

Anti-Candida Effects of Clotrimazole and Fluconazole against Isolated *C. albicans* from Patients of Candidiasis Admitted in Medical Mycology Lab of Special Clinic of Kermanshah University of Medical Sciences, 2014

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ABSTRACT

Candida albicans is the most common and most pathogens agent in candida species. This yeast lives in mucous of body and waiting for opportunity to being pathogen. Today a lot of money is pay for treatment of this disease. Drug resistance is one of the research fields in this disease. Fluconazole and Clotrimazole are two useful drugs in this disease. Several studies have shown drug resistance of candida albicans is different between groups, areas and various drugs. so drug resistances is different between various form of candidiasis. In this study, we collected the personal information of patients with different forms of candidiasis who refers to mycology laboratory special clinic of university in 1393 in .this information's includes age, gender, occupation, location and et. This data were collected by questionnaire. Then all of isolated forms of candida albicans from patients were antibi gram evaluated against Fluconazole and Clotrimazole. This work is done with method concentration in saborad dextrose agar culture. In this evaluation MIC of mention drugs was determinate. In 12 cases medication with Fluconazole and Clotrimazole: Clotrimazole was evaluated with concentration from minimum 0.25 µ/ml to maximum 128 µ/ml That concludeed MIC of Clotrimazole is 2 µ/ml. Also Fluconazole was evaluated with concentration from minimum 8 µ/ml to maximum 4196 µ/ml. That concludeed MIC of Fluconazole is 4196 µ/ml. The results showed that both drugs are but Clotrimazole have better response. Thus clotrimazole have more effect than Fluconazole.

Key words: *Candida albicans*, Clotrimazole, Fluconazole

INTRODUCTION

Candidiasis is the most common opportunistic fungal infection in human that is caused by *Candida* yeast. The source of this fungus is endogenous [1, 2]. This disease has different mucous forms (like oral, vaginal, and digestive), dermal (like the crimping areas of body, paronychia and neonatal period candidiasis) and systemic [3-5]. *Candida albicans* is the most common and most pathogens in *Candida* species. This yeast lives in

the mucous of body and waits for an opportunity to being pathogen. Today, a lot of money is pay for treatment of this disease. Drug resistance is one of the research fields in this disease. Fluconazole and Clotrimazole are two useful drugs in this disease. *Candida albicans* that is drug resistant for treatment, the increase of mortality in patients with systemic forms. Several studies have shown drug resistance of *Candida albicans* is different between groups, areas and various drugs. So drug resistance is different between various forms of candidiasis.

MATERIALS AND METHODS

In this questionnaire, some information like gender, age, marital status, work experience, job status, academic degree and all the information related to person were collected.

To perform the study, data were collected by field method and personal characteristics questionnaire and company testimonial in research were prepared after the study of internal and external scientific resources and obtaining sufficient and required data. Then, the researchers referred to Kermanshah University of medical sciences to obtain the required licenses and letter of introduction. When they obtained the letter of introduction to attend the medical mycology lab under the supervision of this university, they attended the lab and talked to the study group which was comprised of all patients with different forms of *Candida albicans*. The researchers gave complete information about the research project to the patients and completely answered their questions about the research project. The patients were also told that they could ask any kind of questions about the research and could voluntarily attend in the study and the lack of participation in the study will not be an obstacle to receive care and treatment. They were also ensured of the confidentiality of data and the lack of harmful consequences of the study and their findings for job, organizational and social positions. Then, the samples were prepared by using sterile swap and were put inside a sterile glass container by observing the points and principles of Aseptic method. Then, the samples were transferred to lab and by using Aseptic method, these samples were smeared on Sabouraud dextrose agar medium and then the medium was put in incubator. The samples which had a *Candida* cologne on agar were considered positive and the suspicious samples were considered negative. The isolates that were after the growth of *Candida albicans* were kept for anti-biogram tests. Keeping these isolates was done by re-cultivation (passage) method. Among the 27 isolated *Candida albicans*, only 12 cases survived for drug tests. These 12 isolates were examined by anti-biogram in a specific mycology medium called Sabouraud dextrose agar.

The prepared mediums for the study

- A. Sabouraud dextrose agar medium which has the concentrations of 0/25 to 128 micrograms per milliliter fluconazole and 8 to 4096 micrograms per milliliter clotrimazole
- B. Some other pure mediums were used for negative control
- C. Some other solvent containing mediums were used as drug solvents

All samples with negative controls (pure medium and DMSO) were incubated at 25 degrees of centigrade for 72 hours and the results were studied daily. All tests were repeated 3 times to obtain repeatability. Finally, the minimum inhibitory concentration of *Candida albicans* for each isolate was calculated per each drug and was included in the related table. All measures were taken under hood and by observing Aseptic points and rules such as using disposable supplies, glasses, cap, mask and working clothes.

The method of providing antibiogram medium

The medium used in this study is Sabouraud dextrose agar that was provided according to the instruction written on the medium. For this purpose, 65 grams of the medium was mixed to 1 liter of distilled water and then was solved in water with the help of mild heat and continuous stirring. Finally, the medium was transferred into plates after cooling the medium to 50-60 degrees.

The method of providing Fluconazole and Clotrimazole

Providing Fluconazole solution

First, stock solution was provided from the pure powder of Fluconazole by using DMSO 50% solution. Then, the concentrations (8-16-32-64-128-256-512-1024-2048-4096) were prepared by using a shaker.

Providing Clotrimazole solution

First, stock solution was provided from the pure powder of Clotrimazole by using DMSO 50% solution. Then, the concentrations (0/25-0/50-1-2-4-8-16-32-64-128) were prepared by using a shaker.

Table 1: Demographic information and distribution of growth minimum inhibitory concentration of Clotrimazole and Fluconazole on *Albicans* isolates

Conc. of fluconazole	Conc. of clotrimazole	Type of detergent	Previous treatment	Duration of illness	Contact with water	Contaminated place	Cultivation sight	Direct place	Living lesion	Area of	Illness	Job	age	season	gender	person
4096	2	typical	Corticosteroids	1 year	normal	-	calbicans	Yeast groups	Kermanshah	Fingernail	Permphigus	housewife	38	summer	female	235
2048	2	typical	Antibiotics	1 month	little	River - soil - animal	calbicans	Yeast groups + false hyphae	Harsin	Fingernail	-	student	15	summer	male	248
2048	2	typical	Antifungal	2 months	yes	-	calbicans	Yeast groups + false hyphae	Kermanshah	Groin	-	Employee	42	summer	male	269
1024	0.5	—	Bactericidal	10 days	no	-	calbicans	Yeast groups + false hyphae	Kermanshah	Ventriculo-eritoneal fluid	Hydrocephalus	student	9	summer	male	479
4096	1	soap	Topical ointment	2 months	2 times a week	-	Calbicans	Yeast groups + false hyphae	Kermanshah	Groin	-	retired	26	summer	male	485
512	2	soap	-	1 month	normal	-	Calbicans	—	Kermanshah	Fingernail and toenail	-	housewife	42	fall	female	532
1024	4	soap	Topical ointment	3 years	yes	village	calbicans	Yeast groups	Kermanshah	Metatarsus	allergy	housewife	33	fall	female	590
4096	4	soap	-	4 months	yes	-	calbicans	—	Kermanshah	Groin	-	templar	28	fall	male	631
4096	4	liquid	-	4 months	yes	-	Calbicans	Yeast groups + false hyphae	Kermanshah	Fingernail	Diabetes, hypertension	retired	73	winter	male	831
4096	2	soap	-	40 days	no	Boxes of fruits	Calbicans	Yeast groups + false hyphae	Kermanshah	Fingernail	Diabetes	Self-employed	51	winter	male	869
4096	2	typical	-	4 months	little	-	Calbicans	Yeast groups + false hyphae	Kermanshah	Fingernail	hypertension	Employee	54	winter	male	974
4096	2	liquid	-	1 month	yes	-	Calbicans	—	Kermanshah	Palates	cancer	-	4	fall	male	503

Providing drugs solutions and the plates containing them

First, all the used glass and metal supplies were made sterile by an oven (temperature of 120 degree of centigrade) for an hour and all steps of the process were done beside the flame and under clean conditions. After providing anti-biotic solutions, mixing the antibiotic and medium was done before entering the plate in Erlenmeyer and then 10 milliliter of the mixture of drug and medium was poured inside each plate. The medium containing drugs was put in refrigerator for 24 hours. Then, the inoculation of fungus was done in the medium. To study the antifungal effects of each concentration of both Fluconazole and Clotrimazole antibiotics, the blank of each fungus was prepared.

Providing the suspension of inoculation to medium

After sampling the fungi and their cultivation on Sabouraud dextrose agar medium and ensuring the *Candida albicans* of sample, some of it was taken by swap and was solved in normal saline. This method is based on one-unit turbidity test by using macfarlane tube. The final concentration of 1×10^6 cfu/ml was achieved.

RESULTS

Materials and supplies: Fluconazole pure powder from Amin pharmaceutical company, Clotrimazole pure powder from Kish Medipharm Company, Sabouraud dextrose agar medium.

Glasses, gloves, cap, mask and working clothes, test tube and falcon, pipette and syringe, sampler and blue and yellow crystal sampler tip, graduated cylinder and measure, Erlenmeyer flask and beaker and laboratory gas light.

Devises: digital scales, refrigerator, laminar hood, shaker, incubator

DISCUSSION AND CONCLUSION

This study showed that Clotrimazole has a better effect than Fluconazole in terms of growth minimum inhibitory concentration.

Shojaei et al conducted a study on 19 strains of *Candida albicans* isolated from Chronic candidiasis vaginites. The results showed that growth minimum inhibitory concentration for Clotrimazole was 7/05 micrograms per milliliter and for Fluconazole was 7/1 micrograms per milliliter. The results of the present study are consistent with the study of Shojaei et al and in both studies, Clotrimazole was more effective. The results showed that oral Fluconazole had better side effects and results than vaginal Clotrimazole [6].

The results of the present study have no difference from the study of Kariman et al in terms of side effects and the reason for priority of oral Fluconazole than vaginal Clotrimazole is the side effects of less itching and pain in the study subjects. But in terms of laboratory, in this study Fluconazole was introduced as a more appropriate drug than Clotrimazole which is relatively different from the results of this study. Its reason could be the different demographic characteristics of subjects or other effective variables [7].

The study of Najaf Zadeh et al showed that the sensitivity of Clotrimazole and Fluconazole is the same on *Candida albicans* but the implementation method was flow cytometry [8]. The results of this study are not consistent with the study of Najaf Zadeh et al and there is a reason which is not clear but the difference of effective factors can be one of the reasons.

The results of a study by Falahati et al showed that Clotrimazole and Nystatin have higher sensitivity compared to Miconazole [9]. The results of this study are consistent to the study of Falahati because the study of some antifungal drugs introduced Clotrimazole as a more sensitive drug.

The study of Elewski on patients with dermatophytosis showed that growth minimum inhibitory concentration for Fluconazole was 32 and for Terbinafine was 0/25. The results indicated a better and more effective response by Terbinafine [10]. The results of the present study are minimum inhibitory concentration. In this study, growth minimum inhibitory concentration was obtained as 4096 while it was 32 in the present study. Many factors can be effective in this difference.

In a study conducted in Tehran on Fluconazole in patients with tinea capitis, the results showed that Fluconazole has had the minimum effect on the treatment of these patients and other drugs like Ketoconazole and Clotrimazole were more effective [11]. This study is consistent with another conducted study in terms of the obtained results, so that Clotrimazole was a more sensitive drug for the treatment of patients with *Candida albicans*.

CONCLUSION

The results showed that Clotrimazole works on growth inhibition of *Candida albicans* better than Fluconazole.

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