Republic of Iraq
Ministry of Higher Education
And Scientific Research
University of Diyala
College of Medicine

Detection of *Candida* Species Phenotype and Genotype In Diabetes Mellitus Patient In BaqubaTeaching Hospital

A Thesis

Submitted to The Council the College of Medicine , University of Diyala in Partial Fulfillment of The Requirements for The Master Degree Of Science In Medical Microbiology

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2018 A.D. **1440A.H.**

بسم الله الرحمن الرحيم

[أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُواْ الأَلْبَابِ]

صدق اللهُ العظيم

سورة الزمر الآية (9)

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Dedication

♣ *To my beloved parents,*
For their love, endless support, &sacrifices &
Who encouragement me to go on every adventure,
Thank you so much.

♣ *To the light of my life ,*
my beautiful , sisters ,and my support ,
Sura , my eyes Alia ,and Amna

♣ *To my brothers,*
Abdul alrahman ,Ali and too sweet of our life
Youssef

♣ ***Maha***

Acknowledgments

First *and forever, I would like to thank my Lord Allah the most merciful for his blessings and favors to complete this work.*

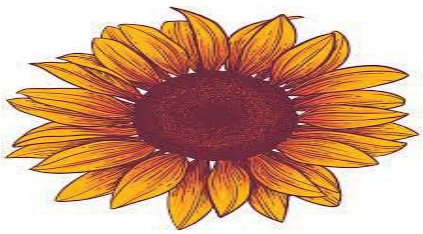
I would *like to express my heartfelt gratitude to my supervisors, Assistant Professor Dr. Luma Taha Ahmeed and Dr.Ahmed Medab for their supervision, scientific guidance, invaluable advice and encouragement.*

My thanks *are extended to the College of Medicine - University of Diyala - Department of Microbiology for providing me with the chance to get the master degree and to Assistant Professor Dr. Areej Atiyah Hussein for kindness and advice.*

I would like *to thanks Diyala teaching hospital and thanks for all patients and I wish them good health and happy life.*

My thanks *to my friends Hala luay for advice and support , Ali Riyadh Hameed for advice and support and assistance in the practical laboratory , and Mohammed mustafa for help and support and many thanks to Dr. Khalid for his help and advice ..*

Maha muhammed



Summary

Summary

Summary:

Candida albicans it resides mainly in the mucosal surfaces of the oral cavities. Oral candidiasis could be a common infection of the mouth caused by an overgrowth of *Candida* spp. species significantly *Candida albicans*. Various risk factors like nutrition, oral hygiene, smoking, dentures, secretion, hydrogen ion concentration disorder, and dry mouth make diabetic patient's additional vulnerable to oral candidiasis.

The aims of this study were detection, isolation and identification of *Candida* species , isolated from patients that have diabetes mellitus this patients had symptom of oral candidiasis in Diyala province by routine laboratory procedures and molecular techniques based on PCR and to identify genotypes distributions of *Candida albicans*.

One hundred oral swab samples of oral candidiasis from diabetic patients were collected. The age's groups their ranged between 20 to 90 years old. There were taken from Baquba Teaching Hospital, in Baquba city , center of Diyala province during the period (from first of December 2017 to the end of February 2018). Among the one hundred study samples collected there were (56%) samples of them were diagnosed as *Candida* spp.

In this study detected them by making a routine and confirmative diagnostic processes by, cultured on Sabouraud dextrose agar (SDA) and then had made sub cultured twice on (SDA) .Then made microscopic examination of colonies and stain with Lacto phenol cotton blue, than use Chromogenic Agar *Candida* (CAC) for Phenotype Identification of *Candida* spp. , and we used germ tube also to identify *Candida albican.*

Then extracted the (DNA) by protocols of kit that mention from company, then makes certain that concentration and purity of extracted (DNA) in the limited rang , next used special primer pairs 25SrDNA followed by programmable thermal controller and Electrophoresis analysis with run the gel and being photographed, using a UV trans illuminatorm . All data were statistically analyzed depending on SPSS (Statistical Package for Social Science) version 18 (2009). Statistical results were considered significant when P-value being under or equal to the 0.05.

The results revealed that, 31 samples (55.35%) of isolates were *Candida albicans;* 12 samples (26.88%) of isolates *Candida globrata*; 6 samples (13.44%) of isolates *Candida parapsilosis*; 4 samples (8.96%) of isolates *Candida krusei* and 3 samples (6.72%) of isolates *Candida tropicalis.* The results of PCR shown that the detect of 25S rDNA gene were 26(83.8%) isolates belonged to the genotype A and 5 (16.1%) genotype C of the *C. albicans.* There were no results for genotype B. The higher infection rates of candidiasis were among female (29) samples (51.8%), while in the male they were (27) samples (48.2%) positive to candidiasis and they were non-significant (P>0.05) relationship between them .In this study found that the higher infection rates were among patients with Type II -31, samples (55.4%) of diabetes in compared with Type -I ,25 samples (44.6%) . There were non-significant (P>0.05) between *Candida* species and type of diabetes. In the case of ages the high infection rates were between ages (61-70) years old there were 18 samples (32.1%) high compared with other ages and there were non-significant (P>0.05) difference between them.

The results showed that *Candida* infection rates were high in weight (50-75) Kg 26 samples (46.4%) in compared to other weigh and it is non-significant (P>0.05) . The rates of the infection were higher among patients who have

hypertension in compared with those that have none have hypertension (64% *vs.* 36%).The infection rates were higher among patients having no kidney problems in compared to patients with kidney problem (66.1% *vs*. 33.9%)while , *Candida* infection rates were higher in non-smoking patients compared to smoking patients (73.2% *vs*. 26.8%).In the case of social economic the rates of the infection were higher among those had good economy (41.1%) compared to those on medium or poor (41.1 %, and 17.9 %) respectively. The rates of the infection were higher among patients that non have family history in compared with patients who have family history (60.7% vs. 39.3%).

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List of abbreviations

Abbreviation	Meaning
AIDS	Acquired immune deficiency
bp	base pair
C. albicans	***Candida albicans***
*C.*non *albicans*	***Candida* non *albicans***
CAC	**chromogenic agar Candida**
Candida spp.	***Candida* species**
CDC	**Center for Disease Control**
EDTA	**Ethylenediaminetetraacetic acid)**
FPG	**fasting plasma glucose**
G	**Gravity**
GIT	**Gastrointestinal**
GT	**Germ tube**
HIV	**Human immunodeficiency virus**
hrs	**hours**
ICU	**Intensive Care Units**
ITS	**Internal transcribed spacer**
LPCB	**Lacto Phenol Cotton Blue**
M	**Molar (mol/L)**
min	**minutes**
OC	**Oral candidiasis**
OD	**Optical Density**
OGTT	**oral glucose tolerance test**
PBS	**Phosphate Buffered Saline**
PCR	**Polymerase Chain Reaction**
pH	**power of Hydrogen**
Rnase	**Ribo nuclease**
rpm	**rounds per minute**

rRNA	**Ribosomal RNA**
SAP	**Secreted Aspartyl Proteinases**
SDA	**Sabouraud Dextrose Agar**
sec	**second**
Sig	**significant**
SPSS	**Statistical Package for Social Sciences**
UV	**Ultra violet**
WHO	**World Health Organization**
YPD	**Yeast extract Peptone Dextrose**
µ	**Micro**

Chapter One: Introduction

1.1. Introduction:

Candidiasis is a mycotic infection caused by members of the genus *Candida*. Chiefly, *Candida albinos* is responsible for about (70–80%) of all *Candida* infection. The *Candida* as an opportunistic yeast pathogen which increases predominantly in patients with predisposing condition, including immunodeficiency such as HIV infections, prolong used of broad-spectrum antibiotics, corticosteroids, diabetic patients and infections with other debilitating disease.(Navarro-Arias *et al*., 2016; AL-Attachi *et al* .,2017) .

Candida infections are ones of the most commonly occurring fungal infections in humans, (Kumar *et al*., 2005).Affecting mucous membrane, skin, nails and internal organs of the body, it additionally a typical opportunist infections in immune compromised patients (Hasan borarialju and ., 2015). *Candida* species belong to the natural micro biota of an individual's mucosal oral cavity, gastrointestinal tract and vagina (Shao *et al*., 2007).

Candida albicans is a common commensal of the human oral cavity and GI tract in the healthy individuals (Calderone claney and ., 2011). Although *Candida albicans* is considered the most agent of fungal infection and the foremost frequently isolated from oral fissure but in recent two decades there has been important increase of other non-*Candida albicans* species such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei, Candida parapsilosis* and *Candida dubliniensisas* a result of different factors like immune-suppressants and the prolonged use of broad spectrum antibiotics and antifungal drugs (Martins *et al*., 2014 ; Patil *et al*., 2015 ;Jain *et al*., 2016). But the majority of fungal infections in humans are caused by the species *C. albicans* and *C. glabrata*. The prevalence

rates of *C. albicans* and *C. glabrata* infections are approximately 70% and 15%, respectively (Kolaczkowski *et al*., 2010; Benedetti *et al*., 2016).

Diabetes mellitus is a common and growing global health problem which causes several complications. Periodontal diseases are considered the sixth complication of this disease. A diabetic has an increased predisposition to the manifestations of oral diseases like candidiasis. Diabetes mellitus is the most common endocrine metabolic disorder (Lotfi-Kamran *et al*., 2009). Nearly 85-90% of diabetic patients are diagnosed with type II diabetes (resulting from insulin resistance) in these patients, salivary dysfunctions like dry mouth, reduced salivary function, lichen, tooth decay, and periodontal diseases are common(Ship, 2003 ; Vijan, 2010).

Candida infections are chronic opportunistic infections related to diabetic patients. The presence of *Candida* spp . in oral cavities of diabetics varies between (50-80 %) (Willis *et al*., 2000; Khosravi *et al*., 2008; and Melton *et al*., 2010). Yeasts commonly inhabit tongue, palate and buccal mucosa, and it has recently been found in the sub gingival sites (Canabarro *et al* .,2013) .

Oral candidiasis is a common profiteering infection of the oral cavity caused by an overgrowth of *Candida* species particularly *Candida albicans* (Guggenheimer *et al*., 2003). This infection is usually accompanied by various symptoms including burning, painful sensation, change of taste, reduced saliva secretion and swallowing difficulty, but it can be also asymptomatic (Nikolic *et al*., 2016).

Among the explanations creating diabetic patients additional susceptible to oral candidiasis (Ship, 2003 ; Vijan, 2010), are high levels of salivary glucose, impaired chemo taxis , low secretion of saliva and defect of phagocytosis because

of polymorph nuclear white blood cell deficiency(Mohammadi *et al* .2016) . The attachment of *C. albicans* to the crystalline hydroxyapatite produces collagen lytic protein, that will increase crystal solubility and consumes chemical element of dentin albuminoid in diabetes mellitus patients (Mohammadi *et al* .,2016 ; Hassan , 2018) .

It Diagnosis can be by different ways, the most common type of diagnosis utilized in the hospitals and labs include direct examination and stain with lacto phenol, culture on SDA , germ tube , chromogenic agar and API kit, and molecular method include conventional PCR and real time PCR ,sequencing ,gene expression (Garner *et al*., 2010; Ahmad *et a l.,* 2012).

Solely 5 major categories of antifungal drugs are presently obtainable including the foremost usually used azloes, polyenes, fluoropyrimidines and the recently generated echinocandins (Basma *et al*., 2009).

1.2. Aims of the Study:

1. Detection, isolation and identification of *Candida* spp. isolated from patients that who diabetes mellitus. In Diyala province by routine laboratory procedures and molecular techniques based on PCR.
2. Identification genotypes distributions of *Candida albicans.*