



CLINICO-MYCOLOGICAL PROFILE OF VAGINITIS IN REPRODUCTIVE AGE GROUP IN KOTA REGION

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ABSTRACT

INTRODUCTION- Fungus is the second most common cause of Vaginitis. Hence the present study was undertaken to investigate the prevalence, risk factors, Isolation, identification & distribution of *Candida* spp., and to determine the susceptibility to commonly used antifungals.

MATERIAL & METHOD- Vaginal swabs collected from 100 patients with history of white discharge and subjecting it to microscopy and culture. Susceptibility of the isolates to antifungal drugs was determined by disk diffusion method.

RESULT- *Candida* as the causative agent of Vulvovaginitis was noted in 36% of cases. The incidence of candida were significantly high in pregnancy (46%), oral contraceptives (35%) & intrauterine devices (38%) users, infertility treatment (29%), post hysterectomy (25%) and diabetes (33%). Out of the 39 isolates, *C.albicans* were most common (69%) species. Overall resistance for *Candida* was 18% for Fluconazole, 26% for Itraconazole, 15% for Clotrimazole, 18% for Ketoconazole, 10% for Nystatin and 0% for Amphotericin B.

CONCLUSION- The prevalence of candida was 36%. Risk factors for VVC are pregnancy, oral contraceptive, intrauterine devices, infertility treatment, post hysterectomy, and diabetes. The predominant species isolated was *C.albicans*. Amphotericin B and Nystatin proved to be the most effective drug in present study.

KEYWORDS :

INTRODUCTION-

Vaginitis (inflammation of vagina) is a term used to describe infectious diseases and other inflammatory conditions affecting the vaginal mucosa and sometimes secondarily involving the vulva.^[1] A change in the amount, color, or smell of vaginal discharge accompanied by pruritus, erythema, edema and sometimes burning, pain, with or without vulvar irritation are characteristics of vaginitis.^[2] It is caused by bacteria, fungus, protozoa, and virus. Fungus is the second most common cause after the bacteria. Vulvovaginal (VV) candidiasis is the second most frequent infection of the female genital tract, representing over 25% of infectious vaginitis.^[3,4] Vaginitis caused by candida is also called as vaginal thrush or moniliasis, vaginal candidiasis, vulvo vaginal candidiasis (VVC) and candidalvaginitis that occurs when there is over growth of the fungus candida which is normally present in the body in small amounts as asymptomatic colonization.^[5] Reduced immunity, prolonged antibiotic therapy, use of steroids, malnutrition, pregnancy, use of high estrogen dose oral contraceptive, hormone replacement therapy, poorly controlled diabetes mellitus and obesity are predisposing factor for VVC.^[6] Diagnosis of VVC based solely on patient history and genital examination is not possible because of the low specificity of symptoms and signs, since other causes mimic VVC, like leukorrhea and pruritus vulvae, therefore, to have a definitive diagnosis of VVC, cultural isolation and identification of *Candida* spp. are crucial. For treatment of fungal infections; many antifungal agent have been used such as polyene antibiotics (nystatin, amphotericin B, hamycin etc.) and azoles (miconazole, ketoconazole, clotrimazole, itraconazole, fluconazole, etc.). But emergence of non-albicans species of candida and random usage of these antifungals in last few years has increased the chances of treatment failure and development of resistance strains to many antifungal agents.^[7] Relatively few epidemiological data on the distribution of *Candida* spp. and antifungal susceptibility of yeast causing VVC are available, and this is in part related to the fact that microbiological tests are not performed routinely.^[8,9]

Hence, this study has been carried out to improve the knowledge

regarding risk factors of vaginal candidiasis, and to determine the prevalence of vaginal candidiasis and speciation of the isolates by conventional methods and to determine the antifungal susceptibility profile by disc diffusion method.

MATERIALS AND METHODS-

A total of 100 patients, age group 18-49 years, with symptoms of vaginitis, attending gynecology and obstetrics department of Govt. medical college, Kota from December 2016 to November 2017 were selected for this study and patient of age under 18 or over 49 years, menstruating female patients, and diagnosed case of bacterial vaginitis were excluded from our study. High vaginal swab specimens were collected carefully and appropriately with sterile cotton swabs from the vagina. While contamination was minimized, by collecting a pair of swabs from each subject. Swabs were used for direct microscopic examination, culture and isolation of strains of candida. Direct microscopic examination of all the specimens was performed by direct wet film examination, 10% KOH mount and Gram's staining^[10] For culture and isolation of strains of *Candida* all the samples were inoculated onto two Sabourauds dextrose agar containing chloramphenicol slant (HiMedia)^[10]. After sample inoculation the inoculated media were incubated at 37°C for 48-72 hours and observed for growth. Those slant which were showing no growth after 72 hours were recorded as no growth and discarded. When the growth appeared on the media inoculated, the colony characteristics were recorded and followed by Microscopy and further processed for speciation by Germ tube test (Reynolds-Braude phenomenon)^[11], Cornmeal agar test (Dalmau plate), Fermentation test, Carbohydrate Assimilation Test^[12] and Hichrom *Candida* differential agar study.^[13,14]

Antifungal sensitivity of various fungal isolates was performed by the disc diffusion method on Mueller Hinton Agar supplemented with 2% glucose and 0.5 mg/l Methylene Blue (HiMedia make).^[15] Antifungal drug discs (HiMedia make) were used in following concentrations: Fluconazole 10µg, Nystatin 100U, Amphotericin 100U, Ketoconazole 10µg, Itraconazole 10µg and Clotrimazole 10µg. After the measurement of zone of inhibition, the results of

antifungal sensitivity were interpreted according to criteria given with HiMedia antifungal discs.

RESULT-

The majority of the cases with white discharge per vagina were seen in the age group of 26 to 35 years (46%) (Table1). Incidence of candida high in pregnancy (46%), oral contraceptive pills users (35%), intrauterine devices users (38%), treatment for infertility (29%), Diabetes (33%) (Table 2). Among the cases positive for vaginal candidiasis, most common clinical symptom in all the study group was white discharge per vagina (100%), followed by pruritus in 23 (64%) of cases, local irritation in 18 cases (50%), soreness was seen in 10 cases (28%) of cases, dyspareunia was seen in 8 cases (22%) and 6 patients (17%) complained of burning micturation. 25 % of the patients with vaginal candidiasis had similar complaints in the past and had been treated for the same. The most common clinical sign on examination was leucorrhoea seen in all the patients (100%), followed by excoriation seen in 13 patients (36%) and vulvar erythema seen in 5 patients (14%). Out of 100 samples Gram staining positive for 20 samples and culture positive for 36 samples. Out of 100 patients, 32 % of the cases had infection due to *Candida* and 28% showed bacterial cause, 4 % of cases showed both mixed growth of *Candida* and bacteria. About 36% of the cases showed no growth (Graph 1). On inoculating the *Candida* isolates on the CHROM agar media, 27 isolates showed light green color indicative of *C.albicans*, 9 isolates showed cream / pale white color suggestive of *C.glabaratas/C.parapsilosis*. Dark blue colored colonies of *C.tropicalis* was seen in 2 cases. Pale pink purple colored colonies of *C.krusei* was seen in 1 case. Among these, isolates from 3 samples showed colonies of more than one color, suggestive of mixed infection. One of these showed light green and pale pink colonies were seen suggestive of *C.albicans* and *C.krusei* mixed infection. Two of the cases showed light green color colonies and cream / pale white color colonies indicating mixed infection due to *C.albicans* and *C.glabrata*. Hence from 36 cases of vaginal candidiasis, total number of isolates was 39. All 39 isolates were subjected to Cornmeal agar test majority showed presence of chlamydo spores (69%) suggestive of *Candida albicans*. The sugar assimilation pattern and fermentation pattern of the isolates correlated with results as in CHROM agar identification and corn meal agar test. Out of the 39 isolates, *C.albicans* was the dominant of the species 27 (69%), 8 isolates were *C.glabrata*(21%), 2 of the isolates were *C.tropicalis*(5%) and one isolate of each of *C.krusei* (3%) and *C.parapsilosis*(3%), (Table 3). Overall resistance for *Candida* in present study was 26% for Fluconazole, 24% for Itraconazole, 29% for Clotrimazole, 18% for Ketoconazole and 10% for Nystatin. No resistance was observed for Amphotericin B (Table 4).

DISCUSSION-

Our study showed that maximum number of cases presenting with leucorrhoea belonged to the age group of 26-35 years, which is comparable to the study done by Puri KJ et al.^[16] (20-40 years). The mean age distribution in our study was 32.71±7.6 years which can be compared to the mean age 30 years in a study done by Norma T. Gross et al ^[17]. This study revealed that patients who were peak of their reproductive years are more vulnerable to infections, because estrogen which induces the lining of the vagina to mature contain glycogen; a substrate on which *Candida* thrives. The prevalence of

candida much more in pregnant patients (46%). This may be probably due to high level of reproductive hormones during pregnancy which provides an excellent carbon source for growth of *candida*, inducing higher glycogen contents in the vaginal epithelial cells and also estrogens have a direct effect on the growth of *Candida* and its adherence to the vaginal epithelium.^[18] Incidence of VVC was much more in patients who were using OCPs, intra uterine device, & treated for infertility. This might be because of high estrogen in these therapies increases epithelial cell receptivity and the glycogen content of epithelial cells, promoting growth of candidiasis. Diabetes enhances *Candida* infection; due to sugar increasing in vagina that make it suitable environment to yeast growth. *C. albicans* (69%) was the major species isolated in vaginitis cases in present study. This correlates with findings of Norma T. Gross et al ^[12] Neeraja Jindal et al ^[19], and Salehei et al. ^[20] *C. glabrata* was the most frequent species among non-*albicans* spp. in the current study. Similar to study done by Neeraja Jindal et al. 2007 ^[19] and Salehei et al 2012. ^[20] Amphotericin B and Nystatin proved to be the most effective drug in present study.

CONCLUSION-

Maximum number of cases presenting with VVC were belonged to the age group of 26-35 years. The prevalence of candida was 36%. Risk factors for VVC are pregnancy, oral contraceptive, intrauterine devices, infertility treatment, post hysterectomy, and diabetes. The predominant species isolated was *C.albicans*. Amphotericin B and Nystatin proved to be the most effective drug in present study.

TABLES-

Table 1: Age (in years) distribution in study group

Age (in years)	Study group	
	Number	Percentage
18-25	18	18
26-35	46	46
36-45	30	30
45-49	6	6
Total	100	100

Table 2: Pre disposing factors

	Number of cases in the study group	Candidial vaginitis	
		Number	Percentage
Pregnancy	35	17	46
Oral contraceptives	12	3	35
Intra uterine devices	16	6	38
Infertility	7	2	29
Diabetes	9	3	33

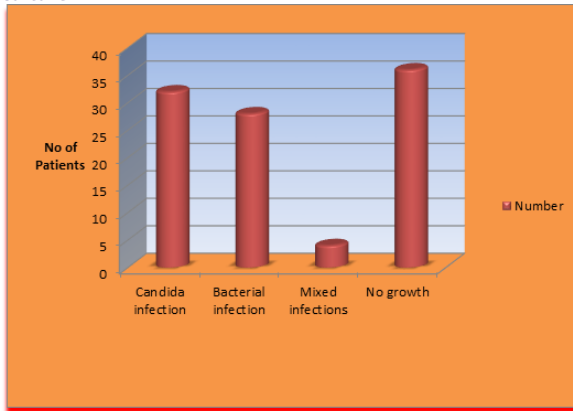
Table 3: Different species of candida isolated

Species Identification	Number	Percentage
<i>C.albicans</i>	27	69
<i>C. glabrata</i>	8	21
<i>C.parapsilosis</i>	1	3
<i>C. tropicalis</i>	2	5
<i>C.krusei</i>	1	3
<i>C.parapsilosis</i>	1	3

Table 4: Antifungal Sensitivity Profile of Candida Isolates (in percentage)

Anti fungal drugs	Fluconazole (25 µg)		Itraconazole (10 µg)		Clotrimazole (10 µg)		Ketoconazole (10 µg)		Nystatin 100 U/disc		AmB 100U	
	S	R	S	R	S	R	S	R	S	R	S	R
<i>C.albicans</i>	85	15	74	26	89	11	81	19	93	7	100	0
<i>C. glabrata</i>	75	25	75	25	75	25	75	25	88	12	100	0
<i>C.tropicalis</i>	100	0	100	0	50	50	100	0	50	50	100	0
<i>C.krusei</i>	0	100	0	100	100	0	100	0	100	0	100	0
<i>C.parapsilosis</i>	100	0	100	0	100	0	100	0	100	0	100	0

Graph 1 : Leucorrhoea due to various causatives based on culture



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