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***Candida* infection and associated risk factors in post-menopausal women: A single center experience form state of Odisha, India.**

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Abstract:

Background: *Candida* infection is a common medical problem in India presenting with many clinical signs and symptoms. Due to changes in the vaginal microbiomes, post-menopausal women are susceptible to many bacterial and fungal infections including *Candida*. This study was undertaken to investigate the prevalence as well as association of risk factors for *Candida* infection in post-menopausal women.

Methods: Post-menopausal women presenting with abnormal vaginal discharge, itching and burning of genitalia, erythema etc. were considered. Vaginal specimens from posterior vaginal fornix were collected and *Candida* infection was investigated by using Albi Quick™ rapid test. Various data including socio-demographic features, sanitation access, and clinical presentations were analyzed statistically for possible association to *Candida* infections.

Results: From 209 post-menopausal women included in this study, *Candida albicans* infection was found in 9.09% of women. *Candida* infections was found to be significantly high in women who were married (P=0.001), who had no formal education (P=0.044) and who were staying with >5 persons in their home (P=0.017). Again, *Candida* infections was significantly high in women characterized with abnormal vaginal discharge (P=0.031) and itching and/or burning in genitalia (P=0.026).

Conclusion: The occurrence of *Candida* infections along with significant association of various risk factors reported in this study call for routine vaginal swab analysis in every women presenting with complaints of abnormal vaginal discharge and itching to rule out lower reproductive tract infections.

Key words: *Candida*; Abnormal vaginal discharge; Post-menopause

Introduction:

Candida infection is caused by an overgrowth of *Candida* species in the vagina disrupting the normal micro flora of the vagina. *Candida* infection can be asymptomatic or symptomatic. The asymptomatic state of *Candida* infections usually referred as colonization, while the symptomatic state of *Candida* infection was diagnosed looking to the local signs and symptoms of inflammation along with the presence of *Candida* species (Sobe, 2007). The symptomatic *Candida* infections are characterized with various signs and symptoms like abnormal vaginal discharge, itching and burning, and erythema etc. Due to the poor sensitivity and specificity of individual clinical symptoms related to *Candida* infection along with the poor performance of many laboratory settings, it is difficult for an accurate diagnosis of *Candida* infections (Anderson et al., 2004).

The prevalence of *Candida* infections in post menopausal women was reported to be different. Hillier et al., (1997) found only 1% of *Candida albicans* species in the vaginal microflora of 73 post menopausal women (Hillier et al., 1997). In a case-control study in Italy, 13.5% of postmenopausal symptomatic women had *Candida* infection (Spinillo et al., 1997). In two different studies, the prevalence of candidiasis was found to be 6-7% (Goldacre et al., 1979; Tibaldi et al., 2009). In a study from Australia, the prevalence of candidiasis was found to be different with respect to hormonal replacement therapy. The presence of *Candida* by culture was reported in 48.5% of women with hormonal replacement therapy while only 3% of women without hormonal replacement therapy. Further, the authors reported 67% of women who had diagnosed with the presence of *Candida* in the hormonal replacement therapy groups had a history of recurrent or chronic candidiasis before menopause (Fischer et al., 2011). During National Social Life, Health and Aging Project (NSHAP) carried out in post menopausal women in the United States, the prevalence of *Candida* infection was 5.94% among women in Wave 1 (57-85 years of women recruited during 2005-2006) compared to 4.98% in wave 2 (recruited during 2010-2011) (Hoffmann et al., 2014). This varied prevalence of *Candida* infections in post-menopausal women can be explained with the association of various factors which like age of the women, hormone replacement therapy, sexual behaviors, personal hygiene, diabetes mellitus etc. (Nwokolo et al., 2000; Hoffmann et al., 2014). With the paucity of literatures on the prevalence of *Candida* infection as well as its risk factors in postmenopausal women in India, this study was carried out with an aim to investigate the prevalence and associated risk factors for *Candida* infection in postmenopausal women in the state of Odisha, India.

Materials and methods:

Study design: This cross-sectional study has been carried out in post-menopausal women attending to the out-patient department (OPD) of Department of Obstetrics and Gynecology (O&G) of Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR), Burla, Odisha, India. The recruitment of study cases was done from December 2016 to November 2018.

Sample size: The prevalence of *Candida* infections in postmenopausal women was reported to be 1-14% in different population worldwide. Considering hypothesized % frequency of outcome factor in the population of 14%, confidence level of 95% and absolute error of 5%, the minimum number of sample required for the study was 185.

Study subjects: Women who had no evidence of menstruation since last 12 months were considered as post-menopausal women. Post-menopausal women presenting various symptoms such as abnormal vaginal discharge, itching and burning of genitalia, dysperunia, lower abdominal pain, back pain etc. were considered for recruitment (Nayak et al., 2020). Some postmenopausal women were excluded in this study like, who were already undergoing treatment; who had a hysterectomy; who had taken a course of antibiotics during the previous three weeks; who had any severe medical disorders requiring immediate health care; and/or refused to be a part of this study.

Collection of data: After the informed consent procedure, a pre-designed questionnaire was asked to each post-menopausal woman. The questionnaire contains the information on sociodemographic features such as age, marital status, religion, education, occupation, number of family member and monthly income etc. Questionnaire on clinical symptoms included abnormal vaginal discharge, burning or itching of the genitalia, and the presence of ulcers at vulva and labia, back pain, abdominal pain etc. Abnormal vaginal discharge can be defined with quantity, odor, color, and consistency of discharge.

Collection of vaginal samples: A speculum examination of vagina and cervix was done by a trained O & G specialist, particularly to look for the presence of any cervical erythema, bleeding, inflammation, with or without discharge, and ulcers at cervix. Vaginal specimens from posterior vaginal fornix were collected using BD BBL swabs (BD, Maryland, USA). The swab was used to determine the presence of *Candida*.

Diagnosis of *Candida albicans*: The diagnosis of *Candida albicans* infection in the vaginal samples was carried out by using Albi QuickTM rapid test (HARDY Diagnostic, CA, USA) as per manufacturer's instructions. Briefly, the vaginal swab was cultured in blood agar and chocolate

agar plate for 48 hours at 37°C. After 48 hours of incubation, sample was taken in a sterile loop and smeared on the test card for 5 min at room temperature. A bright blue-white or blue-purple fluorescence was observed for positive *Candida albicans* infection, while there was no fluorescence for negative samples.

Statistical analysis:

All the generated data was entered in an excel sheet for statistical analysis. Data were presented in number followed by percentage in various groups of post menopausal women. The comparison of categorical data was analyzed by Person's Chi square test. The statistical analysis was carried out by using Stata 11.0 (StataCorp, Stata Statistical Software: Release 11. 2011, StataCorp LP: College Station, TX). A P value of <0.05 was considered for statistically significant. This study was approved by Institutional Ethical Committee of Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR), Burla, Odisha (IEC/IRB-14/16).

Results:

During the study period, a total of 209 post-menopausal women were finally included in this study considering the inclusion and exclusion criterion. The median age of women was 54 years (range of 42 to 64 years).

The socio-demographic features showed, 120 (57.42%) and 89 (42.58%) women were belonging to ≤55 years of age and >55 years of age group respectively. Majority (91.87%, 192/209) of women were belonging to Hindu religion followed by Christian community (4.78%) and Muslim community (3.35%). Among the Hindu women, 50 (26.04%), 57 (29.69%), 57 (29.69%), 28 (14.58%) were belonging to scheduled caste, scheduled tribes, other backwards caste and general caste. Married women who were either separated or became widow during investigations constituted 6.7% (14/209) of women. Postmenopausal women having ≤20 years of marital life and >20 years of marital life constituted 21.05% (44/209) and 78.95% (165/209) respectively. 24.4% of women had no formal education, while 44.98% and 30.62% had attained 5th-10th grade and 12th grade or higher education respectively. Eighteen (8.61%) women were employed whereas 48 (22.97%) and 143 (68.42%) women were self employed and housewife respectively. Number of women living with 1-2 individuals in their home was 19 (9.09%), with 3-5 individuals was 117 (55.98%) and with ≥ 6 individuals was 73 (34.93%). Monthly income of < 5,000.00, 5,000.00-10,000.00 and > 10,000.00 was in 63 (30.14%), 68 (32.54%) and 78 (37.32%) women respectively. Women living in housing colony, urban slum and rural villages were 38 (18.18%), 40 (19.14%) and 131 (62.68%) respectively.

All the postmenopausal women recruited in this study were symptomatic. Various clinical symptoms was abnormal vaginal discharge, burning and itching of genitalia, burning and itching during urination, strawberry cervix, lower back pain and genital sores which were reported in 94 (44.98%), 103 (49.28%), 110 (52.63%), 89 (42.58%), 71 (33.97%) and 9 (4.31%) women respectively. Some of post-menopausal women also had complaint with more than one clinical signs and symptoms.

On investigation, 9.09% (19/209) post-menopausal women had *Candida* infection. The association of various socio-demographic features and *Candida* infection revealed, 73.68% of women with *Candida* infection were married compared to 95.26% in women without *Candida* infection ($P=0.001$). 47.37% of women with *Candida* infection had no formal education compared to 22.11% in women without *Candida* infection ($P=0.044$). Similarly, 63.16% of women with *Candida* infection were staying with more than 5 persons in home compared to 32.11% in women without *Candida* infection ($P=0.017$). There was no statistical significant difference was observed in other socio-demographic features between the women with and without *Candida* infection (Table 1)

There was a significant association of *Candida* infection with various clinical symptoms. Abnormal vaginal discharge was characterized in 68.42% of women with *Candida* infection compared to 42.63% of women without *Candida* infection ($P=0.031$). Similarly, women who had clinical symptoms like feeling of itching or burning of genitalia and/or lower back pain had significantly more *Candida* infection. The comparison of various clinical features among the postmenopausal women with and without *Candida* infection was presented in Table-2.

Discussion:

During the study period, 209 postmenopausal women were included in this study. The median age of the women was 54 years (range: 42 to 64 years). The prevalence of *Candida* infection in postmenopausal women in this study was 9.09% which was found to be consistent with others report (Spinillo et al., 1997; Goldacre et al., 1979; Tibaldi et al., 2009).

Women with *Candida* infection had been found to be presented with various clinical symptoms such as abnormal vaginal discharge, burning and itching of genitalia, and lower back pain. None of the previous studies have analyzed the association of these infections with clinical presentation of the post-menopausal women. Similarly, the prevalence of *Candida* infection was found to be significantly associated with marital status, educational level and number of individuals

living in the house in this study. The high prevalence of *Candida* infection in women with low educational level signified their lack of knowledge about sanitation, use of drugs and others behaviors pattern that affects the occurrence of *Candida*. Again the higher number of individuals living in a house may also impact the sanitation practices due to use of common toilet facilities as well as have the poor sanitation access. Previous studies have reported association of others factors such as sexual behaviors and use of probiotics which have found to be influence the vaginal microbiome in postmenopausal women (Taylor-Robinson et al., 2002; Heinemann et al., 2005; Muhleisen et al., 2016; Kim et al., 2017). As most of the women recruited in this study were unaware about the types and name of the drugs they used, the association of these factors on the occurrence of *Candida* cannot be avoided.

In conclusion, the occurrence of *Candida* infections in symptomatic post-menopausal women was found to be around 10% with varied clinical presentations. As the occurrence of only *Candida albican* has been analyzed in this study, the diagnosis of other *Candida* species and inclusion of asymptomatic post-menopausal women will be helpful for the better understanding of the associated clinical presentations as well as various risk factors in post-menopausal women.

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Declaration of conflicting interests: The Authors declare that there is no conflict of interest

Authorship statement

MN, PD and SS designed the study; MN and OP collected data and samples; MN, SS and AD performed the laboratory works; PP and PD conducted the analysis of the data; MN, PP, PD and SS wrote the manuscript; SS and PD and revised the paper. All the authors have approved the final version of the article.

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Table 1. Association of various socio-demographic features with prevalence of *Candida* infection in post-menopausal women. Data are expressed in Number (%) of Women

Variables	With <i>Candida</i> infection 19 (9.09%)	Without <i>Candida</i> infection 190 (90.91%)	<i>p</i> value
Age (%)			
≤55 years	10 (52.63)	110 (57.89)	0.658
>55 years	9 (47.37)	80 (42.11)	
Marital status (%)			
Married	14 (73.68)	181 (95.26)	0.001
Divorced/separated	5 (26.32)	9 (4.74)	
How many yrs have you been married			
<20 years	3 (15.79)	41 (21.58)	0.555
>20 years	16 (84.21)	149 (78.42)	
Religion			
Hindu	18 (94.74)	174 (91.58)	0.695
Muslim	0 (0)	7 (3.68)	
Christian	1 (5.26)	9 (4.74)	
Caste/Tribe			
Scheduled caste (SC)	6 (33.33)	44 (24.31)	0.668
Scheduled tribe (ST)	5 (27.78)	52 (28.73)	
Other backward caste (OBC)	6 (33.33)	58 (32.04)	
General	1 (5.56)	27 (14.92)	
Education			
No formal education	9 (47.37)	42 (22.11)	0.044
5th – 10th grade	5 (26.32)	89 (46.84)	
12th grade or higher	5 (26.32)	59 (31.05)	
Occupation			
Employed	2 (10.53)	16 (8.42)	0.399
Self-employed	2 (10.53)	46 (24.21)	
Housewife	15 (78.95)	128 (67.37)	
People live in house			
1-2 individuals	2 (10.53)	17 (8.95)	0.017
3-5 individuals	5 (26.32)	112 (58.95)	
>6 individuals	12 (63.16)	61 (32.11)	
Monthly Income			
< 5,000	7 (36.84)	56 (29.47)	0.526
5,000-10,000	4(21.05)	64 (33.68)	
>10,000	8 (42.11)	70 (36.84)	
Where Live			
Housing colony	2 (10.53)	36 (18.95)	0.115
Urban slum	1 (5.26)	39 (20.53)	
Rural/village	16 (84.21)	115 (60.53)	

Table 2. Association of various clinical symptoms with *Candida* infection in postmenopausal women. Data are expressed in Number (%) of Women

Clinical symptoms	With <i>Candida</i> infection 19 (9.09%)	Without <i>Candida</i> infection 190 (90.91%)	<i>p</i> value
Abnormal Vaginal Discharge			
No	6 (31.58)	109 (57.37)	0.031
Yes	13 (68.42)	81 (42.63)	
Burning/ itching in genitalia			
No	5 (26.32)	101 (53.16)	0.026
Yes	14 (73.68)	89 (46.84)	
Burning/itching during urination			
No	7 (36.84)	92 (48.42)	0.335
Yes	12 (63.16)	98 (51.58)	
Strawberry cervix			
No	11 (57.89)	109 (57.37)	0.965
Yes	8 (42.11)	81 (42.63)	
Genital sores			
No	19 (100.00)	181 (95.26)	0.332
Yes	0 (0)	9 (4.74)	
Lower back pain			
No	8 (42.11)	130 (68.42)	0.021
Yes	11 (57.89)	60 (31.58)	