



EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES

MEDICAL ENTOMOLOGY & PARASITOLOGY

E



ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)



Identification of *Candida* Species Isolated from Oral Candidiasis in Cancer Patients using PCR-RFLP in Sulaimani, Iraq

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ARTICLE INFO

Article History

Received:3/9/2022

Accepted:5/11/2022

Available:9/11/2022

Keywords:

Oral Candidiasis,
Cancer patients, PCR-
RFLP, Species of
Candida.

ABSTRACT

Candida species causes oral candidiasis in immunosuppressed individuals such as cancer patients. This study aimed to determine the rate of candida infections in cancer patients (n= 150) and to identify different species of candida using Polymerase Chain Reaction-restriction fragment polymerase length polymorphism (PCR-RFLP), and to find the effect of candidiasis on the PH of the saliva in cancer patients (n=50) and controls (n=50) (Healthy individuals). The rate of candidiasis was different in patients with different types of cancer. A higher percentage of oral Candidiasis was observed in carcinoma (54 %), followed by leukemia patients (17.3 %), sarcoma type (15.3%), and lymphoma (13.33%). The highest prevalence of candidiasis was found in the old age group, 40-60years *Candida albicans* was the most common recorded species,70% in comparison to non- *albicans* species, followed by other species including: *Candida glabrata* (12%), *Candida kefyr* (6.7%), *Candida tropicalis* (5.3%), *Candida krusei* (3.3%), and *Candida dubliniensis* (2.7 %). The pH of saliva was more acidic in cancer patients who have candidiasis in comparison to control individuals (P-value 0.001). In conclusion, this study found that the rate of candidiasis is high among cancer patients and *Candida albican* is the most common species recorded in cancer patients.

INTRODUCTION

Candidiasis is the most commonly observed fungal infection in humans. Among candida species, there are at least 15 distinct *Candida* species that cause human disease, but more than 95% of invasive disease is caused by the 5 most common pathogens: *C albicans*, *C glabrata*, *C tropicalis*, *C parapsilosis*, and *C krusei*. These organisms can result in serious infections that are mostly referred to as invasive candidiasis (McCarty and Pappas, 2016).

Opportunistic fungal infections pose a high threat to immunocompromised patients; Immunocompromise is characterized as the pathologic dysfunction of an organism's defenses against infection. (Harkness, 1987). Many factors, such as low birth weight, cancer, diabetes, AIDS, burns, and organ transplantation, can negatively affect the immune system (Page and Kurtzman, 2005). In addition, both radiation therapy and chemotherapy can lead to irritation and injury to the oral mucosa (mucositis), resulting in hyposalivation and xerostomia, and an increase in oral yeast amplification, and growth (Wahlin, 1991).

The potential of *Candida albicans* to colonize the host defines virulence. *C. albicans* causative agent is determined by a number of factors, such as phenotypic switching, dimorphism, adhesive properties, extracellular enzyme production, and biofilm formation. *Candida sp.*, particularly *Candida albicans*, plays a significant role in the development of oral squamous cell carcinoma through its interaction with epithelial cells, resulting in the production of epithelial cytokines and matrix metalloproteinase, as well as an epithelial pro-invasive phenotype. This role is most likely to occur when *C. albicans* form a biofilm. (Kang *et al.*, 2016).

C. albicans is the most frequently reported species causing human infection, but other species, like *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, have also been reported. Phenotypic methods are considered the gold standard for identifying yeast species isolated from clinical samples in many hospitals. The applications can be seen by eyes including the shape, size, and color of colonies on agar plates as well as microscopic observation of fungal structures in clinical samples and culture. (Montes *et al.*, 2019). Rapid and accurate identification of *Candida* species in clinical specimens is necessary for the initiation of proper antifungal treatments. Even though clinical culture is usually followed by biochemical based on chromogenic media for identifying the infecting species, these methods have some drawbacks, such as the more time required to generate results until the microorganism's identification to complete. Besides the classical methods of identification, they have limited sensitivity, using nucleic acid-based assays such as PCR allows for quick identification of *Candida* species. (Zhang *et al.*, 2016).

Molecular identification of *Candida* species was achieved using a widely used PCR-based method. ITS1 (Internal transcribed spacer1), TS2 (Internal transcribed spacer2), and the 5.8S gene was

directed for identification purposes (Ortiz *et al.*, 2018). The PCR-RFLP strategy has already defined all *Candida species* to species accurately (Mottaghi *et al.*, 2021). In brief, the ITS1-5.8S rDNA-ITS2 was actually used successfully for the identification of candida species after enzymatic digestions, *MSPI*, and electrophoresis. (Mirshekar, Emami and Mohammadi, 2021).

The purpose of this study is to determine the frequency of oral Candidiasis and the identification of species in cancer patients by using Polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP). The second aim of this study was to indicate the effect of candidiasis on the pH of saliva during oral candidiasis in cancer patients.

MATERIALS AND METHODS

Sample Collection:

A total of 150 samples of cancer patients with oral candidiasis were included for the identification of *candid species*. In addition, saliva was collected from 50 healthy individuals and 50 cases to study the change in the acidity of saliva due to candidiasis. The study has been conducted in Hiwa hospital in Sulaymaniyah, Kurdistan Region, Iraq (Fig. 1). The candida samples were taken using a bacteriological swab and the swab stick was immediately replaced with its casing, labeled, and taken to the laboratory for cultural context on Sabouraud dextrose agar. The participants filled out the form with confidence. A questionnaire was filled out for each participant, inquiring about their age, sex, type of cancer, smoking, addresses, occupation and CBC test. We collected saliva in a clean and sterilized container to check the pH in both patients and healthy controls.

Macroscopic Identification:

Each participant's swab was cultured on Sabouraud dextrose agar (Accumedia UK), and prepared slid with chloramphenicol stain (0.1mg/ml Sigma-Germany). The plates were then incubated

for 48 hours at 30 degrees Celsius. Each colony was examined for size, color, and shape. A single colony was picked and streaked on Hichrom agar (Candida differential agar) on each SDA plate, and the plates were incubated at 30 degrees Celsius for 48 hours. The appearance and morphological characteristics of the colonies were also used to approximate, as suggested by the manufacturer. *C. albicans* colonies are light green, *C. tropicalis* colonies are blue, but *C. glabrata* colonies are white to cream, *C. krusei* colonies are purple to pink, and *C. glabrata* colonies are cream to white with a slight purple central colony. (Agarwal *et al.*, 2011).

Molecular Diagnosis:

DNA Extraction:

All isolates Were subjected to molecular diagnosis and characterization using polymerase chain reaction using primers specific to an ITS1-5.8S-ITS2 gene. After overnight culture of candida, DNA was extracted by boiling colony in 40 µl ddH₂O for 20-minute at 95 C according to Lau *et al.* and Alshahni *et al.* The DNA was separated by centrifugation at 12000 rpm for two min, accompanied by the use of 15 µl of the supernatant as the PCR template. (Lau *et al.*, 2008)(AlShahni *et al.*, 2009).

PCR Amplification of ITS1- 5.8S-ITS2 r DNA Regions:

The ITS1 (forward, 5'-TCC GTA GGT GAA CCT GCG G-3') and (reverse primer 5'-TCC TCC GCT TGA TAT GC-3') primers were utilized to amplify ITS1-5.8s-ITS4 region of the gene (Mirhendi *et al.*, 2006). PCR amplification of the ITS1-5.8s-ITS2 region was conducted in a final volume of 30 µl. Each reaction includes 15 µl templates DNA, 1 µl primer (for work of PCR the primer is then diluted to 10pmol/µl by dilution of 10 µl of stock with 190 µl of free nuclease water), 2.5 µl DDH₂O, 12.5% polymerase from 2X Taq PCR master mix. The amplification requirements are as follows: 35 cycles of denaturation at 94 Celsius for 30 seconds, primer annealing at 56 Celsius for 30 seconds, as well as

an extension at 72 Celsius for 30 seconds. The denaturation step in the first cycle was 94°C for 5 minutes, and the extension at 72 Celsius stages in the final cycle was 72 Celsius for 7 minutes. %2 agarose gel electrophoresis in TBE buffer (20mmol/l EDTA, 10 mmol Tris boric pH 8) was used to illustrate amplified products. Blue light gel document was also used to photograph a gel after it was stained with nucleic acid stain (5g/ml).

Restriction Enzymes and Restriction Digestion:

Restriction digestion analyses were used to differentiate candida species using a sequence of ITS1-5.8S-ITS2's rDNA regions. The restriction sites for various restriction enzymes *MspI* and *AvrII* were selected for identification. In a final reaction volume of 10 µl, 15 µl of PCR products obtained by ITS1 and ITS2 primers were incubated with 0.5 µl of each enzyme for 15 minutes at 37 Celsius Agarose gel (35%) in TBE buffer was used to separate DNA pieces for 30 minutes at 100 V. Blue light gel document was also used to photograph the gel after it was stained with nucleic acid stain (5g/ml). The size of DNA fragments was determined directly by using DNA markers. Clinical isolates samples with such a 510-871bp PCR product size arranged *C.albicans* (535bp), *C.glabrata* (871bp), *C.tropicalis* (524bp), *C.keyfr* (722bp), *C.krusi* (510bp), *C.dublinsiensis* (530bp). (Mirhendi *et al.*, 2005).

PCR products were digested with *MspI* and *AVrII* separately. *MspI* enzyme digestion of the ITS1 region of *Candida sp.* created two bands for *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*. The PCR and digestion product lines were equal in shape for *C. keyfr*. Since *MspI* is not able to discriminate between two morphologically similar species, *C. albicans* and *C. dubliniensis*, so we used another enzyme, *AVrII*, which makes DNA cleave where there is a CCTAGG sequence. The digestion DNA product with *AVrII* was analyzed according

to DNA size in different species: for *C. dubliniensis* to 200-335bp but *C. albican* constant 535bp with the same size as PCR fragment (Shokohi *et al.*, 2010).

Acidity Test of Saliva:

After the accumulation of saliva, it's tested with an Electrolytes Analyzer, YACOi (Yarsan-India). Then the acidity was tested to start comparing the PH intervals of patients with oral candidiasis and control cases (Lam-ubol *et al.*, 2021).

Statically Analysis:

Statistical analyses were performed using SPSS, Frequencies, percentages and data compared by using the Chi-square test. P-values below 0.05 were considered significant.

RESULTS

Myeloperoxidase:

The Rate Of Candidiasis Among Cancer Patients:

A total of 200 oral swabs were collected from cancer and control cases in this study. The clinical symptoms of oral

candidiasis in 150 different cancer patients were analyzed including soreness, erythema, ulceration, and the presence of white plugs in the mouth. Of the total of 150 oral candidiasis patients, 108 cases (72%) were residents outside of the city, Sulaimaniyah, whereas 42 cases (28%) come from the center of the city. The age of participants was between 3 to 83 years, but more frequency was observed in (41 to 60 years). We have (50) healthy cases with no clinical symptoms of candidiasis, 27 men (54%) and 23 females (46%).

The rate of candidiasis was different in patients with different types of cancer. Candidiasis was the most frequent among patients who have carcinoma (81 patients, 32 males and 49 females), followed by Leukemia (26 patients, 17 males and 9 females), Sarcoma (23 patients, 13 males and 12 females), and Lymphoma (20 patients, 15 males and 5 females) (Table1).

Table 1: Isolate of Candida species (*Candida albican*, *Candida galabrata*, *Candida kefy*, *Candida tropicalis*, *Candida krusei*, *Candida dubliniensis*) among four types of cancer.

Types of cancer	Number of Cases	Percentage%
Carcinoma	81	54%
Leukemia	26	17.3%
Sarcoma	23	15.3%
Lymphoma	20	13.3%
Total	150	100

Prevalence of Candida Species from Oral Candidiasis In Cancer Patients:

Different types of Candida were observed among four types of cancer patients in Hiwa hospital, Sulaymaniyah, Kurdistan Region, Iraq, including *Candida albicans* (70 percent), *C. glabrata* (12

percent), *C. kefy* (6.7 percent), *C. tropicalis* (5.3 percent), *C. krusei* (3.3 percent), and *C. dubliniensis* (2.7 percent). All samples were obtained from people who had clinical symptoms of oral candidiasis like soreness, erythema, ulceration, and the presence of white plugs in about their mouth. (Fig.1).

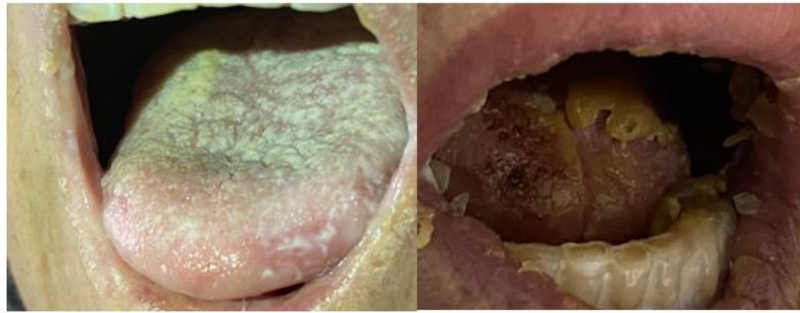


Fig. 1: Oral candidiasis appears when a yeast infection develops on the interior of the mouth and the tongue in cancer patients.

In comparison to control cases, we identified 5 types of candida species with a frequency of 27. *C.albican* (%54), *C.glabrata* 4 cases (%8), *C.tropical*,

C.krusei and *C.dubliniensis* observed one-time frequency (2%), 18 frequency (36%) negative cases (Table 2).

Table 2: Distribution of candida species among cancer patients with oral candidiasis cases and control.

Cancer case with oral candidiasis			Control health		
Candida species	Number of isolates	Percentage %	Candida species	Number of isolates	Percentage %
<i>C. albicans</i>	105	70.0	<i>C. albicans</i>	27	54
<i>C. glabrata</i>	18	12	<i>C. glabrata</i>	4	8
<i>C. kefy</i>	10	6.7	<i>C. tropicalis</i>	1	2
<i>C. tropicalis</i>	8	5.3	<i>C. krusei</i>	1	2
<i>C. krusei</i>	5	3.3	<i>C.dubliniensis</i>	1	2
<i>C.dubliniensis</i>	4	2.7	Negative	16	32
Total	150	100	Total	50	100

Identification of Candida Species by RFLP In Comparison to Routine Methods:

The specimens were all begun to grow on Sabouraud agar plates. This same morphology of colonial possessions was

revealed under a compound light microscope with a magnification of 40X, microscopy of simple smeared cultures obtained from view-based round or ovoid cells with or without budding. (Fig.2).

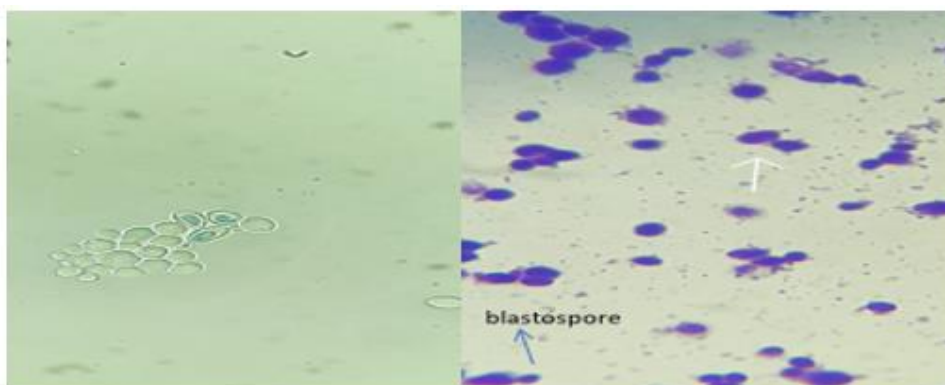


Fig. 2: Budding of candida species was examined at 40x magnification of a microscope. A) Yeast cell Stained with lactophenol cotton blue, B) Stained with simple stain (crystal violet).

All isolates were identified on the Hichrom agar Candida differentiated medium, and we showed different isolates focused on their colors for each candida within 48 hours in 30C⁰. *C.albican* has light green smooth colonies, so even though *C.tropicalis* has blue to metallic blue raised

colonies. *C.glabrata* colonies were also cream to white smooth colonies, whilst also *C.krusei* colonies have become purple fuzzy colonies, *C.keyfr* colonies are cream to white with a slight purple core, and *C.dublinsiensis* colonies were also pale green. (Fig.3).

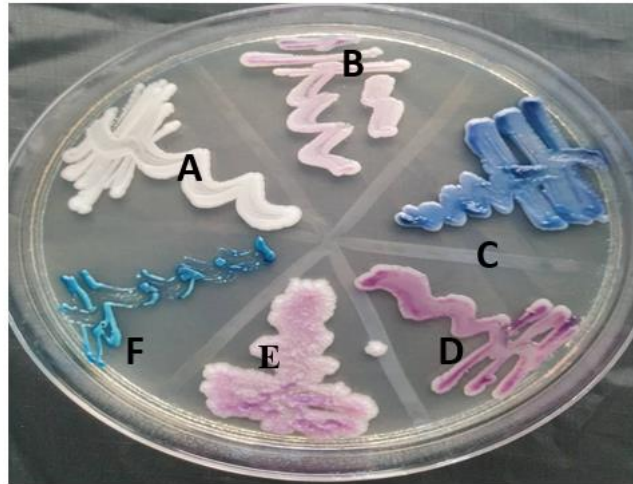


Fig.3: Colonies were grown on Hi Chrome agar (candida differential agar) after 48 h at 30 C. A) *C.glabrata* , B&D) *C.Keyfr*, C) *C.tropicalis* , E) *C.krusei* , F) *C.albicans*.

When *C. albican* and *C. dubliniensis* have become incubated in living thing blood serum at 37°C for 2-3 hours, a spore germination test produces

short, slender tube-like projections called germ tubes. *C.albicans* and *C.dublinsiensis* were also characterized from other yeast species using the germ tube Test. (Fig 4).



Fig. 4: Germ tube formation by *C.albicans* and *C.dublinsiensis*, the yeast has straight walls, without septum and constriction at the junction between the cells light microscope 40 X.

The molecular techniques using PCR were achieved for 6 candida genera. The expected band size was 535bp for *C.albican*, 871bp for *C.glabrata*, 524bp for *C.tropicalis*, 722bp for *C.keyfr*, 510bp for *C.krusei* and 530bp for *C.dublinsiensis*. (Fig.5). The amplicons were further subjected to digestion by *MspI*. (Fig. 6). *C. dublinsiensis* bands were 297-238/100bp,

C.tropicalis 340-190bp, *C.glabrata* 314-557bp, *C.albican* 297-238, *C.krusei*(261-249), and 722bp for *C.keyfr* without cleavage. Also, digestion with *AvrII* was performed and expanded bands for *C.dublinsiensis* was 200-335bp but *C.albican* constant was 535bp after digestion with the same enzyme. (Fig. 7)

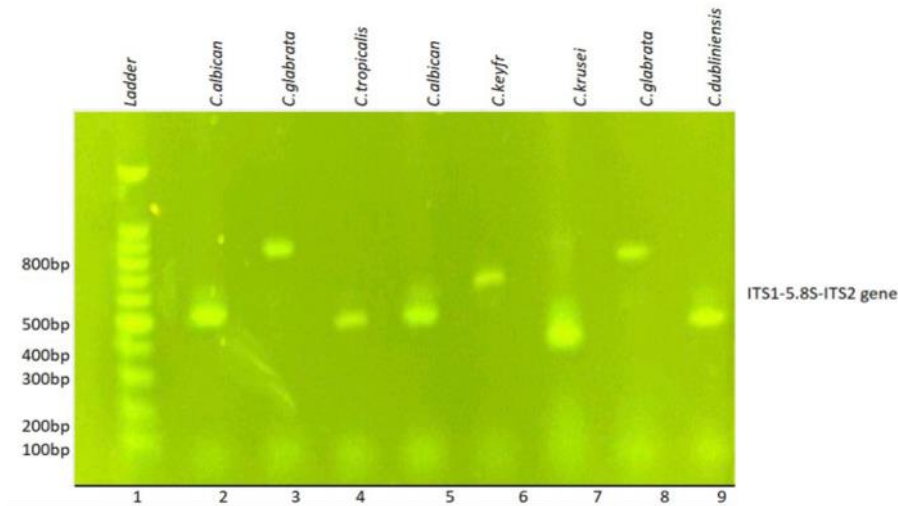


Fig. 5: The PCR amplification of ITS region of different candida species isolated from the mouth of cancer patients, agarose gel (2%) electrophoresis was performed PCR products of ITS regions 1.The marker was 100bp DNA.2&5: *C. albicans*, 3&8:*C. glabrata*, 4:*C. tropicalis*, 6:*C. keyf*, 7:*C. krusi*, 9:*C. dubliniensis*.

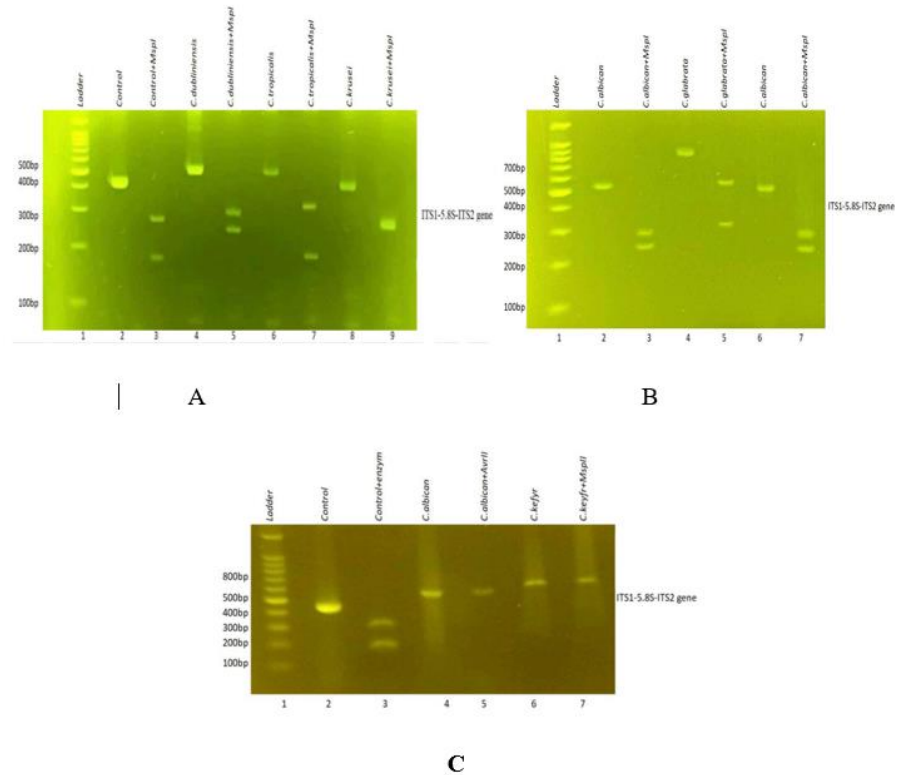


Fig. 6: The PCR amplification of ITS region of different candida species isolated from the mouth of cancer patients, agarose gel electrophoresis of ITS-PCR products of various pathogenic *Candida* species before and after digestion with enzyme *MspI*.

(A).Lanes 1: Ladder 100bp DNA size marker, 2: Control, 3: Control with enzyme, 4: *C. dubliniensis*, 5: *C. dubliniensis* with enzyme *MspI*, 6:*C. tropicalis*, 7: *C. tropicalis* with enzyme *MspI*, 8:*C. krusei*, 9:*C. krusei* with enzyme *MspI*,
 (B) Lanes 1: Ladder 100bp DNA size marker, 2:*C. albicans*, 3:*C. albicans* with enzyme *MspI*, 4: *C. glabrata*, 5: *C. glabrata* with enzyme *MspI*. 6: *C. albicans*, 17:*C. albicans* with enzyme *MspI*.
 (C) Lanes 1: Ladder 100bp DNA size marker, 2: Control, 2: Control with enzyme *MspI*, 3:*C. albicans*, 4:*C. albicans* with *AvrII*, 5: *C. keyfr*, 6:*C. keyfr* with enzyme *MspI*.

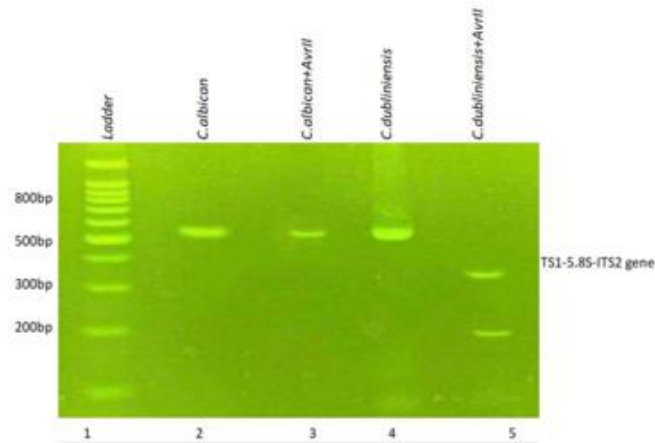


Fig. 7: RFLP patterns of *AVrII* endonuclease on (3 %) of agarose gel; Lane 1: DNA ladder 100bp, 2: PCR product of *C. albican*, 3: *C. albican* with enzyme, 4: *C. dublieniensis* PCR product, 5: *C. dublieniensis* with enzyme.

Acidity test of Saliva

To obtain an insight into the effect of candidiasis in cancer patients on the pH change of saliva, the acidity of saliva was checked in cases and control samples. The min and max PH of patients were 4.3 and 6.5, and the mean PH of saliva in cancer patients was 5.41, with a standard deviation of 0.62.

DISCUSSION

The sharp pathogen *Candida* causes a possibly deadly illness, particularly in immunosuppressed patients (Neppelenbroek *et al.*, 2006). Chemo- and radiotherapy are the most frequently utilized therapy for cancer patients. These cytotoxic treatments have a few unpleasant adverse reactions, such as microsites. Non-hematologic restricted erythema starts to develop into a deep ulcer, causing distress, torment, critical morbidity, and interruption of cancer treatment. (Vasconcelos *et al.*, 2016). Chemotherapy as well creates an imbalance in the standard verbal greenery and the basis of extra-oral vegetation due to oxidative stretch, increased activity of the natural resistant system, and xerostomia. An increase in *Candida* species has been reported all through radiation treatment. (Almståhl, Wikström and Fagerberg-Mohlin, 2008). In developing nations, is little known about candidiasis and *Candida* colonialization in cancer patients. The aims of this research were to

find the rate of candidiasis in cancer patients and then use PCR-RFLP to investigate the rate of different species of candida. This technique is for the first time used in the study area to detect candida species in cancer Patients who suffer from oral Candidiasis in Sulaymaniyah, Kurdistan Reign, Iraq.

As is seen in our findings, most cancer patients are symptomatic patients for candidiasis in their oral mucosa in comparison to healthy control people. This condition might be due to using chemotherapeutics, radiation, and high doses of verbal and systemic corticosteroids. (Teoh and Pavelka, 2016)(Aldossary, Almansour and Abdulraheem, 2016). Carcinoma patients were spoken to as the greatest serious type of cancer in sulaimanyah, suggesting that this type of cancer may be observed across the world in a wide variety of countries. *Candida* sp. has been discovered in (54 percent) of carcinoma patients, owing to the fact that all of those patients received radiotherapy in relation to chemotherapy, or they had a surgical operation in furthermore to chemotherapy, which exacerbated the situation (Jain *et al.*, 2016). Cancer and chemo and radiation are very well known for causing immunosuppression, which enables *Candida* disease to prosper. As a consequence of immunity dysfunction and mucosal harm, yeast diseases such as

mucositis, xerostomia, and candidiasis improvement. (Caira *et al.*, 2015).

Among the leukemia malignancies patients in Sulaymaniyah, Kurdistan Region, Iraq, and the higher incidence of this form was in older life and it may be partly due to the buildup of radioactive material from later wars, (17.3 percent) of leukemia, and lymphoma (13.3 percent) patients appeared verbal Candidiasis may be caused by long the use chemotherapy. It is well known that use of chemotherapy can cause neutropenia, interruption of mucosal obstruction, and general harm to cell-intervened resistance, which raises the risk of contamination. (Kang *et al.*, 2016). This result was consistent with past findings (Dahiya *et al.*, 2003)(Jham *et al.*, 2008)(Hasan and Al-jubouri, 2015), which also found that fungal diseases are more popular in leukemic patients. According to our viewer, the pervasiveness of verbal candidiasis among patients with leukemia and lymphomas had been 32.6 percent. Hamzehee *et al.* detailed comparative results in their show ponder. (28%) of the total.(Hamzehee *et al.*, 2019).

There is indeed a predominance of yeast strains in Europe, including Slovakia, predicated on verbal tests. Moreover, *Candida spp.* collected, *C. albicans* was the most common (61.9 percent), followed by *C. krusei* (14.3 percent), *C. valida* (9.5 percent), and *C. glabrata*, *C. tropicalis*, and *C. intermedia* (all: 4.8 percent)(Černáková *et al.*, 2022). Research in Brazil did find that *C. albicans* colonized approximately half of the patients (42.9 percent) and nonalbicans species colonized 33 percent of the patients (*C. tropicalis*, *P. kudriavzevii* (*C. krusei*), and *C. lusitanae*) (Jham *et al.*, 2007). The prevalence of *Candida* species in our study is comparable to what was published in Mexico. (Sánchez-Vargas *et al.*, 2005).

Six Common *Candida Species* were discovered; even so, *Candida albicans* was the most frequently confined yeast within the show study % 70, the results of this studys predominance were lower than a study conducted in Northwest Ethiopia 82.3

%(Mulu *et al.*, 2013) and higher than a study conducted in central Ethiopia. Mexico reports 58.6 %(Bitew and Abebaw, 2018).Schelenz *et al.*, from the United Kingdom, reported today the distribution of *Candida species* in 400 patients of hematological malignancies, head and neck cancers, and solid tumors. *C. albicans* must have been found to be involved in 74% of case, followed by *C. glabrata* (11.5%), *C. tropicalis* (2.6%), *C. krusei* (2.6%), and *C. parapsilosis* (1.9%)(Schelenz *et al.*, 2011). Another study showed that approximately 85 percent (n = 50) of cancer patients came back positive for *Candida* species culture from the oral mucosa, with *C. albicans* being the most widely distributed species, followed by *C. glabrata* in 14.5 percent of cancer patients(de Sousa *et al.*, 2016)(Aslani *et al.*, 2018). Comparable findings were observed in the present study, and *C.galabrata* was found to be the moment most common separation among the research group. Another earlier research in Iranian people and Basrah, Iraq, noticed that *C. albicans* was the most regularly found in the oral mouth and nose of cancer patients.(Shokohi *et al.*, 2010)(Aldossary, Almansour and Abdulraheem, 2016).

CHROM agar *Candida* medium allowed the development of important clinical yeasts, and also hypothetical recognizable proof of *C. albicans* and other *non-candida albican* (NCA) spp.,(Mehta and Wyawahare, 2016). A few of the variations between the displayed ideas and our opinions led to the appearance of just using CHROMagar due to a mix and undetermined color. This invention could be a strong reason for using molecular methods for determining and trying to identify restoratively essential *Candida sp.* in clinical research labs. In comparison to a phenotypic strategy, which is harsh, appears to require reproducibility and standardization, the investigation of restriction fragment length polymorphic (RFLP) inferred from the DNA of *Candida spp.* has the advantage of being simple,

quick, and solid. (Cirak, Kalkanci and Kustimur, 2003).

We were using a PCR-RFLP method to identify the medically important *Candida spp.* in this study because this profile was also recommended as an easy and accurate method for differentiating between different morphologically similar species, *C. albicans* and *C. dubliniensis*.

In the latest study, the mean PH of cancer patients seemed to be 5.41, standard deviation (of 0.62), compared to 7.11, standard deviation (of 0.43) for normal participants. In a previous study, amplified colonization and poor saliva properties, coupled with an immune-compromised host, put the patient with head and neck cancer at high risk of candidiasis (Fanello *et al.*, 2006). *Candida albicans* are the most commonly found oral species in both healthy and xerostomic people. However, non-*Albicans* species or multiple *Candida* species colonize xerostomic patients, likely to result in a more complex oral environment and treatment difficulty. (Sadeghi *et al.*, 2018).

CONCLUSION

The study concluded that *Candida albican* are the most common of candida spp. in cancer patients in comparison to non-*albican candida* species and it may be due to affected of chemo-radiotherapy treatment. Cancer patients who have carcinoma were the most prevalent type of cancer with oral candidiasis. PCR-RFLP is a rapid, easy and better method than chrome agar to determine species and is also an applicable method in the clinical laboratory for the identification of medically important *Candida spp.*

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