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STUDY OF VAGINAL MICROBIOLOGICAL FLORA IN THE PATIENTS COMPLAINING OF WHITE DISCHARGE IN TERTIARY CARE CENTRE

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ABSTARCT

Background:

Aims: The objective of study is to Study the Microbiological flora of lower female genital tract in patients complaining of Vaginal discharge.

Materials and methods: A total of 100 women of reproductive age group with the complaints of vaginal discharge were selected for this study at random after applying the criteria mentioned above. With the prior consent, a comprehensive history, general examination, and gynecological examination were initially carried out. After making a clinical diagnosis, a sterilized sims speculum was inserted into the vagina to visualize the vagina and cervix.

Results: Bacterial Vaginosis was found in high frequency in the age group of 31-40 years (58.6%) .The most common age group affected by vaginal candidiasis was 20-30(44.8%) .Trichomoniasis was detected in the highest rate at the age group of 31-40years (60%).Normal flora was found seen in high frequency in the age group of >50 (33.3%) followed by 31-40(26.6%). Mixed infection of *Candida* and BV was seen in mainly in the age group 20-30 and 31-40 (40%) followed by 41-50 (20%). 85 (85%) cases were organism positive and 15(15%) cases showed normal Flora. Among 100 patients, BV (46%) was the most common microbiological cause of abnormal vaginal discharge, followed by vaginal candidiasis (29%), trichomoniasis (5%), combined infection (*Candida* and BV) (5%). Characteristics of vaginal discharge of patients differed according to pathogenic agents. The pH of vaginal secretions was >5 in a large proportion of examined cases which included women with BV and TV, but women with *Candida* infection, the pH was slightly below the normal value. Whiff amine test was positive in most of the cases, most of them having BV. Vaginal swab for culture and sensitivity showed prevalence of micro-organism associated mainly with BV.

ISSN 2515-8260 Volume 10, Issue 01, 2023

Conclusion: Clinicians need to be aware of emerging epidemiological data, the different presentations of vaginal discharge, and the approach of their management so that the symptom can be treated according to its etiology.
Keywords:

INTRODUCTION

The female genital tract (FGT) provides a satisfactory environment for many pathogenic microorganisms and multiple infections are therefore common. It is colonized by a variety of species of commensal bacteria causing no harm except under abnormal conditions. At birth, vagina of the newborn is colonized initially by anaerobic and aerobic bacteria acquired during passage through birth canal. The epithelium of the vagina at this time is rich in glycogen as a result of the influence of placental and maternal estrogens. This resulted in a low pH (3.7-6.3). Several weeks after birth the epithelium becomes thin, atrophic and largely devoid of glycogen, the pH rises to 6-8 (remaining so until puberty) and the predominant flora are Gram-positive cocci and bacilli . At puberty the estrogen increases the proliferation of vaginal epithelial cells and glycogen deposit in it. .Lactobacillus proliferates and cause enzymatic breakdown of cellular glycogen, resulting in lactic acid and H2O2 which lower the pH to 3.5-4.5. This indicates normal vaginal environment, which inhibits the growth of pathogenic organisms. [1,2]

After the menopause, lactobacilli again diminish in numbers and mixed flora return. The normal vaginal flora often includes group βhemolytic Streptococci, anaerobic Streptococci (Peptostreptococci), Bacteroids species, Clostridia, Gardnerella vaginalis, Ureaplasma urealyticum, and sometimes Listeria or Mobiluncus species but the cervical canal and fallopian tubes are sterile. Vaginal discharge may be physiological or pathological. Physiological discharge comprises secretions of the Bartholin's gland and the endocervix with cells shed from the vaginal walls. These secretions are affected by hormonal changes during the menstrual cycle. Cervical ectropions, the intra uterine contraceptive device and the combined oral contraceptive may increase physiological discharge. There is a natural increase in vaginal discharge at the time of puberty, ovulation, premenstrual and during pregnancy, this consider under heading of leucorrhoea. The pre-pubertal and postmenopausal vagina, as they are not well estrogenised is more prone to infection.3

Pathological discharge in women of reproductive age, is usually caused by infection and causative organisms may or may not be sexually transmitted. Pathological discharges are due to infections, usually vaginitis (Candida albicans, Trichomonas vaginilis and anaerobic vaginosis) or cervicitis (Chlamydia trachomatis, Neisseria gonorrhoeae and Herpes simplex). Pathological discharge may also due to noninfective disease such as genital tract tumors or fistula and chemical vaginitis as result of the use of perfumed soaps, bath additives, spermicides or antiseptic douche and foreign bodies in vagina (Hudson et al.,1998). Vaginal discharge is a common, but neglected, health problem in women in their reproductive age. It is therefore important to know the exact prevalence of vaginal discharge in women with genital tract infections. The present study carried out to find the characteristics of vaginal

discharge, prevalence of pathogenic agents causing vaginal discharge and the prevalence of pus cells in relation to pathogenic agents. [4]

Patient medical history should include all of the usual gynecologic parameters, including menstrual history, pregnancies, contraception, sexual preference(sexual orientation),past and present sexual relationships, and prior genitourinary infections, underlying medical conditions such as allergies, diabetes, malignancies, and immunodeficiency syndrome (primarily human immunodeficiency virus (HIV) disease) that might be associated with vulvovaginal disease.

MATERIALS AND METHODS

Main source of data for this study are patients who comes with complaint's of white discharge to Gyneac op, Mother and child health care, GGH ,Nalgonda.

Study will be conducted from November 2021 to october 2022 on patients complaining of white discharge in Mother and child health care, GGH ,Nalgonda. Detail information will be taken regarding patient's history, symptoms, other relevant clinical findings and patient's management. Per vaginal Examination is done to know the type of vaginal Discharge and collect samples to study the type of organism under Microscopy. Discharge was collected as per the standard guidelines

Inclusion Criteria : All women > 20 years of age with whitish discharge per vaginum.

Exclusion Criteria: Patients with bleeding per vagina, on treatment with antibiotics -local and systemic and Patients who are pregnant.

A total of 100 women of reproductive age group with the complaints of vaginal discharge were selected for this study at random after applying the criteria mentioned above. With the prior consent, a comprehensive history, general examination, and gynecological examination were initially carried out. After making a clinical diagnosis, a sterilized sims speculum was inserted into the vagina to visualize the vagina and cervix. The amount, color, character, and smell of the vaginal discharge were noted. Vaginal discharge will be collected from the posterior fornix and lateral fornix with three cotton swabs. Speculum will be introduced into vagina without lubricating with antibacterial agent containing cream.

The pH was measured using litmus papers ranging from 2 to 10 by directly dipping pH strip in vagina. Color change was observed and matched against the indicator. One swab was used for making wet mount to look for the motility of TV. The second swab was used for making smears for Gram staining to find out clue cells, The third swab was used to do potassium hydroxide (KOH) mount to look for *Candida*.

For Bacterial Vaginosis:

1. Whiffamine test was done as follows–one or two drops of vaginal discharge were taken on a clean glass slide, and one or two drops of freshly prepared 10% KOH solution were added to it. Both were mixed and smelt immediately.
2. Wet film was examined for the presence of clue cells which are vaginal epithelial cells with a granular surface and blurred margins because of attached bacteria
3. Gram stained smears were examined for the presence of altered vaginal flora in form of Gram-negative coccobacilli studding vaginal epithelial cells instead of normally predominant Gram-positive *Lactobacilli*.

For Candida:

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1. KOHpreparation:adropof10%KOHwasaddedtothevaginalsecretions taken on a clean glass slide and mounted with a coverslip. *Candida* was identified as round or oval budding yeast cells.
2. Gram-stained vaginal smears were examined which showed Gram-positive budding yeast cells with pseudohyphae.
3. Cultures on Sabouraud's dextrose agar (SDA)medium showed a growth of creamy, grayish moist colonies.

Wet mount preparation was immediately made – a drop of discharge was mixed with a drop of normal saline on a clean slide and topped with a coverslip. This was then examined under microscope for flagellate organisms with characteristic motility.

CLINICAL EVALUATION

A detailed history was taken regarding age, parity, medical history, history of vaginal symptoms like vaginal discharge, malodour, obstetric history of previous preterm deliveries and abortions. Baseline parameters like pulse, BP, temperature will be recorded.

After history, general examination done to rule out other symptoms of systemic morbities. Later, Local examination is done which includes

Per abdominal examination which rules out mass per abdomen and other local abnormalities. Per speculum examination is done which shows status of cervix and vagina whether normal or congested or hypertrophied cervix , Vaginal normal or Pale or discharge or congested. Per speculum examination is followed by Per vaginal examination which gives the information about version of uterus, its size, Fornices , laternal wall and any tenderness.

RESULTS:

In this study of 100 cases who presented with abnormal vaginal discharge, the following observations were made. Majority of the cases were present in 31-40 years age group. (48%) followed by 20-30 years age group. (28%) reflecting the strong association of vaginal symptoms in reproductive age group. Followed by 16% in 41-50 years and 8% in >50 years

Table-1: Age wise distribution of cases

Age group	Discharge	Pruritis	Pain abdomen	Urinary symptoms	Backache
20-30	28	10	18	10	06
31-40	48	18	30	24	10
41-50	16	6	14	10	9
>50	8	2	5	2	2

Discharge was predominant symptoms in all the age groups. In the age groups, 31- 40 and 20-30 discharge was followed by pain abdomen as presenting symptom. In age group 41-50 urinary symptoms followed discharge .In >50 age group predominant symptom was white discharge.

ISSN 2515-8260 Volume 10, Issue 01, 2023

Table-2: Showing prevalence of pathogens causing vaginal discharge in different age groups

Age group	Bacterial vaginosis	Candidiasis	Trichomonas vaginalis	Mixed infections	Normal flora
20-30	9	13	1	2	3
31-40	27	11	3	2	4
41-50	8	4	1	1	3
>50	2	1	0	0	5

Bacterial Vaginosis was found in high frequency in the age group of 31-40 years (58.6%) followed by 41-50 years (17.3%), 20-30 (19.5%) and then by >50 (0.04%). The most common age group affected by vaginal candidiasis was 20-30(44.8%) ,followed by 31–40 years (37.9%), 41-50 (13.7%) and > 50 (0.03%) .Trichomoniasis was detected in the highest rate at the age group of 31-40years (60%) followed by 20-30(20%) , 41-50years (20%)
Normal flora was found seen in high frequency in the age group of >50 (33.3%) followed by 31-40(26.6%). Mixed infection of *Candida* and BV was seen in mainly in the age group 20-30 and 31-40 (40%) followed by 41-50 (20%).

Table-3: Aetiology of abnormal vaginal discharge:

Aethiology of abnormal vaginal discharge	**Number (n=100)**	**Percentages (%)**
Bacterial vaginosis	46	46
Candidiasis	29	29
Trichomonas vaginalis	5	5
Mixed infections	5	5
Normal flora	15	15

Of the 100 patients, 85 (85%) cases were organism positive and 15(15%) cases showed normal Flora. Among 100 patients, BV (46%) was the most common microbiological cause of abnormal vaginal discharge, followed by vaginal candidiasis (29%), trichomoniasis (5%), combined infection (*Candida* and BV) (5%).

Table-4:Findings of lab investigations:

Age	**PH**	**Whiff test positive**	**Saline mount positive**	**10%KOH**
20-30	Alkaline	10	10	13
31-40	Acidic	27	29	11
41-50	Alkaline	08	09	4
>50	Acidic	02	03	1

ISSN 2515-8260 Volume 10, Issue 01, 2023

Table-5: Vaginal swab for culture

Age	Clue cells	Gram +ve cocci	Gram -ve bacilli	Vaginal swab
20-30	9	06	06	Ecoli-4, Pseudo-2
31-40	27	07	07	CPS-2, KLEB-1, Ecoli, Pseudo-3
41-50	08	04	04	Ecoli -4, Pseudo-3 CPS-2, KLEB-2
>50	02	02	02	CPS-2 Ecoli-2

(GNB-Gram –ve bacilli, CPS-Coagulase positive staphylococci, CNS-Coagulase negative Staphylococci)

Characteristics of vaginal discharge of patients differed according to pathogenic agents. The pH of vaginal secretions was >5 in a large proportion of examined cases which included women with BV and TV, but women with *Candida* infection, the pH was slightly below the normal value. Whiff amine test was positive in most of the cases, most of them having BV. Vaginal swab for culture and sensitivity showed prevalence of micro-organism associated mainly with BV.

Figure-1: Clue cells in gram stain as seen under microscope

Figure-2: Clue cells in wet mount preparation seen in microscope

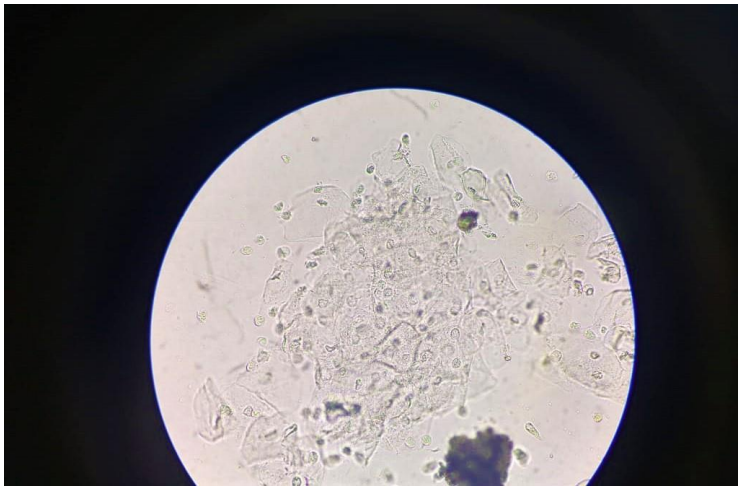
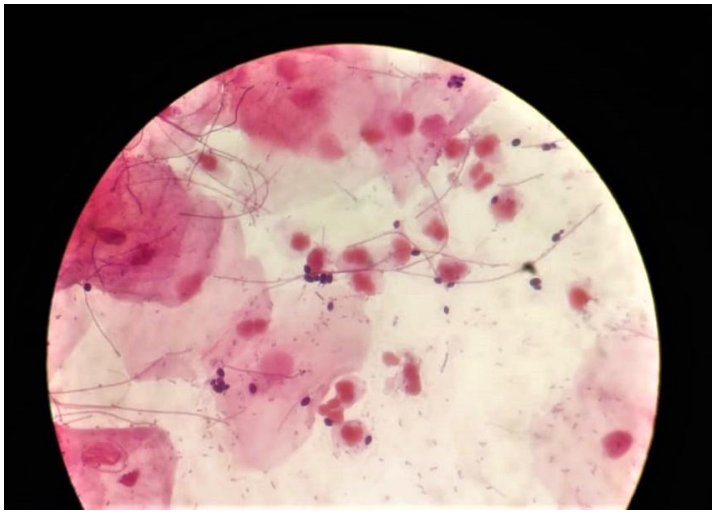


Figure-3: Gram positive budding yeast cells(candida species) under microscope



DISCUSSION

A total of 100 cases who presented with abnormal vaginal discharge were examined during the period of study. Vaginal discharge is a common health problem among women in the reproductive age group. Whether asymptomatic or symptomatic, it is usually neglected by women making the diagnosis more difficult. The incidence of pathogens in vaginal discharge varies in different regions of the world. Vaginal discharge may be either physiological or pathological in origin. It is difficult to know what proportion of discharges belong to either category. Although many cases of abnormal vaginal discharge are not caused by sexually transmitted infections, common curable sexually transmitted infections can present with this symptom.

The present study was done to know the most common cause for Abnormal Vaginal Discharge. This prospective study was conducted at Department of Obstetrics, Mother and child health care, GGH ,Nalgonda during the period of November 2021 to october 2022.

In the present study 100 patients presenting with vaginal discharge were analysed for several criteria. Maximum number of cases were in the age group of 31-40 years (48%) followed by age group 20-30 years (28%) because they belong to sexually active age group. Least number of cases were in the group of age >50years(8%). The commonest symptom in the presentation study is vaginal discharge followed by pain abdomen and pruritis.

In the Present study, the Amsel Criteria for diagnosing bacterial vaginosis were met in Elevated pH>4.5, Positive Whiff test, Presence of clue cells were noted. Litmuss paper test done to know the pH of Vaginal discharge and also Hanging drop method was done to know the motility of Trichomonas vaginalis as only few cases noticed in the study. Discharge was predominant symptoms in all the age groups. In the age groups, 31-40 and 20- 30 discharge was followed by pain abdomen as presenting symptom. In age group 41-50 urinary symptoms followed discharge .In >50 age group predominant symptom was white discharge.

Of the 100 patients, 85 (85%) cases were organism positive and 15(15%) cases showed normal Flora. Among 100 patients, BV (46%) was the most common microbiological cause of abnormal vaginal discharge, followed by vaginal candidiasis (29%), trichomoniasis (5%), combined infection (*Candida* and BV) (5%). Bacterial Vaginosis was found in high

frequency in the age group of 31-40 years (58.6%) followed by 41-50 years (17.3%), 20-30 (19.5%) and then by >50 (0.04%). The most common age group affected by vaginal candidiasis was 20-30(44.8%) ,followed by 31–40 years (37.9%), 41-50 (13.7%) and > 50 (0.03%) .Trichomoniasis was detected in the highest rate at the age group of 31-40years (60%) followed by 20-30(20%) , 41-50years (20%).

Bacterial Vaginosis (27%) was the most common microbiological cause of abnormal vaginal discharge in our study. The following picture 1 shows clue cells seen under microscope after gram staining. This is comparable to the study of Koumans *et al*.[5] who had also found a 29.2% prevalence of BV. In Pawanarkar and Chopra study,[6] BV was the most common cause of genital tract infections as it was prevalent in 19% of women. V. Gupta, et al. presented with 48 cases (40.4%) of bacterial vaginosis,. Monika Rathore, et al. presented with 69 case (26%) , Sujatha Audimulapu,et al.,[7] presented with 48 cases with 46.1% and present study 46% cases. Nessa *et al*.[8] in Bangladesh reported 48.1% cases of BV among the sex workers which is also on the higher side. This high prevalence may be the result of disturbance of vaginal microflora resulting from frequent sexual intercourse and the subsequent frequent washing with water and disinfectant. The variations in the prevalence could be related to geographical distribution or systematic differences in the various population samples; however, there is continuing controversy about its importance as a pathogen and its ability to cause vaginitis.

V. Gupta, et al.[9] presented with discharge symptoms by >50%. Monika Rathore, et al.[10] presented with discharge symptoms by 26%, Sujatha Audimulapu,et all., discharge symptoms by 96.1% and the present study by 100% as only patients with complaint of white discharge are taken in the present study.

Table-6:Comparision of various studies with the present study

Authors	**Percentages**
Discharge symptoms	
V.Gupta et al 2006[9]	>50%
Monika rathore et al 2007[10]	26%
Sujatha Audimulapu et al 2017[7]	96.1%
Present study	100%
Bacterial Vaginosis	
V.Gupta et al 2006[9]	**40.4%**
Monika rathore et al 2007[10]	**26%**
Sujatha Audimulapu et al 2017[7]	**46.1%**
Present study	**46%**
Candidiasis	
V.Gupta et al 2006[9]	**10%**
Monika rathore et al 2007[10]	**14%**
Sujatha Audimulapu et al 2017[7]	**44%**
Ferris DG[11]	**20%**
Present study	**29%**
Trichomonas Vaginalis	
V.Gupta et al 2006[9]	**9%**

Monika rathore et al 2007[10]	**8%**
Haggerty CL et al[12]	**7.4%**
Sujatha Audimulapu et al 2017[7]	**11.53%**
Present study	**5%**

Vaginal candidiasis (29%) was the second most common microbiological etiology of abnormal vaginal discharge in our study. Nwadioha *et al*.[13] and Verbalis *et al*.[14] also reported a similar result of increased prevalence in younger age group because of increased sexual activity in this age group. Candidiasis is not usually a sexually transmitted disease; however, male contacts could be possibly involved.

V. Gupta, et al.[9] presented with 18% of Candidiasis Monika Rathore et al. presented with 14% , Sujatha Audimulapu,et al[7]., presented with 44% , Ferris DG et al.[11], with 20% and present study with 29%. The Incidence of **Trichomoniasis** in earlier similar studies was 9% in Gupta, et al., 7.4% in Haggerty CL, et al.[12] and Monika Rathore, et al. [10] 8% and in the present study it is 5%. The combined infection has been investigated in the present study. In our study, 5% of cases presented with BV + vaginal candidiasis.

In Sujatha Audimulapu et al.[7], study E. Coli was found in 12 cases accounting for 11.53% of Aerobic bacterial vaginiosis. In the present study it was 13 cases associated with Bacterial Vaginosis. Other microorganism also involved in association of BV like Pseudomonas , Klebsiella and Coagulase positive Staphylococcus organism.

All over this accounts for 30% of cases associated with Abnormal Vaginal Discharge . If this associated infection is not treated with appropriate antibiotics , it leads to persistence of symptoms and other complications like cervicitis, cystitis ,UTI , pyelonephritis etc..

The levels of these various organisms vary in each woman. The results showed that the presence of lactobacilli together with other opportunistic pathogens may be due to several factors like effects of antibiotics, type of incubation and antagonism among lactobacilli species to maintain dominance. Also, some studies have reported that there can be chance of overlap of BV and aerobic vaginitis, leading to a mixed condition.

As the vaginal discharge is often polymicrobial in nature, Syndromic management of vaginal discharge allows simple, fast and assured therapy with a high cure rate. Combination kits are cheaper, effective, given in single dose orally with efficacy of 95 - 98%. However, treating these infections by performing Investigations in Outpatient, daily on routine basis can prevent major maternal morbidites like Symptomatic Bacteriuria, chances of preterm labour & delivery, Postpartum puerperal infections etc.,. and fetal morbidities due to preterm delivery and also decreases NICU admissions and preterm complications.

CONCLUSION

The present study was done on 100 patients with signs and symptoms of vaginitis. The causative agents of Bacterial vaginosis , trichomoniasis, and vaginal candidiasis are the most commonly found infectious agents in abnormal vaginal discharge among sexually active women. BV is by far the most common cause of abnormal vaginal discharge in our study followed by vaginal candidiasis and Trichomonas vaginalis was the third most common cause for vaginal discharge.

Out of 100 cases, few cases showed discordance between clinical and laboratory diagnosis.

This discordance can be due to pitfalls in identifying the causative agent clinically or obscuration of the findings due to improper treatment received for other ailments. Thus, the clinico-investigative correlation is more important than considering only the clinical findings. Clinicians need to be aware of emerging epidemiological data, the different presentations of vaginal discharge, and the approach of their management so that the symptom can be treated according to its etiology. It is recommended that prevention, early diagnosis, and prompt treatment of abnormal vaginal discharge especially among the sexually active women should be done to avoid the complications and reduce HIV transmission. There is a need for creating community awareness about health-care facilities and self-concern in women for their own health needs. Hence, this study was done to emphasize the role of laboratory investigations in patients of vaginitis as clinical diagnosis alone can lead to false interpretation. We conclude that standardized bed side diagnostic procedures should be followed in each and every gynaecological practice where in, the unnecessary delay for routine conventional testing procedures is bypassed.

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