Prevalence of oral Candidiasis and Risk Factors in Diabetic Pateints

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Abstract:

Oral candidiasis has a strong association with diabetes. This study investigated the prevalence of candidiasis, effects of age and denture wearing on the isolation rate of *Candida,* virulence factors such as (germ tube (GT), gelatinase assay, phospholipase (PL) activities, biofilm (BF) formation) of different *Candida* species isolated from 40 confirmed diabetic patients with oral candidiasis and susceptibility of *Candida* isolates to antifungal agents. The prevalence of candidiasis was (67%) which is more prevalent in middle age (40-60) years (65%) and among females (65%). Sixty *Candida* isolates were identified among which *Candida albicans* represented (53.3%), followed by *C. glabrata* (31.7%), *C. tropicalis* (10%) and *C. krusei* (5%). In denture wearers, the isolation rate of all *Candida* species was 94.7% while in non denture wearers was 53.7%. The minimun inhibitory concentrations (MICs) of ketoconazle as the lowest concentration of antifungal that inhibited 100% of *C. krusei* while MIC for flucytosine that inhibited 90.6% and 84.2% of *C. albicans* and *C. glabrata* respectively, also the MIC for itraconazole and voriconazole that inhibited 93.5% and 81.2% of *C. albicans*. The MIC of amphotericin B was defined as the lowest drug concentration causing 100% inhibition of all *Candida* species*. Candida albicans and C. tropicalis* isolates were positive for all the virulence factors while *C. glabrata* and *C. krusei* were negative for GT, gelatinase and PL activities. *C. krusei* were positive only for BF formation. In Conclusion, *Candida spp.* in the oral cavity of diabetic patients are potentially pathogenic and can participate in infectious and inflammatory processes since they exhibit most of the virulence factors and resistance to most antifungals. Denture wearing, female sex and middle age are among the risk factors. Controlling serum glucose level and oral hygiene are essential in diabetics.

Keywords: *Diabetes mellitus, Denture wearer, Candida spp., Antifungal susceptibility, Virulence Factors.*

1. Introduction

Diabetes mellitus (DM) is a common disease worldwide. This disease has strong association with wide variety of infections **(Wild *et al.,* 2004).** Candidiasis is the most common

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mycotic infection in the oral cavities **(Abaci and Holiki-Uztan, 2011)** and it has a strong association with diabetes **(Manfredi *et al.,* 2006).** Several systemic or local contributing factors influence the balance between the host and yeast growth. The disturbance of this balance may result in transformation of the commensal *Candida* species to the pathogenic ones that lead to localized as well as disseminated infections **(Janet *et al.,* 2005).** The high prevalence of opportunistic infections especially the oral candidiasis in diabetics had always remained of debate **(Safia, 2010).**

Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to defects in insulin production and/or insulin action. Oral candidiasis has a significant association with the glycemic control and changes in salivary pH due to hyperglycemia **(Kumar and Kannan, 2010).** Numerous oral complications have been related to DM **(Sardi *et al.,* 2011).** The highest rate of colonization by different *Candida* species occurs in patients with poor glycemic control **(Javed *et al.,* 2007).**

Several predisposing risk factors have been demonstrated for oral candidiasis in diabetics such as bad oral hygiene, presence of oral dentures, age, smoking, xerostomia, and poor glycemic control **(Edward, 2011 and Shrimali *et al.,* 2011).** Denture wearers comprise a population commonly described as carrier of fungal species, especially *C. albicans* because of the ability of these pathogens to adhere and form biofilm on denture-fitting surfaces, which become a reservoir of infections **(Gasparoto *et al.,* 2009**). In addition, advances in life expectancy have caused broad interest in factors involved with well-being and health of the elderly people **(Caruso *et al.,* 2009).**

Commensal *Candida* spp. inhabit different sites of the oral cavity. However, given the immunosuppressive condition in DM, these yeasts can become more virulent and express pathogenicity. They have different virulence factors, including mechanisms of cell adhesion and invasion associated with the production of enzymes especially proteinase and phospholipase that aid in tissue degradation and facilitate their proliferation in the oral mucosa **(Sardi *et al.,* 2010).**

There are several antifungal medicines which can be used topically or systemically in the treatment of oral candidiasis polyenes which open channels in the fungal membrane, azoles which inhibits cellular membrane formation by interfering with ergosterol synthesis, 5-fluorocytosine whose entry to cell is mediated by the cytosine permease. Incorporation of 5-fluorouracil into RNA interrupts protein synthesis leading to cell death **(Casalinuovo *et al.,* 2004).**

The objectives of this study were to determine the prevalence of *Candida* infection in the oral cavity of diabetic patients, investigate the effects of age and denture wearing and other predisposing risk factors on the isolation rate of different *Candida* spp., to find out other risk factors

that predispose oral candidiasis, determine some virulence factors of *Candida* isolates, and their susceptibility to antifungal agents.

2. Methods and Materials

2.1 Patients and controls:

The study was carried out at General Department of Internal Medicine at Al-Zahraa-University Hospital, Egypt between June 2013 to August 2014, included 60 adult patients with type 2 diabetes mellitus (T-2DM) suspected to have oral candidaisis. Patients diagnosed based on blood glucose level and clinical examination, and under metabolic control in the form of insulin therapy, age ranged from 30 to 85 years, have signs and symptoms in the oral cavity suggestive of *Candida* infection such as discrete, confluent white plaques on the buccal mucosa, tongue, and sometimes the palate, gingival, and floor of the mouth or wipeable plaques, presence of at least two of erythema, warmth, tenderness or swelling **(Din *et al.,* 2006).** Predisposing factors for *Candida* infection such as maxillary denture wearing, periodontal diseases or other oral pathologies, gingival bleeding were reported. Patients who were on antibiotic, antifungal or corticosteroid therapy during the previous 4 weeks or patients have infections elsewhere in body were excluded from the study. Alcoholism, tobacco use, cancer, symptoms that could indicate systemic diseases, immunodeficiency, cardiovascular complication, neuropathy and pregnancy were also excluded .Complete medical and dental histories were recorded.

According to denture wearing, patients were divided into group I(a), included 19 patients with denture wearing; 7 males and 12 females, their age ranged from 47 to 79 years and group I(b), included 41 non denture wearing patients; 12 males and 29 females; their age ranged from 35 to 86 years. According to their ages patients were divided into: a) middle-aged (40-60) years and b) elder age (>60) years.

All the patients signed an informed consent form to participate in the study, which was approved by the local Ethics Committee. In addition, the study included 20 healthy control subjects (group III); 6 males and 14 females their age ranged from 30 to 58 years. Patients and controls were examined for signs and symptoms of oral candidiasis.

2.2Samples

Oral specimens were collected in the morning from the tongue, angle of the mouth, and buccal mucosa of patients with oral candidiasis. The samples were collected by sterile swabs, which

were rubbed on the lesions. Material was collected from underneath the denture (hard palate) and from the denture fitting surface.

2.3. Microbiological study

Cultures and identification: Oral swab samples were grown on Sabouraud's Dextrose (SD) agar (Oxoid, UK) with 1% chloramphenicol and incubated at 37°C for 48 hr. The obtained growth was described as mild (below 10 colonies), moderate (between 11 and 50 colonies) or heavy growth (over 50 colonies) **(Lamey et al., 1988).** All the isolates were stored in vial tubes containing SD broth plus 10% glycerol in a -80°C.freezer.

Identification was done by Gram stain, subculture on SD agar for purity, followed by inoculation on CHROMagar *Candida*, (BioRad France). Plates were incubated for 48 hr at 37°C. Pink to purple colored colonies indicates *C. albicans,* intense turquoise mat convex colonies indicates *C. tropicalis,* pale turquoise colored colonies with flat, shiny, smooth morphology (fish eye) indicates *C. glabrata* and turquoise-blue colonies with rough irregular outline indicates for *C. krusei.* Microscopic features of slide culture on Cornmeal-Tween 80 agar (Oxoid, UK) and germ tube formation test were done to confirm the identification *of Candida* species **(Kurtzman and Fell, 1998).**

Biochemical identification and Antifungal susceptibility studies

Integral system yeasts Plus *Candida* species (Liofilchem®, Italy) was used for biochemical identification and antifungal susceptibility of the isolates according to the manufacrurer in structions.

Minmum inhibitory concentration (MIC) :

E-test was done for determination of minmum inhibitory concentration (MIC) (Pfaller *et al*., 2002). *Candida* species strains were analyzed with regard to their susceptibility to 7 antifungal agents based on the minimal inhibitory concentration (MIC) in the Etest (bioMérieux SA). The following drugs and their respective minimum and maximum concentrations were used: caspofungin (CS) (concentration range, 0.002 to 32 μg/mL), amphotericin B (AP) (0.002 to 32 μg/mL), itraconazole (IT) (0.002 to 32 μg/mL), and fluconazole (FLU) (0.002 to 64 μg/mL).

The MICs were read as the lowest concentration at which the border of the elliptical inhibition zone of growth intercepted the scale on the Etest strip. For the MIC values that yielded results falling in between conventional serial 2-fold dilution, the next highest dilution was assigned **(Motta et al., 2010).**

Detection of virulence factors of *Candida* isolates

The following virulence factors of different *Candida* spp. were studied: Germ-tube test using fetal bovine serum **(Gatica *et al.,* 2002),** gelatinase production using SDA plates containing 1% gelatin **(Ramesh *et al.*, 2011),** phospholipase activity test using egg yolk agar **(Samaranayake *et al.*, 1984),** and Biofilm formation by the tube adherence method **(Shin *et al.* 2002).**

Statistical analysis

Data were analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16. Parametric data were expressed as mean ± standard deviation and non parametric data were expressed as number and percentage of the total. Determining the extent that a single observed series of proportions differ from a theoretical or expected distribution was done using the Chi-square test. P values were insignificant when >0.05, significant when <0.05, highly significant when <0.01 and Marked significant when P<0.001**(Sokal and Rohlf, 1995)**.

Results

Prevalence of oral candidiasis and Predisposing factors:

Out of the 60 diabetic patients, 19 (31.7%) of them were denture wearers and 41(68.3) of them non denture wearers. 40 patients had culture proven oral candidiasis. A significant difference was found between patients with (67%) and without (33%) oral candidiasis. Prevalence of candidiasis among denture wearers was (18/19) 94.7% while in non denture wearers was (22/41) 53.7%.

The most prominent predisposing factors among diabetic patients with oral candidiasis were old age, female gender, duration of diabetes (p<0.01). Other predisposing factors for oral candidiasis of diabetic patients are inadequate oral hygiene (97.5%), absence of teeth (92.5%), caries (52.5%), removable dentures (45%), filled teeth (17.5%), hypertension (97.5%), and smoking (27.5%).

Frequency of oral candidiasis in relation to age, sex and dentur wearing (Figure 1):

Among the 40 pateints, the frequency oral candidiasis in middle aged (40-60 years) patients was more significant (65%) than old aged patients (35%), (p<0.01) and in females (65%) than males (35%) (p<0.01). However insignificant difference (p>0.05) was detected betweeen denture wearers (45%) and non denture wearers (55%) among diabetics with oral candidiasis.

Candida **load and Frequency of different *Candida* Species**

Candida load in diabetic patients was higher (92.8%) in elder age patients than middle aged patients 77%, among females 84.6% than males 50% and more prevalent 77.8% in denture wearers than non denture wearers 54.5% with significant differences (P< 0.01).

Sixty isolates of different *Candida* spp. were identified: 32 (53.3%) *C. albicans* which is the most common species followed by *C. glabrata* 19 (31.7%), *C.tropicalis* 6 (10%) then *C. krusei* 3 (5%). 52.5% of patients have single *Candida* species and 47.5% of patients have multiple *Candida* species.

Frequency of different *Candida* species in relation to denture

Out of the 60 isolates, 35 (58.3%) of them were isolated from denture weaerer patrients and 25 (41.7%) were isolated from non denture weaerers with insignificant difference between them (P>0.05). Among denture wearers the frequency of *C. albicans* was 94.4%, *C. glabrata* 66.7%, *C. tropicalis* 22.2% and *C. krusei* 11.1% versus 68.2%, 31.8% , 9%, 5% and 4.5% in non-denture wearing respectively (P<0.01) except for *C. krusei,* (Figure 2). .

Frequency of different *Candida* species in relation to age

The frequency of *C. albicans* was 80.7% and 78.6% in middle age and elder patients respectively with insignificant difference while significant higher frequency were observed in old age than middle age patients regarding *C. glabrata* (38.4% versus 64.4%), *C. tropicalis* (3.8% versus 35.7%) and *C. krusei* (3.8% versus14.3%), (Figure 3).

Antifungal susceptibility of the isolated *Candida* species

Among the *C. albicans* isolates (28.1%), were resistant to ketoconazole, (12.5%) to voriconazole, (9.4%) to flucytosine, (9.4%) to fluconazole and (3.1%) to itraconazole. While (93.3%), (47.3%), (36.8%) and (5.2%) of *C. glabrata* isolates were resistant to itraconazole, fluconazole, ketoconazole, voriconazole and flucytosine respectively. Among *C. Tropicalis* isolates; (83.3%), (66.6%), (50%), (33.3%) were resistant to fluconazole, ketoconazole, itraconazole and voriconazole respectively. The only one (33.3%) *C.krusei* isolate was resistant to fluconazole, voriconazole and itraconazole. Beside, amphotercin B was very effective against all *Candida* isolates. Generally high rate of resistance were observed with itraconazole, fluconazole, voriconazle (Table 1).

The minimal inhibitory concentration (MIC) of antifungals:

The MICs of ketoconazle as the lowest concentration of antifungal that inhibited 100% of *C. krusei* while MIC for flucytosine that inhibited 90.6% and 84.2% of *C. albicans* and *C. glabrata*

respectively. Also the MIC for itraconazole and voriconazole that inhibited 93.5% and 81.2% of *C. albicans*. The MIC of amphotericin B was defined as the lowest drug concentration causing 100% inhibition of all *Candida* species (Table 2).

Virulence factors of *Candida* species

Out of 60 *Candida* isolates, 55% were positive for GT formation, 61.7% were positive for gelatinase activity, 58.3% were positive for PL activity, 71.7% were positive for biofilm formation. Most of *C. albicans* were positive for all virulence factors. *C. tropicalis* were also positive for all virulence factors but less frequent than *C. albicans.* While 31.6% of *C. glabrata* and 100% *C. krusei* isolates were positive only for biofilm formation (Table 3).

Among denture wearers 54.5%, 58.1%, 51.4%, 56.8% were positive for germ tube formation, biofilm formation, phospholipase activity, gelatinase activity respectively versus 45.5% , 41.9% , 48.6% , 43.2% in non denture wearers. Significant higher frquency (P<0.05) of all virulence factors were detected among denture wearers compared to non denture wearers except for PL activity, Figure 4.

Among middle age patients 63.6%, 55.8%, 60% and 59.5% of *Candida* spp. were positively for germ tube, biofilm formation, phospholipase activity and gelatinase activity versus 36.4%, 44.2% , 40% and 40.5% in elder age respectively. Significant differences (P<0.05) between middle and elder ages were found in all virulence factors except biofilm formation (P>0.05), Figure 5**.**
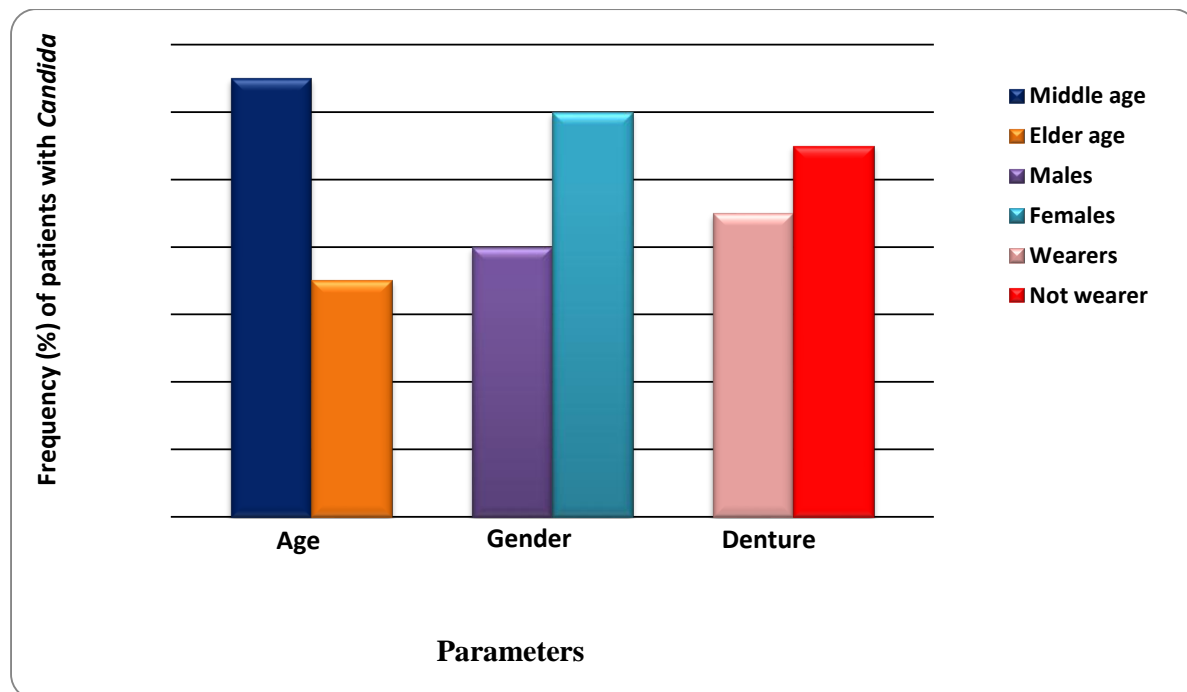

N.B: 18/19 (94.7%) of denture wearers have candidiasis, while 22/41(53.6%) of non denture wearers have candidiasis with a significant difference between both.

Figure (1): Frequency of *Candida* in the oral cavity in relation to age, sex, and denture wearing in diabetics with oral candidiasis.
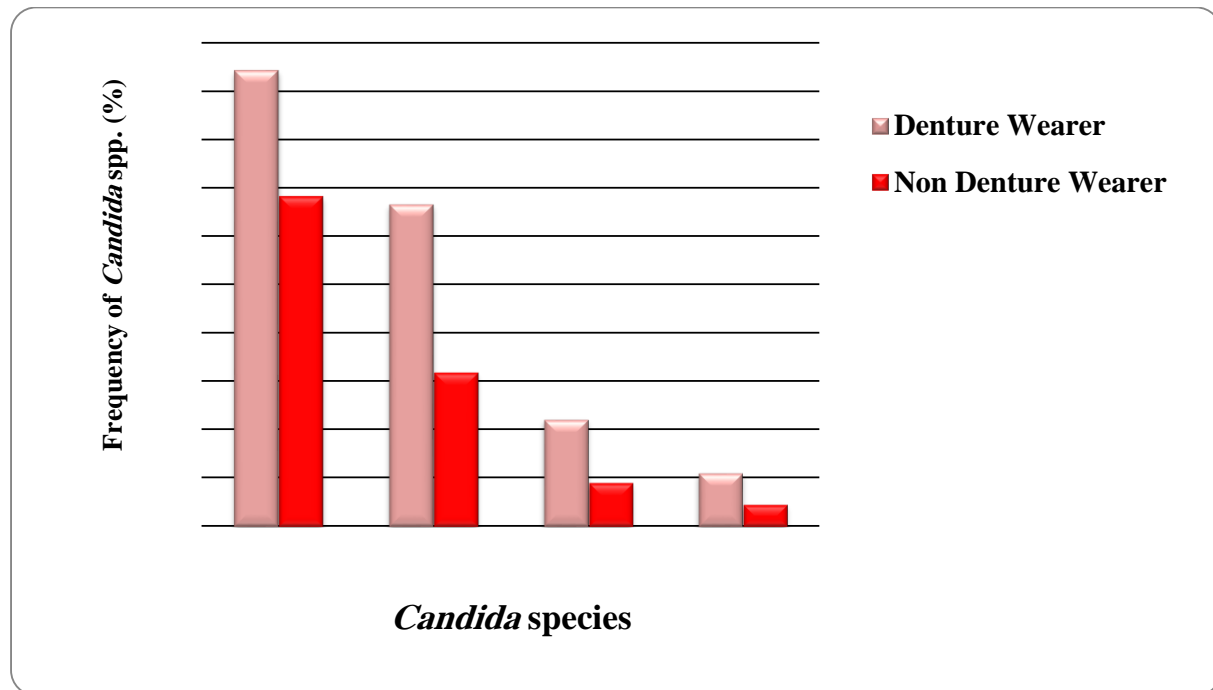
* 35 (58.3%) of *Candida* species isolated from denture weaerer patrients and 25 (41.7%) of *Candida* species isolated from non denture weaerer patients.

Figure(2): Distribution of *Candida* spp. in relation to denture wearing.
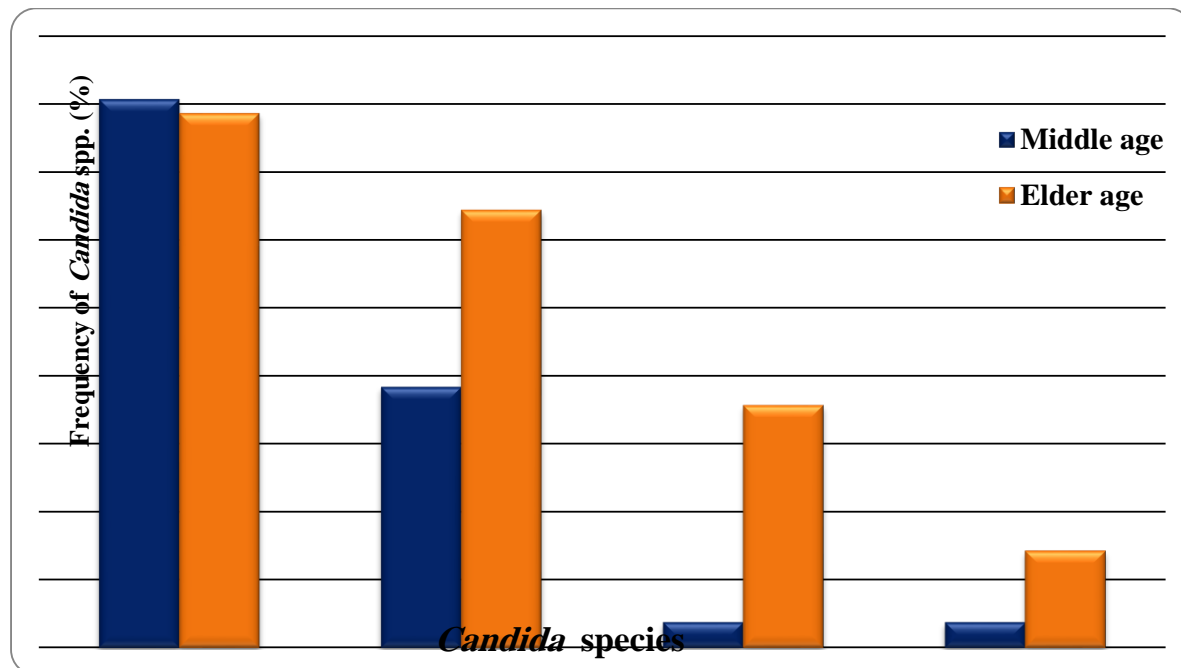
* 33 (55%) of *Candida* species isolated from middle age patients and 27 (45%) of *Candida* species isolated from elder age patients.

Figure (3): Distribution of *Candida* spp in relation to age.

Table (1): The antibiogram patterns of *Candida* species using integral yeast plus system.

Antifungal agent	*Candida albicans* (n=32)						*Candida glabrata* (n=19)						*Candida tropicalis* (n=6)						*Candida krusei* (n=3)					
	R		S		DDS		R		S		DDS		R		S		DDS		R		S		DDS	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
AMB	-	-	32	100	-	-	-	-	19	100	-	-	-	-	6	100	-	-	-	-	3	100	-	-
FCY	3	9.4	29	90.6	-	-	1	5.2	16	84.2	2	10.5	-	-	5	83.3	1	16.6	-	-	2	66.7	1	33.3
KET	9	28.1	21	65.6	2	6.2	7	36.8	9	47.3	3	15.7	4	66.6	1	16.6	1	16.6	-	-	3	100	-	-
ITR	1	3.1	30	93.5	1	3.1	18	93.3	-	-	1	5.2	3	50	2	33.3	1	16.6	1	33.3	2	66.7	-	-
VOR	4	12.5	26	81.2	2	6.2	7	36.8	11	57.8	2	10.5	2	33.3	-	-	4	66.6	1	33.3	2	66.7	-	-
FLU	3	9.4	21	65.6	8	25	9	47.3	2	10.5	11	57.9	5	83.3	1	16.6	-	-	1	33.3	2	66.7	-	-

AMB Amphotericin 2 µg/mL; **FCY** Flucytosine 16 µg/mL; **KCA** Ketoconazole 0.5 µg/mL; **ITR** Itraconazole 1 µg/mL; **VOR** Voriconazole 2 µg/mL; **FLU** Fluconazole 64 µg/ml. R; resistant, DDS; dose dependent sensitive and S; sensitive

Table(2): Mean MIC values for the isolated *Candida* species.

Antifungal agent	Code	Conc (µg/ml)	Breakpoint			
			C. albicans N= 32	*C. glabrata* N=19	*C. tropicals* N=6	*C. krusei* N=3
Amphotericin	AMB	0.002-32	0.315	0.157	0.625	0.625
Flucytosine	FCY	0.002-32	0.014	1.25	0.014	2.5
Ketoconazole	KET	0.002-32	0.014	0.014	0.109	0.625
Itraconazole	ITR	0.002-32	0.024	0.22	0.027	0.875
Voriconazole	VOR	0.002-32	0.014	0.027	0.345	0.21
Fluconazole	FLU	0.002-64	0.22	0.44	1.75	14
Capsofungin	CAS	0.002-32	0.157	0.22	0.44	0.625

Table (3): Distribution of virulence factors among *Candida* species

Virulence factor	Positive isolates		*Candida* species N= 60							
			C.albicans N= 32		*C.glabrata* N=19		*C.tropicalis* N=6		*C. krusei* N=3	
	No	%	No	%	No	%	No	%	No	%
Germ tube formation	33/60	55	32/32	100	-	-	1/6	16.7	-	-
Biofilm formation	43/60	71.7	30/32	93.7	6/19	31.6	4/6	66.7	3/3	100
PL activity	35/60	58.3	31/32	96.8	-	-	4/6	66.7	-	-
Gelatinase activity	37/60	61.7	32/32	100	-	-	5/6	83.3	-	-

* 21/40 (52.5%) of patients have single *Candida* species and 19/40 (47.5%) of patients have multiple *Candida* species.
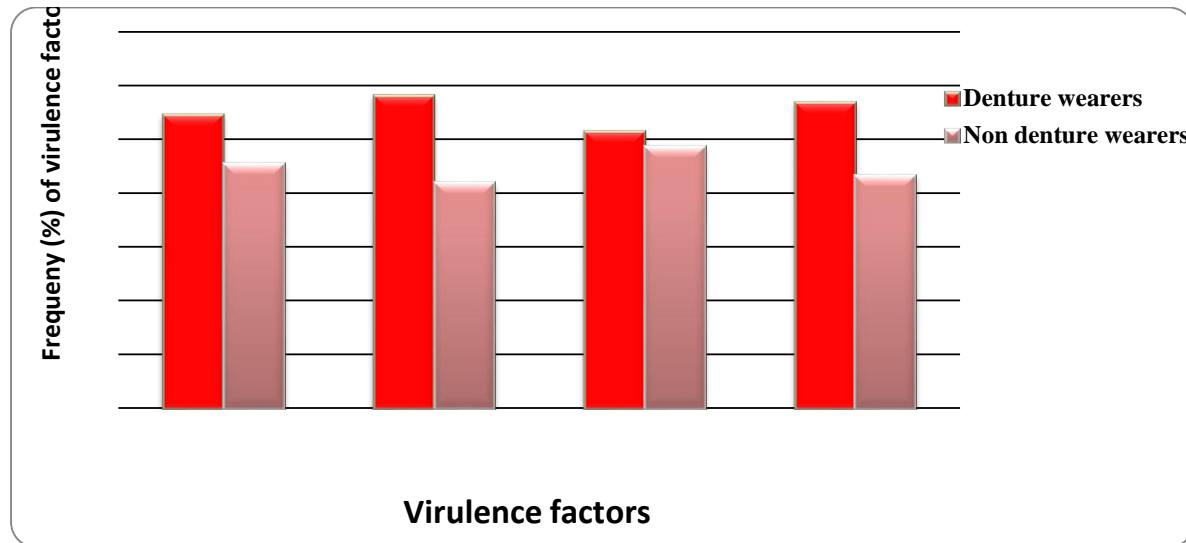

Figure (4): Relationship between virulence factors of *Candida* species and denture wearing.
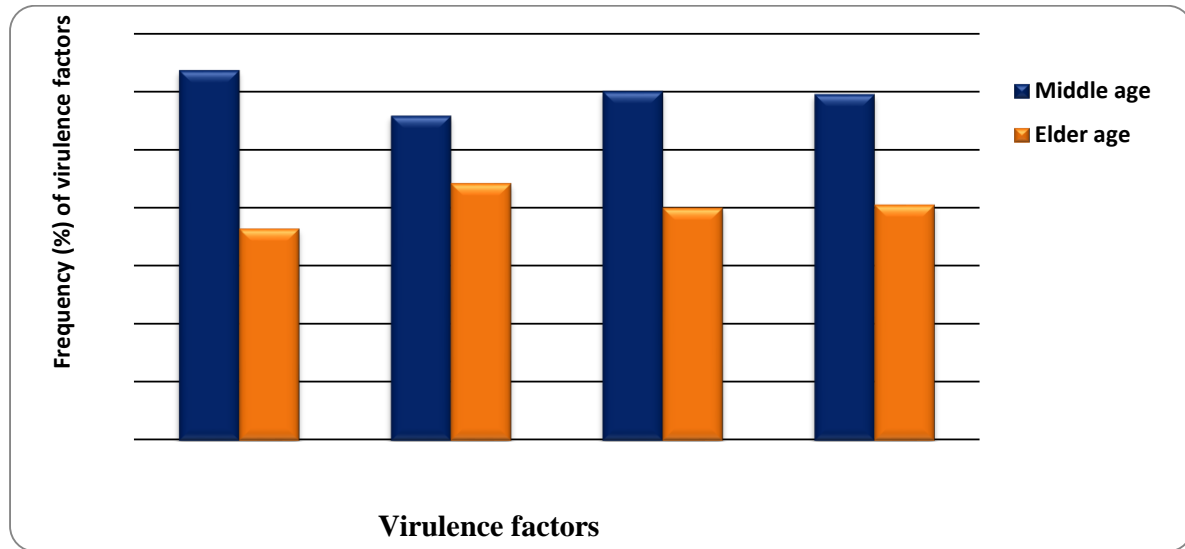
Figure (5): Relationship between virulence factors of *Candida* species and patient's ages

3.2 Discussion

The objectives of this study were to determine the prevalence of *Candida* infection in the oral cavity of diabetic patients, investigate the effects of age and denture wearing and other predisposing risk factors on the isolation rate of different *Candida* spp., determine some virulence factors of *Candida* isolates, and in vitro their susceptibility to antifungal agents,

In the present study the prevalence of *Candida* infection in the oral cavity of diabetic patients was 67%. This finding is in accordance with that of **Soysa *et al.,* (2006)** who reported that the presence of *Candida spp.* in the oral mucosa in diabetic patients, reached up to 80%. In addition our finding is similar to that of **Sardi *et al.* (2013)** and **Belazi (2005),** who studied 128 diabetic and 84 non diabetic patients, *Candida* was isolated from the oral cavity of 64% of the diabetic patients. Incontrast, **Carlos *et al.* (2013)** found that only 25% had a positive result. **Tsang *et al.,* (2007)** found also that *C. albicans* was isolated from 36.2% of patients with DM. Oral candidiasis is explained by the significant association with the glycemic control and changes in salivary pH due to hyperglycemia **(Kumar and Kannan, 2010).**

Slight alterations the physiological condition of the host in can turn a harmless commensal microorganism into a pathogen. The transition is attributable to the suitable predisposing conditions that occur in the host **(Bhat *et al.,* 2011).** The current study revealed that the most common predisposing factors in the oral cavity were inadequent oral hygiene (97.5%), absence of teeth (92.5%), caries (52.5%), and denture wearing (45%). long duration of diabetes >10 years (77.5%), female sex (60%) and the middle aged (40-60 years) patients (65%). In addition, *Candida* load in

diabetic patients was higher (92.8%) in elder age patients than middle aged patients 77%, among females 84.6% than males 50% and more prevalent 77.8% in denture wearers than non denture wearers 54.5% with significant differences.

Frequent oral candidiasis occurs in 45% of denture wearers in this study. However, **Lakshmipriya** *et al.,* **(2014)** found that the prevalence of *Candida* spp. was (93% vs 53%) in denture wearers and non-denture wearers diabetics and explained that candidal colonization in the oral cavity of denture wearing increases and its growth rate was found higher in diabetic denture wearers due to their immune-compromised status. Denture wearing is supportive of growth of species as *C. albicans, C. tropicalis,* and *C. glabrata* **(Hiroyuki** *et al.,* **2007).** Similarly, **Lakshmipriya** *et al.* **(2014)** and **Lotfi-Kamran** *et al.* **(2009)** reported that prevalence of *Candida* in diabetic was 93% in denture wearers and 53% in non denture wearers.

Aging has been believed to cause progressive increases of *Candida* in the oral cavity. Our results indicated that the incidence of *Candida* spp. in middle aged patients was higher (65%) than elderly subjects (35%). In contrast, **Zaremba** *et al.* **(2006)** that found no significant difference between middle aged subjects (67.3%) and the elderly (58.8%). **Lakshmipriya** *et al*. **(2014)** reported also an equivalent rate between middle age (40-60) years old and elder age (>60years) old. However, **Resende Pinho (2002)** reported that the isolation rates of *Candida* species to be high in ages ranging from 60-80 years.

A higher percentage of female patients was observed in the present investigation, and this result agrees with previous studies **(de Resende** *et al.,* **2006). Espinoza** *et al.* **(2003)** who found that elderly women presented more oral lesions than men and that the hormonal factor and the great incidence of iron deficiency in women could be responsible for that disparity **(Figueiral** *et al.,* **2007).**

Sixty isolates of different *Candida* Spp. were identified: 53.3% *C. albicans* which is the most common species followed by *C. glabrata* 31.7%, *C.tropicalis* 10% then *C. krusei* 5%. 52.5% of patients have single *Candida* species and 47.5% of patients have multiple *Candida* species. The most commonly isolated species was *C. albicans*. The present study supported the hypothesis that, although, *C. albicans* was the most common species isolated from patients with candidiasis; yet the *Candida* non *albicans* (*CNA*) species were found to be emerging **(Daniluk** *et al.* **2006),** where it was determined that 43.8% of diabetic patients had *C. albicans*. In agreement with findings of others **(Back-Brito** *et al.,* **2009),** the majority of yeast isolates from oral cavity were *C.albicans*, but it was often recovered in association with other yeasts. These results are in accordance with **Manikandan and Amsath, (2013),** they reported that *C. albicans* was found to be the predominant with 70% followed by *C. glabrata* 16.6%, *C.krusei* and *C. tropicalis* 6.7% for each. Moreover, a study conducted

by **Raju and Shashanka, (2012),** has reported a similar pattern of distribution of species and explained that the more or less similarity with these studies could be due to variation in geographical distribution of various *Candida* species.

Frequent occurrence of multiple *Candida* species sets denture wearers apart from subjects who do not wear dentures. Denture wearing is supportive of growth of species as *C. albicans*, *C. tropicalis*, and *C. glabrata* **(Hiroyuki *et al.,* 2007).**

The findings that most of *C.albicans* have high rates of resistance to itraconazole and all *Candida Non Albicans* species have high rates of resistance to itraconazole, flucytosine and fluconazole. **Sachin (2014)** reported that *C. tropicalis* isolates were found to be more resistant to fluconazole. Resistance to fluconazole and itraconazole was observed relatively high, mainly in isolates of *C. glabrata, C. tropicalis* and *C. albicans* **(Bruder- Nascimento *et al.*, 2010)** while **Baysan *et al.* (2012)** revealed that *C. krusei* is intrinsically resistant to fluconazole. High secondary resistance rates were observed in *C. glabrata* to fluconazole (68%) and itraconazole (88%); this resistance to multiple azoles has been explained by an upreglutation of CDR genes that encode the CDR efflux pumps (Pfaller and Diekema, 2007). *Candida glabrata* strain resistant to itraconazole was recovered in Brazilian tertiary care hospital and similar results with *C. krusei* and *C. tropicalis* isolates were reported in Greece **(Behiry *et al.*, 2010)**.

The findings that all 60 *Candida* isolates were susceptible to amphotericin B, are similar to reports of **Behiry *et al.* (2010)** who found that all 40 *Candida* isolates were susceptible to amphotericin B. Moreover, 81.2% of *C. albicans*, 57.8% of *C. glabrata,* 50% of *C. tropicalis* and 66.7% of *C. krusei* in this study were susceptible to voriconazole with similar result reported by **Baysan *et al.* (2012**) who found that all *Candida* species were susceptible to voriconazole except one *C. glabrata* strain which was intermediate susceptible.

The current work revealed that most of *C. albicans* were positive for all virulence factors. *C. tropicalis* were also positive for all virulence factors but less frequent than *C. albicans.* While 31.6% of *C. glabrata* and 100% *C. krusei* isolates were positive only for biofilm formation. Significant higher frquency of all virulence factors were detected among denture wearers compared to non denture wearers except for PL activity. The study of **Barros *et al.*, (2008)** revealed that 30 to 100% of the oral isolates of *C. albicans* produce phospholipases with variable degrees of enzymatic activity Similarly, **Sardi *et al.* (2013)** reported that phospholipase activity was detected in 95.5 % and proteinase activity in 100 % of *C. albicans* isolates and **Tsang *et al*. (2007)** also found a high proteinase activity in type 2 DM patients. **Manfredi *et al.* (2006)** reported that proteinase expression is not significantly higher in *Candida* isolates from patients with diabetes when compared to healthy patients and that

type 2 DM patients have higher proteinase levels than type 1 DM patients. Deorukhkar **and Saini, (2014)** reported that maximum phospholipase activity was seen in *C. albicans* (81.6%) and among NAC spp. maximum phospholipase activity was noted in *C. tropicalis and C. glabrata*. Interstingly, *Candida* cells in a biofilm exhibit a significant resistance to several antimicrobial drugs, notably the popular azole drugs **(Maia *et al.,* 2008).** High rates of biofilm formation of *Candida* spp. were detected in this study. These results are in agreement with Yigit *et al.* (2011), who found that biofilm production in *Candida* strains isolated from denture stomatitis, were 88.2% of *C. albicans* strains were biofilm producers, while 60% of *C. glabrata*, 44.4% of *C. krusei*, 57.1% of *C. kefyr* and 40% of *C. parapsilosis* positive for biofilm production.

4. Conclusion

Oral Candidiasis is strongly associated with diabetes and is more common among middle aged, female gender denture wearers and it is, hence controlling serum glucose level is essential. Dentures need to be cleaned daily with effective antifungal agents but there comes a time when a denture can no longer be cleaned effectively and must be replaced. So better oral hygiene is necessitated among denture wearers.

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الملخص العربى

معدل تواجد أنواع الكانديدا فى تجويف الفم ومخاطرها لمرضى السكرى

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تمثلت أهداف هذه الدراسة فى معرفة مدى انتشار الإصابة بفطر الكانديدا فى التجويف الفمى لمرضى السكرى ومعرفة العوامل المهيئة لهذه العدوى وفصل سلالات الكانديدا المسببة لها والتعرف على هذه السلالات ومعرفة حساسية سلالات فطر الكانديدا للمضادات الفطرية المختلفة وكذلك معرفة اقل تركيز مثبط للمضادات الفطرية وتقييم عوامل الضراوة للفطر مثل تكوين أنبوبة الإنبات وتكوين الفيلم الحيوى وإفراز إنزيمات الفسفوليبيز والجيلاتينيز.

وشملت الدراسة ٦٠ من مرضى السكرى يشتبه فى وجود عدوى التجويف الفمى بفطر الكانديدا لديهم وبعد عمل المزرعه والعزل تبين ان ٤٠ عينة موجبة لديهم عدوى التجويف الفمى بفطر الكانديدا منهم (١٨ ذو تركيب كامل لطقم الأسنان و٢٢ بدون). ٤٠/٢٦ من متوسطى العمر يتراوح عمرهم بين(٤٠-٦٠) سنة و٤٠/١٤ من كبار السن عمرهم اكبر من ٦٠ سنة.

تم فصل ٦٠ سلالة من الكانديدا (٣٥ من ذوى التركيب و٢٥ بدون، وجد أن الكانديدا أكثر انتشارا بين متوسطى العمروالإناث بنسبة ٦٥% لكل وكان معدل فصل السلالات من العينات الموجبة كالاتى: كانديدا البيكانز ٥٣.٣% يليها كانديدا جلابراتا ٣١.٧% يليها كانديدا تروبيكالز ١٠% ثم ٥% للكانديدا كروزى. وأظهرت الدراسة ان معدل فصل السلالات فى المرضى ذوى التركيب ٩٤.٧% بينما فى المرضى بدون ٥٣.٧%.

وقد أظهرت الدراسة أن أقل تركيز للكيتوكونازول مثبط ل١٠٠% من الكانديدا كروزى بينما اقل تركيز للفلوسيتوزين مثبط ل٩٠.٦% و٨٤.٢% من الكانديدا البيكانز والكانديدا جلابراتا على التوالى وايضا اقل تركيز للايتراكونازول والفوريكونازول مثبطة ل٩٣.٥% و٨١.٢% للكانديدا البيكانزلكل.

وتبين من الدراسة ان الكانديدا البيكانز والكانديدا جلابراتا قادرتان على تكوين انبوبة انبات وتكوين الفيلم الحيوى وانتاج انزيم الفسفوليبيز والجيلاتينيز بينما الكانديدا جلابراتا والكانديدا كروزى غير قادرتان على تكوين انبوبة انبات وانتاج انزيم الفسفوليبيز والجيلاتينيز ولكن الكانيدا كروزى لها القدرة على تكوين الفيلم الحيوى.

وتبين من الدراسة ان الكانيدا البيكانز من اكثرالأنواع انتشارا وشيوعا فى التجويف الفمى لمرضى السكرى حيث أن لها القدرة على احداث الالتهابات وغزو انسجة الفم. وان عدوى التجويف الفمى بفطر الكانديدا شائع فى ذوى التركيب لذا لابد من التحكم فى مستوى السكر فى سيرم الدم و يساهم عدم تنظيف التركيب فى الاصابة بالفطريات التى ترتبط بأمراض الفم والأمراض الجهازية وأن الاصابة بعدوى التجويف الفمى بفطر الكانديدا الاكثر انتشار بين متوسطى العمر والأناث.