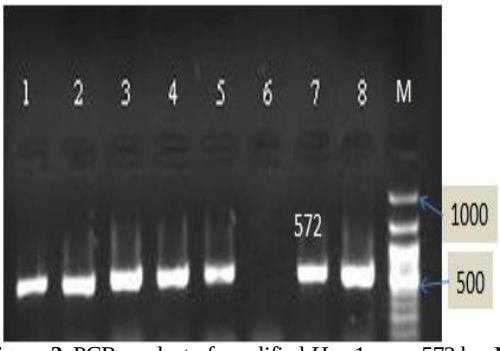

Figure 2. PCR product of amplified *H*wp1genes 572 bp. M: Molecular wight marker (100bp). lane6 negative control. lane1-5 and 7,8 clinical isolates of *Candida albicans*.

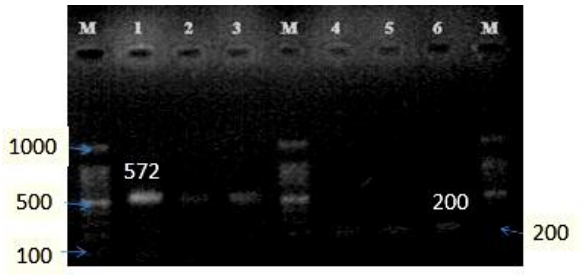

Figure 3. lane1-3 HWP1 gene expression in *candida albicans*. lane 4-6 ACT1 gene amplication.M.Marker 100bp

Table2.The number of colonies (CFU) of yeast cells binding and not binding to vaginal epithelial cells(Hella Cells)

CFU Soup of the yeast supernatant (non-sticky)	CFU yeast sediment ections (adherent cells	Expression of Hwp1	Isolates
10^4×1	10^4×7	+	C1
10^4×1/3	10^4×8/5	+	C2
10^4×1/5	10^4×6/7	+	C3
10^4×0/3	10^4×4/5	+	C4
10^4×0/5	10^4×4	+	C5
10^4×1/5	10^4×7/5	+	C6
10^4×4/5	10^4×1/5	-	C1
10^4×3/4	10^4×1	-	C2
10^4×6	10^4×2	-	C3
10^4×6/5	10^4×2/3	-	C4
10^4×7	10^4×2/4	-	C5
10^4×7/5	10^4×1/2	_-	C6

Table3. Mean yeast cells adherent and non-adherent to vaginal epithelial cells(Hella cells)

CFU Soup of the yeast supernatant (non-sticky)	CFU yeast sediment sections (adherent cells	Isolated
10^4×1	10^4×6/3	Expression of HWP1
10^4×5/8	10^4×1/7	Lacking the gene expression HWP1

4. Discussion

In conclusion, our result indicated that there is a positive correlation between the presence of hwp1 gene in isolates of *C.albicans* and their capacity in cell adherence, A bout 47 (94%) of isolates that positive for hwp1 gene also were resistant to fluconazole, all of *C.albicans* isolates which positive for amplication of *hwp1* gene, were resistant to fluconazole.and more attachment to cells. The obtained data of afore mentioned assays compared with t-test and two way variance analyses. ($p< 0.05$). Previous Studies indicated that hwp1 expression is followed Bcr1 ,which regulator of biofilm formation that is related to adhesion to tissue cells (Nas et al., 2008).

it seems that other regulatory genes have contributed to the adherence mechanisms. Also the role of regulator genes in expression of the relevant virulence genes including *Hwp1*gene family and biofilm formation should not be ignored (Dwivedi et al., 2011).

Some genes including *hwp1* or als gene family caused binding to glycoproteins and mediated the adhesion of *C.albicans* to mucosal surfaces.which adhesion to help colonization. The n-terminal domain of hwp1 roles as a substrate for transglutaminase, then permitted cell to cell attachment (Calderon et al., 2010).

It has been shown that hwp1 gene plays a critical role in regulation of expression of several virulence associated hyphal form and other adhesions in *C.albicans*. Hwp1 experetion which contribut with hyphal formation helps to extanded biofilm, so resistance to antifungal agent, such as fluconazole (Iuliana and Richard, 2009).

According to our result hwp1 gene family in *C.albicans* had a essential role in adherence formation to host cells.

It is suggested that the isolates of *candida* which have the hwp1 genes, is more virulent and their ability to initiate the infection is greater. however, more studies are required to understand the exact mechanisms involved (Ganguly et al., 2011; Staab et al., 1999). In conclusion, Although treatment failure is common in patients with vaginal candidiasis and antifungal resistance is a potential problem, the understanding of correlation between resistance to antifungal treatment and presence of special genes is useful (Sobel, 2007).

Other factors, such as the immune status of the patient and delay in diagnosis, may contribute to the poor response to treatment.

Given that a regulatory network genes contribute to antifungal resistance in *C.albicans*, further investigation is required to explore the correlation between antifungal resistance and virulence potential of *C.albicans* dependent to the Gene.

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