

## Susceptibility of Yeast Species to Different Concentration of Fluconazole in Leukemic and non-Leukemic Cancer Patients

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### ABSTRACT

**Aim :** Isolation and identification of yeast species in leukemic and non leukemic cancer patients and conduct the in vitro fluconazole susceptibility in different concentrations.

**Patients and methods:** This study included 100 oral swabs samples from cancer patients 50 of them were leukemic cancer patients group I, while group II patients were 50 non-leukemic cancer patients. Group I patients attending the hematology department in Ibn-Sina teaching hospital, and group II patients attending oncology and nuclear medicine teaching hospital in Mosul city. The clinical specimens were collected during period (June - December 2013).The patients included in this study were females only. Isolation and identification of yeast species depend on the classical methods. All the isolated yeast species were tested for their susceptibility to fluconazole concentrations (flu.10mcg, flu. 25mg, flu 50mcg) using standard disc agar diffusion method.

**Results:** *C. albicans* 28(52.8%), *C. topocalis* 1(1.9%), *Cryptococcus laurentii* 1(1.9%) all of them were higher in group I than group II, in addition *C.parasilosis* 1(1.9%) was higher in group II than group I, while *C. glabrata* was the same in group I 1(1.9%) and group II 1(1.9%). Fluconazole sensitivity test ranged from 40(38.1%), 34(32.4%), 31(29.5) to fluconazole discs potency 50mcg, 25mcg, 10mcg in both groups of cancer patients.

**Conclusion:** *C. albicans*, *C. topocalis*, *Cryptococcus laurentii* all of them were higher in group I than group II, in addition *C.parasilosis* was higher in group II than group I, while *C. glabrata* was the same in group 1 and group II. The sensitivity test to fluconazole were dose dependent with no significant difference between the three concentration to fluconazole .

**Keywords:** Cancer, *Candida*, Disc agar diffusion method.

## حساسية اجناس الخمائر للتراكيز المختلفة من الفلوكونازول في مرضى سرطان ابيضاض الدم

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### الخلاصة

**الهدف من الدراسة :** عزل وتشخيص اجناس الخمائر من مرضى سرطان ابيضاض الدم ومن مرضى السرطان من غير سرطان ابيضاض الدم و اجراء فحص الحساسية للفلوكونازول بتركيز مختلفة.

**المرضى وطرق الدراسة :** شملت الدراسة 100 عينة من مسحات فم لمرضى السرطان، 50 منهم مصابين بسرطان ابيضاض الدم المجموعة الاولى، بينما المجموعة الثانية تضمنت 50 مريضا من مرضى السرطان من غير سرطان ابيضاض الدم. المجموعة الاولى عزلت من قسم امراض الدم في مستشفى ابن سينا التعليمي والمجموعة الثانية عزلت من مستشفى الاورام والطب النووي في مدينة الموصل. تم جمع العينات السريرية للفترة من حزيران - كانون الاول 2013. الدراسة تضمنت الاناث فقط. عزل وتشخيص اجناس الخمائر تم بالاعتماد على الطرق التقليدية. جميع عزلات اجناس الخمائر اجريت لها فحص الحساسية للفلوكونازول بتركيز (10ملغم، 25ملغم، 50ملغم) باستخدام طريقة الانتشار القرص بالوسط التقليدية.

**النتائج:** 28 *C. albicans* (52.8%), *C. tropicalis* (1.9%), *Cryptococcus laurentii* (1.9%) كانوا جميعا اعلى في المجموعة الاولى منها في الثانية, بالإضافة *C. parapsilosis* (1.9%) اعلى في المجموعة الثانية منها في المجموعة الاولى. بينما *C. glabrata* ظهرت متساوية في المجموعة الاولى (1.9%) والمجموعة الثانية (1.9%). فحص الحساسية للفلوكونازول امتد من 25 mcg, 34 (38.1%), 31 (32.4%), 29.5) للتراكيز الثلاثة المختلفة من الفلوكونازول 50 mcg.

**الاستنتاج:** *C. albicans*, *C. tropicalis*, *Cryptococcus laurentii* كانوا جميعا اعلى في المجموعة الاولى منها في الثانية, بالإضافة *C. parapsilosis* اعلى في المجموعة الثانية منها في المجموعة الاولى, بينما *C. glabrata* ظهرت متساوية في المجموعة الاولى و المجموعة الثانية. فحص الحساسية تجاه الفلوكونازول معتمدا على الجرعة مع عدم وجود فرق معنوي بين التراكيز الثلاثة من الفلوكونازول.

**الكلمات المفتاحية:** سرطان , كانديدا, طريقة الانتشار بالقرص والطبق.

## INTRODUCTION

Most fungi that colonize the body surface are yeasts, mostly find in normal oral commensal in about 20-60% of the population, the most common of which are members of the genus *Candida*, and have a commensal relationship with their hosts. These commensal fungi may find condition conducive to tissue invasion and infections which have increased during the past third decades<sup>1</sup>. Cancer patients have a high prospect for fungal infection especially by yeast species as a result of the immunocompromised state especially those under the effect of the immunosuppression by intensive cytotoxic chemotherapy, recipient hematopoietic stem cell transplant, long term indwelling catheter and with increasing use of broad spectrum antibiotics<sup>2</sup>. Although yeast infection remain an important cause of morbidity and mortality to immunocompromised patients<sup>3</sup>. In recent years there is a shift in the epidemiological characteristics of fungal pathogens toward saprophytic fungal resistance species have placed a greater emphasis on selection of broad spectrum antifungal agents<sup>3</sup>. However the spectrum of infecting species appears to be increasing and many species of the genus *Candida* mainly *Candida albicans*, non-*albicans Candida* species and filamentous fungi of the genus *Aspergillus* have emerged as a possible cause of opportunistic fungal infections in cancer patients<sup>4</sup>.

Fluconazole a safe triazole and a well tolerated antifungal agents that distributes widely in body tissues and is active against the major fungal pathogens in immunocompromised patients with few side effects, its empiric use in onco-haematological patients may result in a higher colonization rate with fluconazole resistant species<sup>5</sup>. Although fluconazole has emerged as the primary treatment option for virtually all forms of susceptible *Candida* infections in both immunocompromised and immunocompetent hosts

given the wide spread use of this agents concern about the development of resistance in yeast have been raised, the in vivo and in vitro susceptibility test of yeast correlated with concentration of fluconazole measured in different compartment of the body<sup>6</sup>.

So, the aim of this research is to isolation and identification of yeast species in leukemic and non leukemic cancer patients, to see any difference between them, and conduct the in vitro fluconazole susceptibility in different concentrations.

## PATIENTS AND METHODS

**Patients :** 100 cancer patients were included in this study, their age ranged from 12-80 years. The patients were females only depend on previous study that yeast count and carriage was higher in females than males<sup>7</sup>. Two groups of cancer patients were included in this study, the first group were 50(50%) leukemic cancer patients (different types of leukemia) group I, while the second group were 50(50%) non leukemic cancer patients (different types of cancer other than leukemia) group II, both groups of patients were under treatment with chemotherapy and different types and duration of treatment with broad spectrum antibiotics.

### Studied Samples

A total of 100 oral swab samples were collected in period ( June - December 2013) from group I patients attending hematology department in Ibn Sina teaching hospital, and from group II patients attending oncology and nuclear medicine teaching hospital in Mosul city.

### Isolation of the Yeast

One oral swab from each patient was used directly for microscopical and macroscopical examinations. Microscopical examination of all specimen were done by Gram stain for presence of

budding yeast cells. Macroscopical examination done by cultured all specimens on double plate of Sabouraud's dextrose agar and blood agar with 5% blood, both are supplemented with (0.5 mcg/ml of chloramphenicol) then incubate for 2-3 days for primary isolation. If positive culture was obtained further identification procedures were applied by study colony morphology by lactophenol mount of portion of the yeast. Germ tube formation test in 0.5 ml of human serum and chlamyospore formation test on corn meal agar for identification of *Candida albicans* from non-*albicans* *Candida* species (NACS). API-10 CS system strip for identification of NACS, *Candida albicans* germ tube negative and other yeast species<sup>8</sup>.

### Antifungal Susceptibility Test

A tested yeast suspension was prepared by taking a small portion of yeast colony, then emulsified in Sabouraud's dextrose broth, then compared to 0.5 McFarland scale. The prepared suspension inoculated onto the surface of Sabouraud's dextrose agar plate supplemented with 0.5 mcg /ml methylene blue dye and leave it for 2-3 minutes. Three concentrations of fluconazole discs (flu.10mcg, flu.25mcg, flu. 50 mcg) were placed firmly on the surface of the inoculated plates, then the plates were incubated for 24-48hr. at 37 °C. The antifungal activity was evaluated by measuring the diameter of inhibition zone (mm) around the discs and the results were recorded as sensitive (S), dose dependent susceptibility (DDS), and resistant (R)<sup>9</sup>.

### Statistical Analysis

The tests used for statistical analysis were, number, percentage, chi-square test, and Z- test.

## RESULTS

Among 100 cancer patients, there were 53(53%) patients showed the presence of yeast in their clinical specimens. From the 53 patients with positive isolated yeast there were 31(58.5%) of them were leukemic patients ( group I), and *Candida albicans* isolates were 28 (52.8 %), while NACS isolates were 2 (3.8%), in addition to one isolate of other yeast species of *Cryptococcus laurentii* 1(1.9%) (Table 1). On the other hand, from 22(41.5%) non leukemia cancer patients (group II), *Candida albicans* isolates were 20 (37.7%), while NACS were 2(3.8 %), there is no significant difference in between group I and group II in the presence of yeast in their clinical specimen at ( P = 0.248) by Z –test, with a significant difference between *Candida albicans* and non *albicans* *Candida* species in between group I at ( P = 0.000) , and in between group II at ( P =0.000) by Z- test. There is a significant

difference between *Candida* species and *Cryptococcus* species at (P= 0.000) by Z-test. (Table1).

### The Isolated Yeasts

From 53 yeast isolates in group I and group II patients, in group I patients the isolated yeast from genus *Candida* were identified into 3 species respectively *Candida albicans* 28 (52.8 %), *Candida glabrata* 1(1.9%), *Candida tropicalis* 1(1.9%), in addition to one isolate from genus *Cryptococcus* was *Cryptococcus laurentii* 1(1.9 %) (Table 1). While the isolated yeast were identified from group II into 3 species respectively *Candida albicans* 20 (37.7%), *Candida glabrata* 1(1.9%), and lastly *Candida parapsilosis* 1(1.9%) (Table1).

### Susceptibility Test

The results of the susceptibility tests to three different concentrations of fluconazole used in this study showed in Figure (1).

A higher number of yeast species were sensitive to fluconazole 50 mcg, followed by fluconazole 25 mcg, then fluconazole 10 mcg (Table 2,3). In group I patients 22 *Candida albicans* isolates were sensitive to fluconazole 50, followed by 18 isolates to fluconazole 25 mcg, in addition to 4 isolates were DDS, then 16 isolates to fluconazole 10mcg, while *Candia glabrata*, *Candida tropicalis*, *Cryptococcus laurentii* were resistant to three fluconazole concentrations (Table2).

For the yeast isolated from group II cancer patients, 17 *Candida albicans* isolates were sensitive to fluconazole 50 mcg followed by 15 isolates were sensitive to fluconazole 25mcg, in addition to 2 isolates were DDS, then 14 isolates were sensitive to fluconazole 10 mcg, while *Candida glabrata* were resistant to 3 fluconazole concentrations, while *Candida parapsilosis* were sensitive to 3 fluconazole concentrations (Table 3).

In this study showed that the sensitivity to fluconazole were 40(38.1%) ,34(32.4%) and 31(29.5%) respectively from both group of patients. Isolates from group I were 22 (39.3%), 18(32.1), and 16(28.6%) respectively to fluconazole 50 mcg,25 mcg,10 mcg concentration, while from group II were 18(36.7%), 16(32.7%), and 15(30.6%) were sensitive to fluconazole 50mcg, 25mcg,, 10 mcg, with no significant difference in between the sensitivity to fluconazole disc 50 mcg and fluconazole disc 25 mcg at (P = 0.859) by  $\chi^2$  test, and there is no significant difference in between the sensitivity to fluconazole disc 10 mcg and fluconazole disc 25 mcg at (P=0.915) by  $\chi^2$  test Table 4.

Table (1) : Fluconazole sensitivity test of isolated yeast species from clinical specimens from both group I and group II.

Isolates	Total isolates		Group I		Group II	
	No.	%	No.	%	No.	%
<i>C. albicans</i>	48	90.5	28	52.8	20	37.7
<i>C. glabrata</i>	2	3.8	1	1.9	1	1.9
<i>C. tropicalis</i>	1	1.9	1	1.9	0	0
<i>C. parapsilosis</i>	1	1.9	0	0	1	1.9
<i>Cryptococcus laurentii</i>	1	1.9	1	1.9	0	0
<b>Total</b>	53	100	31	58.5	22	41.5

There is no significant difference in between group I and group II in the presence of yeast in their clinical specimens at ( P = 0.248) by Z –test.

There is a significant difference between *Candida albicans* and non *albicans Candida* species in between group I at ( P = 0.000) , and in between group II at ( P =0.000) by Z- test.

There is a significant difference between *Candida* species and *Cryptococcus* species at (P= 0.000) by Z-test.

Table 2: Number and percentage of sensitive (S) resistant (R) and dose dependent susceptibility (DDS) of three fluconazole concentrations to different yeast isolated in group I.

isolates	No.	%	Fluconazole 10 mcg			Fluconazole 25 mcg			Fluconazole 50 mcg		
			S	DDS	R	S	DDS	R	S	DDS	R
* <i>C.albicans</i>	28	90.4	16	0	12	18	4	6	22	0	6
<i>C.glabrata</i>	1	3.2	0	0	1	0	0	1	0	0	1
<i>C. tropicalis</i>	1	3.2	0	0	1	0	0	1	0	0	1
** <i>C. laurentii</i>	1	3.2	0	0	1	0	0	1	0	0	1
<b>Total</b>	31	100	16	0	15	18	4	9	22	0	9

\*C = *Candida* , \*\*C= *Cryptococcus*

Table 3: Number and percentage of sensitive (S) resistant (R) and dose dependent susceptibility (DDS) of three fluconazole concentrations to different yeast isolated in group II.

isolates	No.	%	Fluconazole 10 mcg			Fluconazole 25 mcg			Fluconazole 50 mcg		
			S	DDS	R	S	DDS	R	S	DDS	R
<i>C.albicans</i>	20	91	14	0	6	15	2	3	17	0	3
<i>C.glabrata</i>	1	4.5	0	0	1	0	0	1	0	0	1
<i>C. parapsilosis</i>	1	4.5	1	0	0	1	0	0	1	0	0
<b>Total</b>	22	100	15	0	7	16	2	4	18	0	4

Table 4: Number and percentage of yeast species isolates sensitive to three different concentrations to fluconazole in group I and II.

Fluconazole potency in mcg	Isolates sensitive to fluconazole				Total No.	Total %
	Group I		Group II			
	No.	%	No.	%		
Flu. 50 mcg	22	39.3	18	36.7	40	38.1
Flu. 25 mcg	18	32.1	16	32.7	34	32.4
Flu. 10 mcg	16	28.6	15	30.6	31	29.5
<b>Total isolates</b>	56	100	49	100	105	100

There is no significant difference in between the sensitivity to fluconazole disc 50mcg and fluconazole disc 25mcg at (P = 0.859) by  $\chi^2$  - test.

There is no significant difference in between the sensitivity to fluconazole disc 10 mcg and fluconazole disc 25 mcg at (P = 0.915) by  $\chi^2$  - test.

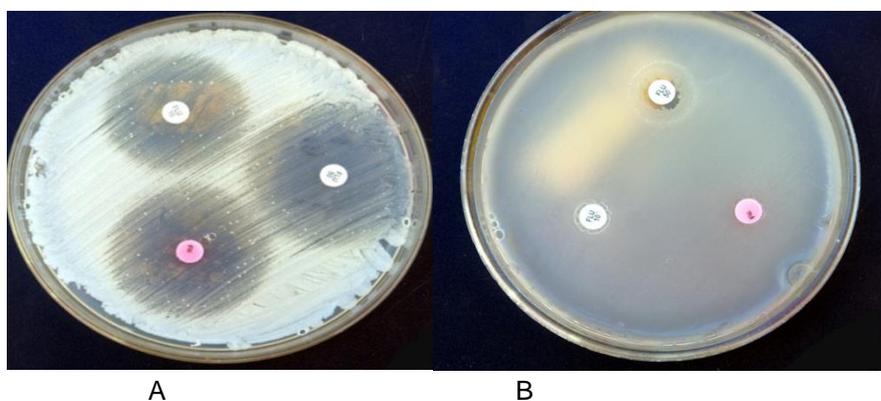


Figure (1) : Disc agar diffusion method of *C. albicans* with three fluconazole concentrations  
 A –Sensitive  
 B –Resistant

**DISCUSSION**

Advances in supportive therapies for continuous growing group of cancer patients have led to increasing rates of fungal infections accompanied with wide spread use of fluconazole for treatment and prevention of oropharangeal yeast infections<sup>10</sup>.

This study was carried out to compare between 2 group of cancer patients under treatment with chemotherapy and antibiotics and perform antifungal susceptibility to 53 (53%) isolates recovered from 100 cancer patients revealed yeasts elements in their clinical specimens, these patients were 31(58.5%) group I patients and 22(41.5%) group II. Leukemia is wide spread globally and in this study this group of cancer patients showed a higher yeast element in their clinical specimen than non leukemic cancer patients, this may be due to prolonged and

intensive use of chemotherapy may lead to neutropenia, disruption of mucosal barrier and over all damage to cell mediated immunity, increase time use for broad spectrum antibiotics, increase time hospital stay than other type of cancer, which increase the risk of yeast infection in such patients<sup>11</sup>. Lone *et al* showed that from 150 patients with various malignancies, a high yeast elements revealed from clinical specimens was in head and neck cancer patients (77%) followed with hematological malignancies (49%), and finally solid tumor malignancies was (40%)<sup>12</sup>.

Two genera of yeast *Candida* 52(98.1%) and *Cryptococcus* 1(1.9%) with four different species of *Candida* were isolated in our study. This study showed that the presence *Candida albicans* isolates was higher in group I 28 (52.8 %), than group II 20 (37.7 %), while NACS isolates were

same in both groups of patients 2(3.8%), with significant difference between *Candida albicans* and NACS in between the two groups of patients at (P=0.000), with no significant difference in between group I and group II in the presence of yeast in their clinical specimen at ( P = 0.248) by Z-test (Table 1). *Candida* is a heterogenous group of organisms, and more than 17 different *Candida* species are known to be etiological agents of human infections, however more than 90% of invasive infections are caused by *Candida albicans*, than NACS<sup>13,14</sup>. A small number of *Candida* species normally present in normal person, but increased when normal flora is altered by antibiotics or when a defect in immune system<sup>15</sup>. Patients with leukemia are susceptible to infection than other type of malignancies due to fact that compromise in immunity by both malignancy and cytotoxic treatment<sup>16</sup>. Although *Candida albicans* is the main *Candida* species isolated from clinical specimen, recent studies showed that there is increase in infection due to NACS such as *Candida krusei* and *Candida glabrata*<sup>17</sup>. Increasing incidence of NACS as *Candida tropicalis*, *Candida parapsilosis* and *Candida glabrata* have been isolated much more frequently as cause of invasive candidiasis worldwide, although *Candida albicans* remain a particular cause for developing candidiasis with attributable mortality around 40% is particularly in onco-hematological patients<sup>13</sup>. One genera of encapsulated yeast *Cryptococcus laurentii*(1.9%), with significant difference in between *Candida* species and *Cryptococcus* species at (P= 0.000) by Z-test were isolated from group I patients, which considered a rare human pathogen, and described as an infected agent in skin, oropharyngeal airways disease, endophthalmitis, keratitis, pulmonary abscess, pneumonia, peritonitis especially in immunocompromised patients<sup>18</sup>.

### Susceptibility Test

This study showed that the sensitivity results of *Candida albicans* were (16,18,22) and (14,15,17) isolates to (flu. 10 mcg,25mcg,50mcg) in both group I and group II respectively, the sensitivity of the isolates increase with increase in dose of fluconazole. Kustimur *et al* showed that the susceptibility of *Candida albicans* to different concentration of fluconazole were (84%) by using

disc agar diffusion method<sup>19</sup>. Four isolates from group I and 2 isolates from group II were DDS in 25mcg fluconazole. El-Mashad *et al* showed that 5 (13.5%) of *Candida albicans* were DDS to fluconazole in 25 mcg<sup>4</sup>. Colombo *et al* showed that 4% of *Candida albicans* isolates considered as DDS to fluconazole 25 mcg and most of these isolates were obtained from oral cavity of HIV infected (AIDS) patients<sup>20</sup>. Dose dependent susceptibility was most commonly reported among *Candida glabrata* and *C.krusei* strains, fluconazole resistant *Candida albicans* isolates are still considered a rare phenomenon<sup>21</sup>.

From group I and group II, *Candida glabrata* in both group, and *Candida tropicalis* were resistant to fluconazole, while *Candida parapsilosis* were sensitive to the 3 concentrations to fluconazole. Resistant to fluconazole was commonly reported among *Candida glabrata* and *Candida krusei* isolates<sup>20</sup>. Study showed that fluconazole resistant *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* isolates were found in 7.7%, 5.8% and 3.3% respectively, this could be due to fact that NCAS is less frequently susceptible to the currently azole antifungal drugs<sup>22</sup>. *Cryptococcus laurentii* in group I were resistant to the 3 concentrations of fluconazole. Study by Lord *et al* showed that eight isolates of *Cryptococcus laurentii* were resistant to fluconazole, but susceptible to other azole<sup>23</sup>. However Ferreira – Pain *et al* did not detect in vitro resistance in 38 isolates, but they find dose dependent susceptibility to fluconazole in 71% of cases<sup>24</sup>.

In our study most of the clinical isolated yeast were sensitive to fluconazole, however it is clear that there are some species differences in susceptibility to this antifungal agents. Sensitivity to fluconazole in both group ranged from 40(38.1) - 34(32.4%)- 31(29.5) of the isolated tested and the susceptibility depending on the species of *Candida* and type of underlying disease considered for analysis, group I show a higher sensitivity than group II. reading the inoculated plates with 10, 25, 50 mcg discs of fluconazole after 24 hr., showed that most of *Candida* species were susceptible to the 3 concentrations of fluconazole. However 10 mcg discs give good results, when increase in dose to 25 mcg increase in the number of isolates sensitive to fluconazole from 31(29.5%) to 34(32.4%) with no significant difference at

( $P=0.859$ ), there is no need to lower the dose than 25 mcg. In the other hand increase in dose from 25 mcg to 50 mcg show increase in number of isolates that sensitive to fluconazole from 34(32.4%) to 40(38.1%) with no significant difference at ( $p =0.915$ ), there is no need to increase in dose of fluconazole disc from 25 mcg to 50 mcg. Other study showed that discs containing 25mcg and 50 mcg fluconazole were compared, with no significant difference was found between the two. According to our result, the disc containing 25mcg, was just as useful as the one containing 50 mcg and 10 mcg for disc diffusion testing within 24 hours incubation on Sabouraud's dextrose agar<sup>19</sup>. Susceptibility test depends on achieving the maximum possible blood level, for fluconazole dose higher than the standard dosing amount (6mg / kg / day) may be needed in adults with normal renal function and body habitus<sup>9</sup>.

## CONCLUSION

*C. albicans*, *C. tropicalis*, *Cryptococcus laurentii* all of them were higher in group I than group II, in addition *C.parasilosis* was higher in group II than group I, while *C. glabrata* was the same in group 1 and group II. *Candida albicans* is the main yeast species revealed from clinical specimen. Fluconazole has a good activity against most strain of yeast species and disc diffusion method appear to be a convenient for susceptibility screening. The fast in detection and diagnosis of fungal infection in Iraq is important in immunocompromised patients to reduction the mortality rate and initiation of antifungal therapy.

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