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Phospholipase and Aspartyl Proteinase Activities of *Candida* Species Causing Vulvovaginal Candidiasis in Patients with Type 2 Diabetes Mellitus

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Few research had investigated the secretion of phospholipase and aspartyl proteinase from *Candida* spp. causing infection in females with type 2 diabetes mellitus. This research aimed to investigate the prevalence of vulvovaginal candidiasis (VVC) in diabetic versus non-diabetic women and compare the ability of identified *Candida* isolates to secrete phospholipases and aspartyl proteinases with characterization of their genetic profile. The study included 80 females with type 2 diabetes mellitus and 100 non-diabetic females within the child-bearing period. *Candida* strains were isolated and identified by conventional microbiological methods and by API *Candida*. The isolates were screened for their extracellular phospholipase and proteinase activities by culturing them on egg yolk and bovine serum albumin media, respectively. Detection of aspartyl proteinase genes (*SAP1* to *SAP8*) and phospholipase genes (*PLB1*, *PLB2*) were performed by multiplex polymerase chain reaction. Our results indicated that vaginal candidiasis was significantly higher among the diabetic group versus non-diabetic group (50% versus 20%, respectively) (p = 0.004). *C. albicans* was the most prevalent species followed by *C. glabrata* in both groups. No significant association between diabetes mellitus and phospholipase activities was detected (p = 0.262), whereas high significant proteinase activities exhibited by *Candida* isolated from diabetic females were found (82.5%) (p = 0.000). Non-significant associations between any of the tested proteinase or phospholipase genes and diabetes mellitus were detected (p > 0.05). In conclusion, it is noticed that the incidence of *C. glabrata* causing VVC is increased. The higher prevalence of vaginal candidiasis among diabetics could be related to the increased aspartyl proteinase production in this group of patients.

Keywords: *Candida*, phospholipases, aspartyl proteinases, vulvovaginitis, type 2 diabetes mellitus

Introduction

Candida is an opportunistic organism and usually not pathogenic except in a compromised host. *Candida* infections cause a wide range of diseases in humans, which range from superficial infections involving the outer layer of the stratum corneum of the skin to disseminated infection involving the lungs and vagina [27].

The range of patients at risk for invasive fungal infections continues to expand, beyond the normal host, to patients with acquired immunodeficiency syndrome; those immunosuppressed owing to therapy for cancer and organ transplantation; those suffering from diabetes mellitus; and those undergoing major surgical operations [7].

Hyperglycemia and diabetes mellitus are commonly encountered in severely ill patients. Increased serum glucose

level may lead to impaired monocyte and neutrophil adherence, and chemotaxis, as well as phagocytosis and pathogen killing. During *Candida* infection, the elevated glucose level in infected tissue increases *Candida* adherence and invasion [25].

Diabetic females are usually at risk of vulvovaginal candidiasis (VVC) [8], with *Candida albicans* as the most common species isolated. Recently, vulvovaginal infection with *Candida* species other than *C. albicans* has been reported with remarkable increase in frequency in non-diabetic groups, possibly due to the widespread empirical use of antifungal drugs [7].

Many virulence factors attribute to enhance *Candida* colonization; adherence, biofilm formation, extracellular enzymes secretion, and dimorphism [16, 23]. Hydrolytic enzyme secretion during infection increases the ability of organisms for adhesion, invasion, as well as destruction of immune factors in the host, in addition to acquisition of nutrients. These enzymes include secreted aspartyl proteinases (SAP), phospholipases (PL), and hemolysins [5].

The phospholipases in general catalyze the hydrolysis of phospholipids, the main part of cell membranes in humans. The phospholipase family is divided into four different classes (A, B, C, D) [16], but only the products of the *PLB1* and *PLB2* genes have been detected extracellularly [20].

Secreted aspartyl proteinases are encoded by 10 members of the gene family (*SAP1* to *SAP10*). All *Candida* species secrete proteinases, but non-*C. albicans* appear to do so at a lower level. These genes exhibit differential expression profiles at different stages and sites of infection [3, 5].

The most highly expressed secreted proteinase is that encoded by the *SAP2* gene, which is able to digest human albumin, keratin, and hemoglobin, and also has the ability to destroy secreted immunoglobulin A. *SAP2* allows *C. albicans* to destroy host barriers by degradation of proteins with subsequent deep penetration into tissues and bloodstream. In addition, *SAP2* has the ability for degradation of extracellular proteins into oligopeptides, which can be taken up by transporters encoded by the *OPT* gene family. Thus, *SAP2* is considered a critical factor for *Candida* growth in the human host that enables the use of host proteins as a nitrogen source [4].

The current study aimed to investigate the prevalence of vulvovaginal candidiasis in diabetic versus non-diabetic females referred to the Gynecology Outpatient's Clinic at Fayoum University Hospital and to compare the ability of identified *Candida* isolates in both groups to secrete phospholipases and aspartyl proteinases *in vitro* with characterization of their genetic profile.

Materials and Methods

Study Population

This study included 80 females with type 2 diabetes mellitus (DM) and 100 non-diabetic females (as a control group) within the child-bearing period referred to the Gynecology Outpatient's Clinic of Fayoum University. Demographic data of each patient were collected. Clinical characteristics were reported, including symptoms of vulvovaginal candidiasis, DM, pregnancy, presence of underling diseases, and previous antibiotics treatment and investigations (*e.g.*, fasting blood glucose level, glycosylated hemoglobin (HbA1c)). Vaginal examination was performed for signs of VVC. Patients with pregnancy, and recent history of antibiotic treatment, corticosteroid or immune-suppressive therapy, or history of other immune-compromising diseases were excluded from the study to rule out any risk factor for VVC other than DM. The study was approved by the Fayoum University ethics committee.

***Candida* Isolates**

Two upper vaginal swabs were obtained from each patient. The specimens were transported and processed within 2 h at the Medical Microbiology and Immunology Department, Faculty of Medicine, Fayoum University. The first swab was used to prepare a smear for Gram staining, and the second swab was inoculated on a Sabouraud Dextrose Agar (SDA) plate (Oxoid Ltd., Hampshire, UK), with chloramphenicol, and incubated at 37°C for 24−48 h. To ensure purity of the *Candida* isolates, separate yeast colonies were subcultured on SDA. Pure *Candida* cultures were suspended in sterile glycerol broth vials and stored at −20°C [13, 26]. Presumptive identification of the isolates was performed by examination of colony morphology, microscopic examination of Gram-stained preparations, and germ tube test [28]. Further identification of gram-positive yeast cells to the species level by API *Candida* (bioMerieux, Marcy-I'Etoile, France) was performed according to the manufacturer's instructions. The API *Candida* strip is composed of 10 tubes with dehydrated substrates, which enable the performance of 12 identification tests (enzymatic reactions and sugar acidification). During incubation, reactions produced and were revealed by spontaneous color changes so it can be read visually according to the reading table. Identification was obtained by using the identification software supplied by the manufacturer.

Phenotypic Screening of Extracellular Phospholipase and Aspartyl Proteinase Enzymes

The fully identified *Candida* isolates were screened for their extracellular phospholipase and proteinase activities using egg yolk and bovine serum albumin media, as described by Mohan and Ballal [19]. All assays were conducted in triplicate in two

occasions for each isolate. *Candida albicans* ATCC10231 was used as a positive control. Proteinase activity (Prz value) or phospholipase activity (Pz value) was obtained by calculating the ratio between the diameter of the well and the total diameter (calculated mean of the replicates) of the precipitation zone for phospholipase or proteolytic unstained zone for proteinase. The Pz or Prz value of 1.0 was evaluated as negative (-), 0.99–0.9 as weak (+), 0.89–0.8 as moderate (++), 0.79–0.7 as relatively strong (+++), and <0.69 as very strong (++++) [21, 28].

Genotypic Characterization of *Candida* Phospholipases and Aspartyl Proteinases

One primer set each for *SAP1* to *SAP8, PLB1,* and *PLB2* was designed according to Naglik *et al*. [20] (Table 1). None of the primer sets amplified any regions containing introns. All the amplified gene products were sequenced at TIBMolbiol, GmbH, Berlin, Germany. Genomic DNA was extracted from 10^8 *Candida* suspensions in glycerol broth by using a High Pure PCR Template Preparation kit (Roche Lifescience, Penzberg, Germany) according to the manufacturer's instructions.

Three multiplex PCR amplifications were performed. The first amplification was for the *PLB1* and *PLB2* genes with initial denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec and extension at 72°C for 30 sec; followed by a final elongation step at 72°C for 10 min. The second multiplex PCR amplification was for the *SAP2, SAP5, SAP6,* and *SAP 8* genes with initial denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 30 sec; followed by a final elongation step at 72°C for 10 min. The third multiplex PCR amplification was for the *SAP1, SAP3, SAP4*, and *SAP7* genes with initial denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 46°C for 30 sec, and extension at 72°C for 30 sec; followed by a final elongation step at 72°C for 10 min.

Amplification was done in a 25 µl total reaction volume

Table 1. Primer sets used to detect phospholipase and aspartyl proteinase genes in *Candida* spp. [20].

Gene primer	Sequence	Product (bp)
PLB1		
Forward	5'-CCT ATT GCC AAA CAA GCA TTG TC-3'	179
Reverse	5'-CCA AGC TAC TGA TTT CAC CTG CTC C-3'	
PLB2		
Forward	5'-GTG GGA TCT TGC AGA GTT CAA GC-3'	270
Reverse	5'-CTC AAA GCT CTC CCA TAG ACA TCT G-3'	
SAP1		
Forward	5'-TCA ATC AAT TTA CTC TTC CAT TTC TAA CA-3'	161
Reverse	5'-CCA GTA GCA TTA ACA GGA GTT TTA ATG ACA-3'	
SAP2		
Forward	5'-AAC AAC AAC CCA CTA GAC ATC ACC-3'	178
Reverse	5'-TGA CCA TTA GTA ACT GGG AAT GCT TTA GGA-3'	
SAP3		
Forward	5'-CCT TCT CTA AAA TTA TGG ATT GGA AC-3'	231
Reverse	5'-TTG ATT TCA CCT TGG GGA CCA GTA ACA TTT-3'	
SAP4		
Forward	5'-TTA TTT TTA GAT ATT GAG CCC ACA GAA A-3'	171
Reverse	5'-GCC AGT GTC AAC AAT AAC GCT AAG TT-3'	
SAP5		
Forward	5'-AGA ATT TCC CGT CGA TGA GAC TGG T-3'	277
Reverse	5'-CAA ATT TTG GGA AGT GCG GGA AGA-3'	
SAP6		
Forward	5'-CCC GTT TTG AAA TTA AAT ATG CTG ATG G-3'	187
Reverse	5'-GTC GTA AGG AGT TCT GGT AGC TTC G-3'	
SAP7		
Forward	5'-GAA ATG CAA AGA GTA TTA GAG TTA TTA C-3'	196
Reverse	5'-GAA TGA TTT GGT TTA CAT CAT CTT CAA CTG-3'	
SAP8		
Forward	5'-GCC GTT GGT GCC AAA TGG AAT AGT TA-3'	256
Reverse	5'-ATT TGA CTT GAG CCA ACA GAA TGG T-3'	

Table 2. Distribution of *Candida* species among diabetic and non-diabetic females.

Species	Non-diabetics (*N* = 20) No. (%)	Diabetics (*N* = 40) No. (%)	*P*-value
C. albicans	15 (75%)	20 (50%)	
C. glabrata	4 (20%)	17 (42.5%)	
C. famata	0 (0%)	2 (5%)	0.175
C. tropicalis	0 (0%)	1 (2.5%)	
C. kruesi	1 (5%)	0 (0%)	

containing 12.5 µl Master Mix PCR buffer (Roche), 6 µl of DNA, 200 mM of each deoxynucleotide triphosphate (dATP, dTTP, dCTP, dGTP), 0.5 µl of Taq polymerases, and 1 µl (20 pmol) of each specific primer (Roche). Distilled water was used to bring the reaction volume to 25 µl. Amplification was carried out in a DNA Thermal cycler (Biometra, Germany). The products of PCR were separated by electrophoresis on a 1.5% agarose gel. The Gene Ruler 100 bp DNA ladder was used as a DNA size marker (Roche).

Statistical Analysis

Data were statistically described in terms of frequencies (number of cases) and percentages. The Chi square (χ^2) test was used to compare between the study groups. The Exact test was used instead when the expected frequency was less than 5. *P* value ≤0.05 was considered statistically significant. All statistical calculations were done using the computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) ver. 15 for Microsoft Windows

Results

The mean age (± SD) of the diabetic group was 31.62 (± 6.23) years versus 29 (± 7.44) years for the non-diabetic group. The mean HbA1c was 8.12 ± 1.09% and the mean fasting blood glucose level was 172.3 ± 27.3 mg/dl for the diabetic group. Vaginal candidiasis was significantly higher among the diabetic group versus non-diabetic group (50% (40/80) versus 20% (20/100), respectively) ($p = 0.004$). *C. albicans* was the most prevelant species followed by *C. glabrata* in both groups. No significant association was found between species of *Candida* causing VVC and diabetes mellitus ($p = 0.175$) (Table 2).

Phenotypic Screening of Extracellular Phospholipase and Aspartyl Proteinase Enzymes

Although *Candida* strains isolated from diabetic females showed strong extracellular phospholipase activities (57.5% and 27.5% for relatively strong and very strong producers, respectively), insignificant associations between DM and phospholipase activities were detected ($p = 0.262$). On the other hand, highly significant strong proteinase activities exhibited by *Candida* isolated from vaginal samples of diabetic females were detected (82.5%) ($p = 0.000$) (Table 3).

Non-significant association was detected between phospholipase and proteinase activities of *Candida* isolated from the diabetic group and HbA1c (p = 0.67 and 0.37, respectively) or fasting blood glucose level (p = 0.8 and 0.88, respectively).

When the association between *Candida* species and their phospholipase and proteinase activities was studied, we found highly significant proteinase production by *C. albicans* and *C. glabrata* in the diabetic group ($p = 0.000$) (Table 4).

Genotypic Characterization of *Candida* Phospholipases and Aspartyl Proteinases

By studying the frequency of the *PLB1* and *PLB2* genes among *Candida* species, non-significant differences were

Table 3. *In vitro* phospholipase and proteinase activities exhibited by *Candida* isolated from vaginal samples of diabetic and non-diabetic group.

Extracellular enzymes	Non-Diabetics (*N* = 20)	Diabetics (*N* = 40)	*P*-value
Phospholipase	No. (%)	No. (%)	
(+)	1 (5%)	0 (0%)	
(++)	6 (30%)	6 (15%)	
(+++)	8 (40%)	23 (57.5%)	0.229
(++++)	5 (25%)	11 (27.5%)	
Total	20 (100%)	40. (100%)	
Proteinase	No. (%)	No. (%)	
(+)	0 (0%)	0 (0%)	
(++)	6 (30%)	1 (2.5%)	
(+++)	7 (35%)	1 (2.5%)	0.000*
(++++)	0 (0%)	33 (82.5%)	
Total	13 (65%)	35 (87.5%)	

*Highly significant.

Table 4. *In vitro* phospholipase and proteinase activities in relation to isolated *Candida* species.

Species	Phospholipase activity	Non-Diabetics (*N* = 20)	Diabetics (*N* = 40)	*P*-value	Proteinase activity	Non-Diabetics (*N* = 20)	Diabetics (*N* = 40)	*P*-value
C. albicans	(+)	0 (0%)	0 (0%)	0.427	(+)	0 (0%)	0 (0%)	0.000*
	(++)	4 (20%)	2 (5%)		(++)	4 (20%)	1 (2.5%)	
	(+++)	7 (35%)	12 (30%)		(+++)	6 (30%)	1 (2.5%)	
	(++++)	4 (20%)	6 (15%)		(++++)	0 (0%)	14 (35%)	
C. glabrata	(+)	1 (5%)	0 (0%)	0.062	(+)	0 (0%)	0 (0%)	0.000*
	(++)	2 (10%)	3 (7.5%)		(++)	2 (10%)	0 (0%)	
	(+++)	1 (5%)	10 (25%)		(+++)	1 (5%)	0 (0%)	
	(++++)	0 (0%)	4 (10%)		(++++)	0 (0%)	17 (42.5%)	
C. non-*albicans* non-*glabrata*	(+)	0 (0%)	0 (0%)	0.513	(+)	0 (0%)	0 (0%)	0.248
	(++)	0 (0%)	1 (2.5%)		(++)	0 (0%)	0 (0%)	
	(+++)	0 (0%)	1 (2.5%)		(+++)	0 (0%)	0 (0%)	
	(++++)	1 (5%)	1 (2.5%)		(++++)	0 (0%)	2 (5%)	

*Highly significant.

detected between the tested groups ($p = 0.361$ and 0.713, respectively). As regards the *SAP1-8* genes, *SAP1* and *SAP2* were the most detected genes in both groups (100% and 100%, respectively, for non-diabetics versus 95% and 95%, respectively, for diabetics) followed by *SAP5* (75% for both groups) and *SAP3* (80% for non-diabetics versus 67% for diabetics). No significant associations between any of the tested *SAP* genes and DM were detected (Table 5). Moreover, we did not find significant association between any of the tested *PLB* or *SAP* genes and *Candida* species ($p > 0.05$).

Discussion

To determine the prevalence of vulvovaginal candidiasis in diabetic females, 80 diabetic and 100 non-diabetic females within the child-bearing period were subjected to clinical and laboratory diagnoses of VVC. We detected a significantly higher prevalence of VVC among diabetics than non-diabetics, as out of 80 diabetic patients, 40 (50%) had VVC versus 20/100 (20%) in the non-diabetic group ($p = 0.004$). Similar to our results, Goswami *et al.* [8] reported a prevalence rate of 46% in the vagina of 78 diabetic women. Gunther *et al.* [10] had reported a significantly higher vaginal colonization rate in diabetic Brazilian women (18.8%) than in non-diabetic women (11.8%) ($p = 0.000$). The diabetic group had more symptomatic VVC and recurrent VVC (66.66%) than non-diabetics (33.33%). Tsang *et al.* [26] reported that the oral colonization of *C. albicans* in the diabetic group (36.2%) was significantly higher than that in the control group (23.8%).

The increased prevalence of *Candida* infection among diabetics is postulated to be due to either host- or pathogen-related factors. The site of infection (a host factor) has direct correlation with pathogen virulence factors. The vulvovaginal tissue has unique conditions that may induce *Candida* virulence. These include pH, temperature, adherence capacity, expression of *Candida*, and nutritional substance. In addition, increase of glucose levels in genital tissues facilitates yeast adhesion and growth. Therefore, vaginal epithelial cells bind to *Candida* with greater tendency in diabetic females than in non-diabetics [15].

Identification of the isolated *Candida* to species level revealed that *C. albicans* was the most commonly isolated species (75% in non-diabetics and 50% in diabetics)

Table 5. Incidence of tested genes among isolated *Candida* species from diabetic and non-diabetic females with vulvovaginal candidiasis.

Gene	Non-Diabetics (*N* = 20)	Diabetics (*N* = 40)	*P*-value
PLB1	19 (95%)	35 (87.5%)	0.361
PLB2	8 (40%)	18 (45%)	0.713
SAP1	20 (100%)	39 (97.5%)	0.476
SAP2	20 (100%)	39 (97.5%)	0.476
SAP3	16(80%)	27 (67.5%)	0.311
SAP4	12 (60%)	24 (60%)	1
SAP5	15 (75%)	30 (75%)	1
SAP6	11 (55%)	13 (32.5%)	0.094
SAP7	6 (30%)	15 (37.5%)	0.566
SAP8	2 (10%)	1 (2.5%)	0.209

followed by *C. glabrata* (20% in non-diabetics and 42.5% in diabetics), with no significant association between species of *Candida* causing VVC and diabetes mellitus ($p = 0.175$). These results partially agreed with Faraji *et al*. [7], who isolated 32 *Candida* strains causing VVC from 100 diabetic women and found that 65.5% were *C. albicans* and 18.7% were *C. glabrata.* Tsang *et al.* [26] reported that *C. albicans* is the predominant species isolated from the oral cavity of diabetic and control groups. The first step in establishing a yeast infection is binding to host mucosa. It seems that *C. albicans* is more adhesive than other non-*C. albicans* species. This could be the likely reason that this species is the most predominant rather than non-*C. albicans* species [9].

Previous studies had reported an increasing frequency in the occurrence of non-*C. albicans* species over time, in particular *C. glabrata*, which appears more commonly associated with VVC in some African and Asian countries [1, 6, 15]. This explains our results that *C. glabrata* prevalence in the diabetic group was 42.5% versus 50% for *C. albicans*.

Our results disagree with another study that found a higher prevalence of non-*C. albicans* species. Ray *et al*. [22] reported that out of 122 diabetic patients, 57% of VVC cases had *C. glabrata.* They explained their findings by the use of oral or local anti-*Candida* regimens to which non-*albicans* species, especially *C. glabrata,* are not sensitive.

The exact association between DM and VVC is still under investigation. Some studies suggest that impaired immune response associated with DM is the cause of recurrent VVC [17]. Previous studies have demonstrated a relationship between the increase in secretions and activities of hydrolytic enzymes and the increase in the pathogenic ability of *Candida* spp., leading to severe candidiasis [2, 11]. Thus, we have studied the ability of clinical strains of *Candida* spp. causing VVC in diabetic females to secrete phospholipase and aspartyl proteinase and tried to investigate the association between DM and the degree of secretion of phospholipase and aspartyl proteinase.

Our study revealed that 100% of *Candida* isolates produced phospholipase, with no significant difference between diabetic and non-diabetic groups ($p = 0.229$). As regards excreted aspartyl proteinase, *Candida* isolated from diabetics express higher significant strong abilities to secrete proteinase than that of non-diabetics (87.7% and 65%, respectively, $p = 0.000$); especially for *C. albicans* and *C. glabrata.* Yildirim *et al.* [30] reported that acid proteinase activities were significantly increased in the uncontrolled diabetic women with VVC in comparison with both the control group and the controlled diabetic group ($p < 0.05$). This may be due to the presence of a high concentration of vaginal glucose, which facilitates the growth of *Candida* and their adherence to vaginal epithelial cells, perhaps by increasing phospholipase activity [15]. The same findings have been reported by Tsang *et al.* [26] and Koga-Ito *et al.* [12], who investigated phospholipase and proteinase activities of *C. albicans* isolated from the oral cavity of diabetic and control groups. Their findings, which agree with the current study, indicated that most of the isolates produce phospholipase, with insignificant difference between both groups, but a significant increase in proteinase activity was linked to the diabetic group.

The present results disagree with Kumari *et al.* [14], who detected phospholipase production in only 57.74% of isolated *Candida* causing vulvovaginitis. Another study has demonstrated no significant increase in proteinase expression from *Candida* isolated from the oral cavity of patients with DM than those isolated from healthy individuals [18]. The diversity between our results and others may be explained by the difference in growth conditions and host factors that affect gene expression in qualitative and quantitative manners [24, 26].

Our results revealed non-significant association between phospholipase and proteinase activities of *Candida* spp. isolated from the diabetic group and HbA1c or fasting blood glucose level. The same findings were reported by Tsang *et al.* [26]. In contrast to our findings, Yildirim *et al*. [30] reported a significant increase in proteinase activities in uncontrolled diabetic women with VVC than in the controlled diabetic group ($p < 0.05$). This conflict between results indicates that the expression of hydrolytic enzymes by *Candida* spp. is a multifactorial process in diabetic patients and the elevated blood glucose level is not the only implicated factor.

Few studies have investigated the incidence of phospholipase B (*PLB*) and aspartyl proteinase (*SAP*) genes in *Candida* isolated from immune-compromised patients, especially diabetic patients. This study, to the best of our knowledge, is the first one to be done on *PLB* and *SAP* genes of *Candida* causing VVC in diabetic women.

Our study revealed insignificant differences in occurrence of *Candida* phospholipase B (*PLB1-2*) genes or aspartyl proteinase (*SAP1–SAP8*) genes between diabetic and non-diabetic women. The *PLB1* gene was positive in 87.5% of *Candida* isolates of diabetics and in 95% of non-diabetics. Whereas the incidence of *PLB2* in the control group was 40%, it was 45% among diabetics ($p > 0.05$). With regards the *SAP* genes, *SAP1* and *SAP2* were the most detected genes followed by *SAP5* in both groups. Our results are in

partial agreement with Naglik *et al*. [20], who analyzed *C. albicans* phospholipase B (*PLB1-2*) and aspartyl proteinase (*SAP1–SAP8*) gene expression during vaginal and oral infections and found insignificant difference of *PLB1* and *PLB2* expression between vaginal-infected patients and carriers. With regards the *SAP* genes, different expression profiles in *SAP* genes were obtained from different *C. albicans* strains isolated during symptomatic disease and in asymptomatic carriers. *SAP2* and *SAP5* were the most frequent genes expressed during both infection and colonization. It has been reported that the expression of *SAP2* is needed for disease development in a vaginitis model [3, 29].

Our results revealed that *SAP8* was detected in only 2.5% of the diabetic group and 10% of the control group. These results disagree with Naglik *et al*. [20], who detected *SAP8* gene expression in 75% of *Candida albicans* isolated from the vaginal-infected group and 32% in the colonized group. The study groups of Naglik and colleagues differed from ours as they compared between infection and colonization, whereas we studied infection only.

Various studies conducted in animal models infected with mutant strains of *Candida* lacking one or more *SAP* genes revealed that model strains deficient in *SAP1* or *SAP2* were attenuated, and those deficient in other *SAP* genes were not [3].

In conclusion, the higher prevalence of vaginal candidiasis among diabetics may be related to increased aspartyl proteinase production in this group of patients. It is noticed that there is an increase in the incidence of *C. glabrata* causing VVC. Awareness of *Candida* species causing vaginitis is needed for proper treatment owing to increased anti-*Candida* drug resistance in non-*C. albicans*, especially *C. glabrata*.

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