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Prevalence and Risk Factors of Bactieral Vaginosis, Trichomoniasis, Candidiasis and Chlamydial infections for women of child bearing age in Benghazi Libya

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Abstract

The abnormal vaginal discharge in women of child bearing age is a common problems in clinical medicine and one of the most frequent gynecological complaints in the clinics following discomfort encountered by such patients.

The Aim of this study is to detect the common microbial causes of abnormal vaginal discharge among pregnant and non pregnant women in primary health care centers. The work also aimed to improve the prevention, early diagnosis and treatment of abnormal vaginal discharge among pregnant women and reduce the transmission of various STDs to the unborn child and other complications. During a period of six months, Bacterial vaginosis, Trichomonas vaginalis ; Candida albicans ; Staph. aureus and Strepto. agalactiae were detected in 141 of 200 pregnant and non pregnant women with abnormal vaginal discharge attended to gynecological department of Benghazi Algadeda Center between December 2010 to May 2011 using wet preparation, Gram stain, Giemsa stain and culture. All women were subjected to clinical examination and interviewed by special questionnaire. The prevalence of Bacterial vaginosis was 99 (49.5%), Trichomonas vaginalis was 38 (19%), Staph. aureus was 25 (13.5%), Candida albicans was 25 (12.5%), other Candida spp was 23 (11.5%) and Strept. agalactiae was 8(4%).

The demographic variables as well as risk factors associated with the prevalence of these agents were analyzed, and compared with other results from the literature. The importance of the prevalence of these agents on pregnancy outcome was discussed and appropriate recommendation for prevention of vaginal infection are suggested.

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BV	Bacterial vaginosis
VVC	Vulvovaginal Candidaiasis
TV	Trichomonas vaginalis
G.Vaginalis	Gardernella vaginalis
STD	Sexually transmitted disease
PLB	Preterm labor birth
PPRM	Preterm prelabor rupture membrane
IUD	Intrauterine devices
PID	Pelvic inflammatory disease
GC	Neisseria gonorrhea
C.Trachomatis	Chlamydia trachomatis
GBS	Group B Streptococci or Streptococcus agalactiae
DIV	Desquamative inflammatory vaginitis
M.Hominis	Mobiluncus hominis
STI	Sexuall transmitted infection
U.urealyticum	Ureplasma urealyticum

CHAPTER ONE

INTRODUCTION

Vaginosis and vaginitis

Vaginitis is an infectious or non infectious inflammation of the vaginal mucosa, which sometimes involves the vulva (external genitals). is a common clinical syndrome being found in 28% of women attending sexually transmitted disease clinics, obstetrics and gynecology units and out patients departments. [(1)]In most cases the vagina is observed to be inflamed with symptoms of itching, severe irritation with discharge. Occasionally however, there may be no discharge or there may be discharge without inflammation. The problem of vaginal discharge is probably the most frequently narrated complaint of women of reproductive age group.[(2)]

Vaginitis falls into many forms: irritant, hormonal, foreign body, sexual transmitted diseases and infective. All types can cause great discomfort to the infected women.[(3)]

Vaginal discharge in the reproductive age group is the most common complaint encountered every day both by gynecologists and general practitioners. Symptomatic vaginal discharge is caused by inflammation due to infection of the vaginal mucosa. It occurs in 1-14% of all women in the reproductive age group.[(4)]

Abnormal vaginal discharge may manifests with an offensive non-bloody discharge in the female lower reproductive tracts. It is a common complaint among women of different age groups in any society whether or not they are sexually active. It may be regarded as any amount of secretion that the patient is worried about. Vaginal discharge may be normal or abnormal. [(5)] Normal vaginal discharge is physiologic, such as occurs during pregnancy, sexual arousal or at specific period in the

menstrual cycle. Physiologic vaginal discharge in pregnancy is colorless or white, non irritating and odorless or has mild odor and is non infective in nature with no squeal. On the other hand, abnormal vaginal discharge may be green, yellow, brown or red in color with foul smelling odor, pruritus, irritation, dysuria or dyspareunia depending on the type of infection. [6]

Abnormal vaginal discharge also predisposes to significant morbidity in the form of pelvic inflammatory diseases, infertility, endometriosis, cuff cellulitis, urethral syndrome, pregnancy loss, preterm labor, to enumerate a few.[3,4]

Vagina is a dynamic ecosystem that normally contains approximately 10^9 bacterial colony forming units per gram of vaginal fluid. The normal bacterial flora is dominated by lactobacilli, but a variety of other organisms, including some potential pathogens, are also present at lower levels. Lactic acids and other organic acids are metabolized from glycogen by the lactobacilli, maintaining the vaginal pH between 3.8 and 4.2. The acidic environment inhibits the overgrowth of bacteria and other organisms with pathogenic potential. The normal vaginal discharge is clear to white, odorless, and of high viscosity.[7]

Microbial agents causing the abnormal vaginal discharges include group affecting the cervix - Chlamydia trachomatis, Group B – streptococcus, Ureaplasma urealyticum and Herpes simplex virus type2. group affecting the vagina – Candida albicans, Trichomonas vaginalis and Gardnerella vaginalis(Bacterial vaginosis).[6]

and other causes of abnormal vaginal discharge or irritation which include atrophic vaginitis, allergic reactions (spermicides, deodorants), vulvar vestibulitis, lichen simplex chronicus and lichen sclerosis (especially pruritis) and foreign bodies (retained tampons).[8]

In a primary-care study,[9] vulvovaginal symptoms including vaginal discharge were due to vulvovaginal candidiasis (VVC) in 27% of patients, bacterial vaginosis (BV) in 21%, trichomoniasis in 8%, Chlamydia trachomatis in 2%, Neisseria gonorrhea(GC) in 1%, and no infection in 34%. Several pathogens may coexist. [10] vulvovaginal candidiasis, Bacterial vaginosis , and Trichomoniasis account for at least 90% of infectious vaginitis.[11]

Bacteria vaginosis is a common gynecological condition, reported in 5–51% of women, depending upon demographics and whether or not they are symptomatic.,[12] The natural history of BV is unclear, as are the risk factors for its acquisition. Race has been identified as one potential risk factor as blacks have higher rates than whites.[12,13] It is unclear, however, if this discrepancy is due to racial differences in vaginal phsiobiology[13,14] or culturally mediated differences in vaginal-cleansing behaviors such as douching.[15,16]Smoking, alcohol consumption, lower socioeconomic status, and lower educational levels have also been associated with BV.[12,14] Oral contraceptives are protective against BV while intrauterine devices have been reported to either increase or have no association with BV risk.[14,16,17] A cause and effect relationship between BV and adverse pregnancy outcome can be better determined in cohort studies, in which BV is diagnosed before the onset of a number of pregnancy complications such as preterm labor birth (PLB) or premature preterm rupture of membranes (PPROM).[18]

Sexually Transmitted Diseases (STDs) and Reproductive Tract Infections (RTIs) often present with vaginal discharge. [17] Presence of an STD increases the likelihood of contracting HIV as these diseases facilitate HIV transmission.[17, 9,20] In order to restrain HIV and AIDS, control of other STDs is imperative.[17,21] Though STD infection rates are similar in both sexes, women bear the major burden of complications and serious

squeal.[9,10,13,19] Hence, STD management in women is particularly important.[23]

Although vaginitis or inflammation of the vagina and cervix generally is both treatable and mild, when left untreated ,is a possible risk for acquisition of HIV/AIDS as well as other complications .Abnormal vaginal discharge in pregnancy poses a greater risk of transmission of HIV to the unborn child and may result to abortion, premature rupture of membrane, prematurity and low birth weight. [14] The relationship between pregnancy and infective vaginal discharge with a particular note on Candidiasis indicates increase in hormonal influences and alteration in vaginal pH .[11,23]

Causes of vaginitis and / or of the abnormal vaginal discharge :

Infectious causes :(Infective vaginitis)

The three infections most commonly associated with abnormal vaginal discharge in adult women are:

Bacterial vaginosis (BV)

Vulvovaginal candidiasis (VVC)

Trichomoniasis

On few occasions, a vaginal discharge may be seen in cervicitis caused by Neisseria gonorrhoeae or Chlamydia trachomatis .[24]

Bactieral vaginosis

Bacterial vaginosis is vaginal condition that can produce vaginal discharge and results from an overgrowth of normal bacteria in the vagina[25]. In the past, the condition was called Gardnerella vaginitis, after the bacteria that were thought to cause the condition. However, the newer

name, bacterial vaginosis, reflects the fact that there are a number of species of bacteria that naturally live in the vaginal area and may grow to excess[26]. Synergistic activity of various anaerobic organisms,including Prevotella spp, Porphyrmonas spp, Peptostreptococci ,Mobiluncus spp Mycoplasmas as well as Gardenerella. vaginalis ,seems to contribute to the pathology of BV [27]. Gardnerella organisms are probably not the sole culprit causing the symptoms. When these multiple species of bacteria become imbalanced, a woman can have a vaginal discharge with a foul odor[28].

Bacterial vaginosis is a common condition, and studies have shown that approximately 29% of women in the US are affected. Bacterial vaginosis is found in about 16% of pregnant women and approximately 60% of women who have a sexually-transmitted disease (STD).[28,29]

About 85% of those affected with bacterial vaginosis actually have no symptoms. When symptoms occur, vaginal discharge and odor are the predominant symptoms. With usually, no other symptoms. The amount of vaginal discharge that is considered normal varies from woman to woman. Therefore, any degree of vaginal discharge that is abnormal for a particular woman should be evaluated.[3,24]

Some women may experience an unpleasant fishy odor with often more noticeable after sexual intercourse.[30]

Symptoms and signs of Bacterial vaginosis

The most common symptoms characterizing a thin, homogeneous vaginal discharge and a malodorous, "fishy" smell. The color and the amount of discharge varies. frequent urination, itching vary from patients to patients .

Burning discomfort around the vagina , often, women with BV have no symptoms at all [4,31].

Mobiluncus

There has been much interest recently in anaerobic ,curved, motile gram-negative bacteria present as components of the vaginal flora, especially as carriage appears to be associated with bacterial vaginosis.[32]These anaerobic curved rods have been given the genus name Mobiluncus, and two species, M . curtisi (curtisii) and M . mulieris[33]. These organisms are extremely fastidious, requiring fresh moist media and extended anaerobic incubation for isolation. Because of the difficulty in isolating these organisms by culture, most researchers attempting to define the prevalence of this organism have used direct techniques such as Gram stain, wet smear, direct staining with monoclonal antibodies or DNA probes[32] and biochemical reactions.

The most useful of these tests were alactosidase activity, arginine and hippurate hydrolysis as well as metronidazole susceptibility.[34]

M. curtisi organisms, referred to as "short forms" are considered to be 1-2 µm long, gram-variable, comma-shaped, resistant to metronidazole, and give positive results in all these differential biochemical tests. In contrast, M. mulieris organisms, referred to as "long forms" (LF), are considered to be 3-4pm long, gram negative ,curved, sensitive to metronidazole, and give negative results in the three biochemical reactions. [33]

Other causes of abnormal vaginal discharge:-

Neisseria gonorrhoeaea and Chlamydia trichomatis , vaginitis and the abnormal vaginal discharge:

Gonorrhea is caused by bacteria called Neisseria gonorrhoeae. It is the second most common sexually transmitted disease in the United States. It where it is estimated that 700,000 cases of gonorrhea occur each year[31]. In women, most gonorrhea infections have no symptoms. that prevents many women from seeking treatment from their physician.[31] Symptoms, when they do occur, include increased vaginal discharge, painful urination, abdominal pain and bleeding after sexual intercourse. The primary site of infection is the cervix, although it can be isolated from the vagina, urethra, rectum and throat. Possible complications include infection of the fallopian tubes, tubo-ovarian abscesses, infertility and pelvic inflammatory disease (PID)[35]. In pregnant women, gonococcal infection can cause spontaneous abortion, fetal death, premature birth, fetal distress, and premature rupture of the membranes.[36]

It was shown in [37], that Mucopurulent cervicitis is most often caused by N. gonorrhoae and C. Trachomatis the two most frequently causes of sexually transmitted infections.

C. trachomatis is the most common cause of cervical infections, with a prevalence ranging from about 9 to 35 percent in asymptomatic adolescents and 20 to 30percent in women treated in sexually transmitted disease clinics. The incidence of chlamydia infection was estimated to be 215 per 100,000 person[38]. Approximately 13 percent of women with chlamydial infection have concurrent gonococcal infection and approximately 30 percent of women with gonococcal infection have associated chlamydial infection .

signs and symptoms of Gonococcal and chlamydial infections:

Initially, chlamydial infections may be asymptomatic, or present with vaginal symptoms (e.g., mucopurulent vaginal discharge, vaginal itching, dyspareunia, dysuria, vague lower abdominal pain), anorectia with sometimes symptoms, and pharyngeal symptoms. However both have the potential to cause pelvic inflammatory disease, the squeal of which include ectopic pregnancy and in fertility. In the clinical examination of the cervix, 3 characteristics have been associated with chlamydial infection: yellow end cervical discharge, easily induced cervical bleeding, and opaque cervical discharge. All of the 3 findings are statistically significant and independently associated with chlamydial infection.[37,38]

Streptococcus agalactiae and abnormal vaginal discharge:

Lancefield group beta-hemolytic streptococci (group B streptococci [GBS] or Streptococcus agalactiae play an important role in the development of neonatal morbidity and mortality. Infections that occur before the seventh day of life so called early onset infections are usually caused by vertical transmission. Between five percent and 40% of women carry GBS. Carriers of cervicovaginal GBS are usually asymptomatic. However, one study showed that in patients with purulent vaginal discharge the prevalence of GBS was increased. Colonization with GBS seems to be more prevalent in patients with excessive vaginal discharge, [39] A correlation has also been demonstrated between the diagnosis of vaginitis and colonization with GBS.[40]

Staphylococcus aureus vaginitis:

Staphylococcus aureus belongs to Those pathogenic bacteria that are usually not present in vagina. It may cause vaginitis, but this is not necessarily so. Treatment is advised in case of vaginitis or if the symptoms are associated with the detection of S. aureus. Because of the limited pathogenicity , one may attempt topical treatment with disinfectants .The pathogen must be eliminated from the vagina prior to surgery or during pregnancy .[39,41]

Staphylococcus aureus has been isolated from vaginal fluids of women with toxic shock syndrome (TSS), a multisystem disease with onset usually during menses. the etiological agent of this condition could be acquired as a result of exposure to predisposing factors include using of hyper absorbable tampons.[42]

Vulvovaginal candidiasis:(VVC)

Yeast are normally found in the vagina. However, if something happens to change the normal environment of the vagina, the yeast organisms flourish and cause a yeast infection. Vaginitis is commonly caused by an overgrowth of yeast (Candida albicans).[43]

Common symptoms may include vaginal itch and/or soreness, burning, a cottage cheese-like discharge, and pain during intercourse. This can be caused by antibiotics, pregnancy, diabetes, birth control pills, or the normal changes during a menstrual period. Other factors associated with increased incidence of yeast infection are sexually transmitted diseases, low estrogen levels accompanying menopause, wearing tight, non-breathing clothing or underwear, and the use of douches or perfumed feminine hygiene sprays.[38]

Signs and symptoms of VVC:

The most frequent symptom is pruritus or burning. Abnormal discharge is a complaint for majority of symptomatic women with VVC confirmed by culture. In addition, women may complain of a thick, odorless, cottage cheese–like discharge A thick, curdled-appearing discharge points to a diagnosis of Candida because it is rarely present with BV or Trichomoniasis.[44]

Trichomonas vaginalis:

Another common cause of abnormal vaginal discharge is Trichomonas vaginalis. This is a sexually transmitted disease caused by a protozoan parasite called Trichomonas vaginalis.[45]It usually cause the following Symptoms: a foul-smelling, yellowish-green vaginal discharge and itching. Symptoms worsen during menstruation.[38]

Signs and symptoms related to trichomonaisis:

There is usually a profuse and offensive greenish- yellow frothy discharge which produces irritation and soreness but in a few cases the discharge is less. the Vaginal wall is intensely red and inflamed and numerous minute punctuate ulcers(strawberry spots) may be seen .[38]

Aerobic vaginitis

Aerobic vaginitis is a term proposed to describe purulent vaginal discharge with predominance of abnormal aerobic flora.[31] Aerobic vaginitis which may be severe, has been reported as the cause of 5% of cases in a series from a specialty vaginitis clinic.[46]The usual abnormal aerobic flora 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.[47] The case series referred to above also reported good therapeutic response to 2% topical clindamycin .[46]

Non infectious vaginitis:

Noninfectious causes of abnormal vaginal discharge include physiologic, irritant, allergic, cytolytic vaginitis, desquamative inflammatory vaginitis, collagen vascular diseases, and idiopathic vaginitis.[48]

Irritant and allergic vaginitis:

This may result from sensitivities to topical medications, the active or base ingredients of spermicidal products, douching solutions, or the latex of condoms or diaphragms. If a woman showed persistent symptoms due to using such intravaginal products, she should stop its use.[46,49]

Cytolytic vaginitis

Is characterized by overgrowth of lactobacilli and cytolysis of squamous cells, including presence of cytoplasmic fragments and intact cells with naked nuclei [50]. 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.[50, 51] Symptoms often mimic VVC and may include a white, cheesy discharge, with vaginal pH ranges from 3.5 to 5.5. Recurrences during luteal phase of the menstrual cycle have been described.[52] Intra vaginal anti-fungal if used, should be discontinued. Baking soda baths or douches are often used, but clinical trial data to support this practice are lacking.[43]

Noninfectious desquamative inflammatory vaginitis (DIV)

DIV is an uncommon vaginitis characterized by profuse purulent discharge with epithelial cell exfoliation.[53]

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 plans.[54]Treatment is usually difficult, though there may be some response to local or systemic corticosteroid therapy .[53]

Abnormal vaginal bacterial flora :

The absence of vaginal lactobacillary morphotypes is associated with anaerobic bacterial vaginosis[8,55] with certain sexually transmitted diseases,[56] and with over growth of facultative pathogenic commensal bacteria of intestinal origin. [57] The presence of abnormal flora (bacterial vaginosis) has been associated with elevated concentrations of selected bacteria, an elevated sialidase level, a high pH, and elevated cytokine levels in the cervix and vagina.[50,59]

Risk factors predisposing to vaginitis:

Symptomatic yeast vaginitis has been associated with condom and diaphragm use, recent antibiotic use, receptive oral sex , oral contraceptive use, spermicidal use, diabetes, and immune suppression including AIDS.[60, 61] Although pregnancy has been postulated as a risk factor for symptomatic vulvovaginal candidiasis, prevalence of yeast on culture in pregnant women is similar to that of non-pregnant women.[60]

Certain factors have been identified that increase the chances of developing bacterial vaginosis. These include multiple or new sexual partners, intrauterine devices for contraception, endometrial biopsy or uterine curettage, antibiotic use, vaginal douching. Cigarette smoking associated with intrauterine device (IUD), endometrial biopsy or uterine curettage,[61] and Infection with Trichomonas vaginalis were associated with Multiple partners.[62]

Vaginitis and Adverse pregnancy outcomes

Vaginitis during pregnancy is associated with premature rupture of the membranes, chorioamnionitis, preterm labour, lower birth weight, preterm birth and post-cesarean delivery endometritis, The presence of BV during an invasive procedure, such as placement of an intrauterine device (IUD), endometrial biopsy or uterine curettage. has been associated with post-procedure pelvic inflammatory disease and vaginal cuff cellulites.[6,63]

The presence of bacterial vaginosis at a particular gestational age may be a factor in the subsequent development of pregnancy complications, and the risk for disease may change based on BV positivity during different stages of gestation.[25]For example, the risk of preterm delivery due to BV in the first trimester, during early fetal and placental development, may be different compared with the risk of preterm delivery in the second and third trimesters, during profuse placental functioning. These relations currently are unknown and need further development.[25,29,64]

Relative benefits of diagnostic tests:

A gold standard test has not been established for BV. In about 50% of asymptomatic women, culture results are positive for flora such as Gardnerella vaginalis. Amsel's criteria are often used as a reference and generally suffice for the evaluation of symptomatic women .The best candidate for a gold standard test is probably Gram stain assessment using Nugent's criteria[65] Lack of leukocytes in the vaginal fluid supports a diagnosis of BV. A finding of white blood cells in excess of the number of vaginal epithelial cells suggests an inflammatory process .[47]

Amsel's criteria with wet mount preparations:

The diagnostic approach most commonly used in clinical laboratory and officesis Amsel's criteria which consist of : homogenous discharge, positive whiff-amine test, pH >4.5, and clue cells found on wet-mount microscopy.[65] Three of 4 criteria deemed positive is considered diagnostic. If Gram stain is used as the reference standard, then Amsel's criteria have 70% sensitivity and 94% specificity for diagnosing BV.[66]
Homogenous discharge : A thin, homogenous, grayish discharge is traditionally associated with BV. However, it is not specific to BV, being found commonly also in women with culture result positive for VVC or with negative diagnosis of vaginitis. It is the criterion least likely to be consistent with the whole group, seen in about half of BV positive women and over one third of women BV-negative using Amsel□s criteria as the reference standard.[67]

Vaginal pH:

More commonly, pH when increased above 4.5, prompt further evaluation. Even in the absence of symptoms, routine pH testing increases the detection of trichomoniasis and bacterial vaginosis in a primary-care setting by enhancing microscopy in a significant proportion of asymptomatic cases.[68]

The sensitivity of vaginal pH > 4.5 for the diagnosis of bacterial vaginosis is 88.3%, whereas specificity is much less (58.6%).In cases of Trichomonas vaginalis infection or severe aerobic vaginitis, the pH may be vastly increased to 6.5 or more.[69]

Theoretically, many non-infectious conditions may alter the normal vaginal pH including menstruation, recent unprotected sexual intercourse with deposition of semen, use of local antifungal agents or antibiotics. Therefore, the finding of an increased pH should be followed by microscopy and/or cultures to confirm a presumptive diagnosis ,in order not to erroneously treat a common non-infectious condition with antimicrobial agents.[69]

The pH of the vagina should be measured directly in the vagina, on the speculum, on the swab or on the glass slide prepared for microscopy, but addition of saline for fresh microscopy causes the pH to rise and should be discouraged.[43]

Office dipstick tests can be used and show a good correlation of increased pH with lactobacillary grades, cervicitis ,trichomonas infection, bacterial vaginosis and aerobic vaginitis.[69]Merck's as well as Machery Nagel's dipstick can be used efficiently in this pH range, but in difficult cases the latter are more user-friendly and less time -consuming.[70]

Whiff test.

The advantage of doing Whiff test is that it requires less training and can be done without speculum, but antibiotic use should be restricted. Compared to speculum examination, it will not diagnose conditions like cervicitis, cervical erosion and PID. Results of pH test and Whiff test are comparable to microscopy and these tests are cost-effective and less time consuming as compared to microscopy.[67][69]

Gram stain

Because of it higher sensitivity in diagnosing BV ,it is the most widely used scoring system as mentioned in[71]. A score of 1–4 of lactobacillary morphotypes, as well as a score of 1–4 of Gardnerella morphotypes, and a score of 1 or 2 for Mobiluncus morphotypes has to be added to obtain a global Nugents score .[71]

Nugent score is well suited to diagnosing BV (score of ≥7) and normal flora (score 0-3), but the interpretation of the so-called 'intermediate flora' (score 4–6) remains controversial. Fig(1). In many studies the intermediate flora was associated with undefined microbial correlate[72]and a different set of complications during pregnancy,[72,73] and classic therapy for BV (metronidazole) did not cure most cases with this type of flora.

Figure (1): Nugent□s gram stain score for bacterial vaginosis

Organism morphotype	Number\oil Immersion field	score
Lactobacilli-like(parallel-sides, gram-positive rods)	>30 5-30 1-4 □1 0	0 1 2 3 4
Mobiluncus-like(curved ,gram-negative rods)	>5 □1-4 0	2 1 0
Gardnerella\Bacteriods-like (tiny ,gram-variable coccobacilli and gram-negative rods with vacuoles	>30 5-30 1-4 □1 0	4 3 2 1 0

score	interpretation
0-3 4-6 7-10	Normal Intermediate, repeat test later Bacterial vaginosis
Review each of the three rows in the above box , assigning scores according to exam findings. Add the points for all 3rows for a final sum. A score of 7 or higher indicates bacterial vaginosis.	

Culture techniques:

Gardenella vaginalis:

Cultures of Gardnerella vaginalis are not useful for BV diagnosis, as up to 50% of healthy women have positive cultures due to low numbers of G. vaginalis in the vagina without any sign of BV. However, when no wet mounts or Gram stains are available and a clinical diagnosis is doubtful, massive growth of BV-associated bacteria or of Escherichia coli, group B streptococci or Staphylococcus aureus can help in distinguishing aerobic vaginitis from BV.[29,30] Furthermore, cultures for T. vaginalis and Candida may be extremely helpful in doubtful cases and in cases with mixed infections.

Mycoplasma cultures may help to delineate the pathogenicity of certain types of abnormal vaginal flora, especially in pregnancy, where there is evidence that the concomitant infection of Mycoplasma. hominis and Uraeplsma. urealyticum with BV may cause a more sever eset of complications such as miscarriage or preterm birth.[74-76]

Trichomonas vaginalis:

Culture for trichomoniasis is the gold standard, and several culture media have been used, most commonly the Diamond medium.

Recently introduced is a transport and culture medium for detection of Trichomonas, which performs specifically as well as Diamond medium.[77–80] Sensitivity varies from 42%-70% depending upon the experience of the microscopic and specimen collection technique. White blood cells are frequently seen.[77]

Vulvovaginal candidiasis:

Visualization of pseudohyphae (germ tube) and/or budding yeast (conidia) on 10% KOH examination which is preferred .Saline wet mount, or Gram stains was usually performed too.

When pH is abnormally high (>4.5), concurrent BV or trichomoniasis is suspected.

Cultures are not useful for routine diagnosis, since positive cultures may detect colonization rather than clinically significant infections and therefore should not be treated. Cultures may be useful to detect non-albicans species or resistant organisms in women with recurrent disease.[24,61]

Chlamydial infection:

The isolation of C. trachomatis on McCoy cells and monkey kidney cells culture from the, vagina, or rectum ,A although its specificity approaches 100%, the sensitivity of culture has been estimated at between 70% and 90% in experienced laboratories.[87,80]

Giemsa stain:

Cytological examination has been used to evaluated end cervical and urethral scrapings, including those obtained for pap smears. C.trachomatis inclusion bodies can be detected microscopically by immune fluorescence or in Giemsa stained preparation. Giemsa staining method is insensitive compared with culture or other methods.[80]

Biochemical, Serological and Molecular diagnostic methods:

Nucleic acid amplification assays have been developed for a variety of yeast species and Trichomonas vaginalis , although they are relatively expensive.[81]

Five commercial assays using nucleic acid amplifications are approved for the laboratory diagnosis of C. trachomatis infection. These assays use different formats: polymerase chain reaction (PCR), ligase chain reaction, standard displacement amplification, and transcription-mediated

amplification. Studies clearly indicated that these tests are more sensitive than culture and other non-nucleic acid amplification assays.[82-84]

DNA probes have been developed too to directly detect the presence of Candida, Trichomonas and Gardnerella, thus providing a more objective as well as sensitive diagnosis. [83]

Since Gardnerella is a normal part of the vaginal flora, the DNA probe test that is designed were relatively insensitive, detecting only pathogenic levels of Gardnerella. The Affirm VP III Microbial Identification System which is a commercially available DNA probe office-based test kit that simultaneously detects the presence of Gardnerella, Trichomonas and Candida [85] .The test's sensitivity for detecting Trichomonas vaginalis is high, and it can provide results in as little as 45 mins. Trichomonas can also be detected by DNA probes amplified by polymerase chain reaction. Sample is treated with enzymes that amplify specific regions of Trichomonas vaginalis' DNA. After amplification, the number of DNA fragments are quantified. Polymerase chain reaction has proven to be the most accurate diagnostic method in recent studies. Moreover, it is currently used only in research, not clinical settings.

Serological tests using monoclonal antibodies with direct immune fluorescence or enzyme-linked immunoassays are the current laboratory methods utilized for the diagnosis of C. trachomatis.[80,81]

A rapid and reliable laboratory test for diagnosis of bacterial vaginosis would be helpful in the clinical detection of this disease. Elevated prolineaminopeptidase activity has been identified as a reliable enzymatic marker for bacterial vaginosis. Prolineaminopeptidase assay has been shown to predict accurately women with a clinical diagnosis of bacterial

vaginosis. However, this assay has significant practical disadvantages, the most notable of which is the production of a carcinogenic end product, alpha-naphthylamine. Development of a modified assay for this bacterial vaginosis enzyme marker with L-prolinep-nitroanilide, a substrate that does not yield a carcinogenic end-product is now introduced [85].The new prolineaminopeptidase assay is a one-step test that is analyzed colorimetrically with microsomal leucineaminopeptidase used as a standard enzyme.[86]

Aims of the study:

Detection of common microbial causative agents of the abnormal vaginal discharges in risk group patients, will improve early diagnosis, prompt treatment and prevention of infective vaginal discharge in line with current syndrome management. This study which was undertaken in Benghazi area for fulfilling some of the aims :

1- To determine the prevalence of the most common infectious agents causing vaginitis and vaginosis among women of child bearing age in Benghazi area.
2- To determine the risk factors for acquiring these infectious agents.
3- To determine the best diagnostic algorithm for diagnosis and management of vaginitis and vaginosis in this area.

CHAPTER TWO

LITERATURE REVIEW

In a Cohort study performed in a rural area of Shandong province in china, about 439 married women of reproductive age was cluster randomly selected from the local birth control registry, the rate of bacterial vaginosis was 29% . [87]

In another study,[88] a total of 1223 pregnant women with gestational age of 16 –36 weeks from Amir-Almomenin General Hospital in Semnan, Iran were recruited they characterized by having no known medical risk factors for preterm labor birth were examined to identify the rate of bactieral vaginosis. Bacterial vaginosis was positive in 65 (33.1%) patients at 16 – 20 weeks of gestation. All patients with bacterial vaginosis had a vaginal pH of ≥5. There was a significant correlation between bacterial vaginosis and vaginal pH of ≥5, with preterm labor birth and preterm pre labor rupture of membranes.

Between June 1998 and July 2000, in 32 general practices and five family planning clinics in south London ,the prevalence of bacterial vaginosis was 14.5% (174 women). Bacterial vaginosis was more common in women under 25 years, those of Africo-Caribbean or black African ethnic groups. Those in social classes 3-5. Single women and those who had previously used oral contraception, those who smoked during pregnancy, as well as those with a history of termination, and those with concurrent Chlamydia infection were also proved to be risk factors.[89]

Bacterial vaginosis was very high in Turkish women using intrauterine devices (IUD). The infection rate was also very high in sexually transmitted disease clinics 60%. There was a significant negative

association between BV and oral contraceptives and condom use, and a significant positive association between BV and IUD use.[90]

Bacterial vaginosis was studied in 965 Peruvian women and was (27%) and significantly associated with having a bacterial sexually transmitted disease or trichomoniasis. age, marital status, and a history of sex work. No relation between BV and sexual experience, frequency of intercourse, or unprotected intercourse, were arrived.[91]

As cited in, [92] the prevalence of bacterial vaginosis was (20.7%) of 289 Brazilin women with abnormal vaginal discharge and were the most frequent manifestation Results also revealed a high prevalence with little relation to symptoms.

In a study in mysore, India, [93] an investigation of 838 women of 15-30 of reproductive age for bacterial vaginosis, Neisseria gonorrhoeae Trichomonas vaginalis infection and candidiasis was reported. They found that 391(34%)of these women were positive for bacterial vaginosis.

The overall prevalence of bacterial vaginosis according to the Amsel□s criteria in Thai women was 20.3%..The most common complaints were the abnormal vaginal discharge (41.0%) and pelvic pain(41.0%), whereas 32% had no symptoms. The only significant factor associated with BV was the duration of the Intrauterine Device Uses. Women with BV were more likely to have used IUD for a longer period than women without BV, especially more than 15 years.[94]

As shown in, [95] the presence of Gardnerella. vaginalis with the absence of lactobacilli in vaginal secretion are characteristics of patients with bacterial vaginosis.

Diagnosis was possible when a patient complains of non –irritating grayish-white, malodorous profuse vaginal discharge which may become a life-long nuisance to the women if it is not diagnosed correctly and

adequately treated.[41,96] An elevated vaginal pH (5-6.5) and the amine test odor in normal estrogenized patients is always associated with bacterial vaginosis.[97,98,99] However, G. vaginalis was most often observed adhering to the surface of exfoliated vaginal epithelial cells (clue cells) in higher number than anaerobic bacteria suggesting that G. vaginalis is responsible for clue cell formation which may be due to the presence of glycocalyx layer[100] as detected by wet mount or gram's stained smears.[100,48]

The incidence of abnormal vaginal discharge in 587 females of reproductive age is the commonest problems in Kano (Nigeria) and one of the most frequent gynecologic complaints in the clinics following discomfort encountered by such patients. Their mean age of was 25.9 years and Gardnerella vaginalis was detected in 62(24.5%) [101] of them. Out of 413 vaginal swabs examined from pregnant women in Basra, Iraq Gardnerella vaginalis was identified in 32 (7.7%) In cases where Gardnerella vaginalis was detected, 93.1% of women had vaginal pH greater than 4.5 as compared to those with no Gardnerella vaginalis where only 10.2% had greater than 4.5. All women with positive cultures had positive amine test (100%). Clue cells were a constant finding in the discharge of pregnant women with positive Gardnerella vaginalis cultures.[102]

The diagnosis of bacterial vaginosis (BV) by direct gram-stained smear of vaginal discharge was evaluated in 90 consecutive patients in Guinea-Bissau. Only 35(38.9%) of the patients with vaginal discharge satisfied the clinical diagnosis of BV. The vaginal gram-stained smear from all these 35 patients contained typical gardnerella morphotypes characteristic of BV and G .vaginalis was isolated from all of them as well as from a further 10 patients who did not satisfy the criteria for diagnosis of BV.[103]

Curved rods were detected in gram stained smears of vaginal fluid by Spiegel et al [104] in 51 % of 61 women with bacterial vaginosis (that is, nonspecific vaginitis), and by Cristiano et al [105]in 39% of 163 women with bacterial vaginosis and 0.3% of women without bacterial vaginosis. Hallen et al[106] detected Mobiluncus spp. with monoclonal antibodies in 20% of 455 women attending a sexually transmitted disease clinic, including 50% of the 107 women with BV. Roberts et al [107] detected Mobiluncus by gram stain and DNA probe in 33% of 92 consecutive female STD clinic patients, including 68% of women with BV.

Mobiluncus has been less highly associated with BV among pregnant women. Krohn[108] reported finding Mobiluncus morphotypes by Gram stain in 28% of 122 pregnant women with BV compared with 3% of 471 pregnant women without clinical signs of BV.

In a Population-based data on sexually transmitted infections (STD), carried out in a resource-poor community in Northeast Brazil, the infection rate with Neisseria gonorrhea was1.2%. [109]

Gonococcal infection represented 34% in752womenwith many urogenital complaint who presented consecutively to sexually transmitted disease (STD) clinic of the comprehensive Health centre in Kingston, Jamaica .[110]

As seen in,[111] in a primary-care study, it was reported that vulvovaginal symptoms including vaginal discharge due to Neisseria gonorrhea (GC) in 1%of more than 100 women .several pathogens may coexist.

In a Population-based data on sexually transmitted infections (STD), carried out in a resource-poor community in Northeast Brazil, the infection rate of Chlamydia Trachomatis was4.5% (0.3-6.6).[109]

A study was also done in 1994 on women with urogental complaint treat at a Jamaican sexually transmitted disease (STD) clinic, they find that Chlamydial infections was 34% in these women .[110]

A primary-care study, reported that vulvovaginal symptoms including vaginal discharge were due to Chlamydial trachomatis in 2%of more than 100 women .[111]

finally the overall prevalence of Chlamydial infection in 32 general practices and five family planning clinics in south London was 2.4% (29 of 1214, 1.5% to 3.3%), but 8.5% (13 of 152, 4.1% to 12.9%) in women under 25 and 14.3% (6 of 42, 3.7% to 24.9%) in teenagers. [90]
discharge[66], A correlation has also been demonstrated between the diagnosis of vaginitis and colonization with GBS.[67]

The prevalence of GBS colonization was high in pregnant women , it represented 25.4% of 405 Brazilian patients between 35 and 37 weeks of gestation, this study revealed that maternal age, number of sexual intercourse\week, occurrence of previous spontaneous abortion, presence of candidosis and cyctoltic vaginosis were associated with streptococcus colonization.[112]

Group B streptococcus was detected in 5% of 100 Pakistanian women of reproductive age group with complaint of vaginal discharge[113]While in study was done in India Streptococcus agalactiae was detected in 13 of 100 women with per partum fever and puerperal sepsis.[41]
Staphylococcus aureus was detected in 21(35%) of 102 women with abnormal vaginal discharge which were included in analytical cross-sectional study done in Goiania state of Goias.[114]

In a retrospective study done in Atlanta 1980, staphylococcus aureus was isolated from sixty two of 64 women with toxic shock syndrome and seven of 71 vaginal cultures obtained from healthy controls.[115]

In another study Staphylococcus aureus constituted 18.9% of total isolates of 110 vaginal swabs among married women.

Signs and symptoms consisted of :pus discharge, itch, painful urination and sexual intercourse and bad odor.[116]

Cohort study was performed in a rural area of Shandong province in china ,about 439 married women of reproductive age was a cluster of randomly selected from the local birth control registry. Infection rate with candida was 6.6% of these cases.[90]

A prospective study of female genital swabs from pregnant women which was performed in Nigeria, showed that Candida species were at the peak of the group of causative agents with 80.0% of the 380positive genital swab samples.[117]

In a cross sectional study conducted in public primary care service unites in botucatu Brazil from 2006-2008 included 289 pregnant women, the prevalence of vaginal Candidiasis was (11.8%).[92]

Simple laboratory tests identified etiologic agent in 64(91%) patents attending the outpatients department in a large public hospital in Katmandu, Nepal. The most frequent infection were Moniliasis (78%) .[118]

Furthermore, in a prospective study done over a period of one year at a teaching tertiary care hospital in Bangalore ,candidiasis constituted 9% of total isolates. [119]

The major pathogens found in Kano, Nigeria were Candid albicans 177(70.0%) of about 587 women.[101]

A total of 1223 pregnant women from Amir-Almomenin General Hospital in Iran, who had no known medical risk factors for preterm labor birth were examined to identify the cause of abnormal vaginal discharge . T.vaginalis was detected in 5.5% of these women, The frequencies for T.vaginalis which were 20 (29.9%) and 47 (70.1%) patients,

respectively.[88]

A prospective study of female genital swabs from pregnant women which was performed in Mongolia, showed that on rate of Trichomonas was (19%). [120]

In study when used algorithms (flowcharts) for syndromic management of abnormal vaginal discharge to treat 752 women who presented at a Jamaican sexually transmitted disease (STD) clinic, they find that trichomoniasis was documented for 25% the recruited women.[111]

In Michigan, in a primary-care study, vulvovaginal symptoms including vaginal discharge were due to trichomoniasis in 8%, BV and trichomoniasis account for at least 90% of more than 100 women of infectious vaginitis. [112]

In across sectional study performed on 683 women attending private outpatient clinics in Hamadan, western Iran, only 2.2% of patients with clinically diagnosed trichomonal vaginitis were positive for this agent vaginalis by wet smear and culture, the majority of them were married and non pregnant.[121]

In Libya ,in a cross sectional study that included 2450 women was done in Libya at Benghazi city by Kassem and Majoud (2006). A prevalence rate was 1.2% noted for trichomoniasis.[122]

A Saudi study conducted to reported the prevalence of trichomoniasis among total STDs recorded during 6 yr in a traditional society28%(10967 women of 39049).[123]

The prevalence of trichomonias is infection was geographically different in the various parts of Iran. For example, prevalence of trichomoniasis infection was 17% (12/141of patients) in Zahedan[124], 9.9% (100/1010) in Kashan[49], and 10.75%(43/400) in Bandar Abbas.[125]

CHAPTER THREE

Material and methods

Study populations

The present study was conducted at Benghazi Algadeda center (one of the poly clinics at Benghazi city). A total of 200 pregnant and non-pregnant women at their 15-50years of age (mean=33.7) were included in this study. They were selected from those attended the gynecology department between December 2010 to May 2011.Their main complain was symptoms of abnormal vaginal discharges and signs of vaginitis. Pregnant ladies at any gestational age were also included in this study from antenatal follow-up group whereas unmarried patients were excluded from this study. All women were subjected to gynecological examination by one of the experienced obstetricians of the center and interviewed by special questionnaire (attached).

Specimen collection

High vaginal swabs (HVS) were taken after standardized vaginal speculum examination . An un moistened speculum is inserted before any another vaginal examination was preformed. Vaginal fluid was collected from the posterior vaginal vault and put in the Amie's transport medium The following characteristics of the discharge were reported :

quantity, color , smell and the pH of the discharge which was determined directly with a pH indicator paper. An immediate fishy odor on addition of 1-2 drops of10% KOH was noted (positive amine test)Four sterile vaginal swabs were collected from each patient , swab is rubbed and rotated in the posterior post vaginal fornix .

- **First swab** was used immediately to prepare a wet mount and examined for motility of Trichomonas vaginalis ,yeasts, pseudohyphal and clue cells. It was also used for pH determination and whiff testing.

A diagnosis of bacterial vaginosis was made when three of the following parameters were detected:

(i) Vaginal pH greater than 4.5

(ii) Thin homogenous discharge

(iii) Presence of "clue" cells by wet mount, gram or giemsa staining

(iv) Positive amine test.

patients were considered negative for bacterial vaginosis if 2 or 3 of these criteria were not met. also if two parameters met.

- **Second swab** was immediately sent to the laboratory for gram's and Giemsa staining and reporting .
- **Third swab** was inoculated directly on chocolate ,blood MacConkey's agars and Sabouraud's dextrose agar.
- **Fourth swab** was used for the diagnosis of Chlamydia infection which was determined by a serological (CTK biotech Inc .)

The diagnostic Laboratory procedures: Fig(2)

The characteristics of the vaginal discharge :

The following parameters were reported for each sample:

Volume :small ,moderate, profuse .

Consistency: clumpy, thin, homogeneous, watery .

Color : white, yellow, creamy, grey, greenish, mixed with blood .

Fresh wet-mount examination:

Wet mount prepared with one to two drop of a normal saline on a glass slide and examined by light microscopy for:

actively motile ,pear shaped Trichomonas vaginalis. pus cells, budding yeast cells, pseudohyphae and clue cells were also looked for in the same wet mount .

Trichomonas vaginalis is pear shaped, with a short undulating membrane lined with flagellum and four anterior flagella. The organism moves with a characteristic wobbling and rotating motion. [(36)]

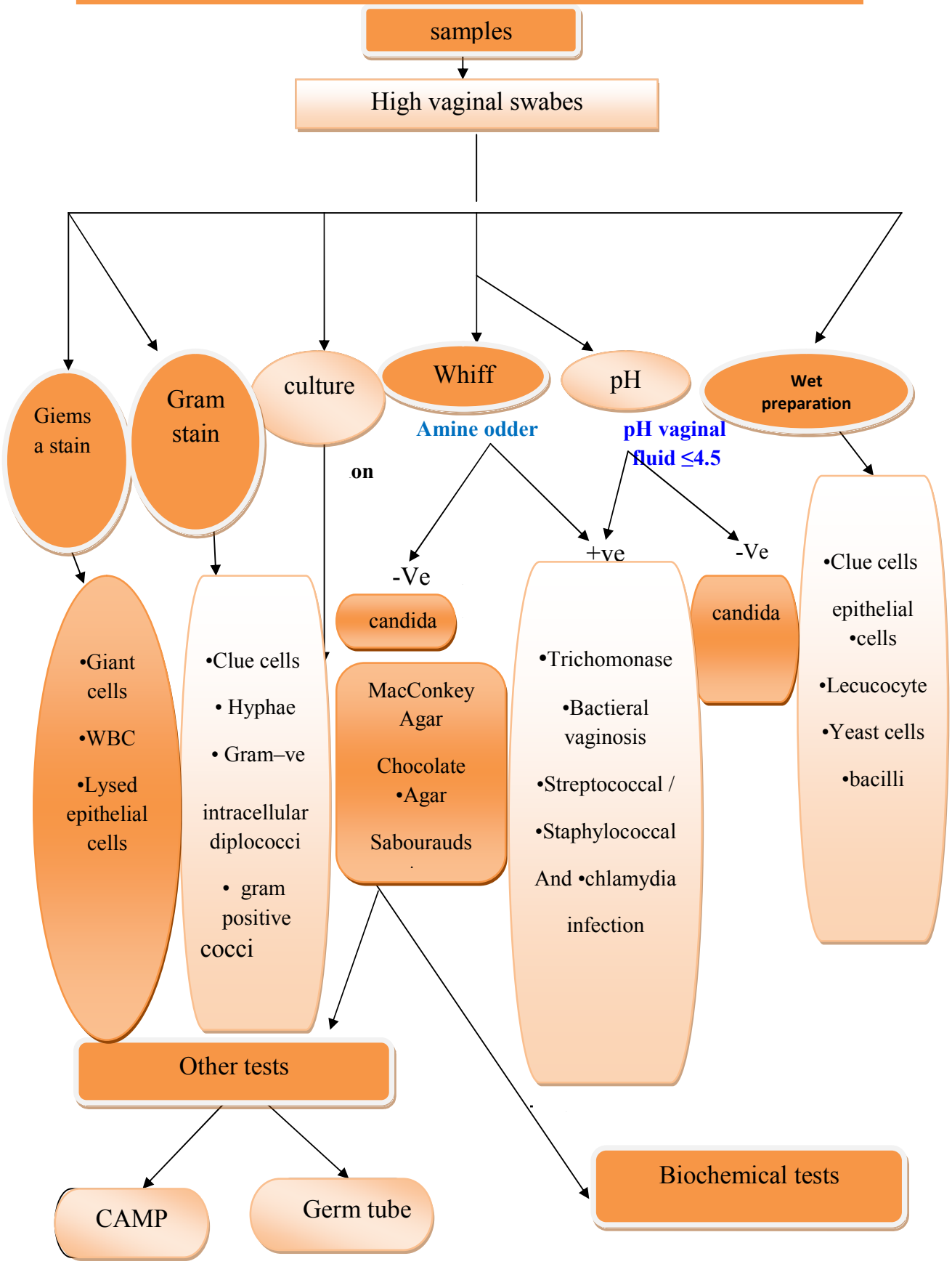
PH Determination:

Vaginal pH was determined with a pH indicator paper (range 5 – 9) (Riedel-De Haenag ,jahre; india) held with forceps and dipped into the vaginal discharge, color developed in 30 seconds was registered .Care was taken to avoid contamination with cervical secretion as it falsely change pH. strip was color-fixed indicator sticks obtained from (Riedel-De Haenag ,jahre; india) .

Whiff (amine odor) test:

Whiff test was done by adding 1-2 drops of KOH solution (10 %) on vaginal discharge. As reported in [(81)]. Presence of fishy odor was interpreted as positive whereas the absence of such odor indicate negative result.

Figure (2) : STEPS OF LAB DIAGNOSIS OF VAGINAL DISCHARGE



Gram stain and microscopic examination:

Vaginal swab is first rolled and spread as thin smear on a clean glass slide. Smears allowed to air- dry in safe place then fixed by using gentle heat . Smears were Gram stained, and looked especially for:

Pus cells, epithelial cells with attached gram variable coccobacilli that usually considered as Gardenerella vaginalis (clue cells). Fig(3).

Large gram positive yeast cells that could be Candida albicans or other candida species. Fig(4).

Pus cells containing Gram negative diplococci that could be Neisseriae gonorrheae .

Gram positive streptococci that could be Streptococcus agalactiae .

gram negative rods that could be bacteroides species or coliforms and gram positive cocci resembling Staphylococcus aureus .Fig (5).

Gram positive bacilli that could be lactobacilli .Fig(6).

Grading of vaginal gram smear:

Gram stained vaginal smears are usually graded by the improved Nugent's score [81]. This grading system was chosen because of its value in clinical practice during pregnancy. The scoring system that uses the most reliable morphotypes from the vaginal smear was proposed for diagnosing bacterial vaginosis. Fig(1). The scoring system that consisted of (0 to 10) figures scattered on 3 grades was described as a weighted combination of the following morphotypes:

lactobacilli, Gardnerella vaginalis or bacteroides (small gram-variable rods or gram-negative rods), and mobiluncus (curved gram-variable rods). Smears were graded in the following manner: grade I : (0-3) (normal flora), lactobacillus morphotype only.

grade II:(4-6) (intermediate flora), reduced lactobacillus morphotype with mixed bacterial morphotypes.

grade III:(7-10) (Bacterial vaginosis), mixed bacterial morphotypes with few or absent lactobacillus morphotypes .

Giemsa stain :

Vaginal swab is rolled on a clean glass slide, dried and fixed by covering it with methanol for 3 minutes. Smears were allowed to air dry and stained by the routine Giemsa staining technique for 30 minutes.

Slides were evaluated for :

Giant multi-nucleated cells, WBC or epithelial cells dominance, lactobacillary grades, WBC-epithelial ratio, clue cells, cellular debris, lysed epithelial cells, grading of lactobacillary morphotypes(small rods and curved long rods), acidophilic, basophilic cells and RBC. Fig (7,8,9).

Fig.(3): Gram stained smears showing clue cells coated with <u>Gardenerella vaginalis</u>

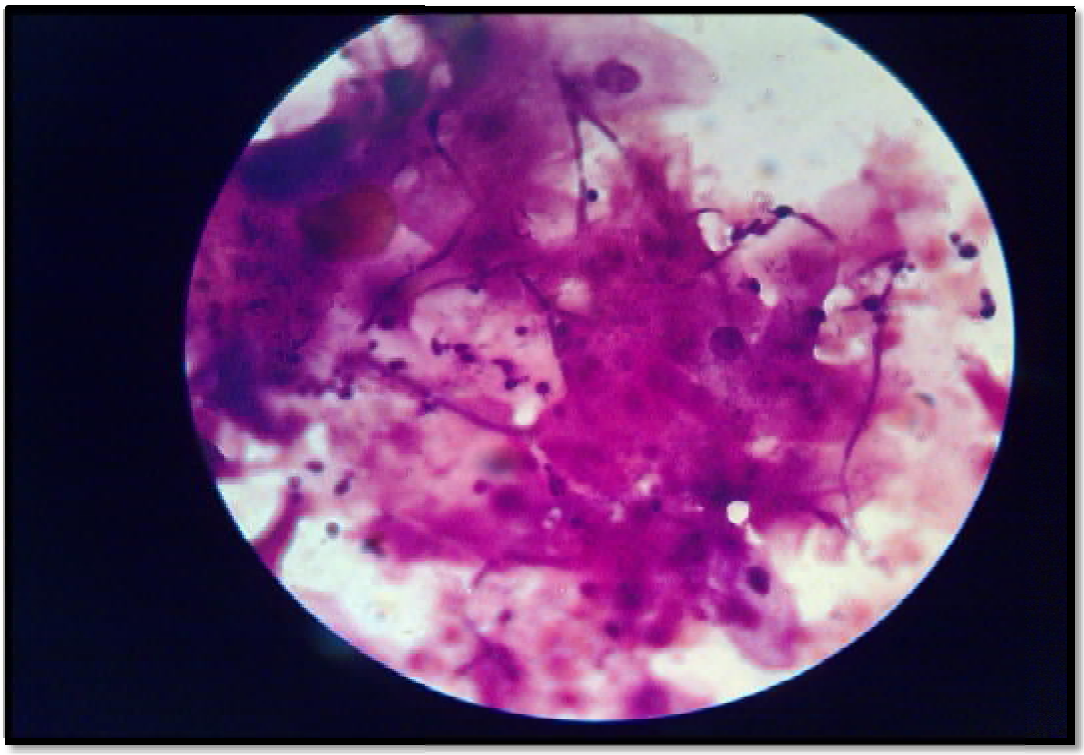


Fig.(4): Gram stained smears showing the presence Candida albicans

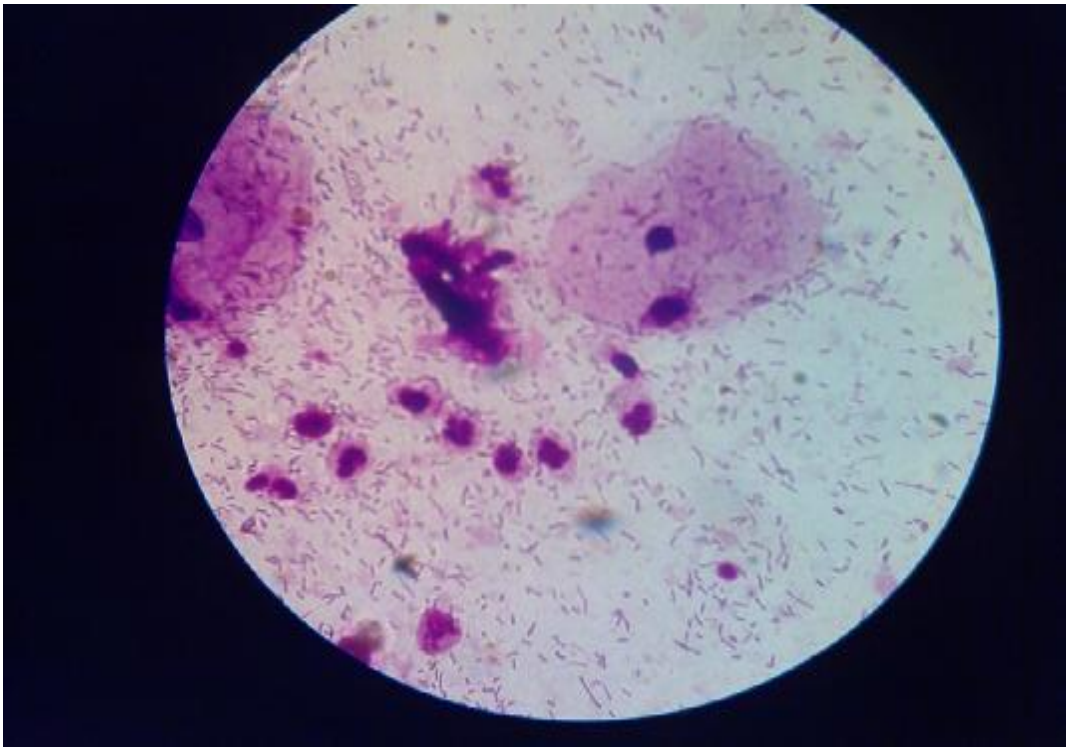


Fig.(5): Gram stained smear showing coliforms , Bacteroides and gram positive cocci

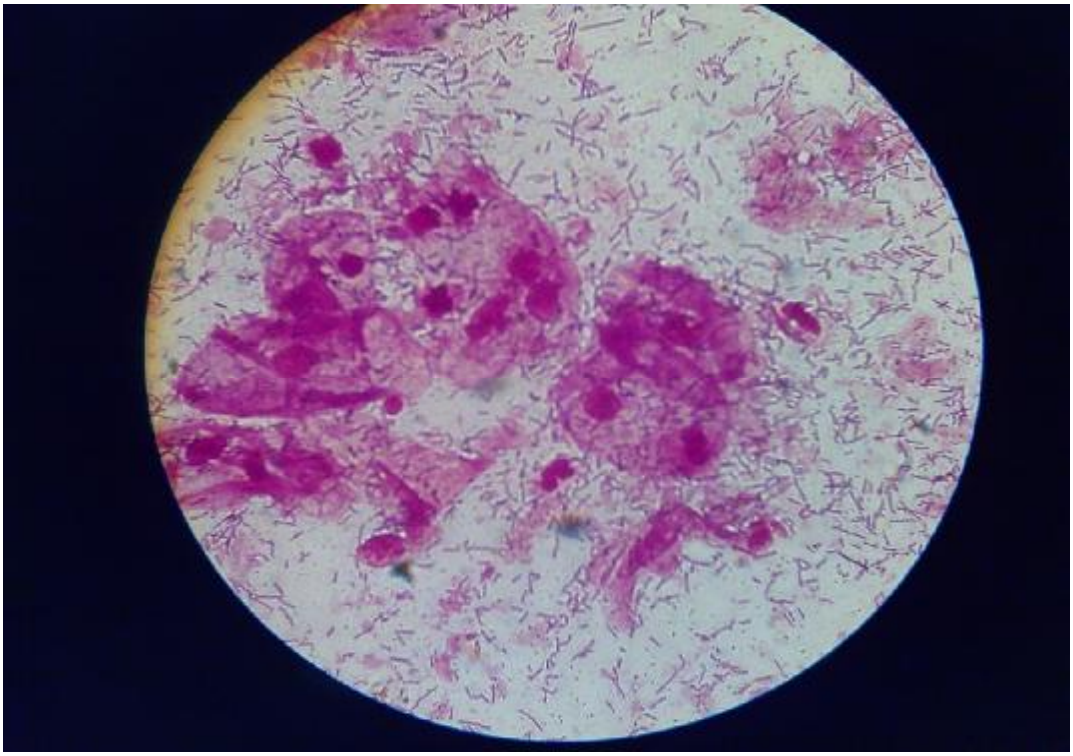


Fig.(6): Gram stained vaginal smear showing lactobacilli and clue

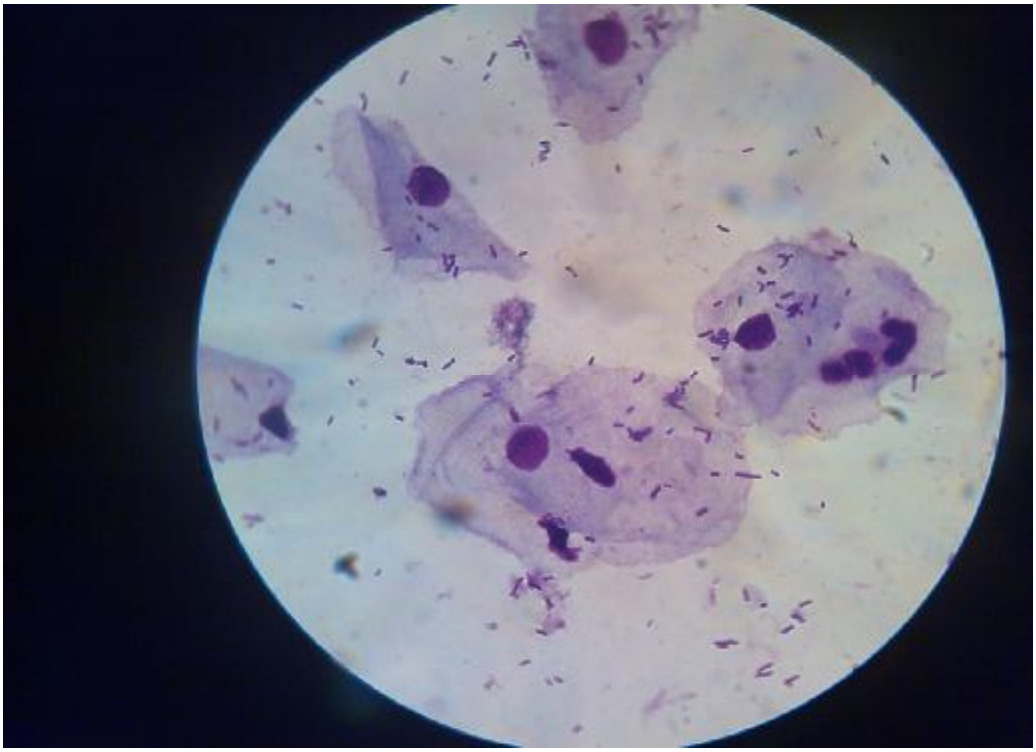


Fig.(7):Giemsa stained vaginal smear showing curved rods and small rods

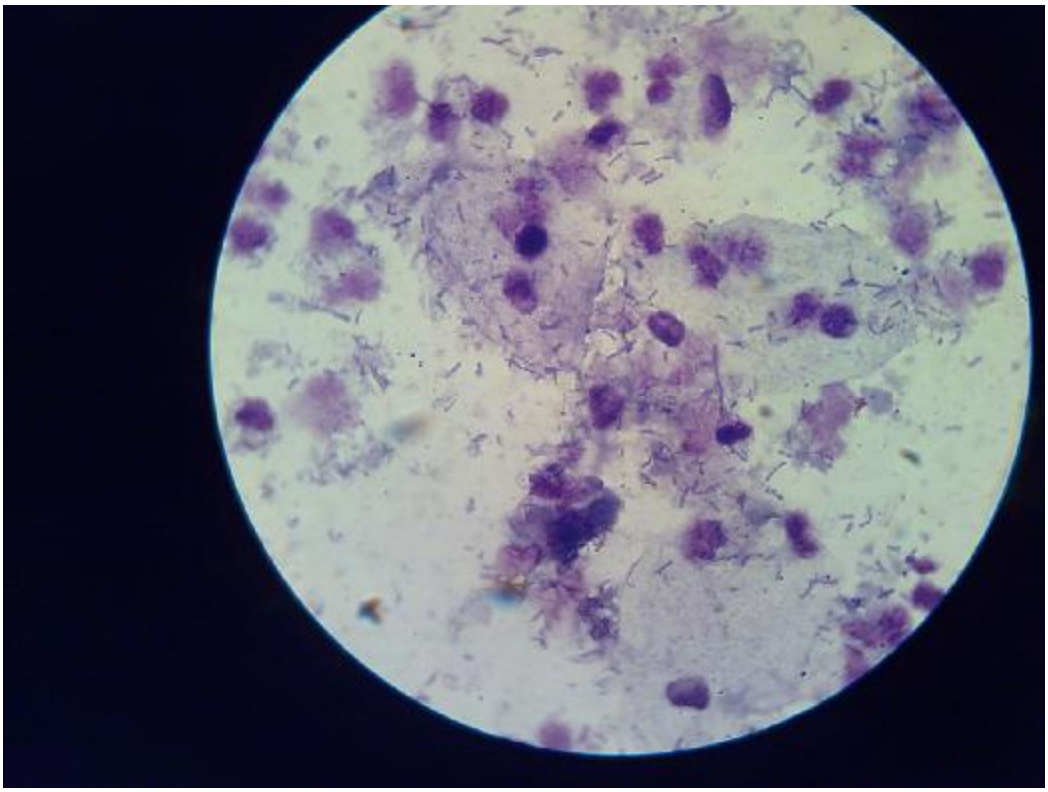


Fig.(8): Giemsa stained vaginal smear showing acidophilic cells:

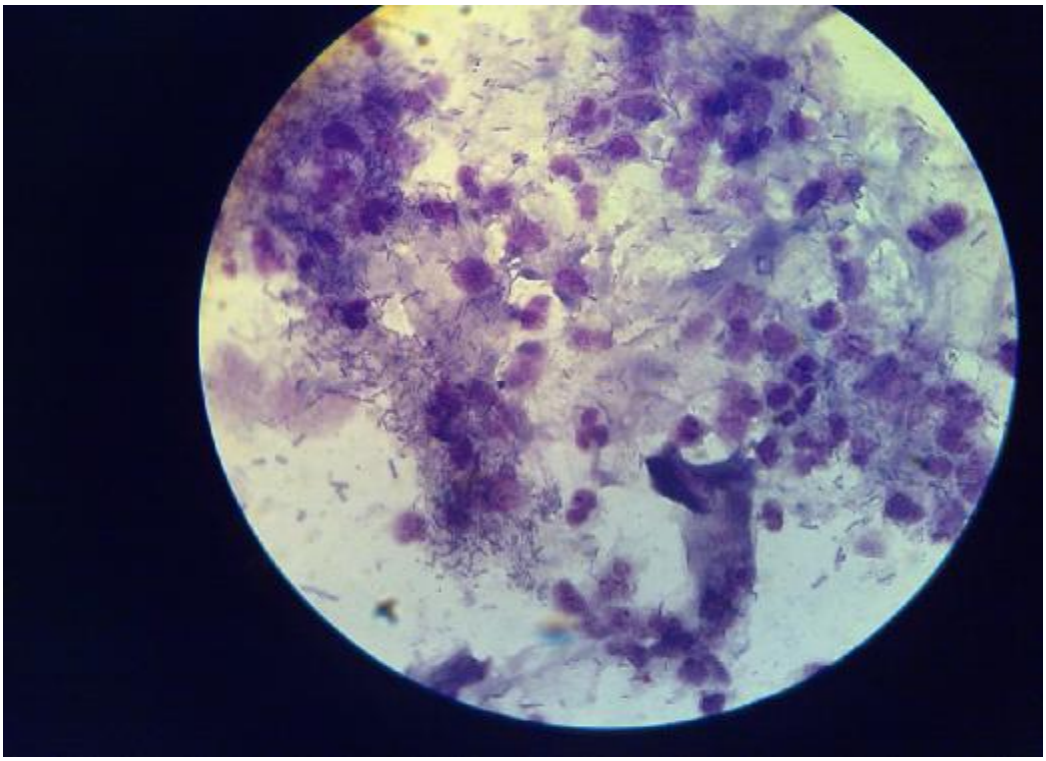


Fig.(9):Giemsa stained vaginal smear showing basophilic cells and RBC

Cultivation of the vaginal discharge:

vaginal swabs were inoculated directly on to: chocolate, blood, MacConkey's and Sabouraud dextrose agars. Chocolate and blood agar plates were incubated at 37 ºC for 48 hours. MacConkey's and Sabouraud's agar plates were incubated at 35 ºC±2 aerobically for 48 hours for the growth of gram negative bacilli and Candida respectively. Further chocolate and blood agars were incubated in candle jars to provide an increased CO2 tension (5-10%) required for the optimal growth of many pathogenic bacteria

Identification of isolates:

Group B streptococcus agalactiae:

On blood agar, small colonies produced beta-heamolysis after incubation at 5-10% CO2 environment. Colonies were identified by their morphology and resistance to Bactracin disc. they were picked up and subjected to Gram staining (showed gram positive cocci arranged in chains) and were CAMP test positive test. Fig (10).

Staphylococcus aureus and Staph. saprophyticus:

Golden yellow colonies appeared on blood and chocolate agar after incubation at 35-37C for 24 hours at 5-10% CO2. Colonies that produced complete hemolysis on blood agar were picked up and subjected to Gram staining (gram positive cocci arranged in group) and biochemical reactions(positive catalase and positive coagulase productions). On the other hand, the presence of large white colonies which appeared non-

hemolytic on blood agar and are coagulase negative indicate the finding of staph. saprophyticus

Candida albicans

On Sabouraud's dextrose agar and blood agar large cream colored colonies with a yeasty odor developed after 2-3 days incubation at room at 37C. Fig(11).

These colonies were identified by films stained with gram stain and showed budding yeast cells and Germ tube test positive.

Biochemical reactions for identification of the isolates:

Catalase test:

This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide .[126] It is used to differentiate between staphylococci and streptococci. Suspected colonies were immersed in a drop of 3% of hydrogen peroxide solution in a test tube. the production of gas bubbles indicates a positive reaction .[126]

Coagulase test:

A-Bound coagulase (slide test) :

As reported in [127] , on a clean microscopic slide one- two colonies of the culture to be tested were emulsified in saline and mixed with a drop of undiluted plasma added and gently mixed. The appearance of coarse clumping visible to the naked eye within 5-10 second means positive reaction while the absence of clumping or any reaction more than 10 seconds to develop is considered as negative reaction .

B-Free coagulase (tube test) :

The procedure given in [127] was followed.

One in ten dilution of the plasma in saline was prepared and 0.5 ml of the diluted plasma was placed in a small tube then 0.1 ml of an 18-28 broth culture of the strain under test was inoculated into the diluted plasma.

Then the tubes were incubated at 37C, and examined for coagulation at 1,3 and 6 h, up to 24 h ..the plasma has been converted into a stiff gel, in positive test while in negative test the plasma remains wholly liquid and free flowing.

CAMP test (Christie, Atkins, and Munch Peterson):

It was for the identification of Streptococcus agalactiae that produce diffusible extracellular protein (CAMP factor) which acts synergistically with the beta-lysin of Staphylococcus aureus to cause enhanced lysis of red blood cells.[81] It was done by single straight streaks of the streptococcus to be tested and a B-lysed –producing staph. aureus strain were made perpendicular to each other ,and 4mm apart on the surface of blood agar plate. After 24-48 hrs incubation at 35˚C, a positive test result appeared as an arrow head –shaped zone of complete hemolysis in the area into which both staphylococcal B-lysin and CAMP factor have diffused. The hemolysis is enhanced in the vicinity of the B-lysin producing staph. aureus . Fig(12).

Germ tube test:

It is confirmatory test of Candida albicans. A germ tube is defined as an appendage that is half the width and 3 to 4 times the length of the yeast cell from which it arises .There is no point of constriction at the origin of the germ tube from the cell. The test was done as mentioned in [127]

A light inoculum from the colony was inoculated on 0.5 ml human serum and incubated at 37˚C for 1-2 hrs. A drop of the serum yeast culture transferred to a glass slide and covered with a cover glass, on microscopic examination under low power magnification, pseudo-germ tubes seen as extensions from the yeast cells (drum stick appearance). Fig(13).

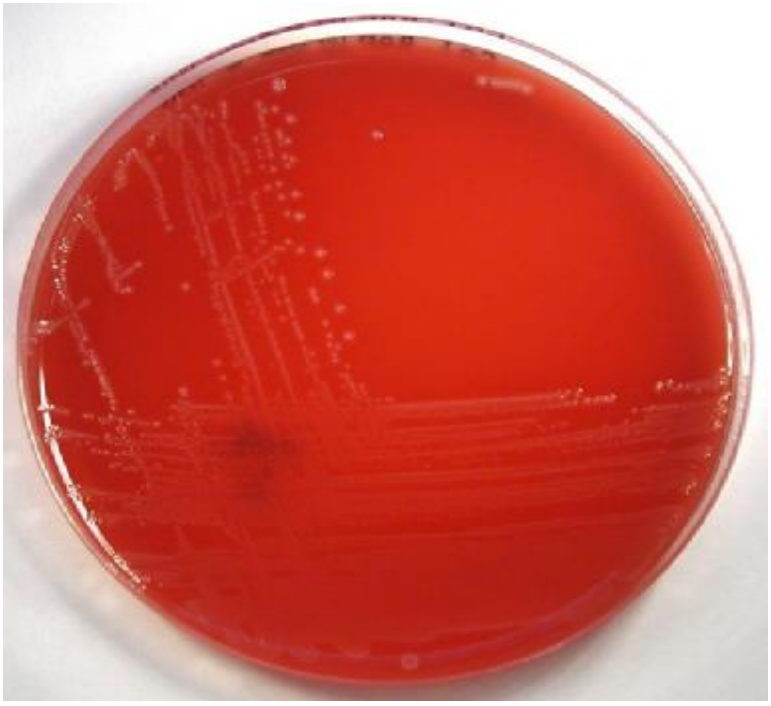


Fig.(10): Growth of Streptococcus agalactiae on blood agar

Fig.(11): Growth of <u>Candida albicans</u> on sabouraud's dextrose agar

Rapid Chlamydia test- cassette (swab):

Chlamydia Rapid test is a lateral flow chromatographic immunoassay based on the principle of the double antibody-sandwich technique. It utilizes a unique pair of the monoclonal antibodies to selectively identify Chlamydia Trachomatis antigen in the specimen. (CTK Biotech, Inc.). The test was performed according to the supplied instruction.

Test procedure:

Extraction tube for each patient was labeled and placed in a tube holder or rack. Four drops (200µl) of extraction buffer A to the extraction tube containing the specimen was added and twirled briefly to mix reagent. Tubes were left at room temperature (15˚C-30˚C) for 5 to 10 minutes , and then 4 drops (200µl) of extraction buffer B was added to the extraction tube containing the swab and twirled briefly. Tubes were incubated for 1 minute then pressed to remove as much liquid as possible from the swab. This was done by pressing and rotating the fiber portion against the wall of tube. Swabs discarded in a safety manner and tube caped and contents mixed by gentle swirling. Chlamydia Rapid test device was removed from its protective pouch and placed on a clean, flat surface. Two drops (100µl) of liquid from the extraction tube was dispensed to the sample pad and the result read within 15 minutes. Negative result indicated by only control band showed color development , test band showed no color development. Fig(14)(15). Both control and test bands give colors validate the test as positive.

Statistical analysis

Frequencies and percentages were executed by SPSS (version 17.0) .The statistical significance of differences between data was assessed using the chi- square test .

All statistical tests were two-sided, and differences between groups were considered significant for probability of less than 0.05 .

Fig.(12): CAMP test

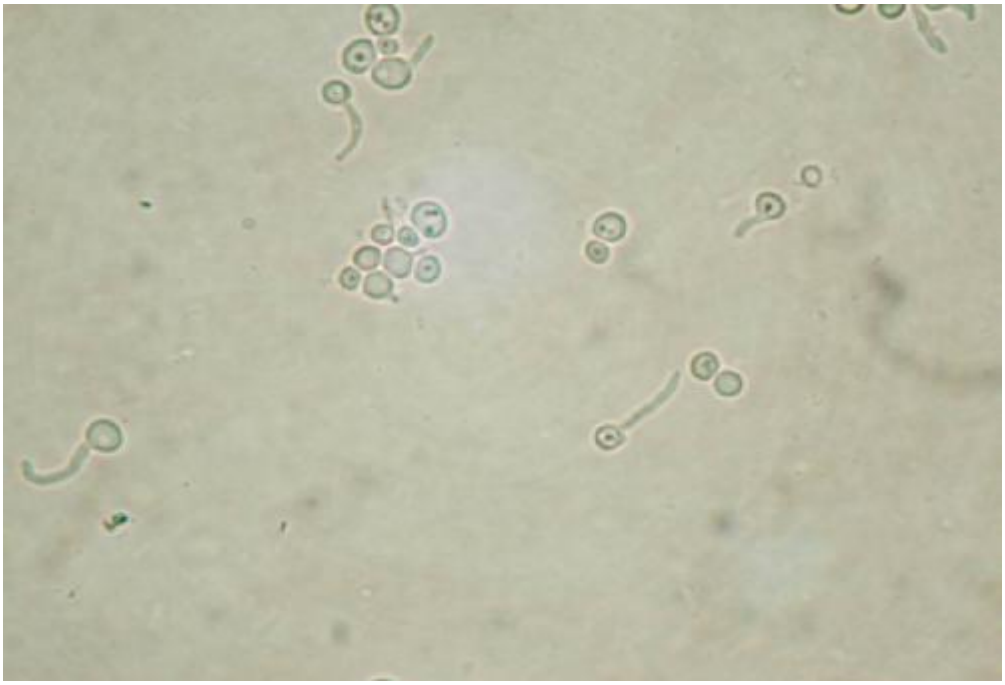


Fig.(13): Germ tube test

1

2

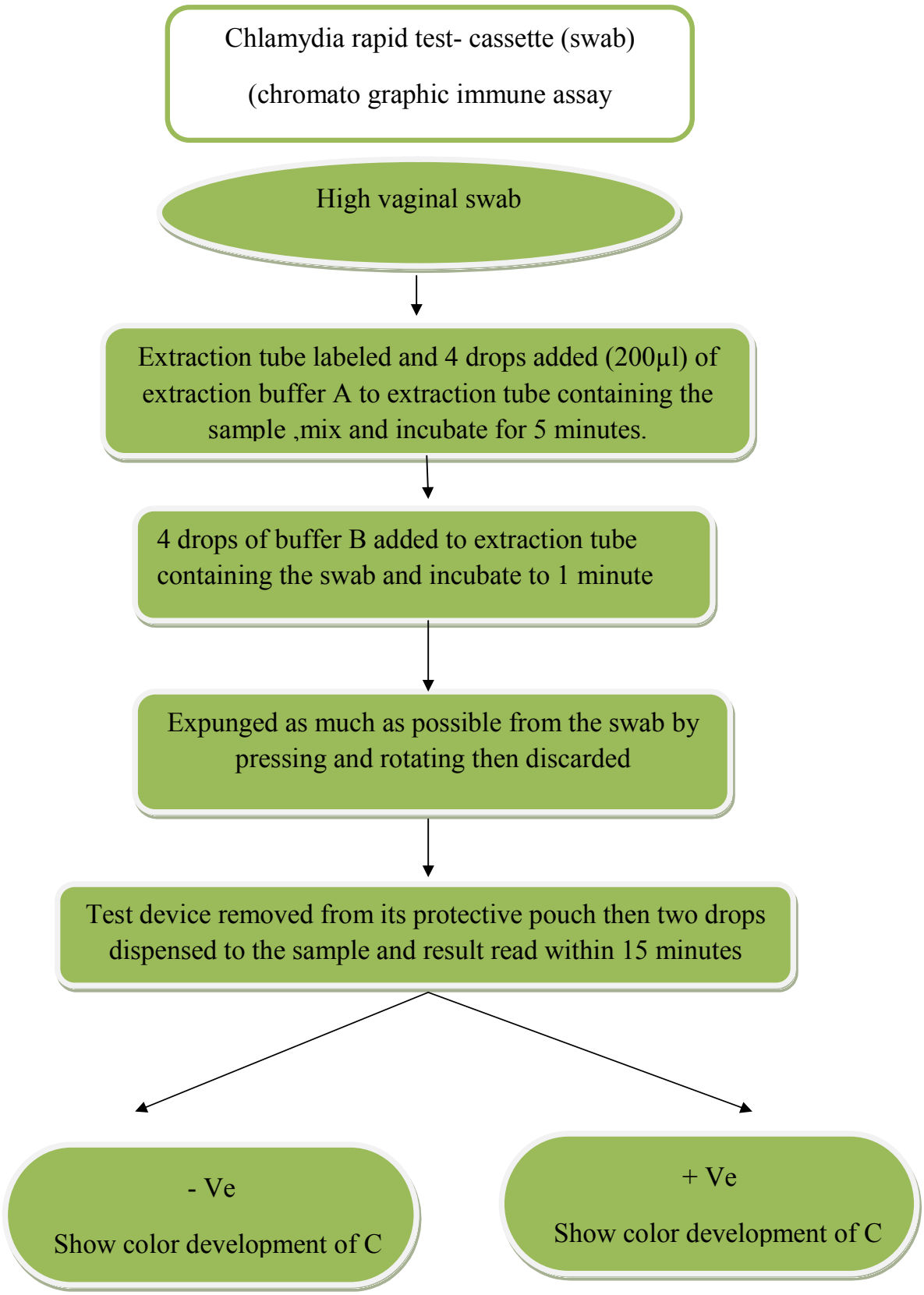
3

5

4

Fig.(14): Rapid Chlamydia test

Figure (15) : STEPS OF CHLAMYDIA TRACHOMATIS DETECTION



CHAPTER FOUR

Results

A total of 200 pregnant and non-pregnant women with abnormal vaginal discharges attended gynecology department in Benghazi, Algadeda center during a period of six months were included for this study. The finding of the common microbial causes of the abnormal vaginal discharge was allocated. Vaginal swabs were collected from them and assayed using wet preparation, Gram stain, Giemsa stain and culture assays .

Age of the patients:

In Table (1) and figure (17) the age of the recruited patients is depicted. They ranged between 16- 45 years (mean= 33.5). Thirty (15%) were in the age group of 16-25 years, 95 (47.5%) were 26-35 years in age, whereas 61(30.5%) were in the age group of 36-45 years. The remaining 14 patients (7%) aged over 45 years.

Demographic characteristics of patients :

In Table (2), the socio-economic characteristics was presented. Of all of the 200 patients , only 8 (4%) were illiterate, compared to 53 (26%) who finished their primary school, 72(36%) completed their high school and 67 had the Bachelor degree or higher. One hundred and ninety seven of women were married (98.5%), one is divorced and two are widows. Forty- five were employees compared to155 which were house wives. One hundred ninety eight of the 200 patients were white compared to two which were black .

Their husband socioeconomic status were as follow:

Educated husband were 75 (37.5%) who completed high school compared to 65(32.5%) whom completed primary school, whereas 48 (24%) had been Bachelor degree and higher. Only12 (6%) of them were illiterate Husbands who married one or two wives were 191(95.5%),only three (1.5%) married three or four .

Obstetric history:

As shown in table (3), the number of non-pregnant women were 143 (71.5%) compared to pregnant patients which were 57 (28.5%) . Pregnant women in their first trimester of gestation (≤13 weeks) were 21(10.5%) in contrast to 18 (9%) which were in their second trimester (13-37 weeks) and third trimester (27weeks to term). Concerning history of abortion seventy four (37%) women gave positive response in contrast to126 (63%)women who reported no history of abortion their method of delivery was as follow 143 (71.5%) reported vaginal, 12 (6%) delivered by cesarean section, 19 (9.5%) practiced mixed method of deliveries (vaginal and cesarean). Twenty six (13%) cases were primigravid hence no method of delivery is shown for them .

Table (1): Study population by age groups

Age-group	Total Number	Percent %
16- 25	30	15
26-35	95	47.5
36-45	61	30.5
More than 45	14	7
Total	200	100

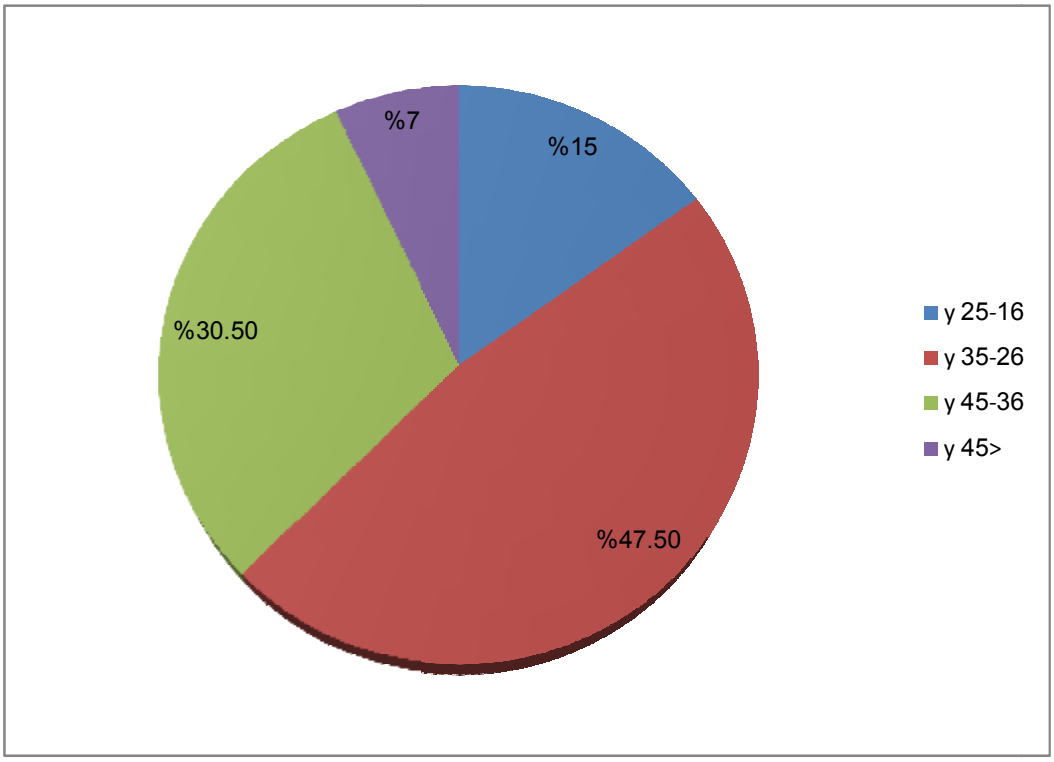


Fig.16:Distribution of studied population according to their age.

Table 2 : Demographic characteristics of patients

Education	Studied population	
	No.	%
illiterate	8	4
Primary school	53	26.5
Completed High school	72	36
University certificate	67	33.5
Marital status		
married	197	98.5
Divorced	1	0.5
widowed	2	1
Pregnant	57	28.5
Occupation		
House wife	155	77.5
worker	45	22.5
Husband education		
illiterate	12	6
Primary school	65	32.5
High school	75	37.5
University certificate	48	24
Number of wives		
1-2	191	95.5
3-4	3	1.5
Race		
Black	2	1
white	198	99

Table(3): Obstetrical history of patients.

Characteristic	Studied population	
	No (out of 200)	%
pregnant	57	28.5
Non pregnant	143	71.5
Gestational Age First trimester Second trimester Third trimester	 21 18 18	 10.5 9 9
Abortion Yes No	 74 126	 37 63
Method of delivery Vaginal Cesarean Vaginal +cesarean Primigravida	 143 12 19 26	 71.5 6 9.5 13

Risk factors of patients with vaginitis:

As depicted in table(4), 28(14%) of the 200 women gave history of oral pills used whereas those used condom mode were 7 (3.5%). Twenty five (12.5%) gave history of intrauterine devices use compared to 20 (10%) who used two types of contraception, and 13 (6.5%) gave history of practicing the rhythm method only .

Daily Hygienic care consisted using of intra -vaginal douching was practiced by 36 (18%) of women. Almost 186 ladies (93%) gave history of pad/ reusable towel use, whereas 121(60.5%) indicated the current use of toilet soap, 42 (21%) women used traditional medicine chemical preparations.

Concerning passive smoking, 89 (44.5%) showed positive response and 105 (52.5%) gave history of drinking of 1-2 cups of coffee daily.

Ten (5%) of the recruited women were diabetic, compared to 90 (45%) gave a history of evacuation and curettage and 70 (35%) who were under various current medications .

Table 4: Risk factors determined among the studied patients.

Risk factors	Studied population	
	No	%
Mode of Contraception		
Oral pills Condom IUD Two methods Rhythm	28 7 25 20 13	14 3.5 12.5 10 6.5
History of Evacuation and curettage	90	45
Pad/ reusable towel use	186	93
Vaginal douching	36	18
Current use of soap	121	60.5
Chemical preparation use	42	21
Current medication	70	35
Diabetes	10	5
Smoking passive	89	44.5
Caffeine consumption	105	52.5

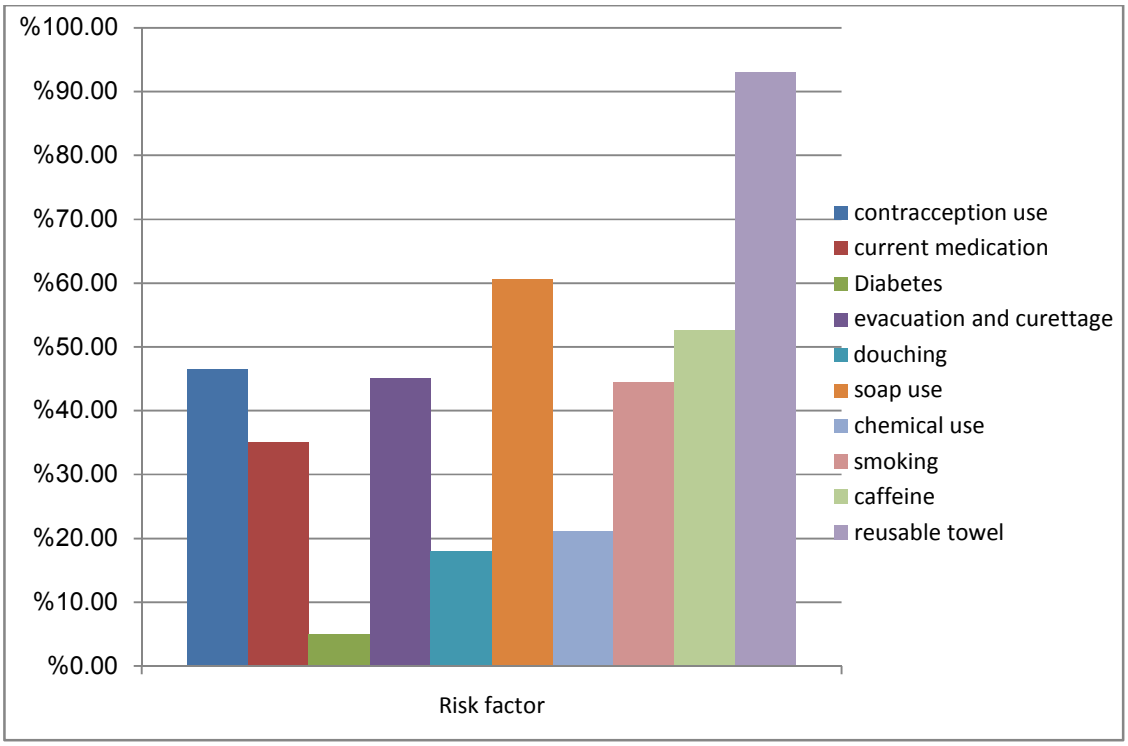


Fig.17:Risk factors detected for the determined patient

Vaginitis in relation to the demographic features of the patients:

1-Vaginitis by age:

Table (5) shows the prevalence of abnormal vaginal discharge in relation to the age group of patients. In the age range of 26-35 years, 95 (82.1%) of women were positive compared to 40 (65.5%) in the age group of 36-45 years . In the Age range of 16-25 years, 30(56.6%) showed positive results whereas in the age of over 45 only 8(57.1%) were with abnormal discharge. Statistical differences between these data were significant (p=0.012).

2- Vaginitis and educational level of patients:

Abnormal vaginal discharge was detected in all of the 8 illiterate cases(100%) contrasted to 53 (79.1%) of women who had the bachelor degree or more. Of the 72 women who completed high school only,46(63.8%) were positive. In the low education level (primary school), of the 53 women,36(66.6%) were positive. Differences were, however, statistically insignificant (0.058).

3- Vaginitis and marital status :

Of the 197 married women, 140 (71%) were positive compared to one divorced women positive and one widowed women which were positive. These data were, however, statistically insignificant (p= 0.545)

4- Vaginitis and occupation :

Of the 45 employees, 33 (73.3%) were positive compared to 110 (70.9%) of the non-employees who were positive too. Despite, these differences were statistically insignificant .(p=0.727).

5- Vaginitis and Race:

Of the 198 white women, 142 (71.7%) were positive for abnormal discharge compared to two black women, 1(50%) of them was positive. Differences in relation to race were statistically insignificant .(p=0.489)

6- Vaginitis and monthly income:

In the low social economic status, 123 (72.7%) of 169 women were positive compared to 20 (66.5%) the median social economic status which were positive. In the high social economic status one of them were positive . Differences in relation to monthly income were statistically insignificant p = (0.224).

7- Vaginitis in relation to husband education:

Of the 48 husband who had bachelor degree and higher,38(79.1%) of their wives were positive. In contrast, 50 (66.6%) of those who completed high school, and 45 (69.2%) of the low level of education were positive . In respect to illiterates, 10 (69.2%) of them were positive . Differences in respect to this parameter were statistically insignificant

(p=(0.357)

Table 5 : Vaginitis in relation to the socio-economic status of the patients.

characteristic	T. No	No. of positive	%	No of Negative	%	Statistical significance
Age						
16-25	30	17	56.7	13	43.3	χ^2=10.9
26-35	95	78	82.1	17	17.9	
36-45	61	40	65.6	21	34.4	P = 0.012
>45	14	8	57.1	6	42.9	
Education level						
illiterate	8	8	100	0	0	χ^2=7.46
Primary school	53	36	66.6	17	32	
High school	72	46	63.8	26	63.1	P = 0.058
University certificate or higher	67	53	79.1	14	20.8	
Marital status						
married	197	139	69.5	61	30.5	χ^2=1.214
Divorced	1	1	100	0	0	
widowed	2	2	100	0	0	P = 0.545
Occupation						χ^2=0.096
House	155	110	70.9	45	29	
worker	45	33	73.3	12	26.6	P = 0.727
Race						χ^2=o.458
Black	2	1	50	1	50	
white	198	124	71.7	56	28.2	P = 0.498
Monthly income						χ^2=2.989
Low SES	169	123	72.7	46	27.2	
Median SES	30	20	66.6	10	33.3	P = 0.224
High SES	1	0	0	1	50	
Husband education						
illiterate	12	10	38.3	2	6.6	χ^2=3.233
Primary school	65	45	69.2	20	30.7	
High school	75	50	66	25	33.3	P = 0.357
University certificate	48	38	79.1	10	20.8	

vaginitis in relation to clinical and previous medical history :

Table (6) shows patient□s clinical presentation and their previous health history.

1-prevalence of vaginitis in relation to gravidity status:

Out of 57 of pregnant women, 42 (73.7%) were positive compared to 101 (70.6%) in the non-pregnant women who were positive. Differences, however, were statistically insignificant (p=0.666)

2- Vaginitis and history of abortion:

Out of the 47 women who had history of habitual abortion, 26 (55.3%) were positive compared to 90 (71.4%) of 126 women with no history of habitual abortion which were vaginitis positive. Differences in relation to abortion were statistically insignificant (p=0.977)

3- Vaginitis and method of delivery:

Of the 143 women who delivered by vaginal method, 102 (76.1%) were positive, in contrast to 10 (83.3%) of the 12 women who delivered by cesarean section. Within the 18 of those who had more than one method of delivery 13 (68.4%) were positive . Primigravid women that consisted of 26 cases, 18 (69.2%) of them were positive. Differences were statistically insignificant (p=0.806)

4- Vaginitis and current medical history:

Of the 10 Diabetic women, 5 (50%) were positive, compared to 2 hypertensive (100%) which were both positive. In respect to the 181 women who gave no history of medical problem, 133 (73.4%) were positive. Differences however were statistically insignificant (p=0.1

Table (6): Prevalence of vaginitis in relation to the patient□s clinical presentations and previous medical history

characteristic	T. No	No. of positive	%	No of Negative	%	Statistical significance
Gravidity status						
pregnant	57	42	73.7	15	26.3	χ^2=o.187
Non pregnant	143	101	70.6	42	29.4	P = 0.666
History of Abortion						
Yes	47	26	55.3	21	44.7	χ^2=0.001
No	126	90	71.4	36	28.6	P = 0.977
Method of delivery						
vaginal	143	102	76.1	41	30.6	χ^2=0.981
cesarean	12	10	83.3	2	16.7	P = 0.806
Vaginal+ cesarean	19	13	68.4	6	31.6	
No cases	26	18	69.2	8	30.7	
Past and current medical history						
Diabetes	10	5	50	5	50	χ^2=8.511
Hypertension	2	2	100	0	0	P = 0.130
Diabetes+ Hypertension	2	1	50	1	50	
Others	4	1	25	3	75	
No cases	181	133	73.5	48	26.5	

Prevalence of vaginitis in relation to personal hygiene and habits:

As demonstrated in table (7), 70(75.2%) of the 93 women with history of contraception use were positive. This is contrasted to, 65(72.2%) of the 90 women who gave past evacuation and curettage were positive . Furthermore, of the 186 women who gave history of reusable towel use, 131(70.4) were positive, compared to 31 (86.1%) of the 36 who gave history of vaginal douching use, 90 (74.3%) of the 121 women who frequently used soap . these who reported the use of traditional medicine chemical preparations consisted of 42 women, 36 (85.7%) were positive. Concerning history of husband smoking, 65 (73%) of the 89 women were positive , compared to all of the 105 (100%) who gave history of drinking caffeine were positive. Difference however, were statistically insignificant ($p>0.05$). In respect to patients who registered history of current medication, 70 (75.2%) showed to be positive.

Table (7) : Prevalence of vaginitis in relation to the patient's personal hygiene and habits.

Risk factors	T. No	%	NO. of positive	%	Statistical significance
Contraception use	93	46.5	70	75.2	P=0.656
Evacuation and curettage	90	45	65	72.2	P=0.838
Pad/ reusable towel use	186	52.5	131	70.4	P=0.222
Vaginal douching	36	18	31	86.1	P=0.032
Current use of soap	121	60.5	90	74.3	P=0.264
Traditional chemical preparations use	42	21	36	85.7	P=0.022
Passive smoking	89	44.5	65	73	P=0.667
Caffeine consumption	105	52.5	105	100	P=0.789

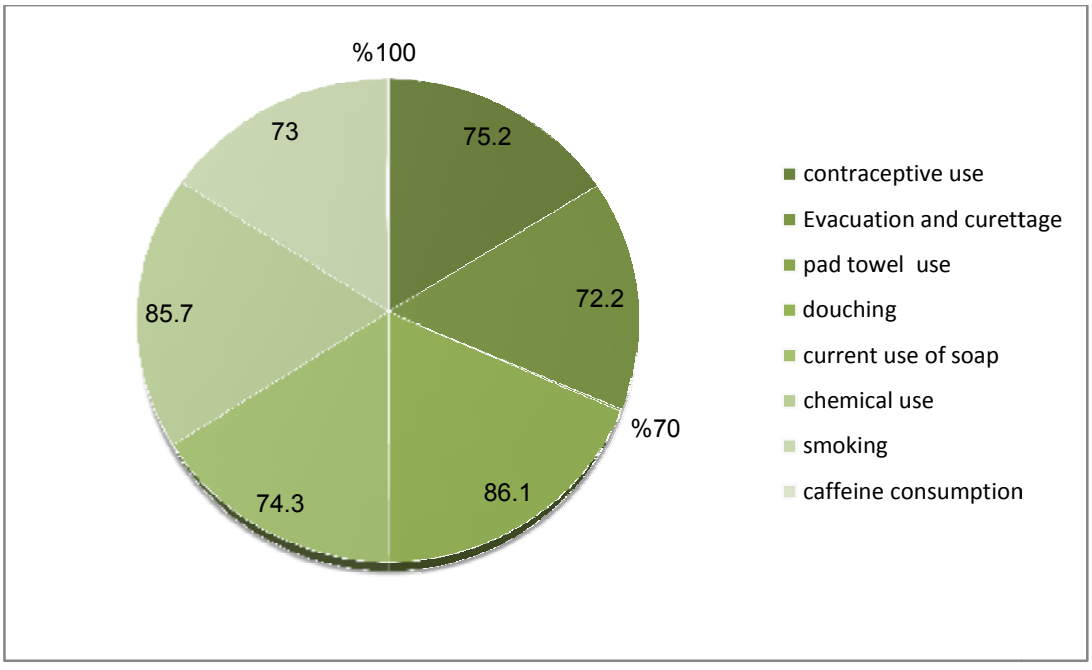


Fig.18:Etiologic agents of vaginitis in relation to Risk factors.

Causes of vaginitis and bacterial vaginosis :

As given in table (8), Bacterial vaginosis was reported in 99 (49.5%) of patients followed by Trichomonas vaginalis which was demonstrated in 38 (19%) of patients. Staph. aureus was seen in 27 (13.5%) of cases , in contrast to Candida albicans which was proved in 25(12.5%). Other Candida spp was documented in 23 (11.5%) of patients .In respect to Streptococcus agalactiae, 8 (4%) cases were positive compared to 12 (6%) of patients gave Staphylococcus saprophyticus positivity . Mixed infections were detected in 58 (29%) of patients.

Frequencies of vaginal infection:

Table 9 depicted that 141 (70.5%) of women with abnormal vaginal discharge showed determined causative agents compared to 59 (24.5%) which were negative for any causative agent.

Table 8: Bacterial, parasitic and fungal causes of vaginitis

Agent	frequency	%
Bacterial vaginosis	99	49.5
Trichomonas vaginalis	38	19
Staphylococcus aureus	27	13.5
Candida albicans	25	12.5
Candida spp	23	11.5
Staphylococcus saprophyticus	12	6
Streptococcus agalactiae	8	4
Mixed infections	58	29

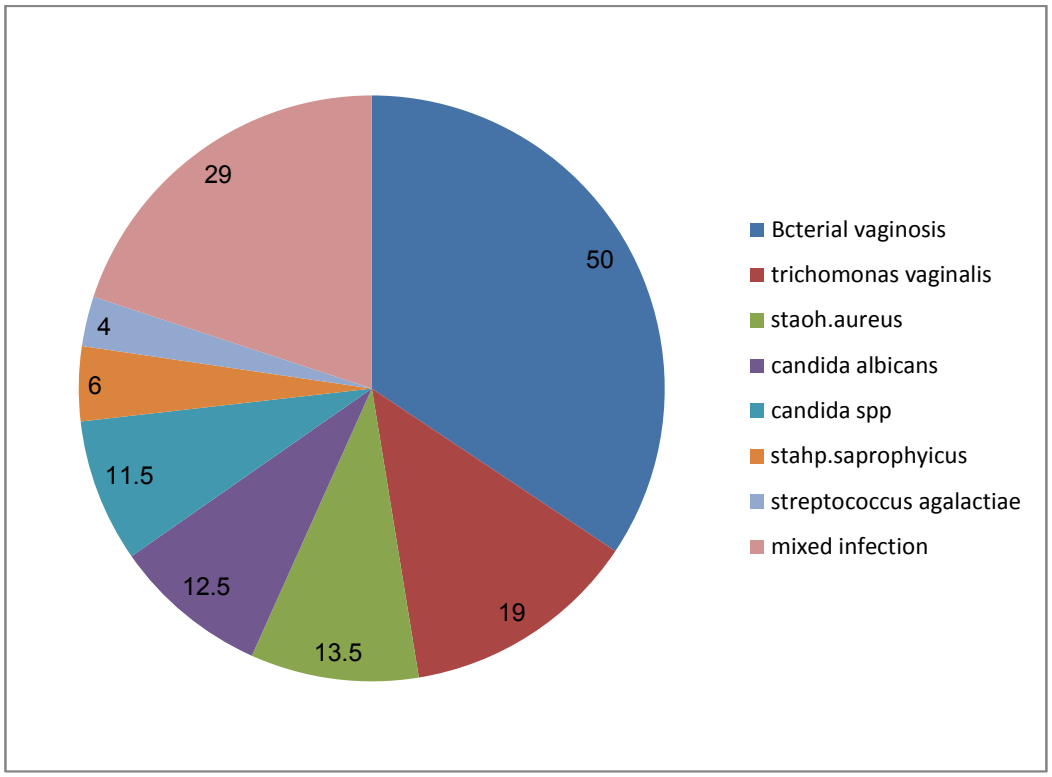


Fig.19: Frequency of different etiological agents of abnormal vaginal discharge

Table 9 : Frequencies of vaginal infections whether the cause is defined or unidentified etiology.

	Studied population	
	No	%
Positive for causative agent	141	70.5
Negative for any causative agent	59	29.5

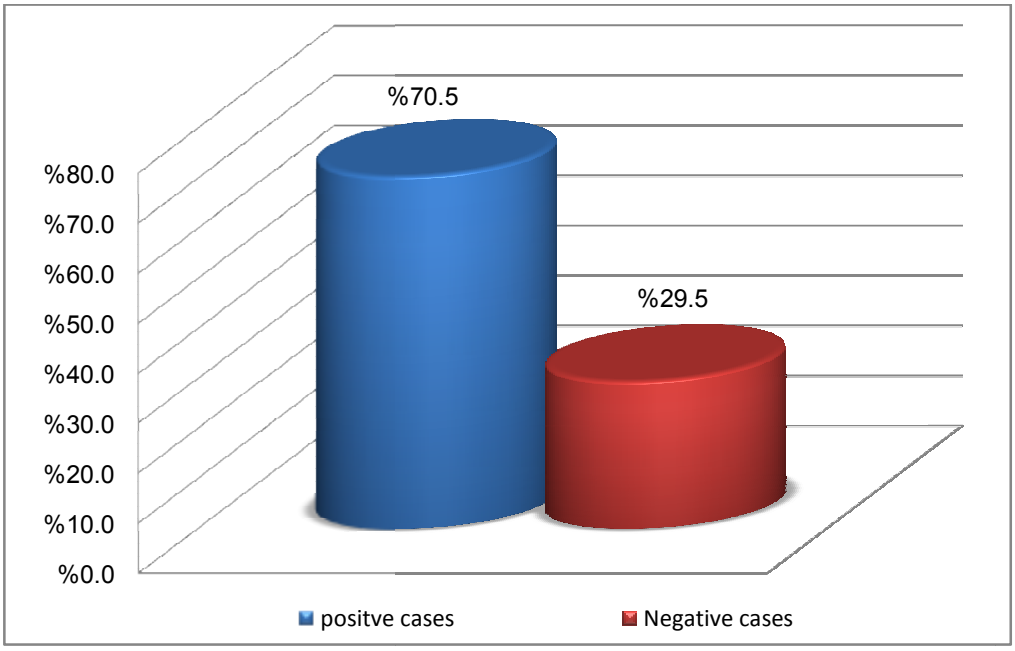


Fig.20: Frequency of vaginal infections in the determined women.

Data obtained from cultural characteristics

Table (10) depicts that no growth results were seen in 67 (33.5%) of total 200 vaginal samples. Difference in respect to this parameter were statistically significant (p□0.05) Lactobacilli were obtained in 60 (30%) of the cases, with difference which were statistically insignificant (p>0.05). Candida albicans appeared in 25 (12.5%) of cultures, whereas other Candida spp. were demonstrated in 23 (11.5%). Differences were statistically significant (p=0.05). Concerning Staphylococcus aureus, they were cultivated in 27 (13.5%) of the samples, compared to Staph. saprophyticus which were reported in 12 (6%) instances. Streptococcus agalactiae were successfully grown from 8 (4%) of cultivation trials, with difference were statistically insignificant (p>0.05) .

Table 10 : Microorganisms obtained after cultivation of the vaginal discharges.

organism obtained	T. No	%	Statistical significance
No growth	67	33.5	χ^2 =10.806 p=0.001
Lactobacilli	60	30	χ^2 =1.777 p=0.128
Candida albicans	25	12.5	χ^2 =11.69 p=0.003
Other Candida spp.	23	11.5	χ^2 =10.359 p=0.01
Coagulase positive Staphylococcus aureus	27	13.5	χ^2 =9.853 p=0.002
Staphylococcus saprophyticus	12	6	χ^2 =5.089 p=0.024
B-hemolytic streptococci(Strept.agalactiae)	8	4	χ^2 =2.891 p=0.089

Causes of vaginitis and methods of contraception:

Table (11) depicts that bacterial vaginosis were reported in 17(60.7%) of cases who used oral pills, in 4 (57.1%) who practice condom use, In those cases who used two methods 13 (65%) were positive. In women applying the rhythm method of contraception, 7 (53.8%) were positive compared to 45 (42 %) of cases who don't practiced any contraception method .

In respect to Trichomonas vaginalis, they were reported in 4 (14.2%) of cases using oral pills,4 (57.1%) women using condom,3 (12%) with IUD use. In patients who used two methods, the rate was 4 (20%) and only 1 (7.6%) was positive in those with rhythm method .On the other hand, 25 (23.3%) were positive in those don□t use any contraception .

In the present study, Candida albicans was diagnosed in 1 (3.5%) of female using oral pills , contrasted to none of those using condom was positive. With IUD or rhythm methods use, 2 (8%) and 3 (23%) were noticed positive. With no application of any contraception, 14 (1%) were positive.

With concern to other Candida spp. 5 (17.8%) of women using oral pills,1 (14.2%) women using condom, and 5 (20%) of IUD method were positive. For cases who used two methods, the level was 2 (10%), compared to 1 (7.6%) for rhythm method women and 9 (8 %) for cases who don't use contraception .

Vaginitis caused by staph. aureus was seen in 2 (7.1%) of cases who used oral pills compared to no cases because of condom use. For IUD use, the rate was 4 (16%), whereas in two methods or rhythm practice 1

(5%), and 3 (23%) was positive respectively. In cases who don't use contraception, 17 (15.8%) were suffered of vaginitis .

Cases due to Streptococcus agalactiae the following data were obtained : 1 (3.5%) of cases using oral pills, 3 (12%) of those using condom, 3 (12%) of IUD use and none in those practicing the rhythm method with 3 (2.8%) was reported in cases who don□t use contraception.

Figures for Staphylococcus saprophyticus were as follow : 1 (3.5%) of cases who used oral pills, 1 (14.2%) in cases using condom, 2 (8%) with IUD use. None was recorded in two methods use whereas in using of rhythm method the rate was 2 (15.3%) compared to 7 (6.5%) in cases who don't use contraception. Differences for all of the above data were statistically insignificant (p=0.650) .

Table (11) : Etiologic agents of vaginitis based on methods of Contraceptive.

contraceptive	Total No. (%)	BV (%)	TV (%)	CA (%)	CA spp (%)	S.aureus (%)	Strept agalac (%)	Staph. sapro (%)
Oral pills	28 (14%)	17(60.7)	4(14.2)	1(3.5)	5(17.8)	2(7.1)	1(3.5)	1(3.5)
Condom use	7(3.5%)	4(57.1)	1(14.2)	0(0)	1(14.2).	0(0)	0(0)	1(14.2)
IUD	25 (12.5%)	13(52)	3(12)	2(8)	5(20)	4(16)	3(12)	2(8)
Two methods	20(10%)	13(65)	4(20)	2(10)	2(10)	1(5)	0(0)	0(0)
Rhythm method	13(6.5%)	7(53.8)	1(7.6)	3(23)	(7.6)	3(23)	0(0)	2(15.3)
No use	107 (53.5%)	45(42)	25(23.3)	14(1)	9(8.4)	17(15.8)	3(2.8)	7(6.5)
Total	200	99	38	25	23	27	7	

$X^2=3.327$

P=0.650

BV= Bacterial vaginosis TV=Trichomonas vaginalis CA=candida albicans
CAssp=candida spp , Staph=staphylococcus aureus Strept=streptococcus agalactiae

Staph. sapro= saprophyticus

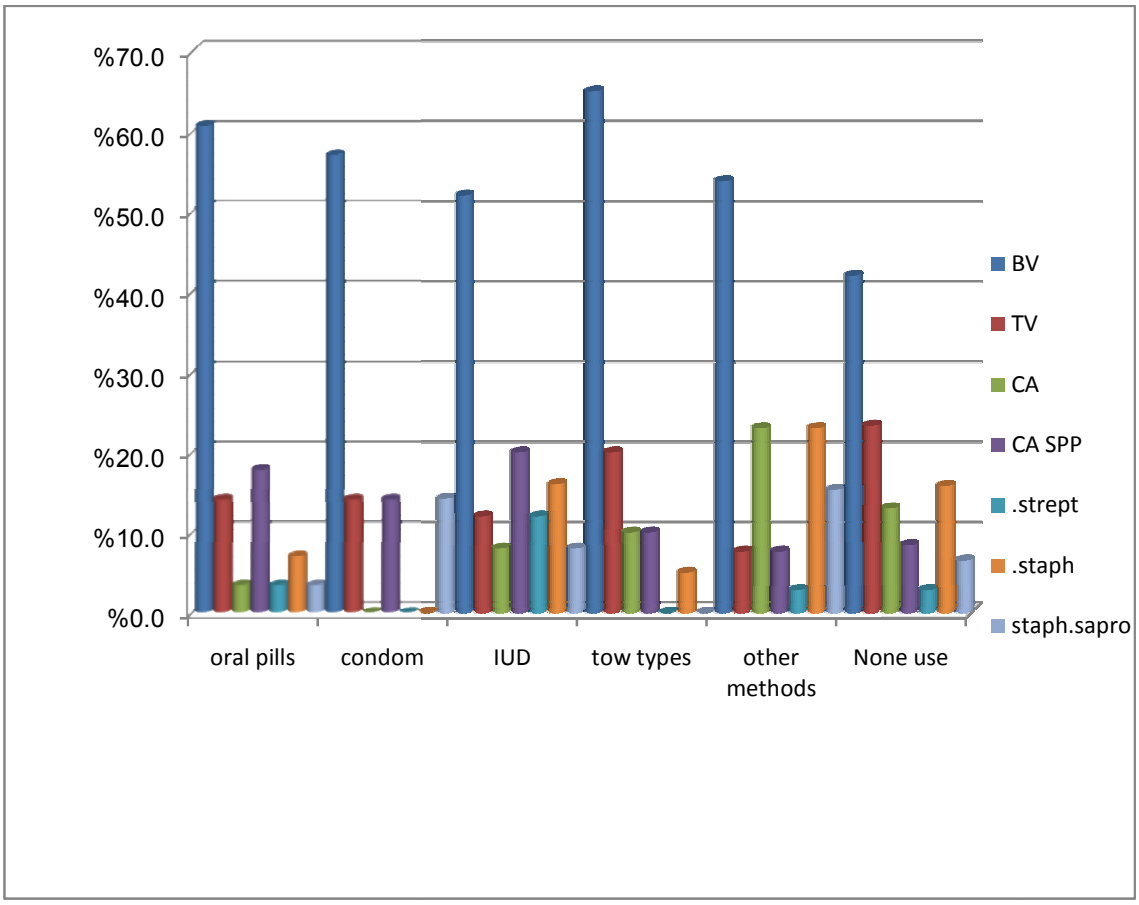


Fig.21:Etiologic agents of vaginitis in relation to contraceptive Methods.

Trimester of gestation and causes of vaginitis :

Table (12) demonstrate that bacterial vaginosis in the first trimesters was reported in 15 (71.4%) of cases, in second trimester the rate was 9 (50%) , and 8(44.4%)in the third trimester. In the non-pregnant women the level was 67 (46.8%). Differences was statistically insignificant (P=0.841).

In cases due Trichomonas vaginalis , in the first trimester, 4 (19%) were positive compared to 2 (11.1%) in second trimesters and 3(16.6 %) in third trimester. In the non-pregnant women 29 (20.2%) were with sign of vaginitis.

Infection with Candida albicans was seen in 2 (9.5%) in the first trimesters ,4 (22.2%) in the second trimesters, 6 (33.3) in third trimester and 13 (9%) of the non-pregnant women .

Affection with Candida spp in the first trimesters was reported in 7 (33.3%) , in the second trimesters it was 2 (11.1%) ,1(5.5%) was in third trimester and in non-pregnant women it was 13 (9%).

Vginitis caused by staph. aureus in the first trimesters was demonstrated in 1 (4.7%) , in the second trimesters it was seen in 4 (22.2%) , in 1 (5.5%) in third trimester and in 21 (14.6%) of the non-pregnant women.

Concerning Strept. agalactiae cases none was reported in the first trimester compared to1 (5.5%) encountered both in second and third trimesters. For the non-pregnant women , 5 (4.3%) were positive.

Staphylococcus saprophyticus infection was not diagnosed in first and second trimester and reported only in the third trimester 5 (27.7%) and in

7 (4.8%) of the non-pregnant women . Differences between all of the above data were statistically insignificant (p=0.841)

Wet mount preparations :

As depicted in table (13) pus cells was detected in 121 (72.8%) cases with determined causative agents of the 166 patients with sign of pus cells .Difference statistically were, however insignificant (p>0.05).

Epithelial cells were recorded in all of the 200 patient as well as 134 (71.5%) of the cause positive cases.

The presence of Trichominas vaginalis was demonstrated in 34 (17%) patients of them 32 (94.1%) were culture positive. This is contrasted to 111 (66.8%) showed culture positive results in the Trichomonas vaginals negative cases .the difference statistically were significant (p=0.05).

Motile bacteria was recognized in 120 (70.5%) culture positive women of the total 170 women positive to this parameter. only 23 (76.6%) positive women showed no motile bacteria. Difference statistically were insignificant (p>0.05).

Yeast cells were detected in 159 (79.5%) cases, of them 111 (69.8%) were with a determined causative agent through culture. Difference statistically were in insignificant (p>0.05).

Hyphae or germ tubes were found in 19 (9.5%) of patients, 18 (94.8%) of them were culture positive Difference statistically were insignificant (p>0.05).

clue cells was detected in 99 (75.5%) cases with determined causative agents of the 131 patients with sign of pus cells . difference statistically were significant ($p<0.05$).

Direct gram□s stain features:

As indicated in table (14), Pus cells were counted in 94 (83.9%) of culture positive cases out of the total positive 112 samples compared to 49 (55.6%) culture positive results of the total 88 samples where pus cells were absent. Differences concerning this parameter were statistically significant (P□0.05)

Gram positive cocci were detected in 66 (33%) women, of these 57 (86.3%) were culture positive contrasted to 86 (64.1%) which were culture positive in the gram positive cocci negative group. Differences were statistically significant (P=0.05) too.

In respect to the presence of gram negative coccobacilli which was recorded in 38 cases ,of them 27 (71%) were culture positive. By comparison , absent gram negative coccobacilli cases were 162, 116 (53.8%) of them were culture negative. Differences between these parameters were statistically insignificant (P>0.05).

Lactobacilli was present in 84 (67.2%) of the culture positive samples out of the total 125 women which carry these microorganisms. In those negative for this parameter, culture positive cases were 59 (78.6%) .Differences were, however, statistically insignificant (P >0.05).

Out of the 108 samples that showed the presence of clue cells, 65 (60.1%) were culture positive whereas 78 (84.7%) of the clue cells

negative samples were culture positive. Differences between these data were statistically significant (P□0.05).

Curved rods were demonstrated in 69 (34.5%) patients, of them culture positive results were 54 (78.7 %) , compared to negative cases which were 131, of them culture positive were 89 (87.9%). Differences were statistically insignificant (P>0.05)

Table (12): Etiologic agents of vaginitis in relation to the gestational age of patients .

Gravidity status	Total. No (%)	BV	TV	CA	CA spp	staph	Strept agalact	Staph sapro
First trimester	21 (10.5)	15 (71.4)	4(19)	2(9.5)	7 (33.3)	1(4.7)	0(0)	0(0)
Second trimester	18(9%)	9(50)	2(11.1)	4(22.2)	2 (11.1)	4(22.2)	1(5.5)	0(0)
Third trimester	18(9%)	8(44.4)	3(16.6)	6(33.3)	1(5.5)	1(5.5)	1(5.5)	5 (27.7%)
Non pregnant	143 (71.5%)	67 (46.8)	29 (20.2)	13(9)	13(9)	21 (14.6)	5(4.3)	7(4.8)
Total	200	99	38	25	23	27	7	12

$X^2=0.834$

P=0.841

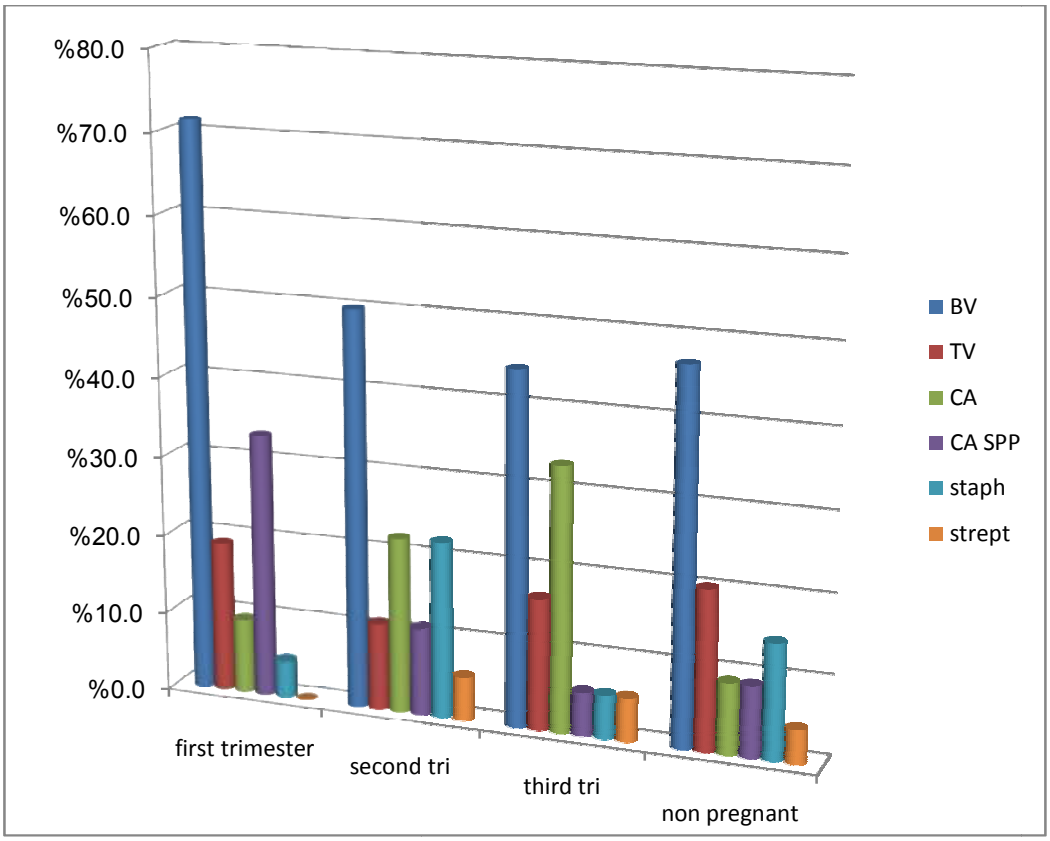


Fig.22: Etiologic agents of vaginitis in relation to gravidity status.

Table (13):Data obtained from Wet- mount preparations

Diagnostic criterion	Total No	%	No. of culture positive cases	%	No. of culture Negative cases	%
Pus cells present						
yes	166	83	121	72.8	45	27.1
No	34	17	22	69.7	12	35.2
χ^2=0.928 p=0.335						
Epithelial cells						
yes	200	100	134	71.5	57	28.5
No	0	0	0	0	0	0
Trichomonas vaginalis						
yes	34	17	32	94.1	2	5.9
No	166	83	111	66.8	55	33.1
χ^2=10.284 p=0.001						
Motile Bacteria						
yes	170	85	120	70.5	50	29.4
No	30	15	23	76.6	7	23.3
χ^2=0.462 p=0.497						
Yeast cells						
Yes	159	79.5	111	69.8	48	30.1
No	41	20.5	32	78	9	21.9
χ^2=1.085 p=0.297						
Hyphae or germ tube						
yes	19	9.5	18	94.8	1	5.2
No	181	90.5	125	69	56	30.9
χ^2=5.563 p=0.18						
Clue cells						
yes	131	65.5	99	75.5	32	24.4
No	96	48	41	42.7	28	29.1
χ^2=5.61 p=0.018						

Table (14): knowledges obtained through direct Gram stain examinations

Diagnostic criterion	Total No	%	No. of culture positive samples	%	No. of culture Negative samples	%	Statistical significance
Pus cells present							χ^2=19.296 p=0.000
yes	112	56	94	83.9	18	16	
No	88	44	49	55.6	39	44.3	
Gram- positive cocci							χ^2=10.680 p=0.001
yes	66	33	57	86.3	9	13.6	
No	134	67	86	64.1	48	35.8	
Gram-Negative coccobacilli							χ^2=0.005 p=0.946
yes	38	19	27	71	11	28.9	
No	162	81	116	53.8	46	28.3	
Lactobacilli							χ^2=3.025 p=0.082
yes	125	62.5	84	67.2	41	32.8	
No	75	37.5	59	78.6	16	21.3	
Clue cells							χ^2=14.751 p=0.000
yes	108	54	65	60.1	43	39.8	
No	92	46	78	84.7	14	15.2	
Curved gram positive rods							χ^2=2.363 p=0.124
yes	69	34.5	54	78.7	15	21.7	
No	131	65.5	89	67.9	42	23	

Giemsa stain features.

Table (15) indicates that Giant cells was seen in 17 (70.8%) culture positive cases of the total 24 smears with giant cells ,compared to 126 (71.5%) culture positive result of the 176 smears showed no giant cells . Results were, however, statistically insignificant (P >0.05).

Clue cells were present in 99 (71.7%) of the culture positive cases of the 138 clue cells positive samples, contrasted to 44 (70.9%) culture positive cases showed no clue cells. Statistical analysis indicated that differences were insignificant (P>0.05).

WBC dominance was noted in 49 slides, comprising 37 (75.5%) culture positive . Epithelial cell dominance was evident in 131, including 95 (72.5%) culture positive results. Differences between these parameter were, however statistically insignificant (P>0.05).

Equal WBC- epithelial cells ratio were shown in 20 patients, with 11 (55%) of them culture positive instances.

WBC- epithelial ratio index(1) (1\1-1\5) occurred in 87 cases, of them 64 (73.5%) were culture positive . Index (2) (1\6-1\10) was seen in 60 smears with 42 (70%) culture positive ones. Differences were statistically insignificant.(P>0.05)

Index (3) (2\1-5\1) as well as index (4) (6\1>6\1) where WBC were more predominant than epithelial cells were noted in 33 and 24 respectively. Culture positive cases within these 2 indices were 24 (72.7%) and 14 (66.6%) respectively. Differences were, however, statistically insignificant . (P>0.05)

Smears with lactobacilli grades(1&2) were 127, of them 86(67.7%) were culture positive, in contrast grades (3 & 4) were 66 , of them 48 (72.7%) of these were culture positive. Statistical analysis of these parameters indicated that differences were non-significant. ($p>0.05$).

Smears that show curved rod morphotypes at ratio(1-5) were 124, comprising 102(82.7%) culture positive cases ,contrasted to 58 smear that gave curved rods morphotypes at ratio >5 , with 41(70.6) that were culture positive. Differences were, however, statically insignificant ($P>0.05$).

Smears that appeared with small rod morphotypes at ratio (1-5) were 122, where 84 (68.8%) were culture positive. In comparison, those with small rods morphotypes at ratio >5 were 77 including 59 (76.6%) culture positive cases. Statistically, differences were, however, insignificant ($P>0.05$).

Basophilic cells were detected in 11smears, 8 (72.7%) of them were culture positive. Basophilic cells negative smears were 189 with 135 (79.9%) culture positive smears. Differences were statistically insignificant ($P>0.05$).

Acidophilic cells were recorded in 2 instances only one of them were culture positive .

RBCs recognition was possible in 23 smears,18 (78.2%) of these were culture positive , whereas those with no RBCs were 177, comprised 125 (70.6%) which were culture positive. Differences were, however, statistically insignificant ($P>0.05$).

Table (15 A) : Features gathered from Giemsa stained smears

Diagnostic criterion	T .No	%	NO. of culture positive samples	%	NO. of culture Negative samples	%	Statistical significance
Giant cells present							χ^2=0.006 p=0.939
yes	24	12	17	70.8	7	29.1	
No	176	88	126	71.5	50	28.4	
Clue cells present							χ^2=0.12 p=0.911
yes	138	69	99	71.7	39	28.7	
No	62	31	44	70.9	18	29	
WBC-Epithelial dominance							χ^2=3.126 p=0.211
WBC dominance	49	24.5	37	75.5	12	24.4	
Epithelial dominance	131	65.5	95	72.5	36	13.7	
Equal number	20	10	11	55	9	45	
WBC-Epithelial cell ratio :(index)							
1\1- 1\5: (1)	87	43.5	64	73.5	23	26.4	χ^2=0.322 p=0.571
1\6- 1\10: (2)	60	30	42	70	18	30	χ^2=0.095 p=0.758
2\1- 5\1: (3)	33	16.5	24	72.7	9	27.2	χ^2=0.029 p=0.864
6\1- > 6\1: (4)	21	10.5	14	66.6	7	χ^2=0.269 p=0.604 33.3	

Table (15 B) : Features seen from Giemsa stained smears.

Diagnostic criterion	T. No.	%	No. of positive	%	No. of Negative	%	
Lactobacilli grades							χ^2=0.513 p=0.916
1-2	127	63.5	86	67.7	38	19	
3-4	66	33	48	72.7	18	9	
Curved rods/field							χ^2=0.236 p=0.627
1-5	124	71	102	82.7	40	32.2	
>5	58	29	41	70.6	17	29.3	
Small rods/ field							χ^2=0.026 p=0.871
1-5	122	61	84	42	38	31.1	
>5	77	38.5	59	29.5	18	23.3	
Basophilic cells							χ^2=0.009 p=0.926
yes	11	5.5	8	72.7	3	27.2	
No	189	94.5	135	72.9	54	28.5	
Acidophic cells							χ^2=0.458 p=0.498
yes	2	1	1	50	1	50	
No	198	99	142	7	56	28.2	
RBC present							χ^2=0.583 p=0.445
yes	23	11.5	18	78.2	5	21.7	
NO	177	88.5	125	70.6	52	29.3	

Bacterial vaginosis diagnosis by the modified Amsel s score :

Table (16) depict the rates of BV cases diagnosed by the modified Amsel score as done in the present study. Number of those cases showed positive results by all of the six test were 28 (14%) compared to those which were positive by 5 tests which were 32 (16%). Respecting 4 test positive cases, they were 36 (18%) whereas those positive by only 3 tests were 37 (18.5%).Cases positive by 2 as well as 1 tests which were not considered as affirmed BV cases were as shown in the table.

Bacterial vaginosis by the extended Amsel s criteria:

As depicted in table (16), homogeneous thin discharge was noticed in 86 patients (43%) compared to 114 (57%) vaginal samples which does not showed this characteristic. Statistical analysis indicated that differences between those positive and negative cases were insignificant respecting this parameter(P >0.05).

pH was more than 4.5 in 156 (79.5%) cases compared to 41 (20.5%) samples which was associated with normal pH and less than 4.5 .Statistical differences were insignificant (P>0.05).

Whiff test (fishy amine odor) was positive in 125 (62.5%) cases with 75 (37.5%) samples were negative. Differences were statistically insignificant (P>0.05).

In wet mount preparation , clue cells were detected in 121 (60.5%) in contrast to 79 (39.5%) cases which were negative. Statistically, differences between these changes were insignificant (P>0.05).

Table (16): Diagnostic tests allocated for detection of BV by the present study modified Amsel s score.

No. of test positive	Total. No (%)
6 tests + ve	28 (14 %)
5 test + ve	32 (16 %)
4 test + ve	36 (18 %)
3 tests + ve	37 (18.5 %)
2 tests + ve	37 (18.5 %)
1 tests + ve	29 (14.5 %)

Table (17): women with bacterial vaginosis scored by the extended Amsel□s and Nugent scores (using 3 of 6 tests as positive cutoff)

Diagnostic criterion	Positive Number(%)	Negative number(%)	Statistical significance
Homogeneous thin discharge	86(43)	114(57)	χ^2=4.796 p=0.029
PH > 4.5	156(79.5)	41(20.5)	χ^2=0.100 p=0.752
Whiff test	125(62.5)	75(37.5)	χ^2=0.025 p=0.873
Clue cells by wet mount preparations	121(60.5)	79(39.5)	χ^2=1.777 p=0.278
Clue cells by Gram staining	108(54)	92(46)	χ^2=14.751 p=0.000
Clue cells by Giemsa staining	138(69)	62(31)	χ^2=0.012 p=0.911

Bacterial vaginosis examined by Nugent score.

As depicted in table (18), 53 (26.5%) of 200 women demonstrated normal score compared to 54 cases (27%) which was with intermediate score . the remaining 93 (46.5%) cases were fit with bacterial vaginosis criteria

Physical characteristics and etiological agents.

As depicted in table (19), erythema was noticed in 57 (57.5%) of women diagnosed as bacterial vaginosis , in 20 (52.6%) with cases of Trichomonas vaginalis, and in 3 (52%) of Candida albicans cases. Furthermore, 12 (52.1%) of Candida spp. Cases, 17 (62.9%) of Staph. aureus infected cases,6 (75%) of Streptococcus agalactiae cases, 5 (41.6%) of Staph. Saprophyticus and 15 (29%) of cases diagnosed with no any causative agents showed the erythematous features.

Itching was the main symptoms seen in 70 (70.7%) of patients infected with bacterial vaginosis, compared to 17 (44.7%) of cases due Trichomonas vaginalis, in 22 (88%) also It was reported in Candida albicans cases, in 16 (69.5%) of other Candida spp. cases . 16 (59.2%) of Staph. aureus infected cases, in 6 (75%) Streptococcus agalactiae patients, in 7 (58.3%) Staph. saprophyticus infected women and in 17 (33.3%) of cases diagnosed as not attributable to any causative agents.

Low abdominal pain was noted in 82 (82.8%) women diagnosed with bacterial vaginosis , in 16 (42.1%) of cases due to Trichomonas vaginalis, and 14 (56%) of Candida albicans cases. It was reported in 15 (65.2%) of Candida spp Cases, 14 (51.8%) of Staph .aureus infected cases, 6 (75%) complained of Streptococcus agalactiae, 9 (75%) of Staph. saprophyticus infected patients as well as in 25 (49%) of cases diagnosed with no found causative agents .

Sixty five (65.6%) of women diagnosed as suffering of bacterial vaginosis was complained of bad odor, together with 18 women (47.3%) with Trichomonas vaginalis. These symptoms were also noticed in 15 (60%) of Candida albicans cases, in 14 (60%) of other Candida spp. cases , in 12 (44.4%) with Staph. aureus infection , in 4 (50%) of Streptococcus agalactiae patients. It was demonstrated and by 7 (58.3%) of Staph. saprophyticus patients and in 16 (31.3%) of cases diagnosed with no determined causative agents.

Profuse thin discharge was detected in 49 (49.4%) patients infected with bacterial vaginosis, contrasted to 9 (23.9%) of cases of Trichomonas vaginalis, as well as 3 (12%) of Candida albicans cases. Such symptom was noted in 6 (26%) of other Candida spp. Cases, in 15 (55.5%) of Staph. aureus infected cases, in 3 (37.5%) of Streptococcus agalactiae

women, in 8 (66.6%) of Staph. saprophyticus and in 25 (49%) of cases showed no any shown causative agents.

Another complain noted was the thick white discharge which was evident in 28 (28.2) cases of bacterial vaginosis, in 5 (5.2%) cases with Trichomonas vaginalis, and in 16(64%) of Candida albicans cases. This lesion was also demonstrated in 6 (26%) of other those with Candida spp. cases , in 8 (29.6%) of Staph. aureus infected cases, in 2 (25%) of those diagnosed as Streptococcus agalactiae infected, in 3 (25%) of Staph. saprophyticus and in 6 (11.7%) of cases with no detected causative agents.

Frothy greenish- yellow discharge was of the symptoms in 24 (24.2%) of cases diagnosed as bacterial vaginosis, in 14(13.1%) of cases with Trichomonas vaginalis, and in 5 (20%) of Candida albicans cases. It is demonstrated too in 8 (34.7%) of the other Candida spp. Cases, in 4 (4.8%) of Staph .aureus infected cases ,in 2 (25%) of Streptococcus agalactiae infection, in 2 (16.6%) of Staph. saprophyticus patients as well as in 4 (7.8%) of the cases who complained of no found causative agents.

Table (18): Women with bacterial vaginosis evaluated by the Nugent score.

finding	Number	Percentage %
Normal	53	26.5
intermediate	54	27
Bacterial vaginosis	93	46.5

Table 19: physical characteristics of vaginal discharge of patients in relation to the etiological agents.

characteristics	BV N(%)	TV N(%)	CA N(%)	CA SPP N(%)	Staph aureus N(%)	Strept N(%)	staph saproN (%)	No causative agent N(%)
Redness	57(57.5)	20(52.6)	13(52)	12(52.1)	17(62.9)	6(75)	5(41.6)	15(29)
Itching	70(70.7)	17(44.7)	22(88)	16(69.5)	16(59.2)	6(75)	7(58.3)	17(33.3)
Low abdominal pain	82(82.8)	16(42.1)	14(56)	15(65.2)	14(51.8)	6(75)	9(75)	25(49)
Bad odor	65(65.6)	18(47.3)	15(60)	14(60)	12(44.4)	4(50)	7(58.3)	16(31.3)
Profuse thin discharge	49(49.4)	9(23.6)	3(12)	6(26)	15(55.5)	3(37.5)	8(66.6)	25(49)
Thick white discharge	28(28.2)	5(5.2)	16(64)	6(26)	8(29.6)	2(25)	3(25)	6(11.7)
Frothy greenish, yellow discharge	24(24.2)	14(13.1)	5(20)	8(34.7)	4(4.8)	2(25)	2(16.6)	4(7.8)

Wet preparation and etiological agents:

Table (20) allocated that pus cells was recorded in 56 (56.5%) cases of bacterial vaginosis compared to 13 (34.2%) women with Trichomonasis. Seven (28%) of patients with Candida albicans were positive for pus cells as well as 7 (30.4%) Candida spp. Women infected with staph. aureus

Demonstrated of this problem in 4 (14.8%) of them compared to those infected with Streptococcus agalactiae which were 3 (37.5%) cases. Eleven (91.6%)of those with Staph. saprophyticus and 34 (57.6%) of women diagnosed with no detected causative agents were positive. Statistical analysis proved the significance when rates given by bacterial vaginosis were compared to those infected with the other causes. ($P \leq 0.05$).

Epithelial cells were noticed in all of the BV cases as well as those infected with Staph. saprophyticus .They were 99 (100%) and 12

(100%), respectively. They were also recorded in 13 (34.2%) of cases with Trichomonas vaginalis, in 10 (40%) of patients with Candida albicans, in 8 (37.7%) of other Candida spp. It was also recorded in 9 (33.3%) of women infected with Staph. aureus, in 3(37.5%) of Streptococcus agalactiae cases and in 34 (57.6%) of women who diagnosed with no causative agents. Statistical differences were significant between BV cases compared to other causes ($P \leq 0.05$).

Trichomonas vaginalis was noted in 11 (11.1%) of women diagnosed as bacterial vaginosis compared to all of the 38 (100%) women with Trichomonasis . Three of patients(12%) with Candida albicans infection ,

1 (4.3%) with the other Candida spp, 2 (7.4%) of the women infected with staph. aureus, 1 (12.5%) of Streptococcus agalactiae cases as well as in none of those without causative agents .Statistical analysis were significant when BV cases were contrasted to cases with Trichomonas vaginalis ($P \leq 0.05$).

Motile Bacteria were noticed in all of the BV cases and as well as Staph. saprophyticus women compared to 9 (23.6%) of trichomonasis patients . Eight of patients (32%) with Candida albicans infection, 8 (37.7%) with the other Candida spp, and 8 (29.6%) women infected with Staph. aureus showed motile bacteria . In 3 (37.5%) of Streptococcus agalactiae cases, 12 (100%) of Staph. saprophyticus cases and 39 (66.1%) women diagnosed as negative to any causative agents were also motile bacteria carriers. Statistical analysis proved that motile bacteria were significantly associated with BV as well as Staph. Saprpphyticus infections ($P \leq 0.05$) compared to the other proved causes.

Yeast cells were reported in 3 (3%) cases diagnosed as having bacterial vaginosis compared to 2 (5.2%)women with Trichomonasis vaginalis, 3 (12%) of patients with Candida albicans infection , 4 (17.3%) with other Candida spp. They were seen too in 2 (7.4%) of women infected with Staph. aureus, in none of those with Streptococci, in 1 (8.3%) of Staph. saprophyticus cases and in 5 (8.4%) of women diagnosed with no causative agents.

Hyphae were shown in 1 (1%) cases diagnosed as BV , in none of those as Trichomonasis, Staph. aureus ,Streptococcus as well as in women with no causative agents. They were recorded in 1 (4.3%) of cases due to

Candida spp. and in 1 (8.3%) of case infected with Staphylococcus saprophyticus.

Clue cells were reported in 68 (68.6%) of women diagnosed as bacterial vaginosis compared to 21 (55.2%)women with Trichomonasis . nine of patients (36%) with Candida albicans infection , 6 (26%) with the other Candida spp, 11 (4.7%) of the women infected with Staph. aureus, 11 (4.7%) of Streptococcus agalactiae cases as well as in none of those without causative agents. Statistical analysis were significant when BV cases were contrasted to cases with clue cells ($P \leq 0.05$).

Direct gram□s stain smear and etiological agents:

Table(21) depicts that pus cells was recorded in 56 (56.5%) of BV cases compared to 13 (34.2%) of women with Trichomonas vaginalis. Pus cells were also shown in 7 (28%) of Patients with Candida albicans, in 3 (11.1%) of women infected with Staph. aureus, in 3 (37.5%) of Streptococcus agalactiae cases, in 12 (100%) of Staph. saprophyticus patients and in 30 (50.8%) of women diagnosed with no determined causative agents. Statistical analysis indicates that differences between figures of BV cases and the rest of data were significant ($P \leq 0.05$).

Concerning clue cells, they were seen in 50 (50.5%) of BV women as well as in 2 (5.2%) of women with trichomoniasis. Remaining patients gave the following data concerning clue cells : 5 (20%) Candidasis cases, in 2 (8.6%) of women infected with Staph. aureus, and in none of Streptococcus agalactiae cases. They were demonstrated in 6 (50%) of Staph. saprophyticus cases and in 12 (20.3%) of women with no proved causative agents.

Statistical differences were significant when BV cases were compared with the figures of the causes. ($P \leq 0.05$).

gram positive cocci were noticed in 14 (14.1%) of BV cases contrasted to 5 (13.1%) of women with trichomoniasis. Respecting Candida albicans infection 1(4%) was positive , in none of Candida spp. Cases , in 9 (29.6%) of Staph aureus infection. women in 2 (25%) of Streptococcus agalactiae cases. It was seen in all of 12 (100%) Staph. saprophyticus infected cases and in 4 (6.7%) of women with unknown causative agents. Statistical analysis indicated that differences between Staph. saprophyticus cases and the data of the remaining affections were significant ($P \leq 0.05$).

Gram Negative coccobacilli was reported in 21 (21.1%) of BV cases , in none of trichomonaisis and as well as candidasis cases . Moreover, it was recorded in 2 (7.4%) of cases infected with Staph. aureus, in 1 (12.5%)case of streptococci, in 2 (16.6%) of Staph. saprophyticus cases and in 9 (15.2%) women with no causative agents. Statistical differences were , however, insignificant($P \geq 0.05$).

Lactobacilli was demonstrated in 29 (29.2%) of BV cases ,compared to 7 (18.4%) of women with trichomonaisis. Six (24%) of patients with Candida albicans, and 5 (21.7%) of women infected with Candida spp. Showed lactobacilli this features was present in 4 (14.8%) of cases due Staph. aureus, but not seen in Streptococcus agalactiae cases. Lactobacilli also reported in 3 (25%) of Staph. saprophyticus cases and in 32 (54.2%) of women with no known causative agents. Differences were, however, statistically insignificant ($p \geq 0.05$).

With respect to Gardnerella vaginialis, they were seen in 40 (40.4%) of BV cases, in 3 (7.8%)of women with trichomonaisis, and in 4 (16%) of patients with Candida albicans. They were as well detected in 3 (13%) of women infected with other Candida spp. in 3 (11.1%) of cases with Staph. aureus infection, in (12.5%) of Streptococcus agalactiae cases, in 5 (41.6%) of Staph. saprophyticus cases and in 7 (11.8%) women with no determined causative agents. Statistical differences between data of BV cases and the rest of etiologies were differing significant ($P \leq 0.05$).

Gram positive curved rods were obtained in 26 (26.2%) of BV cases , in 3 (7.8%) of trichomonaisis women, and in 4 (16%) of Candida albicans cases. They were demonstrated too in 3 (13%) of cases of other Candida spp, in 2 (7.4%) of cases infected with Staph. aureus, in 1 (12.5%) of streptococci cases, in 8 (66.6%) of Staph. saprophyticus women and in 7 (11.8%) of patients with no identified pathogen . Statistical analysis demonstrate that differences between Staph. Saprophticus cases and the other affections were statically significant ($P \leq 0.05$).

Table (20): Wet mount preparation findings allocated by the causative agents.

Diagnostic criterion	BV N(%)	TV N(%)	CA N(%)	CA SPP. N (%)	Staph aureus N(%)	Strept N(%)	staph sapro N (%)	No causative agents N (%)
Pus cells present	56(56.5)	13(34.2)	7(28)	7(30.4)	4(14.8)	3(37.5)	11(91.6)	34(57.6)
Epithelial cells present	99(100)	13(34.2)	10(40)	8(37.7)	9(33.3)	3(37.5)	12(100)	34(57.6)
Trichomonas vaginalis	11(11.1)	38(100)	3(12)	1(4.3)	2(7.4)	1(12.5)	2(16.6)	0(0)
Motile Bacteria	99(100)	9(23.6)	8(32)	8(37.7)	8(29.6)	3(37.5)	12(100)	39(66.1)
Yeast cells	3(3)	2(5.2)	3(12)	4(17.3)	2(7.4)	0(0)	1(8.3)	5(8.4)
Hyphae	1(1)	0(0)	2(8)	1(4.3)	0(0)	0(0)	1(8.3)	0(0)
Clue cells	68(68.6)	21(55.5)	9(36)	6(26)	11(4.7)	2(25)	8(66.6)	0(0)

Table (21) :Findings obtained from direct Gram□s stain classified by etiological agents.

Diagnostic criterion	BV N(%)	TV N(%)	CA N(%)	CA SPP N(%)	Staph N(%)	Strept N(%)	Staph sapro N(%)	No causative agent N(%)
Pus cells present	56(56.5)	13(34.2)	7(28)	7(28)	3(11.1)	3(37.5)	12(100)	30(50.8)
Clue cells	50(50.5)	2(5.2)	5(20)	2(8.6)	3(11.1)	0(0)	6(50)	12(20.3)
Gram- positive cocci	14(14.1)	5(13.1)	1(4)	0(0)	9(29.6)	2(25)	12(100)	4(6.7)
Gram- Negative coccobacilli	21(21.1)	0(0)	0(0)	0(0)	2(7.4)	1(12.5)	2(16.6)	9(15.2)
Lactobacilli	29(29.2)	7(18.4)	6(24)	5(21.7)	4(14.8)	0(0)	3(25)	32(54.2)
Gardnerella vaginialis	40(40.4)	3(7.8)	4(16)	3(13)	3(11.1)	1(12.5)	5(41.6)	7(11.8)
Gram +ve ,curved rods	26(26.2)	3(7.8)	4(16)	3(13)	2(7.4)	1(12.5)	8(66.6)	7(11.8)

Giemsa stained smears result in relation to etiological agents:

Table (22, A,B) shows that Giant cells were demonstrated in 5 (5%) of BV cases, in 4 (10.5%) of trichomonaisis patients, and in none of candidasis cases. They were detected in 1 (3.7%) of smears from Staphylococcus aureus cases, in none of Streptococcus agalactiae and Staph. saprophyticus case. They were noticed too in 6 (10.1%) of cases with no determined causative agents.

Clue cells were reported in 43 (43.4%) of BV patients ,in 10(26.3%)of trichomonasis cases, in 7 (28%) of Candida albicans cases. They were also demonstrated in 3 (13%) of cases with other Candida spp, in 5

(18.5%) of cases infected with Staph. aureus, in 1 (12.5%) of streptococci cases. clue cells were also detected in 11 (11.1%) of Staph. saprophyticus cases and in 25 (42.3%) women with no identified pathogen. Statistical analysis of these differences indicated that they were insignificant ($P \geq 0.05$).

WBC dominance was noted in 15 (15.1%) of BV conditions, in 5 (13.1%)of trichomonasis cases, in 3 (12%) and in1 (4.3%) of Candida albicans cases, and other Candidiass, in none of Staph. aureus patients.

They were occured in 3 (37.5%) of streptococci cases , in 4 (4.0%) of Staph. saprophyticus cases and in 9 (15.2%) of women with no identified pathogen.

Epithelial cell dominance was evident in 35 (35.3%) of BV smears, in 7 (18.4%)of Trichomonasis cases and in 5 (20%) of Candida albicans cases. They were also seen in 6 (26%) of cases of other Candida spp , in 6 (22.2%) of cases infected with Staph. aureus , in 1 (12.5%) of

streptococci cases , in 9 (9.1%) of Staph. saprophyticus patients and in 29 (49.1%) of women with no identified pathogen. Statistical differences were , however, insignificant ($P \geq 0.05$).

Equal ratio of WBC- epithelial cells were shown in 7 (7.0%) of BV smears, in 1(2.6%)of trichomonasis cases, in 2 (8%) of Candida albicans women. They were shown in 1 (4.3%) of cases with other Candida spp in 3 (11.1%) of cases infected with Staph. aureus, in 1 (12.5%) of streptococci cases, in none of Staph. saprophyticus cases and in 3 (5.0%) cases with no determined causative agent .

WBC- epithelial cells ratio index (1) occurred in 23 (23.2%) of BV cases, in 6 (15.7%)of trichomonasis patients , in 4 (10.5%) of Candida albicans cases. They were recorded in 5 (21.7%) of cases due to other Candida spp, in 8 (29.6%) of cases infected with Staph. aureus, in 1 (12.5%) of streptococci cases, in 5 (5.1) of Staph. saprophyticus cases and in 17 (28.8%) of cases with no determined causative agent .

Index (2) was reported in 16 (16.1%) of BV smears of cases, in 3 (7.8%)of trichomonasis cases, in 2 (8%) in Candida albicans women, It was recorded in 3 (13%) of cases caused other Candida spp, in 1 (3.7%) of ladies infected with Staph. aureus, in 12 (25%) of streptococci cases, in 3 (3.0) of Staph. saprophyticus cases and in 12 (20.3%) cases with no determined causative agents .

WBC-epithelial cells ratio index (3) was reported in14 (14.1%) of BV cases, in 1 (2.6%) of trichomonasis cases, in 3 (12%) in Candida albicans cases, in none of other Candida spp, Staph .aureus and streptococci patients. It was recorded in 2 (2%) of Staph. saprophyticus ladies and in 7 (11.8%) cases with no known causative agent .

Respecting, it Index (4) was seen in 4 (4.0%) of BV cases, in 4 (10.5%)of trichomonasis cases, in 1 (4%) of Candida albicans women, in none of other Candida spp, Staph .aureus and streptococci cases. it was recorded in 2 (2.1) of Staph. saprophyticus cases and in 8 (13.5%) of cases with no identified pathogen .

Smears with lactobacilli grades(1&2) was seen in 34 (34.3%) of BV as well as 10 (26.3%) of women with trichomoniasis. Other patients gave the following data : 7 (28%) and 6 (26%) of Candida albicans and other Candida spp respectively, 6 (22.2%) of women infected with Staph. aureus, and 2 (25%) of Streptococcus agalactiae cases. They were demonstrated in 9 (9.1%) of Staph. saprophyticus cases and in 29 (49.1%) women with no detectable causative agent. Furthermore, Smears with lactobacilli grades(3&4) was seen in 21 (21.2%) BV cases, in 2 (5.2%) of trichomonaisis ,in 2(8%), and 2 (8.6) of both Candidasis cases. They were localized in 2 (7.4%) of smears of Staphylococcus aureus cases , in none of Streptococcus agalactiae cases, in 3 (3.1%) of Staph. saprophyticus cases and in 14 (23.7%) of patients with no determined causative agents.

Smears that show curved rod morphotypes at ratio (1-5) occurred in 37 (37.3%) of BV as well as 8 (21%) of women with trichomoniasis. These features were reported in 8 (32%), and 5 (21.7) of both Candidasis categories in 6 (22.2%) of women infected with Staph. aureus and in 2 (25%) of Streptococcus agalactiae cases. They were demonstrated in 9 (9.1%) of Staph. saprophyticus patients and in 28 (47.4%) women with unknown causative agent.

Curved rod morphotypes at ratio(>5) was seen in 19 (19.1%) of BV cases, in 5 (13.1%) of trichomonaisis women, in 2 (8%) and 3(13) of the 2 Candidasis agents. They were demonstrated too in 3(11.1%) of Staphylococcus aureus cases, in 1 (12.5) of Streptococcus agalactiae ladies, in 3 (3.1%) of Staph. saprophyticus cases and only 1 (25.4%) cases with no determined causative agents.

Small rod morphotypes at ratio (1-5) was reported in 37 (37.3%) of BV and 9 (23.6%) of women with trichomoniasis. They occurred too in 6 (24%) and 7 (30.4) Candidasis causes, in 5 (18.5%) of women infected with Staph. aureus, in 2 (25%) of Streptococcus agalactiae cases. They were demonstrated in 8(8.0%) of Staph. saprophyticus cases and in 30 (50.8%) women with known causative agent.

Small rod morphotypes at ratio (>5) was seen in 20 (20.2%) BV cases, in 4 (10.5%) of trichomonaisis and Candida albicans cases, in 1 (4.3%)of the other Candida cases. They were demonstrated in 4 (14.8%) of staphylococcus aureus cases, in 1(12.5%) of Streptococcus agalactiae cases, in 3 (3.1%) of Staph. saprophyticus cases and in 13 (22.0%) cases with no determined causative agents.

Basophilic cells were detected in 5 (5.0%) BV cases, in 2 (5.2%) of trichomonaisis, It was not seen in any of Candida albicans , other Candida spp, Staphylococcus aureus, streptococci and, Staph. saprophyticus cases. They were noticed in 1 (1.6%) of cases with no determined causative agents.

Acidophilic cells were detected in 2 (2.0%) BV cases, in 1 (2.6%) of trichomonaisis, in none of Candida albicans, in 1 (4.3%) of other Candida

spp, It was not seen in Staphylococcus aureus, streptococci and, Staph. saprophyticus cases and cases with no determined causative agents.

In respect to RBC they were noticed only in 6 cases of BV (6.1%) cases, in None of trichomoniasis cases. And they were detected in 4 (10.5%) Candida albicans cases, but in none of other Candida spp cases. Staphylococcus aureus, streptococci or Staph. saprophyticus cases . They were noticed in however, 2 (3.3%) cases with no determined causative agents.

Culture result in relation to the etiological agents:

Table(23) indicates that finding a detectable growth was failed in 28 (28.2%) of bacterial vaginosis as well as 7 (18.4%) of cases with trichomonasis. In Candidasis, Staphylococcus aureus , Streptococci as well as Staph. saprophyticus cases none failed to find a cause. No growth on the other hand were noticed in 16 (27.1%) cases only with no determined causative agents. Statistical analysis of these differences proved their significance ($P \leq 0.05$).

Lactobacilli was obtained in 24 (24.2%) of BV cases, in 4 (10.5%) of women with trichomonaisis, in 3 (12%) of Candida albicans cases, in 1 (4.3%)of other Candida spp cases. They were demonstrated too in 3 (11.1%) of Staphylococcus aureus cases, in 1(12.5%) of Streptococcus sagalactiae cases, in 4 (33%) of Staph. saprophyticus cases and in 21 (35.5%) of cases with no determined causative agents.

Candida albicans complicated 15 (15.1%) of cases diagnosed as BV, as well as 3 (7.8%) of trichomonasis. It was obtained too in 1 (3.7%) of Staphylococcus aureus, but none of Streptococcus agalactiae, Staphylococcus saprophyticus and cases with unknown causative agents.

Other Candida spp appeared with 8 (8.0%) BV cases, in 1 (2.6%) of trichomonaisis and in none of Candida albicans cases, They were demonstrated within 1 (3.7%) of Staphylococcus aureus cases but in none of streptococcus, Staph. saprophyticus and cases with no proved causative agents.

Concerning Staphylococcus aureus, they accompanied 10 (10.1%) of BV cases, 2 (5.2%) of trichomonaisis, in 1 (4%) of Candida albicans cases , 1 (4.3%) of other Candida spp Cases. in 4 (50%) of Streptococcus agalactiae, in none of Staph. saprophyticus as well as cases with no determined causative agents.

Streptococcus agalactiae occured in 2 (2.0%) BV, and in 1 (2.6%) of trichomoniasis , but in none of candidasis patients. They were seen in 4 (50%) of Staphylococcus aureus , in 8 (100%) of streptococci and in none of Staph. saprophyticus and cases with no determined causative agents.

Table(22 A:) Giemsa stain result in relation to the etiological agents of vaginitis.

Diagnostic criterion	BV N(%)	TV N(%)	CA N(%)	CA SPP. N(%)	Staph N(%)	Strept N(%)	Staph sapro N(%)	No causative agent
Giant cells present	5(5%)	4(10.5)	0(0)	0(0)	1(3.7)	0(0)	0(0)	6(10.1)
Clue cells present	43(43.4)	10(26.3)	7(28)	3(13)	5(18.5)	1(12.5)	11(11.1)	25(42.3)
WBC-Epithelial cell dominance								
WBC dominance	15(15.1)	5(13.1)	3(12)	1(4.3)	0(0)	3(37.5)	4(4.0)	9(15.2)
Epithelial domain	35(35.3)	7(18.4)	5(20)	6(26)	6(22.2)	1(12.5)	9(9.1)	29(49.1)
equal	7(7.0)	1 (2.6)	2(8)	1(4.3)	3(11.1)	1(12.5)	0(0)	3(5.0)
WBC-Epithelial cell ratio								
1\1- 1\5 (1)	23(23.2)	6(15.7)	4(10.5)	5(21.7)	8(29.6)	1(12.5)	5(5.1)	17(28.8)
1\6- 1\10 (2)	16(16.1)	3(7.8)	2(8)	3(13)	1(3.7)	2(25)	3(3.0)	12(20.3)
2\1- 5\5 (3)	14(14.1)	1(2.6)	3(12)	0(0)	0(0)	0(0)	2(2.0)	7(11.8)
6\1- > 6\10(4)	4(4.0)	4(10.5)	1(4)	0(0)	0(0)	0(0)	2(2.1)	8(13.5)

Table(22 B:) Finding gathered from Giemsa staining in relation to the etiological agents of Vaginitis

Diagnostic criterion	BV N(%)	TV N%	CA N(%)	CA SPP	Staph N(%)	Strept N(%)	Staph Sapro	No causative agent
Lactobacilli grades								
1-3	34(34.3)	10(26.3)	7(28)	6(26)	6(22.2)	2(25)	9(9.1)	29(49.1)
3-4	21(21.2)	2(5.2)	2(8)	2(8.6)	2(7.4)	0(0)	3(3.1)	14(23.7)
Curved rods								
1-5	37(37.3)	8(21)	8(32)	5(21.7)	6(22.2)	2(25)	9(9.1)	28(47.4)
> 5	19(19.1)	5(13.1)	2(8)	3(13)	3(11.1)	1(12.5)	3(3.1)	1(25.4)
Small rods								
1-5	37(37.3)	9(23.6)	6(24)	7(30.4)	5(18.5)	2(25)	8(8.0)	30(50.8)
> 5	20(20.2)	4(10.5)	4(10.5)	1(4.3)	4(14.8)	1(12.5)	3(3.1)	13(22.0)
Basophilic cells present	5(5.0)	2(5.2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.6)
Acidophilic cells present	2(2.0)	1(2.6)	0(0)	1(4.3)	0(0)	0(0)	0(0)	0(0)
RBC present	6(6.0)	0(0)	4(10.5)	0(0)	0(0)	0(0)	0(0)	2(3.3)

Table(23): Culture result of etiologic agent of abnormal vaginal discharge

Diagnostic criterion	BV N(%)	TV N(%)	CA N(%)	CA SPP	Staph N(%)	Strept N(%)	Staph sapro	No causative agent
No. growth	28(28.2)	7(18.4)	0(0)	0(0)	0(0)	0(0)	0(0)	16(27.1)
Lactobacilli	24(24.2)	4(10.5)	3(12)	1(4.3)	3(11.1)	1(12.5)	4(33)	21(35.5)
Candida albicans	15(15.1)	3(7.8)	25(100)	0(0)	1(3.7)	0(0)	0(0)	0(0)
Candida spp	8(8.0)	1(2.6)	0(0)	23(100%)	1(3.7)	0(0)	0(0)	0(0)
Gram positive staphyloccci	10(10.1)	2(5.2)	1(4)	1(4.3)	27(100%)	4(50%)	0(0)	0(0)
B-hemolytic streptococci	2(2.0)	1(2.6)	0(0)	0(0)	4(50%)	8(100%)	0(0)	0(0)

CHAPTER FIVE

Discussion

Research on Vaginitis has a peculiar problem in developing countries like Libya where many people consider collection of vaginal swabs as a taboo and something unthinkable especially among unmarried women. Those who report to the hospital with complaints of abnormal vaginal discharge seem to volunteer information with some difficulties. As a result of frequent recurrences, some families believe that their patients have acquired an incurable disease and then resort to traditional medicine. Most of these inherent problems in this area of research make the actual incidence of vaginitis largely unknown in tropics with sketchy data.

The problem of abnormal vaginal discharge is a frequently encountered gynecologic disorder that manifests with an offensive non-bloody or sometimes tingled with blood discharge in the female lower reproductive tracts. It is a common complaint among women of different age groups in any society whether or not they are sexually active . It may be regarded as any amount of secretion that the patient is worried about. Vaginal discharge may be normal or abnormal. Normal vaginal discharge is physiologic, such as that occurs during pregnancy, sexual arousal or at specific period in the menstrual cycle. Physiologic vaginal discharge in pregnant women is colorless or white, non-irritating and odorless or has mild odor and is non-infective in nature with no sequel. On the other hand, the abnormal vaginal discharge may be green, yellow, brown or red in color with foul smelling odor, purities, irritation, dysuria or dyspareunia depending on the type of infection**.**

In Libya, to the best of our information, only single very preliminary study was found on the role of Trichomonas vaginalis in vaginitis in

Benghazi area (82). Their findings indicated that the prevalence of Trichomonas was 1.2%.

Therefore, basically the present study was set to detect the most common microbial causes of infective genital discharge among pregnant as well non-pregnant women in primary health care unit in Benghazi Algadeda polyclinic .The work also aimed to improve the prevention, early diagnosis and proper management of abnormal vaginal discharge among complaining women .

The success rate of determined causes of the abnormal vaginal discharge of the present study was 141 (70.5%) of the recruited cases (200 ladies) whereas the number of undetermined causes was 59 (29.5%). These findings were higher than what was shown by two previous studies on general female populations too.[21,128] They recorded a figure range of 35% and 46.2%, respectively for their series. Moreover, current results were in agreement with two other studies .[129,130]

Reasons behind the inability to resolve the causes the complaint in 59.5% of cases of the current study is probably due to a number of technical difficulties. It is possible that some women might harbor agents like Ureaplasma urealyticum as well as other organisms like viruses which were not easily and routinely detectable by current classical and preliminary tools .Another explanation might be that large number of the recruited patients indicated the practice of traditional medicine or physical ways to alleviate their manifestation which could be contributed killing, inhibition or even complication of the problem.

During this work ,the following microbial agents were detected in descending order : Gardernella vaginalis (bacterial vaginosis) (49.5%),

Tichomonas vaginalis (TV) (19%) ,Staph. aureus (13.5 %), Candida albicans (12.5%), Non- candida albicans spp. (11.5 %), Staphylococcus saprophyticus (6%)Streptococcus agalactiae (4%), Chlamydia trachomatis (zero %). Mixed infections were notice in 29 % The three most commonly encountered agents consisted of Gardenerella vaginalis (49.5%), TV (19%) and Candida albicans (12.5%) . Much higher rates were noticed in a study on Nepalese women [80] who had an overall rate of 91% concerning Candida albicans ,55% for Gardenerella vaginalis and 20% with Trichomonas vaginalis . The present result were, however, higher than the findings from Brazil [106] which showed the following figures (20% for both BV and Candida, 4.1% for TV) and higher than that from India (38.5.1 for BV , 9% with Candidiasis and 6% for TV). [131] Although, differences are expected due to various ecological and geographical effects, concordance on the importance of these most common causes whether for Libyan or other populations indicate the significant value of the agents as a cause of abnormal vaginal discharge.

Vaginal infections maximally occurred in women of reproductive age between 26-36 years as noted in [132,133] as well as the present work This may have important implications because women in this age range are at higher risk for STDs and low birth outcomes. Other age groups (16-25) and women of more than 45 years showed lower percentages of infections (56.6% , 57.1 % , respectively) . An increase, however, at age group (36-45) years were demonstrated (65.5%). A significant association between age and vaginal infections was indicated here in this study as well as in many other previous published data. [130,134,135] Moreover, the probability coefficients for all vaginal infections seems to

depend on the type of population studied and the prevalence in the population studied as shown by the same above trials .

By analyzing the socio-demographic variables of the recruited patients of the present study , education seems to significantly associated with vaginal infections .Indeed, high rates of infection was observed with illiterate (100%) and low education women (66.6%) . Increased rate was also surprisingly seen in women with university certificate (74.1%). These results probably indicate the assumption given in[130,134,135] on that the probability coefficients for all vaginal infections seems to depend on the type of population studied and the prevalence of the population studied. A look should be better directed on the personal hygiene practiced by the patient and taken as a risk factor for each the patient alone.

The highest percentage of abnormal vaginal discharge was demonstrated in patients married to non-educated or low educated husbands (69.2%). These results agreed with many other studies. [136,133] Other socio-demographic factors such as low monthly income, occupation (employees) and marital state (Divorced and widowed) all were also associated with relatively high prevalence of infections , although no statistical significant differences with respect to these factors were noted. Similar observations were given by various previous researches. [89,130,136]

The group of the non- pregnant women of the present work were higher in number than pregnant ladies (143 versus 57 women). A vaginitis prevalence rate of 70.6% was documented among the non-pregnant compared to a prevalence value of 73.6% for the pregnant group . These findings were in accordance with those mentioned in the study

from north westerns Nigeria. [5] These observations are a good indication that pregnancy is a predisposing factor for the occurrence of the abnormal vaginal discharge or a higher chance of infections.

Present research proved that no significant association between vaginal infections and abortion and an increased rate of infection was connected in women with no history of habitual abortion which were 90 (71.4%) cases . These data were in agreement with those given in [137] . Disagreement in contrast were also existed [138] which allocated that vaginal infections were linked with habitual abortion for 73% in their series . Contradiction with respect to the role of abortion in vaginitis in the present as well as those in previous works probably related to their small number of recruited patients. Another probable reason is that the cause of their illness together with many further complication not available in the present group of patients could contributed to these differences. Further studies seems mandatory to elucidate the positive or negative association.

In the present study, the abnormal vaginal discharges were the optimal in women delivered by cesarean sections (83.3%) followed by vaginal delivery (76.1%), prim gravid(69.2%) and women who delivered by more than one method of delivery during their gestation life (68.4%) . Identical results were noted in [87], who demonstrated that cesarean surgery as well as vaginal delivery might predispose to more chances of repeated and complicated local situations leading to reduced resistance of the genital tract . These procedures seems also to lead to more ability to colonization of various infectious agents in these places.

Although ,data in Table (6) generally indicated that no significant correlations between past medical history and rate of infections was

noticed , hypertension seems probably to be associated with high rates of infection (100%) compared to cases given no history of any medical problems (73.4 %) as well as diabetic cases (50%) . These results were in total agreement with those demonstrated in [89] which proved that the abnormal vaginal discharges are unrelated to the general health status of women complaining of vaginitis. Number of hypertensive women suffered of vaginitis of the present study were too small to allow a clear cut conclusion at this time(two cases) .

Present findings revealed that BV is the commonest cause of the abnormal vaginal discharges (reported in 99 women (49.5%) out of the total 200 samples) . In a previous study [90] , a record of 60% of BV complaints was documented . The later figures are relatively higher than the ones of the present study probably because of various geographical as well as ecological differences . On the other hand, many other studies e.g. from Basra (Iraq) , Iran , and China only arrived to 7% - 16 % of BV rates. [102,88,87] Further other comparable data are those reported in [103, 42] which stated a BV accounts of 39.1% - 38.9% in a few hundred women with abnormal vaginal discharges .

The insertion of intrauterine devices (IUD) , oral pills, and condom use are the most prevalent and effective reversible methods of contraception worldwide . However , they are used for less often than experts believe it should be , probably due to the concerns of risk of complications linked to their uses, particularly pelvic inflammatory diseases (pip) and subsequent squeals.[139] Other common medical problems associated with the contraceptive use are bleeding and low pelvic pain along with genital infections. [139]

The practice contraception have been shown to be associated with BV in many studies. [89,110,140] The current data too showed that 65% of those applied two type of contraception contrasted to 60.7% of oral pills users, 57% of condom users and 52% of intrauterine device appliers suffered of abnormal vaginal discharges. Similar figures were noted in [89,110,141] who mentioned that BV were frequent in women who previously used oral contraception as well as significant positive association between BV and IUD application. They further suggested that women with IUD and BV may be at higher risk for pip especially if BV is presented prior to the insertion. Present authors belief , that the association between BV and contraception use might probably due to the change in vaginal flora in favor of BV-associated bacteria on the expense of normal flora lactobacilli .

Women of the present study with gestational age range of ≤13 weeks demonstrated the maximum rate of Bacterial vaginosis (71.4%) compared to 50% of women with gestational age of (13 -27) weeks . The rate was, however, lower in patients at their third trimesters(27 weeks to term) (44.4%) contrasted to non-pregnant women values which were (46.8 %). Comparable findings were given in [136] who noted that 44.5% rate of abnormal vaginal discharges in first trimester women and 39.5% in second trimester series. These observations seems to land on that first trimester as well as early second trimester pregnancy changes had a drastic effects on the ecology of the female genital leading to the appearance of suitable conditions favoring BV infections.

Previous authors as well as the authors of the present investigation advise the screen for infective vaginal discharges during pregnancy more often at the extremes of the gestational ages with more concentration in cases with multigravidity than primigravidity .

Another agent which is Trichomonas vaginalis that is associated with good lot of ranges of adverse reproductive health outcomes , including preterm birth , cervical neoplasia , atypical pelvic inflammatory disease vaginitis and cervicitis in women. [142]

Trichomoniasis is recognized as a major sexually transmitted disease (STD) worldwide with the highest prevalence of all of the other STDs. Its prevalence is strongly related to cultural and social norms indifferent societies , in relation to sexual partnership , monogamy , or polygamy. [135]

An overall prevalence of 19% for Trichomonas vaginalis of this studyis higher than the percentages reported in [109] (2.9%) and in (4.1 %)The current results were, however, lower than the values shown in [110] ,where a prevalence of 25% in Jamaican women attending the STD clinic were observed . In a third study reported in [111] where T.

vaginalis was seen in 90% of women complained of infectious vaginitis. Free Sexual behavior trends in Jamaican women might be the reason behind that very high occurrence of Trichomoniasis. The use of different contraceptive policies, however, have been shown to be lessassociated with Trichomonas vaginalis in some studies [90,140] compared to BV infections. In the present study 20% rate of positivity was seen in women used two types of contraception . In oral pills and condom users, the rate was 14.2 % compared to (23.3%) figures for women who do not practiced any contraception . These results were comparable to those reported in [89] , where 14.5%, 16% rates of Trichomoniasis was shown in women who had previously used oral pills and condom .

Moreover, trichomoniasis was positive in 19.1 % pregnant women With the peak prevalence during the first trimester and in third trimesters (16.6 % both) contrasted to the non-pregnant women of(20.2 %) . It decreases with second trimester to11.1 %. These observations were in agreement with those demonstrated in [117]

where they found that 44.5% rate of infective vaginal discharges were possible in the first trimester and 54.5 % in third trimester .

Vaginitis due to Staphylococcus aureus of the present study is 13.5% which were concordant to those noted in [114] . Lower figures, however, were shown in [143 ,115] indicating the importance of the search for these organisms in genital infections.

Staphylococcus aureus infections have been shown to be only weakly associated with the use of contraception, and were more related to the absence of use of any contraception (15.8 %). These findings were in agreement with those given in [115] and probably further prove that since Staphylococcus aureus are not a part of the normal vaginal flora no effect to contraception is possible.

Women of the present study in their second trimester suffered of the highest rate of Staphylococcus aureus vaginal infections (22.2%). On the other hand, low figures were observed in patients at their first trimesters (4.7 %) as well as third trimesters (5.5 %) .These goes with the data documented in[5], but disagreed with those shown in [71] . Although, The exact reasons behind these figures is unknown, but one explanation can be the low resistance that might occurred during the second trimester compared to the other trimesters. Staphylococcus aureus infections in those who were non pregnant were (14.6 %).

The present study showed the involvement of Stathylococcus Saprophyticus in vaginitis. Indeed , in 12(6%) of women these bacteria were isolated. Although, Staph. saprophyticus is principally a uropathogenic bacterium that causes acute uncomplicated urinary tract infections, particularly in female outpatients, it also sometimes cause vaginitis as shown in the present trail. Concordant data are available that indicated a 4.8% rate for this bacteria. [144]

Candidiasis due to Candida albicans as well as other Candida spp. was the second leading cause of the abnormal vaginal discharges in the current study with 25 (12.5 %) of the total 200 female genital samples caused by Candida albicans and 23 (11.5 %) vaginitis infections due to other Candida spp . Comparable data were reported in [35] concerning different Candida spp. roles in vaginitis. Previous studies shown in[109] [76] recorded much higher (78%) candidiasis cases as well as Candida albicans (20 %).Much lower figures shown by were also available (9%). [131]

Many practitioners believe that nylon underwear and tight insulating clothing predispose to vaginal candidiasis by increasing the temperature and moisture of the perineum . One study [101] proved that many African woman wearing tight clothes reported higher prevalence of Candida albicans than those wearing loose clothing. Many other reports have shown that several factors show some degree of association with candidiasis including pregnancy ,disorders affecting the immune system , uncontrolled diabetes ,and recent treatment with antibiotics. [61] The present data seems to indicate the relatively significant importance of Candida spp. in the abnormal vaginal discharges for Libyan females.

The association of Candidiasis and contraception use was detected , where the rate was 20% for IUD users followed by 17.8% for the oral pills consumers and 14.2% for the condom user .These results are in agreement with those given in [23] . Moreover, these trails also documented that Candida albicans infections were more prevalent in pregnant women at their third trimesters (33.3%) and second trimesters (22.2%) in contrast to non-Candida albicans spp. where the infection was generally high during the first trimesters (33.3%) . These data were concordant with those shown in [5] . The relationships between pregnancy and infective vaginal discharges with particular note on candidiasis indicates an increase in hormonal influences as well as alteration of the vaginal pH as demonstrated in [111] .

Streptococcus agalactiae accounted for 4% of vaginitis cases in the present study. Similar finding was reported in [112] who showed that 5% of bacterial isolates were diagnosed as Strept. agalactiae. Disagreement on the other hand was showed in [145,146] who attributed streptococci in 13% of their series. The association between streptococcal infections and contraception use , however, was as much as 12% in 1UD users and 3.5% in oral pills group .Identical data was demonstrated in [89] .

A Good sum of data exist that document the important role of Chlamydia trachomatis in the causation of the abnormal vaginal discharge. Between half and three- quarters of all of women with Chlamydial infection of the cervix (cervicitis) showed no symptoms and do not known that they are infected . [147] Symptomatic Chlamydial cervicitis in a female patient is characterized by mucopurulent cervical discharge, erythema and inflammation .

The present research tried to find the participation for Chlamydia trachomatis in vaginitis using the Chlamydia rapid immune chromatographic test kit for these purposes. None of the recruited 200 patients were positive for this agent despite the indications obtained via Giemsa staining studies that suspected the presence of the chlamydial inclusion bodies in a number of the vaginal smears.

Different literatures cited that diagnosis of genital chlamydial infections is best accomplished by using the nucleic acid implication tests(NAAT) preformed on swab specimens collected from the cervix such as polymerase chain reaction(PCR).[148] Because of improved test accuracy, ease of specimen management, convenience in specimen transport and ease of screening sexually active men and women, NAATs have largely replaced culture, the gold standard for chlamydial diagnosis.

In the present work, the accuracy of the rapid test used for screening for chlamydial infections failed in doing this purpose . Another factors behind this failure might be due to sampling collection, transport and handling. In conclusion, genital chlamydial infections need to be tackled again through using more sensitive assays as well as more reliable sampling procedures.

In the current study, risk factors for acquiring abnormal vaginal discharges were elucidated .As depicted in Table 4, the prevalence of vaginal infections seems to increase with caffeine consumption(100%), vaginal douching (86.1%) , as well as traditional medicine use (85.7%). Furthermore, contraception use (75.2%) , current use of soap (74.3%), smoking (73%) ,evacuation and curettage (72.2%) , reusable towel use (70.4%) and diabetes (50%) . Of these risk factors, statistical differences were significant with the practice of traditional medicine ,

pad towel and vaginal douching. These results were in concordance with the conclusions shown in a number of works. [59-61] Significant association with vaginal douching and traditional medicine use might probably due to the change in the pH of the vagina that lead to the disturbances in the vaginal flora . Vaginal douching have been shown to be an independent risk parameter for vaginal infection by several other studies. [95,140] The influences depend on several other situations e.g. douching frequency, ,recentness, reasons for douching, preparations, if solution, powders or gels. One previous study reported in [90] found strong association with the applications of commercial antiseptic products to vulvae mucosa .

Vaginal infections are only sufficiently diagnosed by the microscopic examination of wet preparations of vaginal secretion . In deed the present study (Tables 13 and 20) demonstrated the existence of pus cells in 72.8% of all of the 200 cases . Around half of these cases suffered of BV (56.5%) as well as trichomoniasis (34.2%). Pus cells were present in 28% of Candida albicans and in 30.4% of other Candida spp. These findings were comparable to data noted in [149] that showed a significantly high prevalence of pus cells in BV as well as trichomoniasis (70.2%), (66,5%) .

Epithelial cells on the other hand, were noticed in all BV cases. The presence of clue cells is one of the BV diagnostic criteria. [131,102] Furthermore, concerning the existence of pus cells in that much of the BV cases probably indicate that these women suffered of mixed infections rather than BV only(mixed infections was noticed in 29% of cases,(table 8).

Yeast cells were detected in 159 (79.5 %) of 200 women with abnormal vaginal discharges, in 111 (69.8 %) of culture positive cases. It was demonstrated in 29.3 % cases of candidaisis only, in 12% of Candida albicans and in 17.3% of Candida spp. cases. The low rates of chances of demonstration of yeast cells in candidal vaginitis series cannot be explained with certainty , but sampling as well as working technical errors are possible. Further studies seems mandatory to solve some of these contradictions as well the improvement in this respect.

Microscopic examination of wet preparation of all of the thirty eight (100%) smears with Trichomonas vaginalis showed the presence of these agents, with 11 of them (11.1%) recorded the occurrence of BV infections too. This procedure showed good sensitivity and reproducibility for the detection of T. vaginalis infections as noted by the present study .Wet mount microscopy examination proved easily applicable and practical method for the routine diagnosis of T. vaginalis. It is , however, of low sensitivity thus cultural procedures have been advised by a number of studies. PCR was also suggested as a more effective way for the detection of T. vaginalis than wet mount microscopy, culture and Giemsa staining tests. [85]

Diagnosis of vaginal infections and mainly BV manifestations by gram stain following Nugent criteria has been adopted as the gold standard for the routine investigations as well as research purposes, including studies of prematurity. [36,96] In the present work, gram stained vaginal smears had been allocated as probably a sensitive non cultural diagnostic technique for the documentation of the cause of the abnormal vaginal discharges . Gram stain was reliable for diagnosis of Candidiasis through the finding of budding yeasts and pseudohyphae.

Similar information were given in one published research. [60] In the present study , diagnosis of BV by the examination of gram stained smear by detection of the clue cells. This was also subjected to evaluation by the standard diagnostic methods consisted of pH , fish odor test, nature and consistency of the discharge and the clinical presentation . All of the 99 cases by Amsel□s score or the 93 women by Nugent score with confirmed diagnosis of BV (tables 17 and 18) showed clue cells by gram stained smear examinations . With respect to the other parameters , table (17) depicts that pH was more than 4.5 in 156, whiff test was positive in 125 , homogenous discharge was detected in 86 of the BV patients whereas clue cells by wet mount smears were 121.

Regarding the method for the diagnosis of bacterial vaginosis, although Amsel's criteria are more often used in the clinical setting [104], the Nugent scoring system has a higher reproducibility [102], with comparable sensitivity and specificity to the former criteria [63, 65,66]

Nugent score is 7 or more is usually diagnostic for BV which appears qualitatively as dominant morph types other than Lactobacilli .

The present work in table (17) searched the number of cases showing clue cells by wet mount , gram staining and Giemsa staining smears in all of the 200 recruited women. Giemsa staining gave the top sensitivity (138 case s), followed by the wet mount smears (122 women) whereas gram stained smears gave the lowest values (108 cases). Clue cells are one of the diagnostic criteria of BV which routinely detected by wet mounts or gram staining [66,68] The present work proved the better sensitivity and reproducibility of Giemsa staining for these purposes

and recommend its inclusion in Amsel□s and Nugent scores together with the other tests for more consistent conclusion.

As shown in Tables (14 and 21) an increased prevalence of Gram negative coccobacilli (21.1%), Gram variable coccobacilli (40.4%), curved rods (26.2%) and clue cells (50.5%) was observed in the smears from patients with Bacterial vaginosis. Comparable rates were noticed in smears obtained from patients with no causative agents which might probably been better included within cases of BV .This augment the number of BV cases from around 50% (99 out of 200) to the values detected by Giemsa staining which were 138 / 200 as demonstrated by clue cells figures (table 17).

High and consistent numbers of gram positive cocci was noticed in smears from patients infected with staphylococci (29.6%) and streptococci (25%). Trichomonas vaginalis and BV infected women demonstrated these cocci in 13.1% , 14.1% of cases, respectively . Agreed data were shown in [67,149,131] proving that significant number of vaginitis cases are mixed infections making confirmed diagnosis a difficult task. This also force investigators to try many tests rather than a single one for a correct and save diagnosis.

In general Gram staining seems more sensitive, objective and reproducible in comparison to wet mount examinations ,with a sensitivity of 93% and specificity of 70% when Amsel□s criteria were used as the gold standard for BV diagnosis. [64,48] It is especially useful for the evaluation of asymptomatic women and provides durable record of the patient compared with Nugent criteria which tend to under diagnose large number of cases. The current work , however, clearly demonstrated that superiority of wet mounts in the search for clue cells

over gram staining (table 17). This problem mandate more studies to elucidate these aspects of great value for the routine diagnosis of BV. The current study frequency of BV by Amsel□s criteria was 49.5% whereas it was 46.5% with the use of Nugent s criteria. These observations were in accordance with data shown in [48] [150] that clearly demonstrated the superiority of the former over the later.

The use of Giemsa stained vaginal smears to diagnose vaginitis and to gathering valuable information has been attempted in the present study and compared to other diagnostic procedures. This staining methodology proved very useful in the diagnosis of T. vaginalis and evaluation of vaginal smears to provide deeply interesting clues.

The present study showed Giant cells in 70.8% of the recruited cases (Table 15 and 22). It was demonstrated in 10.1% trichomniasis cases , in 5% of BV patients and in 10.1% of cases that showed no causative agents. The presence of Giant cells in these cases of vaginal infections seem to be associated with mixed or viral infections as noticed in [111]. Further studies on these aspects accompanied with more specific techniques are required to test these conclusions.

Basophilic cells was observed in 5% of BV cases, in 5.2 % of TV infected women and in 1.6% smears that diagnosed as without causative agents . Basophilic cells during vulvovaginal pathology are mostly associated with atrophic vaginitis changes as cited by [50, 69] . Basophilic as well as acidophilic cells represent vaginal changes under the various hormonal changes during menstruations.

RBCs were recorded in 6% cases of BV patients, in 10.5% of Candida albicans infected women and 3.3% cases showed undetermined causative agents. The presence of RBCs outside menstruation are

frequently associated with many noninfectious vaginitis cases. Current findings were in agreement with those shown in a number of investigations. [140,149,151]

Culture procedures to ascertain of Gardnerella vaginalis infections is considered as not very much useful for the BV diagnosis in comparison to the non-cultural methods ,as up to 50% of healthy women have positive culture due to low numbers of G. vaginalis in the vagina without any sign of BV manifestations. [30,76] However , when no wet mounts or Gram stains are available and a clinical diagnosis is doubtful , massive growth of BV associated bacteria, group B streptococci and Staphylococcus aureus can help in distinguishing aerobic vaginitis from BV. Culture are also useful to delete the non albicans species which are non-routinely encountered in these cases. [131, 138] In the present study, however, culture s were assayed and proved essential for diagnosis of large number of vaginitis cases especially those suffered of infectious agents other than Gardnerella vaginalis.

In the present study , organisms obtained by culture techniques (table 10 , 23) were greatly associated with tremendous amounts of abnormal vaginal discharges. Almost all cases due to Candida albicans, other Candida spp, staphylococci and B-hemolytic streptococci infections. Scanty amounts of discharges, on the other hand, are produced when no growth (33.5%) or lactobacilli was diagnosed (30 %). Identical in formations were given in [106,131] and landed on the requirement of powerful inflammatory inducers for the induction of a profuse abnormal vaginal discharges.

No growth characteristics in the actual trial were only observed in 28.2% of BV cases and in 18.7% of TV patients. Normal flora lactobacilli were

demonstrated in culture of 24 (24.2%) of BV cases and in 4 (10.5%) of TV cases . Moreover, in candidiasis , staphylococcal and Streptococcal infections, cultural results gave the predominance of those etiological agents. These findings clearly demonstrated the essential value of the direct gram staining in directing the suitable diagnostic approaches.

High percentages of mixed infections were especially reported in BV cases, in staphylococcus and streptococcal infections. these results were comparable to those reported in many previously shown Investigations **(104 ,137,136,152)** .

Abnormal vaginal discharges were the chief complaint in the majority vulvovaginal inflammations in various studies**(132 , 135 , 99)** . In the present study , concentrations on a number of signs and symptoms together with the abnormal vaginal discharges which consisted of low abdominal pain, itching , bad odor and erythematic symptoms. These manifestations were the commonest features and were find in almost all of the patients infected with the major six types of agents documented in the present study . These four main symptoms accompanied BV infections as follow: 82.8%, 70.7%, 56.6%, and 57.5%, respectively. In trichomoniasis infections, they were in the following order :42.1 % , 44.7% , 47.3% , and 52.6% , respectively. In candidiasis, the rates were 56% , 88% , 60% and 52%, respectively . Concerning staphylococcal infections figures were 51.8% , 59.2% , 60% and 62.9% , respectively. The main complain in streptococcal infections was the bad odor which was obtained in 75% of these infections. these symptoms were noticed in 50% of patients who diagnosed with undetermined microbial agents. symptoms and signs reported in the current trail were similarly given in **(153,154)** .

Most of the women presented with homogenous vaginal discharges suffered of bacterial vaginosis. On the other hand profuse thin discharges were the feature evident in 49.4% of staphylococcus infected patients, in 55.5% of streptococcus infections m and in 42.3% of women with undetermined microbial cause.

In respect to candidiasis, 64% of the patients resented with thick white cheesy discharge compared to 13.1% of trichomoniasis cases which came with frothy yellow discharges. The characteristics of the nature and consistency of the abnormal vaginal discharge given by the present work were in agreement with those reported in [153,155] .

Finally, based on the characteristics obtained by the present work as well as various other previous data. [132, 129,154], authors of the present work proposed the nature and the physical features of the abnormal vaginal discharges as a possible and helpful criterion in differentiating the types of vaginal infection. The following table demonstrates these parameters :

Comparative finding of the causes of vaginitis.

cause	Physical charactristic	Gram stain	Giemsa stain	Wet mount
Bacterial vaginosis	50% asymptomaticWhite or thin homogenous discharge coating vaginal wall	Clue cells, Predominant gram negative coccobacilli	Curved rods, Giant cells, Lactobacilli grade 3-4	Clue cells and motil bacilli
Candida vaginitis	Rendness, inflammation of vulva skin and white discharge	Gram positive mycelia and spores	WBC dominance	Leukocytes± Pseudohyphae or budding yeast
Non candida yeast vaginitis	Erythema and curdy vaginal discharge	Gram positive yeast cells	Epithelial dominance	Usually negative
Trichomonas	Itch and 50% asymptomatic Profuse frothy greenish yellow discharge	Often not seen	Lysed epithelial cells and WBC dominance	Leukocytes± Motile trichomonads
Aerobic vaginitis	Often Sever and Abundant purulent discharge	Gram positive staphylococci and streptococci	Epithelial dominance and few RBC	Leukocytes Cocci or coarse rods
Cyctolytic vaginitis	Itch, Profuse discharge, often cheesy	Predominant of Gram positive lactobacilli	Lysed epithelial cells lactobacilli grade 3	Overgrowth of lactobacilli squamous cell fragments
Irritant and allergic vaginitis	Variable often erythema and offensive purulent discharge	Few gram positive bacilli	Lysed epithelial and few RBC	Leukocytes±

Conclusions

1- Bacterial vaginosis, Candida albicans and the Trichomonas vaginitis were the most frequent microbial causes of vulvovaginitis among married women in the primary health care centers in Benghazi.

2-Bacterial vaginosis was the major clinical entity of the various microbial causes of vulvovaginal infections resulting in the abnormal vaginal discharge.

3- Abnormal vaginal discharges together with low abdominal pain , itching , bad odor and erythematic symptoms was the chief complaint with almost all the vaginal infections.

4- Vaginal infections increased with risk factors such as caffeine , traditional medicine use ,contraception and vaginal douching .

5- Diagnosis of bacterial vaginosis, by Amsel's criteria are more often used in the clinical setting , the Nugent scoring system has a higher reproducibility , with comparable sensitivity and specificity to the former criteria .

6- High positive correlation between microscopy and clinical features was obtained in the detection of BV , candidacies and trichomoniasis. .

7- Physical properties of the vaginal discharges was helpful criterion in differentiating the types of the vaginal infection .

8- Measuring the vaginal pH and whiff test can correctly and inexpensively identify pregnant women at risk of developing BV as well as preterm labor .

9- Socio-demographic factors such as education, low monthly income, age and multiple of marriage are associated with frequent occurrence of vaginal infections .

Recommendations

1- Deep need for further studies to determine whether prevention or control of vaginal infections particularly by approaches that rely not only on antibiotic treatment , but also on maintenance of healthy vaginal ecosystem to reduce the adverse health outcomes.

2-Further studies on the treatment of infection in pregnant women with BV and the impact of this strategy on the prevention of preterm labor birth (PLB) and preterm pre labor rupture membrane (PPROM) and rate of persistent form of these diseases are required

3-Pregnnant women should be advised to practice good personal hygiene and avoid drinking of caffeine. They are also advised to limit the use of traditional medicine and intra vaginal douching practices.

4- Health education to consider the essential factors for prevention of bacterial , viral and parasitic infections is mandatory.

5- Screening and treatment for BV in all pregnant women with a previous preterm birth is hotly recommended.

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جامعة بنغازي

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دراسة معدل انتشار والعوامل المساعدة على حدوث العدوى البكتيرية المهبلية , المشعرات المهبلية , التهابات الفطرية وعدوى الكلاميديا في السيدات عند عمر الانجاب

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الملخص العربي

حدوث الإفرازات المهبلية الغير طبيعية في السيدات عند عمر الإنجاب تعتبر من المشاكل الطبية الأكثر شيوعا والشكاوي الأكثر تكرارا بقسم أمراض النساء في وحدات الرعاية الصحية. تهدف هذه الدراسة تحديد الأسباب الميكروبية الشائعة للإفرازات المهبلية الغير طبيعية ودورها في الإصابة بالأمراض المنتقلة مشيميا وما تسببه من نتائج عكسية أثناء الحمل فضلا عن بعض الإخماج التي تقود إلى العقم . كما درسنا تأثير بعض العوامل المؤهبة للالتهاب المهبل الجرثومي مثل استعمال موانع الحمل التاريخ الطبي للمريض استعمال بعض المواد الكيمائية –استعمال المتكرر للغسول المهبلي والتدخين وأيضا تناول بعض المنبهات مثل القهوة. تم تجميع 200 مسحة مهبلية من نساء حوامل وغير حوامل تراوحت أعمارهن 16و45 سنة تعاني من إفرازات مهبلية غير طبيعية في اللون والرائحة والحكة , تم عزل وتشخيص المسببات البكتيرية والفطرية والطفيلية في 141 سيدة حامل وغير حامل بقسم أمراض النساء والحوامل في المركز الصحي بنغازي الجديدة , امتدت الدراسة لمدة ستة اشهر ابتداء من شهر ديسمبر 2010 إلى مايو 2011. تم التعرف على المسببات للالتهابات المهبلية عن طريق الفحص ألمجهري بواسطة صبغة الجرام وصبغة جيمسا وطريقة المسحة الرطبة وأيضا عن طريق زرع المسحات المهبلية. كل هذه السيدات اخضعوا إلى فحص إكلينيكي دقيق مع الاستفسار عن أعراض ومشاكل تدل على إصابات مهبلية حسب استمارة استبيان معينة.

تم الحصول على النتائج التالية:

1- أعلى نسبة تواجد للأحياء المجهرية المهبلية لدى النساء في مجتمع الدراسة الحالية كانت متمثلة بالبكتيريا المهبلية Bacterial vaginosis 49.5% وتليها الإصابة بطفيلي المشعرات المهبلية Trichomonasa vaginalis 19% والمكور العنقودي الذهبي Staph .aureus بنسبة 13.5% ويتبعها فطركانددا المبيضات البيض Candida albicans 12.5% بينما كانت نسبة انواع كانددا الاخرى Candida spp 11.5% إما نسبة الإصابة بالميكروب السبحي Strept. agalactiae كانت بنسبة 4% .

2- من خلال دراستنا للعوامل المؤدية الى الاصابة لوحظ وجود علاقة إحصائية هامة بين العمر وزيادة نسبة الإصابة وكذلك وجود علاقة بين نسبة الإصابة والمستوى التعليمي ولوحظ أيضا هناك ارتباط بين معدل تناول القهوة واستعمال بعض المواد الكيمائية واستعمال الغسول المهبلي ونسبة الإصابة ولم تكن هناك أي دلالة إحصائية هامة بين العوامل الأخرى المؤدية للإصابة.

3- دراستنا بينت أن هناك ارتباط دقيق بين التشخيص المعملي والفحص الإكلينيكي للعينات المهبلية ودورهما في تشخيص المسببات البكترية والفطرية والطفيلية , وأيضا كذلك طبيعة الإفرازات المهبلية من حيث الشكل واللون والرائحة ساهم في التفريق بين انواع التهابات المهبلية .

4- التشخيص المعملي بواسطة فكرة Amsel's والفحص المجري بواسطة صبغة الجرام والتي تم فيها استعمال Nugent's score يعد الطريقة التشخيصية الأكثر أهمية في تشخيص المسبب للالتهابات المهبلية البكترية Bacterial vaginosis