Diagnosis of *Trichotomies Vaginalis* in reproductive age group Libyan Ladies in Benghazi City using OSOM rapid test

Abstract

Trichomonas Vaginosis is a protozoal infestation considered among sexually transmitted diseases with a prevalence rate variable across societies and regions. Such infection is associated with some gynecological trouble and affects adversely the quality of life of women. The aim of the study is to study the prevalence of *Trichomonas vaginalis* infection using OSOM test ™. Two hundred women were investigated with history, speculum examination and OSOM test. The prevalence of Trichomonas infection was 4.0% with another 0.5% inconclusive result. The conservative nature and hygienic practice among the Libyan population may help in the lower prevalence of TV infection. But the prevalence is still high in comparison to some other countries in the Mediterranean region. History of irregular cycle and also Co-morbidities with hypertension, diabetes mellitus, polycystic ovarian syndrome and thyroid disorder as well as findings of whitish discharge and Odorous discharge have no statistically significant association with positive result. Frothy discharge was higher in positively testing with a statistically significant association and sensitivity as well as specificity and negative predictive value of the finding of frothy secretion were 100.0%, while the positive predictive value was 72.7%. The OSOM test should be available for use in maternity centers and women health clinics. A careful history and clinical findings should be obtained from the clinician. A further well designed study may be worthy to examine other characteristics might be related to the Trichomonas infection across different groups of women.

List of Appendices

List of Abbreviations

Abbreviation	Meaning
AV	Aerobic vaginitis
AVF	Abnormal Vaginal Flora
BV	Bacterial Vaginosis
CI	Confidence Interval
DIV	Desquamative Inflammatory Vaginitis
HIV	Human Immunodeficiency Virus
I.V.	Intra Venous
L	Litre
Ml	Milliliter
MV	Mixed vaginitis
N / A	Not Applicable
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio
RR	Risk Ratio
STD	Sexual Transmitted Disease
STI	Sexual Transmitted Infection
TV	Trichomonas Vaginosis
VVC	Vulvovaginal candidiosis
WHO	World Health Organization

Chapter I

1. Introduction:

1.1 Introduction

Sexually transmitted infections (STIs) are group of infectious diseases caused by many varieties of organisms belong to different categories like bacteria, viruses, protozoal organisms and also fungi. Such infections are contagious and spread mainly through their habitation in genital tract and existence in genital secretions. That's why they got their name as they are transmitted through intimate sexual contact, although STIs are important as a public health problem all over the world, There are some difficulties in prevention and control programs of STIs due to clinical and laboratory diagnostic problems. The most common STIs are Chlamydia trachomatis infections, trichomoniasis and gonorrhoea (Al-dunate *et al.*, 2015).

Vaginal infections and STI are responsible for a large proportion of gynaecological outpatient visits, from 10 to 20 % of consultations, and are typical in woman of childbearing age. Further important issues with these vaginal infections are the very high rate of recurrences and a variety of associated serious adverse outcomes in pregnancy. Finally, they have a major impact on the quality of life and driving these women exhausted. Types of vaginal infections include three main well defined vaginal infections (Mendling *et al., 2016).*

1- bacterial vaginosis (BV),

2- vulvovaginal candidosis (VVC), and

3- Trichomoniasis/Trichomonas vaginitis (TV).

Additionally, there are other vaginal infections which have not yet been completely characterised and recognised. Especially the ‘intermediate flora’ (based on Nugent score 4–6) and the not well defined ‘mixed flora’ or ‘abnormal vaginal flora’ (AVF) could possibly play a bigger role in the development of preterm birth than expected before. Moreover, aerobic bacteria are also involved in vaginal infections but their role is not yet understood. Based on this knowledge a fourth, still poorly characterised type of vaginal infections has been defined: aerobic vaginitis (AV), which is probably the same entity as desquamative inflammatory vaginitis (DIV), where severe inflammation is observed and complains are more intense. Additionally, mixed vaginitis (MV) and co-infection with coexistence of BV, VVC, and TV, is possible, and thus diagnosis and treatment of vaginal infections is not always easy.

Trichomonas vaginalis (TV) is a common motile protozoan parasite, which is the most prevalent non-viral sexually transmitted infection (STI) in the world and was estimated by the WHO to cause 276.4 million new infections per year in persons between the ages of 15 and 49 years in 2008. *Trichomonas vaginalis* is more prevalent than *Chlamydia trachomatis, Neisseria gonorrhoeae*, and syphilis combined. The global prevalence of TV has been estimated at 8.1 % for women and 1.0 % for men. In general, Africans or persons of African descent have higher rates of TV, as evidenced by higher rates in Sub-Saharan Africa.

The prevalence of the infection in Africa ranges from 5% to 74% in women and 5% to 29% in men, depending on the populations studied and the diagnostic methods used to detect the infection.

Among pregnant women, *T. vaginalis* infection has been associated with adverse birth outcomes such as preterm delivery, premature rupture of the membranes, and low birth weight. In addition to these reproductive health complications, mounting evidence suggests that *T. vaginalis* may be a cofactor for HIV-1 transmission and acquisition. *T. vaginalis* infection is generally given less emphasis than other STIs and is not a reportable infection. As a result, the infection is infrequently studied and little information exists about the risk factors for it; consequently, its public health importance remains poorly understood, and issues like partner management, antimicrobial resistance, associated conditions (eg, human immunodeficiency virus [HIV], pregnancy complications, and others), diagnostic methods, screening, reporting, and prevention of trichomoniasis take a considerable part of research in Western societies (Meites *et al.*,2015**)**.

Bacterial vaginosis is a common lower genital tract syndrome defined as a shift from normal hydrogen peroxide–producing lactobacilli to mixed anaerobes, such as Gardnerella species, Prevotella species, and Atopobium species (Yazısız H *et al*, 2020) and (Olson KM *et al*, 2018). Lactobacillus species comprise between 90 and 95 percent of the total bacteria count in the healthy vaginal flora and play a key role in maintaining balance and host defense against pathogens by producing several substances that inhibit the growth of deleterious microorganisms (Ling Z *et al*, 2010) and (Wood BA *et al*, 1975).

. Symptoms of bacterial vaginosis typically include off-white, thin, homogenous discharge or vaginal “fishy” odor, or both; however, many women with bacterial vaginosis are asymptomatic.

Worldwide bacterial vaginosis prevalence estimates range from 12% in Australian women and 29 percent in North American women to more than 50 percent in women from Eastern and Southern Africa (Tweats *et al.*,2012***)*** The prevalence of bacterial vaginosis in the United States is estimated to be 29.2 percent among all women ages 14 to 49 years (some of whom are pregnant), corresponding to 21 million women, according to National Health and Nutrition Examination Survey (NHANES) data from 2001 through 2004, the most recent years for which nationally representative estimates are available (Tarrant *et al.***,**2014). Prevalence varies most notably by race/ethnicity. The NHANES data from 2001 through 2004 showed significantly higher rates among African Americans (52.6%) and Mexican Americans (32%) than among non-Hispanic whites (23%) (Tarral A *et al*, 2014).

Among five studies published between 1995 and 2014, a higher prevalence of bacterial vaginosis (range, 25% to 50%) was observed among women who have sex with women (Bosserman *et al., 2011).* In the United States, the prevalence of bacterial vaginosis among pregnant women ranges from 5.8 to 19.3 percent and is influenced by the study population and the diagnostic criteria. The prevalence is higher in some races/ethnicities (Sobel *et al., 2001).*

Studies conducted betweUpregnancy loss before 22 weeks (RR range, 3.1 [95% CI, 1.4 to 6.9]),(Obiero *et al.,2012*) pelvic inflammatory disease (magnitude not well defined), (Newman *et al.,2015*) postabortion sepsis (magnitude not well defined),(Ashshi *et al.,2015*) postpartum endometritis (odds ratio [OR], 5.8 [95% CI, 3.0 to 10.9]),(Joseph *et al.,2016*) and low birth weight (OR, 1.4 [95% CI, 1.1 to 1.8]).(Rowley *et al.,2019*).

1.2. Importance of the study:

Up to the best available knowledge, no published similar work in Libya. Use of OSM test

1.3. Aims of the study:

The aim of the study is to study the prevalence of *Trichomonas vaginalis* infection using OSOM test ™.

Chapter II

2.Review of literature:

2.Review of literature:

2.1. Epidemiology of STI and vaginosis

Based on prevalence data from 2009 to 2016, the estimated pooled global prevalence of chlamydia in 15–49-year-old women was 3.8% (95% UI: 3.3–4.5) and in men 2.7% (95% UI: 1.9–3.7), with regional values ranging from 1.5 to 7.0% in women and 1.2 to 4.0% in men. For *gonorrhoea,* the global estimate was 0.9% (95% UI: 0.7–1.1) in women and 0.7% (95% UI: 0.5–1.1) in men, with regional values in women ranging from 0.3 to 1.9% and from 0.3 to 1.6% in men. For syphilis, the global estimate in both men and women was 0.5% (95% UI: 0.4–0.6) with regional values ranging from 0.1 to 1.6%. The WHO African Region had the highest prevalence for *chlamydia* in men, *gonorrhoea* in women and men, *trichomoniasis* in women and syphilis in men and women. The WHO Region of the Americas had the highest prevalence of *chlamydia* in women and of *trichomoniasis* in men.

These prevalence estimates correspond to the totals of 124.3 million cases of *chlamydia*, 30.6 million cases of *gonorrhoea*, 110.4 million cases of trichomoniasis and 19.9 million cases of syphilis (available from the data repository) (Rowley J *et al*, 2019).

2.2. Epidemiology of trichomoniasis:

The estimates for trichomoniasis were 5.3% (95% UI: 4.0–7.2) in women and 0.6% (95% UI: 0.4–0.9) in men, with regional values ranging from 1.6 to 11.7% in women and from 0.2 to 1.3% in men.

According to WHO's 2012 estimates were based upon literature reviews of prevalence data from 2005 through 2012 among general populations for genitourinary infection with ***chlamydia***, ***gonorrhoea***, and ***trichomoniasis***, and nationally reported data on syphilis seroprevalence among antenatal care attendees (Newman L *et al*, 2015).

Newman L, *et al* (2015) upon data during 2012, among women aged 15–49 years, the estimated global prevalence of chlamydia was 4.2% (95% CI(UI): 3.7–4.7%), gonorrhoea 0.8% (0.6–1.0%), trichomoniasis 5.0% (4.0–6.4%), and syphilis 0.5% (0.4–0.6%); among men, estimated chlamydia prevalence was 2.7% (2.0–3.6%), gonorrhoea 0.6% (0.4– 0.9%), trichomoniasis 0.6% (0.4–0.8%), and syphilis 0.48% (0.3–0.7%). These figures correspond to an estimated 131 million new cases of chlamydia (100–166 million), 78 million of gonorrhoea (53–110 million), 143 million of trichomoniasis (98–202 million), and 6 million of syphilis (4–8 million). Prevalence and incidence estimates

varied by region and sex. According to Newman L, *et al* (2015) upon data during 2012, trichomoniasis global estimate was 5.0% (4.0–6.4%) and the regional values ranged from 1.0% to 11.5%. The lowest prevalence was reported in the European and South Asian regions. In eastern Mediterranean Region the prevalnec of trichomoniasis among women was around 6% while among men was 1% (Newman L *et al*, 2015).

In a case control study by Ashshi AM *et al* (2015) in Saudi Arabia including comparison of 7 STDs between normal and ectopic pregnancy, the prevalence of co-infections was significantly higher compared with single infection in the study participants and it was associated with 5 times greater risk of developing ectopic pregnancy including *Trichomonas vaginalis* (Ashshi AM *et al*, 2015).

Trichomonas vaginalis is considered as a curable STD despite the potential complications it may contribute to. A systematic review by Joseph Davey DL *et al* (2016) described studies performed in Kenya, Tanzania, Somalia, Ethiopia, Uganda, and Sudan for prevalence of *Trichomonas vaginalis* among pregnant women. The adjusted mean prevalence was similarly high for TV in 3 studies at 6.8% (95% CI, 4.6– 9.0) (Joseph Davey DL *et al*, 2016).

Rowley J *et al* (2019) published a meta-analysis including 76 data points regarding Prevalence and incidence of *Trichomonas vaginalis* along with some other sexually transmitted diseases. The global estimate for *Trichomonas vaginalis* infection among men was 0.6% (95% CI: 0.4 – 0.9). The highest prevalence among WHO regions was reported in African region; for women 11.7% (95% CI: 8.6 – 15.6) and for men 1.2% (95%CI: 0.7 – 1.8). While the Eastern Mediterranean Region (EMRO) which include Libya reported the lowest prevalence, for men0.2% (95%CI: 0.1 – 0.3) and for women 1.6% (95% CI: 1.1 – 2.3) (Rowley J *et al*, 2019).

Chemaitelly H *et al* (2019) demonstrated in a meta-analysis for studies including female sex workers in Middle East (Egypt, Iran and Pakistan) and found that the infection prevalence ranged from 0%-19.3%, with a median of 7.0%. The highest prevalence was in Egypt (Chemaitelly H *et al*, 2019).

According to (Crespillo-Andujar C *et al*, 2018), T. vaginalis protozoa are the most common nonviral STI in the world, and incidence is increasing. The genital tract of humans is the natural habitat for this parasite, which can cause urogenital tract infection.

T. vaginalis has been identified in seminal fluid and has been related to decreased sperm quality.

2.3. Taxonomic classification of *Trichomonas vaginitis*

. Domain : Eukarya

. Kingdom : Protista

. Phylum : Metamonada

. Class : Parabasilia

. Family :Trichomonadida

. Genus : Trichomonas

. Species : *Trichomonas* vaginalis

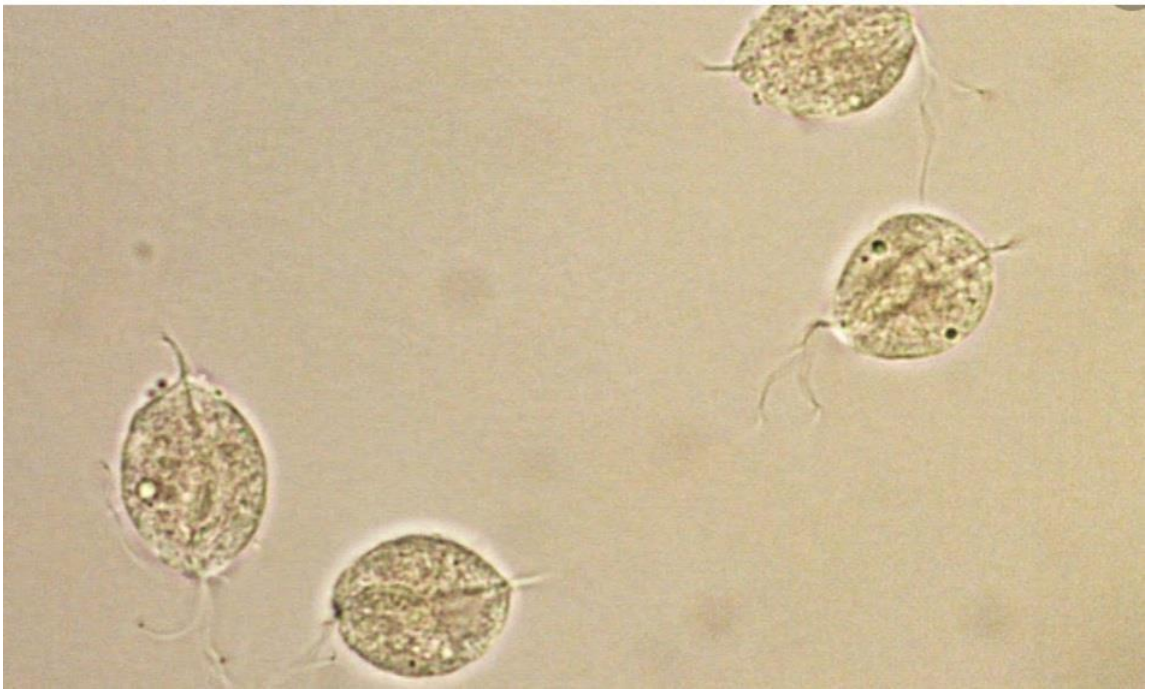

Figure 1: Trichomonas virgin power 4^{o}

2.4. Pathogenesis of TV

TV is a flagellated parasitic protozoan, typically pyriform (Harp *et al., 2011*) the individual organism is 10–20 μ m long and 2–14 μ m wide.

Four flagella project from the anterior portion of the cell (Carlton *et al,* .2007) TV is a highly predatory obligate parasite that phagocytoses bacteria, vaginal epithelial cells and erythrocytes. TV uses carbohydrates as its main energy source. Incubation period is generally between 4 and 28 days (Petrin *et al., 1998*). TV primarily infects the squamous

epithelium of the genital tract. TV resides in the female lower genital tract and the male urethra and prostate, where it replicates by binary fission. TV is transmitted among humans, its only known host, primarily by sexual intercourse. Infection may persist for long periods, months to even years, in women and generally less than 10 days in males (Krieger *et al., 1995*).

Studies show an association between TV and vaginitis, cervicitis, urethritis, bacterial vaginosis, candidiasis, herpes simplex virus *al.,2009*).TV has also been associated with poor birth outcomes such as low birth weight, preterm delivery, pelvic inflammatory disease, and Premature rupture of membranes (Silver *et al.,2014*). One study showed an association between maternal TV infection and intellectual disability in children (Mann *et al., 2009*). Rarely,TV infection can be transmitted perinatally and (Schwandt *et al.,*2008).cause vaginal and respiratory infections in neonates (Carter *et al.,*2008 & Temesvari *et al.,* 200).

2.5. HIV acquisition and transmission

Several cross-sectional and cohort studies that have indicated a higher risk for HIV acquisition among TV+ compared to TV- women (Kissinger *et al.,2013*).

This greater susceptibility is biologically plausible for three reasons: inflammatory response to TV infection results in the increased appearance of HIV target cells (Sardana *et al., 1994*). TV infection can impair the mechanical barrier to HIV via punctate mucosal hemorrhages (Guenthner *et al.,* 2005) and TV infection may change the normal vaginal flora rendering it more permissive for bacterial vaginosis (Moodley *et al., 2002*). Which, in turn, can increase the risk of HIV acquisition (Van *et al.,* 2009). These consequences facilitate HIV in TV-infected women. Several studies have also demonstrated increased HIV expression among HIV+/TV+ women. Some estimates that in a community with a high prevalence of TV, as much as 20 % of HIV could be attributed to TV infection (Sorvillo *et al.,* 1998 & Chesson *et al.,* 2004). Control of TV, therefore, may provide a cost-effective strategy for reducing HIV transmission especially in settings where TV is common (McClelland *et al.,* 2008 & Price *et al.,* 2006). Or among subgroups who are at higher risk for TV such as African Americans (Sorvillo *et al., 2001*).

Among HIV+ women, TV has been associated with increased HIV vaginal shedding in several studies. Fortunately, treatment for TV has demonstrated reductions in HIV genital

shedding in several studies. HIV+ men with urethritis in Malawi, with TV diagnosed by NAAT, experienced a decrease in seminal HIV after MTZ treatment (Price *et al.,* 2003).

HIV vaginal shedding was decreased after treatment in one cohort of women, diagnosed by microscopy and culture in Kenya (Wang *et al.,* 2001). And another, diagnosed by culture, in Louisiana, US (Kissinger *et al., 2009*). These data underscore the importance of screening and treatment among HIV positive persons.

2.6. HSV-2:

TV appears to have a similar bi-directional association with Herpes Simplex virus II (HSV-2) as it does with HIV-1. Concomitant infection with TV has been associated with HSV-2 shedding (Boselli *et al.,* 2005) and women have been found to have TV have a higher incidence of HSV-2 (Gottlieb *et al., 2004*).

2.7. Neoplasia:

Evidence that TV is associated with HPV acquisition, thus there may be in indirect link between TV and cervical neoplasia. Some studies found that TV was associated with a 1.9 fold risk of cervical neoplasia (Zhang *et al., 1994*). Studies of Finnish, Dutch, Belgian and Chinese women have all found elevated odds (1.4–2.0) of cervical neoplasia among women who have TV or vice versa (Depuydt *et al., 2010 &*Viikki *et al.,* 2000 & Yap *et al., 1995*). Sutcliffe et atfound an association between TV and prostate cancer in one study but not in other studies (Sutcliffe *et al.,* 2009 & Sutcliff *et al.,* 2006).

Among women, common sites of infection include the vagina, urethra and endocervix. Symptoms include vaginal discharge (which is often diffuse, malodorous and yellow-green), dysuria, itching, vulvar irritation and abdominal pain. The normal vaginal pH is 4.5, but with TV infection this increases markedly, often to >5 (Petrin *et al., 1998*).

Ther complications include infection of the adnexa, endometrium, and Skene and Bartholin glands. In men, it can cause epididymitis, prostatitis, and decreased sperm cell motility (Martinez *et al.,* 1996).

Trichomoniasis has been associated with poor reproductive health outcomes such as low birth weight (LBW) and premature birth (Cotch *et al.,* 1997 & Silver *et al.,* 2014) .TV infection is also associated with twofold to threefold increased risk of HIV

acquisition and pelvic inflammatory disease (PID) among HIV-infected women (Van *et al.,* 2008 & Kissinger *et al.,*2013) .

Because infection with TV is so common and can be associated with such serious adverse events, diagnostic testing for detection of TV and treatment of TV infections are recommended for symptomatic women and men. For asymptomatic individuals, screening is only recommended for HIV-positive women and is only encouraged for persons in such locations as sexually transmitted disease clinics and correctional facilities (Workowski *et al., 2015*).

The conventional methods to detect TV in vaginal swabs are wet mount microscopy and culture techniques. Wet mount microscopy is the most common method for detection of TV, and although this technique is rapid and inexpensive, it is only about 36%–75% sensitive compared with culture even in the hands of trained microscopists NAATs (Nye *et al.,* 2009) may detect a prevalence of threefold to fivefold higher than wet preparation microscopy (Schwebke *et al.,2004*).

2.7 Chemical and physical environment of female genital tract:

The vagina and ectocervix and the lower female reproductive tract in general, comprise a good chemical and physical barrier to invading exogenous organisms, partially because of the structure of the stratified vaginal epithelium along with the excretion of cervicovaginal fluid (CVF). The CVF is eubiotic and viscoelastic serving as an effective lubricant to facilitate the trapping of exogenous organisms and also, it is an acidified medium in which there is many of antimicrobial molecules (antibodies, defensins etc.). The adhesion of those vaginal microbiota (Boris *et al*., 1998) .enabled by the mucosal layer (mucus and layers of dead epithelial cells). In asymptomatic women, microbiota acidify the vagina through lactic acid-production whilst bacterial vaginosis-associated bacteria (BVAB) produce many short chain fatty acids (SCFAs) that contribute the development of a dysbiotic vaginal environment ((Aroutcheva *et al*., 2001a,b; Valore *et al*., 2002; Yeoman *et al*., 2013)

2.8. Diagnosis of TV

1-Microscopic examination of a wet mount of vaginal (Schwebke *et al.,* 2004).

2-Fluid, looking for trichomonads sensitivity, ranges from 44–68% compared to culture (Hobbs *et al.,* 2013).

3-Culture was the gold standard for diagnosis of *T. vaginalis*, with a sensitivity of 81–94% (Garber *et al.,2005 &* Ohelmeyer *et al.,1998*). Diamond's medium is the traditional

culture method used for the isolation of *T. vaginalis* (Garber *et al.,*1987). Itis time consuming and contamination with vaginal bacteria is common, making this technique difficult.

4-T. vaginalis NAATs for use in female urine, endocervical swab, as well as male urine. Diagnostic sensitivity and specificity for the NAAT range from 99.5–100% and 99.4–99.9% for female genital specimens and 97.2–99.9% for male urine specimens (Schwebke *et al.,* 2018).

5- The OSOM® Trichomonas Rapid Test (Sekisui, Framingham, MA), this trichomonas rapid antigen test is an immunochromatographic capillary-flow enzyme immunoassay based on trichomonas membrane proteins, which can detect trichomonas in 10 min.

Compared with wet preparation and culture, OSOM Trichomonas test has a good sensitivity, excellent specificity and compares favourability to NAAT assays with reported sensitivities of 83%– 90% (Nye *et al.,*2009 & Huppert *et al.,*2007 & Huppert *et al.,*2005).

An early study compared the sensitivity and specificity of the OSOM test, wet mount and culture performed on vaginal swabs from 449 sexually active women. The overall prevalence of TV was 23.4% by the reference standards of either wet mount or culture test being positive. For the vaginal swabs, the OSOM test displayed 83.3% and 98.8% sensitivity and specificity, respectively, and it performed better than wet preparation.

The OSOM assay is the only POC assay that is CLIA waived, meaning it does not need to be performed in a laboratory, and is the only one that does not require special instrumentation. Its high sensitivity (83%–90%) and excellent specificity make it an ideal assay for resource limited settings (Madhivanan *et al.,* 2013). It is currently recommended in Q women only.

2.9 Diagnosis and clinical presentation of pathogens in vaginal infections:

Anaerobic bacteria Gardnerella (facultative anaerobic), Atopobium, Prevotella, Mobiluncus, etc. Aerobic bacteria, Staphylococci, Streptococci, etc. Anaerobic and/or aerobic bacteria and/or *Candida*. C. *albicans* (80–90 %), C. *glabrata* (2–5 %), C. *krusei* (1-2 %) and *Trichomonas vaginalis* (Mendling W *et al*, 2016).

Symptoms and signs of vginosis include the following: Vaginal dysbiosis or infections are usually characterised by an abnormal vaginal discharge and other vaginal signs and symptoms. They can be differentiated into vaginosis, which does not show inflammation

signs, and vaginitis (usually candidosis or aerobic infection), which leads to inflammation (Aldunate M *et al*, 2015).

Schmidt *et al*; 1994; Denmark; Nonpregnant, nonmenstruating women who did or did not complain of vaginal discharge and were gynecologically examined at a general practice; 31.6% complained of vaginal discharge.

Sonnex *et al*; 1995; United Kingdom Women attending three general practices or a hospital-based genitourinary clinic in the Cambridge area 135 (45.5) had vaginal discharge; 46 (15.5) had vaginal discharge and malodor from general practice; 54 (32.9) had vaginal discharge from genitourinary clinic.

Confirmed diagnosis with Gram stain Byun *et al*; 2016 South Korea; 34.9% (90) Cartwright *et al*; 2013; United States ; 64.6% (95) Schwebke *et al* (2018); 50.5% (99)

Criteria for diagnosis of vaginosis include: In clinical settings, at least three of four Amsel criteria are necessary for a diagnosis of BV:

- homogenous, thin, grayish-white vaginal discharge;
-vaginal pH >4.5, positive whiff-amine test; and clue cells present on a wet mount of vaginal fluid. In research settings, BV is defined by the Nugent score.
- Scores of 0–3 are graded as lactobacillus-predominate normal vaginal flora, 4–6 as intermediate flora with the emergence of *G. vaginalis/Bacteroides* morphotypes and 7–10 as BV flora with disappearance of lactobacillus species, numerous *G. Vaginalis / Bacteroides* and curved Gram-variable anaerobic rods. The appearance of curved Gram-variab le anaerobic rods results in Nugent scores of 9–10 (**Kristin *et al*.,2018).**

Molecular diagnosis:

Among molecular fingerprinting methods, PCR-DGGE represents a rapid and reliable technique to identify the predominant microbiota in various ecological niches.

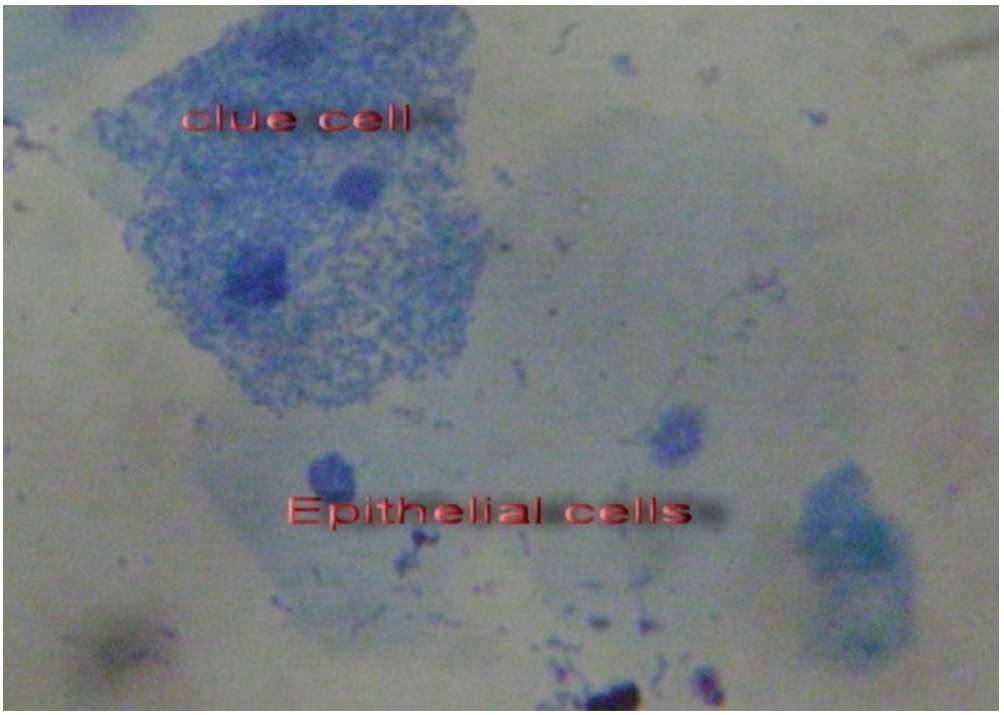

Figure 2: Clue cell under light microscope

Sequencing of 16S rRNA genes from different samples by constructing clone libraries, (typically at most a few thousand clones from a low number of individuals), has revolutionized our understanding of microbial systematics and diversity (Ling *et al*.,2010).

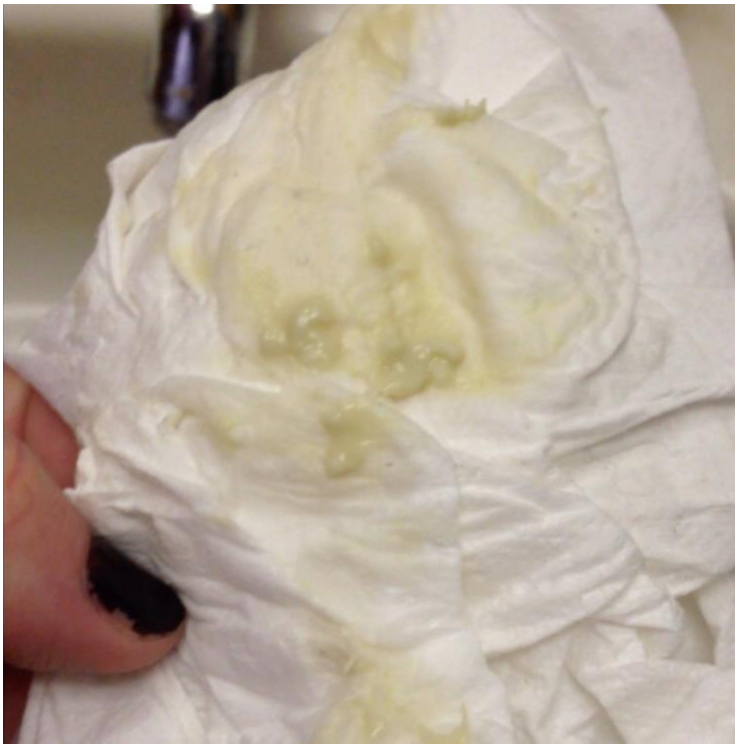

Figure 3: Greenish Vaginal discharge in patient with TV

2.10 Treatment of vaginosis:

The choice of an anti-infective compound depends on the type of infection: According to various guidelines, 5-nitroimidazoles (e.g., metronidazole, tinidazole) are used for the treatment of BV and TV. VVC is treated with orally or locally administered imidazoles or triazoles (e.g., clotrimazole, miconazole, and fluconazole), polyenes or ciclopiroxolamine. Thus depending on the pathogens involved, different antimicrobial agents (antibiotics, antiseptics, antimycotics, etc.) are used as first line therapy, and controlled trials have shown cure rates of 70–80 % after 4 weeks of treatment. For the treatment of BV, anti-infectives with activity against anaerobes are indicated, and routine treatment of sexual partners is usually not recommended. Currently, metronidazole and clindamycin taken orally or applied vaginally are the mainstays of BV therapy. They have different spectra of antimicrobial activity but equivalent efficacy with regard to short-term and long-term cure rates (Meites E *et al*, 2015).

2.11 Treatment of trichomoniasis:

Medications approved by the US Food and Drug Administration (FDA) for treatment of trichomoniasis include metronidazole (since 1963) and tinidazole (since 2004). Standard therapy consists of either metronidazole or tinidazole in a single 2-g dose taken orally, or, if necessary, intravenously. The CDC also recommends an alternative regimen of metronidazole 500 mg orally twice a day for 7 days. Tinidazole has a half-life of approximately 12.5 hours, compared with a half-life of 7.3 hours for metronidazole. Also they exhibit higher serum and genitourinary tract drug levels (Wood BA *et al*, 1975), (Viitanen J *et al*, 1985) and (Mannisto P *et al*, 1984).

. Tinidazole is approximately 10 times more expensive than metronidazole. Both metronidazole and tinidazole are 5-nitroimidazoles, which is currently the only class of antimicrobial medications approved for effective treatment of trichomoniasis and ***T. vaginalis*** infections. Other nitroimidazoles, such as secnidazole and ornidazole, have been used as antiparasitic agents in other countries but have not been approved for use within the United States. Another nitroimidazole called fexinidazole was favorably evaluated for toxicity and is undergoing research as a potential novel antiparasitic agent (Tweats D *et al*, 2012) and (Tarral A *et al*, 2014).

Persistent or recurrent infection due to antimicrobialresistant ***T. vaginalis*** or other causes should be distinguished from the possibility of reinfection from an untreated or insufficiently treated sex partner. The CDC's Division of STD Prevention and Division of Parasitic Diseases and Malaria have accumulated experience with testing and treatment of nitroimidazole-resistant ***T. vaginalis*** and can offer susceptibility testing and management recommendations upon request. Alternative treatment options are limited as no other FDA approved therapies are available. Combination regimens have not been systematically evaluated. The most anecdotal experience has been with intravaginal paromomycin in combination with high-dose tinidazole. Some studies demonstrated treatment success with agents including intravaginal paromomycin, intravaginal boric acid, nitazoxanide, and intravaginal metronidazole/miconazole (Bosserman EA *et al*, 2011), (Sobel JD *et al*, 2001), (Nyirjesy P *et al*, 2011),Tayal SC *et al*, 1998), (Muzny C *et al*, 2012), (Aggarwal A *et al*, 2008), (Dan M *et al*, 2007) and (Schwebke JR *et al*, 2013).

Toxicities with any of the topical regimens are not high, despite painful vulvar ulcers can occur uncommonly as a self-limited side effect of paromomycin. Other attempted treatments that have been reported with a < 50% success rate include intravaginal betadine (povidone-iodine), clotrimazole, acetic acid, furazolidone, gentian violet, nonoxynol-9, and potassium permanganate. To date, no topical microbicide has shown an effect on trichomoniasis (Obiero J *et al*, 2012).

Chapter III

3. Methods:

Study design:

Descriptive cross sectional.

Patients and settings:

Pregnant women during antenatal care in selected clinics.

Patient selection criteria:

All pregnant women attending antenatal care with symptoms of vaginal discharge or itching.

Sample:

Purposive sample.

Techniques and materials:

The patients were interviewed and clinically examined by certified physician. Then a swab was taken through speculum examination.

Microscopic examination of the swabs was undertaken with light microscope??? And OSOM™ trichomonas rapid test was also applied for vaginal washes.

OSOM™ trichomonas rapid test (by SEKISUI®) is a qualitative test for detection of Trichomonas vaginalis based on immunochromatographic, capillary flow, dipstick technology.

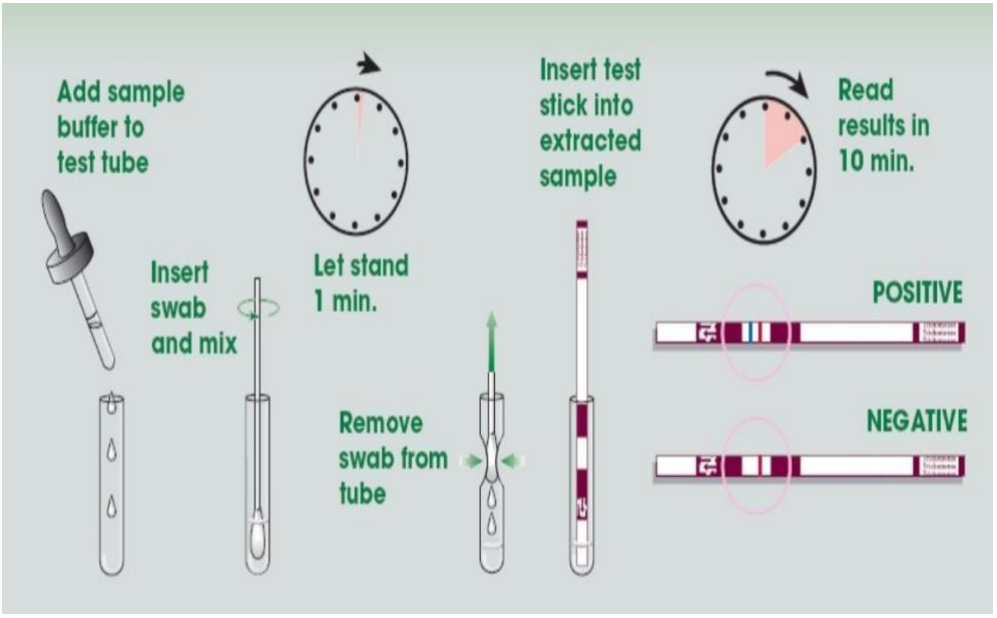

Figure 4: Procedure of OSOM test

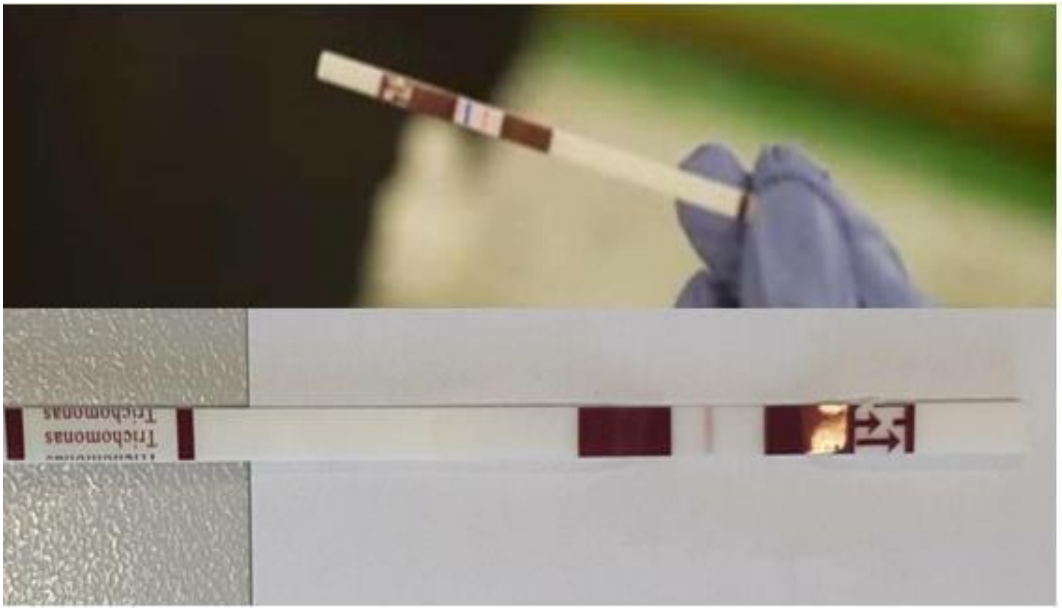

Figure 5: Positive OSOM Test (Above) AND Negative OSOM test (Below)

Data synthesis:

Data were registered on data collection form and transferred to Excel Microsoft® electronic register.

Statistical methods:

Statistical Package for Social Science (SPSS IBM v. 23) was used to analyze data. Descriptive statistics as frequency and percentage.

Inferential statistics were used when needed Chi-square (X^2) to find the difference in the distribution of the variables between the two groups, P-value were considered significant when ≤ 0.05.

Data were presented in form of tables and figures, were the figures done by Microsoft Excel 2010.

Chapter IV

4.Results

4.1. Health Characteristics of the Study Population:

Figure 6 shows the rates of different comorbid conditions among the population of the study. Most frequent conditions were PCOS and irregular cycles.

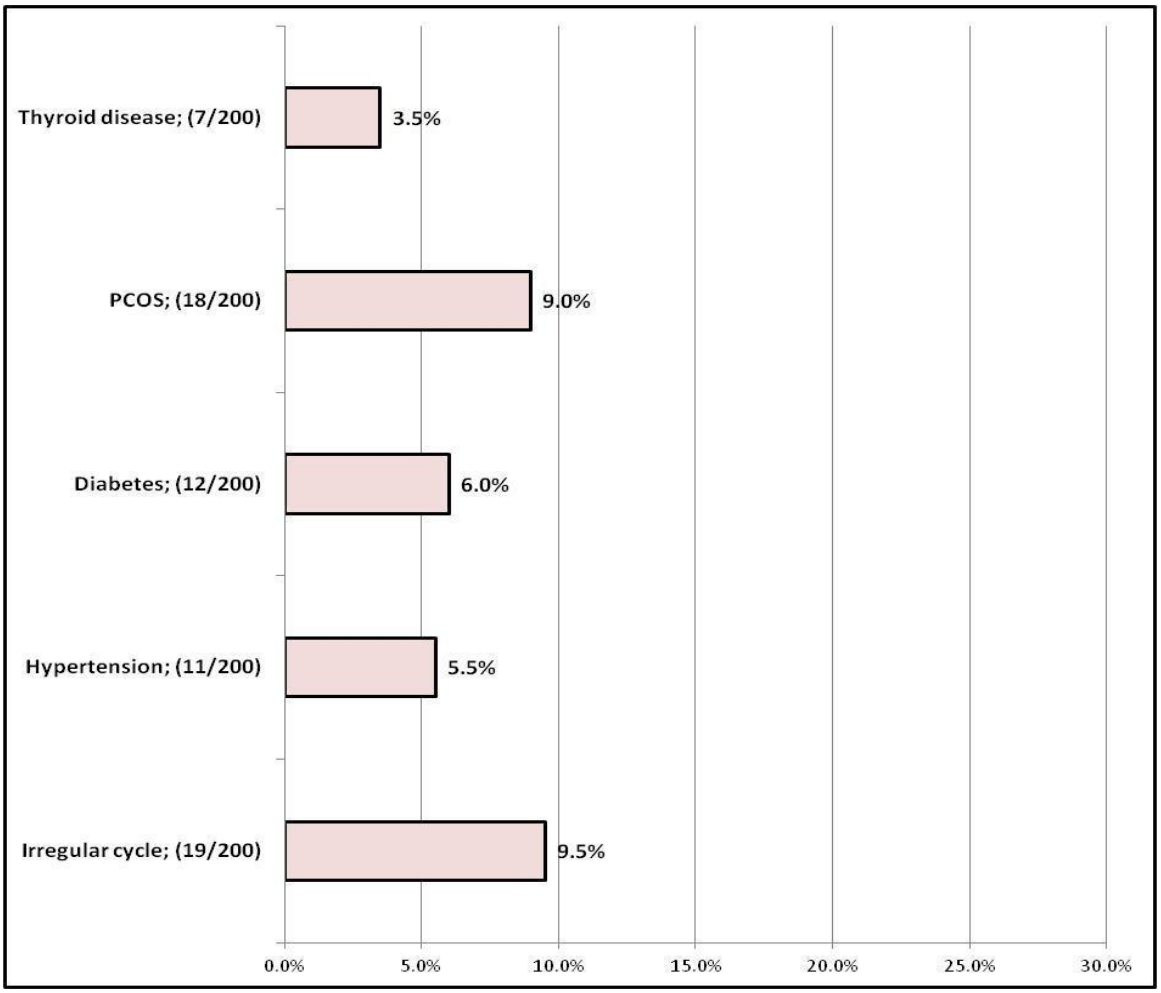

Figure 6: Rates of co-morbid conditions among study population

4.2. Symptoms distributions among the Study Population:

Figure 7 shows the distribution of different types of vaginal discharge according to the history. Sticky and whitish discharge were the most frequent.

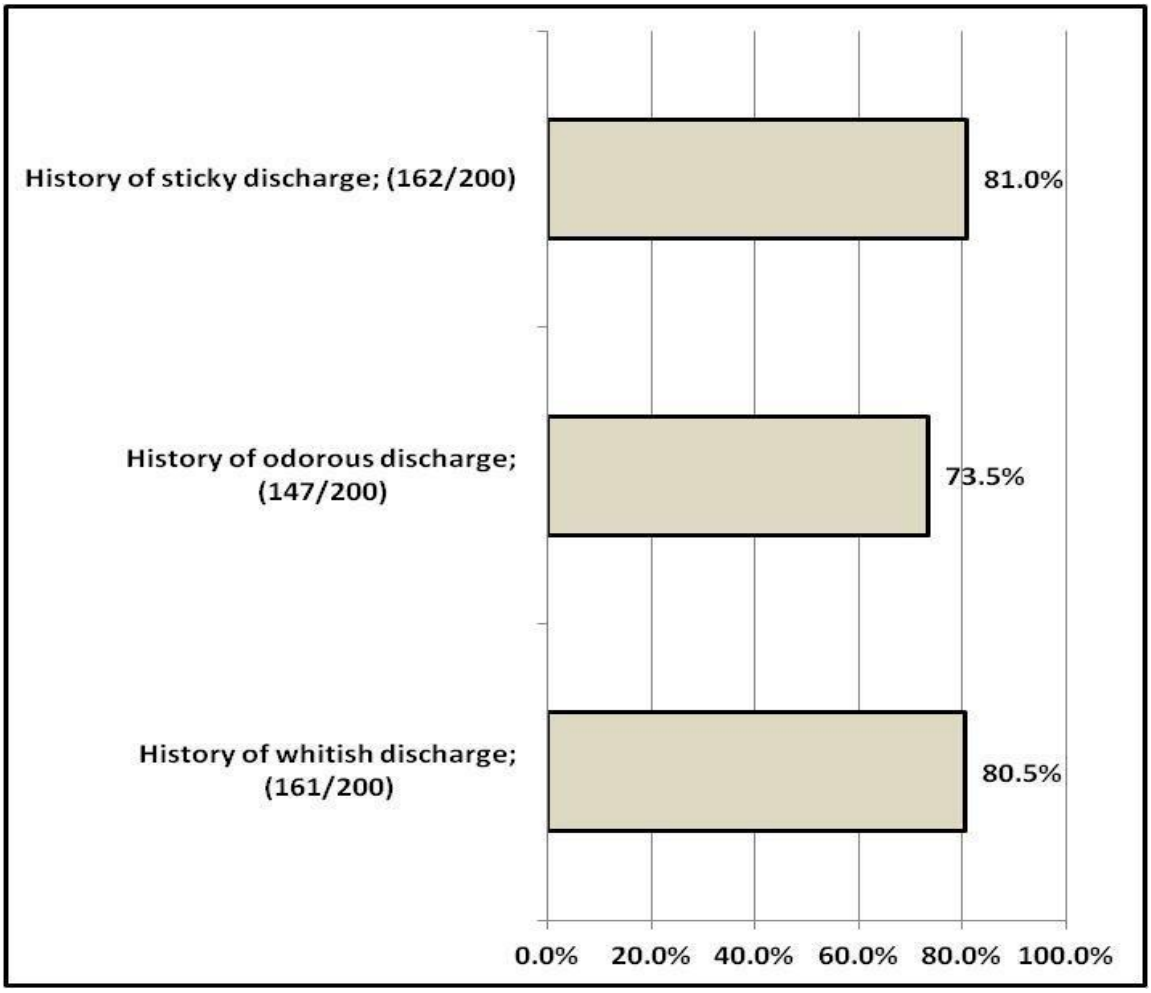

Figure 7: Rates of different vaginal discharge characteristics among study population, history wise

Figure 8 shows the rate of lower abdominal pain. Out of 200 cases 168 (84.0%) with lower abdominal pain , 32(16.0%) were without lower abdominal pain.

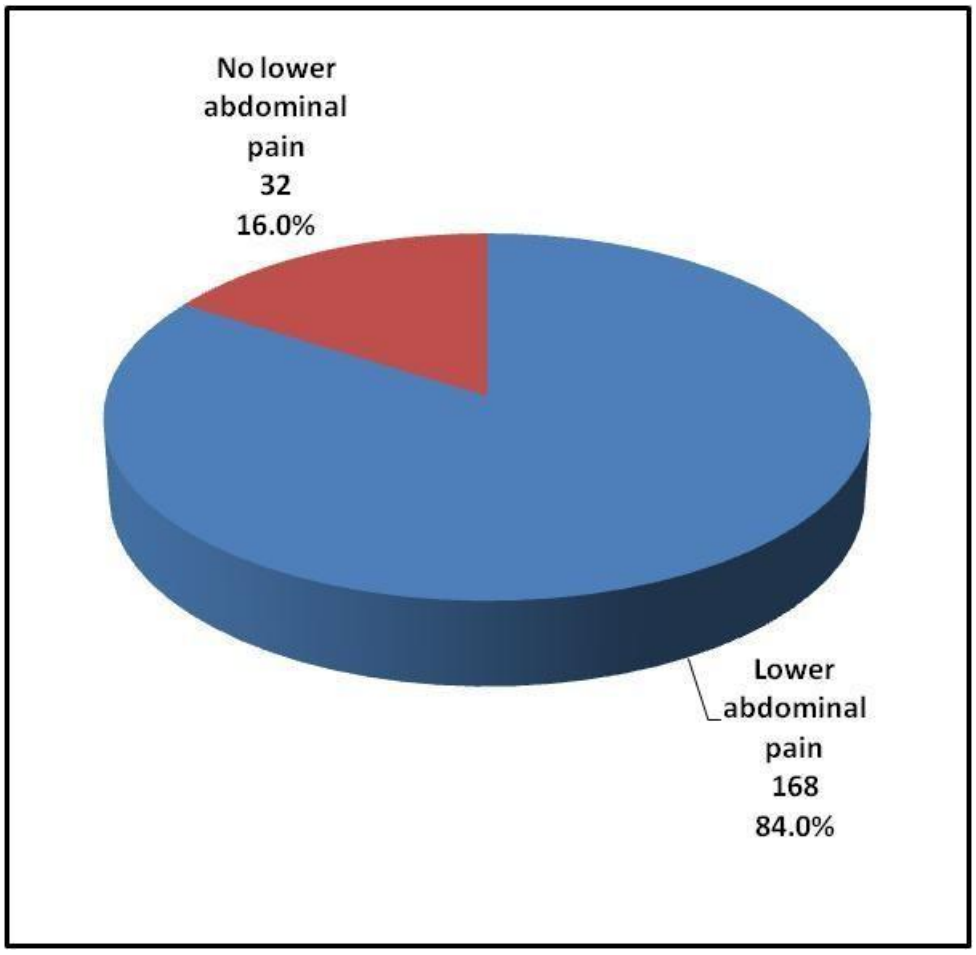

Figure 8: Distribution of study population according to symptom of lower abdominal pain

Figure 9 indicates that only 4.5% of the study population complains of per vaginal (PV) bleeding (9/200) and 191/200 (95.5%) without P.V bleeding.

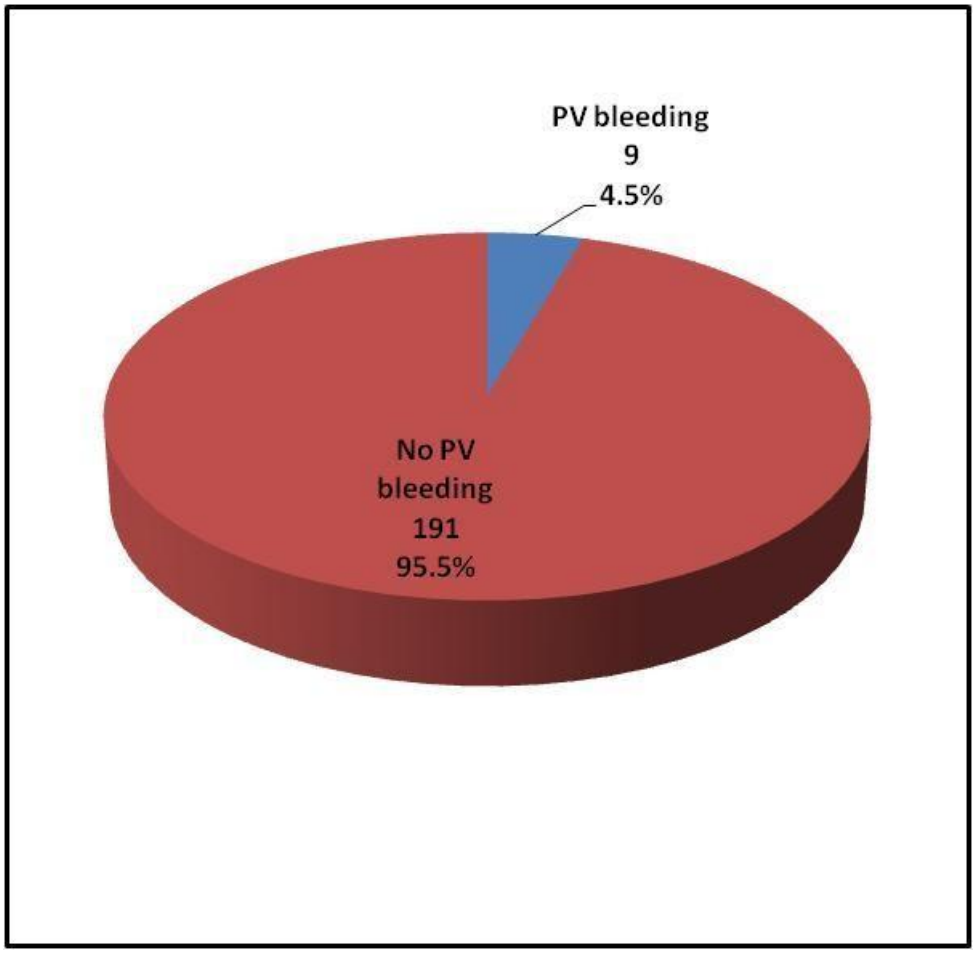

Figure 9: Distribution of study population according to symptom of per vaginal bleeding

Figure 10 shows that 187/200 cases (93.5%) had itching, 13/200 (6.5%) gave history\y of itching

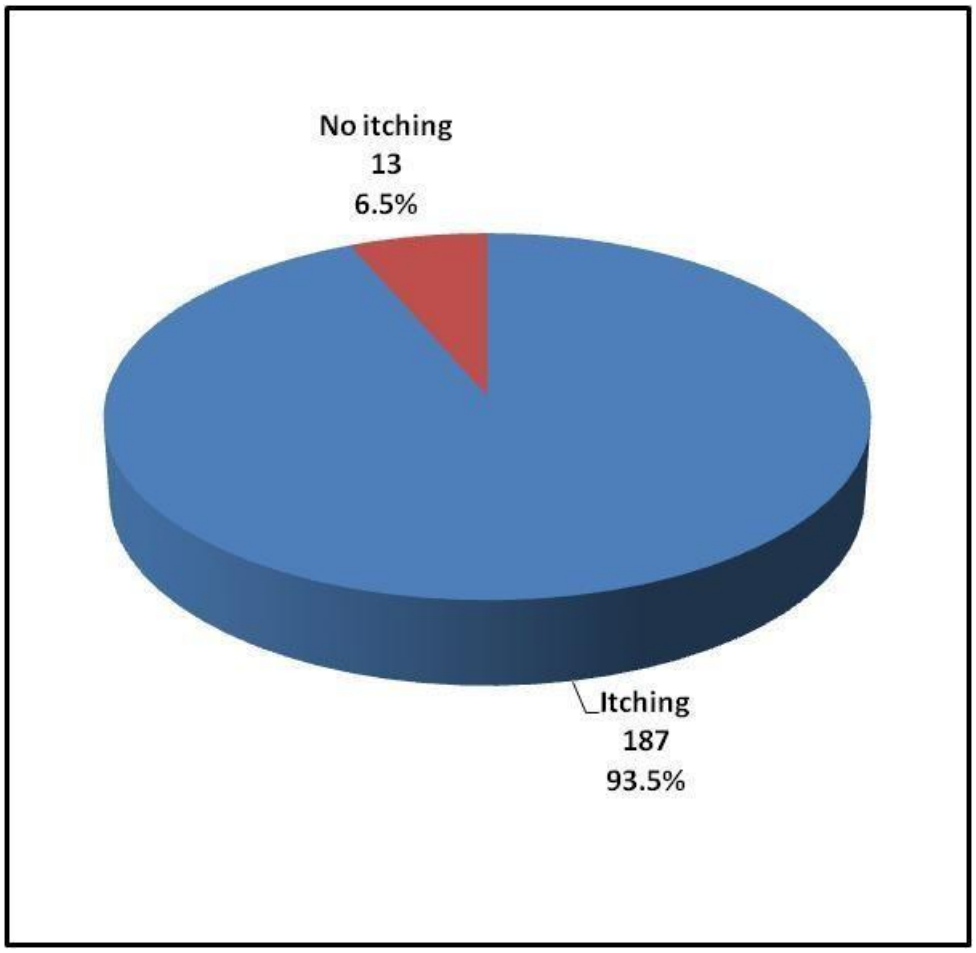

Figure 10: Distribution of study population according to symptom of vulvar itching

187/200 cases (93.5%) had itching, 13/200 (6.5%) gave history\y of itching

Figure 11 shows that Out of 200 87 (43.5%) had history of UTI, 113 (65.5%) had not UTI.

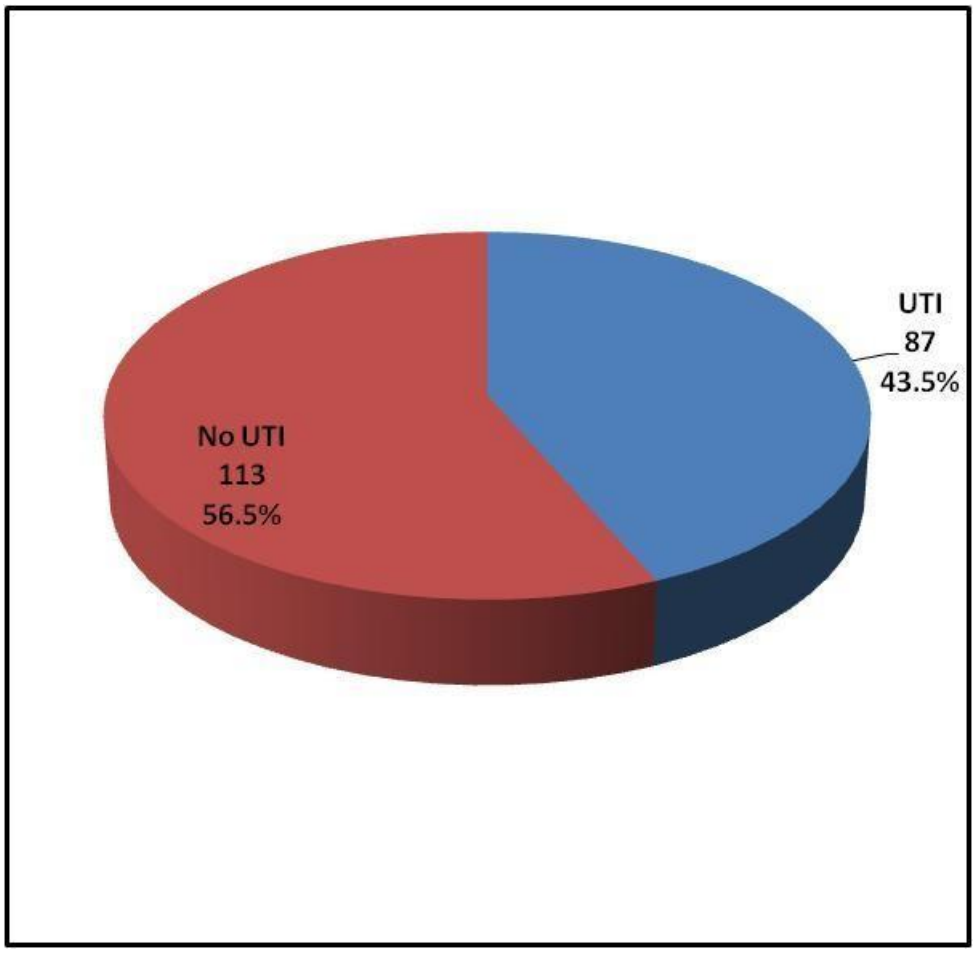

Figure 11: Distribution of study population according to history of urinary tract infection

4.3. Speculum findings distributions among the Study Population:

Figure 12 shows that 105/200 (52.5%) were with whitish vaginal discharge , 95/200 (47.5%) without whitish vaginal discharge

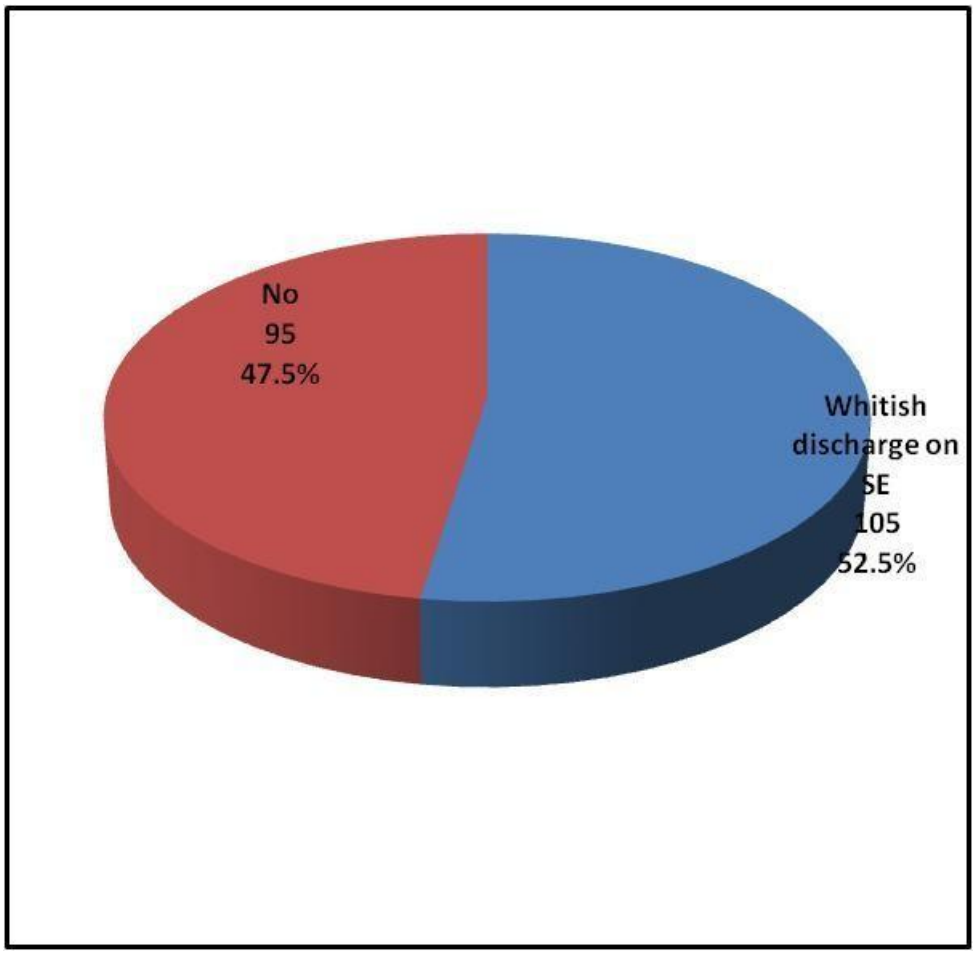

Figure 12: Distribution of study population according to whitish vaginal discharge in speculum examination

Figure 13 shows that out of 200 173 (86.5%) cases gave history of odorous vaginal discharge in speculum examination, 27 cases (13.5%) without.

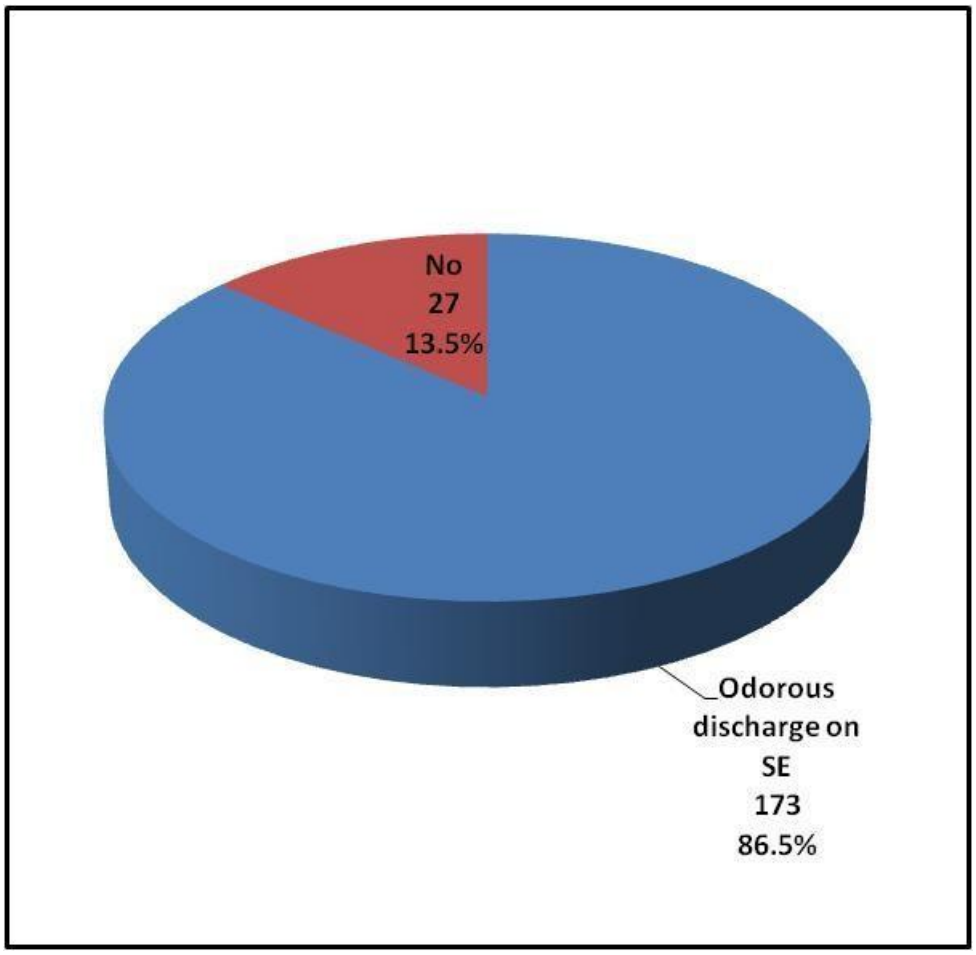

Figure 13: Distribution of study population according to odorous vaginal discharge in speculum examination

As in figure 14, frothy discharge and OSOM test result out of 200 cases 11(5.5%) case with frothy secretion thick discharge 72 cases (36.0%) and 117 cases (85.5%) with liquid discharge

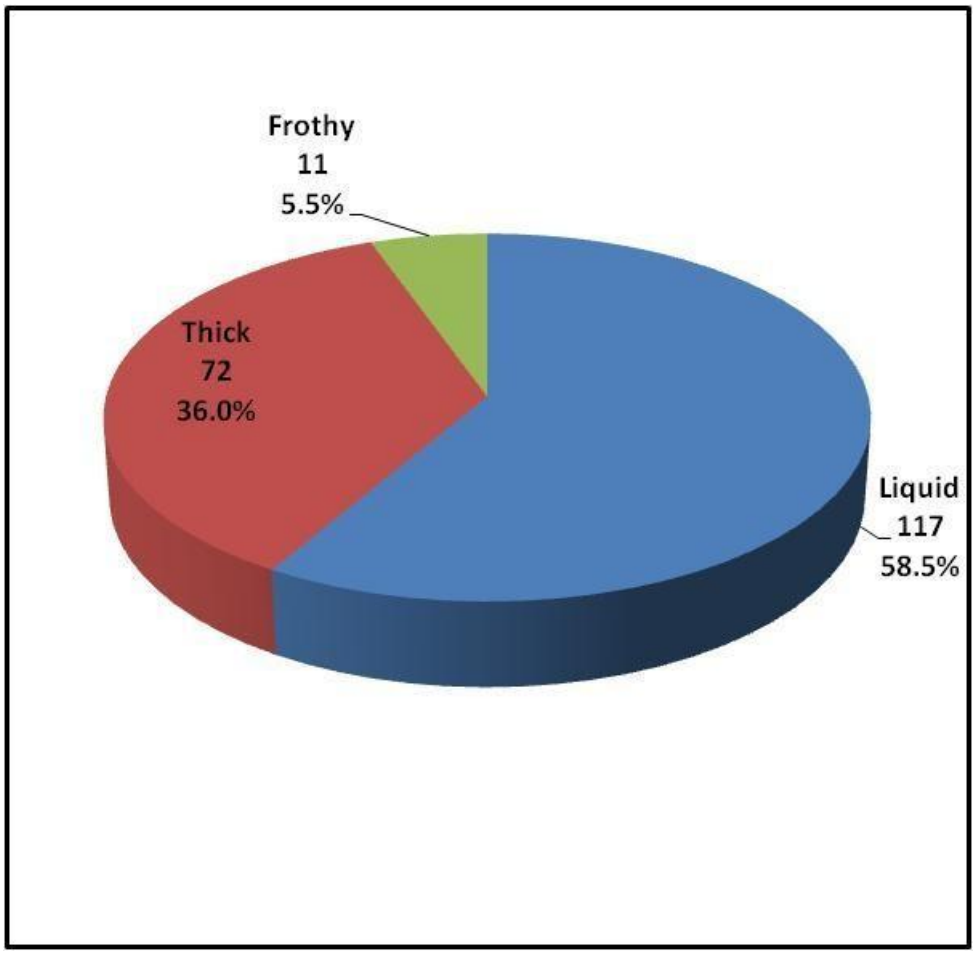

Figure 14: Distribution of study population according to frothy vaginal discharge in speculum examination

4.4. Analysis of Trichomonas findings the Study Population:

In this study out of 200 cases only 8cases (4.0%) were positive for OSOM test One case is invalid (0.5%), 191 (95.5%) were negative. See figure 15

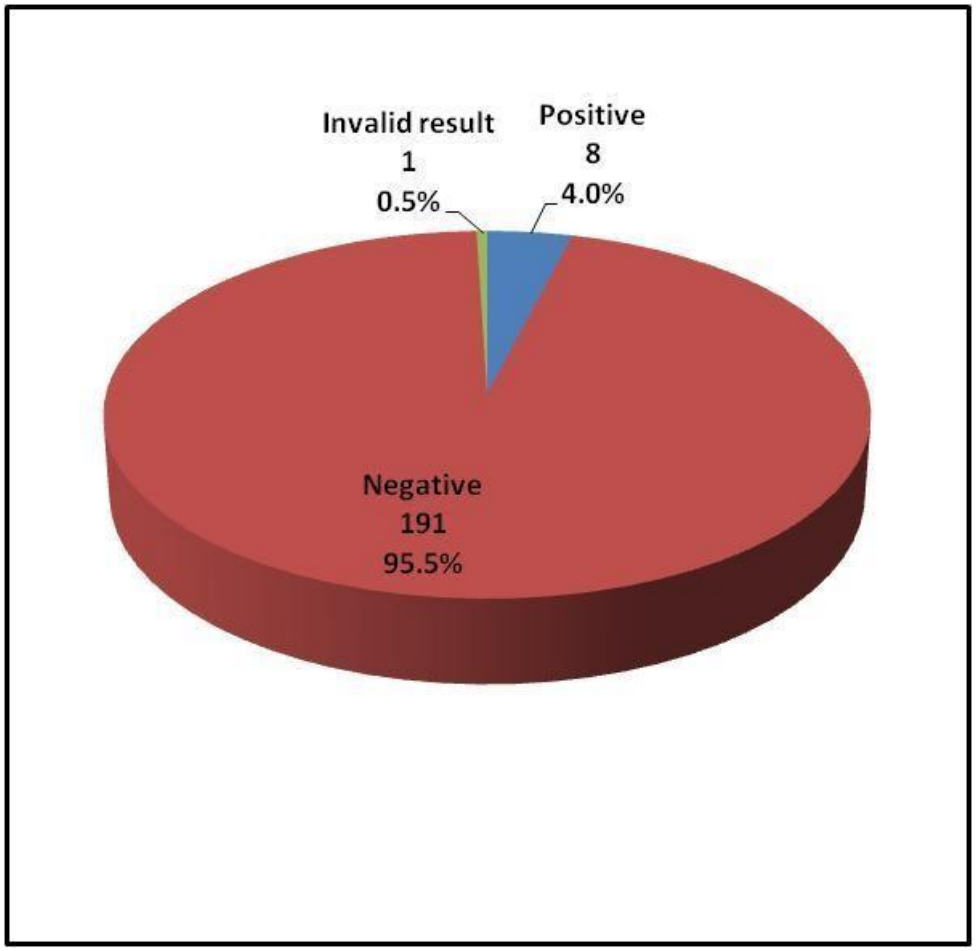

Figure 15: Distribution of study population according to OSOM™ test result

Out of 200 cases, 120 cases (60.0%) were Candida, 25 cases (12, 5%) were Bacteria, 47 cases (23, 5%) were mixed organisms and Trichomonas was in 8 cases (4.0%). See figure 16

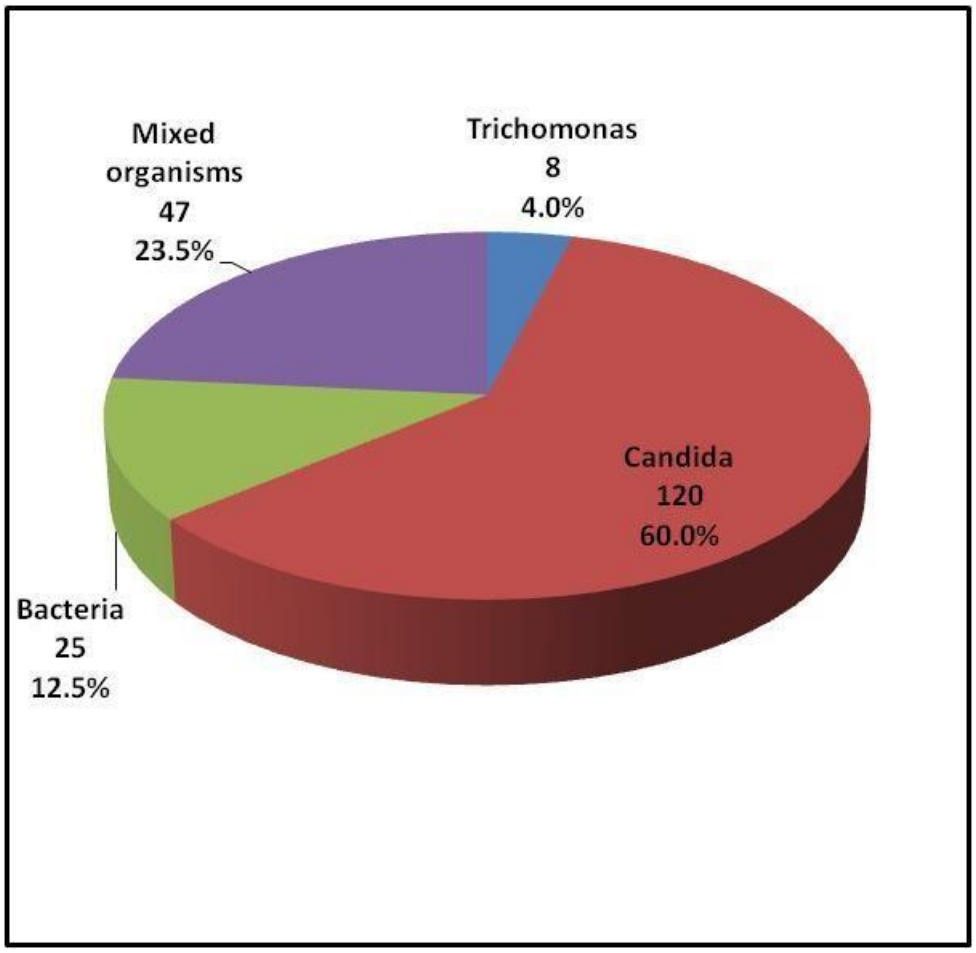

Figure 16: Distribution of study population according to result of microscopic examination of vaginal discharge

4.5. Analysis of OSOM test results among the Study Population:

Out of 200 cases 172 were from Benghazi 7/172 (4.1%) were positive, 165/172 (95.5) were negative. 28/200 (14.0%) cases were from areas out of Benghazi city. 1/28 (3.6%) were positive, 27/28 (96.4%) were negative. The difference was not statistically significant. See table 1

Table 1: Residency and OSOM test result

Residency	OSOM test result		Total
	Positive	Negative	
Benghazi city	7	165	172
	4.1%	95.9%	100.0%
Out of Benghazi city	1	27	28
	3.6%	96.4%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, $P = 1.000$

Out of 200, 19 cases with irregular cycle, one case (5,3%) was positive OSOM; 18 cases (94.7%) were negative, 181 cases with normal cycle, 7 cases (3.9) were positive OSOM, 174 cases (96,1%) were negative OSOM. The difference was not statistically significant. See table 2

Table 2: Irregular cycle and OSOM test result

Irregular cycle	OSOM test result		Total
	Positive	Negative	
Yes	1	18	19
	5.3%	94.7%	100.0%
No	7	174	181
	3.9%	96.1%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, *P* = 0.557

Out of 200, 11 cases (100%) were hypertensive 189 cases with normal blood pressure. 8 cases (4.2%) were positive OSOM, 181 cases (95.8%) were negative. The difference was not statistically significant. See table 3

Table 3: Hypertension and OSOM test result

Hypertension	OSOM test result		Total
	Positive	Negative	
Yes	0	11	11
	0.0%	100.0%	100.0%
No	8	181	189
	4.2%	95.8%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, *P* = 1.000

Out of 200, 12 cases (100%) were diabetic. 188 cases with normal blood sugar, 8 cases (4.3%) were positive OSOM. 180 cases (95.7%) were negative OSOM. The difference was not statistically significant. See table 4

Table 4: Diabetes and OSOM test result

Diabetes	OSOM test result		Total
	Positive	Negative	
Yes	0	12	12
	0.0%	100.0%	100.0%
No	8	180	188
	4.3%	95.7%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, $P = 1.000$

Out of 200 18 cases were PCOS. One case (5.6%) was positive OSOM, 17 cases (94,4%) were negative, 182 cases without PCOS, 7 cases (3.8%) were positive OSOM and175cases (96.2%) were negative OSOM. The difference was not statistically significant. See table 5

Table 5: PCOS and OSOM test result

PCOS	OSOM test result		Total
	Positive	Negative	
Yes	1	17	18
	5.6%	94.4%	100.0%
No	7	175	182
	3.8%	96.2%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, *P* = 0.536

Out of 200, 7 cases were thyroid disease. One of case (14.3%) was positive OSOM, 6 cases (85.7%) were negative, 193 cases were Euthyroid, 7 cases (3,6%) were positive and 186 cases (94.4%) were negative. The difference was not statistically significant. See table 6

Table 6: Thyroid and OSOM test result

Thyroid	OSOM test result		Total
	Positive	Negative	
Yes	1	6	7
	14.3%	85.7%	100.0%
No	7	186	193
	3.6%	96.4%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, *P* = 0.252

No positive test result with patients with no symptoms of whitish vaginal discharge. This result was not statistically significant. See table 7

Table 7: Symptom of Whitish discharge and OSOM test result

Whitish discharge	OSOM test result		Total
	Positive	Negative	
Yes	8	153	161
	5.0%	95.0%	100.0%
No	0	39	39
	0.0%	100.0%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, *P* = 0.359

More proportion of positive test result with patients with odorous discharge. The result was not statistically significant. See table 8

Table 8: Symptom of Odorous discharge and OSOM test result

Odorous discharge	OSOM test result		Total
	Positive	Negative	
Yes	7	140	147
	4.8%	95.2%	100.0%
No	1	51	52
	1.9%	98.1%	100.0%
Total	8	191	199
	4.0%	96.0%	100.0%

Fisher's Exact Test, *P* = 0.683

Frothy discharge and OSOM test result out of 200 cases 11 case with frothy secretion, 8/11 (72.7%) were positive, 3/11 (27.3%) were negative. other discharge (liquid & thick) were in 189. 100% were negative by OSOM test. The result was statistically significant. See table 9

Table 9: Symptom of Frothy discharge and OSOM test result

Frothy discharge	OSOM test result		Total
	Positive	Negative	
Frothy discharge	8	3	11
	72.7%	27.3%	100.0%
Other types of discharge	0	189	189
	0.0%	100.0%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test, $P = 0.001$ (Significant)

Positive test results were found only with patients without whitish discharge on speculum examination. The result was statistically significant. See table 10

Table 10: Whitish discharge on speculum examination and OSOM test result

SE Whitish discharge	OSOM test result		Total
	Positive	Negative	
Yes	0	105	105
	0.0%	100.0%	100.0%
No	8	87	95
	8.4%	91.6%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test $P = 0.002$ (Significant)

Positive test results were found only with those with odorous discharge was found on speculum examination. The result was not statistically significant. See table 11

Table 11: Odorous discharge on speculum examination and OSOM test result

SE Odorous discharge	OSOM test result		Total
	Positive	Negative	
Yes	8	165	173
	4.6%	95.4%	100.0%
No	0	27	27
	0.0%	100.0%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test 0.601 (Non-significant)

Positive test results were found only with those with frothy discharge was found on speculum examination. The result was statistically significant. See table 12

Table 12: Frothy discharge on speculum examination and OSOM test result

Frothy discharge	OSOM test result		Total
	Positive	Negative	
Frothy discharge	8	3	11
	72.7%	27.3%	100.0%
Other types of discharge	0	189	189
	0.0%	100.0%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Fisher's Exact Test $P < 0.001$ (Significant)

Patients with vaginal discharge with liquid or thick consistency on speculum examination show no positive test result. The result was statistically significant. See table 13

Table 13: Consistency of discharge on speculum examination and OSOM test result

Consistency of discharge	OSOM test result		Total
	Positive	Negative	
Liquid	0	117	117
	0.0%	100.0%	100.0%
Thick	0	72	72
	0.0%	100.0%	100.0%
Frothy	8	3	11
	72.7%	27.3%	100.0%
Total	8	192	200
	4.0%	96.0%	100.0%

Pearson Chi-Square 143.182 *P* <0.001

Chapter V

5. 1. Discussion:

The OSOM® Trichomonas Rapid Test (Sekisui, Framingham, MA) is a rapid antigen test which can detect trichomonas in 10 min. Compared with wet preparation and culture, OSOM Trichomonas test has a good sensitivity, excellent specificity and compares favourability to NAAT assays with reported sensitivities of 83%– 90% (Nye *et al.*,2009 & Huppert *et al.*,2007 & Huppert *et al.*,2005) .

In the present study, regarding OSOM test results, the prevalence of Trichomonas infection was 4.0% with another 0.5% inconclusive result. This result is comparable to findings of global estimates by Newman L, *et al* (2015) upon data during 2012 of 5.0% (4.0–6.4%) with regional variations from 1.0% to 11.5%. The eastern Mediterranean Region estimate of the prevalence of trichomoniasis among women was around 6% while among men was 1%.

The present study estimate compared to a systematic review by Joseph Davey DL *et al* (2016) described studies from Kenya, Tanzania, Somalia, Ethiopia, Uganda, and Sudan for prevalence of *Trichomonas vaginalis* among pregnant women. The adjusted mean prevalence was similarly high for TV in 3 studies at 6.8% (95% CI, 4.6– 9.0). The pregnancy condition of participants in those studies made comparison questionable as the pregnancy may affect the physiology of genital tract and increase susceptibility.

More recently, Rowley J *et al* (2019) published a meta-analysis including 76 data points investigating prevalence of TV infection and found that the highest prevalence among WHO regions was reported in African region; for women 11.7% (95% CI: 8.6 – 15.6). While the Eastern Mediterranean Region (EMRO) which include Libya reported the lowest prevalence for women 1.6% (95% CI: 1.1 – 2.3). The finding in the present study is still lower than the global and African estimate but higher than the EMRO one. Chemaitelly H *et al* (2019) demonstrated in a meta-analysis for studies including female sex workers in Middle East (Egypt, Iran and Pakistan) and found a high prevalence in Egypt approaching 19%.

The conservative nature and hygienic practice among the Libyan population may help in the lower prevalence of TV infection. But the prevalence is still high in comparison to some other countries in the Mediterranean region.

Among those 81.0% revealed a history of sticky vaginal discharge, 73.5% complained of an odorous discharge and 80.5% reported a whitish discharge. Also, 84.0

of participating women gave the history of lower abdominal pain and only 9/200 (4.5%) had vaginal bleeding. Urinary tract infection was a manifestation in the history of 43.5% of the study population.

Itching was reported by 93.5% of participating women in the study population.

History of irregular cycle and also Co-morbidities with hypertension, diabetes mellitus, polycystic ovarian syndrome and thyroid disorder has no statistically significant association with positive OSOM test result.

Findings of Whitish discharge ($P = 0.359$) and Odorous discharge ($P = 0.683$) had no significant association with OSOM test result.

Frothy discharge was found only in 11 cases and OSOM test result was poisitve among 8 of them (72.7%). Other types of discharge had no any OSOM test result. The difference indicates a statistically significant association ($P = 0.001$). Sensitivity as well as specificity and negative predictive value of the finding of frothy secretion were 100.0%, while the positive predictive value was 72.7%.

Compared to Hobbs *et al.,* 2013, looking for trichomonads sensitivity by vaginal discharge examination which was found ranged from 44–68% compared to culture; Garber *et al.,2005 &* Ohelmeyer *et al.,1998* using traditional culture method with a sensitivity of 81–94%. Anyhow, the finding of frothy secretion in the present study indicated much higher test performance. This may be due to the less prevalence of other sexually transmitted disease in our community.

The promising use of NAATs for *T. vaginalis* diagnosis proved diagnostic sensitivity and specificity range from 99.5–100% and 99.4–99.9% for female genital specimens and 97.2–99.9% for male urine specimens (Schwebke *et al.,* 2018). This is should be considered within the daily practice once economic value taken in account.

5.2. Conclusion and Recommendations:

Prevalence of Trichomonas Vaginosis in Libyan women estimate is 4.0% which is reasonable comparable to global and regional figures. OSOM test is a quick and helpful tool in screening and diagnosis of Trichomonas Vaginosis. The finding of frothy secretion can be used to predict the infection before performing the test.

The OSOM test should be available for use in maternity centers and women health clinics. A careful history and clinical findings should be obtained from the

clinician. A further well designed study may be worthy to examine other characteristics might be related to the Trichomonas infection across different groups of women.

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