ROLE OF LABORATORY TESTS IN DETERMINING THE ETIOLOGY OF VAGINAL DISCHARGE AND THE IMPACT ON ITS MANAGEMENT

By

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Dissertation submitted to the
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In partial fulfilment of the requirements for the degree of

MASTER OF SURGERY IN OBSTETRICS AND GYNAECOLOGY

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LIST OF ABBREVIATIONS

BV	-	Bacterial Vaginosis
C.albicans	-	Candida albicans
C.trachomatis	-	Chlamydia trachomatis
CDC	-	Centres for disease control
CIN	-	Cervical intraepithelial neoplasia
DIV	-	Non-infectious desquamative inflammatory vaginitis
HIV	-	Human immunodeficiency virus
HPV	-	Human papilloma viruses
HSV	-	Herpes simplex virus
IUD	-	Intrauterine device
KOH	-	Potassium hydroxide
OCs	-	Oral contraceptives
N.gonorrhoeae	-	Nisseria gonorrhoeae
PAP	-	Papanicolaou
PCR	-	Polymerase chain reaction
PID	-	Pelvic inflammatory disease
PMNLs	-	Polymorphonuclear leucocytes
STD	-	Sexually transmitted disease
VVC	-	Vulvo vaginal candiasis
P/S	-	Per-speculum examination
P/V	-	Per-vaginal examination
FT	-	Fornicial tenderness
FF	-	Fornicial Fullness

ABSTRACT

Objectives:

1) To find out the clinical profile of women presenting with vaginal discharge and outcome of treatment based on clinical diagnosis.
2) To study the interventions in these patients.

Material and methods:

This study was carried out from December 2009 to May 2011, on all patients with complaints of vaginal discharge at the outpatient department of Obstetrics and Gynecology in R.L. Jalappa hospital and research centre, Kolar.

A 100 patients were included in the study in a period of 1 ½ years. A comprehensive history, general examination and gynecological examination were initially carried out. Based on the clinical findings including the characteristics of vaginal discharge, a provisional clinical diagnosis and treatment initiated. Vaginal exudate was obtained for the following investigations: pH estimation, saline mount for Trichomonas vaginalis and 10%KOH mount for yeast cells, Pap smear was obtained for all the patients. Mid stream urine sample was taken for microscopy.

Treatment was initiated on the basis of clinical findings and modified, if needed on the basis of laboratory findings. Follow-up was advised. Response to treatment was assessed primarily by relief of symptoms.

Results:

The most common age group was between 20-30 years (44%).The most common associated symptom was pruritus (30%), followed by lower abdominal pain (21%), and lower back pain (9%). 55% patients had no associated symptoms. Bacterial

vaginosis was the most common infective cause of Vaginitis (49%) followed by candidiasis (12%) and Trichomoniasis (11%). There were 29 cases out of 100 in the study which had clinical signs of infection but no organism was detected on laboratory tests.

These organisms might be Chylamydia, Mycoplasma, Ureaplasma, Anaerobic bacteria (Prevotella), Herpes simplex virus and other organisms, for which no specific tests were done in the study. These patients were treated according to syndromic management 20 were relieved of their symptoms on follow up.

10 patients could not be followed up, probably because the study is based in a rural setup and temporary relief was useful for the patients. Following initial treatment based on clinical diagnosis, 65% patients were completely relieved of symptoms, treatment was modified following laboratory diagnosis in 15 patients and the partners needed to be treated in 11 cases.

Conclusion:

Many of the diagnostic techniques to detect cause of vaginal discharge can be done as office procedures which avoid unnecessary delay in initiating treatment and indiscriminate use of antibiotics. Clinicians need more education in the laboratory diagnosis of vaginal discharge, as many of the diagnostic techniques can be done as office procedures for diagnosing the etiology of vaginal discharge.

Keywords

Vaginal discharge, Bacterial Vaginosis, Candidiasis, Trichomonas Vaginalis, endocervicitis, vaginal pH, Whiff test, wet mount, KOH mount, Pap smear, grams staining, culture, clinical diagnosis, laboratory diagnosis.

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INTRODUCTION

Vaginitis is an ancient disease and was described by Hippocrates. Leucorrhoea is one of the major problems encountered in Gynaecological practice. The most common cause of leucorrhoea is vaginal infection due to bacteria, virus, fungi and parasites. Other causes include foreign bodies, cervicitis and atrophic vaginitis [1].

Vaginitis is the most common gynecologic problem encountered by physicians providing primary care to women. It affects all age groups and has a variety of causes it can be a significant source of physical and emotional distress for the patient and frustration for the physician, especially if it is recurrent or chronic .Vaginitis may be difficult to establish because of the various causes. Initial episode should be properly evaluated, diagnosed, and treated as recurrent episodes are more may be more difficult to manage.[2]

Having some amount of vaginal discharge is normal, especially in the child bearing age. Glands in the cervix produce a clear mucous. Vaginal discharge that suddenly differs in colour, odour or consistency, significantly increases or decreases in amount, may indicate an underlying problem like an infection.

About 30% of women with vaginal complaints go without a diagnosis even after a complete evaluation using techniques more comprehensive than those usually available. Perhaps this explains why many clinicians appear to manage patients without performing a pH examination of the discharge or microscopy. In actual clinical practice, diagnoses of vaginal complaints do not show good agreement with diagnoses based on cultures.[2]

Women who present with vaginal complaints often receive tests for gonorrhea or chlamydia. However, the association between gonorrhea, chlamydia, and vaginal discharge is not confirmed. Additional less common causes of vulvovaginal symptoms are infection with herpes simplex, allergic reactions

to chemical irritants, latex, or semen, mechanical irritation due to lack of lubrication; and atrophic vaginitis in postmenopausal women. [3]

Current recommendations for the diagnosis of vaginal complaints in premenopausal women involve a vaginal examination and microscopy. The evaluation has traditionally been oriented toward the detection of vaginal candidiasis, bacterial vaginosis, and trichomoniasis, which are the 3 most common causes of vaginitis in this age group. Prevalence of these 3 conditions will vary depending on the clinical setting. [3]

Although diagnosis and therapy of vulvovaginits is straightforward in most cases, many patients experience persistence or recurrence, and still others who have less distinctive types of vaginitis do not receive satisfactory therapy.

Developments in the last five years have included improved knowledge of the complications associated with vaginitis, new diagnostic techniques, and refinements of therapy.[4]The crux of the diagnosis of vaginal infections and discharge rests with the microscopic examination of the vaginal discharge.

The importance of reproductive tract infections in causing morbidity and long term sequelae has been recognized by the world health organization which has recommended syndromic approach at the community level. The availability of facilities for accurate laboratory diagnosis in peripheral centres is lacking especially in developing countries and patients are thus denied the benefit of prompt therapy in preventing sequelae.

AIMS AND OBJECTIVES

The aims and Objectives of the study are as follows

1. To find out the clinical profile of women with vaginal discharge and the outcome of treatment based on clinical diagnosis.

2. To study the treatment interventions in these patients.

REVIEW OF LITERATURE

Vaginal discharge is a common presenting symptom of vaginitis, seen by Gynaecologists. It causes annoyance as well as apprehension to the patient. The word leucorrhoea means running white substance in excessive amount.

A study by Bourne and Williams 1945 described it as a functional excess of normal secretion of cervix and vagina or existence of white discharge containing pus and other abnormal elements due to infection.[4]

Earlier, vague terminology such as nonspecific vaginitis or non specific lower genital infections often was used to describe conditions that produce vaginal discharge. Recently, careful definitions of clinical syndromes and increased knowledge about the specific agents that cause genital infections in women have made more precise diagnosis, possible.

NORMAL VAGINAL FLORA

The microbiology of the female genital tract is indeed complex. In healthy women, the vagina contains 10^9 bacterial colony-forming units per gram of secretions**.** Vaginal flora of a normal asymptomatic reproductive aged woman includes multiple aerobic or facultative species as well as obligate anaerobic species. Of these, anaerobes are predominant and outnumber aerobic species approximately 10:1.[5]

One study of 55 asymptomatic reproductive aged women found a mean of 4.2 and 2.1 bacterial species recovered from the endocervix and the endometrial cavity, respectively **.**[6]

Of the species recovered, 17% were recovered from the endometrium, 50% were recovered from the endocervix, and the remainder was recovered from both sites. This implies that there is access to the upper reproductive tract by certain bacterial species normally found in the vaginal flora.

The function and the reason for bacterial colonization of the vagina remains unknown. Bacteria do exist in a symbiotic relationship with the host and are alterable, depending on the microenvironment. These organisms localize where their survival needs are met, and have exemption from the infection preventing destructive capacity of the host. Within this vaginal ecosystem, some microorganisms produce substances such as lactic acid and hydrogen peroxide that inhibit non-indigenous organisms .[9]

In addition several other antibacterial compounds, termed bacteriocins, provide a similar role and include peptides such as acidocin and lactacin. Moreover, some species have the ability to produce proteinaceous adhesions and attach to the vaginal epithelial cells. For protection from many of these toxic substances, the vagina secretes leucocyte protease inhibitor. This protein protects local tissues against toxic inflammatory products and infection.

The first extensive study of the vaginal flora in adult woman was reported by Doderlein, who emphasized the prominence of facultative lactobacilli. The normal mature vagina contains multitude of organisms. Hydrogen peroxide producing lactobacilli are predominant and suppress the growth of anaerobes. A variety of species of commensal bacteria colonize the surfaces, they cause no harm to the host except under abnormal circumstances. The flora of the female genital tract varies with the pH and estrogen concentration of the mucosa, which is dependent on the age of the host.[5]

Lactobacillus acidophilus, which are the dominant bacteria (more than 95%) in a healthy vaginal ecosystem. It suppresses the growth of the Gram-negative and Gram-positive facultative and obligate anerobes and maintains normal pH (3.8-4.2) through the production of lactic acid. In addition, these bacteria produce hydrogen peroxide & bacteriocins, which is toxic to the anerobes.

Vaginitis occurs because the vaginal ecosystem has been altered, either by the introduction of an organism or by a disturbance (e.g. antibiotics, hormones & exposure) that allows the pathogens normally residing in this environment to

proliferate. An altered vaginal ecosystem usually results from a combination of factors.[10]

Vaginal pH

Typically, the vaginal pH ranges between 4 and 4.5. Although not completely understood, it is believed to result from *Lactobacillus species* due to production of lactic acid, fatty acids, and other organic acids. In addition, amino acid fermentation by anaerobic bacteria results in organic acid production as does bacterial protein catabolism. Glycogen present in healthy vaginal mucosa is believed to provide nutrients for many species in the vaginal ecosystem. Accordingly, as glycogen content within the vaginal epithelial cells diminishes after menopause, this decreased substrate for acid production leads to rise in vaginal pH.

Calillouette and associates (1997) showed that vaginal pH of 6.0-7.5 was strongly suggestive of menopause in the absence of symptoms. Moreover, serum follicle stimulating hormone (FSH) levels and vaginal pH were positively correlated, whereas an inverse relationship was noted between those two and serum estradiol levels.[5]

Altered Flora

Changing any element of this ecology may alter the prevalence of various species**. Delliverd and colleagues (2004)** reported that hormone replacement therapy restored vaginal lactobacilli populations, which protected against reproductive tract pathogens. The menstrual cycle may also alter normal flora.[10]

Transient changes are observed, predominantly during first part of the menstrual cycle, and are presumed to be associated with hormonal changes .[10]

Several other events predictably alter lower reproductive tract flora and may lead to patient infection. Treatment with a broad-spectrum antibiotic or

menstruation may result in symptoms attributed to inflammation from *Candida albicans* or other *Candida* species. Hysterectomy with removal of the cervix may also alter lower reproductive tract flora with or without prophylactic antimicrobial administration.[5]

VAGINAL SECRETIONS

Sources

Vulval secretions- Bartholin's, Skene, sweat & sebaceous glands.

Vaginal transudate from the vaginal wall & exfoliated vaginal epithelial cells.

Cervical secretion-exfoliated cervical cells & mucous

Uterine secretion

Fallopian tubal secretion

Causes of vaginal discharge

- Physiological
- Leucorrhea
- Inflammatory

-Vulvovaginitis

-Cervicitis, gonococcal, chlamydial, anerobic, pueperal, & secondary infection of an erosion.

- Endometritis, puerperal, senile & pyometra
- Non specific- Secondary infections

-Burns & chemical injuries

Infected cervical polyps/leiomyomas/ prolapse

- Surgeries, conization, cauterization,

-Vaginal hysterectomy & pelvic floor repair

- IUCD

-Foreign body

- Genital tract malignancy
- Fistulas- Vesico-vaginal and recto-vaginal

Physiological increase in vaginal secretion

1. at ovulation
2. after coitus
3. Oral contraceptive pill use

Leucorrhoea

Refers to excessive amount of normal vaginal discharge mainly of cervical component although white/cream when fresh, it dries to leave a brownish yellow stain on clothing , if patient fails to bathe frequently, causes excoriation & soreness of vulva.

Characteristics

- Non-purulent
- Non-offensive
- Non-irritant
- Never causes pruritis

Causes

- At birth – in new born babies
- Before and after menarche
- Prolonged ill health, anxiety states & neurosis, sedentary occupation & standing for long periods in hot atmospheres
- Cervical erosion /ectopy
- Vaginal adenosis
- Combined OCPs
- Regular douching

Causes of Infective vaginal discharge[11]

Infective

Bacteria

Aerobic organisms

Gram positive cocci

Staphylococcus aureus *Staphylococcus epidermidis*

Staphylococcus saprophyticus *Enterococcus fecalis*

Streptococcus agalactiae *Streptococcus pneumonia*

Streptococcus pyogenes *Viridans group streptococci*

Gram positive bacilli

Cornybacterium sp *Gardenella vaginalis*

Diptheroids

Lactobacillus sp *Listeria monocytogens*

Gram negative cocci

Nisseria gonorrhoeae

Gram negative bacilli

Enterobacter sp *Escherichia coli*

Klebsiella pneumonia *Proteus mirabilis*

Pseudomonas aeruginosa

Anaerobic Bacteria

Gram positive cocci

Pepto streptococcus anaerobius *Preptostreptococcus*

Pseudostreptococcus asaccharolyticus *Peptostreptococcus magnus*

Gram-positive bacilli

Actinomyces *Propionibacterium sp*

Clostridium perfringes *Clostridium sp*

Clostridium difficile

Gram-negative bacilli

Bacteriodes (Bacteriodes fragilis group) *Bacteriodes sp,other*
Fusobacterium sp *Porphyromonas*
asaccharolytica
Provotella bivia Provotella disiens
Provotella melaninogenica

Mycoplasmas

Mycoplasma hominis *Ureaplasma urealyticum*
Mycoplasma genitalium

Viruses

Cytomegalovirus *Herpes simplex virus*
Human papilloma virus *Hepatitis B virus*
Human parvovirus *Human immunodeficiency*
virus

Intracellular bacteria

Chlamydia trachomatis

Parasites

Trichomonas vaginalis *Toxoplasma gondii*

Yeasts

Candida albicans *Candida (Torulopsis)*
glabrata
Candida tropicalis *Candida pseudotropicalis*
Saccharomyces cerevisiae

ABNORMAL VAGINAL DISCHARGE

The most common symptom of vaginitis is an abnormal vaginal discharge that is excessive, malodorous or purulent in nature. The conditions associated with abnormal vaginal discharge are bacterial vaginosis, mycotic Vulvovaginitis and Trichomoniasis.

A study by **Renkonen et al** (1970) reported bacteriological studies on vaginal discharge from 200 patients, in which trichomoniasis were 18.5 %, candidiasis 32.5 %, Haemophilus vaginalis infection 30.5 %, and enterococcus infection 52.5%.

Haemophilus vaginalis is never found as a single organism to cause vaginitis but always found in association with cocci. [12]

Study was done by **John G et al (1977)** on two patient's population; the first group consisted of 17 healthy college students who were sampled during a routine vaginal examination. The gynaecologist also completed a form concerning observations on the pelvic examination in the age group ranging from 18 to 28 years.

Reasons for attending the gynaecology clinic included contraceptive counselling and cervical cytological examination. Pelvic examination was normal in all cases. The second group consisted of 5 healthy women age ranging from 22 to 38 years. All were in good health. The predominant facultative organisms were staphylococcus epidermidis, streptococcus and lactobacillus.[13]

A study was done by **Richard Amsel** 1983 on 397 patients, out of which 140 patients had symptoms of vaginitis, 93 patients were for contraception consultation, 51 patients on annual examination, pelvic pain in 29 patients, menstrual disorder in 24 patients and 15 pregnancy cases. Gram staining showed organisms present on epithelial cells to be gram variable coccobacilli.[14]

In 1985 a study was done by **Nancy B et al** to analyze microbiological and clinical correlates of inflammatory and epithelial cell changes on papanicolaou stained cervical smears. Yeast infection remained significantly associated with minimal epithelial cell changes in the random group, Condylomata with moderate nuclear changes and dyskeratosis and koilocytotic changes, C.trachomatis with reactive or atypical metaplastic cells.[15]

TABLE 1

CARACTERISTICS OF NORMAL VAGINAL SERECTIONS AND VAGINITIS

Feature	Normal	Bacterial vaginosis	Trichomoniasis	Yeast
Appearance	White, Floccular; high viscosity	Gray, white, milky/creamy	Gray, yellow, greenish, or white; homogenous, often frothy	White, Often curdy
pH	<4.5	>4.5	>4.5	<4.5
Amine odour	Absent	Present	Absent	Absent
Clue cells	Absent	Present	Absent	Absent
Trichomonads	Absent	Absent	Present	Absent
Mycelia	Absent	Absent	Absent	Present

Approximately 8-10% of patients with abnormal vulvovaginal symptoms will not be diagnosed by the tests described. In these patients, cervicitis due to gonorrhoea or chylamydia should be excluded by specific testing. In addition a saline preparation of an endocervical smear should be examined microscopically to detect evidence of cervicitis which is often nongonococcal and nonchlamydial in origin.

SITE OF INFECTION

The vagina, ectocervix and endocervix are all susceptible to various pathogens depending on the type of epithelium present and other factors in the micro environment. The squamous epithelium of the vagina and

ectocervix is susceptible to infection with candida species and T. vaginalis, and the columnar epithelium of the endocervix is susceptible to infection with N.gonorrhoeae and C.Trachomatis. Herpes simplex virus may infect both types of epithelium. [16]

EVALUATION

History

Details about the type of discharge such as onset, duration, amount, colour,blood staining,consistency odour,previous episodes should be elicited.

Presence of associated symptoms such as itching, soreness,dysuria, intermensutral bleeding, post coital bleeding, lower abdominal pain,pelvic pain,dysparenuria should be enquired about.Relationship with menstruation & coitus, recent use of antibiotics, hormonal use, prior treatment & self-medication, douching/application of antiseptics

History of exposure to new sexual partners, previous STDs, any symptoms in the male partner and any history of allergy to deodorants & soaps should be asked.

CLINICAL EXAMINATION

- General – any ill health, anemia
- Abdominal examination – tenderness or any mass
- Examination of external genitalia—care full inspection of perivulval, vulva, perineum, perianal & anal regions for excoriations, ulcers, erythema, edema & growths. Urethral discharge if any noted.
- Speculum examination to inspect vagina & cervix for any discharge, erythema, edema, ulcerations, cervical erosion & growths.
- Bimanual examination – to rule out any pelvic mass, tenderness.

LABORATORY EVALUATION OF A PATIENT WITH VAGINAL DISCHARGE[3]

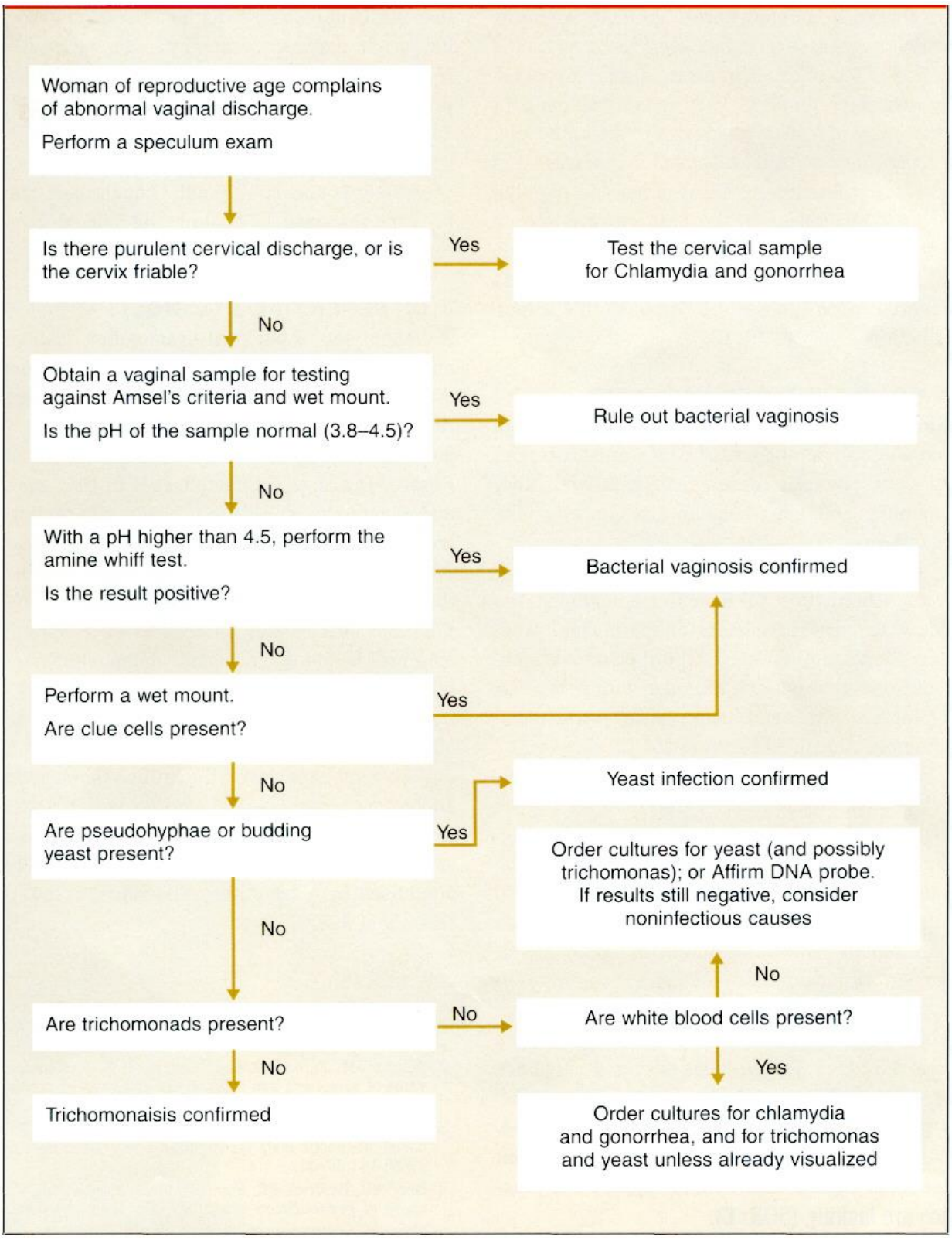


Figure:1

BACTERIAL VAGINOSIS

Bacterial vaginosis is an abnormal condition of the vaginal ecosystem caused by the overgrowth of both aerobic and anaerobic vaginal bacterial flora. It is the most prevalent vaginitis and is responsible for approximately one third of all cases of vulvovaginitis in women of reproductive age group. The hall mark symptoms are discharge and odour. The diagnosis of this condition is likely when a patient complains of a malodorous, non irritating discharge, and on examination reveals homogeneous grey white secretions, but more than one half of patients with demonstrable signs have no symptoms.[5]

A type of vaginitis produced by a previously unrecognised bacterium was reported by Gardner and Dukes in (1955). They described the clinical aspects the appearance of the organism in wet amount, Gram stain, and culture, some of the biochemical properties of the bacterium, and its pathogenicity. To it, they assigned the name Haemophilus vaginalis.[17]

In time, the bacterium could not be considered taxonomically Haemophilles, because it had no absolute requirement for hemin. It was eventually moved to the new genus Gardenrella. It became evident over time that Gardenerella was not the only etiological cause of bacterial vaginosis, largely because of the inability to eliminate Gardnerella in patients who were cured and its isolation from vaginal secretions in many women without bacterial vaginosis. Eventually the current designation, bacterial vaginosis was agreed on by international convention. The replacement of the term vaginitis by vaginosis denotes the presence of vaginal discharge without an apparent inflammatory response.

One of the controversies regarding bacterial vaginosis concerns its mode of transmission. It is not clear that bacterial vaginosis is sexually transmitted. There is no significant difference in the prevalence of bacterial vaginosis or in the prevalence of isolation of Gardnerella vaginalis between the sexually active and virginal groups.[18]

Epidemology

In unselected populations, the prevalence of BV is 10 to 20%, but it may be as high as 36% in women attending STI clinics and 28% in those seeking elective termination of pregnancy

TABLE: 2

BACTERIAL VAGINOSIS RISK FACTORS[6]

• Oral sex
• Douching
• Black race
• Cigarette smoking
• Sex during menses
• Intrauterine device
• Early age of sexual intercourse
• New/Multiple sexual partners
• Sexual activity with other women

Microbiology of Bacterial Vaginosis

Bacterial vaginosis is a polymicrobic synergistic infection. The normally predominant lactobacilli population is reduced in the vagina, while populations of *Gardnerella vaginalis* and other anaerobes are increased.*G vaginalis* is the only member of its genus.

Originally, it was known as *Haemophilus vaginalis* and then as *Corynebacterium vaginale.*

It is a nonmotile, nonflagellated, nonsporeforming, facultative anaerobic, and nonencapsulated bacteria. Although *G vaginalis* appears microscopically as a gram-variable rod, it is officially categorized as a gram-negative rod.

The pH required for the growth of anaerobes is 4.5 or less. **Gardner and dukes** demonstrated that G. Vaginalis parasitizes the surface of vaginal epithelial cells, many of which became so heavily massed with these bacteria that they present as clue cells.[19]

TABLE: 3

MICROBIAL SHIFTS IN BACTERIAL VAGINOSIS[2]

Normal vaginal secretions	**Bacterial Vaginosis**
pH4.0-4.4	pH>/ 4.7
10^4CFU/mL	Up to 10^{11} CFU/Ml
Lactobacilli predominate	Abundant anaerobes, Gardenella vaginalis, and genital mycoplasmas

Diagnosis

The chief symptom is vaginal discharge rather than pruritus. Some patients may note an offensive odour which may be accentuated after coitus. Upon examination, the typical discharge, characterized as whitish, creamy or milky and homogenous (compared with normal secretions which are heterogeneous and floccular), often is evident at the introitus. Vulvovaginal irritation is less marked than with trichomoniasis or candidiasis.

Findings on clinical examination

- Vaginal discharge most often gray, thin, and homogeneous, adherent to the vaginal mucosa
- May not visualize pooling of discharge in the posterior fornix because of adherence to the vaginal mucosa
- May observe small bubbles in the discharge fluid
- An increased light reflex of the vaginal walls may be observed, indicating a very wet appearance; however, typically, no or little evidence of inflammation is apparent.
- The labia, introitus, cervix, and cervical discharge appear normal.
- Evidence of cervicitis should prompt a workup for concomitant infection with *Neisseria gonorrhoeae, Chlamydia trachomatis,* or herpes simplex virus (HSV)

The diagnosis is established by detecting at least three of the following criteria (Amsel's criteria):[10]

1. A thin homogenous vaginal discharge
2. A vaginal pH of > 4.5
3. Amine test – a characteristic "amine" odour released when alkali (10% KOH wt/vol) is added to a specimen of vaginal fluid.
4. Appearance of clue cells in a wet mount of vaginal fluid.

The best two combinations of parameters for rapid accurate clinical diagnosis are clue cells and odour on alkalinization.

Vaginal pH is considered the most sensitive but least specific. Characteristic is Amsel's criteria with its sensitivity 98.2 % and specificity 94.3 %.[10] Variations in the pH of vaginal secretions can occur because of many reasons. Including recent intercourse, menses, and different sampling times within the menstrual cycle.

As such, the use of pH > 4.5 alone as an indicator of bacterial vaginosis would result in many erroneous diagnosis .Clue cells, that is , squamous vaginal epithelial cells covered with attached bacteria and obscuring borders are the most sensitive and specific sign of bacterial vaginosis.

Direct Gram Stain

Gardner and Dukes (1959) and Dunkelberg (1965) described that vaginal fluid from bacterial vaginosis patients had many small gram negative organisms resembling Gardnella vaginalis, in the absence of lactobacillus morphotypes.[13]Spiegel et al showed that the diagnosis of bacterial vaginosis was possible from a Gram stain of vaginal secretions if other morphologic types outnumbered lactobacilli even if Gardnerella and lactobacillus morphologic types were present at >/= 3+ levels. Gram staining of vaginal secretions may be used to diagnose Bacterial vaginosis. Specimens from patients with BV have numerous mixed bacteria and a paucity of lactobacilli. Clue cells may be seen clearly. In comparison, patients with normal secretions have fewer bacteria which are predominantly lactobacilli cell types.

There are a considerable number of patients with an intermediate type of flora on gram stain. These patients seem to be developing BV of perhaps resolving it spontaneously.

The Gram stain interpretation was as follows

(1+) Less than one organism per field

(2+) One to five organisms per field

(3+) Six to thirty organisms per field

(4+) > 30 organisms per field

When large gram-positive lactobacillus morphologic types were present alone or greatly exceeded other bacterial morphotypes, the Gram stain was interpreted as negative for bacterial vaginosis.[20]

TABLE:4

Nugent et al 1991 – proposed an improved scoring system: [21]

Organism Morphotype	**Number / oil immersion field**	**Score**
Lactobacillus like (Parallel sided Gram + ve rods)	> 30	0
	5–30	1
	1–4	2
	<1	3
	0	4
Mobiluncus like (Curved Gram –ve rods)	> 5	2
	< 1 – 4	1
	0	0
Gardnerella / Bacteroid like (Tiny, Gram-variable coccobacilli And rounded pleomorphic Gm –ve rods with vacuoles)		
	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

Score

0 – 3 Normal

4 – 6 Intermediate repeat test

7 – 10 Bacterial vaginosis

Studies of comparison of the diagnosis by compound criteria with that done by Gram Stain criteria have yielded a high rate of sensitivity of 97 % and specificity of 79 – 100 %.[22]

SPIEGEL's criteria[23]

Bacterial vaginosis is diagnosed if lactobacillus morphotypes are fewer than 5 per oil immersion field & if there were 5 or more other morphotypes (gram + ve cocci, small gram – ve rods, curved gram variable rods or fusiforms) per oil immersion field.

If 5 or more lactobacilli & fewer than 5 other morphotypes were present per oil immersion field, gram's stain is considered to be normal.

Other diagnostic tests for Bacterial vaginosis

Other diagnostic tests include the Pap smear, oligonucleotide probes, and detection of amines, short chain volatile acids (such as putrescine, cadaverine, and trimethylamine), and enzymes (such as proline aminopeptidase). Standardized criteria have not been routinely applied to the pap smear for diagnosis of bacterial vaginosis; BV should be diagnosed on the basis of vaginal, not cervical, secretions.

DNA testing for Gardnerella is accurate for detection, but it is not synonymous with a diagnosis of BV, as described. DNA testing is further described under "Differential Diagnosis." Gram staining is more reliable than gas-liquid chromatography and an assay for proline aminopeptidase (a metabolic product of some of the bacteria associated with BV).Latex agglutination testing for vaginal lactoferrin is a nonspecific marker for leukocytes, and thus inflammation. It is of little clinical utility in the diagnosis of vaginal discharge.[23]

Sequelae

Bacterial vaginosis frequently produces serious infectious sequelae. Several bacteria can cause the infection. Those most often mentioned are the various staphylococci, streptococci, coliform bacilli, micrococci and diphtheroids. 40% of G. vaginalis was isolated in IUCD users.

A recently published hypothesis suggests that bacterial vaginosis can cause neoplasia of the cervix. There is a strong development of vaginal cuff infections following hysterectomy and pelvic inflammatory disease has been postulated.[24] One study shows an association between premature labour with intact membranes and amniotic fluid infected with bacteria.[25]

In the patients with gynaecologic disease, bacterial vaginosis is associated with laparoscopically proved pelvic inflammatory disease, urinary tract infections, endometritis, post partum endometritis and chorioamnionitis.[26]

Treatment

The standard treatment for bacterial vaginosis (BV) has been oral Metronidazole 500 mg twice daily for 5 to 7 days. Intravaginal 0.75% metronidazole gel has been shown to be as effective as oral metronidazole.[28,29] Clindamycin, orally or in vaginal cream, for 5 days is also effective for BV. [30,31]

It is said that bacterial vaginosis need not include routine treatment of the male sexual partner; however, one study using more objective diagnostic criteria demonstrated that treating partners significantly increased cure rates in woman .[27]

Recurrent Bacterial vaginosis

The initial regimen or an alternative regimen may be used. A longer, 10- to 14-day, course of antibiotic therapy with Metronidazole has been recommended by one expert for treating relapses. Suppressive therapy such as intravaginal metronidazole twice weekly may also be considered as maintenance therapy to prevent recurrences.

TABLE: 5

Recommended Treatment of Bacterial Vaginosis

Agent	Dosage
Metronidazole	500mg orally twice daily for 7 days
Metronidazole gel 0.75%	5 g (1 full applicator) intravaginally once daily for 5 days
Clindamycin cream 2%	5g (1 full applicator) intravaginally at bedtime for 5 days.

(From centres for Disease Control and Prevention, 2006).

Other forms of therapy such as introduction of lactobacilli, acidifying gel, and use of probiotics have shown inconsistent benefits.

VULVOVAGINAL CANDIDIASIS

Yeasts are commonly isolated in the female lower genital tract, with rates of 22% among asymptomatic college women, 26% among patients in an STD clinic, and 39% among women with vulvovaginal symptoms.When conditions in the vagina favour the growth of yeast over bacterial flora, vaginal candidiasis may result.

Candida is often present in women with no symptoms, and is probably part of the normal flora. Vulvo vaginal candidiasis includes the spectrum of patient both with and without symptoms that have positive cultures and who phenotypically may range from having a total absence of symptom to the presence of florid, severe disease. The term vulvar was introduced to emphasize the often dominant vulvar component of the symptomatic infection.[32]

Epidemology

1. Vulvo vaginal candidiasis is the second most common cause of vaginitis and vaginal discharge after bacterial vaginosis.

2. Recurrent vulvo vaginal candidiasis is present in less than 5% of women in the child bearing age.

3.The life time incidence of vulvo vaginal candidiasis is estimated at50–75 %.

4. The prevalence of vulvo vaginal candidiasis is not known, but the disease is diagnosed in 5 – 15 % of women who attend sexually transmitted disease and family planning clinics.

5. Candida albicans is found in 10 – 20 % of woman of reproductive age in the absence of symptoms. These women do not require treatment.

6. Women of child bearing age are most likely to develop vulvo vaginal candidiasis, as candida albicans proliferates in an oestrogen – rich environment.

7. Vulvo vaginal candidiasis is much less common in prepubertal girls and postmenopausal women. Vulvo vaginal candidiasis is usually classified as either sporadic or recurrent on the basis of episodic frequency.[32]

Pre Disposing Factors[33]

A. Diabetes Mellitus

B. Medications

- Corticosteroids
- Immunosuppressant medications
- Broad spectrum antibiotics
- Oral Contraceptives – increases frequency of candida carrier state.
- Doesn't increase symptomatic vulvovaginits

C. Heat and moisture retaining clothing

D. Pregnancy

E. Premenstrual phase of menstrual cycle.

F. Depressed cell medicated immunity eg. AIDS.

TABLE: 6

Classification of Vulvovaginal candidiasis [34]

Uncomplicated VVC (all of the listed criteria)
Sporadic or infrequent vulvovaginal candidiasis
Mild to moderate vulovaginal candidiasis
Candida albicans infection
Normal, non-pregnant women
Complicated VVC(any of the listed criteria)
Recurrent episodes (>4 episodes per year)
Severe symptoms or findings
Non-albicans yeast species
Abnormal host (eg. diabetes, immunosupression)

Preliminary data suggest that women undergoing natural menopause who receive exogenous estrogen replacement therapy may be at higher risk of vulvovaginal candidiasis. Vulvo vaginal candidiasis is not a reportable disease and prevalence estimates have relied mainly on self reported history of physician diagnosis.

Regrettably, vulvovaginal candidiasis is routinely diagnosed without benefit of microscopy or culture, and in as many as half of the cases so diagnosed, the women may be uninfected or have other conditions.[35]

Microbiology

Yeasts causing vulvovaginal candidiasis are *Candida Albicans, Candida Glabarata, Candida Krusei, Candida Lusitaniae, Cnadida Parapsilosis, Candida Tropicalis, Candida Cerevisiae.*[36]

85 – 90 % of yeast isolated from the vagina is candida albicans strains. Several investigations have recently reported that cases of sporadic and recurrent vulvovaginal candidiasis caused by non albicans species of candida are increasing. In specialized clinics > 10 % and, occasionally, > 20 % of patients were infected with non albicans candida organism.[37,38] The dominant nonalbicans candida species reported is C.glabrata. Increasing non-albicans

candida vulvovaginitis is due to the increase use of over-the-counter antimycotics with incomplete course of therapy. [39]

Candida albicans is a dimorphic fungus, which can take two forms most of the time it exists as oval single yeasts cells and it reproduces by budding. Normal room temperature favour the yeast form of the organism, but under physiological conditions like body temperature, pH and the presence of serum it may develop into a hyphae form called pseudohyphae, is composed of chains of cells. Candida is a commensal organism found in 40 – 80 % normal humans and is present in the mouth, gut and vagina. For candida to colonize the vaginal mucosa, they first must adhere to the vaginal epithelial cells. C. albicans adhere in significantly higher numbers to vaginal epthelial cells than the nonalbicans candida species.[41]

Pathogenesis

Candida organisms gain access to the vaginal lumen and secretions predominantly from the adjacent perianal area. It was concluded that C.albicans was never a commensal in the vagina and was always a pathogen in one study. A change in the vaginal environment is necessary before the yeast exerts a pathological action. Candida associated vaginitis is seen predominantly in women of child bearing age.

A variety of factors have been implicated in triggering episodes of candidiasis that is transformation of asymptomatic carriage to symptomatic vaginitis. [35]

Factors associated with Vulvovaginal candidasis

Studies have focused on immunopathogenesis, antibiotic use and hormonal influences. Women with VVC should be managed sympathetically as it can be a distressing and embarrassing condition.

Sexual factors

There is increase in the frequency of VVC at the time when most women begin regular sexual activity. VVC is not a sexually transmitted infection, but there is limited evidence that women receiving oral sex may be at a greater risk of contacting the disease.[38]

Yeasts usually are not acquired through sexual intercourse. Evidence supporting sexual transmission in yeast vulvovaginitis includes :[39]

(a.) a fourfold increase in yeast colonization in male partners of infected women and
(b.) isolation of the same strains in infected couples.

Contraception

The role of oral contraceptives (OCs) is controversial. **Oriel et. Ala**.[39] found that OC users were more likely to have yeast isolates in vaginal cultures (32% of 241 women taking OCs vs.18% of women not taking OCs), but symptoms or signs of vaginal yeast infections were not increased (23% OC vs.26% no OC). Other investigators also reported increased vaginal carriage among OC users. However recent studies of low-dose OCs have consistently reported no increase in Candida isolation among OC users.

Proposed mechanism include increased adherence or receptivity, increased vaginal glycogen, and increased yeast virulence.

HIV

Candida is the most common cause of opportunistic mucosal infection in human immunodeficiency virus positive women. Intra Uterine Device – probably increases the incidence of VVC. [40]

Pregnancy

VVC is more common in pregnant women, with a higher prevalence of both asymptomatic colonization with candida albicans and symptomatic candidiasis throughout pregnancy.

Study made by Haussmann showed that when a pregnant woman who is free from infection is inoculated with infected material, she develops symptoms of vaginitis. High incidence of cases has been found in the women in the later part of pregnancy harbouring the organism without symptoms.

The high levels of reproductive hormones by providing higher glycogen content in the vaginal environment provide an excellent source for candida to grow and germinate.[35]

Hygiene and sanitation

Spinillo et al 1995 25 reported douching as a risk factor in women who are predisposed to recurrent bouts of VVC caused by candida glabrata. Tampons –There is no evidence that tampons cause VVC. Sanitary towels – There is limited evidence that sanitary towels may cause episodes of thrush.[36] It is commonly suggested that wearing tight fitting undergarments predisposes to yeast infection by increasing local humidity and temperature.

Diabetes

Diabetes is known to increase the risk of candida infection in the skin and vagina, possibly because a high glucose environment encourages the proliferation of candida.[32] It has been suggested that improving glucose control in these women may reduce the risk of VVC.

Antibiotics

It has been identified as a risk factor for VVC in some women. A prerequisite for infection appears to be vaginal colonization by candida organism.

An association between lack or loss of vaginal lactobacilli and hydrogen peroxide production, and susceptibility to VVC as not been established in women in whom infection develops while they are taking antibiotics. [32]

Relapses

Recurrent VVC is defined as four or more episodes of candidiasis in one year. It is present in > 5 % women of child bearing age. Treatment failure in uncomplicated VVC is unusual, if symptoms have cleared up after seven to fourteen days, then high vaginal swab is taken and sent for culture. [36]

Clinical features

The main symptom of vaginal candidiasis is itching of the vulva and perivaginal mucosa. External dysuria, caused by urine contacting the irritated vulva is sometimes present. Examination reveals cervical erythematic.

Extreme pruritus may lead to excoriations of the vulva. A curdy white discharge may be present, and satellite papules are occasionally seen. The adherent white cottage cheese discharge in vagina has 50 % sensitivity and 90 % specificity.[42]

Diagnosis

The relative lack of specificity of symptoms and signs precludes a diagnosis that is based on history and physical examination alone. The most common candida specific symptom is pruritis without discharge.[35]

Vaginal fluid in the normal pH range of 4.0 – 4.5 is indicative of VVC. A higher pH is suggestive of bacterial vaginosis or trichomoniasis.

Wet Mount or saline preparation should be done routinely, not only to identify the presence of yeast and mycelia, but also to exclude the presence of clue cells and Trichomonads. It is not sensitive or specific for yeast.

KOH preparation (10 %) is useful and the test sensitivity is 50 %. It is done to look for pseudohyphae or budding yeast forms.

Culture – Fungal culture is rarely performed. It is very helpful in certain cases:

a.) confirm asymptomatic carrier of vaginal candida

b.) Identify cause of recurrent vaginitis

PAP staining is unreliable as a diagnostic test. Diagnosis requires a correlation between clinical findings, microscopic examination and finally culture of vaginal secretion. There is no reliable serologic technique for the diagnosis of symptomatic candidial vaginitis.

Treatment

Oral treatments are popular, most commonly a single dose of fluconazole. Oral itraconazole and ketoconazole have also been used successfully [43-46]. A systematic review of oral vs. vaginal azoles showed similar efficacy, but more side effects occurred with oral therapy[47]. Gastrointestinal side effects occur in up to 15% of women[48].CDC recommends oral treatment with150 mg tablet single dose. Intravaginal agents recommended are butoconazole 2% cream 5g for 3 days or clotrimazole 1% cream 5g for 7-14 days or clotrimazole 100 mg vaginal tablet, single application or miconazole 2% cream for 7 days or miconazole 200mg suppository one suppository for 3 days.

About 5% of women diagnosed with VVC will have frequent recurrences, 4 or more per year. Current therapies are fungistatic rather than fungicidal, so the yeast are reduced but not eradicated. Hypersensitivity and allergic reactions to topical preparations may be confused with recurrences.

Experts recommend that, if wet mount or culture results confirm recurrent vaginitis, topical therapy should be increased from 5 to 7 days up to 10 to 14 days, or that a second oral fluconazole tablet be given 3 days after the first.[45] Women with severe cases of VVC also benefit from 2 sequential doses of fluconazole given 3 days apart.[50]

Suppressive therapy may be used after initial treatment for 6 months or more. Suppressive therapy options include oral fluconazole 150 mg or vaginal clotrimazole 500 mg once a week, oral or intravaginal nystatin twice weekly, and oral itraconazole 200 mg monthly.[49-51]

Non-*albicans* species tend to be more resistant to oral and topical azoles .[52-54] If this species is detected on culture, a long course of suppressive therapy should be attempted .[49]

TABLE:7

Recommended Treatment of Vulvovaginal Candidial Infection

Intravaginal agents
Butoconazole 2% cream5g for 3 days **Or** (5g sustained release) once
Or
Clotrimazole cream 1% for 7-14 days Or 1% cream,5g intravaginally 7-14 days Or 100mg tablet intravaginally for 7 days Or 100 mg tablet intravaginally,2 tablets for 3 days
Or
Miconazole 2% cream5g intravaginally, for 7 days Or 100 mg suppository intravaginally for 7 days Or 200 mg suppository intravaginally for 3 days Or 1200 mg suppository intravaginally once
Or Nystatin100,000-unit tablet for 14 days
Or Tioconazole 6.5% ointment,5g once
Or Terconazole 0.4% cream, 5g for 7 days **Or** 0.8% cream,5g for 3 days **Or** 80 mg suppository for 3 days
Oral agent
Fluconazole 150mg oral tablet once

(From centres for Disease Control and Prevention, 2006).

Recurrences

Incidence is 5% of women during their reproductive age. Women with RVVC have a modest increase in the frequency of attacks caused by non-*albicans Candida* species reaching approximately 10–15%.

Many women will attribute a persistent vaginal discharge to recurrent thrush infection. However, diagnosis based on clinical features alone is unreliable

and as many as half of women who believe they have thrush will have a different condition causing their symptoms.

After cessation of maintenance suppressive therapy, which is usually recommended for approximately 6 months, 60–70% of patients will relapse, symptomatically, within 1–2 months of stopping the antifungal therapy. [52]

The failure of the fungistatic azoles to eliminate organisms from the lower genital tract, co-exist with the persistence of the immunodeficiency or aberrant immunoresponse in the vagina to the offending pathogen is the mechanism.

With proven recurrences caused by identical organisms, one has no alternative but to reintroduce an induction regimen followed by the maintenance regimen, which should then be given for approximately 12 months. [53]

TABLE: 8

Treatment and Prevention of recurrent Vulvo vaginal candidiasis[51]

Step-1 Eradication regimen
Fluconazole 150 mg orally on day 1,4,and 8
Or Intravaginal Azole for 14 days
Or Ketoconazole 400mg daily for 14 days
Step-2 Prevention regimen for 3-6 months
Fluconazole 150 mg orally weekly
Or Ketoconazole 100 mg orally daily
Or Itraconazole 100 mg orally every alternate day
Or Clotrimazole 500 mg vaginally weekly
Or Any topical azole, daily

TRICHOMONIASIS

The Trichomonas Organism is a protozoon, actively motile, slightly larger than a leucocyte and is anaerobic. Three types of Trichomonas identified are T.buccalis which is a normal inhabitant of the mouth, T-hominis habitant of the anal canal and rectum and T vaginalis, which is found in the vagina. It is a sexually transmitted disease.

Epidemology

- Causes 25% vulvovaginitis
- Almost always sexually transmitted & fomite transmission is rare.
- Incubation period 3-28 days
- Causative agent-Trichomonas vaginalis, a flagellated anerobic protozoan.
- Trichomonads are identified in 30% to 80% of male sexual partners of infected women. In men, it is most often an asymptomatic carrier state. It causes non gonococal urethritis (10%) & these infections are mostly asymptomatic.
- Prevalence in pregnancy -7-13% & associated with Preterm rupture of membranes, Preterm loss, low birth weight infants, post-hysterectomy cellulitis & increased acquisition of HIV.

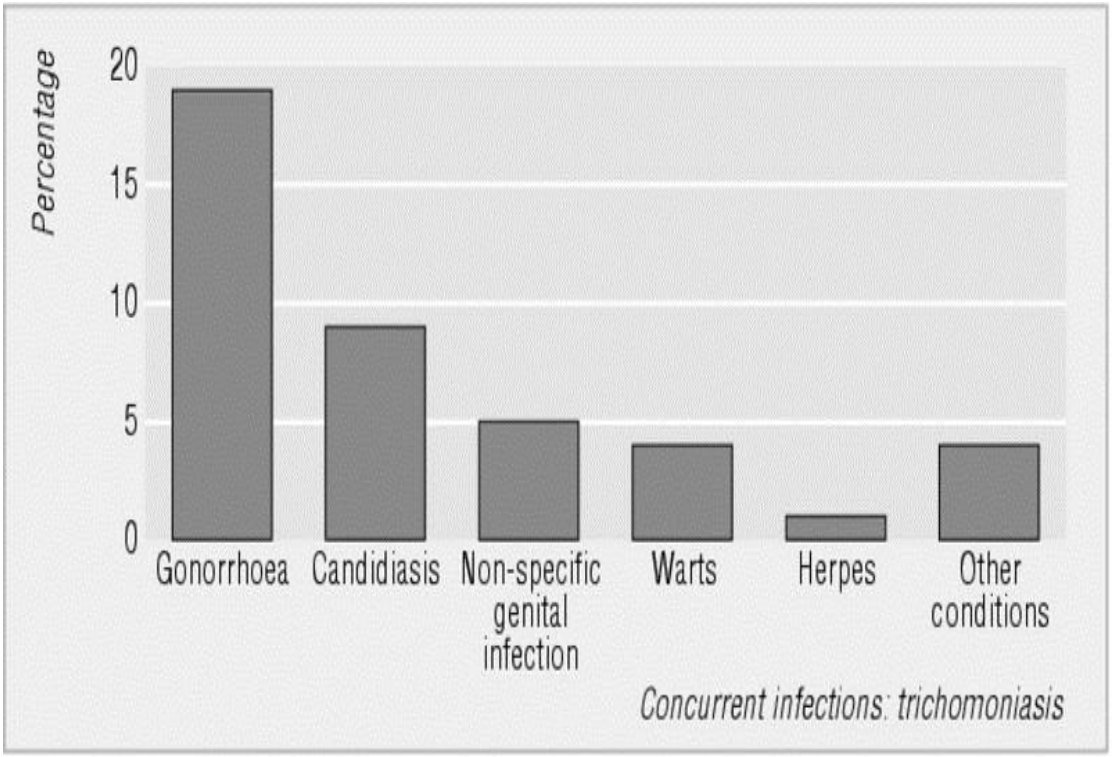


Figure: 2

Concurrent STIs found in women with *T* vaginalis (Mitchell 2004)

Pathogenesis

(Donne in 1837)[54] first identified T vaginalis as motile organisms in women presenting with vaginal discharge. Its adherence to the vagina is favoured by a low resistance and when pH is raised as during menstrual period, pH is within the range of 5.5 to 6.0. **Robinson and Mirchandani's** [56] studies showed that one or both the organism i.e., Trichomonas and candida could be found.

Trichomonads bind equally well to live or dead vaginal epithelial cells (vecs).[57] No difference in cytoadherence level was detected by different isolates to vecs from the same patient compared with adherence to vecs from normal individuals.

Trichomoniasis has long been considered as a STD. Recent studies by Barbone F et al 1990 have demonstrated the absence of Trichomonas in the vagina of adolescent females.[58] During pregnancy due to increased

hyperaemia and glycogen content of the vagina there was growth of Trichomonos.[59]

Clinical Manifestations

T. vaginalis infects the vagina and ectocervix, it is often symptomatic. The urethra, bartholin's and skene's glands are other common sites of infection.

A frothy yellow-green discharge is often cited as the typical finding, but in objective series, frothiness was detected in only 12%-34%.Gardner and Dukes described the colour of the discharge as gray in 46% of cases, yellow-green in 36%,and yellow-gray in 10%. Vaginal discharge (more than 50%) often dating from menstruation is copious, frothy (35%), homogenous, gray or yellow-green & discharge may be malodorous.Pruritus is less common than discharge, rarely vaginal, vulvar soreness, dysuria and dyspareunia.

The vaginal walls and vulva are erythematous and punctate haemorrhages are sometimes seen on the ectocervix which is known as "**strawberry cervix**" or "**flea-bitten cervix**". Colposcopic evidence of strawberry cervix had 45 % sensitivity and 99 % specificity for the presumptive diagnosis of T. vaginal infection **Paavonen. J 1988.**[60]

Approximately 50% of women with T.vaginalis are asymptomatic, but about 30% of these will asymptomatic women will develop symptoms when they are observed for 6 months.

Laboratory Investigations

1. Vaginal Saline Mount

It is in use for more than 150 years long and the main stay for the diagnosis. Approximately 60 to 70 % of infections are detected by this method. However, sensitivity of the wet mount is up to 60 % in the hands of microbiologist and up to 92 % in those of a highly trained researcher. This test is inexpensive and has 100 % specificity.[61]

2. Papanicolaou smear

The diagnosis of trichomoniasis is possible on a Papanicolaou (pap) smear, but the sensitivity of this method is modest (52-67%).There is low specificity, even when the smear is prepared with a vital stain such as acridine orange.

3. Culture

Culture is considered as the gold standard in the diagnosis of Trichomonas infection. They grow best under anaerobic condition. Diamond or modified Kupferberg media is considered superior however the greater cost of the procedure, and the need to allow three to seven days for growth of the organism before the diagnosis is confirmed, is disadvantageous.

4. Direct immunofluorescence assay

It has been found to be more sensitive than the wet mount i.e., 80 to 90 %.

5. Direct Enzyme immunoassay

Sensitivity is 80 to 90 %. Fast turnaround diagnosis is possible. Specificity is 99 to 99.5 %, some false positive cases occur. It is more expensive than culture.

6. Urine

Urine sediment can be examined for trichomonads, and this is the preferred site for detection in males. The sediment can be cultured as well as inspected microscopically. Women with trichomonal infection should be tested for other sexually transmitted infections. Additionally, sexual contact should be referred for evaluation.

Treatment

Current treatment for trichomoniasis is oral metronidazole, given as a 2-g single dose, 250 mg, 3 times daily for 7 days or 500 mg twice a day for 7 days. Treatment should also be given to the woman's partner .[62] Intravaginal therapy of metronidazole is not effective, probably due to the parasite's presence in inaccessible areas such as the vaginal glands and urethra.[63, 64]

Short-term treatment is, comparable with long term treatment, with similar rates of nausea and vomiting .[62] A 1.5-g single-dose treatment has been shown to be equivalent to 2 g .[65] The incidence metronidazole resistant trichomoniasis has been estimated at 5%.[66] In such cases, higher-dose therapy may be still be effective.

TABLE: 9

Recommended Treatment of Trichomoniasis: [68]

Primary therapy
Metronidazole single 1-g dose orally
Or
Tinidazole single 2-g dose orally
Alternative regimen
Metronidazole 500mg orally twice daily for 7 days

(From centres for Disease Control and Prevention, 2006).

Adverse effects may include a metallic taste and a disulfiram like reaction if combined with alcohol during use and for 24 hours following metronidazole therapy and for 72 hours after Tinidazole. For low to moderate resistance, 2 to 2.5 g daily for 3 to 10 days has been recommended.[67]

Intravenous high-dose metronidazole, 2 g every 8 hours for 3 days, has been reported to successfully treat highly resistant trichomonas.[68]

Patients who become asymptomatic or who are asymptomatic do not require re-evaluation. However, recurrence occurs in approximately 30% patients. Condom use may be protective.

CHLAMYDIA TRACHOMATIS

Chlamydia is obligatory intracellular bacterial pathogens. They are unique with characteristics of both viruses and bacteria. Although they were first isolated in 1930, it was not until 1957 that Chlamydia was recognised as the causative agent for a triad of infections. Chlamydia Trachomatis is a common

sexually transmitted organism found in 1 – 3 % of the general female population, 15 – 40 % of the woman seen in sexually transmitted disease clinics and 45 – 68% of the female partners of men who are culture positive for Chlamydia trachomatis.[69]

Three species of Chlamydia produce human disease. C.psittaci causes acute respiratory infections. Infection, Serotypes L1, L2 and L3 causes Lymphogranuloma venereum. A, B1, B2 and C cause's Trachoma. TWAR produces respiratory disease.
Serotypes D, E, F, G, H, I, J, K causes female and male genitourinary infections.

Symptoms

This obligate intracellular parasite is dependent on host cells for survival. It causes columnar epithelial infection. These presenting symptoms reflect endocervical glandular infection, with resultant mucopurulent discharge or endocervical secretions. If infected the endocervical tissue commonly oedematous and hyperaemic. Urethritis is another lower genital infection that can develop, and dysuria is prominent.

Chlamydial Cervicitis

The three major causes of cervicitis are Gonorrhoea, HSV2 infections and C.trachomatis. Clinically they manifested as yellow endocervical mucopus. The term hypertrophic follicular cervicitis was introduced by Dunlop et al to describe the characteristic oedematous and friable transformation zone frequently seen in woman with chlamydial cervicitis.

It was also reported that colposcopic features of immature squamous metaplasia of the cervix was associated with chlamydial infection. There is a reported incidence of increase risk of C.cervicitis with uses of oral contraceptive pills.[60]

Diagnosis

Microscopic inspection of secretions following a saline preparation typically reveals 20 or more leucocytes per high power field. More specifically culture NAAT, and enzyme linked immunosorbent assay (ELISA) are available for endocervical specimens. Alternatively a combined gonococcal and chlamydial test is widely used. If *C trachomatis* is diagnosed or suspected, then screening for other STDs is indicated. Moreover sexual partner should be counselled, tested, and treated or referred for evaluation.

Role of cytology

The use of routine, papanicolaou stained cervical smears to diagnose C.trachomatis is attractive and has been advocated for many years. The concept of detecting C.trachomatis in cervical papanicolaou smears was first advanced by Naib in 1970. He described the cytology of the TRIC agent in both papanicolaou stained conjunctival smears from infants with neonatal inclusion conjunctivitis and cervical smears. The smears demonstrated perinucluear, thin, membrane – lined vacuoles from 0.5 – 3 micro meters in diameter that contained numerous small inclusions.

Type I inclusions correspond to the coccoid body described by Gupta et al. They are composed of small, poorly defined, single or multiple vacuoles with, small, granular inclusions. Type II are, inclusions which were defined as a medium to large intracytoplasmic vacuoles with a crisp border. Type III inclusions were medium to large intracytoplasmic vacuoles containing multiple small inclusions. C.trachomatis was associated with transformed lymphocytes, hystiocytes and PMNLs.

Inclusion containing vacuoles were seen predominately in the metaplastic cells and occasionally in endocervical type cells. C.trachomatis is an obligatory organism with a tendency to induce chronic inflammation and cervical carcinogenesis.[70]

High incidence of Chlamydia infection in mothers having preterm birth has been reported.[71] C.trachomatis is associated with cervicitis, salphingitis and endometritis.[72]

TABLE: 10

Recommended Treatment of Chlamydial Infection[73]

Primary treatment
Azithromycin 1g orally once
Or
Doxyclclin 100mg orally twice daily for 7 days
Alternative treatment
Erythromycin base 500mg daily four times for 7 days
Or
Erythromycin ethysuccinate 800mg orally four times for 7 days
Or
Ofoxacin 300 mg orally twice daily for 7 days
Or
Levofloxacin 500 mg orally for 7 days

(From centres for Disease Control and Prevention, 2006).

Following treatment resting is not recommended if symptoms resolve. To prevent further infection, abstinence is recommended until a woman and her partner are asymptomatic.

ACTINOMYCES

Actinomyces is a predominant pathogen. In humans, actinomycosis is a normal inhabitant of the oral cavity. Actinomyces is a micro aerophilic filamentous pleomorphic bacterium. It is difficult to identify it in the female genital tract because of the abundant other filamentous and an aerobic bacteria in the female vagina.

In pelvic actinomycosis the roots of infection discovered have been haematogenous,by direct spread from an abdominal focus or ascending via the lower genital tract after orogenital contact or from intestinal colonization.[74]

The disease presents with one of the three regional forms: Cervicofacial, abdominal or thoracic. Gupta et al described collection of mucus, leukocytes and bacteria that he believed could help to make the diagnosis of actinomycosis by cytologic means .[75] Actinomycetes has been recognised in association with pelvic inflammatory disease – PID. [76]

The association of actinomyces like organism on cervicovaginal papanicolaou smear with the use of an intra uterine device is well established. [77]

Treatment

Prolonged courses of penicillin for 6-12 months are recommended. Tetracycline is recommended for patients allergic to penicillin. Erythromycin, minocycline, clindamycin and cephalosporin's can also be used.[73]

GONOCOCCI:

Gonorrhoea is a common sexually transmitted disease which is caused by Nisseria gonorrhoeae. N - gonorrhoeae is a Gram negative kidney shaped diplococcus belonging to Genus Nisseria. Gonococcus was first described in pus by Neisser in 1879.Gonococcal infection among women is frequently asymptomatic. For this reason, it is essential that women at risk be screened periodically.Risk factors for gonococcal carriage and potential upper reproductive tract infection are: age less than 25 years, the presence of other sexually transmitted infections, a history of previous gonococcal infection, new or multiple sexual partners, lack of barrier protection, drug use, and in commercial sex workers.

Morphology

These organisms are diplococcus with slightly concave adjacent surfaces. Diplococci are often seen inside polymorphonuclear leucocytes. There are pili on the surface which promotes virulence by adhesion and inhibiting phagocytosis.

Clinical features

In woman aged less than 25 years 60 to 80 % of pelvic infections are caused by Gonorrhoea. PID (Pelvic Inflammatory Disease) can occur by ascending spread of genital commensals following surgical trauma, pregnancy, and IUD insertion. Symptomatic gonorrhoea may present as vaginitis or cervicitis. Those with cervicitis commonly describe a profuse odourless, non-irritating, and white-to-yellow vaginal discharge. Gonococcus can also infect the Bartholin's and Skene glands, the urethra, and ascend into the endometrial and fallopian tube to cause upper reproductive tract infection.[78]

Culture

Gonococci are fastidious in their growth requirements. Most of the time these are present along with normal flora. Growth occurs at pH of 7.2 – 7.6, temperature 35ºc to 36ºc and 5 to 10 percent carbon dioxide. Thayer Martin medium is the most common culture media used. Typical colonies are 0.5 – 1mm in diameter, grey, translucent and slightly raised. Presumptive diagnosis can be made by performing Grams stain, oxidase test and sepuroxol test. For definitive identification the isolate can be sent to reference laboratory. Urine sample for NAT (Nucleic Acid Testing) for Gonorrhoea.[78]

Complications

PID and acute salphingitis. Gram stain remains the simplest and quickest method for diagnosis. It has a high sensitivity and specificity of 90 %.

Treatment

Several of the newer quinolones, e.g. ciprofloxacin and ofloxacin have been used successfully as single- dose regimens, 500 mg and 400 mg respectively. Quinolone resistant strains have emerged in some Pacific and Asian countries, but CDC continues to recommend these in areas where there is no problem of resistance. Ceftriaxone is recommended by CDC for the treatment of uncomplicated gonorrhoea in a single, intramuscular injection of 125mg.Ceftixime is a useful oral alternative.[73]

TABLE: 11

Recommended Single-dose Treatment of Uncomplicated Gonococcal Infection

Ceftriaxone 125 mg IM
Or
Cefixime 400mg orally
Or
Ciprofloxacin 500 mg orally
Or
Ofloxacin 400mg orally
Or
Levofloxacin 250 mg orally
Plus
Treatment for Chlamydia infection if not excluded

(From centres for Disease Control and Prevention, 2006).

HUMAN PAPILLOMA VIRUSES (H P V)

Human Papilloma viruses are DNA Tumour viruses belonging to the family of Papovaviridae. It is a common infection; it disappears without causing any disease and treatment, only when the infection persists it can cause cervical cancer. Certain HPV types have been associated frequently with benign and malignant proliferatory lesions of adult genital tract epithelium. Eighty types of HPV have been isolated.

Some important ones are Condylomata acuminata – type 6 and 11. Dysplastic and Squamous cell carcinoma of uterine cervix - type 16 and 18, Leukoplakia – type 9,Oral Papillomas – type 10 [79].

HPV infection of the uterine cervix is one of the most common sexually transmitted disease, which is usually acquired around the time sexual activity begins. The most common cytopathologic manifestation of cervical HPV infections are low grade squamous intraepithelial lesions i.e., CIN – I. In a study of one year by Falls K R et al 1991 showed that Grade I CIN regresses spontaneously.[80]

Koilocytes are the predominant cellular features of infection with HPV. The Patho-gnomonic change in the koilocytotic cell is the large perinuclear halo with irregular clear cut edges with hyaline cytoplasm. These lesions occur in the transformation zone. Early detections of HPV can be done by using DNA hybridization techniques (Southern Blot hybridization, filter in situ hybridization). PCR was performed on exfoliated cervical cells.[81] HPV and HIV – woman infected with HIV are at a greater risk of being co-infected with HPV.[82]

HPV and Malignant transformation

For yet unknown reasons, when the infection doesn't resolve, high grade squamous intra epithelial lesions can develop and progress to cancer over a period of several years.

Herpes Simplex Virus (HSV)

It is plausible that HSV- 2 is a factor, but not the factor, in increasing the risk of cervical cancer said Horowitz.

He also underlined the fact that there may be a lot of other risk factors as well and HSV – 2 may be one of the many promoters of cervical cancer.[83]

Two serotypes of the virus infecting humans and sharing antigens between types 1 and 2 make serologic differentiation difficult. Type I is seen in the oral cavity and upper part of the body. Type II infection produced genital lesions. The prevalence of HSV – 2 has been steadily increasing. Primary infection with HSV – 2 lasts around three weeks. This infection may be asymptomatic or mild. It presents symptoms in 70 % of cases, like pain, localised pruritis, and dysuria, vesicular and ulcerative lesions, situated on the outer genitilia or cervix.

The most consistent risk factors for HSV – 2 seropositivity are female sex and cumulative risk of exposure including age, years of sexual activities and multiple life time sexual partners.[84]

In 1960's and 1970's some authors showed that HSV – 2 caused cervical cancer, but further studies did not prove the link. A recent study showed that there is a relationship between HSV – 2 and cervical cancer. Both HSV – 2 and HPV infect the tissues on the surface of the cervix, where invasive cervical cancer starts.

Herpes virus causes ulcerated lesions, they may serve has cofactors for HPV infections and subsequent cervical cancer by making it easier for the HPV virus to get deeper in to the cervical tissue. [83]

HIV – HSV interaction

The prevalence of HSV infections is considerably higher in HIV positive patients. Santos et al found 73 % positivity for HSV- 2 in serological testing of HIV positive patients.

Diagnosis

Cytopathology of exfoliated cells may permit diagnosis, but this depends on adequate sample collections. General sensitivity of this method is around 60 to70 %. Enzymatic serological tests for HSV antibodies (ELISA) are also available, but they present low sensitivity and specificity. The definitive epidemiological test has been the western blot.

ATROPHIC VAGINITIS

Atrophic vaginitis is a skin condition of the vulva and vagina. It occurs when a hormone called estrogen is lacking in the body. [85]

Clinical features

Problems with urination such as burning, frequency and pain. Vulvar itching, bleeding / spotting, pain with intercourse. Diagnosis is made by elevated pH, predominance of parabasal cells. Condition is treated by estrogen therapy.

AEROBIC VAGINITIS

Aerobic vaginitis is a term proposed to describe purulent vaginal discharge with predominance of abnormal aerobic flora.[86]

Aerobic vaginitis, which may be severe, has been reported as the cause of 5% of cases in a series from a specialty vaginitis clinic. The usual predominant microorganisms are Group B Streptococci, *Escherichia coli*, and *Staphylococcus aureus*. It is likely that less severe cases of aerobic vaginitis are not recognized in the primary care setting and are treated as BV or resolve spontaneously. The case series referred to above also reported good therapeutic response to 2% topical Clindamycin .[87]

NONINFECTIOUS VAGINITIS

Non-infectious causes of vaginal discharge include physiologic, irritant and allergic, cytolytic vaginitis, desquamative inflammatory vaginitis, collagen vascular disease, and idiopathic vaginitis.

Irritant and allergic vaginitis may result from sensitivities to topical medications, the active or base ingredients of spermicidal products, douching solutions, and the latex of condoms or diaphragms. If a woman with persistent symptoms has been using such intravaginal products, she should stop.

Cytolytic vaginitis is characterized by overgrowth of lactobacilli and cytolysis of squamous cells, including presence of cytoplasmic fragments and intact cells with naked nuclei.[88]

The cause is uncertain but may include a reaction to intravaginal medications or other products such as tampons. It can be found in up to 5% of women with symptoms and signs of vaginitis.[88,89]

Symptoms often mimic VVC and may include a white, cheesy discharge. Vaginal pH ranges from 3.5 to 5.5. Recurrences during luteal phase of the menstrual cycle have been described.[90]

Intravaginal antifungals should be discontinued. Baking soda sitz baths or douches are often used, but clinical trial data to support this practice are lacking.

Non-infectious desquamative inflammatory vaginitis (DIV)

Although desquamative inflammatory vaginitis was first described nearly 50 years ago, this entity has not been well studied nor widely recognised.[91] The diagnosis is based upon the presence of a heavy, often frothy discharge, an elevated pH, and a purulent vaginitis with mild vaginal erythema. Wet mounts are diagnostic and show large numbers of polymorphonuclear leucocytes and parabasal cells, but no clue cells. In addition there are few lactobacilli in the background bacteria, but there are abundant coccoid bacilli.

The differential diagnosis of desquamative vaginitis is extensive. Because of the outpouring of white blood cells, trichomoniasis must be ruled out, and the laboratory picture often looks like atrophic vaginitis. In any patient who has abundant white blood cells, cervical origin (from cervicitis) must be considered. The presence of foreign bodies must be determined, as well as necrosis and exudates secondary to a vaginal or cervical cancer. The dermatologic condition of erosive linchen planus also enters into the diagnosis.

Although there is not much overlap in the clinical picture between desquamative inflammatory vaginitis and BV, both entities have an elevated pH and a complaint of a discharge.[85]

It may occur at any time during the reproductive years or after menopause. There is probably a heterogeneous group of causes of DIV. Some cases may correspond to a disorder within the spectrum of lichen planus.[91]

Most observers have identified a good response to Clindamycin vaginal cream when used in a usual course, but relapse is common. In postmenopausal or other estrogen deficient women, supplemental hormone replacement therapy may be helpful, especially when there is relapse. There may be some response to local or systemic corticosteroid therapy .[84]

Iatrogenic

Drug induced

Drugs may induce a vaginal discharge either by alteration of the hormonal environment or by means of an allergic or inflammatory process. Hormonal contraceptives have long been associated with increased vaginal discharge secondary to the activity of oestrogen on the vaginal epithelium. Preparations such as the use of douches, vaginal creams, antiseptics, latex condoms or spermicides may precipitate an allergic reaction, resulting in chemical desquamation and facilitating the development of secondary infection.

Foreign bodies

The description of a highly offensive vaginal discharge, especially if blood-stained, should alert the physician to enquire about the possibility of a retained foreign body in the vagina. Patients are often too embarrassed to volunteer such information, or may have forgotten such an event having occurred. A foreign body is a well-recognized cause of vaginal discharge in

children. Vaginal ulceration and the development of subsequent infection, usually predominantly with anaerobic bacteria, can result. If present for some time, subsequent vaginal stenosis can shield the object from view during speculum examination, and plain X-ray or ultrasound examination may fail to identify certain non-metallic materials. Management is removal of the foreign body together with antibiotic cover where appropriate. [91]

Malignancy

The possibility of a genital tract malignancy should always be considered in women presenting with a persistent vaginal discharge, but particularly when a blood-stained discharge is reported in an older woman. Cervical, uterine, vaginal and ovarian malignancies have all been reported in association with vaginal discharge.

Other

Fistula

Vaginal discharge may be the presenting complaint associated with either a recto-vaginal or vesico-vaginal fistula. A history of pelvic surgery, radiotherapy or inflammatory conditions such as Crohn's disease should increase suspicion of a structural defect. Even a careful examination of the genital tract in clinic may fail to locate a small fistula, and full investigation should be instigated if there is a high index of suspicion.

Dermatological conditions

Although rare, vaginal discharge may be associated with dermatological conditions, particularly those causing ulceration or epithelial disruption affecting mucous

membranes. Pemphigus vulgaris, pemphigoid, Behçet's disease, idiopathic ulceration and erosive lichen planus have all been described presenting in this manner.

Pemphigus and pemphigoid are immunobullous diseases causing blistering of the skin and mucous membranes. Treatment is with high-dose steroids or alternative immunosuppressive agents.

Behçet's disease is a systemic vasculitis of unknown origin that is associated with oral and genital ulceration.

A cell-mediated immune reaction is thought to be the pathological process involved in erosive lichen planus. On examination of the vagina, ulceration or erosion of the vaginal epithelium is seen. Treatment is with topical or systemic steroids and regular dilation of the vagina to prevent the development of vaginal adhesions.[90]

MATERIALS AND METHODS

Source of Data

This study was carried out on 100 women presenting with vaginal discharge from December 2009 to May 2011 (1 ½ years) at the outpatient Department of Obstetrics and Gynecology in R.L. Jalappa hospital and research centre, Kolar.

Method of collection of Data

A prospective study of 100 women with vaginal discharge. A comprehensive history, general and gynecological examination was initially carried out. Formal consent was obtained prior to examination and collection of the specimen for investigations.

Selection criteria

Patients in the reproductive age group who presented with vaginal discharge for a variable period of time with or without associated vaginal discomfort, pruritus and burning sensation.

Exclusion criteria

1) Pregnancy.

2) Patients who had undergone treatment for the same symptoms within prior 48 hours.

3) Patients who were taking the prescribed treatment at the time of presentation.

4) Patients who had intercourse within prior 48 hours.

The clinical diagnosis was made on the basis of presenting complaints, associated symptoms and the characteristics of vaginal discharge on per speculum examination such as colour, quantity, consistency, odour, and signs of vaginitis or cervicitis.

Vaginal exudates were obtained for the following investigations

- pH estimation
- Amine test
- Wet mount/saline mount
- KOH mount
- Grams stain

Patients were treated on the basis of clinical diagnosis made at the first visit and modified, if needed, on the basis of laboratory findings. The treatment prescribed was according to the CDC regimen.

Follow up was done in all but 10 patients. Response to treatment on follow up was assessed primarily by relief of symptoms. The findings of clinical diagnosis, laboratory findings, and response to treatment on follow up documented and analyzed statistically.

Descriptive statistical analysis was carried out in the study. Chi square test/Fisher exact test was used to find the significance of study parameters on categorical scale between two or more groups. Diagnostic statistics such as sensitivity, specificity, PPV, NPV and accuracy have been computed to find the correlation of various tests/criteria with BV/TV/Candida.

The following tests were performed on the vaginal exudates

pH Estimation

The pH was measured using indication papers (Ranbaxy lab) ranging from 4.5 to 7.5 (in units of 0.5). A small strip of the roll was detached and touched to small amounts of discharge or gently rubbed against the vaginal wall. Color change was observed and matched against the indicator. Thus the pH of vaginal fluid was measured.

Amine Test (Whiff test)

One or two drops of vaginal discharge were taken on a clean glass slide and one or two drops of freshly prepared potassium hydroxide solution was added to it. Both were mixed and smelt immediately. The presence of a fishy (seminal) odor was taken as a positive test.

Wet Mount/Saline mount:

A drop of discharge was mixed with a drop of normal saline on a clean slide and topped with a cover slip. This was then observed first under low power and then under high power.

The presence of the following were identified and noted down:

I) Motile Trichomonads

II) Clue cells

III) Pus Cells (Leukocytes)

IV) Lactobacilli

V) Hyphae

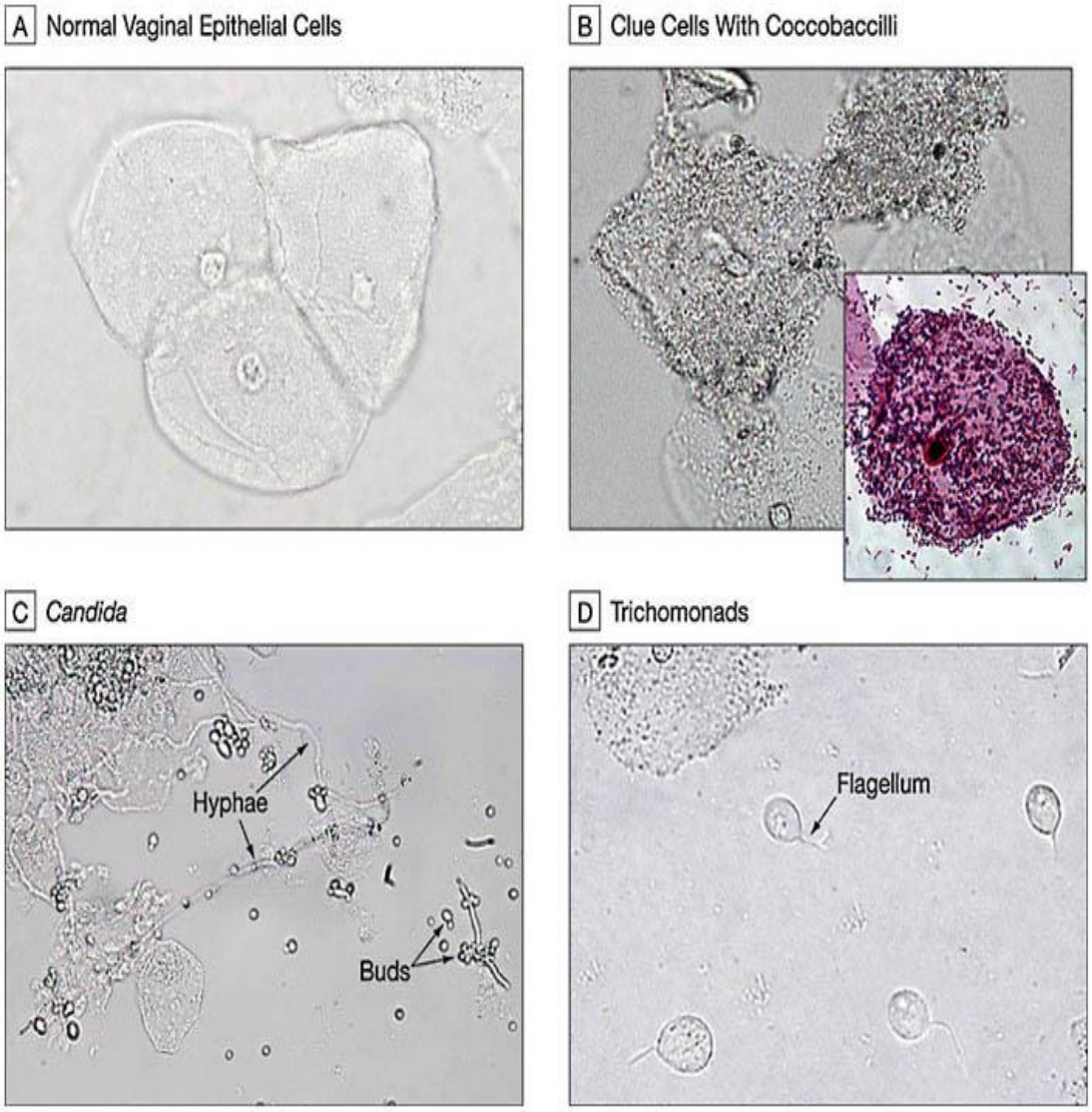


Figure: 3

Microscopic Examination of Vaginal Samples

A) Normal saline wet mount showing a clump of 3 normal vaginal epithelial cells

Normal saline wet mount showing 2 clue cells

B) Gram stain demonstrating how coccobaccilli on the surface of vaginal epithelial cells create the characteristic granular appearance and indistinct borders of clue cell.

C) Normal saline wet mount showing numerous *Candida* hyphae and buds.

D) Normal saline wet mount showing 4 trichomonads. Trichomonads can often be identified easily because of their characteristic jerky motility

KOH Mount

A small amount of secretion was placed on a slide and mixed with 10 % potassium hydroxide. The presence of fungal hyphae, spores and yeast cells were looked for, using the high power of the microscope.

Pap Smear

Smears were taken from the vaginal wall, posterior fornix and endocervix and, spread in sterile glass slides, dropped immediately into 95 % methanol for fixation and stained with Papanicolaou stain. Sterile cotton swabs were used for vaginal smears and Ayres spatula with endocervical brush were used to obtain cervical smears. In all cases, Papanicolaou smears were carefully evaluated as per the Bethesda System (2001).The presence of infecting organisms as well as cytological features, were noted.

Gram Stain

A smear of the vaginal discharge was made on a clean glass slide and heat fixed and gram stain was performed. The normal bacterial flora and associated anaerobic morphotypes were scored as per Nugent's criteria (1991). Also presence of other organisms like gonococcus, Candida were noted.

Culture

Vaginal swabs from the same discharge were also sent for culture and sensitivity. Bacterial culture was done on Mc Conkey Agar, Chocolate Agar , Blood agar and Thioglycolate broth, and Sabourauds dextrose agar for candida.

Diagnosis based on investigations

Trichomonas vaginalis was identified through microscopy of a wet mount, Pap smear and acridine orange staining of vaginal smear. Candidiasis was diagnosed when budding yeast cells and pseudohyphae were seen on a wet mount, KOH mount or Gram stain of a vaginal smear. Bacterial vaginosis was defined by Amsel's criteria (presence of at least three of the following: homogenous vaginal discharge, positive whiff test, pH > 4.5, and clue cells observed on a gram stained vaginal smear). *Neisseria gonorrhoeae* was detected through examination of a gram stain of a cervical smear and Pap smear.

OBSERVATION AND RESULTS

A total Number of 100 cases of women with vaginal discharge were studied during the period from December 2009 to May 2011 attending the outpatient department.

Age distribution

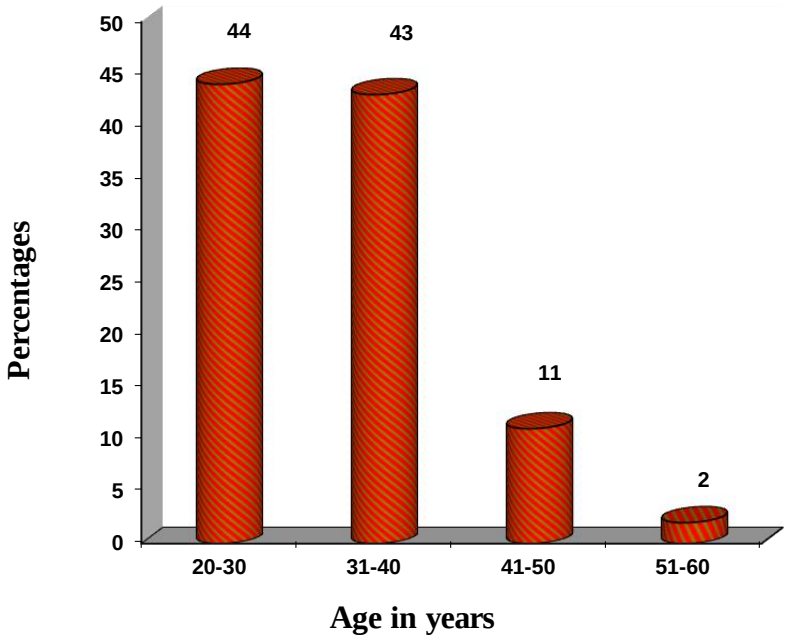
TABLE : 12

Age in years	Number of cases	%
20-30	44	44.0
31-40	43	43.0
41-50	11	11.0
51-60	2	2.0
Total	100	100.0

Mean ± SD: 33.31±7.47

Majority of the women were between 20-30 years of age**.**

Figure : 4



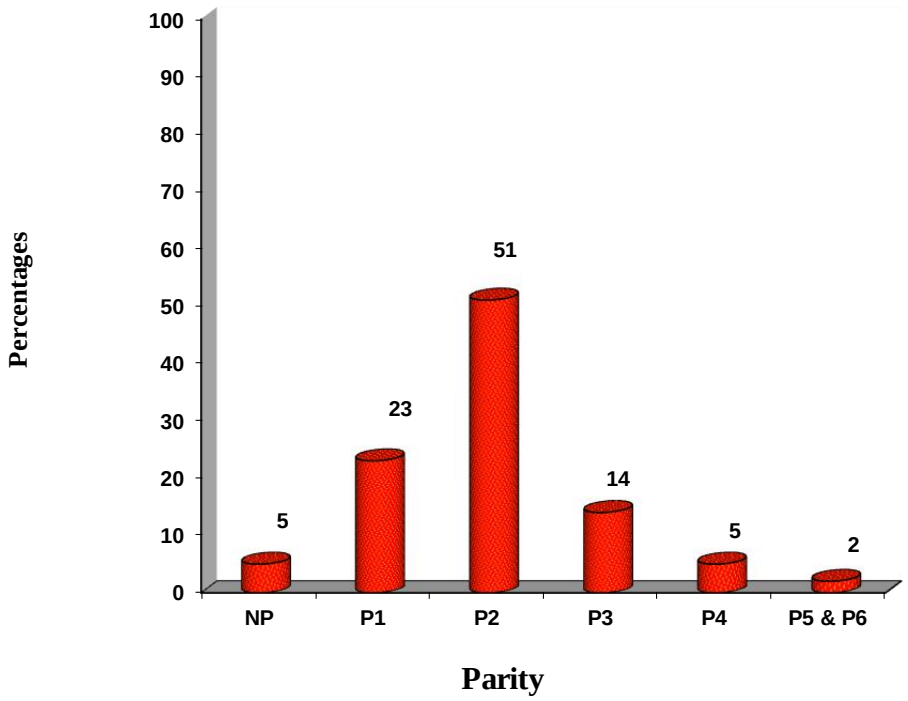
Parity distribution of patients studied

TABLE: 13

Parity	Number of cases	%
NP	5	5.0
P1	23	23.0
P2	51	51.0
P3	14	14.0
P4	5	5.0
P5 & 6	2	2.0
Total	100	100.0

5% of the women were nulliparous, while 23% had only 1 living issue 22% of whom were not tubectomized or taking any contraception.

Figure: 5



Clinical Features

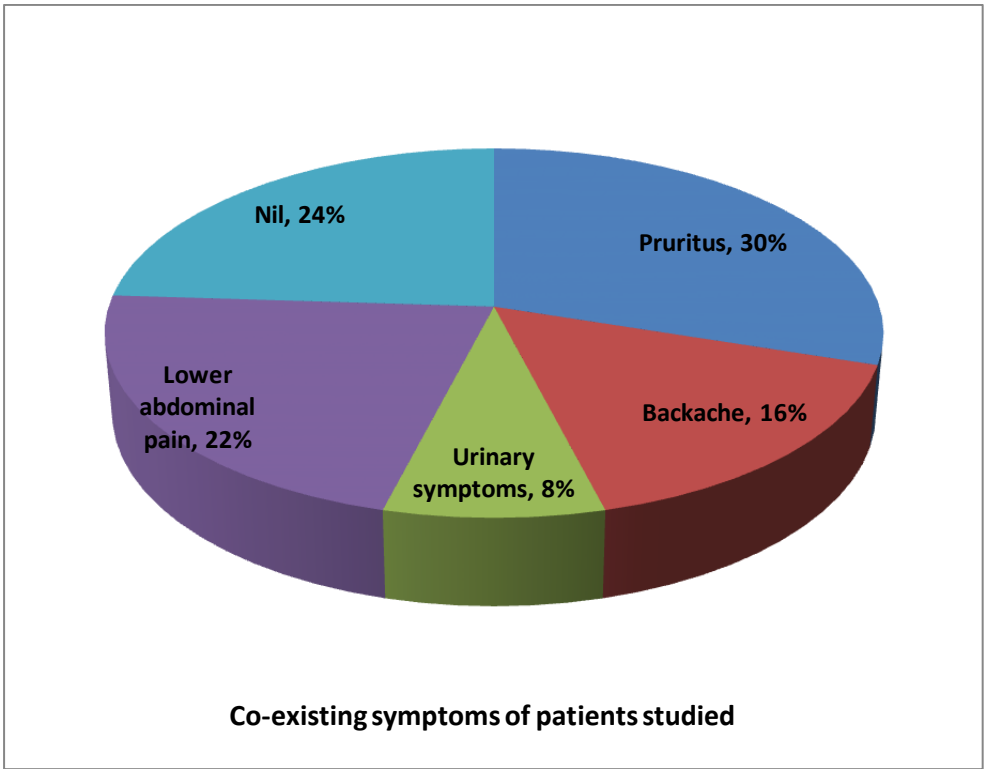
TABLE: 14

Co-existing symptoms

Co existing	Number of cases	%
Pruritus	30	30
Lower abdominal pain (LAP)	22	22
Backache (BA)	16	16
Urinary symptoms (BMU)	8	8

One or more additional symptoms were reported by 46 patients (46%). Most common co existing symptom was pruritus 30% followed by pain in lower abdomen (22%), lower backache (9%) and urinary symptoms (8%).

Figure: 6



Characteristics of vaginal discharge

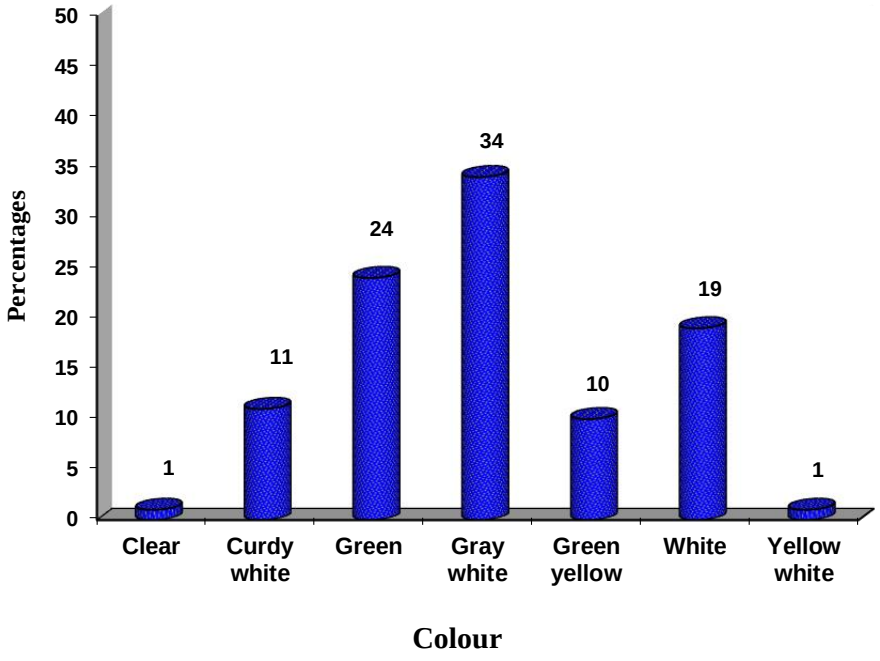
COLOUR OF DISCHARGE

TABLE: 15

Colour	Number of cases	%
Clear	1	1.0
Curdy white	11	11.0
Green	24	24.0
Gray White	34	34.0
Green Yellow	10	10.0
White	19	19.0
Yellow white	1	1.0
Total	100	100.0

34% patients had gray white discharge followed by green discharge.

Figure: 7



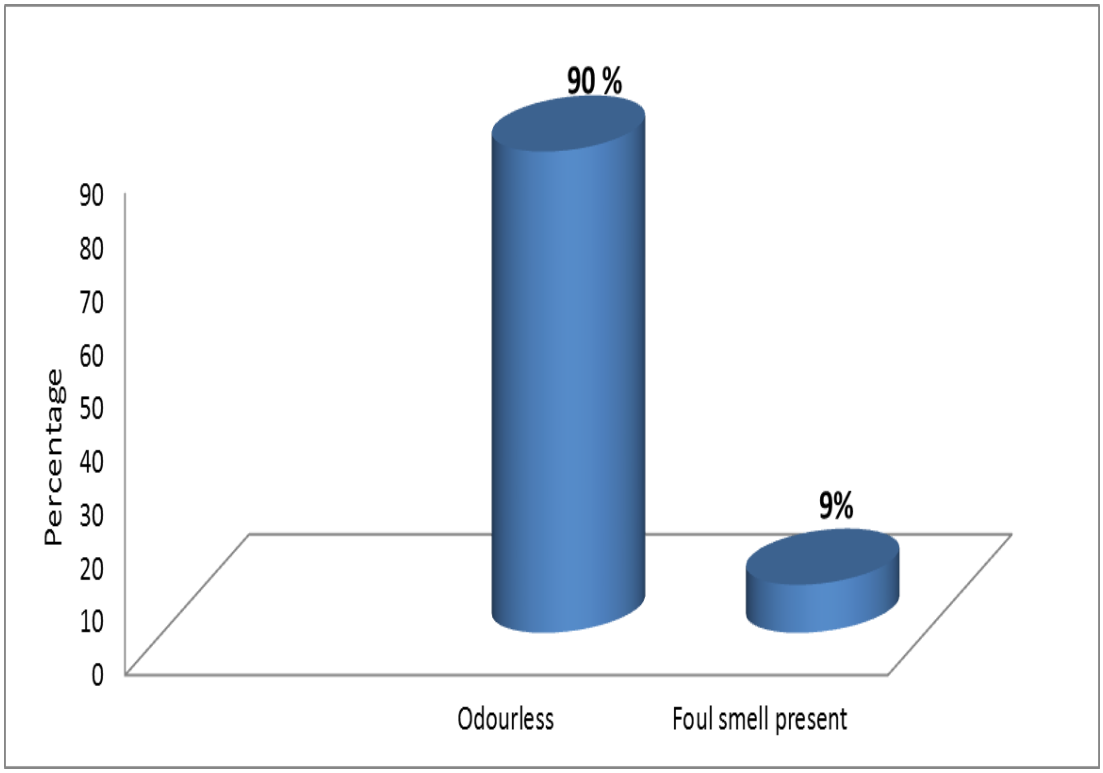
ODOUR OF DISCHARGE

TABLE: 16

Odour	Number of cases	%
Odourless	90	90.0
Foul smell present	10	10.0
Total	100	100.0

90% of the patients studied odorless vaginal discharge.

Figure : 8



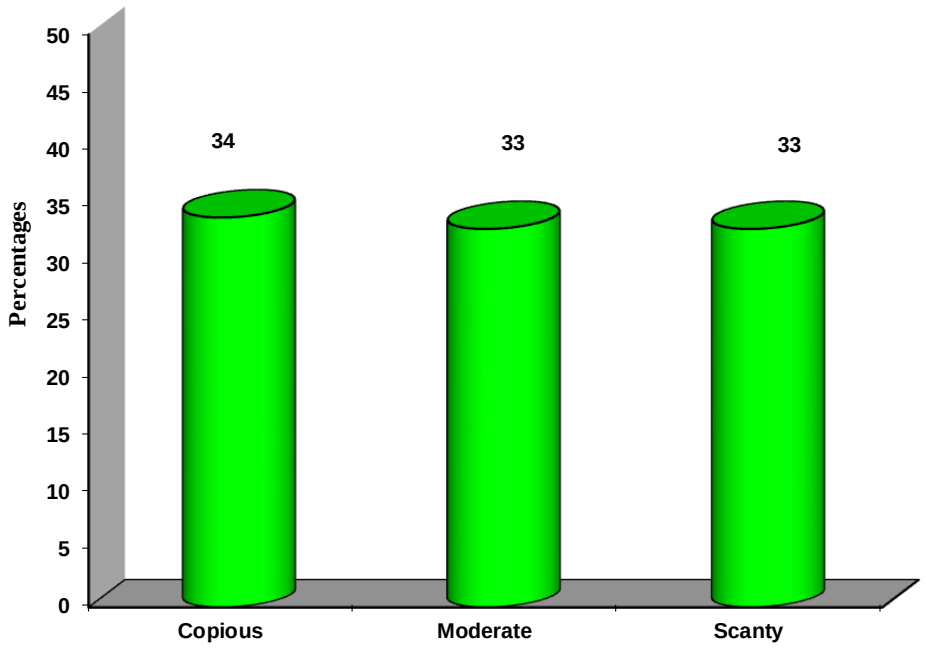
AMOUNT OF DISCHARGE

TABLE: 17

Amount	Number of cases	%
Copious	34	34.0
Moderate	33	33.0
Scanty	33	33.0
Total	100	100.0

34% of patients studied had copious discharge, 33% had moderate discharge whereas 33% had only scanty vaginal discharge on speculum examination.

Figure: 9



CLINICAL EXAMINATION

TABLE: 18

Clinical Examination	Number of cases (n=100)	%
P/S		
• Vaginitis	55	55.0
• Cervicitis	13	13.0
• Cervicitis and Vaginitis	24	24.0
• Cervix Vagina healthy	3	3.0
• Strawberry cervix	5	5.0
Total	100	100.0
P/V		
• FF, FT+	21	21.0
• FF, NT	79	79.0
Total	100	100.0

55% patients studied had features of vaginitis, and 13% had cervicitis, 24% patients had both cervicitis and vaginitis,5% patients had characteristic strawberry cervix or flea-bitten cervix suggesting trichomoniasis, whereas cervix and vagina was healthy in 3% patients. 21% patients had fornicial tenderness on bimanual examination.

Figure: 10

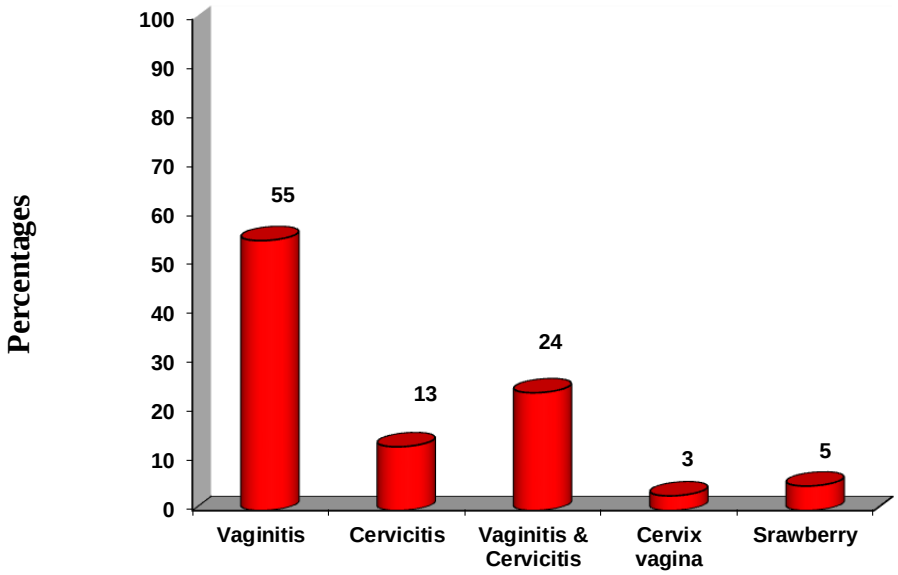
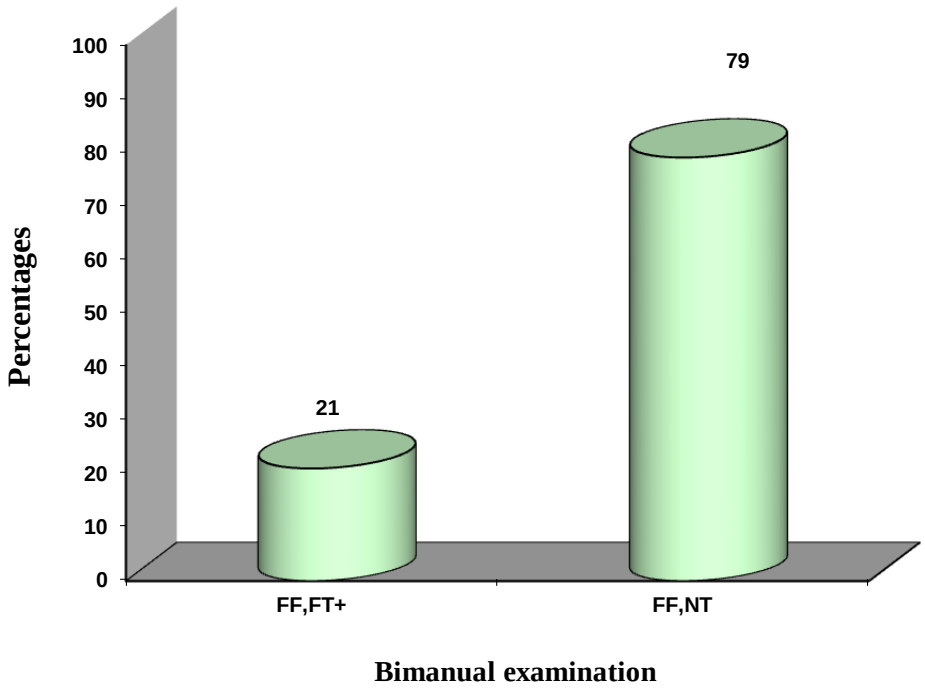


Figure: 11



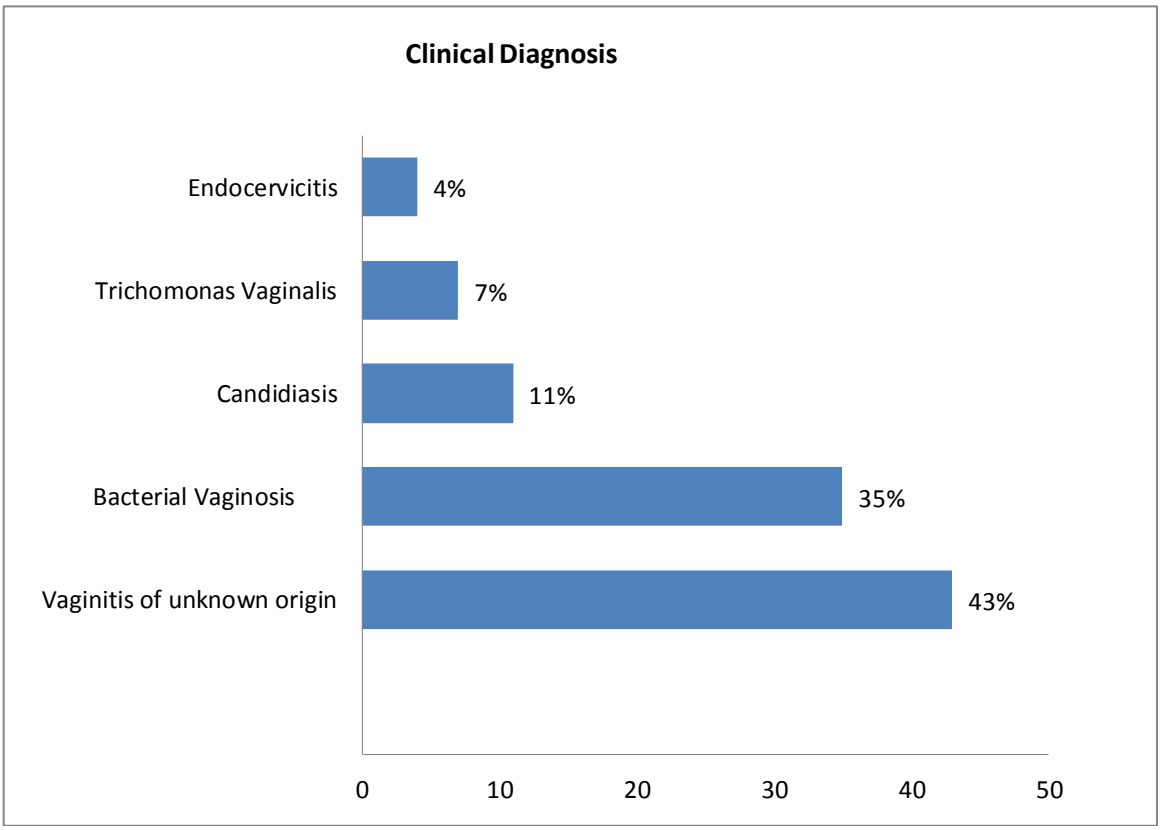
CLINICAL DIAGNOSIS

TABLE: 19

Clinical Diagnosis	Number of cases	%
Vaginitis of unknown origin	43	40.0
Bacterial Vaginosis	35	35.0
Candidiasis	11	11.0
Trichomonas Vaginalis	7	7.0
Endocervicitis	4	4.0
Total	100	100.0

A clinical diagnosis of Bacterial vaginosis was made in 35 cases, candidiasis in 11 patients, trichomosiasis in 7 cases, 4 cases of endocervicitis, and 43 cases of vaginitis of unknown origin.

Figure: 12



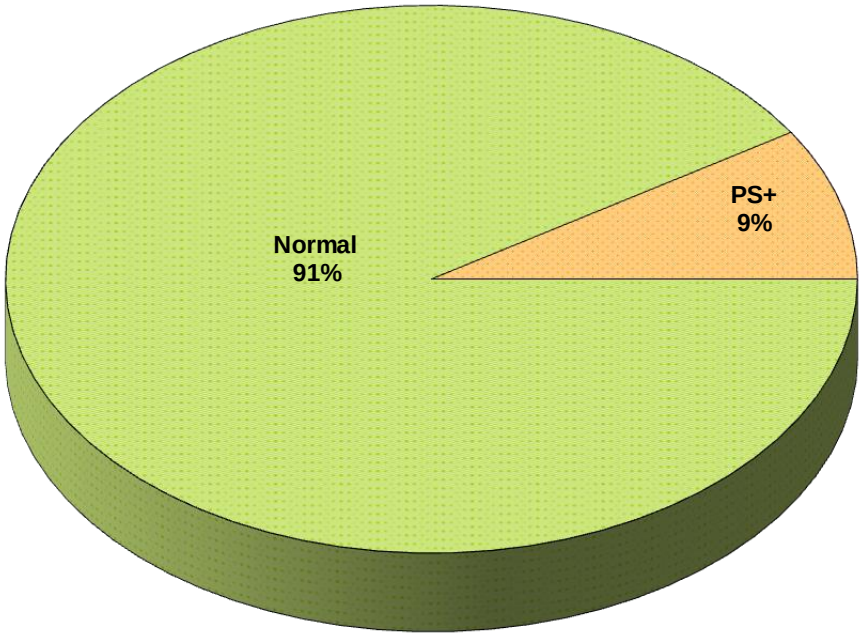
URINE ROUTINE

TABLE: 20

Results	Number of cases	%
Normal	91	91.0
Pus cells present	9	9.0
Total	100	100.0

9 patients had pus cells in their urine sample on microscopy.

Figure: 13



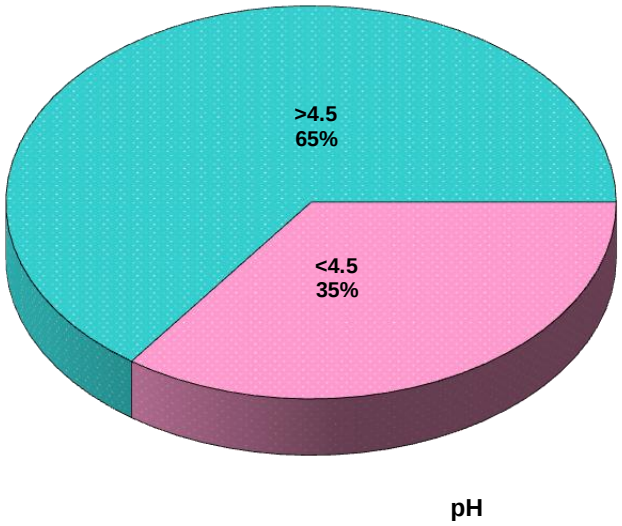
pH OF VAGINAL DISCHARGE

TABLE: 21

pH	Number of cases	%
• Less than or equal to 4.5	35	35.0
• >4.5	65	65.0
Total	100	100.0

65% of the patients in the study group had pH more than 4.5

Figure: 14



10% KOH MOUNT FOR YEAST CELLS

TABLE: 22

KOH Mount	Number of cases	Percentage
• Negative	90	90.0
• Positive	10	10.0
Total	100	100.0

10% KOH mount for yeast cells was positive in 10 cases.

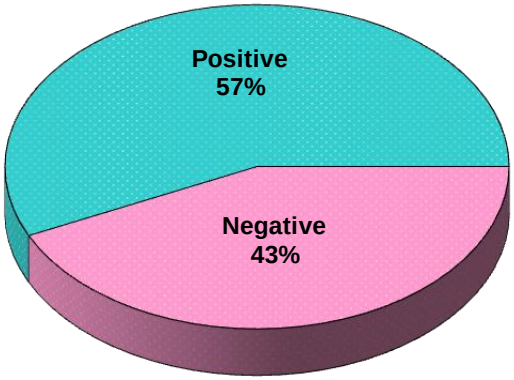
SALINE MOUNT

TABLE: 23

Saline Mount	No of cases	Percentage
• Negative	43	43.0
• Positive	57	57.0
Total	100	100.0
1. Clue cells	48	48.0
2. Hyphae	5	5.0
3. Motile Trichomonads	4	4.0

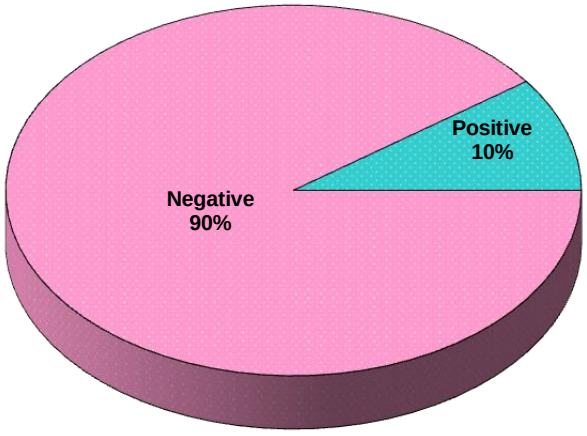
Saline mount was positive in 57 cases, detected clue cells in 48%cases, hyphae in 5% and motile trichomonads in 4 cases.

Figure: 15



Saline Mount

Figure: 16



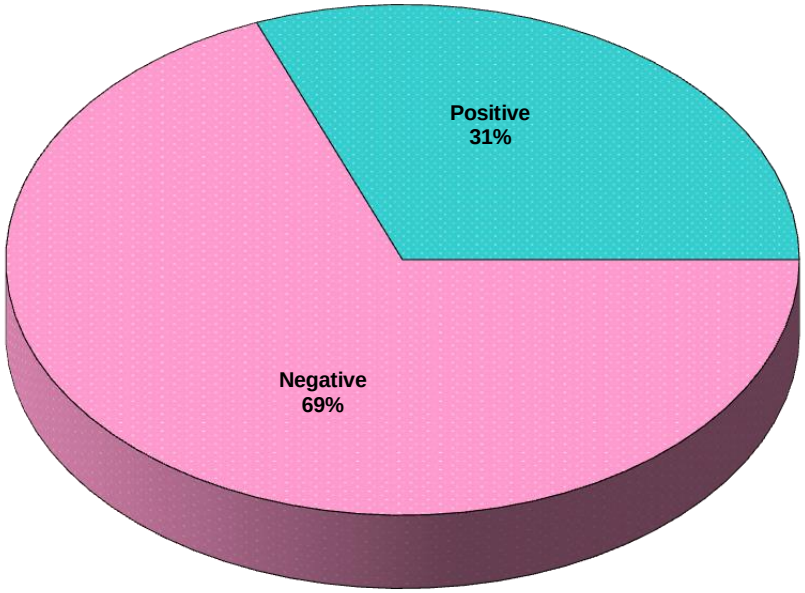
KOH Mount

WHIFF TEST FOR BACTERIAL VAGINOSIS

TABLE: 24

Whiff Test	Number of cases	Percentage
• Negative	69	69.0
• Positive	31	31.0
Total	100	100.0

Whiff test or Amine test was positive for Bacterial vaginosis in 31 cases.



Whiff Test for Bacterial vaginosis

Figure: 17

PAP SMEAR FINDINGS

TABLE: 25

Pap smear results	Number of cases	%
Inflammatory smear	52	52.0
Bacterial Vaginosis	24	24.0
Trichomonas Vaginalis	11	11.0
Candidiasis	8	8.0
Normal Smear	4	4.0
Leptothrix	1	1.0
Total	100	100.0

Pap smear detected bacterial vaginosis in 24 cases, trichomoniasis in 11 cases, candidiasis in 8 patients. One case of Leptothrix was reported and 52 patients had inflammatory smears indicating infection.

Figure : 18

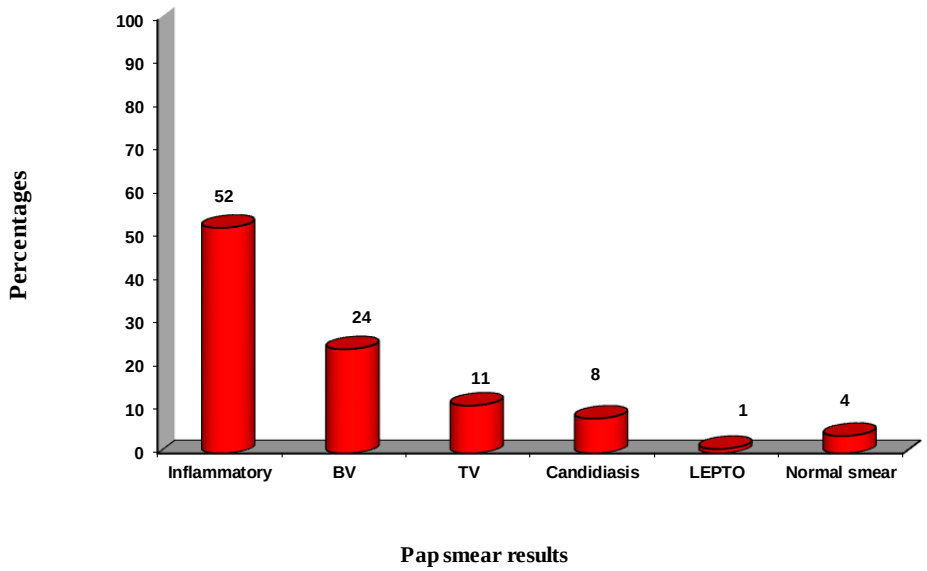


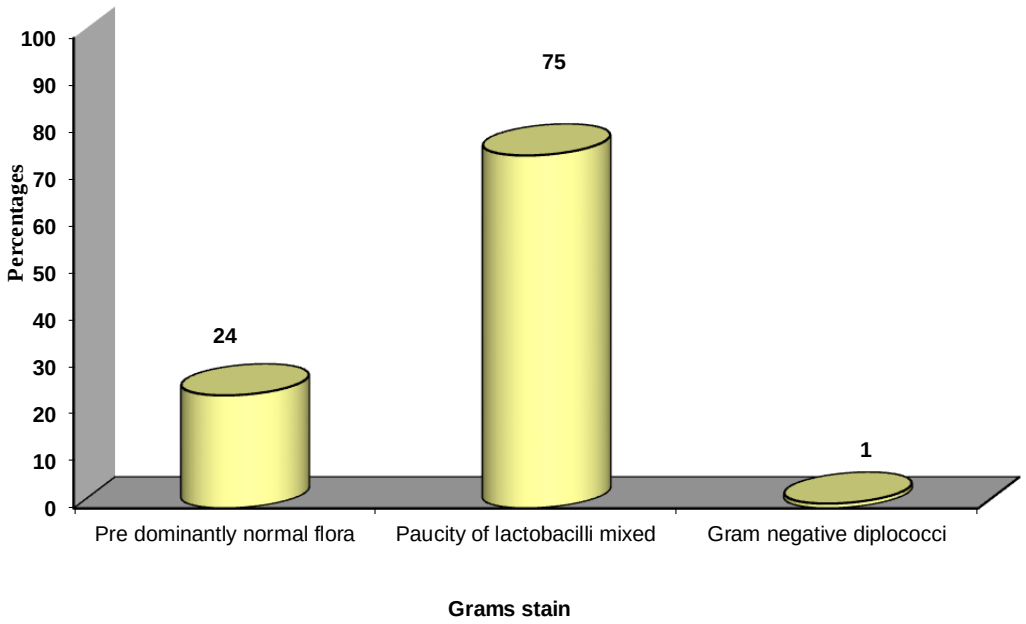
TABLE: 26

GRAMS STAINING

Grams stain	Number of cases	%
Pre dominantly normal flora	24	24.0
Paucity of lactobacilli mixed with Gram variable coccobacilli	75	75.0
Gram negative diplococci	1	1.0
Total	100	100.0

Predominantly normal flora was seen in 24 patients, whereas gram variable coccobacilli with paucity of lactobacilli was found in 75% patients, one case of gonorrhoea was diagnosed on the basis of identification of gram negative diplococci.

Figure: 19



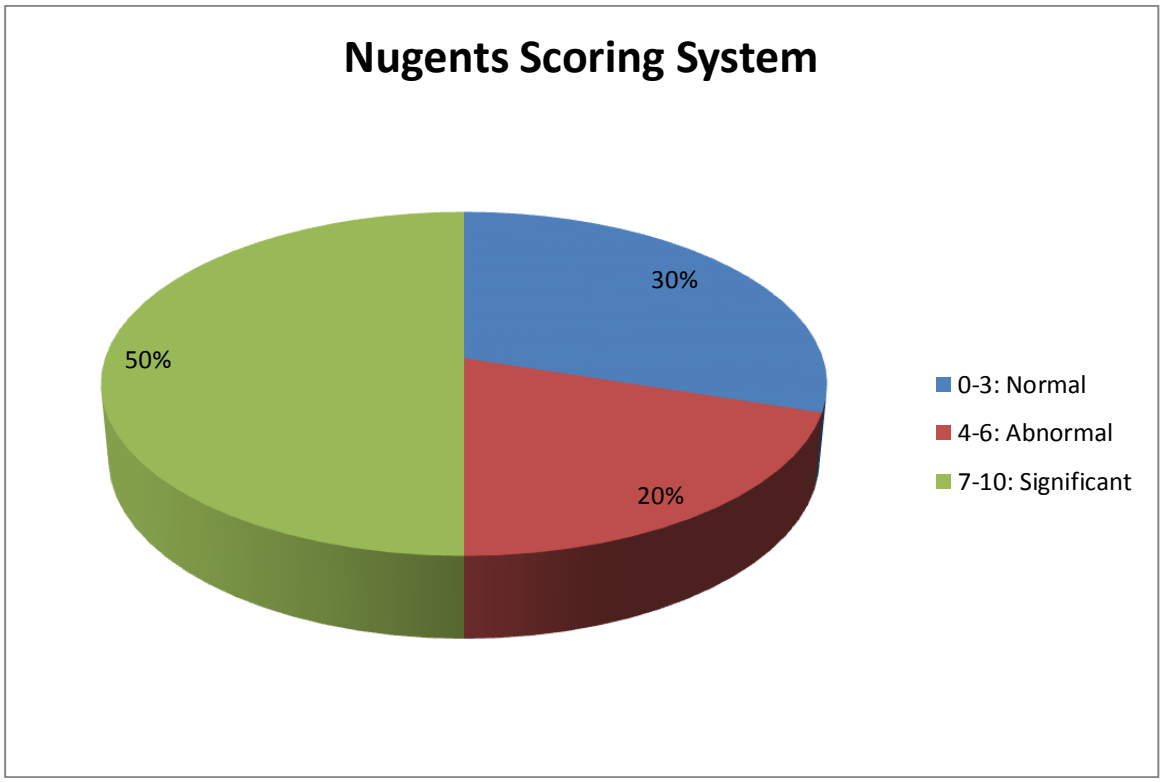
NUGENTS SCORING SYSTEM

TABLE: 27

Nugents scoring system	Number of patients	%
0-3: Normal	30	30.0
4-6: Abnormal	20	20.0
7-10: Significant	50	50.0
Total	100	100.0

Scoring was, abnormal in 70 patients and significant in 50 cases. Final diagnosis of bacterial vaginosis was made in 49 cases. Grams stain was significant in 1 woman without Bacterial vaginosis.

Figure: 20



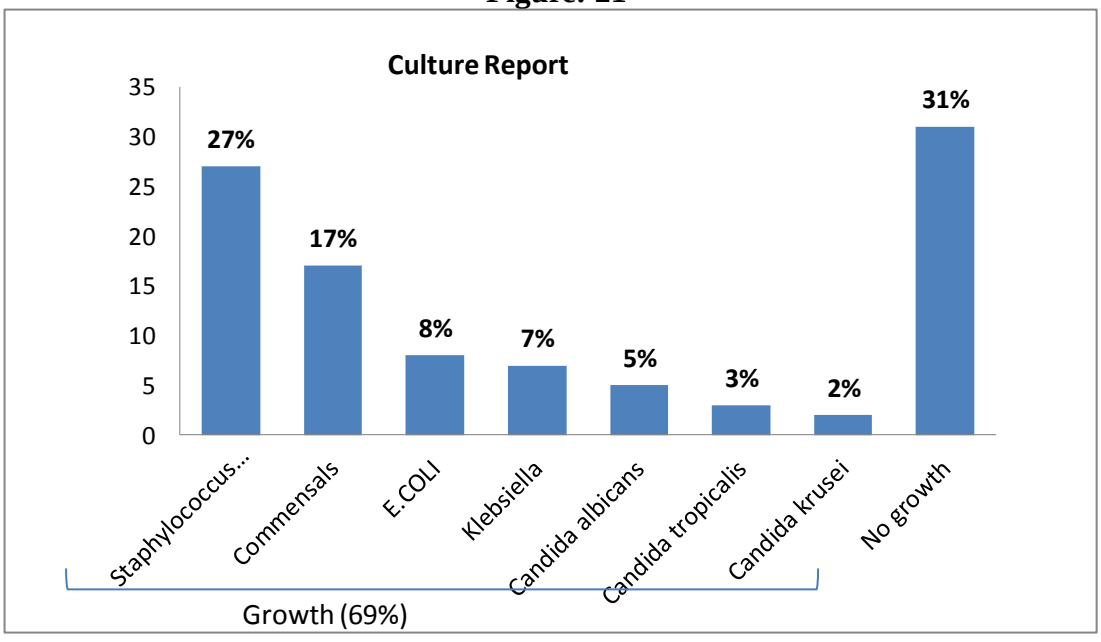
CULTURE RESULTS

TABLE: 28

Culture Report	Number of cases	%
No growth	31	31.0
Growth	69	69.0
• **Staphylococcus aureus**	27	27.0
• Commensal	17	17.0
• E.COLI	8	8.0
• Klebsiella	7	7.0
• Candida albicans	5	5.0
• Candida tropicalis	3	3.0
• Candida Krusei	2	2.0

Culture showed staphylococcus was detected in 27% cases, E.coli in 8 patients, Klebsiella in 7 cases. Three species of candida were identified, Candida albicans in 7 women and Candida tropicalis in 3 cases, and Candida Krusei in 2 cases.

Figure: 21

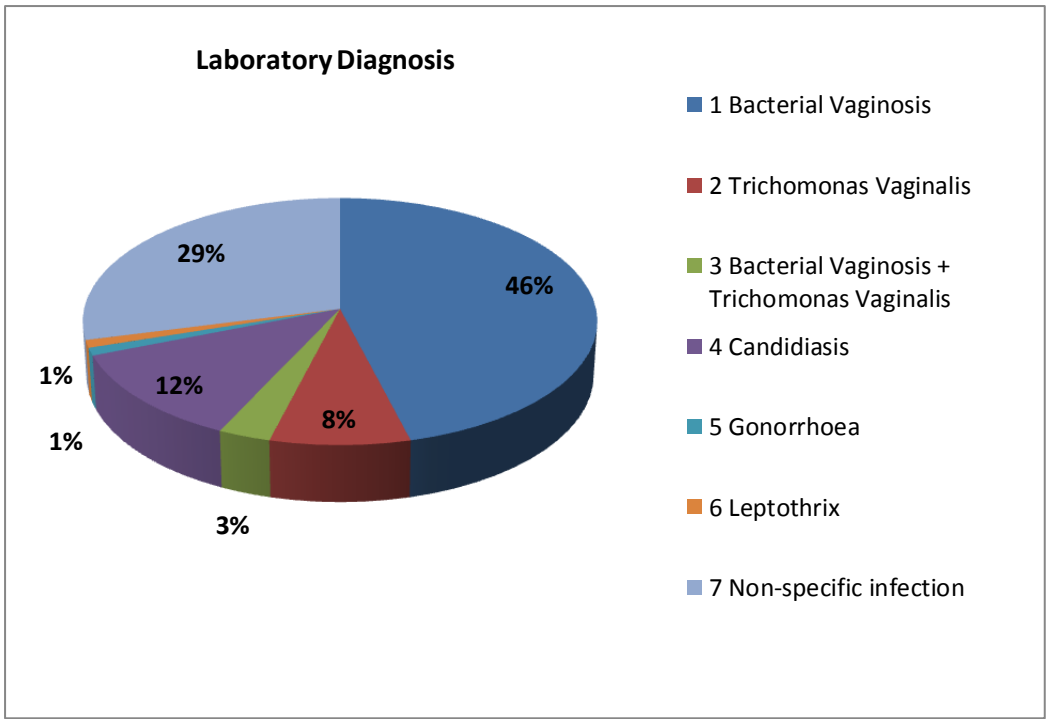


LABORATORY DIAGNOSIS

TABLE: 29

Laboratory diagnosis	Number of cases	%
Bacterial Vaginosis	46	46.0
Non-specific infection	29	29.0
Candidiasis	12	12.0
Trichomonas Vaginalis	8	8.0
Bacterial Vaginosis + Trichomonas Vaginalis	3	3.0
Gonorrhoea	1	1.0
Leptothrix	1	1.0
Total	100	100.0

Figure : 22



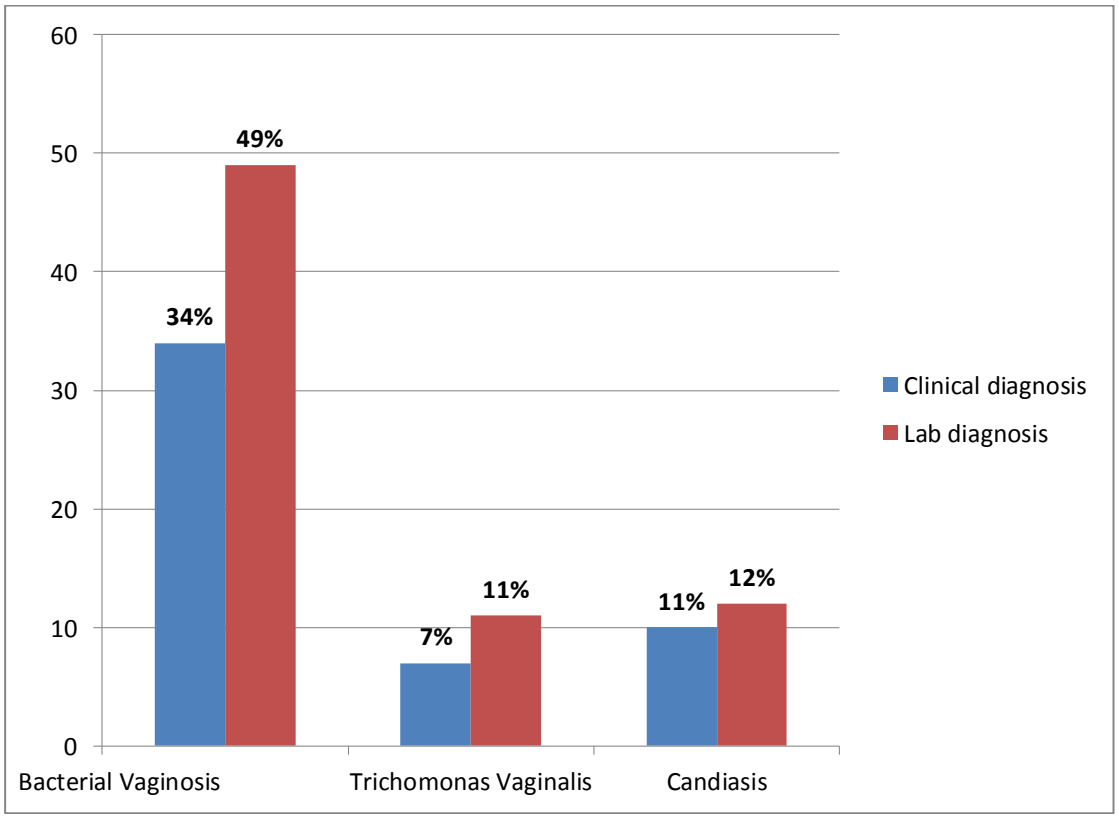
CORRELATION OF CLINICAL AND LABORATORY RESULTS

TABLE: 30

Etiology	Clinical diagnosis %	Lab diagnosis %
Bacterial Vaginosis	35%	49%
Trichomonas Vaginalis	7%	11%
Candidiasis	11%	12%

Clinical diagnosis of bacterial vaginosis was made in 35 out of 49 cases, Trichomonas was detected in 7 out of the 11 cases and 11 out of 12 cases of candida were detected clinically.

Figure: 23



EFFICACY OF SIMPLE TESTS

TABLE: 31

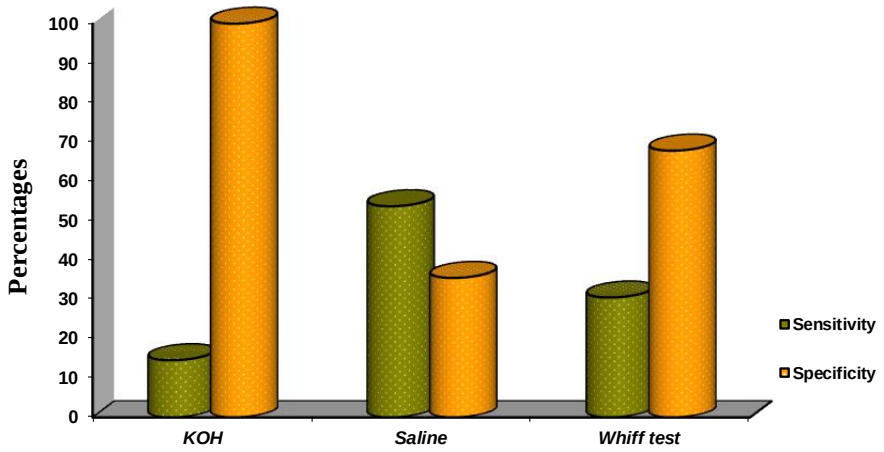
	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
KOH mount	14.49	100.00	100.00	34.44	41.00	0.025*
Saline mount	53.62	35.40	64.91	25.58	48.00	0.309
Whiff test	30.43	67.74	67.74	30.43	42.00	0.855

Of the simple tests used the sensitivity of KOH mount was 14.9% and specificity was 100%, with a positive predictive value of 100% and negative predictive value of 34.44%.

Saline mount had a sensitivity of 53.62% and specificity of 35.4%, positive predictive value for this test was 64.91% and negative predictive value was 25.58%.

Whiff test had a sensitivity of 30.43%, and specificity of 67.74%, a positive predictive value of 67.74% and negative predictive value of 30.43%.

Figure: 24



EFFICACY OF PAP SMEAR

TABLE: 32

	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Bacterial Vaginosis	48.98	100.00	100.00	67.11	75.00	<0.001**
Trichomonas Vaginalis	100.00	100.00	100.00	100.00	100.00	<0.001**
Candidiaasis	66.67	100.00	100.00	95.65	96.00	<0.001**

The pap smear results were most sensitive for Trichomonas vaginalis with a specificity of 100% a positive predictive value of 100% and negative predictive value of 100%, and 100% accuracy. Sensitivity for bacterial vaginosis was 48.98% with 100% specificity and 75% accuracy. Candidiasis was detected with a sensitivity of 66.67% and specificity of 100%, negative predictive value of 95.65% and 96% accuracy.

Figure: 25

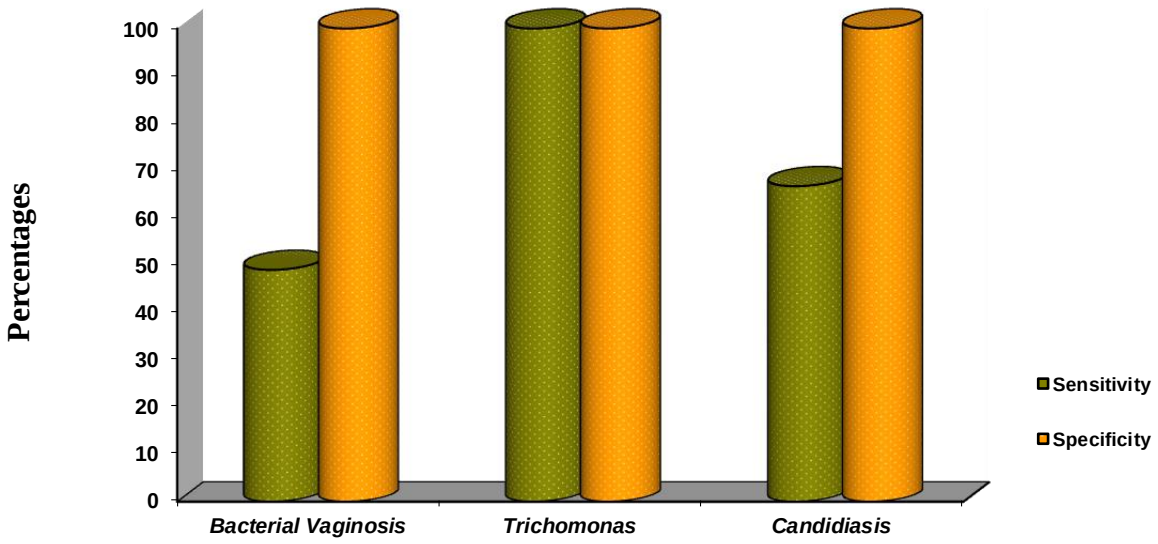


TABLE: 33

Efficacy of Clinical Diagnosis for Bacterial vaginosis

Criteria	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Clinical diagnosis of BV with Lab diagnosis for BV	71.43	100.00	100.00	78.46	86.00	<0.001**

In diagnosing Bacterial vaginosis clinical criteria was 86% accurate.

TABLE: 34

Efficacy of Clinical Diagnosis of for Trichomoniasis

Criteria	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Clinical diagnosis of TV with Lab diagnosis for TV	63.64	100.00	100.00	95.70	96.00	<0.001**

The sensitivity of clinical diagnosis was 63.64% and accuracy was 96%

TABLE: 35

Efficacy of Clinical Diagnosis of for Candidiasis

Criteria	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Clinical diagnosis of Candidiasis with Lab diagnosis for Candidiasis	83.33	100.00	100.00	97.78	98.00	<0.001**

In the diagnosis of Candidiasis clinical criteria had an accuracy of 98% with 83.3% sensitivity.

Bacterial vaginosis:

- A final diagnosis of bacterial vaginosis as the cause of vaginal discharge was made in 49 cases.
- 35 cases of bacterial vaginosis were identified by clinical examination.
- 37 cases had a characteristic thin homogenous discharge out of the 49 cases, 91% of the patients had odorless discharge, in 40% patients the discharge was copious, 91% patients with bacterial vaginosis had no pruritus.
- 8 cases had features of both cervicitis and vaginitis, whereas all other cases had features suggestive of vaginitis, except one where cervix and vagina appeared healthy.
- Nugents scoring was the most sensitive test for diagnosis of bacterial vaginosis being significant in all 49 cases.
- pH was more than 4.5 in 42 cases.
- Whiff test was positive in 31 out of 49 cases.
- Saline mount detected clue cells in 48 out of the 49 cases.
- Pap smear made a diagnosis of bacterial vaginosis in 24 cases.
- 3 cases were diagnosed to have both bacterial vaginosis and trichomoniasis.

TABLE: 36

CLINICAL SIGNS AND SYMPTOMS IN BACTERIAL VAGINOSIS

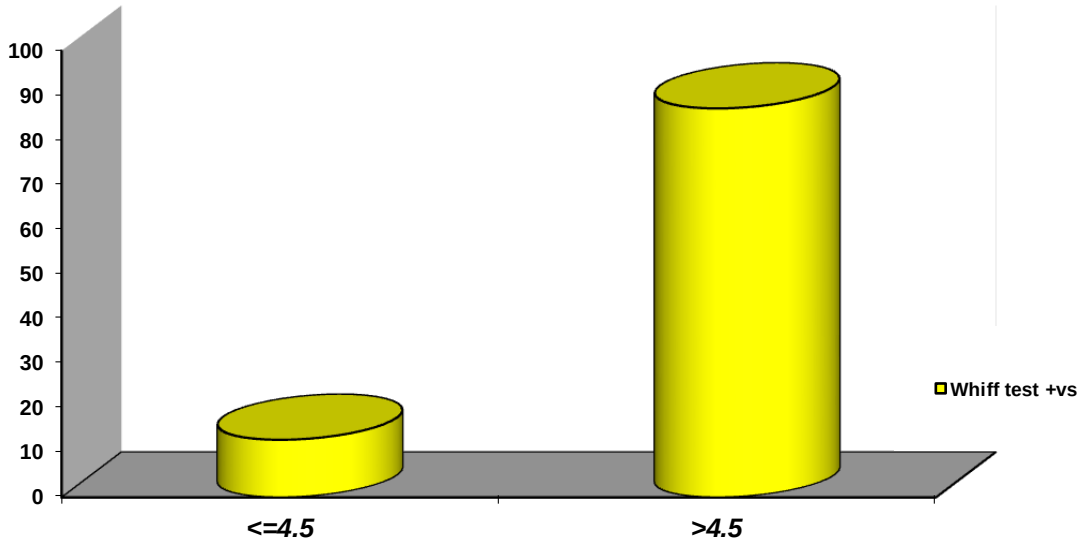
Variables/Criteria	Number of cases (n=49)	%
Odour		
• Odourless	45	91.8
• Foul smell present	4	8.2
Consistency		
• Thin	48	97.9
• Thick	1	2.0
Amount		
• Copious	20	40.8
• Moderate	19	38.8
• Scanty	10	20.4
Pruritus		
• Absent	45	91.8
• Present	4	8.2
Burning micturation(BMU)		
• No	47	95.9
• Yes	2	4.1
P/S		
• Cervicitis (cxitis)	2	4.1
• Vaginitis (vaginits)	38	77.6
• Both (CxVgnits)	8	16.3
P/V		
• FF, FT+	4	8.2
• FF, NT	45	91.8

CORELATION OF pH WITH WHIFF TEST
TABLE: 37

pH	Whiff test positive		Whiff test negative	
	No	**%**	**No**	**%**
≤4.5	4	12.9	31	44.9
>4.5	27	87.1	38	55.1
Total	31	100.0	69	100.0
Inference	pH >4.5 is significantly associated with Whiff test +ve with χ^2=9.64; P=0.002**			

87.1% with whiff test positive had pH more than 4.5, pH more than 4.5 was significantly associated with positive whiff test with a p value of 0.002.

Figure: 26



NUGENTS SCORING IN BACTERIAL VAGINOSIS

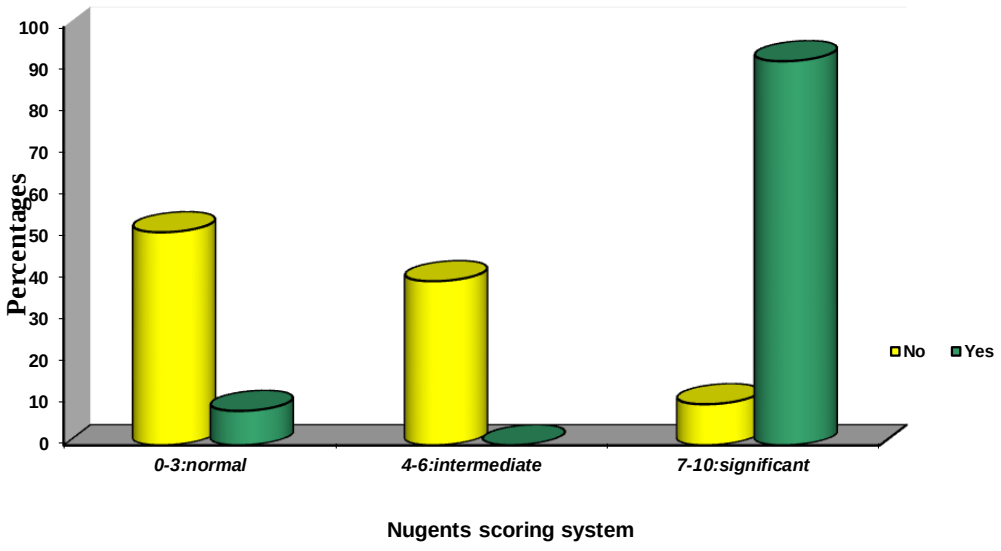
TABLE: 38

Nugents scoring system	Total Number of cases	Bacterial Vaginosis	
		No	Yes
0-3: Normal	30	26(50.9%)	4(8.2%)
4-6: Intermediate	20	20(39.2%)	0
7-10: Significant	50	5(9.8%)	45(91.8%)
Total	100	51(100.0%)	49(100.0%)
Inference	Nugents score >7.0 is significantly associated with incidence of BV with p<0.001**		

2x3 Fisher Exact test

A significant Nugent score on grams staining was reported in 50 patients. It was significant in all patients with bacterial vaginosis.

Figure: 27

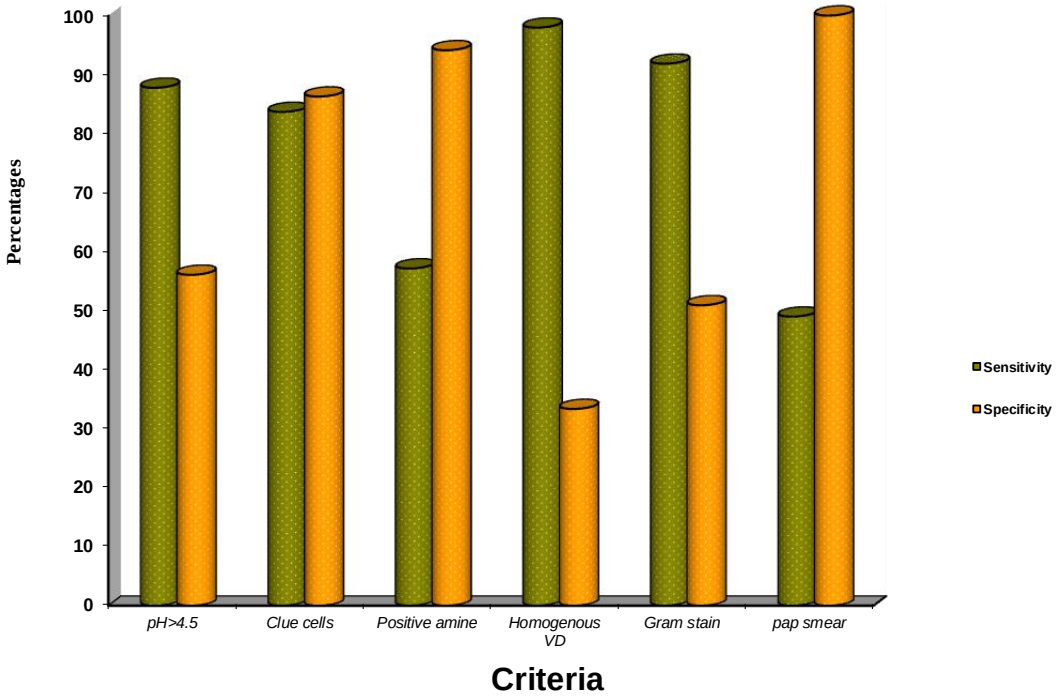


EFFICACY OF SIMPLE TESTS IN DIAGNOSING BACTERIAL VAGINOSIS

TABLE: 39

Criteria	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
pH > 4.5	87.76	56.06	66.15	82.06	72.00	<0.001**
Clue cells	83.67	86.27	85.42	84.62	85.00	<0.001**
Positive amine test	57.14	94.12	90.32	69.57	76.00	<0.001**
Homogenous vaginal discharge	97.96	33.33	58.54	94.44	65.00	<0.001**
Gram stain	91.84	50.90	64.29	66.67	71.00	<0.001**
Pap smear	48.98	100.00	100.00	67.11	75.00	<0.001**

Figure: 28



Pap smear was the least sensitive criteria whereas thin homogenous discharge was the most sensitive in the diagnosis of bacterial vaginosis. Positive amine test had the highest specificity. Presence of clue cells was most accurate criteria in making the diagnosis.

Candidiasis

- The incidence of candidiasis in this study was 12, out of which 11 cases were detected by clinical evaluation.

- Clinical diagnosis was made on the basis of history, characteristics of vaginal discharge on examination and features of vaginitis.

- 10 of the 12 women diagnosed with candidiasis complained of pruritus, a characteristic curdy white discharge was seen in 11 women, while yellow white homogenous discharge was seen in one patient, discharge was scanty in 50% of the cases.

- 6 cases had features of both cervicitis and vaginitis on clinical examination.

- Laboratory diagnosis was made on the basis of presence of hyphae/pseudohyphae on KOH mount, Saline mount, Pap smear, and grams staining and culture.

- pH was less than or equal to 4.5 in 11 out of the twelve cases.

- Hyphae and pseudohyphae were seen in 5 cases on wet mount.

- Pap smear reported candidiasis in 8 cases.

- Culture on chrome agar showed 5 cases of C. Albicans and 3 cases of C. Tropicalis and 2 cases of *Candida Krusei* were identified.

CLINICAL SIGNS AND SYMPTOMS IN CANDIDIASIS

TABLE: 40

Variables/Criteria	Number of cases (n=12)	%
Colour		
• White (W)	1	8.3
• Curdy white (CW)	11	91.7
Consistency		
• Thin	1	8.3
• Thick	11	91.7
Amount		
• Copious	2	16.7
• Moderate	4	33.3
• Scanty	6	50.0
Pruritus		
• Absent	1	8.3
• Present	11	91.7
P/S		
• Vaginitis	7	58.3
• Cervicitis and Vaginitis	5	41.7
P/V		
• FF, FT+	0	0.0
• FF, NT	12	100.0

Trichomoniasis

- The incidence of Trichomonas vaginalis in the study was 11 cases (11%).

- Clinical diagnosis was made on the basis of history, and characteristics of vaginal discharge on examination, and presence of vaginitis 7 cases of Trichomonas vaginalis were diagnosed clinically.

- Pruritus was a common associated symptom in this group as all 11 patients complained of pruritus.

- 90% patients had green yellow discharge and one case presented with gray white discharge on examination.

- 5 cases had features of strawberry cervix or flea bitten cervix on examination.

- pH was more than 5 in all cases diagnosed with Trichomonas vaginalis.

- Motile trichomonas were detected by saline mount in 4 cases.

- All 11 cases were reported in Pap smear staining.

- 3 cases had co-exiting Bacterial vaginosis.

CLINICAL SIGNS AND SYMPTOMS IN TRICHOMONIASIS

TABLE: 41

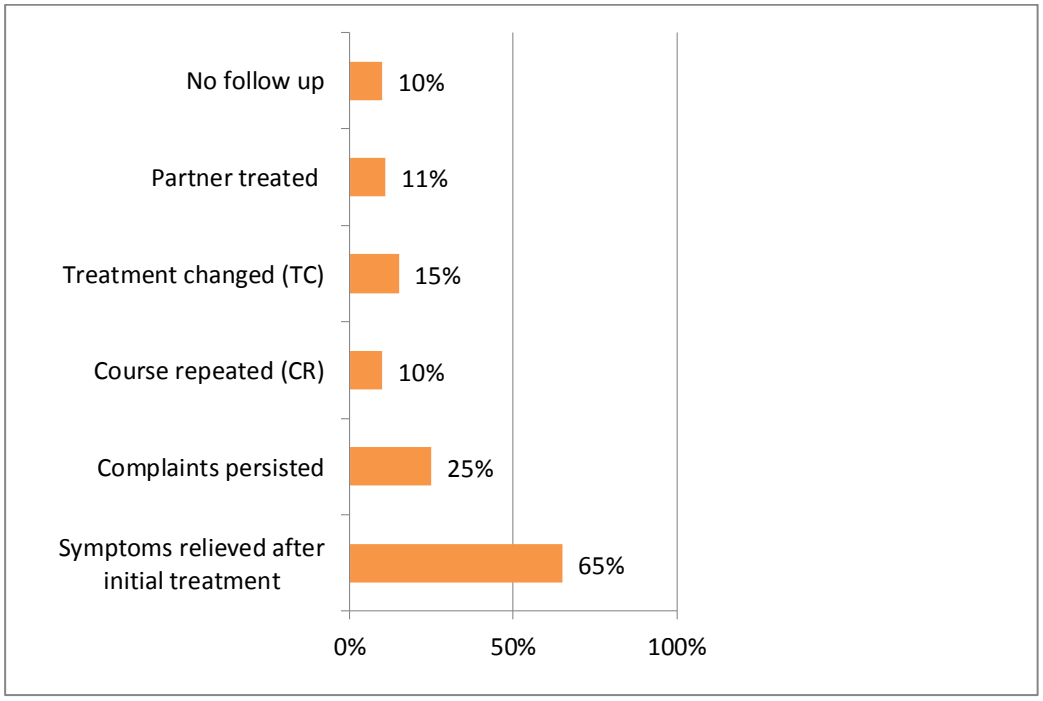
Variables/Criteria	Number of cases (n=11)	%
Colour		
• Green	0	0.0
• Gray White	1	9.1
• Green Yellow	10	90.9
Consistency		
• Thin	11	100.0
• Thick	0	0.0
Amount		
• Copious	8	72.7
• Moderate	2	18.2
• Scanty	1	9.1
Pruritus		
• Absent	0	0.0
• Present	11	100.0
P/S		
• Strawberry cervix	5	45.5
P/V		
• FF, FT+	9	81.8
• FF, NT	2	18.2

FOLLOW UP OF CASES

TABLE: 42

Follow up status	Number of cases (n=100)	%
Symptoms relieved after initial treatment	65	65.0
Complaints persisted	25	25.0
Course repeated (CR)	10	10.0
Treatment changed (TC) after lab diagnosis	15	15.0
Partner treated	11	11.0
No follow up	10	10.0

Figure: 29



10 patients could not followed-up after initial treatment. Following treatment 65% patients had complete relief of all symptoms. 25% patients had persistent symptoms following which, treatment was modified in 17 cases following laboratory results, the antibiotic course was repeated in 15 %cases suspecting re-infection or persistent infection since the clinical diagnosis was confirmed by laboratory diagnosis.

Partners were treated in 11% cases following laboratory diagnosis.

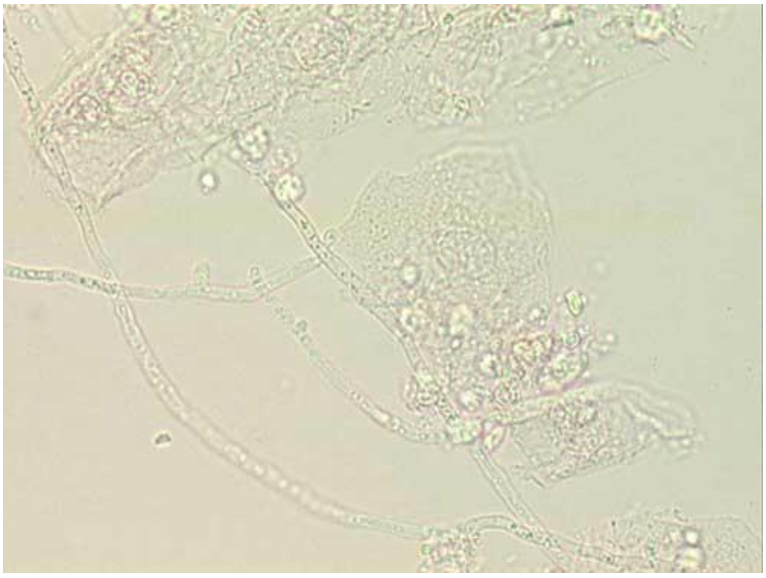


Figure: 30 Hyphae seen in saline mount

Figure: 31 Culture plate showing *Candida Krusei*

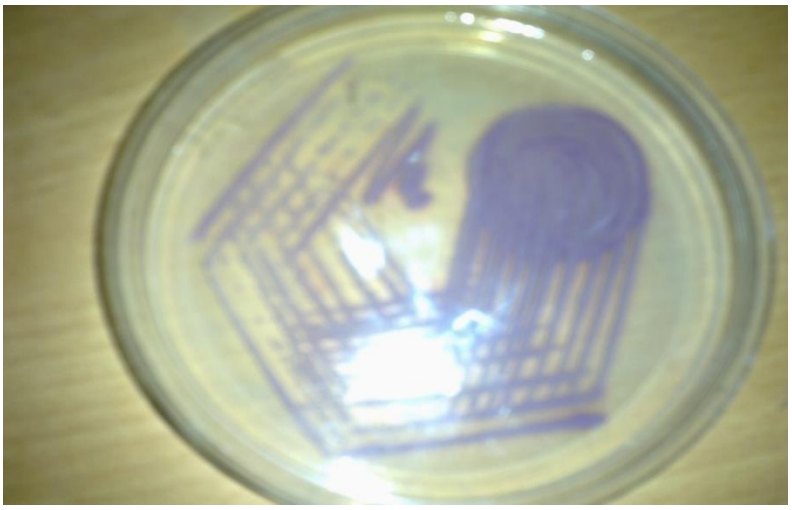


Figure: 32 Culture plate showing Candida *Tropicalis*

Figure: 33 Strawberry cervix in trichomonas vaginalis

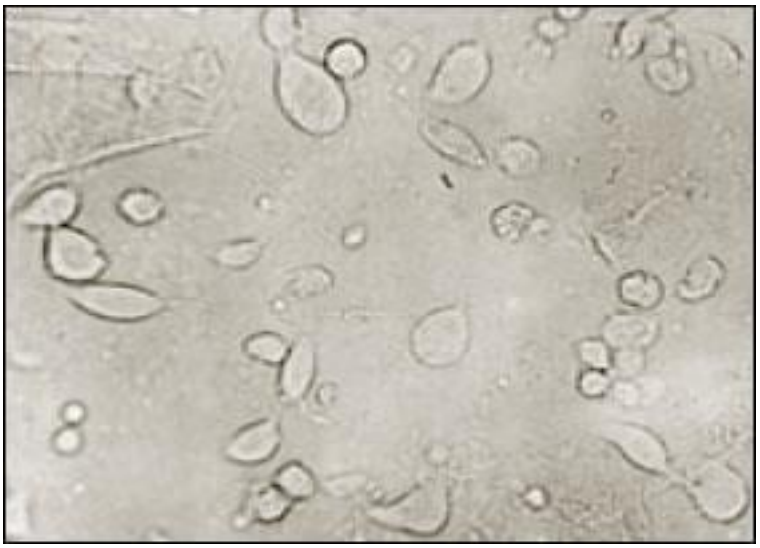


Figure: 34 Trichomonads in wet mount

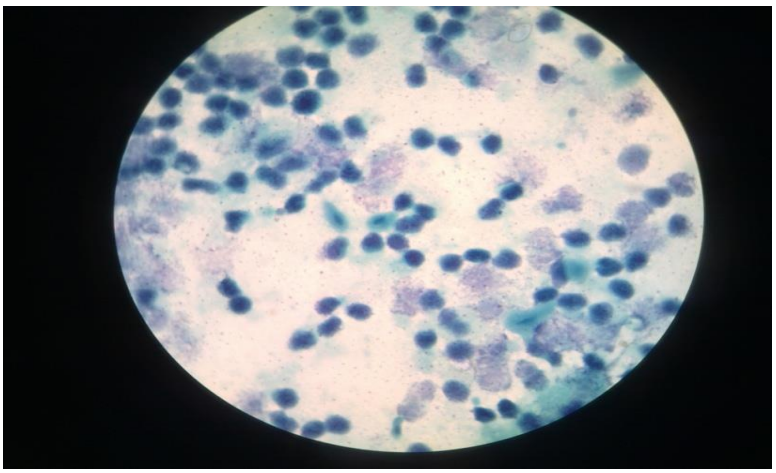


Figure: 35 Pap smear showing Trichomonas Vaginalis

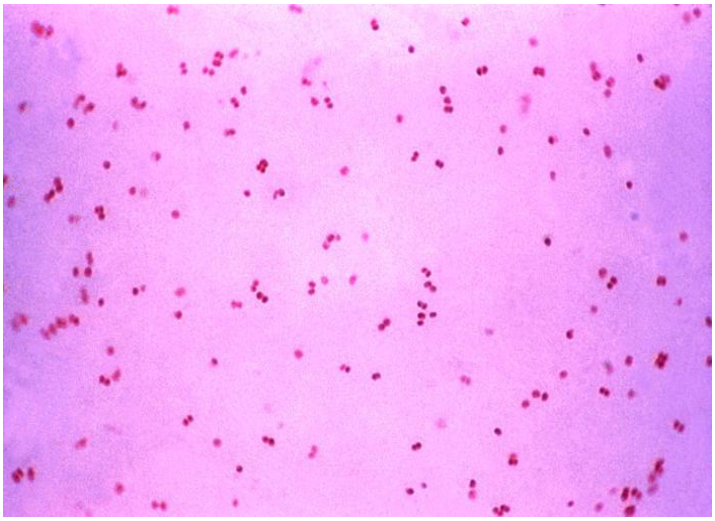


Figure: 36 Gram negative gonococci

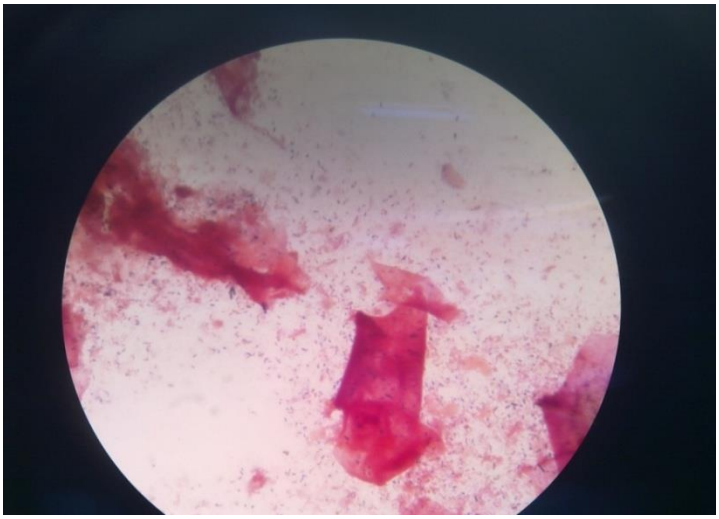


Figure: 37 Clue cells in grams staining

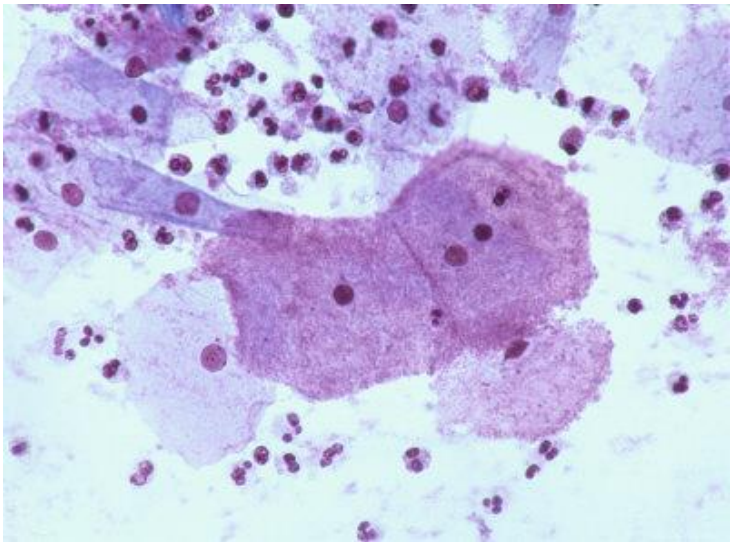


Figure: 38 Clue cells in Pap smear

DISCUSSION

Vaginal discharge is a common presenting symptom seen by general practitioners, Gynecologists, and those working in family planning clinics and departments of genitourinary medicine. It is often a pathognomonic sign of underlying pathology.

The commonest group under the causes of pathological vaginal discharge includes infective causes. A variety of genital pathogens are sexually transmitted.

Various clinicomicrobiological studies have revealed that bacterial vaginosis is the commonest cause of vaginal discharge**.**

Prevalence of G.Vaginalis, T. Vaginalis and Candida in different studies:

Table: 43

S.N	STUDY GROUP	YEAR	B.VAGINOSIS	T.VAGINALIS	CANDIDA
1.	Brewer J I Halpern et al[90]	1957	42.18%	11.3%	4.26%
2.	Holst et al [91]	1987	34%	9%	23%
3.	Bleker et al [93]	1989	38%	13%	25%
4.	Schaaf et al [94]	1990	22%	7%	26%
5.	Abbott [95]	1995	41%	7%	32%
6.	Mahadani et al [74]	1998	32.90%	16.45%	12.45%
7.	Abu Shaqua [96]	2001	30%	3%	26%
8.	Jyoti Thulkar [97]	2008	39.01%	6.74%	7.62%
9.	Vineeta Gupta[98]	2009	43.6%	9.1%	10%
10.	Present study	2011	49%	11%	12%

The variations in the results are consistent with the fact that all the authors have not used identical methods in diagnosing the cases.

The most common associated symptoms in the study by **Vineeta gupta et al** were pruritus(54%), pain in lower abdomen(60%), urinary symptoms(46%), and backache(34.5%).[98] In the present study also pruritus was the most common symptom (30%) followed by lower abdominal pain (22%) ,backache (16%) and urinary symptoms(8%).

BACTERIAL VAGINOSIS

BV is a syndrome marked by an increased vaginal pH, thin homogenous discharge, amine or fishy odour. Microbiologically, bacterial vaginosis is characterized by a shift in the vaginal flora from the dominant lactobacillus species to a mixed vaginal flora that includes G.vaginalis, Bacteroides spp, mobiluncus species and mycoplasma hominis. A study by **Eschenbach** in 1988 revealed 35% prevalence of bacterial vaginosis in women sexually transmitted diseases clinics, and 5-15% women attending gynecological clinics.[18] In this study also, bacterial vaginosis was the commonest cause of vaginal discharge, seen in 49%patients.A study by **Vineeta Gupta et al** in 2009 also showed the incidence of Bacterial vaginosis as 43.6%[98] which co-relates with our study**.** This also correlates with findings of **Joshi et al** [99] and **Bandi et al** [100].

Clinical diagnosis of bacterial vaginosis had a sensitivity of 71.43% and specificity of 100%, positive predictive value of 100% and negative predictive value of 78.46%.it's accuracy was 86%. Of the 49 patients with bacterial vaginosis 37 presented with thin

homogenous discharge, 5 with thick frothy discharge, 3 patients had thin foul smelling discharge and 4 had associated pruritis.

The comparison of genital symptoms in cases in this study with that of Amsel et at (1983) shows the following data .[11]

TABLE : 44

Discharge	Odour	Irritation	Amsel et al	Present Study
+	-	-	1.4%	100%
-	+	-	2.95	Nil
+	+	-	26%	11.11 %
+	-	+	4.3%	4%
-	+	+	1.4%	Nil
+	+	+	7.2%	37.77 %
-	-	-	53.6%	Nil

Amsel et al, have diagnosed G.vaginalis infection in 53% of their patients who were totally asymptomatic. The present study has not been able to co-relate with this criteria, as only symptomatic patients were included in this study.

Comparison of Sensitivity, Specificity, Positive and Negative Predictive Values of the present study with that of Mahadani et al :[74]

TABLE : 45

Diagnostic Criteria		Sensitivity	Specificity	PPV	NPV
pH>4.5	Previous Study	92%	62.26%	54.54%	94.28%
	Present Study	87.76%	56.06%	66.15%	82.06%
Clue Cells	Previous Study	100%	95.28%	91.23%	100%
	Present Study	83.67%	86.27%	85.42%	84.62%
Positive Amine Test	Previous Study	100%	97.17%	94.54%	95%
	Present Study	57.14%	94.12%	90.32%	69.57%
Thin V.Discharge	Previous Study	96.15%	80.19%	70.42%	97.07%
	Present Study	97.96%	33.33%	58.54%	94.44%

In the present study 46 women had Bacterial Vaginosis as per Amsel's criteria that are 3 of the 4 parameters. Considering each criteria, positive amine test was the least sensitive parameter. Mahadhani et al[74] showed sensitivity of 100% and specificity of 97.17% for this sign. This present study showed a sensitivity of 57.14% and specificity of 94.12%.

In this study raised pH has a sensitivity of 87.76 % and specificity of 56.06% making it an unreliable indicator, as it may lead to wrong diagnosis when considered alone, as it has high positive predictive value. This shows that B.Vaginosis cannot be ruled out when a women presents with purulent discharge and increased pH.

Presence of clue cells in a saline mount is an excellent denominator of BV, but it depends on:

1) The quality of the microscope

2) Adequacy of specimen

3) Skill of the observer.[18]

These clue cells are epithelial cells studded with coccobacillary organisms obscuring the nucleus and edges of the cells. In the present study clue cell identification shows a sensitivity of 83.67% and specificity of 86.27% which was comparable with the results by **Mahadani et al**.[74]

Evidence of an association of non specific vaginitis with anaerobic bacteria has reported by **Pheifer et al** it was increased in patients with non specific vaginitis as compared with normal patients[101]. **Spiegel et al** demonstrated increased concentration of Bacteroides and peptococcus species in non-specific vaginitis.[16]

Gram Stain assessment, independent of standard criteria for B. Vaginosis - comparison of present study with previous studies.

TABLE : 46

	Sensitivity	Specificity	PPV	NPV
Eschenbach D.A et al, 1988 [18]	97%	79%	69%	
P.E.Hay et al, 1992 [20]	100%	100%	-	-
Mahadani et al 1998 [78]	88.46%	100%	100%	94.64%
Present Study 2011	97.78%	100%	100%	98.40%

The specificity of BV was higher than the study done by **Eschenbach** 1988[18] as the diagnosing method used there was Spiegel criteria and in the present study we have used Nugent's Gram stain scoring.

Nugent et al developed a scoring system. Each Gram stained smear was evaluated for lactobacilli Gram positive rods, Gram negative rods, Gram variable cocci, under oil immersion.

Gram stain evaluation correlates well with clinical diagnosis and presents a more reliable and reproducible method of diagnosing bacterial vaginosis. Gram stain preparation has an advantage that smears can be stored and reviewed later.[17] It is also inexpensive and widely available in many laboratories.[90]

Papanicolaou Smears:

Pap smears is done for two reasons; for identification of infection and to study the occurrence of epithelial atypia in vaginal smears from women with BV. Clue cells are seen as they are seen in saline mount and Gram stain.

In Pap smears, the squamous cells have thin, transparent grayish granular cytoplasm covered by tiny coccobacilli. The cell borders become indistinct. A variable amount of acute inflammation may be present in the background.

When compared to clinical diagnosis and Gram stain the identification of clue cells in Pap smear has a sensitivity of 48.98 % and specificity of 100 %.

The infection may be associated with atypical squamous cells of undetermined significance. In the present study 100% specificity was obtained regarding clue cells and there was a cocobicillary background. In the study of epithelial cell changes, it was seen that there was reactive cell changes in 40 (88.88 %) of the 45 cases.

CANDIDIASIS

Candidiasis is a common problem among woman who have pain abdomen, vaginal discharge and dyspareunia. 25% of asymptomatic woman harbor C.albicans in the vagina.

No symptoms or signs either alone or in combination are specific for the diagnosis of VVC. Other infections particularly BV (which is more common) can present in a similar way. Microscopy alone may lead to misdiagnosis in up to 50% of cases. The symptoms of vaginal candida are familiar to most physicians and do not help to distinguish *C. albicans* from the non-albicans species. They include vulval itching or soreness, curdy white vaginal discharge without a smell, dysuria or superficial dyspareunia. Vulval itching is the most common symptom and the discharge might be absent in 50% of cases. The diagnosis of a candida infection can be made entirely clinically, based on the symptoms and signs above. It is helpful to test the pH of the discharge, as this is usually normal with candidiasis (4.0–4.5) and a raised pH might suggest another cause for the discharge, such as trichomoniasis.

Incidence of Candidiais in the study was 12%, which correlates with the findings of **Vineeta Gupta et al**[98] (10%), **Joshi et al** [99] (11%) and **Bandi et al** [100](12%).

Sobel et al in 1998 said that that patient whose history was compatible with the disease and yeast was identified microscopically; culture was not required for confirmation. [28]

As with the study by **Mahadani** KOH mount when compared with culture had sensitivity of 93.33 % and specificity of 100%.[74]In the present study culture was done on all the cases showing hyphae, as a routine procedure. Culture and speciation of the organism may be helpful for those patients harboring organisms that do not respond to treatment and in those instances when there is a question of antimicrobial resistance. However, uniform methods for fungal antimicrobial sensitivity have not been established and can be relied upon only if obtained from a few reference laboratories. It has been found that *C tropicalis* and *C glabrata* tend to be more difficult to treat than *C albicans*.

A study by Jack Soble showed the following result indicating high sensitivity and specificity b/w KOH and culture:[28]

TABLE: 47

	SYMPTOMS	**SIGNS**	**KOH**	**CULTURE**
Candida				
70 -80 %	++	++	+	+
20 -30%	++		-	+

In the present study also 91%patients with candidiasis had characteristic signs and symptoms.KOH had an accuracy of 83.3% culture had an accuracy of 90% in detecting candidiasis.

TRICHOMONIASIS

Symptoms traditionally associated with Trichomonas infection in women are vaginal discharge and dysuria. Leucorrhoea, as identified is equally common in those patients with and without Trichomoniasis. In a study by Anthony et al it was ascertained that T.V. was seen in both symptomatic and asymptomatic patients. All the patients were in the 3rd decade and presented with greenish yellow frothy discharge and thin homogeneous discharge.

Fouts et al 1980 concluded that Trichomoniasis could be seen with any kind of discharge and need not be present only with the characteristics greenish yellow frothy discharge.

The same was with our study, two out of six patients had the characteristic discharge and they revealed pathognomonic strawberry hemorrhages on the cervix on per speculum examination. All the findings were similar to the study done by **Fout et al**.[51]As our study included only the symptomatic group, the asymptomatic patients were not examined.

Since 1837 Donne's microscopic visualization of Trichomonads is the most useful test for the confirmation of T. vaginitis.Culture techniques are regarded as the gold standard in diagnosing trichomonas infection. But the disadvantage is that the diamond media which is highly sensitive is not readily available and is very expensive.

Lossick and Kent showed that T.V causes inflammatory tissue reaction. Supporting this theory, our study showed increased vaginal pH and positive amine test[63]. In the presence study 3 out of 5 cases was identified on pap smears. All the five cases were

positive on saline mount. The advantage of Pap smear is that it can be preserved and reviewed later.

Rein et all 1975 stated that the sensitivity of pap smears ranged from 60 - 80%.[75] Highest rate of Trichomonads confirmation can be achieved by fonotana smear than pap technique.In the present study Pap smear was used and the sensitivity of Pap smear was 100% they appeared as small round to oval structures with nucleus of a leukocyte to that of a parabasal cell. The staining varies according to the pH of the vagina, but they have taken a cytoplasmic color.

In the present study 11(11%) cases were diagnosed as having Trichomoniasis. Four cases were diagnosed by the presence of trichomonads on saline mount.

OTHERS

One case of Gonococci was detected on Gram smear, but repeat confirmation with pap and culture was not possible.

One case of Leptothrix was detected. Patient presented with history of thick foul smelling discharge. Pap smear showed features consistent with the organism.

There are limitations in the existing diagnostic approach and it fails to diagnose approximately 30% of women with vaginal symptoms. The time is ripe for new approaches to these complaints.

Other than simple and inexpensive tests like wet mount, KOH, Gram stain, the vaginal smears were also stained by pap stain and all the smears were reported as per the Bethesda system 2001. In the present study on pap smears we diagnosed of BV, 2.72% of candidiasis, 2.72% of TV. Gonococci were not able to be identified on pap smears, and one case of Leptothrix was seen. By this we can conclude that the diagnosis of vaginal infections requires a simple test as mentioned earlier.

In the present study 52% of cases were associated with inflammatory cells in cases of vaginitis, 9.09% were associated with epithelial abnormalities.

We found that Whiff test, vaginal pH estimation, wet mount for T.vaginalis 10% KOH for yeast cells and urine routine and microscopic examination are simple investigations which can inexpensive procedure which can be used as routine office procedures for working up vaginal discharge.

Pap smears are very useful and sensitive techniques which should be done in all patients with vaginal discharge, especially if they fail to respond to treatment based on syndromic approach. In these study 15.4% patients, vaginal discharge was due to endocervicitis and urinary infections. Therefore, these should not be ignored when diagnosing a patient with vaginal discharge.

TREATMENT AND FOLLOW UP

Follow up of 10 patients was not possible, as the temporary relief of symptoms was useful for the patients. Complete relief of symptoms was seen in 65% patients. The same course of antibiotic was repeated in 10% patients as the clinical diagnosis was confirmed by the laboratory diagnosis and the patient complained of symptoms persisting. Treatment was modified following laboratory investigations in 15% patients. The partner was treated in 11 % cases after laboratory diagnosis.

Management of recurrent BV is controversial and largely unexplored. Most practitioners repeat therapy, avoiding short-course regimen and possibly switching from one class of antimicrobials to another and including a trial of tinidazole therapy [102]. There is no evidence that combination therapies benefit. Vaginal lactobacillus replacement is an attractive solution but remains a clinical research endeavor.

Therefore, in the absence of any available effective therapy for recurrent disease, clinicians rely mainly on the use of maintenance antimicrobial suppressive therapy. After an initial pilot study showing reasonable efficacy of maintenance metronidazole vaginal gels, 0.75%. A large multicenter study was recently completed that showed considerable efficacy achieving in excess of 70% protective efficacy on a twice-weekly regimen of metronidazole vaginal gel.[103]

The principal benefits of treating vaginal infections are the relief of symptoms of these conditions, thereby meeting a major expectation of patients, as well as the prevention of gynecological and obstetric complications associated with vaginal infections. At the same time, other approaches for the control of cervical infections are required to ensure quality care in antenatal and family planning clinics serving populations with moderate to high prevalence, given the potentially severe consequences of these infections to a women's health.

The control of STI's in resource poor settings remains a major challenge. The development of simple and affordable diagnostic tests that can be used for case finding is of highest priority. Other approaches such more aggressive treatment of these infections in men with effective partner management are required.

Recognizing that the clinical examination is a limited tool in this setting presents the challenge of finding ways to better diagnose and manage patients with vaginal symptoms. Vaginal symptoms may be the most common gynecological complaint in primary care, but much remains to be learned about their clinical diagnosis.

CONCLUSIONS

Based on our present prospective study the following conclusions were drawn

1. Vaginal infections are common gynecological problems. This study was intended to use simple, rapid and inexpensive methods of diagnosis of vaginits.
2. Vaginal infections are common in reproductive age group.

3. The most common associated symptom is pruritus (30%) followed by lower abdominal pain (21%) and lower back pain (9%).

4. Bacterial vaginosis was the most common infective cause of vaginal discharge followed by Candidiasis and Trichomoniasis.

5. Clinical diagnosis of vaginal discharge can be done on the basis of history and clinical findings and can be more accurate if accompanied by simple laboratory tests for diagnosis.

6. Among the four standard criteria for diagnosis of bacterial vaginosis as per Amsel's criteria, clue cells on the saline mount were the most sensitive and specific with an accuracy of 85%.

7. For Bacterial vaginosis Grams stain interpretation had a sensitivity of 97.78 % and specificity of 100 %. Nugent's score more than 7 was significantly associated with incidence of bacterial vaginosis with a $p<0.001$.

8. Candidiasis is mostly associated with a vaginal pH less than 4.5. Pruritus is more commonly associated with candidiasis. 10% KOH mount has high specificity and sensitivity in detecting hyphae and pseudohyphae. Pap smear had an accuracy of 100%.Culture is informative about the species of candida and thus differentiating complicated candidiasis from uncomplicated candidiasis.

9. Most common associated symptom with trichomoniasis was pruritus, a vaginal pH of more than 5 was associated with all cases of trichomoniasis, and Pap smear has high sensitivity and specificity in detecting Trichomonas vaginalis.

10. We found that Whiff test; vaginal pH, wet mount, KOH mount and urine for routine and microscopic examination are simple and inexpensive and can be done in the outpatient department as office procedures.

11. Treatment based on clinical diagnosis is effective in treatment of vaginal discharge and can be more effective if simple tests are used as office procedures in aiding clinical diagnosis, syndromic management can be done in cases of vaginitis of unknown etiology or non-specific infections where diagnosis has not been made.

12. It is neither essential nor practical to perform certain tests as office procedures in all patients. But the samples may be collected and when required sent to appropriately equipped laboratory the results may be useful in subsequent follow up of the cases.

13. Pap smear is a very useful and sensitive technique which should be done in all patients with vaginal discharge.

14. Many of the diagnostic techniques can be done as office procedures which avoid unnecessary delay in initiating treatment.

15. Clinicians need more education in the laboratory diagnosis of vaginal discharge, as many of the diagnostic techniques can be done as office procedures for diagnosing the etiology of vaginal discharge.

SUMMARY

Vaginal discharge is a common symptom in women of reproductive age. The causes can be divided into those that are infective or non-infective, the most common being the infective agents. Initial assessment of a patient with vaginal discharge requires a thorough history, including sexual history, examination, and testing for these common infections.

The non-sexually transmitted infections (STIs)—bacterial vaginosis and candidiasis—are the most frequently encountered and these can often be diagnosed immediately by the clinical findings and simple bedside tests. Persistence or recurrence of these infections is also seen and might require repeated or prolonged courses of treatment.

The sexually transmitted diseases such as chlamydia, gonorrhoea and trichomoniasis—can also cause vaginal discharge and diagnosis requires appropriate laboratory tests to be performed. Partner notification and treatment is an essential part of the management.

The aim of the present study was to study the prevalence of vaginal infections in the women of reproductive age group to find out the clinical profile of women with vaginal discharge and the outcome of treatment based on clinical diagnosis and to study the treatment interventions in these patients.

It is a prospective study of 100 patients with vaginal discharge, a comprehensive history, general and gynaecological examination was initially carried out. Formal consent was obtained prior to examination and collection of the specimen for investigations.

Based on clinical findings including characteristics of vaginal discharge, a provisional diagnosis was made and treatment was initiated. Patients were advised to follow up after one week.

Vaginal exudates were obtained for laboratory tests including simple diagnostic tests such as pH estimation, Whiff test, saline mount, KOH mount. Pap smear was done in all patients and also grams staining and culture.

Follow up was done in all but 10 patients; response to treatment was assessed primarily by relief of symptoms. The findings of clinical diagnosis, laboratory findings, and response to treatment on follow up was documented and analyzed.

Bacterial vaginosis was the most common infective cause of Vaginitis (49%) followed by candidiasis (12%) and Trichomoniasis (11%). Clinical diagnosis of vaginitis was correct in 35 of cases of BV, 11 in candidiasis and 7 in Trichomoniasis.

We concluded that many of the diagnostic techniques can be done as office procedures which avoid unnecessary delay in initiating treatment. Clinicians need more education in the laboratory diagnosis of vaginal discharge, as many of the diagnostic techniques can be done as office procedures for diagnosing the etiology of vaginal discharge, which will avoid unnecessary delay in initiating treatment.

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ANNEXURE-1

PORFORMA

Name: I.P.No:

Age: D.O.E:

Occupation:

Address:

Husband's occupation:

Socio economic status:

Clinical history:

Vaginal discharge:

Amount:

Color:

Odour:

Itching:

Consistency:

H/O Associated symptoms:

Menstrual history:

Age of menarche

Length of cycles:

Duration of bleeding:

L.M.P:

Obstetric history:

Married life: Para/Living:

Mode of delivery: Last child birth:

Past history:

H/O STD/PID: Treatment for similar complaint:

Surgeries:

Family history:

Personal history:

Sleep: Diet:

Appetite: Bowel and Bladder:

GENERAL PHYSICAL EXAMINATION:

Built: Nourishment:

Pallor/Icterus/clubbing/cyanosis/lymphadenopathy/edema:

Vitals:

Breast: Thyroid:

Systemic examination:

Cardiovascular system: Respiratory system:

Per abdomen examination:

Local examination:

Inspection:

Per-speculum:

Per-Vaginal:

Investigations:

Hb%: Blood Gp & Typing:

Urine Routine:

TC: DC:

Specific microscopic tests to be performed on vaginal exudutaes:

a.)Whiff test b.) pH estimation:

c.)Saline mount d.)Acridine orange stain smear for T.vaginalis.

e.) Gram stain smear for pus cells, candida, N gonorrhoae.

f.) Culture

Pap smear:

Diagnosis:

a.) Clinical

b.) Laboratory:

Treatment:

Follow up:

ANNXEURE II

Method of Staining Smears Gram's

1. Smears are air dried and heat fixed.
2. They are flooded with gentian violet and left for 1 minute.
3. Then they are washed in running tap water.
4. 1 % Lugol's (Gram's) Iodine is applied for 1 minute.
5. Iodine is then washed off by running tap water.
6. Acetone is then applied over the smears for about 10 - 30 seconds.
7. They are then washed with running tap water.
8. Counter staining with Dilute Basic fuchsin is done for 30 seconds. Slides are again washed with water, blotted and dried.

Ref: Mackie & McCartney: Practical Medical Microbiology, 14th Edition, Churchill Livingstone.

Clinical criteria to distinguish Vaginal discharge

ANNEXURE III

Papanicolaou Staining Method

1. After fixation the slides are transferred to absolute to absolute alcohol and run down through 80%, 70% and 50% alcohols to distilled H20.

2. The slides are put in Harris Haemotoxylin for 4 minutes.

3. The slides are rinsed in distilled water. All rinsing is done gently to prevent smears from being washed off.

4. The slides are dipped in 0.25% acid alcohol about 3 times.

5. The slides are then placed in running water for 6 minutes.

6. Rinsed in distilled water and run through 50%, 70% and 95% alcohol respectively.

7. The slides are put in OG-6 1 % minutes.

8. The slides are then rinsed in 95% alcohol two changes.

9. The slides are then transferred to EA-36 for 1 % to 4 times.

10. The slides are dipped in 95% alcohol two changes.

11. The slides are dehydrated and cleaned by running through absolute alcohol, mixture of alcohol and xylol , and mounted by using DPX.

ANNEXURE-IV

The 2001 Bethesda System

SPECIMEN ADEQUACY

Satisfactory for evaluation

Unsatisfactory for evaluation ... (specify reason)

GENERAL CATEGORIZATION

Negative for Intraepithelial Lesion or Malignancy

ORGANISMS:

Trichomonoas vaginalis

Fungal organisms morphologically consistent with Candida spp Shift in flora suggestive of bacterial vaginosis Bacteria morphologically consistent with Actinomyces spp. Cellular changes consistent with Herpes simplex virus.

OTHER NON-NEOPLASTIC FINDINGS

Reactive cellular changes associated with

- inflammation
- radiation
- intrauterine contraceptive device (IUD)

Glandular cells status post hysterectomy Atrophy

OTHER

Endometrial cells (in a woman > 40 years of age).

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

Atypical squamous cells (ASC - US)

Low grade squamous intraepithelial lesion (LSIL)

Encompassing: HPV/mild dysplasia/CIN 1

High grade squamous intraepithelial lesion (HSIL)

Encompassing: moderate and severe dysplasia, CIS/CIN 2 and CIN 3

SQUAMOUS CELL CARCINOMA

GLANDULAR CELL

A typical

- endocervical cells
- endometrial cells
- glandular cells

Atypical

- endocervical cells, favour neoplastic
- glandular cells, favour neoplastic

Endocervical adenocarcinoma in situ

Adenocarcinoma

- endocervical
- endometrial
- extrauterine

ANNEXURE V

KEY TO MASTER CHART

BA: Backache

BV: Bacterial Vaginosis

BMU: Burning micturation

CAND: Candiasis

CAND-A:Candida albicans

CAND-T:Candida tropicalis

C/D:Clinical Diagnosis

Co E symptom: Co existing symptom

CHY: Chylamydia

CW: Curdy white

COMM: Commensals

CP: Complaints persisted

CR: Course repeated

Cxitis: Cervicitis

Cxstraw: strawberry cervix

CxVgitis: Cervicitis and Vaginitis

CxVH: Cervix Vagina healthy

FF: Fornices free

FS+: Foul smell present

FT +: Fornicial tenderness present

G: Green

GHEA: Gonorrhoea

GW: Gray white

GV: Gardenella Vaginalis

GY: Green yellow

Hos.No.: Hospital Number

IFS: Inflammatory smear

KLEB: Klebsiella

L/D: Laboratory diagnosis

LAP: Lower abdominal pain

LEPTO: Leptothrix

MT+: Motile trichomonas present

ML (yrs): Married life (years)

NG: No growth

NP: Nulliparous

NSV: Non-specific vaginitis

NT: Non-tender fornices

OL: Odourless

PL: Para Living

P/S=Per speculum examination

PS+: Pus cells present

P/V: Per vaginal examination

SAUR = Staphylococcus aureus

SR: Symptoms relieved

STREP = Streptococci

TV: Trichomonas vaginalis

TC: Treatment changed

Urine RE: Urine routine

UTI: Urinary Tract infection

Vgiitis: Vaginitis

VUO: Vaginitis of unknown origin

W: White

X: Pre dominantly normal flora

Y: Paucity of lactobacilli mixed with Gram variable cocobacilli

Z: Gram negative diplococci