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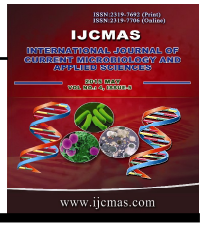

Original Research Article

Prevalence of vaginal candidiasis in diabetic women of Madhya Pradesh, India

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A B S T R A C T

In a healthy women, vaginal ecosystem acts to provide a barrier to both new colonization by pathogenic organisms and overgrowth of organisms that are otherwise commensals. Diabetes is a proven predisposing factor for the imbalance in vaginal microbiota resulting vulvovaginal candidiasis. The present study was undertaken to find out prevalence of vaginal candidiasis in diabetic women. For the study, 119 diabetic women were recruited and categorized with symptomatic and asymptomatic group of vaginal infections. Vaginal swab samples were collected and processed in laboratory. The patients were asked to fill the questionnaires related to personal introduction, history of diabetes, duration of diabetes, blood sugar level, socioeconomic status, education, etc. For the isolation of *Candida* species, the swabs were streaked on Sabouard Dextrose agar plates containing chloramphenicol and incubated at 27^0C for 48 h. After incubation the plates were observed and pure cultures of yeast were obtained and characterized for species level. Proteinase and haemolysin activity of Candida species were also determined. Total 57 (47.5%) yeast were isolated from 119 diabetic women. Out of 57 yeast culture total four candida species were identified viz. 12 (21.1%) *Candida albicans*, 15 (26.3%) *Candida glabrata*, 24 (42.1%) *Candida krusei* and 6 (10.5%) *Candida tropicalis*. In symptomatic group of patients *Candida albicans* was predominant as 10 (17.5%) followed by *Candida glabrata* 8 (14%), while in asymptomatic group of patients *Candida krusei* 19 (33.3%) was predominant. *C.albicans* has shown the maximum extent of proteinase activity range from 0.096-0.325 with average 0.179±0.05, haemolysin activity range from 0.256-0.568 with an average value of 0.352±0.08 among the other species.

K e y w o r d s

Vaginal candidiasis, SDA, *C.albicans*, *C.glabrata*, *C.krusei*, *C.topicalis*, diabetes

Introduction

The vaginal vault is colonized within 24 hours of a female child's birth and persists until death, (Marshall, et al., 1981) comprising an ever-changing and fine-tuned ecosystem with numerous factors that have the potential to destruct the vaginal ecosystem. Diabetes is a proven predisposing factor for the imbalance in

vaginal micro flora resulting vulvovaginal candidiasis. Symptomatic vulvovaginal candidiasis has been proven to be more prevalent in diabetic women than in the general population (Scudamore et al., 1992 and Sobel 1993)**.** Hyperglycemia is the major reason of increased susceptibility of diabetic women with vulvovaginal candidiasis. Increased glucose levels in genital tissues, enhance yeast adhesion and growth. Opsonic activity may also be affected in patients with diabetes. An impairment result when glucose binds to the biochemically active site of the third component of complement C3 and thereby inhibits attachment of this protein to the pathogen (Hostetter, 1990), as well as impair the function of neutrophils and other polymorphs thus hinder the innate immunity of vaginal cells.

An estimated 75 percent of women will have at least one episode of vulvovaginal candidiasis, and 40 to 45 percent will have two or more thus recurrent. *Candida albicans* is responsible for 80 to 92 percent of episodes of vulvovaginal candidiasis (Odds, 1988). Recently, an increased frequency of other candida species, particularly *C. glabrata,* has been reported possibly due to widespread use of over-the-counter drugs, long-term use of azoles, and the use of short courses of antifungal drugs (Horowitz *et al.,* 1992).

Currently, more than two hundred ascomycetous yeasts are included into the genus *Candida*, but only a few species are opportunistic pathogens of humans. Nowadays, *Candida albicans* is thought to be the major fungal pathogen of humans because it shown a number of virulence factors that attribute the candidiasis viz. phenotypic switching, phospholipase, proteinase and hemolytic activity. *C. albicans* is isolated in 85-90% of all cases of vulvovaginal candidiasis, followed by C. *glabrata* (5-10%), C. *tropicalis* (3-5%), and other species (Peters *et al.,* 1966). In diabetic patients, limited data suggest an increased prevalence of *Candida* species other than C. *albicans*, particularly C. *glabrata*, as the primary causative species. Moreover, the geographical distribution of pathogens and environmental conditions may vary from one place to other, thus the present study was undertaken to evaluate the prevalence of pathogens in vagina of diabetic women in this region.

Materials and Methods

Vaginal samples and data collection from diabetic women

Sterile Hi-vaginal swabs of 130mm x12mm diameter were purchased from Hi-media, for the collection of vaginal samples from diabetic women. Vaginal samples were collected from the diabetic women from Jan 2011 to 2013 who attended Gwalior diabetic centre from last two years. After presumptive diagnosis of symptomatic and asymptomatic vaginal infection of patients on the basis of their sign and symptoms, the clinicians collected the samples and immediately sent to the laboratory for microbiological examination. The patients were asked to fill the questionnaires related to personal introduction, history of diabetes, duration of diabetes, blood sugar level, socioeconomic status, education, etc. Clearance was taken from the local ethical committee for this study.

Isolation and characterization of *Candida* species

For the isolation of *Candida* species, the swabs were streaked on Sabouard Dextrose agar plates containing chloramphenicol and incubated at 27^0C for 48 h. After incubation

the plates were observed and yeast like colonies were transferred onto fresh plate to obtain the pure culture. The yeast was characterized on the basis of gram staining; color on chromogenic media, growth on cornmeal agar, germ tube formation test, sugar fermentation and other biochemical test.

Characterization of *Candida* species for their virulence factors

Proteinase activity

Extracellular proteinase activity of *Candida* isolates was analyzed in terms of bovine serum albumin (BSA) degradation according to the technique described by Staib *et al*., (1965). In short, 24 h old yeast suspension of $1X10^6$ cells/ ml was prepared, and 10 µl suspension was inoculated onto a 1% BSA medium plate (Dextrose 2%, KH2PO4 0.1%, MgSO4 0.05%, agar 2% mixed after cooling to 50^0C with 1% BSA solution). The plate was incubated at 37^0C for 5 days. The plates were then fixed with 20% trichloracetic acid and stained with 1.25% amidoblack. Acetic acid was used for decolourisation. Degradation of the protein was seen as opaqueness of the agar, corresponding to a zone of proteolysis around the colony which could not be stained with amidoblack. The assay was done in duplicate on three different occasions for each *Candida* isolate tested. Proteinase activity (Prz) was determined as the ratio of the colony to the diameter of the proteolytic unstained zone. A Prz value of 1 signifies no activity, and less than one (Prz<1) means proteinase activity. The lower the Prz value, the higher the enzymatic activity.

Hemolysin activity

Haemolysin activity was evaluated with a blood agar plate assay as described by Manns *et al.,* (1994) with slight modification. Media were prepared by adding 5 ml fresh blood to 100 ml SDA supplemented with glucose at a final concentration of 3% (w/v). Each Candida isolate was inoculated in centre within 6mm diameter on separate blood agar plate. The plate was then incubated at 37^0C for 48 h.The ratio of the diameter of the colony to that of the translucent zone of haemolysis (in mm) was used as the haemolytic index (Hz value) to represent the extent of haemolysin activity by different Candida isolates. The assay was conducted in duplicate on three separate occasions for each yeast isolate tested.

Results and Discussion

A total 119 women suffering from the diabetes mellitus were enrolled for this study. The vaginal swab samples were collected from all 119 patients and they were asked to fill the questionnaires related to personal introduction, history of diabetes, socioeconomic status, education and religion etc. The data were analyzed and summarized in Table 1. The patients were allocated to one of two groups (i) symptomatic group (those with complaints of vaginitis) - 45 (37.8%) women; and (ii) asymptomatic group – 74 (62.3%) women. Out of 45 women shown symptoms of vaginitis, 12 (26.6%) having recurrent infection in vagina.

The age of the patients varied from 30-64 years (mean age 49±9 years). The mean age of symptomatic patients was higher (50±10 years) than those of asymptomatic patients (48±8 years).

Isolation and characterization of candida species

From the vagina of 119 diabetic women 57 (47.5%) yeast were isolated and distributed

as 24 (53.33%) cultures were isolated from the women who complained for vaginitis while 33 (44.51%) culture were isolated from the women with the asymptomatic infection. Pure culture of yeast were maintained on SDA plate (Fig.1) and in glycerol stock at -4^{0}C and characterized on the basis of gram staining (Fig.2.); color on chromogenic media (Fig.3.), growth on cornmeal agar, germ tube formation test (Fig.4.), sugar fermentation and other biochemical test. Table 2 shown the biochemical and morphological characterization of candida species.

Out of 57 yeast culture total four candida species were identified viz. 12 (21.1%) *Candida albicans*, 15 (26.3%) *Candida glabrata*, 24 (42.1%) *Candida krusei* and 6 (10.5%) *Candida tropicalis*. Table 3 and Graph 1 had shown the distribution of Candida species in symptomatic and asymptomatic group of patients. In symptomatic group of patients *Candida albicans* was predominant as 10 (17.5%) followed by *Candida glabrata* 8 (14%), while in asymptomatic group of patients *Candida krusei* 19 (33.3%) was predominant.

Characterization of *Candida* species for their virulence factors

Proteinase activity

The extent of proteinase activity of all candida specie was represented as Pr_z value (the ratio of the diameter of the colony to that of the zone of proteolysis (in mm) was used as an index). Out of 57 Candida species 35 (61.4%) had shown the various extent of proteinase activity. *C.albicans* has shown the maximum extent of proteinase activity range from 0.096-0.325 with average 0.179±0.05 among the Candida species. (Fig.5) (Table 4)

Haemolysin activity

Like the proteinase activity, haemolysin activity was expressed as Hz value (The ratio of the diameter of the colony to that of the translucent zone of haemolysis (in mm) was used as the haemolytic index). Out of 57 Candida species 39 (68.4%) had shown α-haemolytic activity. Among the Candida species *C.albicans* shown the maximum haemolysin activity range from 0.256-0.568 with an average value of 0.352±0.08. (Fig.6.) (Table 4.)

Diabetes is a proven predisposing factor for vulvovaginal infection especially vaginal candidiasis, along with pregnancy, use of broad-spectrum antibiotics, high-estrogen-dose oral contraceptives, obesity, and drug addiction (Rajawat *et al*., 2013). In this study 119 diabetic women were recruited and allocated one of two group (i) symptomatic group-45 (37.8%) women (ii) asymptomatic group-74 (62.3%) women. Out of 45 women shown symptoms of vaginitis, 12 (26.6%) having recurrent infection in vagina. The possible etiology of recurrent infection is the poor management of glucose level in diabetic women that impair several aspects of humoral host defense, resulting in decreased random motion of neutrophils, chemotaxis, phagocytosis, and microbial killing (Bohanon, 1998).

Previous studies on diabetic women in developed countries have found widely varying prevalence rates ranged from around 7 to more than 50% (Bohanon, 1998; Davis, 1969; Malazy *et al*., 2007), and most of which was attributed to *C. albicans* (Duerr *et al*., 1997; Malazy *et al*., 2007). Goswami *et al*., (2006) reported the prevalence rate of 46% in 78 diabetic women. Peer *et al*. (1993) reported the prevalence rate of the vulvovaginal candidiasis infection 24% in

111 diabetic women. These statistics show that different prevalence rates of vulvovaginal candidiasis in diabetic women are seen

Table.1 Summary of diabetic women data

Variables	Symptomatic Patients	Asymptomatic Patients	Total
No. of Patients	45 (37.8%)	74 (62.3%)	119
Mean age (years)	50±10	48±8	49±9
Range (years)	33-64	30-64	30-64
Religion			
Hindu	25 (28.7%)	62 (71.3%)	87 (73.1%)
Muslims	17 (73.9%)	06 (26.1%)	23 (19.3%)
Others	3 (33.3%)	06 (66.7%)	9 (7.6%)
Occupation			
Working	1(20%)	4 (80%)	5 (4.20%)
House wife	44 (38.6%)	70 (61.4%)	114 (95.8%)
Education			
Literate	18 (32.7%)	37 (67.3%)	55 (46.2%)
Non-Literate	27 (42.2%)	37 (57.8%)	64 (53.7%)
Socioeconomic status			
Rural	17 (34%)	33 (66%)	50 (42.7%)
Urban	28 (41.8%)	39 (58.2%)	67 (57.3%)
Food habits			
Vegetarian	23 (30.3%)	53 (69.7%)	76 (63.9%)
Non-Vegetarian	22 (51.2%)	21 (48.8%)	43 (36.1%)
Type of diabetes			
Type-1	3 (33.3%)	6 (66.7%)	9 (7.6%)
Type-2	42 (38.2%)	68 (61.8%)	110 (92.4%)
Mean Plasma glucose level (mg/dl)	R (238±40) PP (290±70) F (212±62)	R (185±31) PP (229±27) F (143±26)	R (204±43) PP (279±68) F (165±50)
Range of glucose level (mg/dl)	R (156-289) PP (210-420) F (158-280)	R (145-268) PP (210-249) F (101-180)	R (145-289) PP (210-420) F (101-280)
Mean duration of diabetes (years)	3.77±2.6	3.67±3	2.8±3
Yeast Isolate	24 (53.33%)	33 (44.51%)	57 (47.5%)
Bacterial Isolates	9 (45 %)	11 (55 %)	20 (16.8%)
Yeast and Bacteria	3 (15%)	5 (25%)	8 (40%)

Table.2 Morphological and Biochemical Characterization of Candida spp

	Test	C2, 4,6,11,15, 17, 22, 27, 33, 34,39,44, 49, 51, 56	C3,7,8,10, 13,16,18,19,21,23, 25,28,29,31,35,36,40,42,43,46,50,52,54,55	C9,32,38, 45,47,57	C1,5,12,14,20,24, 26,30,37,41,48,53
Morphological Test	**Gram staining**	+ve oval shape	+ve oval shape	+ve oval shape	+ve oval shape
	On Hi-crome candida agar	White smooth	Pink to purple	Purple fuzzy	Light green
	Corn meal agar	-ve	-ve	-ve	+ve
	Germ tube test	-ve	-ve	-ve	+ve
Biochemical Test	**Dextrose fermentation**	-ve	-ve	+ve	+ve
	Sucrose fermentation	-ve	+ve	+ve	+ve
	Xylose fermentation	+ve	+ve	+ve	+ve
	Mannose fermentation	+ve	+ve	+ve	+ve
	Trehalose fermentation	+ve	+ve	+ve	+ve
	Lactose fermentation	-ve	+ve	+ve	+ve
	Mannitol fermentation	+ve	-ve	+ve	+ve
	Maltose fermentation	-ve	+ve	+ve	+ve
	Suspected species	***C.glabrata***	***C.krusei***	***C.tropicalis***	***C.albicans***

+ve= Posotive, -ve= Negative

Table.3 Distribution of Candida spp. in symptomatic and asymptomatic group of patients

Yeast Isolates	Symptomatic	Asymptomatic (Carriers)	Total
Candida albicans	10 (17.5%)	2 (3.5%)	12 (21.1%)
C. glabrata	8 (14%)	07 (12.3%)	15 (26.3%)
C. krusei	5 (8.7%)	19 (33.3%)	24 (42.1%)
C. Tropicalis	1(1.8%)	5 (8.7%)	6 (10.5%)
Total	**24 (42.1%)**	**33 (57.9%)**	**57**

Table.4 Proteinase and Haemolysin activity of Candida species

S.No.	Candida Species	Proteinase activity (Pr_z)		Haemolysin activity (H_z)	
		Range	Mean	Rang	Mean
1.	*Candida albicans*	0.096-0.325	0.179±0.05	0.256-0.568	0.352±0.08
2.	*Candida glabrata*	0.117-0.0.489	0.232±0.08	0.356-0.632	0.428±0.06
3.	*Candida krusei*	0.216-0.685	0.450±0.10	0.558-0.689	0.598±0.04
4.	*Candida tropicalis*	0.259-0.675	0.365±0.60	0.598-0.758	0.601±0.51

Fig.1 Pure culture of Candida species

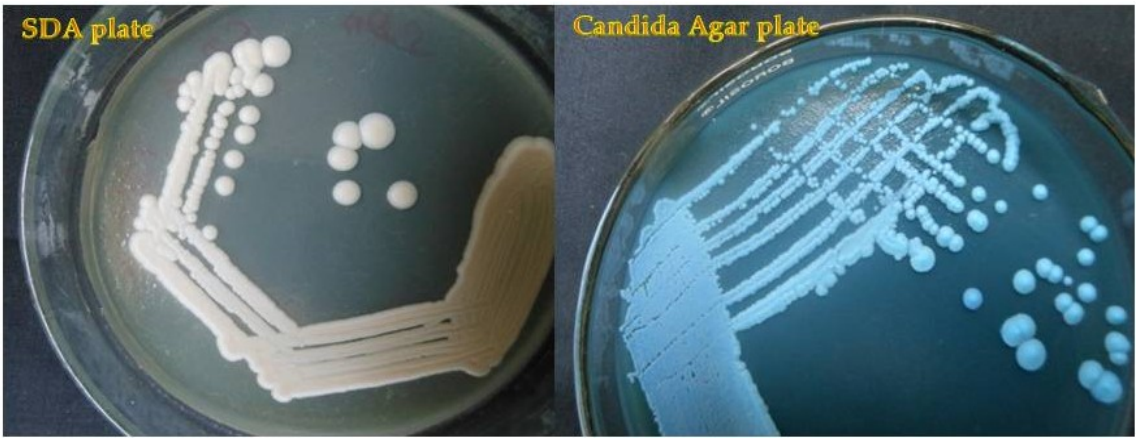


Fig.2 Microscopic morphology of Candia species

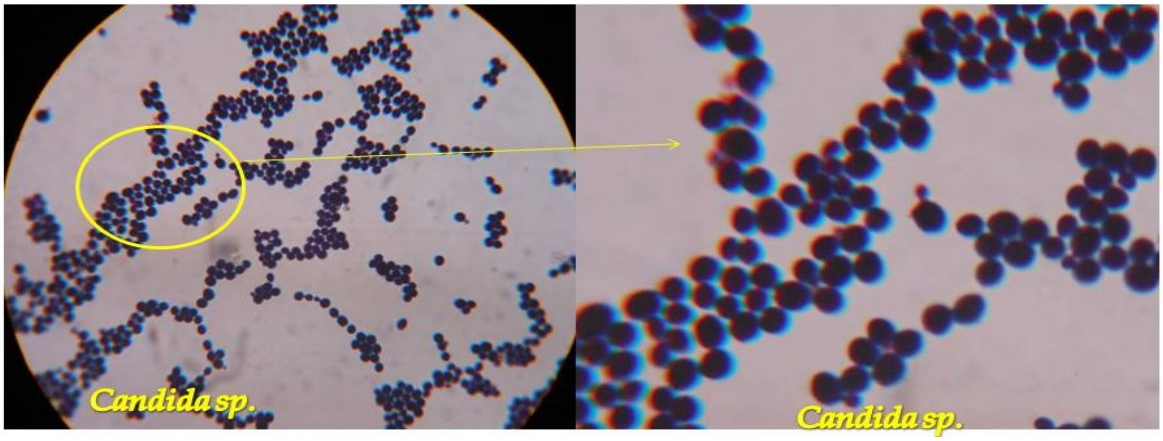


Fig.3 Identification of Candida species on Hi-Chrome agar plate

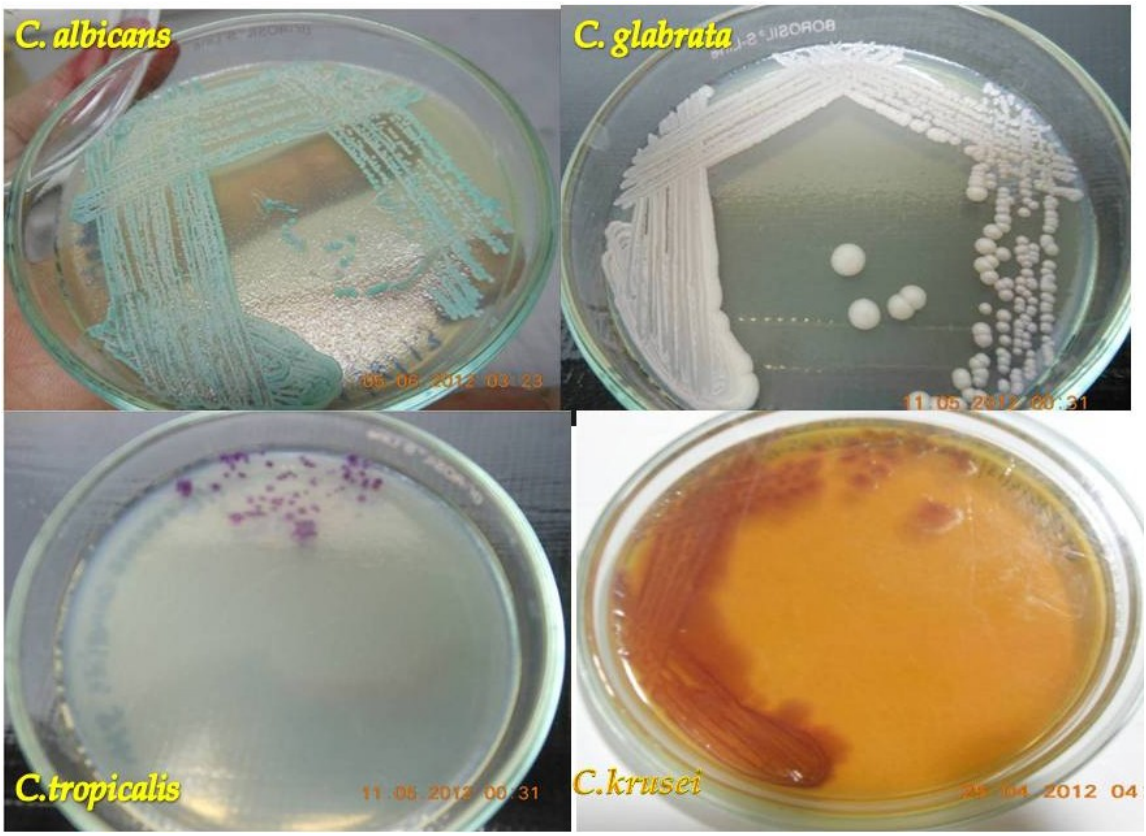


Fig.4 Chylamydospore formation and Germ tube test for identification of Candida albicans

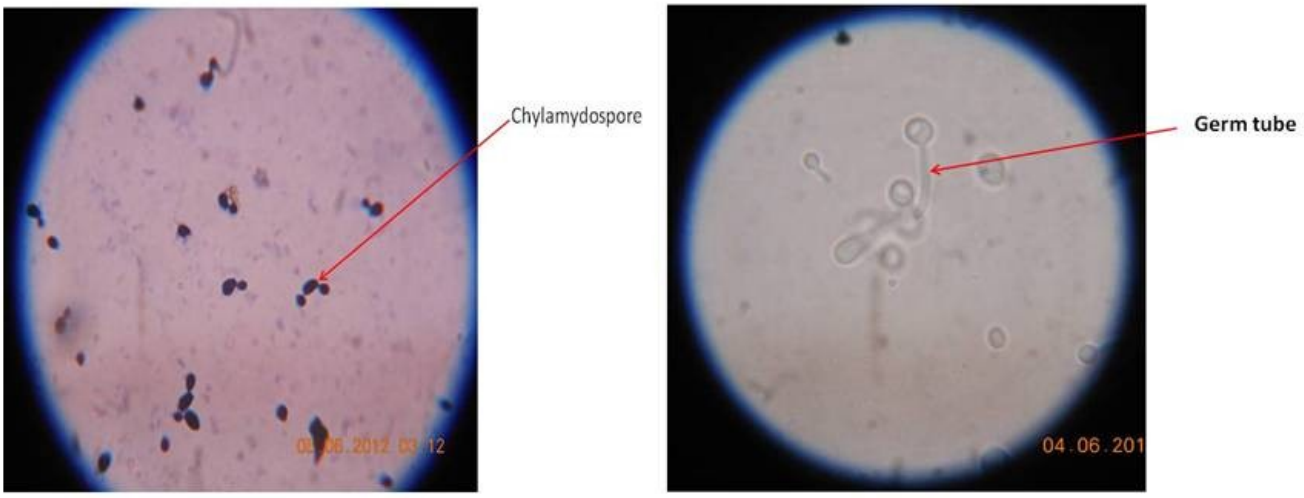


Fig.5 Proteinase activity of Candida species

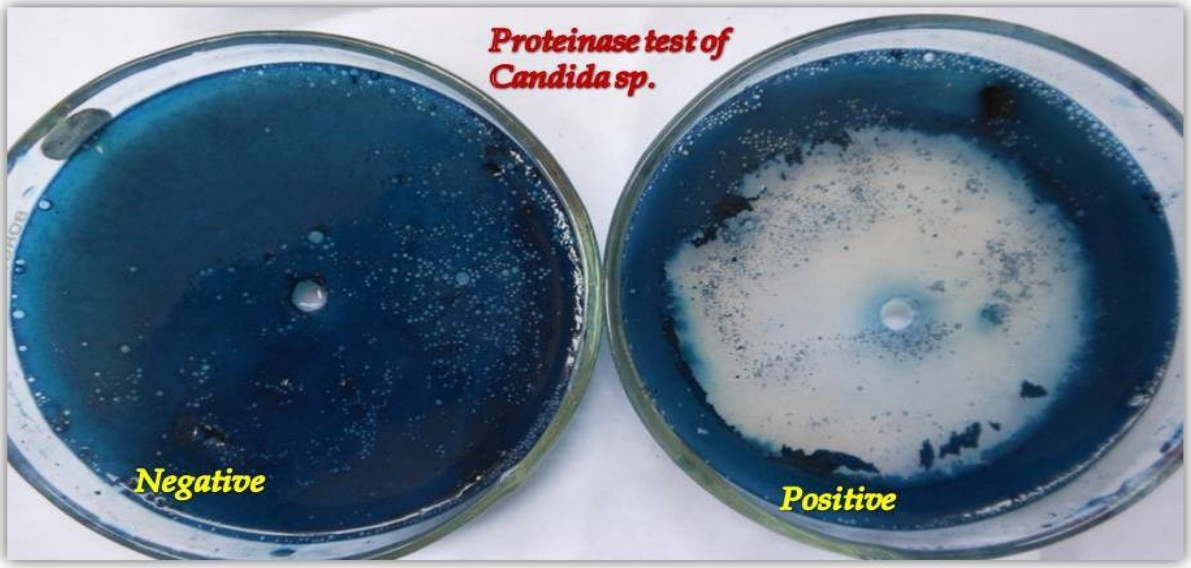
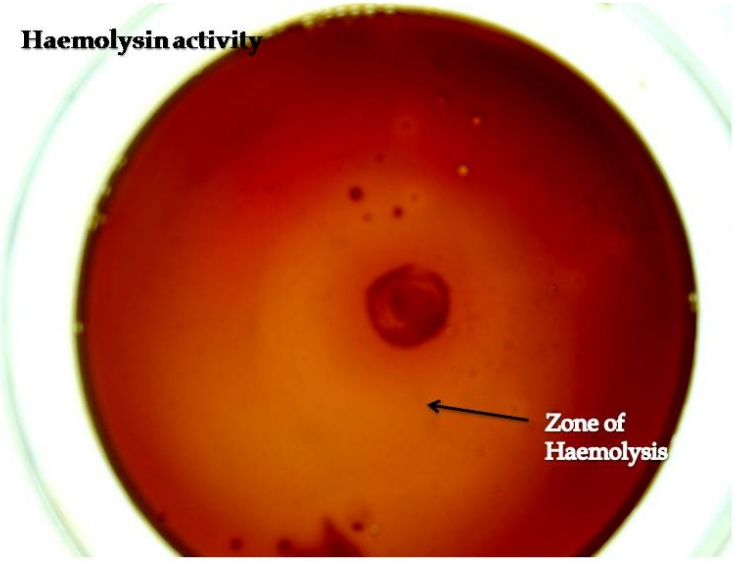
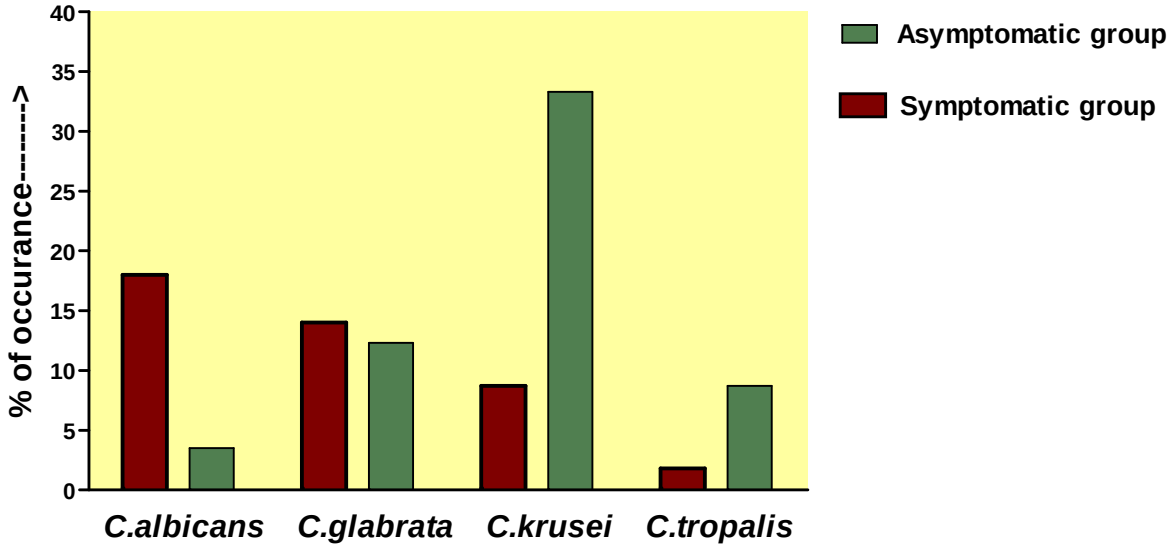


Fig.6 Hemolytic activity of Candida species



Graph.1 Shown the distribution of various Candida species in diabetic women



In the present study, the prevalence rate of candida infection in 119 diabetic women was found 47.5%. The rate of candidal infection was higher in symptomatic vaginitis (53.33%) as compare with asymptomatic infection (44.51%). The data revealed the positive association between candida infections in symptomatic vaginitis of diabetic women. Likely reasons for this difference could be attributed to increase the glucose level in genital tissue enhance yeast adhesions and growth and also to the use of unsuitable antifungal agents (Bohanon, 1998, Reza Faraji *et al*., 2012).

In this study, which was similar to other studies like Antony *et al*., (2009), Corsello *et al*., (2003), Grigoriou *et al*., (2006), the most isolated species from symptomatic group of patients was *C. albicans* with the occurrence rate 17.5% followed by *C.glabrata* (14%). The first step in establishing a yeast infection is bonding to the vaginal mucosa. It seems that *C. albicans* is more adhersive than other non-*C. albicans* species. This could be considered as one of the likely reasons that this species are predominant rather than non-*C. albicans* species in symptomatic group of patients.

In the present study, *C.krusei* was found predominant species among the non-albicans in asymptomatic group of patients which is quite dissimilar as Goswami *et al*., (2000) reported the prevalence rate of non-*C. albicans* species 39% with the predominant species *C. glabrata* in diabetic women. Sobel and Malazy (1998, 2007) stated the probable causes of higher non-*C. albicans* species: the short duration of use for oral or local anti-*Candida* regimens; the widespread use of over-the-counter antifungal agents.

The pathogenicity of *Candida* depends on several putative virulence factors, including germination, adherence to host cells, phenotypic switching and production of extracellular hydrolytic enzymes (Chakrabarti *et al*., 2000). Among these virulence attributes of *Candida* species, the present study targeted extracellular, proteinase and haemolysin activities in Candida species isolated from various clinical samples. In the present study, Out of

57 Candida species 35 (61.4%) had shown the proteinase activity. *C.albicans* has shown the maximum extent of proteinase activity range from 0.096-0.325 with average 0.179±0.05 among the Candida species. Similarily high rates of proteinase production in *C.albicans* have been reported by other workers like Tsang *et al*., 2000 and Koelsch *et al*., 2007. The secreted proteinases (SAPs) are responsible for the adhesion tissue damage and invasion of host immune responses. Their proteolytic activity has been associated with tissue invasion.

Studies on the activity of haemolysin in Candida are limited. Manns *et al*., (1994) defined the condition under which *C.albicans* can display haemolytic activity, but found that haemolysis is non-existent when no glucose is available in the culture medium.

On the other hand, Luo *et al*., (2001) have tested 80 *Candida* isolates from clinical sources in different geographical locales and detected only alpha, and no beta haemolysis in experiments with glucose-free sheep blood agar. Similarly, in this study, Out of 57 Candida species 39 (68.4%) had shown α- haemolytic activity. Among the Candida species *C.albicans* shown the maximum haemolysin activity range from 0.256-0.568 with an average value of 0.352±0.08 followed by *C.glabrata*.

Candida albicans was found to be a predominant and the most virulent species in symptomatic group of patients followed by *Candida glabrata*, while in asymptomatic group of patients *Candida krusei* was predominant.

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