EFFECT OF PREGNANCY AND DEMOGRAPHY ON SUSCEPTIBILITY PATTERN OF AZOLE ANTIFUNGAL AGENTS AGAINST CLINICAL ISOLATES OF CANDIDA SPECIES RECOVERED FROM VULVOVAGINAL CANDIDIASIS PATIENTS

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ABSTRACT

Objective: To determine the in vitro susceptibility of vaginal Candida species isolates retrieved from both pregnant and non-pregnant vaginal candidiasis patients belonging to urban and rural areas of Peshawar against commonly used azole antifungal agents.

Material and Method: Susceptibility assays were performed on isolates collected and differentiated.40 isolates were recovered from 50 pregnant and 50 non-pregnant (urban=25 and rural=25) vulvovaginal candidiasis patients, 28 isolates from pregnant women (urban=22 and rural=6) and 12 isolates from non-pregnant women. (urban=10 and rural=2) were retrieved.

Results: Overall susceptibility order of test agents in urban population was clotrimazole>ketoconazole=fluconazole>itraconazole. In rural population it was clotrimazole>itraconazole>ketoconazole however fluconazole showed highest resistance. Overall susceptibility order of test agents in pregnant patients was clotrimazole>ketoconazole>fluconazole>itraconazole. While in non-pregnant patients it was clotrimazole>itraconazole>fluconazole, whereas ketoconazole showed highest resistance.

Conclusion: All strains from urban as well as rural population were susceptible to clotrimazole. Isolates from urban and rural areas showed extreme resistance against itraconazole andfluconazole respectively. The most susceptible drug in pregnant vaginal candidiasis patients was clotrimazole while itraconazole remained highly resistant. While in non-pregnant patients C.albicans was mildly susceptible to clotrimazole, however, ketoconazole and fluconazole remained highly resistant.

Keywords: Candida, albicans, glabrata, krusei, Clotrimazole, Fluconazole, Ketoconazole, Itraconazole.

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INTRODUCTION

The yeast Candida is normal commensal microflora in healthy individuals. However, sometimes it becomes opportunistic and causes candidiasis. [1] About 75% of the women experience vulvovaginal candidiasis (VVC) at some point in their reproductive age[2] of which 60% is due to Candida albicans while the rest is due to non albican speicies.[3] Vertical transmission of VVC can occur. [4] The abnormal vaginal discharge (ranges from a slightly watery to thick white chunky), dysuria, vulvovaginal soreness and itching are pathagnomic of VVC.[5]

Naturally a balance in the microbiota of the vagina is maintained by the Candida and other normal flora like lactobacilli[6] yet conditions like diabetes mellitus[7, 8], gestation[8, 9] obesity[7], immunodeficiency[8] oral contraceptive pills[10] and use of antimicrobials[8] predispose to VVC. Lactobacillus prevents VVC by keeping vaginal pH low and is found in natural food products like yogurt and cheese[11]. Rural population consume more natural dairy products as compare to people of urban and sub urban areas[12]. Use of IUCDs predispose to VVC in urban areas.[13]

Data regarding the antifungal susceptibility of Can-

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dida Species plays an important role in predicting the possible efficacy of consequent treatment. Candida species have been reported to be susceptible to many antifungal agents, of which clotrimazole is most effective.[14] Resistance of Candida species against azole antifungal agents has become a major health issue due to their indiscriminate use. [15]

Varied antifungal use, difference in diet and life style and unpredictable susceptibility patterns of Candidain both pregnant and non-pregnat women of rural versus urban population, has made it indispensable to carry out antimicrobial susceptibility testing, to prevent resistance and make data available to clinicians regarding the appropriate antifungal treatment.

MATERIAL AND METHODS

This descriptive study of six months duration (Feb-Jul 2013) was carried out on samples collected from patients fulfilling the inclusion criteria at Khyber Teaching Hospital, Peshawar, and processed at Pharmacology laboratory, PMC, Peshawar and Microbiology Laboratory, Sarhad University. Equal number of samples were obtained from both pregnant as well as non-pregnant females (50 each) and rural as well as urban population (25each).The Ethical committee of PMC approved the study. Married and sexually active either pregnant or non-pregnant women between (18-45) years of age who presented to the tertiary health care centre with self-reported symptoms of vaginal discharge and/or genital itching and/or dysperunia during the study period were included. Women who were immunocomprimised, diabetic, using antibiotics, corticosteroids and/or oral contraceptive pills, sexually menstruating, never been sexually active or had a hysterectomy were all excluded. Written informed consent along with detail history proforma was taken by all subjects.

Sterile high vaginal swab was taken from each participant by a trained gynaecologist.[16] The swabs taken from the patients labelied and kept at temperature (2–8°C), were transported to Microbiology laboratory, Sarhad University, within 2 hours of collection for culturing. The collected samples were streaked on Sabouraud's Dextrose agar supplemented with 0.005% (w/v) Chloramphenicol (MM1067, HiMedia Lab. Ltd, India). After incubation period of 48 hrs at 37°C plates were observed for presence of fungal growth. Positive cultures were differentiated tospecies level by germ tube test [17] and HiCrome candida differential agar (M1297A, HiMedia Lab. Lt, India). [18]

The suspensions of isolated Candida spp were prepared by suspending them in sterile saline and adjusted to match the turbidity of 0.5 McFarland. [19] Test antifungals included Clotrimazole, Ketoconazole and Itraconazole (Janseen, Beerse, Belgium) and Fluconazole (Pfizer, Surrey, UK). The stock solutions were diluted, such that six dilutions for each antifungal agents; Fluconazole (256–16μg/ml), Ketoconazole (16–1μg/ml), Clotrimazole (0.24–0.015μg/ml) and Itraconazole (4–0.25μg/ml) were obtained. The MIC of each isolate was determined by broth micro dilution method recommended by NCCLS M27-A guide lines. [19] Data analysis was conducted with statistical software (SPSS) version 20. Susceptibility of the antifungal agents was determined by descriptive median statistics.

RESULTS

Among 50 urban patients 32 isolates were retrieved (22 pregnant and 10 non-pregnant). Out of these 32 isolates, C.albicans(n=16) was predominant followed by C.glabrata(n=11) and C.krusei(n=5). However in case of 50 rural patients 8 isolates (6 pregnant and 2 non-pregnant) were retrieved of which C.albicans(n=5), C.glabrata(n=2) and C.krusei(n=1).

Good susceptibility of clotrimazole was observed as fifteen isolates of C.albicans, from urban patients while, five isolates of C.albicans from rural patients were susceptible to it. High resistance against itraconazole was observed in isolates retrieved from urban patients however ketoconazole susceptibility was fair to pooron the basis of the MIC value of each isolate which was determined by broth micro dilution method recommended by NCCLS M27-A guide lines.

In case of isolates retrieved from rural population ketoconazole and itraconazole susceptibility was fair to poor however all the isolates were resistant to fluconazole as shown in table I. C.albicans (n=15) was predominant in pregnant VVC patients followed by C. glabrata(n=7) and C.krusei(n=6). In non-pregnant patients 6 cases each of C.glabrata and C.albicanswere retrieved.Clotrimazole susceptibility was very good in pregnant vaginal candidiasis patients as all the fifteen C.albicans, five C.kruseiand four C.glabrata showed sensitivity to it while, in non-pregnant patients its susceptibility was less than 50%. C.glabrata and C.krusei from both pregnant and non-pregnant were highly resistant to itraconazole while C.albicans showed poor susceptibility to the drug. Ketoconazole susceptibility was fair in case of pregnant VVC patients whereas high resistance in case of isolates retrieved from non-pregnant VVC. Fluconazole susceptibility was also poor in both cases as shown in Table II

DISCUSSION

In this study prevalence of VVC was 70% in pregnant while 30% in non-pregnant patients. Out of 70% preg-

Table 1: Susceptibility pattern of Candida isolates recovered from urban as well as rural population against test azole antifungal agents.

Candida Species	Area	Clotrimazole		Ketoconazole		Fluconazole		Itraconazole	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
C.albicans (n=16)	Urban (n=32)	15	1	3	13	5	11	8	8
C.glabrata (n=11)		4	7	4	7	4	7	0	11
C.krusei (n=5)		4	1	4	1	2	3	0	5
Total		23	9	11	21	11	21	8	24
C.albicans (n=5)	Rural (n=8)	5	0	0	5	0	5	2	3
C.glabrata (n=2)		0	2	0	2	0	2	0	2
C.Krusei (n=1)		1	0	1	0	0	1	0	1
Total		6	2	1	7	0	8	2	6

Table 2: Susceptibility pattern of Candida isolates recovered from pregnant as well as non-pregnant vaginal candidiasis patients against test azole antifungal agents.

Candida Species	Area	Clotrimazole		Ketoconazole		Fluconazole		Itraconazole	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
C.albicans (n=15)	Pregnant (n=28)	15	0	2	13	3	12	6	9
C.glabrata (n=7)		4	3	4	3	4	3	0	7
C. krusei (n=6)		5	1	5	1	2	4	0	6
Total		24	4	11	17	9	19	6	22
C.albicans (n=6)	Non-pregnant (n=12)	5	1	1	5	2	4	4	2
C.glabrata (n=6)		0	6	0	6	0	6	0	6
C. Krusei (n=0)		0	0	0	0	0	0	0	0
Total		5	7	1	11	2	10	4	8

nant patients 53.5% were Calbicans positive while 47.5% had vaginitis due to non-albicans species. In non-pregnant vaginitis patients 50% isolates were C.albicans while remaining 50% were C.glabrata. This finding is in conformity to the earlier finding in which VVC is significantly higher in pregnant than non-pregnant patients. [20]

In our study the prevalence of C.albicans and non-albicans species in urban population remained about 50% each, in contrast to the Chinese study in which C.albicans (80.5%) was common specie while non-albicans species were 19.5% causing VVC. [21] In present study 62.5% isolates from rural area were C.albicans positive while 37.5% were non-albicans that is in contrast to an Indian study that give prevalence of 60% non-albicans infections and 40% C. albicans vaginitis. [22]

Susceptibility to Clotrimazole was 71.8% and 75% in VVC urban and rural patients respectively. 63.6% isolates of C.glabrata from urban area and 100% from rural area were resistant to clotrimazole, a finding in conformity with Japanese data that gives more susceptibility of C.albicans for Clotrimazoleas compare to C.glabrata.[23] Results of the rural area are in favor of this study yielding 90.4% susceptibility of clotrimazole against C.albicans while in contrast to the same study as far as the susceptibility of clotraimazole against C.glabrata (78.2%)[24]

In this study, 34.3% patients from urban area and 12.5% patients from rural area showed response to ketoconazole. Both C.albicans and C.glabrataisolates from rural patients showed complete resistance while 18.7% C. albicans and 36.3% C.glabrataisoaltes retrieved from urban patients responded to it. All isolates of C. krusei from rural patients and 80% from urban patients responded to

this drug. This finding is contrary to an Iranian study which states that 90.6% urban isolates to be sensitive to ketoconazole. [25] The resistance rate of this agent forC.glabrata isolates was 15% and that of C.albicans was 9.6%[25], which is contrary to our report. In a study done in rural area both C.albicans and non-albican species were found to be highly susceptible toketococnazole which is in contrast to our study. [22] 34.3% isolates retrieved from urban VVC patients responded to fluconazoole while allisolates from rural patients showed resistance, contrary to Brazalian study reporting 93.6% susceptibility of C.albicans while 81.8% of C.glabratato fluconazole[26] In present study resistance to Fluconazole in all species from rural patients is in contrast to the report yielding 100% susceptibility of fluconazole to all candida isolates from VVC patients visiting a rural primary health care centre in north India.[27]

Fifty percent of C. albicans from urban patients were susceptible to itraconazole while all isolates of C. glabrata and C. krusei showed resistance to it. So in urban area the drug susceptibility was 25%, in contrast toa finding reported that 10% of C.albicansand 88% C.glabratato be resistant against Itraconazole.[28] 85.7% pregnant VVC patients showed susceptibility for Clotrimazole of which 100% of C.albicansand 57.1% of C.glabrata were found susceptible to it which is in favor of a study done in Uganda presenting resistance of 0.61% and 36.67% againstC. albican and C.glabrataspecies respectively.[29] However 83.3% non-pregnant VVC patients responded to this agent and only C.albicanswere susceptible to clotrimazole while C. glabrata were highly resistant to it which is in favor of a Japanese study yielding high resistance of C.glabrata against clotrimazole. [23]

In case of pregnant VVC patients, 39.3% were susceptible to Ketoconazole which is contrary to a studyreporting highest sensitivity of ketoconazole against C.albicans in pregnant patients.[30]

In our study out of all isolates from pregnant vulvovaginal candidiasis patients only 32.1% were found susceptible to fluconazole which is in favor of another study done in Peshawar yielding 33.3% of candida isolates sensitive to fluconazole. [31]

CONCLUSION

This study demonstrated the importance of specie identification and antifungal susceptibility testing as the results were different among urban and rural population, showing high resistance against itraconazole and fluconazoe in urban and rural population respectively.

The high prevalence of VVC with multiple species in pregnant patients was found to be most susceptible to clotrimazole while highest resistance was observed against itraconazole.

In case of non-pregnant patients high resistance was observed against ketoconazole and fluconazole.

RECOMMENDATIONS

If a similar study is done on a large sample size with better study design it will give more clear picture as one study can provide grounds for other researches.

CONFLICT OF INTEREST:

This study is a part of my M.Phil thesis in which clotrimazole, fluconazole, ketoconazole and itraconazole susceptibilities of candida species recovered from vulvovaginitis patients in a tertiary care hospital of peshawar, Pakistan was established. The seminal paper has already been published. This auxiliary data has some more insight results and is reported in this article. I am thankful to my supervisor, co. supervisor and my colleagues for their support.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Zafar S: Main idea.

Javaid A: Data collection.

Salman F: Searching relevant article.

Khan AZ: Laboratory work.

Haq M: Data entrance.

Khurram M: Supervised the whole project.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.