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Original Research Article

Clinicomicrobiological profile of abnormal vaginal discharge in sexually active females of reproductive age group in a tertiary care hospital of Southern Odisha

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Abstract

Introduction: Vaginal discharge is the most frequent complain of the females of reproductive age group attending Gynaecology & STI OPDs. Even though it can be physiological or pathological in origin, its clinical presentation varies amongst different aetiological agents.
Aims & Objectives: 1. To determine the prevalence of abnormal vaginal discharge in sexually active females attending the tertiary care hospital, 2. To isolate & identify the causative pathogens and perform their antimicrobial susceptibility tests.
Materials and Methods: A prospective study was carried out in the Department of Microbiology, MKCG MCH, Berhampur on 210 sexually active females of reproductive age group attending outpatient departments (OPDs) of Obstetrics & Gynaecology and Dermatology, presenting with abnormal vaginal discharge. Clinicodemographic datas were obtained and samples were collected with aseptic procedures. Various aetiological agents were identified based on the presentation, macroscopic, microscopic examination and culture of the samples as per the standard procedures. Antibiotic susceptibility of the bacterial and fungal isolates were performed as per the CLSI guidelines.
Results: Out of 210 clinically diagnosed STI cases with abnormal vaginal discharge, 44.2% were from age group 26-35 years. Candida spp was the most predominant pathogen isolated (31.43%). Staphylococcus aureus was the most common Gram positive bacterial pathogen to be isolated (8.09%) while E. coli was the prevalent Gram negative bacilli (2.85%).
Conclusion: There has been upsurge of drug resistance candidiasis & bacterial vaginosis in reproductive tract infection associated with abnormal vaginal discharge in sexually active females. Early diagnosis and management of such cases by clinico-demographic studies and laboratory tests can help in reducing the occurrence.

Keywords: RTI, Abnormal Vaginal discharge, Candidiasis, Bacterial vaginosis.

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1. Introduction

Reproductive health plays a crucial part on a women's mental, physical and reproductive health which can adversely affect her family life and socioeconomic development of the society.[1,2] In India, reproductive age group females constitute 22.2% of the total population.[3] Vaginal discharge is a frequent complaint among women attending sexually transmissible infections (STIs) clinics. Physiological or pathological factors attribute to it. Pathological causes can be of infectious or non-infectious origin. The most common physiological aetiology of vaginal discharge is Bacterial vaginosis (BV), while pathological aetiological agents comprises Candida species, Neisseria gonorrhoeae (3.7%– 27.77%), herpes simplex (7.9%– 14.6%), and Mycoplasma. The reproductive hygiene of females varies according to various socio-cultural factors, their literacy rate and awareness. As the prevalence of various etiological agents defers from community to community, it is imperative to focus on what preventive measures to be taken to improve public awareness about the health care services and their sexual and contraceptive practices.[4]

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Health education regarding proper personal hygiene, menstrual hygiene, sexual practices are the important component of STI prevention. Hence, reproductive health of women is an issue that warrants high priority in developing country like India.[2]

Not much studies identifies the regional variation in the presentation of vaginal discharge amongst sexually active females of reproductive age group. Hence this study will depict the prevalence of abnormal vaginal discharge in sexually active females and the aetiological agents associated with it.

2. Materials and Methods

2.1. Place of study & duration

A collaboration between Department of Microbiology, MKCG Medical College Hospital, Berhampur with Department of Obstetrics & Gynaecology and Department of Dermatology was done. A prospective study was conducted from August 2015 to July 2017.

2.2. Study population

A total of 210 sexually active female patients within age group 15 to 45(child bearing age group) attending outpatient department (OPD) of Obstetrics & Gynaecology and Dermatology participated in this study. Patients with complaints of abnormal foul smelling vaginal discharge, itching, lower abdominal pain, burning micturition, pain during sexual intercourse were included in the study group.[1]

A detailed socio demographic, personal, gynaecological history of each patient and their high risk partners in form of a semi-structured questionnaires was taken.[1,2] This study was approved by institute's research and ethical committees. Before collecting samples, informed written consent was recorded from each patients.

Statically analysis of the data collected was done by SPSS software version 26 (IBM corporation, New York, USA) software.

2.3. Exclusion criteria

Any patient with pregnancy, on antimicrobial therapy, menstruating female, patients who had undergone hysterectomy, patients with carcinoma cervix, uterine fibroids and uterine prolapse were debarred from the study.[2,5]

2.4. Collection of specimens

Samples were collected using ready-made cotton swab stick of 22cm.[6] Three high vaginal swabs or endo cervical swab were collected after labelling patient details on adhesive tape of cotton swab stick. The high vaginal swabs were inserted into upper part of vagina, rotated to collect exudates both from upper and lower vaginal wall. The endocervical swab was collected by inserting one to two cm into endocervical canal, rotated for 10-30 seconds, and then retracted as such without touching the vaginal wall. The swabs were transported in stuart' s transport media to microbiology laboratory. From first, second, third swab normal saline mount, gram staining and culture was done respectively.[7] Specimen transported at room temperature were processed immediately otherwise stored at 4^0C, if there was delay in processing for up to 48 hours.

2.5. Specimen examination

1. Macroscopic examination
   a. Observation of sample: Specific colour and odour of samples were observed. Curd like white discharge indicates Candidiasis. In cases of trichomoniasis there is greenish frothy discharge. Foul smelling discharge is characteristic of bacterial vaginosis.
   b. pH of vaginal discharge: Vaginal discharge pH was recorded using standard pH indicator paper. Vaginal pH more than 4.5 was diagnostic feature of bacterial vaginosis.
   c. Amine test/whiff test: Few drops of 10% KOH (potassium hydroxide) solution was added directly over the collected swab. Presence of amine like odour was diagnostic of bacterial vaginosis.
2. Microscopic examination
   a. Wet mount: In normal saline mount examination, motile trophozoites of Trichomonas vaginalis, pus cell and budding yeast cells were observed.[8]
   b. Gram's staining: A smear was made from second swab over a clean glass slide and stained using Gram´s staining technique. The smear was examined in 100X for presence of clue cells, Gram negative intracellular diplococci, budding yeast cells, polymorphs and Trichomonas vaginalis. Clue cells are squamous epithelial cells covered with small Gram variable bacilli as described by Gardner & Dukes (1955).[9] Clinico-microbiological diagnosis of bacterial vaginosis was made on the basis of presence of any three among the four criteria described by Amsel et al (1984). Scoring of vaginal flora pattern was done according to scoring system of Nugent RP et al, 1991.
3. Microbiological Culture
   a. Culture of aerobic bacteria: Culture of bacteria was done by streaking third swab on blood agar, MacConkey agar, and chocolate agar. The plates were examined for any growth of microorganism after 24 hours of incubation at 37^0c and re-incubated in case of no growth for another 24hrs. The blood agar and chocolate agar plates were placed in candle jar and then incubated.[7] Standard protocol was followed for identification of isolated organism by gram staining, morphology, cultural and biochemical characteristics.
   b. Culture of fungus: Fungal culture was done by inoculating third swab on Sabouraud ´s dextrose agar (SDA) media and incubating in BOD incubator

at 25^0c and 37^0c. Culture was confirmed by studying colony morphology, growth on CHROMagar, Gram stain and formation of germ tube.[7]

2.5.1. Germ tube test

Colonies of yeast was inoculated into 0.5 ml of human pooled sera and incubated for 2hrs at 37^0c. After 2hrs, one to two drops of suspension was added on glass slide, covered with cover slip and was observed for germtube formation.

*2.6. Fungal culture & Identification (**Figure 1**)*

2.6.1. Antimicrobial susceptibility testing

As per CLSI guidelines, antibiotic susceptibility testing was performed using Kirby Bauer disc diffusion method. Further antifungal susceptibility test was done for the candida isolates by Kirby Bauer disc diffusion method using commercially available HiMedia discs (clotrimazole, fluconazole, itraconazole, amphotericin B, voriconazole) as per the CLSI guidelines.

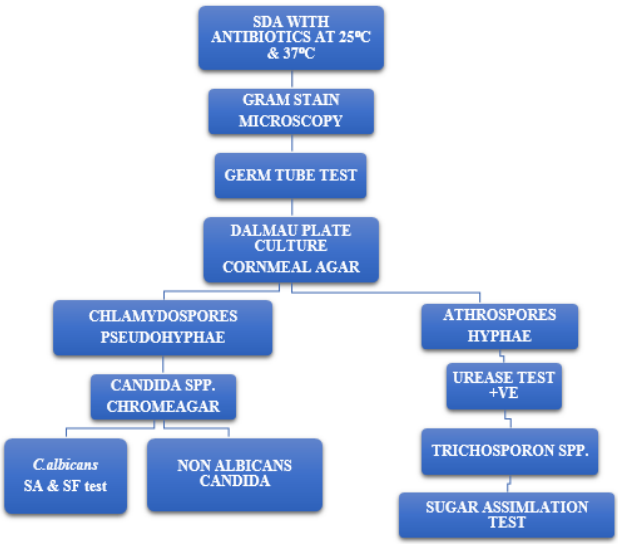

Figure 1: Fungal Culture & Identification

3. Results

210 cases of RTI with symptoms of abnormal vaginal discharge were included in this study. Maximum of these cases were between 26 to 35 age group (44.2 %) and were found to be higher in rural areas i.e 61% than urban areas. Comparing the marital status of these patients, higher prevalence was seen among married women i.e 46.2%.Out of 210 clinically diagnosed RTI cases with abnormal vaginal discharge, maximum number of women were educated up to primary school and most of the cases were housewife followed by daily wage worker and the prevalence is quite low among service women. . These manifestation was more prevalent among multigravida women than primigravida and nulliparous women and was higher among women who have undergone medical procedure like abortion. RTI with abnormal vaginal discharge was most prevalent among women using IUDs and least among those using condom (**Table 1**). However most patient with vaginal discharge are associated with pruritus, dysuria, dyspareunia and low abdomen pain. (**Table 2**). Most cases of clinically diagnosed RTI patient presented with curdy white discharge followed by greyish discharge with fishy smell. (**Table 3**). The diagnosis of bacterial vaginosis depend on replacement of predominantly lactobacilli flora by a mixture of aerobic and anaerobic organism, shift of pH to alkaline and presence of clue cells which were squamous epithelial cells studded with large number of gram variable coccobacilli(G.vaginalis).[7]

The presence of polymorphonuclear cells embedded with gram negative diplococci (ICDC) was considered as diagnostic of gonorrhoea which was confirmed by culture on chocolate agar.[7] The presence of 5 or more pus cells in the absence of Gram negative diplococci was considered as presumptive diagnosis of nongonococcal cervicitis (NGC).[8]

Out of 210 clinical diagnosed RTI cases with abnormal vaginal discharge, gram stain revealed budding yeast cell in 66(31.4%), clue cells in 49(23.3%),gram positive cocci in 22(10.4%),gram negative rods in 18(8.57%), gram negative intracellular diplococci in 5(2.3%) cases (**Table 4**). Out of 210 clinical diagnosed RTI cases, Candida spp was the most predominant pathogen isolated (31.43%).Staphylococcus aureus was the most common Gram positive bacterial pathogen to be isolated on culture (8.09%) while E. coli was the common Gram negative bacilli (2.85%) followed by Neisseria gonorrhoea (2.38%) (**Table 5**, **Table 6**).

Ampicillin was the most susceptible drug followed by cefepime for gram positive cocci (**Table 7**), while Gentamycin was the most sensitive drug followed by Ceftriaxone for gram negative organisms (**Table 8**). Clotrimazole was found to be the most effective drug among the antifungals (**Table 9**).

Table 1: Sociodemographic and associated risk factors associated with Vaginal discharge (n=210)

Risk factors	Cases	Percentage (%)
1.Age		
15-25	46	21.9
26-35	93	44.2
36-45	71	33.8
2.Residence		
Urban	82	39

Rural	128	61
3.Marital Status		
Unmarried	46	21.9
Married	97	46.2
Divorced/widow/ separated	67	31.9
4. Educational Status		
Illiterate	52	24.76
Primary School	66	31.43
High School	40	19.05
Higher Secondary School	35	16.67
Degree	17	8.09
5. Occupation		
Service	21	10
Business	13	6.1
Daily wage worker	43	20.4
Housewife	92	43.8
Student	41	19.5
6. Obstetrics history **a) Parity**		
Nulliparous	34	16.2
Primigravidae	59	28.1
Multigravidae	117	55.7
b) History of abortion		
No abortion	98	46.6
Had abortion	112	53.4
7. Contraceptive Status		
None	46	21.9
Oral Contraceptive pills	51	24.2
Copper T	92	43.8
Condom	9	4.2
Tubal ligation	12	5.7

Table 2: Clinical presentation of study group

Clinical feature	**Cases (n=210)**	**Percentage (%)**
Vaginal discharge (VD) only	80	38.1%
VD + Pruritus + Dysuria	55	26.19%
VD + Pruritus + Low abdominal pain	52	24.76%
VD + Dysuria +Dyspareunia	23	10.95%

Table 3: Macroscopic examination of samples (Vaginal Discharge)

Colour of discharge	**Cases(n=210)**	**Percentage%**
Curdy white discharge	119	56.7
Greenish / frothy discharge	13	6.2
Gray colour discharge with fishy smell	72	34.2%
Mucopurulent discharge	6	2.8%

Table 4: Microscopic examination of samples

Wet Mount	**Cases(n=210)**	**Percentage%**
Motile trophozoite of *Trichomonas vaginalis*	5	2.3%
Gram Stain Bacteria		
Clue cells	49	23.3%
Intracellular diplococcic (ICDC)	5	2.3%
>5 PUS cells & No ICDC(NGC)	27	2.8%

GPC	22	10.4%
GNR	18	8.7%
GPR(Lactobacilli)	41	19.5%
Fungus		
a. Gram positive budding yeast cells	66	31.4%
b. Hyphae with athrospores	3	1.4%

Table 5: Bacterial isolates (n=43)

Bacteria	**Cases**	**Percentage%**
GPC		
Staphylococcus aureus	13	30.23%
Streptococcus spp.	3	6.97%
Enterococcus spp.	4	9.30%
GNR		
E.coli	7	16.28%
Klebsiella spp.	4	9.30%
Psuedomonas aeruginosa	3	6.97%
Acinetobacter spp	2	4.65%
Citrobacter spp.	2	4.65%
Neisseria gonorrhoea	5	11.64 %

Table 6: Fungal Isolates (n=69)

Fungus	**Number**	**Percentage%**
Candida spp 66		
Candida albicans	42	60.87
Candida glabrata	24	34.78
Trichosporon asahii	3	4.35

Table 7: Antimicrobial Susceptibility test for Gram Positive Cocci (n=30)

Bacteria	**Erythromycin**		**Ampicillin**		**Clindamycin**		**Cefepime**		**Linezolid**		**Vancomycin**	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Staphylococcus aureus (n =17)	10	7	13	4	9	8	11	6	10	7	9	8
Gr.B *Streptococcus* (n =7)	4	3	5	2	4	3	4	3	3	4	3	4
Enterococcus spp (n =6)	3	3	4	2	3	3	4	2	3	3	4	2
Total (30)	17	13	22	8	16	14	19	11	16	14	16	14

Table 8: Antimicrobial Susceptibility of Gram Negative Bacteria

Bacteria	**Ampicillin**		**Gentamycin**		**Cefepime**		**Ceftriaxone**		**Ciprofloxacin**		**Tetracycline**	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Escherichia coli (n=6)	2	4	4	2	3	3	4	2	4	2	3	3
Klebsiella spp. (n=5)	2	3	4	1	3	2	3	2	3	2	2	3
Pseudomonas aeruginosa (n=3)	1	2	2	1	1	2	2	1	1	2	2	1
Acinetobacter spp (n=2)	1	1	1	1	2	0	2	0	1	1	1	1
Citrobacter spp (n=2)	1	1	1	1	1	1	2	0	1	1	0	2
Neisseria gonorrhoea (n=5)	2	3	3	2	3	2	3	2	1	4	3	2
Total(23)	9	13	15	8	13	10	16	7	11	12	11	12

Table 9: Antifungal susceptibility test

Fungus isolated	Clotrimazole		Itraconazole		Fluconazole		Ketoconazole		Amphotericin	
	S	R	S	R	S	R	S	R	S	R
Candida spp (n=73)	51	22	38	35	41	32	33	40	32	41
Trichosporon spp(n=3)	3	0	2	1	2	1	2	1	1	2

4. Discussion

In our study, women of age group 26-35 years mostly presented with abnormal vaginal discharge accounting for (44.2%)(**Table 1**) as most women have highest sexual and reproductive activity during this period. This finding is similar to Kosambiya et al. &Sharma et al study.[10,11]

In a multicentric study conducted by Latha et al.(1997) in rural West Bengal, Gujarat, Baroda and Mumbai found RTI prevalence ranging from 19 – 71%.[12] Similar finding was observed in our study. Crucial factors attributing to increased prevalence of RTI cases in rural areas being use of cloth/ homemade napkins during menstrual cycle, non-usage of sanitary toilets, illiteracy, poor menstrual hygiene, unawareness about reproductive health, health care services available, rural women being unexposed to social media and communication. This finding was discordant with study of Kosambiya et al. in Surat in which higher RTI /STI cases was seen in urban areas. This was due to higher migratory population inhabiting the urban areas of Surat.[10]

Abnormal vaginal discharge was found to be associated with married women as they are more sexually active, have higher risk of acquiring RTIs.[13] In this present study, mostly married women presented with abnormal vaginal discharge (46.1%) than widow /separated/divorced/unmarried women. Thus increase tenure of married life predisposes them to higher risk of RTIs.[14] The prevalence of RTI with abnormal vaginal discharge was low among widow /separated/ divorced/unmarried women due to orthodox nature of society while the cause of abnormal vaginal discharge in unmarried women /students being lack of personal hygienic practices during reproductive age group which fascilitates endogenous infections.[15] In recent times urbanization, education, separation from the influence of family had led the younger generation to engage in high risk behaviours like drug abuse, sexual activity, frequent changing of partners resulting in increased incidence of abnormal vaginal discharge.[2]

Education plays crucial role in enlightening women about their reproductive health. In this study, prevalence of RTI cases was inversely proprtional to level of education being highest among women educated upto high school (31.4%), lowest among women with degree (8.1%).This finding matches with Sreelatha CY et al. Study in which illiterates have higher prevalence (40.9%) than women with degree (20.8%).[16] This was because of poor knowledge of reproductive health and sexual needs & low health seeking behaviour among illiterate women.

In this study, RTI cases was highest among housewives (43.8%) followed by daily wage workers (20.4%) and lowest among service women (10%). This is probably due to lack of awareness about proper personal and menstrual hygiene, poor living condition, illiteracy, low socioeconomic status, psychological distress, fear of rejection and social stigmas associated with RTIs.[2] Moreover housewives are economically dependent on their life partners, have little freedom about their lives, reproductive health and fertility.[1] While study conducted by Jasmin Helen Prasad et al. in South India and J.Bogaerts et al. in Dhaka showed that STI among women with husband having no permanent place of occupation were 1.6 times higher, as migratory occupation, makes them more vulnerable to have multiple sexual partners thereby increasing the chance of contracting STIs.[17,18]

In this study, multigravida (45.7%) have more prone to RTIs than primigravida and nulliparous women. The cause may be due to multigravida have increased number of deliveries, exposed to more contraceptive devices, gynaecological surgeries.[13] This study was in accordance to Rathore et al. where multigravida have 28.5% RTIs and primigravida and nulliparous women have 13%, 2.4% respectively.[19] Maximum symptomatic RTI/STI cases were among women who had undergone abortion (53.4%) because of iatrogenic introduction of microorganism into the reproductive tract during the medical procedure. This finding is supported by Verma et al. who observed the highest prevalence (56%) in those who had a history of abortion and lowest (35%) in those with no risk factors of abortion.[20]

There was significant association between contraceptive status of the females and abnormal vaginal discharge. In this study the prevalence of RTI with abnormal vaginal discharge was highest among women using Copper T (43.8%) and lowest among those using condoms (4.2%). This confirms the fact that contraceptive methods like condom have protective role in prevention of RTI/STI.[2] This coincides with finding by Prasad et al. where women using IUCD have increased RTIs (67%).[14] Women using Copper T have increased prevalence due to improper sterilization practice during IUCD insertion, lack of follow up care after insertion and the string of IUD facilitating access of pathogen to upper genital tract.

In this study most RTI patients presented with vaginal discharge along with were also associated with low abdominal pain, pruritus, burning micturation, dyspareunia (**Table 2**). This coincides with study conducted by Nandan et al. in Uttar Pradesh where mostly women (53.4%) presented with abnormal vaginal discharge.[21] Study of Patel et al.,[22]

Samanta et al., Kosambia et al.[10] and Acharya et al., in different parts of rural India showed most common symptom of RTI patients was vaginal discharge.[2]

The common presentation in these patients was curdy white vaginal discharge (43.8%) followed by gray colour homogenous discharge with fishy odour (31.4%) (**Table 3**). This was in accordance to study conducted by Sreelatha et al. in rural areas of Karnataka in which curdy white vaginal discharge was the most common symptom (63.2%).[16] This observation was contrary to study conducted by Masand DL et al. in which homogenous gray discharge was the most common symptom (52%) followed by mucopurulent discharge in 23% of women.[23]

The gram staining finding in present study reveals gram positive budding yeast cell (31.4%), clue cells (23.3%), gram positive cocci (10.4%), gram negative rods (8.7%), gram negative intracellular diplococci (2.3%) and hyphae with athrospores (1.4%). In normal saline mount, (2.3%) motile trophozoite of Trichomonas vaginalis was observed (**Table 4**).

In present study culture finding reveals Candida spp. (31.4%), *Staphylococcus aureus* (6.1%), *Escherichia coli* (5.2%), Klebsiella spp. (3.3%), gram negative intracellular diplococci (2.3%), *Enterococcus* spp. (1.9%), Group B β haemolytic *Streptococcus* (1.4%), *Trichosporon asahii* (1.4%). (**Table 5**).

Candida spp. was the most prevalent isolate from abnormal vaginal discharge (31.4%) followed by bacterial pathogens (20%). Our study finding was similar to Murugesan M et al. in which Candida spp. accounted for (35.42%)24 followed by Escherichia coli (20.8%).[24] This study was also in accordance with observation made by Parikh et al. and Ranjan et al. where majority of clinically diagnosed RTI cases with abnormal vaginal discharge were having candidiasis accounting for 17% and 26.3% respectively.[25,26]

In cases of gram positive cocci Ampicillin was the drug of choice followed by Cefepime (**Table 6**). Amongst gram negative bacteria Gentamycin was the most susceptible antibiotic followed by Ceftriaxone (**Table 7**). In antifungal susceptibility testing, most of the fungal isolates were sensitive to Clotrimazole followed by Fluconazole (**Table 8**).

5. Conclusion

Vaginal discharge plays an important role in assessing the reproductive health of females. There has been rise in prevalence of candidiasis associated with drug resistance in females presenting with abnormal vaginal discharge. Hence, proper evaluation of abnormal vaginal discharge with respect to presentation, characteristic features and appropriate laboratory diagnosis can aid in early diagnosis and treatment and prepare health strategies to reduce its occurrence.

6. Source of Funding

None.

7. Conflict of Interest

None.

Reference

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