Role of Fluconazole prophylaxis in decreasing *Candida* colonization among neutropenic children with Hematological Malignancies

Enas A.Daef[1], Michael N. Agban[1], Mona H. Mohammed[1], Mohammed H. Ghazally[2], Salah S. Abdelhadi[3], Amany M. Ali[4], Mazen A.A. Gwass[5]

[1]Department of Microbiology and Immunology, Assiut University, [2]Department of Pediatrics, Assiut University, [3]Department of Pediatrics Oncology, National Cancer Institute – Cairo University, [4]Department of Pediatrics Oncology, South Egypt Cancer Institute – Assiut University, Egypt. [5]Department of Pediatrics, Aden University, Yemen. agbanmichael@yahoo.com

Abstract: Invasive fungal infection is a leading cause of infection related mortality among patients with hematological malignancies, associated with prolonged and severe neutropenia. So, antifungal prophylaxis may be a good approach for neutropenic patients undergoing intensive myelosuppresive chemotherapy. **Aim of the study**: to study the prevalence and types of Candida colonization among neutropenic pediatric patients with hematological malignancies, to study the role of antifungal prophylaxis in decreasing Candida colonization and infection and in prevention of invasive Candida infection among severe and prolonged neutropenic children with hematological malignancies. Methods: the study included 64 children patients with severe and prolonged neutropenia associated with hematological malignancies. Group I: 32 patients received Fluconazole prophylaxis and Group II: 32 patients received placebo. Candida colonization and types was identified using phenotypic methods (Sabouraud's Dextrose agar, Hichrome Candida Differential agar, Cezpek Dox Agar with Tween 80, Germ tube test and Sugar assimilation test) and genotypic methods using PCR to detect type and species of Candida; for different samples [oral, rectal, blood, and urine] at base line and end of the study. Antifungal susceptibility test using agar disc diffusion method was used to test isolated strains. IgM for Candida albicans was done for all patients. Results: Pediatric patients (N = 64) with age 1.5 – 16 years old, 45 males and 19 females, with hematological malignancies and severe prolonged neutropenia. Colonization by Candida species was found in 54 samples (42%) in group I at base line against 47 samples (37%) in group II (P = 0.08), most of them from oral and rectal samples. These isolates were C. albicans (33), C. glabrata (6), and C. tropicalis (3) in group I, against 31, 7, and 2 isolates respectively in group II at base line (P= 0.295). For all types of samples; C. albicans was the most sensitive isolate to fluconazole followed by C. glabrata and C. tropicalis. There was reduction of overall colonization in group I from 42% to 38% while increase in colonization from 37% to 56% in group II (P = 0.08). IgM for C. albicans was statistically significant (P= 0.02) with the subgroups of hematological malignancies.Conclusion:Candida species colonization is a common problem in children with hematological malignancies and severe neutropenia. C. albicans is the most common species encountered in isolated samples. It's also the most sensitive to fluconazole. Fluconazole plays a major role in reduction of colonization, so it's recommended to use it in such cases.

[Enas A.Daef, Michael N. Agban, Mona H. Mohammed, Mohammed H. Ghazally, Salah S. Abdelhadi, Amany M. Ali, Mazen A.A. Gwass. **Role of Fluconazole prophylaxis in decreasing *Candida* colonization among neutropenic children with Hematological Malignancies.** *J Am Sci* 2013;9(2):403-409]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 55

Key words: Candida infection, prolonged neutropenia, hematological malignancies.

1. Introduction

Invasive fungal infection is a leading cause of infection-related mortality among patients with cancer and prolonged neutropenia (1). The main risk factor for all types of fungal infections in the oncology patients is prolonged and severe neutropenia (2).

Neutropenia due to cancer chemotherapy is defined as (absolute neutrophil count (ANC) nadir < 1 x 10^9 / L), while severe neutropenia is defined as (ANC nadir < 0.5 x 10^9 / L) (3).

Fungi are common causes of secondary infection among neutropenic patients who have received courses of broad-spectrum antibiotics and may also cause primary infections (4).

Early diagnosis of invasive fungal infections is difficult (5) and prevention of superficial infections is considered important because of its possible role in the development of systemic fungal infection (6), so antifungal prophylaxis could be the best approach for neutropenic patients undergoing myelosuppresive chemotherapy (5).

Candida species are responsible for more than 50% of oral infections that occur during antileukemic chemotherapy, and for almost two thirds in patients taking antineoplastic drugs for solid tumours (7). Candida albicans is the most common fungal pathogen (6).

Aims of the study: to study the prevalence and types of Candida colonization among neutropenic pediatric patients with hematological malignancies, to study the role of antifungal prophylaxis in decreasing Candida colonization and infection and in prevention of invasive Candida infection among severe and prolonged neutropenic children with hematological malignancies.

2. Patients, Material and Methods:

This study was a controlled clinical trial. It was carried out at Pediatric Department in South Egypt Cancer Institute-Assiut University during the period from (December 2008 to June 2010). It was conducted on 62 patients with hematological malignancies and suffered from severe neutropenia (ANC < 0.5 cells/ µl) either induced by chemotherapy or due to malignancy. The patients included in the study presented to Cancer Institute for diagnosis or during relapse, and were expected to remain >7 days. The patients were divided into two groups: Group I (32 patients received fluconazole as a prophylaxis) and Group II (32 patients received placebo). The study was approved by the Ethical Committee and written informed consent was obtained from each patient parent prior to study entry.

Inclusion criteria:

Patients age (0-16 years) mean (6.40 ± 3.83) suffered from neutropenia. Patients with absolute neutrophiles count less than 500 cells / ml^3, 7 days or more from start of chemotherapy. Also neutropenic patients presented during relapse after first remission of hematological malignancies.

Patient who have no contraindication to fluconazole.

Group I received antifungal prophylaxis with intravenous fluconazole (Diflucan®, Pfizer) at dose of 3 mg/kg/dose once daily plus the traditional antibiotic therapy. Group II received only the traditional antibiotic therapy.

All patients were subjected to:

Full clinical history for any symptom suggesting Candida infections and history of drug intake (antibiotics, antifungal, chemotherapy).

Meticulous physical examination with special focus on signs of infections such as fever, skin rashes, oral mucosa for rash, vaginal discharge. Peripheral or central intravenous catheterization of the selected patients.

Laboratory investigations in the form of: complete blood count, liver function tests, renal function tests and Candida isolation from the following specimens; Oropharyngeal swabs, mid-stream urine specimens, anorectal swabs/or stool samples and blood culture.

From each patient included in the study, four specimens at baseline and four specimens at end of the study were collected.

Phenotypic examination: all the samples were cultured onto Sabouraud's Dextrose Agar (SDA) (HiMedia, Mumbai, India) plates supplemented with chloramphenicol. Suspected Candida colonies were identified phenotypically by the conventional methods including: Gram's staining [8], germ tube test [9], Culture on chromogenic HiCrome Candida Differential agar (HiMedia ™ M1297A Mumbai, India) [10], Culture on Czapek Dox agar (HiMedia ™ M075) with Tween 80 (Dalmau test), urease utilization and sugar assimilation test using Yeast nitrogen base agar (HiMedia ™ M139) and suger assimilation discs (Glucose, Maltose ,Sucrose, Lactose, Galactose, and Raffinose) [11].

Table (1) Primers for type and species identification:

Primer	Nucleotide sequence (5'-3')	Amplification step	Location
CTSF	CGCATCGATGAAGAACGCAGC	1ST round PCR	5.8S rDNA universal 5' primer
CTSR	TCTTTTCCTCCGCTTATTGATATGC	1ST round PCR	28S rDNA universal 3' primer
CADET	ATTGCTTGCGGCGGTAACGTCC	snPCR	ITS region of C. albicans
CPDET	ACAAACTCCAAAACTTCTTCCA	snPCR	ITS region of C. parapsilosis
CTDET	AACGCTTATTTTGCTAGTGGCC	snPCR	ITS region of C. tropicalis
CGDET	TAGGTTTTACCAACTCGGTGTT	snPCR	ITS region of C. glabrata
CKDET	GGCCCGAGCGAACTAGACTTTT	snPCR	ITS region of C. krusei
CDDET	GCTAAGGCGGTCTGGCGTCG	snPCR	ITS region of C. dubliniensis

Genotypic confirmation and identification of Candida species:

was done by seminested PCR (snPCR) using universal fungal primers (CTSF; forward primer; 22-bp) and (CTSR; reverse primer ; 25-bp), capable of amplifying the 3' end of 5.8S rDNA and the 5' end of 28S rDNA, including the intervening spacer region (metabion international, Germany), and species specific oligonucleotide primers derived from the internal transcribed spacer (ITS2) regions of C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. krusei and C. dubliniensis.

Primers for PCR and snPCR [12, 13].

Extraction of Candida DNA: DNA was extracted from liquid culture of Candida grown for 20

- 24 hs at 30°C in YPD (1%yeast extract, 2% peptone, 2% dextrose) according to Harju et al. [13].
Amplification of target DNA was carried out in a total volume of 50 µl containing: 1 µl AmpliTaq PCR buffer I, 1U of AmpliTaq DNA polymerase, 10 pmol each of CTSF and CTSR primers, 1 µl of extracted DNA from culture and 0.1 mM each deoxynucleoside triphosphate.

After amplification in the 1st step, 1 µl of the product was further amplified using the initial reverse primer (CTSR) and a species specific forward primer in six separate tubes corresponding to each of the *Candida* species to be detected. For snPCR, the reaction mixture consisted of: 1 µl AmpliTaq PCR buffer I, 1 U of AmpliTaq DNA polymerase, 5 pmol of CTSR together with 5 pmol of CADET, CPDET, CGDET, CTDET, CKDET, or CDDET, 1 µl of the first PCR product and 0.1 mM each deoxynucleoside triphosphate.

PCR cycling was carried out in a Perkin-Elmer cycler (GeneAmp PCR system 2400) under the following conditions:

An initial denaturation step at 94°C for 1 min.

PCR cycle: Denaturation at 94°C for 1 min, Annealing at 60°C for 30 sec., and Extension at 72°C for 1 min.

Final extension step at 72°C for 10 min.
Optimum amplification was determined to be obtained with 30 cycles of the first PCR followed by 20 cycles of the snPCR for DNA extracted from cultures of *Candida* species. Appropriate negative controls were included in each test run.

Amplified DNA fragments were detected using agarose gel electrophoresis (2%) (Sigma, Germany) stained with ethidium bromide.

Antifungal susceptibility: of the isolates were determined by disc diffusion method [14] using Muller Hinton Agar (HiMedia ™M173) +2% Glucose and 0.5µg/ml Methylene Blue Dye (GMB) Medium, and Fluconazole 25 µg Antifungal Susceptibility discs. The interpretive criteria for the fluconazole disk diffusion tests were: sensitive (S), zone diameters of ≥19 mm; susceptible dose-dependent (SDD), zone diameters of 15 to 18 mm; and resistant (R), zone diameters of ≤14 mm[14].

IgM to *Candida albicans*: was detected for all patients using the RIDASCREEN® *Candida* IgM test, article no. K9031 *Candida* IgM (96 determinations) (R-Biopharm Germany).

Statistical analysis: was performed using SPSS software version 16 (SPSS Inc., Chicago, USA), and expressed as mean, standard deviation (SD), number and percentage. Statistical methods were applied including descriptive statistics (frequency, percentage, mean and SD) and tests of significance (X2 and Fisher exact tests for categorical variables and Student t test and Mann Whitney tests for continuous variables). Statistical significance was assumed when $P < 0.05$.

3. Results:

This study included 64 pediatric patients with hematological malignancies and severe prolonged neutropenia. There were two groups: Group I (32 patients, that received fluconazole as a prophylaxis), Group II (32 patients, that didn't receive antifungal prophylaxis). There age ranged from 1.5 to 16 years old, 45 males and 19 females, 38 cases were diagnosed as Acute Lymphoid Leukemia, 14 as acute myeloid leukemia and 12 as non Hodgkin's lymphoma. There were no significant difference between Group I and II in demographic data.

Table (1) shows the distribution of colonization of *Candida* species at different sites. There was statistically significant difference between both groups in colonization at one site, in colonization of oral and rectal sites and in colonization of oral, rectal and blood together.

Table (1) Distribution of *Candida* Colonization in different sites in both groups at base line and end of therapy

Variable	Study group (32)		Control group(32)		*P* value
	Baseline	End	Baseline	End	
1 site only	7(23.33 %)	9(45%)	13 (40.33 %)	3(9.38 %)	0.01*
≥ 2 sites	23(76.67%)	17(65%)	17(56.67%)	29(90.62%)	0.02*
2 sites		13(76.47%)	17(100%)	20(68.96%)	0.243
Oral +Rectal	22(95.65%)	12(92.30 %)	17(100%)	20(100%)	0.03*
Oral+ urine	-	1(7.70%)	-	-	
3 sites	1(4.35 %)	4(23.53%)		7(24.14%)	0.253 n.s
Oral+ Rectal +urine	1(100 %)	2(50%)	-	5 (71.43 %)	0.392 n.s
Oral+ Rectal + Blood	-	2(50%)	-	2(28.57%)	0.01*
4 sites				2(6.9%)	-
Oral + Rectal+ urine +Blood	-	-	-	2(100 %)	

There was reduction of overall colonization rate in study group from 42% to 38% while increase in colonization from 37% to 56% in control group.

In study group: The highest rate of colonization was detected in oral samples. There was reduction in oral colonization (carriage) from 28 (52%) to 22(46%) which was more than rate of reduction in rectal colonization (from 46% to 44%). There was increase in colonization in urine culture from1% to 3%, and acquisition of candidaemia from 0% to 4% in the studied cases at end of study. There was no statistical significant difference between both groups.

When confirming the phenotypic methods by PCR, there was 99.3% agreement for *C. albicans* and 97.6% agreement for *C. glabrata* and 100% agreement for both *C. tropicalis* and *C. krusie.*

Table (2) Distribution of Candida species in study and control group at base line and at end of study (Each species to different sites)

Variable	Study group (32)		Control group(32)		*P* value
	Baseline	End	Baseline	End	
C. albicans	33	18	31	52	0.000***
Oral	19(57.57%)	6(33.33%)	15(48.39%)	20(38.46%)	0.001**
Rectal	13(39.40%)	8(44.44%)	16(51.61%)	23(44.23%)	0.000***
Urine	1(3.03%)	3(16.67%)	-	6(11.54%)	0.001**
Blood	-	1(5.56%)	-	3(5.77%)	0.251
C. glabrata	6	16	7	11	0.000***
Oral	4(66.67%)	7(43.75%)	4(57.14%)	5(45.45%)	0.293
Rectal	2(33.33%)	8(50%)	3(42.86%)	5(45.45%)	0.02*
Blood	-	1(6.25%)	-	1(9.1%)	-
C. tropicalis	3	3	2	2	0.391
Oral	2(66.67%)	2(66.67%)	2(100%)	2(100%)	-
Rectal	1(33.33%)	1(33.33%)	-	-	-
C. krusie	-	2	-	-	-
Oral	-	2(100%)	-	-	-
C. albicans + *C. glabrata*	12	8	7	7	0.02*
Oral	3(25%)	4(50%)	3(42.86%)	5(71.42%)	0.251
Rectal	9(75%)	4(50%)	4(57.14%)	1(14.29%)	0.01*
Urine	-	-	-	1(14.29%)	-
C. glabrata + *C. krusie*	-	1	-	-	-
Oral	-	1(100%)	-	-	-

There was statistical significant difference in distribution of *C. albicans* in all types of samples except for blood samples. Also, significant statistical difference was detected in distribution of *C. glabrata* isolates among rectal samples only and in distribution of mixed *C. albicans* and *C. glabrata* found in rectal samples among both groups as shown in Table (2).

Table (3) Fluconazole susceptibility patterns of *Candida* isolate in Study and Control groups.

Variable	Study group (32)		Control group(32)		P value
	Baseline	End	Baseline	End	
C. albicans					
S	32(96.97%)	9(50%)	30(96.78%)	47(90.38%)	0.02*
DDS	1(3.03%)	9(50%)	1(3.22%)	5(9.62%)	0.03*
C. glabrata					
S	3(50%)	4(25%)	3(42.86%)	4(36.37%)	0.492
DDS	3(50%)	8(50%)	4(57.14%)	5(45.45%)	0.302
R	-	4(25%)	-	2(18.18%)	0.214
C. tropicalis					
DDS	3(100%)	1(33.33%)	2(100%)	2(100%)	0.603
R	-	2(66.67%)	-	-	
C. krusie					
R	-	2(100%)	-	-	-
Total					
S	35(64.81%)	13(27.08%)	33(70.21%)	51(70.83%)	0.01*
DDS	7(12.96%)	18(37.5%)	7(14.89%)	12(16.67%)	0.02*
R	-	8(16.67%)	-	2(2.78%)	0.001*

N.B.: S = sensitive, DDS = dose dependant susceptibility, R = resistant, * statistically significant.

Table (3) shows statistical significant difference in susceptibility pattern of *Candida* species between both groups at base line and end of the study.

Table (4) Pattern of sensitivity of solitary *C. albicans* isolates in different samples.

Variable		Study group (32)		Control group(32)		*P* value
Sample		Baseline	End	Baseline	End	
Oral	S	18(94.74%)	2(33.33%)	15(100%)	18(90%)	0.000***
	DDS	1(5.26%)	4(66.67%)	-	2(10%)	0.01*
Rectal	S	13(100%)	4(50%)	15(93.75%)	22(95.65%)	0.000***
	DDS	-	4(50%)	1(6.25%)	1(4.35%)	0.000***
Urine	S	1(100%)	2(66.67%)	-	6(100%)	0.01*
	DDS	-	1(33.33%)	-	-	
Blood	S		1(100%)	-	1(33.33%)	0.001**
	DDS	-	-		2(66.67%)	-

There was statistical significant difference in sensitivity pattern of *C. albicans* strains isolated from different samples between both groups as detected in Table (4).

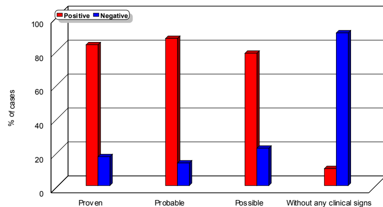

Figure (1): shows the pattern of *Candida albicans* IgM in different cases.

Table (5) Correlation of subgroups of hematological malignancies patients according to EORTC/MSG criteria and results of Anti-*Candida albicans* IgM ELISA

Patients characteristics by EORTC/MSG criteria	Anti-*Candida albicans* IgM ELISA				Total
	Positive		Negative		
	No	%	No	%	
Proven	5	83	1	17	6
Probable	13	86.7	2	13.3	15
Possible	18	78	5	22	23
Without any clinical signs	2	10	18	90	20
Total	38	59	26	41	64
P value	0.02*				

EORTC/MSG: European Organization for Research and Treatment of Cancer.There was significant correlation between subgroups of hematological malignancies patients according to EORTC/MSG criteria and the results of Anti-*Candida albicans* IgM ELISA test as shown in Figure (1) and Table (5).

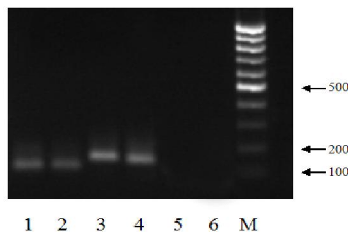

Figure (2): Lanes 1 to 6, snPCR amplification of DNAs from *C. tropicalis, C. albicans*, *C. glabrata*, and *C. krusei*, respectively, using primer CTSR with primers CTDET, CADET, CGDET, CKDET, CDDET, and CPDET respectively. Lane M, 100-bp molecular size marker.

The PCR amplification with the universal primers (CTSD and CTSR) and species specific primers of genomic DNA from *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. Krusei* resulted in amplification of DNA fragments of 344, 334, 423 and 335 bp respectively. Meanwhile, non of the isolated strains gave specific bands with the species specific primers of *C. dubliniensis* or *C. parapsilosis* (Lane 5& 6 in Figure 2).

Table (6): Agreement between phenotypic and genotypic methods for identification of *Candida* species.

Candida species	No. of isolates identified by		% of agreement
	Phenotypic methods	Genotypic methods	
C. albicans	133	134	99.3%
C. glabrata	41	40	97.6%
C.tropicalis	10	10	100%
C. krusie	2	2	100%

Table (6) shows the percent agreement between phenotypic and genotypic methods for identification of *Candida* species which was 99.3% for *C. albicans*, 97.6% for *C. glabrata* and 100% for each of *C. tropicalis* and *C. krusei.*

4. Discussion:

C. albicans is a normal human commensal, so it's not surprising that this organism is the most common opportunistic fungal pathogen in immunocompromised patients.

Colonization of the oropharynx and/or the alimentary tract often precedes invasive yeast infections. Strategies for antifungal prophylaxis in cancer patients receiving intensive cytotoxic chemotherapy have been based on the principle of reducing fungal colonization in the alimentary tract in order to reduce systemic invasive fungal infections during the period of greatest risk due to profound neutropenia [15].

In our study, we found high rate of colonization by *Candida* species ranging from 42% in study group to 37 % in control group at base line. Oral samples showed the highest rate of colonization, with

reduction in oral colonization from 52% to 46% which is slight more than rate of reduction of rectal colonization. On the other hand, there was increase in colonization in urine culture from1% to 3%, and acquisition of candidaemia from 0% to 4% of isolated cases at end of study (one isolate of *C. albicans* and one isolate of *C. glabrata*). This is concomitant with Parisa *et al*. [16] who found *Candida* colonization in 55.2% of all patients, with one positive case only of candidaemia, and Laverdiere *et al.*, [17] who found two cases of candidaemia due to *C. krusei.*

C. albicans colonization was reduced from 33% to 18% in the fluconazole recipients, while it increased from 31 to 52% in the placebo patients. Also by the end of prophylaxis, colonization with non-*albicans Candida* species increased from 9 to 21% and 9 to 13% in the fluconazole and placebo patients respectively. These figures agreed with those of Laverdiere *et al*., [17]. Our results disagreed with Laverdiere *et al*., [17] in which we found that the use of fluconazole enhanced the emergence of *C. glabrata* and *C. krusei* but not *C. tropicalis*. Variations of *Candida* spp. distribution may be dependant on the hospital, type of clinical specimen, and the geographical region in which the study was conductedIt was found that colonization of multiple sites (76.6%) was more common than colonization of single site only (23.3%). This disagreed with Parisa *et al*., [16] and Ana *et al*., [18] who found only that 16% of colonized patients had infection at more than one site.In our study, *C. albicans* was the most frequent isolate (72.04%), followed by *C. glabrata* (21.5%), *C. tropicalis* (5.38%) and lastly C. krusie (1.08 %) respectively which agreed with Parisa *et al*., [16] who found *C. albicans* was the most frequent isolate, followed by *C. tropicalis* and lastly *C. krusie*, but they couldn't detect *C. glabrata.*

For all types of isolates, oral colonization was the most frequent, followed by rectal, urinary and blood respectively. There was statistically significant difference in distribution of *C. albicans* in all types of samples, but there was statistical significant difference in distribution of *C. glabrata* found in rectal samples, and in mixed *C. albicans* and *C. glabrata* found in rectal samples too. This is concomitant with Parisa *et al*., [16] who found oral colonization in 33.5% of neutropenic patients. This is in contrast to Ana *et al.*, [18] who reported that candidimia was more common followed by colonization in oral, rectal and urinary sites respectively.

Our study showed that only two patients in the fluconazole group had candidemia (6.25%), one is *C. albicans* and the other is *C. glabrata*, while in the placebo group four patients had candidemia (12.5%); three *C. albicans* and one *C. glabrata* at end of the study. This shows a clear role of antifungal prophylaxis with fluconazole in decreasing the rate of candidemia and invasive fungal infections in fluconazole group in comparison with placebo group. This is concomitant with Pagano *et al*. [19] who found candidemia is 1.6% of cases.

In our study we found that for all types of samples; *C. albicans* was the most sensitive isolate to fluconazole followed by *C. glabrata* and *C. tropicalis*. This is concomitant with the results of Schelenz [20].

In the present study; 96.97% of *C. albicans* strains were sensitive while 3.03% showed dose dependent susceptibility at baseline in the fluconazole group, but at end of study the sensitive strains decreased to 50% while the dose dependent susceptibility stains increased to 50%, there is no resistant strains detected. Our results are in agreement with other studies [21], which conclude that long-term clinically relevant decrease in *C. albicans* fluconazole susceptibility undoubtedly occurs and accompanies prolonged fluconazole chemoprophylaxis.

In our study, there was significant correlation between subgroups of hematological malignancies patients according to EORTC/MSG criteria and the results of Anti-*Candida albicans* IgM ELISA test. This concludes that serological tests as Anti-*Candida albicans* IgM ELISA method can potentially serve as a useful tool for the early diagnosis and management of patients suffering from hematological malignancies and at risk for systemic candidiasis. These results are in agreement with Badiee *et al*. [22]

In our study, there was 97.6% – 100% agreement between phenotypic and genotypic methods. These results were similar to Girgis *et al*., [23]. The discrepancy was among one isolate which was phenotypically identified as *C. glabrata*, but was identified as *C. albicans* when tested with the species specific primers used in the study. Although the precise reason for this discrepancy remains unclear, it could be related to inadequacy of the presently available phenotypic Candida identification methods. Further confirmation by DNA sequencing may be required to correctly identify these isolates.

Conclusion

Patients with severe neutropenia resulting from chemotherapy for malignant diseases, are at high risk of difficult to treat and often fatal invasive fungal infections. Fungal prophylaxis is a commonly used treatment strategy, because the diagnosis of fungal infection is often delayed or difficult to establish with certainty and a delay in antifungal treatment increases mortality. Serological tests as Anti-*Candida albicans* IgM ELISA method can potentially serve as a useful tool for the early diagnosis and management of patients suffering from hematological malignancies and at risk for systemic candidiasis. Although PCR

gives more accurate results, it has the disadvantage of high cost in comparison with ELISA.

References:

1. Segal BH, Almyroudis NG, Battiwalla M, Herbrecht R, Perfect JR, Walsh TJ, and Wingard JR (2007): Prevention and Early Treatment of Invasive Fungal Infection in Patients with Cancer and Neutropenia and in Stem Cell Transplant Recipients in the Era of Newer Broad-Spectrum Antifungal Agents and Diagnostic Adjuncts. CID:44 (1 February)
2. Mays SR, Bogle MA, Bodey GP(2006): Cutaneous fungal infections in the oncology patient: recognition and management. Am J Clin Derm.; 7(1):31-43.
3. Crawford J, Wolff D, Culakova E, Poniewierski M.S, Selby C, Dale D,and Lyman G.H for the ANC Study Group (2005): First-Cycle Risk of Severe and Febrile Neutropenia in Cancer Patients Receiving Systemic Chemotherapy: Results From a Prospective Nationwide Study. J of Supportive Oncology .Vol.3, No.2, Supp.1…..
4. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T,Feld R, Pizzo PA, Rolston KV.I,Shenep J.I, and Young LS (2002): Guidelines for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer. J. Clini. Infect. Dis.; 34:730–51.
5. Cornely OA, Ullmann AJ , Karthaus M (2003): Evidence based assessment of primary antifungal prophylaxis in patients with hematologic malignancies .Blood; 1; 101(9):3365-72.
6. Ship J A, Vissink A, and Challacombe S J (2007): Use of prophylactic antifungals in the immunocompromised host. Surg Oral Med Oral Pathol Oral Radiol Endod;103 (suppl 1): 1-14.
7. Soysa NS, Samaranayake LP, Ellepola AN. (2004): Cytotoxic drugs, radiotherapy and oral candidiasis. J. Oral Oncol ; 40(10): 971 -8.
8. Mitchell T.G. (2004): Medical mycology. In: Jawetz, Menick and Adelberg's Medical Microbiology, 23rd ed. Ch.45 , pp: 623-660. Brooks G.F.,Butel J.S. and Morse S.A. eds. McGraw-Hill, USA.
9. Ellis ME, Clink H, Ernst P, Halim MA, Padmos A, Spence D, Kalin M, Hussain Qadri SM, Burnie J, Greer W (2007); Controlled study of fluconazole in the prevention of fungal infections in neutropenic patients with haematological malignancies and bone marrow transplant recipients. Eur J Clin Microbiol Infect Dis 13:3–11.
10. Baradkar VP, Mathur M, and Kumar S.(2010): Hichrom candida agar for identification of Candida species. Indian J Pathol Microbiol. 2010 Jan-Mar;53(1):93-5.
11. Barnett JA., Pane RW., and Yaeeow D. (2000): Yeasts: Characteristics and Identification. 3rd edition. P; 26-27.
12. Fujita S., Lasker BA., Lott TJ., Reiss E., and Morrison CJ. (1995): Microtitration plate enzyme immunoassay to detect PCR- amplified DNA from Candida species in blood. J. Clin Microbiol; 33; 962 – 967.
13. Khan Z., Mustafa AS., and Alam FF. (2009); Real-time light cycler polymerase chain reaction and melting temperature analysis for identification of clinically important Candida species. J. Microbiolo Immunol Infect; 42; 290-295.
14. Wilheim A.B. , Miranda-Filho D. B., Nogueira R.A., Rêgo R.S.M., Lima K.M., and Pereira L.M.M.B (2009): The resistance to fluconazole in patients with esophageal candidiasis. Arq Gastroenterolv. 46 – no.1 – p32-37.
15. Schulze J. and Sonnenborn U (2009); Yeasts in the gut; from commensals to infectious agents. Dtsch Arztebl Int. 106 (51-52); 837-842.
16. Parisa B., Parivash K., Abdolvahab A., Maryam Z., and Pedram H. (2009); Early detection of systemic candidiasis in the whole blood of patients with hematological malignancies. Jpn. J. Infect. Dis., 62, 1-5.
17. Laverdiere M., Rotstein C., Bow E. J., Roberts R. S., Ioannou S., Carr D., and Moghaddam N. (2000); Impact of fluconazole prophylaxis on fungal colonization and infection rates in neutropenic patients. Journal of Antimicrobial Chemotherapy; 46; 1001-1008.
18. Ana M., Luciana R., Vera L., and Rejane P., (2009); Candidiasis in pediatric patients with cancer interned in a university hospital. Brazillian Jour. Of Microbiology. 40:321-4.
19. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, Pastore D, Picardi M, Bonini A, Chierichini A, Fanci R, Caramatti C, Invernizzi R, Mattei D, Mitra ME, Melillo L, Aversa F, Van Lint MT, Falcucci P, Valentini CG, Girmenia C, Nosari A.(2006): The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica; 91(8):1068-75.
20. Schelenz S(2008): Management of candidiasis in the intensive care unit.Journal of Antimicrobial Chemotherapy 2008 61(Supplement 1):i31-i34.
21. Shahid Z and Sobel J.D. (2009): Reduced fluconazole susceptibility of Candida albicans isolates in women with recurrent vulvovaginal candidiasis: effects of long-term fluconazole therapy. Diagnostic Microbiology and Infectious Disease; 64; 354–356.
22. Badiee P., Kordbacheh P., Alborzi A., Zakernia M., and Haddadi P. (2009):Early Detection of Systemic Candidiasis in the Whole Blood of Patients with Hematologic Malignancies. Jpn. J. Infect. Dis., 62; 1-5.
23. Girgis SA., El-Mehalawy AA., and Rady LM.: (2009): Comparison between culture and non-culture based methods for detection of Nosocomial fungal infections of Candida species in intensive care unit patients. Egypt Acad J. Biolog. Sci; 1; 37 – 47.

1/12/2013