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RESEARCH ARTICLE

Etiological trends in vaginal discharge syndrome in patients attending the regional centre for sexually transmitted infections in central India: 2017-2021

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ABSTRACT

Objectives: Vaginal discharge is one of the commonest symptoms in women attending the clinic for sexually transmitted infections (STIs). We have aimed to study the etiological trends of vaginal discharge syndrome (VDS) in patients attending the regional STI centre in central India. **Materials and methods:** Three viscose swabsticks were used to collect vaginal secretions, from 13933 patients of VDS between April 2017 to March 2021 at Regional STI centre, Govt. Medical College & Hospital, Nagpur. One swab stick was transported into 0.5 ml of sterile normal saline for gram staining and KOH preparation. The other two swabsticks were transported in Stuart's medium for culture. **Results:** Of 13933 cases of VDS, 3332(23.9%) patients had VVC followed by 2630 (18.9%) patientswith BV and 188(1.3%) patients were positive for TV. Chrome agar identified 5 species of candida. The commonest was *C. albicans* accounting for 844(25.3%) infections followed by *C. glabrata* in 317(9.5%) infections, and *C. parapsilosis* in 308(9.2%) infections. The least encountered was *C. krusei* in 91(2.8%) infections. Our data has shown that mixed vaginal infections were also encountered. Candidiasis was associated with BV in 157 (2.6%) patients, with TV in 6 (0.09%) patients, BV with TV in 8(0.13%) patients and candidiasis with both BV and TV in 6(0.09%) patients. **Conclusions:** We found vulvovaginal candidiasis was the commonest vaginal infection followed by BV and TV. Amongst the mixed vaginal infections,the occurrence in decreasing order of frequency were candidiasis with BV followed by candidiasis with TV,then BVwith TV and least was candidiasis with both BV and TV.

Keywords: Vaginal discharge syndrome, candidiasis, bacterial vaginosis, trichomonal vaginitis, mixed vaginal infections.

Normal vaginal discharge is clear and nonoffensive but becomes thicker and sticky during certain phases of the menstrual cycle, during and after sexual activity, during pregnancy and lactation [1]. In general, women pay attention to vaginal discharge only if they perceive it as abnormal, or if it causes itching or discomfort. In vaginal discharge syndrome (VDS), symptoms are abnormal vaginal discharge, vaginal itching, dysuria and dyspareunia. Abnormal discharge may have a different color, odor, consistency or an increased quantum. Three common etiological causes of VDS are vulvovaginal candidiasis (VVC), bacterial vaginosis and trichomoniasis.

VVC is an issue of global concern due to its association with sexually transmitted infections and ascending genital tract infections [2,3]. *Candida albicans* is the most common etiology of VVC followed by candida non albicans like *C. glabrata* and *C. tropicalis.* Bacterial vaginosis (BV) is a common vaginal dysbiosis among women in reproductive age group. It is also consistently associated with sexually transmitted infections (STIs), including HIV [4]. BV is associated with reduction in the normal protective vaginal microbiome and concurrent increase in vaginal anaerobic

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flora [5].

Trichomonas vaginalis (TV), an STI, afflicts an estimated 2.6 million cases in United States in 2018 [6]. Trichomoniasis might increase the risk of getting or spreading HIV infection. The World Health Organization (WHO) has estimated that this infection accounts for almost half of all curable sexually transmitted infections [7]. Trichomonal vaginitis is often under diagnosed and self-treatment by patients is also found to be more common. In many cases, the physicians often do not test the sexual partner and thus, the cycle of transmission goes on [8]. In trichomoniasis, clinical syndrome in females vary from asymptomatic presentations (more commonly) to vaginitis with copious discharge. We present our study on etiological trends in VDS patients attending the regional STI centre.

Materials and methods

Government Medical College and Hospital, Nagpur is a regional centre for the diagnosis, treatment, control, training and research for STI in central India. The centre is sponsored by Maharashtra States AIDS Control Society (MSACS), Mumbai. The Institute is running STI clinics in the Department of Dermatology and Venerology & Obstetrics and Gynaecology (OBGY). The RSTRRL (Regional STI Training, Research and Referral Laboratory) in the Department of Microbiology is receiving samples for etiological diagnosis of STI. The present study was in association with STI clinic at OBGY department only. From January 2017 to December 2021, a total of 13933 non duplicate vaginal swabs were received in RSTRRL, GMC, Nagpur.

Sample collection: Three viscose swab sticks were used to collect vaginal secretions from the lateral and posterior fornix of the vagina of each patient. One swab stick was transported in 0.5 ml of sterile normal saline for gram stain and KOH preparation. The other two swab sticks were transportedin Stuart's medium for culture.

Processing of the samples: Vaginal pH, wet mount, grams stain, Nugent's scoring and culture was done in the RSTRRL.

- One swab stick was used to prepare a 10% KOH preparation and smear for gram stain.
- Second swab stick was used for culture on Sabouraud's Dextrose agar (SDA, Hi media, Mumbai) for candida species.
- Third swab stick was put in Kupferberg medium (Hi media, Mumbai) for culture of *T. vaginalis.*

Identification of candida: KOH mounts of all samples were examined under a low power (10x) and high power (40x) magnifications for presence of typical yeast cells with fungal hyphal form. Colonies on SDA were identified as candida species by gram stain. Species identification was done on chrome agar (Hi media, Mumbai).

Diagnosis of bacterial vaginosis (BV): This was made by a positive Whiff's test (fishy odor on addition of 10% KOH), Ph > 4.5, presence of clue cells in wet mount and Nugent's score of 7-10 in gram stain. A score of 0-3 was considered as normal vaginal microbiome and a score of 4-6 was considered as transition towards BV.

Identification of *T. vaginalis: T. vaginalis* was identified as pear shaped trophozoite with jerky motility in smears from Kupferberg medium after incubation of 7 days [9].

Results

Out of 13933 patients of VDS, etiological agent could be determined in 6150 (44.1%) patients. In the remaining 7783(55.9%) patients no etiological agent was identified.

Table 1: Etiological diagnosis of vaginal discharge syndrome

No of samples	Infective VD n (%)			Non infective VD n (%)
13933	Candidiasis 3332 (23.9%)	BV 2630(18.9%)	TV 188(1.3%)	7783 (55.9%)
Total	6150 (44.1%)			7783(55.9%)

VD - Vaginal discharge, BV - Bacterial vaginosis, TV - Trichomonal vaginitis

Candidiasis was diagnosed in 3332(23.9%) patients, bacterial vaginosis in 2630(18.9%) patients and Trichomoniasis in 188(1.3%) patients (table 1). We found culture more sensitive than microscopy. Microscopy for candidiasis had missed 101(0.6%) patients while in trichomoniasis, microscopy had missed 9(0.3%) patients.

Table 2: Identification of Candida species on chrome agar :2017-2021

Total positive	*Candida albicans*	*Candida glabrata*	*Candida parapsilosis*	*Candida tropicalis*	*Candida krusei*	Unidentified Candida species
3332 (%)	844 (25.3)	317 (9.5)	308 (9.2)	122 (3.7)	91 (2.8)	1650 (49.5)

Table 3: Laboratory based diagnosis of bacterial vaginosis

Number of samples	Normal vaginal microbiome	Transition towards BV	BV
13933 (%)	11303 (81.1)	752 (5.4)	1878 (13.5)

Chrome agar identified 5 species of candida as shown in table 2. The commonest was *C. albicans* accounting for 844(25.3%) infections followed by *C.glabrata* in 317(9.5%) infections, and *C. parapsilosis* in 308(9.2%) infections. The least encountered was *C. krusei* in 91(2.8%) infection. In 49.5% patients candida infection was could not be speciated.

Bacterial vaginosis accounted for 1878(13.5%) infections as shown in table 3. A small proportion of patients (5.4%)

showed transition from normal vaginal flora to bacterial vaginosis.

Our data has shown that mixed vaginal infections were also encountered as shown in table 4. We found mixed vaginitis showing candidiasis with BV in 157(2.6%) patients, candidiasis with TV in 6 (0.09%) patients while BV with TV in 8(0.13%) patients and candidiasis with both BV and TV in 6(0.09%) patients.

Table 4: Mixed vaginal infections

Total infective	Candida+BV(%)	Candida+TV(%)	BV+TV(%)	Candida+BV+TV(%)
6150	157(2.6)	6(0.09)	8(0.13)	6(0.09)

BV - Bacterial vaginosis, TV - Trichomonal vaginitis

Discussion

Sexually transmitted infections are a major global burden for both developing and developed countries. The WHO developed syndromic treatment guidelines for curing symptomatic STIs on clinical evaluation without a laboratory diagnosis [10]. VVC is the most common mucosal infection of the female genital tract, caused by the opportunistic fungus *Candida albicans*, affecting the physical as well as mental health of women. A member of the normal vaginal microbiota, *C. albicans* generally inhabit the lumen of vagina asymptomatically. However, symptomatic infection can result from inflammation, soreness and pruritis of the vaginal mucosa.

We found the prevalence of VVC among the STI attendees to be 23.9%. The commonest species was *C. albicans* (25.3%). The least encountered was *C.krusei*. Candida causing vulvovaginitis is a major health problem due to unhygienic conditions and lack of attention to the vaginal discharge. Anandi et al [11] reported that the commonly isolated species of VDS were *Candida albicans* (43.66%). Rao et al [12] showed the prevalence of VVC among nonpregnant women attending a tertiary care teaching hospital in Karnataka, India to be 18.3%. Sasikala et al [13] reported isolation of *C. albicans* as 46.1% in a tertiary care teaching hospital in Karnataka. Waikhom *et al* [14] reported 30.7% total prevalence of vulvovaginal candidiasis, out of which nonalbicans candida (NAC) and *C. albicans* had a prevalence of 74.1 and 25.9%, respectively. Among the candida nonalbicans, *C. glabrata* was the most common species, followed by *C. albicans, C. krusei*, and *C. parapsilosis*. Vos T et al [15] have documented that recurrent VVC probably affects more than 130 million women, with a global annual prevalence of 3871 per 1000000 females. Vaginal candidiasis indirectly causes the reduction of normal vaginal microbiota (lactobacilli) and alters the vaginal pH that facilitates the initiation of BV and the transmission of STIs [16].

Bacterial vaginosis (BV) is second most common vaginal infection among women of reproductive age, causing physical and psychosocial discomfort. BV is associated with an increased risk of adverse outcomes including preterm birth, pelvic inflammatory disease (PID), endometritis, and transmission of human immunodeficiency virus type 1 (HIV-1) and other sexually transmitted infections (STIs) [17]. We found the prevalence of BV in STI clinic attendees to be 18.9% which included 5.4% (Intermediate by Nugent's score) and 13.5% (BV by Nugent's score). Djomand et al [18] reported a prevalence of 47.8% of BV in HIV infected women in Kenya. In a study conducted by Chen et al [19] reported prevalence of BV as 42.1% among women in sub-Saharan Africa while Raghubanshi et al [20] found the prevalence of BV as 24.4% in India. Vaca et al [21] have documented that the occurrence of BV is common in sexually inactive females. This proves that sexual activity is not a prerequisite for BV. Yen S et al [22] stated that the lack of attention towards vaginal discharge and menstrual hygiene, unhealthy diet, smoking and alcohol intake might alters the vaginal microbiome thereby causing bacterial vaginosis in unmarried women. BV varies in prevalence with respect to STI which are associated with HIV, and IUD users. Ekanem et al [23], Patel et al [24] and Vanechoutte et al [25] discussed the manifestation of many STI which gets altered during pregnancy that affects maternal immune competence, thereby increasing vaginal pH. Alteration in vaginal flora increases their susceptibility for bacterial and yeast infections.

The laboratory diagnosis of *Trichomonas vaginalis* infection by wet mount preparation is simple, rapid yet cost effective method in contrast to culture. In our study low prevalence of trichomonas infection (1.33%) was observed among the STI attendees. McClelland et al [26] have documented that with most STIs, *T. vaginalis* infection is largely associated with an increased risk in human immunodeficiency virus (HIV) infection. Singh et al [27] detected 1.96% prevalence of *T. vaginalis* in women presenting with vaginal discharge in Gynaecological Out Patient Department (GOPD) of tertiary care hospital. Madhivanan P et al [28] observed 8.5% cases of trichomoniasis, while Rowley et al [29] reported 0.6% cases. These reports are comparable to our findings. Flagg et al [30] have reported that

TV infection among women aged 15 to 59 years was higher than those aged 15 to 24 years. Fan et al [31] studied that mixed vaginitis is the simultaneous presence of minimum of two types of vaginitis resulting in the abnormal vaginal milieu. The clinical presentation of mixed vaginitis may be variable. Consequently, simply diagnosing the types of vaginitis does not demonstrate a cause–effect relationship with clinical symptoms and signs. Sober et al [32] analysed mixed vaginitis overall which included desquamative inflammatory vaginitis (DIV) or aerobic vaginitis (AV), vulvovaginal candidiasis (VVC), bacterial vaginosis (BV), trichomonas vaginalis (TV) infections and cytolytic vaginitis (CV). The concept of mixed vaginitis has escaped clinical scrutiny and definition. The pathogenic mechanism of mixed vaginitis remains understudied and the association between the signs and symptoms have not been clearly elucidated. Dai et al [33] discussed that the diagnostic criteria to determine the mixed vaginitis includes physical examination, microscopic examination, and culture methods, which are usually performed in hospitals and STI laboratories. Nyirjesy et al [34] reported that mixed vaginitis may be hard to eradicate, and recurrence is frequent. This is due to the diverse behaviour of the pathogenic vaginal microbiome which affects the immune response of the host. We found mixed vaginal infection on a very low scale. In contrast Wang et al [35] found a relatively high rate of mixed vaginitis (35.06%) in Shanghai.

In our study, in 55.9% patients, no infective cause could be determined. Noninfectious causes of VDS are well known like atrophic vaginitis, foreign body, malignancy and chemical irritants.

Conclusion

From our study we found vulvovaginal candidiasis was the commonest vaginal infection followed by BV and TV. Amongst the mixed vaginal infections,the occurrence in decreasing order of frequency were candidiasis with BV followed by candidiasis with TV, then BV with TV and least was candidiasis with both BV and TV. Standard treatments should be chosen to minimize the abuse of unnecessary antimicrobials for which definitive laboratory diagnosis is important.

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