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Detection of Bacterial Vaginosis, Human *Cytomegalovirus* and *Trichomonas vaginalis* in Aborted Women in Maysan Governorate

A Thesis Submitted to

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By

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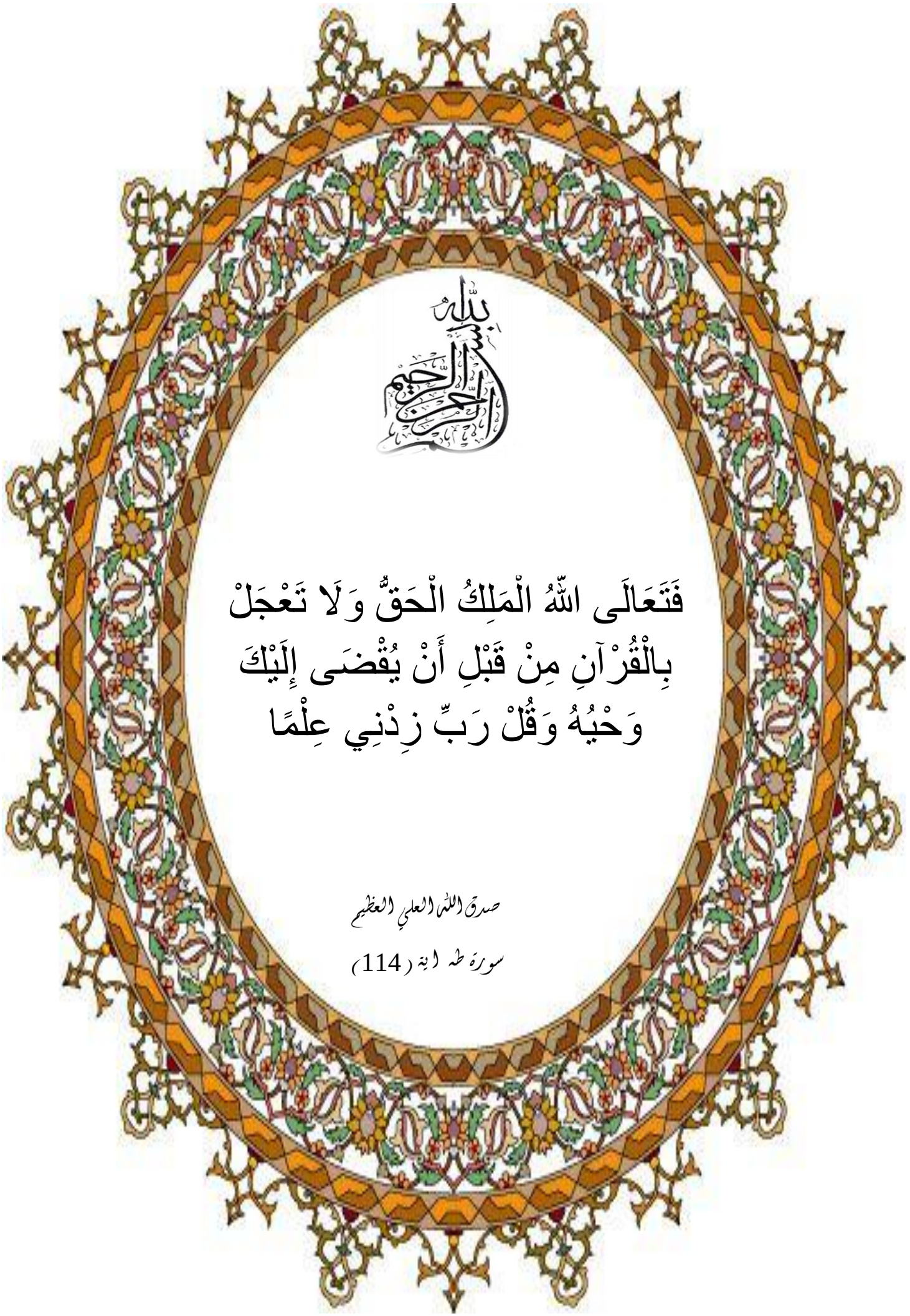

بسم الله الرحمن الرحيم

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ
بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ
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صدق الله العلي العظيم

سورة طه اية (114)

" Supervisor Certification"

This is to certify that this thesis entitled (**Detection of Bacterial Vaginosis,Human *Cytomegalovirus* and *Trichomonas vaginalis* in Aborted Women in Maysan Governorate**)was prepared under me supervision at the Department of Biology, College of science, University of Misan. as partial requirement of the degree of Master in Biology.

Signature

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Date: / / 2021

Recommendation of Head of Biology Department

In View of the available recommendations I forward this thesis for debate by the examining committee.

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First of all, great thanks for Allah the most merciful the most compassionate, who gave me health, strength, and facilitated the ways for me to accomplish this work, and the prayer and peace of Allah be upon our Master and prophet Muhammad , who guided us to get out of darkness of nescience.

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Dedication

The search locomotive passed many obstacles, however, I tried to steadily overcome it, thanks to **Allah** and from Him.

To My parents, My brothers and my friends, they were support me in order to complete the search.

To the lives lost during this course of study

And I should not forget **my teachers** who had the greatest role in my work .

I present to you my graduate thesis.....

Praying to the Almighty to prolong your life and bless you with bounties

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Summary

Abortion is one of the most common problems in pregnant women, which is a spontaneous loss of pregnancy before 12 weeks and is called an early miscarriage, or within 12-24 weeks and is called a late miscarriage. It occurs in one in five cases of women.

About (339) samples were collected from hospitals in Maysan Governorate / Iraq (226 cervical swabs) and (113 blood samples) from aborted women in the obstetrics and gynecology department, according to the sample size (which was cervical swabs and serum) from the November 2020 to May 2021, in this study set of paramets that are closely related, including age, education, place of residence, and common diseases with abortion were studied.

Statistical analysis was carried out using the spss program version 23 and the chi-square test was used to determine the relationship between the incidence rate and some factors and the P value <0.05 was also considered statistically significant.

The relationship between age groups 14-45 years with abortion was determined, where the highest percentage of the age group was recorded (24-33), with a frequency of 48 case (42.5%)while, the lowest percentage was for the older age groups in this study, which was seven percentage. Primary education record the highest percentage reached (59%) and the lowest percentage in higher education (12%).

Swabs were obtained from the cervix to diagnose bacterial vaginosis from aborted women, using microscopic examination methods Gram staining, and by biochemical test vitek-2 compact, the highest percentage of pathogenic bacteria was *Klebsiella* spp. 23.6% and the lowest percentage were the species *Acinetobacter* spp. *Staphylococcus sciuri*, *Enterobacter cloaca* and *Proteus* spp with a percentage of 0.9%.the bacterial infection with *Staphylococcus aureus* was documented at a rate of 9% as well as, the anaerobic bacterial infection with *Neisseria spp*. with a percentage of (3.6%).

Regarding the immunoglobulin for *Cytomegalovirus*, the highest percentage of IgG was recorded by (86%),which indicates chronic infection (previous) and the virus may be able to reactivate while, the

lowest percentage of immunoglobulin IgM was recorded by (3.7%), It refers to a cute infection .

The virus was diagnosed by using the rapid test as an initial test, then using the minividaus device as a confirmatory test, where blood samples were separated using a centrifuge.

With regard to *Trichomonas vaginalis* which, is the most common sexually transmitted disease worldwide 49 women were diagnosed with this parasite from 113 aborted women by direct microscopy and watching the parasite's movement under a light microscope (40x magnification power by preparing several slides for each sample, Gram Giemsa stains were used, as were amies transport, and InPouch medium incubated at a temperature of 37 °C and examined daily for 5 days to monitor parasite growth and to confirm the results of the microscopic examination.

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List Of Abbreviations

Abbreviations	Key
BHI	Brain Heart Infusion
HPV	Human Papilloma Virus
HVS	High Vaginal Swabs
IUD	Intrauterine Device
KOH	Potassium Hydroxide
NPIs	Negleced Parasitic Infections
NEN	Necrotizing Enterocolitis
P.bivia	Prevotella bivia
PSI	Pounds Per Sequare Inch
STI	Sexually Transmitted Infection
TMA	Tri Methyle Amine
UTI	Urinary Tract Infection

Chapter One

Introduction

1.1 Introduction:

The abortion is a big problems to a woman may face during early pregnancy, it is causes morbidity and mortality significant social and psychological on women based on estimates about 6-15% of clinically identified pregnancies end in spontaneous abortion (Rouse *et al*., 2017) there are two stages of mischarges early miscarriage as pregnancy lack in less than 12 weeks or in first trimester late miscarriage occurs in the second trimester 12-24 weeks from pregnancy and it`s unusual, about (1–2)% of pregnancies(Gikoumelou *et al*., 2016) bacterial vaginosis is the most publicity which was causes the vaginal secretions and characterized by a malodorous vaginal discharge, at the side of womankind childbearing age (Hay .,2017) all patients have deeply suffered from fishy smelling discharge, frequently during period of menstrual cycle (Kafi, 2012) bacterial vaginosis may be transient and asymptomatic in nature (Hay, 2019) it is the most spread cause of unnatural vaginal secretions in women of birth age, others may have bacterial vaginosis in passing and without symptoms it usually responds to antibiotic treatment but can relapse quickly, and recorded average of relapse are furthermore than 50% within three to six months, there is need for replacement to treatments to find a way to stop relapse, some modern studies appoint that it`s transport by sexual contact, with more cause diseases strains of *Escherichia coli*, *Streptococcus* spp., *Gardnerella vaginalis* and *Atopobium vaginae Leptotrichia aminionii*, *Mobiluncus* spp., *Prevotella* spp., *Mycoplasma hominis*, *Bacteroides* spp. Being determined (Swidsinski and Mending, 2005) *Trichomonas vaginalis* a flagellate parasite, is the causal factor of the almost universal no viral the infection is transmitted sexually, the most widespread sexual transport infection (STI) in the creation is *Trichomonas vaginalis*(Rowley *et al*., 2019).

The uses of glucose as its primary carbon source in both anaerobic and aerobic conditions, as a result, of power production via breaking down sugars critical for the survival from trichomonal chronic infection, the glycogen generated of vagina epithelia cell by glucosidase and amylase is the main origin from glucose in vagina liquid, *Trichomonas* can cause major health problems such as low giving birth weighting babies and early rupture of the placental membrane, as well as, pelvic inflammatory

illness, cervical cancer, and infertility *Trichomonas* has also been linked to raise the risk of HIV transfer in prior research (Goldstein *et al*., 1993; Cherpes *et al*., 2006; Bala and Chonker, 2018).

Viruses can infect and kill foetus by the transplacental or ascending routes the most commonly virus as which was cause by *Cytomegalovirus*, who can induce old or frequent maternal hit of cervical mucosa. *Cytomegalovirus* be infect placenta through cervix or via viremia after both first and repeated maternal contagion, causing blood vessels failure, texture destruction, and transport at the foetus(Yamamoto *et al*., 2004). Infections that occur during pregnancy which effect on the fetus for example, congenital abnormalities caused by *Cytomegalovirus*, rubella virus, and herpes simplex virus congenital *Cytomegalovirus* infection affects perception, kinetics role, hearing, language evolution, vestibular function, sight and leading cause of the central nervous system and sensual abnormalities (Zalei *et al*., 2017).

1.2 The aim of the study

-Detecting of bacterial vaginosis, *Cytomegalovirus* and *Trichomonas vaginalis* in aborted women.

Chapter two

Literature review

2.1 Miscarriage:

Miscarriage defined as a common complication in the life cycle of pregnancy, it knows as spontaneous abortion is define as an expulsion of embryo or extraction of fetus weight (500) gm or less weight in age 20-22 week (Brody *et al*.,1997).

Based on the american college of obstetricians and gynecologists, miscarriage is a common cases in women because of, women's health concerns and common type of pregnancy loss regarding to some studies about 10-20% of all clinically document pregnancies have data in miscarriage, diagnosis the main causes and providing a management which was help to decrease a miscarriage and reflect on healthy fetus (Dugas and Slane, 2020)miscarriage is one of the most common difficulties of woman during the first trimester pregnancy, is not only linked to morbidity and death, but also has a significant social and psychological impact on women (Rouse *et al*., 2017).

Some factors like the psychological state of the mothers, stress, having a non-steroid antiphlogistic drug, tobacco, and spirit these factors which linked together upper percentages averages of abortion(Coste *et al*., 1991) first miscarriage in pregnant women lead to a high riskiness of the latter gestation pregnancy termination compare with pregnant with live childbirth (Kashanian *et al*., 2006).

Infection which associated with miscarriage (Benedetoo *et al*., 2004) about, 15% of early aborted and 66% of late miscarriages have related with infections (Srinivas *et al*., 2008) repeated spontaneous abortions mostly frequent pregnancy implications and is regarded like one of the world's almost serious reproduction trouble, abortion known as the

ending of a pregnant for a variety of reasons, repeated abortions also, known as repetive gestation loss, know such as loss of three or more sequence pregnancies previous to the 20th weekly deliver has an impact one percent from couple (Laxmi *et al*., 2012).

2.2 Bacterial vaginosis:

In female of productive age bacterial vaginosis it`s often prevalent lower general channel diseases (Kenyon *et al*., 2013)vaginal discomfort, itching, irregular vaginal discharge, and burning sensation as common symptoms of bacterial vaginosis (Onderdonk *et al*., 2016).

Asymptomatic in up to (50%) other symptoms such as fishy odor and gray discharge(Klebanoff and Turner, 2014)bacterial vaginosis is a vaginal disease in which a variable mixture of aerobic and facultative bacteria replaces the dominant stable *Lactobacilli* (Nasioudis *et al*., 2017).

The exact composition of a woman's bacterial normal flora is not the same count from one woman(Malaguti *et al*., 2015) *Gardnerella*, *Atobobium*, *Prevotella*, *Megasphaera*, *Snethia*, and *Mycoplasma* are typical bacterial vaginosis-associated pathogens (Machado *et al*., 2015).

Bacterial vaginosis a popular vagina infectious in ladies from childbearing years, It`s an imbalance of the vaginal flora, where the abundance of H_2O_2 usually produces *Lactobacilli* is rare and other bacteria such as *Escherichia coli* are abundant (Hemalatha *et al*., 2013).

It has been demonstrated as risk factor for negative pregnancy outcomes such as premature labor recurrent miscarriage post-abortion sepsis, early miscarriage and stillbirth (Africa *et al*., 2014).

Bacterial vaginosis was linked together with an increased extreme of sexually transition diseases(Wiesenfeld *et al*., 2003; Bortman and Zenilman, 2010; Wijgert, 2017).

Decreased the number of *Lactobacilli* and an overgrowth of large numbers of *Gardnerella vaginalis* it is caused bacterial vaginosis (Balashov *et al*., 2010;Shipitsyna *et al*., 2013).

Many female don`t have a symptomatic of bacterial vaginosis to theirs provider, until a clinical sings or signs of infection are present (Masson L *et al*., 2007) bacterial vaginitis has been linked to abortion and premature labor, according to microorganisms detected in the amniotic fluid and the chorion derived of the cervical vaginal mucosal(Fukuta *et al*., 2020).

Bacterial vaginosis is linked to a threefold increase in the chance of spontaneity aborting in the first trimester of pregnancy(Giakoumelou *et al*., 2016) however, recurrent spontaneous abortion increased fourfold in the late trimester(Fukata *et al*., 2020).

2.3 Diagnosis of Bacterial Vaginosis:

To diagnose bacterial vaginosis: two gold standard criteria been using: one diagnosis procedure is Amsel's clinically criterion, and second Nugent Gram smearing examination in the laboratory.

2.3.1 Diagnosis by Amsel’s Criteria

Clinical criteria includes the presence of a homogeneous vaginal discharge, pH of the vaginal being >4.5 the presence of clue cells in Gram stained vaginal discharge smears and a positive whiff test according to Amsel, if three of the four criteria are positive the patient has bacterial vaginosis (Amsel *et al*., 1983).

A) modular secretion with bacterial vaginosis soft atypical secretion is floccular in nature.

b) The pH indicator paper is used to determine the pH, the sensitivity of pH measurement for bacterial vaginosis diagnosis is excellent, but the specificity is low the investigation by hallén *et al*., discovered of 98.8% sensitivity and a of specificity 71% (Hallen *et al*.,1987) and only 81 % of the women with bacterial vaginosis in Amsel's study having pH >4.5 (Amsel *et al*.,1983).

When the pH of the discharge is low amines for example trimethylamine (TMA) are rapidly released and melted as an acid in the discharge to detect odor a trimethylamine the whiff test has a high sensitivity and specificity, according to published studies Hallén *et al*, found that their method has a of sensitivity 95 percent specificity and a of 100% (Hallen *et al*.,1987).

Whiff test is perform by addition 1 droplet from (10%-20%) of KOH to the dump on the endoscopy, or by dumping drop from discharge upon microscope slide and additional one drip of the (10% -20% KOH mixture and swirling the microscope slide. the odor of moldy fish is caused by TMA, which allow found with vagina specimen from bacterial vaginosis patients(Gardner and Dukes,1955)

The adhesion of adherent strains is thought to be the cause of the Clue cell phenomenon(Scott *et al*., 1987) it`s vaginal epithelia cell to those boundaries are difficulty to identify due to the abundance of germs on their surfaces, introduced the clue cell as one of their clinical criteria to diagnosis bacterial vaginosis (Gardner and Dukes,1955).

In Vitro adhesions and biotype of *Gardnerella vagina*lis species, in relation to the occurring of clue cell in vaginal discharge, *Lactobacilli* produce hydrogen peroxide which helps to maintenance the acidic pH of healthy vagina, and inhibits the development of anaerobic microbes(Eschenbach *et al*., 1988).

Some factors that cause the normal flora to change are not well understood, menstruation, sexual active, contraception measures, and antibiotic using are among the postulates (Saidi *et al*., 1994).

Most beneficial singular methods for diagnosing bacterial vaginosis the detection of clue cells, bacterial vaginosis is responsible for 10- 30 of all instances of infections vaginitis (Sobel *et al*., 2011).

On wet mount, more than 20% of clue cells had a high sensitivity and specificity for bacterial vaginosis diagnosis(Chandeying *et al*.,1998) the presence of clue cell in vaginal discharge was also used to predictive infection following surgery (Larsson *et al*.,1991).

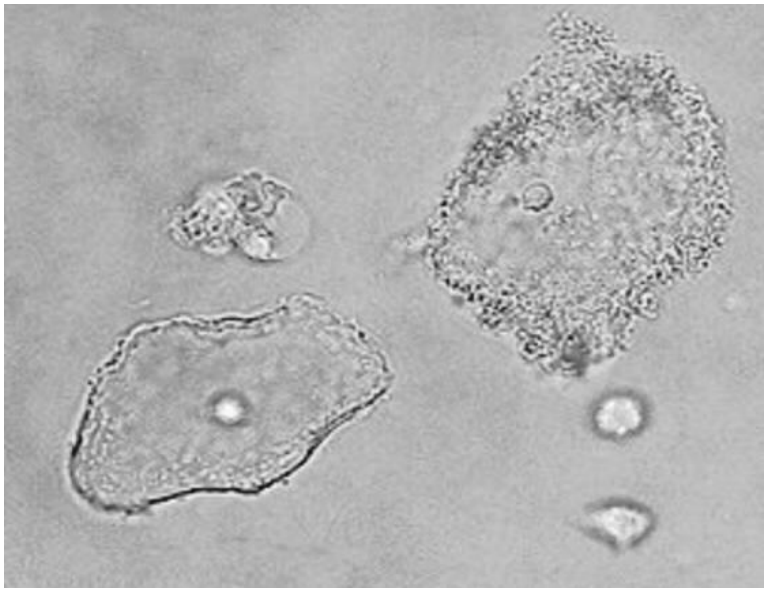


Figure (2-1):Clue cell in wet amount.

2.4 Epidemiology of Bacterial Vaginosis:

The reasons of bacteria vaginal is unknown, as the explanation for the disparity in prevalence around the world (Kenyon *et al*., 2013) the prevalence rate of bacterial vaginosis during pregnancy about 4.9-49% (Ishaque *et al*., 2011) the spread of these in women affected by variety of factors, including the clinical environment, sociodemographic factors, medical criteria, gestational period, and others (Trabert and Misra, 2007) In western countries spread in pregnancy woman ranges from (14-21%), while in Asia countries, the prevalence ranges from (13.6-18%) (Ishaque *et al*., 2011) researchers attempted to reported the global epidemiology of bacterial vaginosis and using data for Nugent scoring criteria to diagnose bacterial vaginosis patient southern and eastern parts of africa had the highest bacterial vaginosis prevalence was found 58% Gambia had the second-highest bacterial vaginosis prevalence, was 37%, Uganda with 34% (Kenyon *et al*., 2013)in maternal woman in south africa, the proliferators was stated to be 17.6% (Redelinghuys *et al*., 2013).

2.5 Pathogenicity:

The forming of microbial biofilm on vaginal epithelial cell is a noteworthy hallmark of bacterial vaginosis due to, the present of O_2 gradual within the membrane-mediated biological presence of this membrane creates a favorable habitat for anaerobic bacteria, afterward primary colonized kind of clings into the roof polymicrobes biofilm, like the bacterial vaginosis biofilm integrate accessory bacteria synergized connection together in this species permit the bacterial biofilm to bloom and grow up (Machado and Cerca, 2015).

The first stage of bacterial vaginosis formation is adherence to the vaginal epithelium (Swidsinski and Mendling, 2005) and the first stage in the formation of a bacterial vaginosis biofilm (Joo, 2012).

A reduction *Lactobacillius* and an increasing other stringent anaerobic bacterial vaginosis-bound with bacteria by lowering the oxidation possible in the vagina microbiome such as, *Prevetolla bivia* and *Atobium vagina* (Schwebke *et al*, 2014) which are gained of maternity and environment source are usually present in extremely low numbers (Hyman *et al*., 2005).

Many other bacterial vaginosis associated bacteria, including *Mycoplasma* spp., *Staphylococcus* spp., *Brevibacterium mcbrellneri*, and *Enterococcus* spp., grew as biofilms as well, this suggests that when bacterial vaginosis-associated bacteria come into touch at human cells line a small quantity of *bacteria* isn't enough to cause biofilm development hydrogen peroxide, lactic acid material produce by vaginal *Lactobacilli* that lower the vaginal pH (4.5) and prevent colonization of pathological anaerobes (Patterson *et al*., 2007).

2.6 Clinical manifestations

The clinical definition of bacterial vaginosis also includes women without symptoms (Ledger,1993) researchers from one study (Mcgrgor *et al*.,1995) reported a 50 percent reduction in preterm birth and premature rupture of the membranes associated with bacterial vaginosis after women with the infection had been treated with oral clindamycin in view of potentially serious sequelae, the question of whether to screen for bacterial vaginosis in pregnancy has been raised (Eltabbakh *et al*.,1995) Further, study is needed to determine if screening for vaginal infections should become a routine part of prenatal care.

Any woman presenting with symptoms of vaginal discharge should be evaluated with a physical examination, wet mount and potassium hydroxide preparation to determine the cause of the discharge so appropriate treatment can be initiated.

Increase excreta that smelling similar fish and is consistent, thin, and grey in color are clinical indications of bacterial vaginosis(Oduyebo *et al*., 2009).

Inflammatory symptoms of the vaginal mucosa are uncommon in bacterial vaginosis dueto, almost complete absence of polymorphonuclear neutrophils, suggesting that the microorganisms do not invade the subepithelium (Li *et al*., 2015).

About three of the clinical criteria are meet, clinical diagnosis of it certainty these are some of the standards as uniform and homogeneous vaginal discharges that is gray, whiten, yellowish color, pH of 4.5 or higher in vaginal discharge existence of clue cell in the vagina mucus smears sample and a positive whiff test amine odor after adding ten per cent potassium hydroxide to vaginal discharge at least one to five cell of the vaginal epithelial (Armstrong *et al*., 2009).

2.7 *Cytomegalovirus* :

Human *cytomegalovirus* is a member from the herpsviridae family based and the beta herpsviridae subfamily it`s found in all of the world and infects human in all ages and socioeconomic groups (Richard *et al*., 2007).

In humans, it is greatest prevalent cause of creation infected (Lamichhane *et al*., 2007).

Human *Cytomegalovirus* infection is a very common across the world with seropositivity rates about (40%) in developed countries was (100%) (Chakravarti *et al*.,2009).

The result some of the studies on role *Cytomegalovirus* infectious in recurrent abortion were mixed when comparing women with chronic pregnancy loss to normal pregnant women, and found a higher prevalence of antibodies to *cytomegalovirus*, in serum while others found a similar or even lower prevalence.

As well as, the fact that several experiments have discovered elevated levels of *cytomegalovirus* antigens in abortion tissues (Virol, 2015).

2.7.1 Classification of Cytomegalovirus:

CMV was been classified as the flowing:

Kingdom: Heunggongvirae

Phylum: Peploviricota

Class: Herviviricetes

Order: Herpesvirales

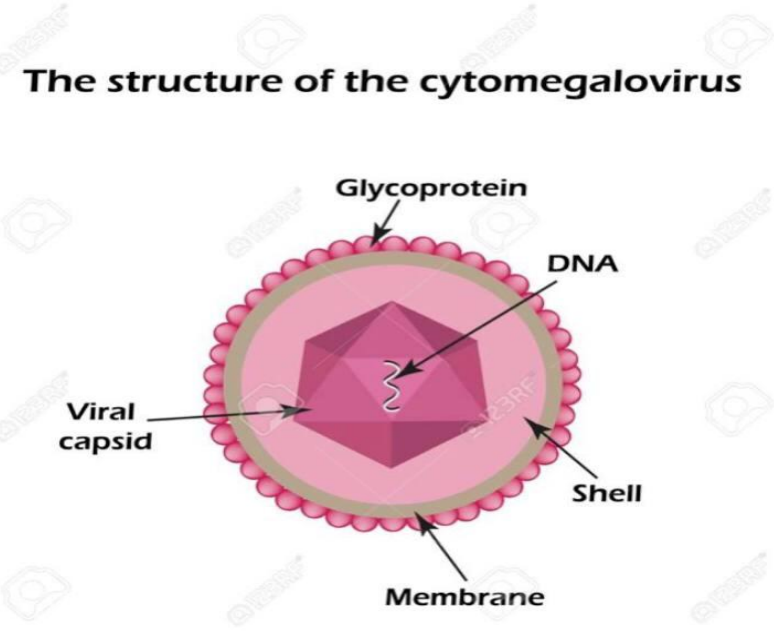
Family :Herpesviridae

Subfamily :Betaherpesvirina

Genus :*Cytomegalovirus*

2.7.2 Structure of Cytomegalovirus:

The enveloped of *Cytomegalovirus* was icosahedra, spherical to pleomorphic, and round geometries the diameter range between 150-200 nanometers genome are linear and non-fragmented, around 200 kb in length (Virol, 2015) also Known as human herpes virus-5 (Koichi *et al*., 2007) herpesviruses abiggest genomes, which was encoding hundreds of protein and as a result, it has the longer genomics and one from the longest genome of any humans virus generally (Sijmons *et al*., 2014).



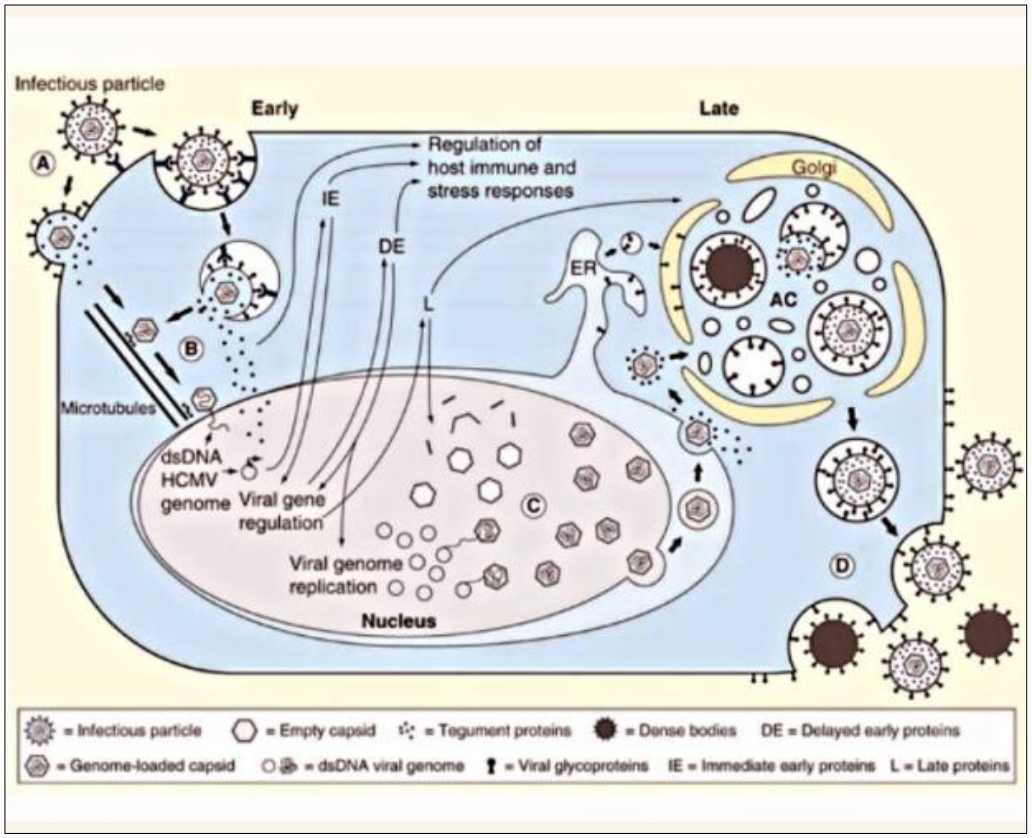
Figure(2-2):*Cytomegalovirus* (CMV) structure.

2.7.3 Life cycle of *Cytomegalovirus*:

Viral replication is lysogenic and nuclear the connection of viral glycoprotein to host receptor, whom causes endocytosis allow virus to enter the host cell the dsDNA bidirectional replication model is used for replication method of transcription is DNA template transcription with an alternate splicing mechanism after egress and budding, the virus leaves the host cell the natural hosts are humans and monkeys contact with body liquid like salivary, urinary, and vaginal secretion of persons affected is required for transmission (Cannon *et al*., 2010).

Human CMV is strictly species-specific and infects cell cultures of fibroblasts and to a lesser extent certain epithelial cells and B-and T-lymphocytes, latent infection *In vivo* are found in leucocytes and possibly endothelial cells, a great number of genetic variants of CMV have been

demonstrated by the use of restriction endonuclease assays, the virus contain 33 structural proteins and codes for unknown number of non-structural proteins the glycoproteins of the envelope are important antigens(Haaheiml and Whitley, 2002).



Figure(2-3):Overview of the human cytomegalovirus life cycle. (A) Virions enter the cell through interaction with cellular receptors. Tegument and capsid proteins are delivered to the cytoplasm. (B) The capsid travels to the nucleus, then the genome is delivered and circularized. Tegument proteins regulate host cell responses and initiate the temporal cascade of the expression of viral immediate early (IE) genes, followed by delayed early (DE) genes, which initiate viral genome replication, and late (L) genes. (C) Late gene expression initiates capsid assembly in the nucleus, followed by nuclear egress to the cytosol. Capsids associate with tegument proteins in the cytoplasm and are

trafficked to the viral assembly complex (AC) that contains components of the endoplasmic reticulum (ER), Golgi apparatus, and endosomal machinery. The capsids further acquire tegument and viral envelope by budding into intracellular vesicles at the AC. (D) Enveloped infectious particles are released along with non-infectious dense bodies. Figure and figure legends are adapted from (Jean Beltran & Cristea, 2014) and modified.

2.7.4 Pathogenesis and transmission of *Cytomegalovirus*:

Cytomegalovirus in early life its transport way by vertical and horizontal, congenital infectious occurs before birth perinatal infection occurs during childbirth, and postnatal infection occurs some other time on living lateral move is mostprevalent the majority of injuries are gained across online closely call at people for example little kids under the age of three a shedding of viral into the body fluid each slaver or urinalysis, bloody transfusions (a Post perfusion Syndrome was recognized in open heart surgery patients who received large quantities of blood which were contaminated with *Cytomegalovirus*, sexual contact(it`s found in Semen and in the Cervix), bone marrow transplants, and strong organ transplants are also possible routes of transmission infected people (Gulia *et al*., 2007) it seems that CMV is transmitted by infected cells in the donor Kidney.

2.7.5 Clinical features of Cytomegalovirus infection:

At the time of pregnancy (40 to 60 %) from pregnancy women are vulnerable risk this virus, one to four proportion of these women will contract *Cytomegalovirus* in gestation period and about 40–50% percent of afffected we`ll pass the virus on the their unborn children ,the transmission rate is lowest in the first three month about (35%)

percentage, and (73%) percent for female who infected virus infection in the last few months (Bodeus *et al*., 2010).

About 33% of infants infected with symptoms, neurological deficiency this neonatal disease prevalence a highest among children be born of mothers on with a primary incidence with in first half pregnancy (Alder ., 2011)also, sins of in childbirth and may suffer long complications including hearing loss(Ross *et al*., 2006).

About 90% of congenitally infected babies are asymptomatic at birth, with (5-17)% developing symptoms such as, sensorineural hearing loss, choriortinitis, or neurological deficiency in the first two years (Revello and Gerna, 2002).

Infection with *Cytomegalovirus* during pregnancy can real in abortion, intrauterine growth retardation, mental retardation, hepatosplenomegaly, intracranial calcification, jaundice, thrombocytopenia, hepatitis, microcephaly, and neonatal death (Chakravarti *et al*., 2009) liver dysfunctional, bleeding, disseminated intravascular clotting and secondary bacteria casualties are the most common causes of death, dental problems may start as early as the age of two (Nayeri and Thung, 2013).

2.7.6 Diagnosis of *Cytomegalovirus*

In a primary *Cytomegalovirus* infection in the mother is made, *Cytomegalovirus* testing which help and determine the risk of transmission to the fetus, medical signs alone cannot be used to diagnosis maternal primary *Cytomegalovirus* infection because they are nonspecific as fever, nausea and headache about, (25-50)% of mothers without symptoms (Revello and Gerna, 2002) a primary C*ytomegalovirus* infection in the mother is suspect diagnosis test need to be include

seeking this virus type IgG in serum of previous seronegative pregnancy woman Since, inadequate baseline *Cytomegalovirus*-specific IgG negative samples is often unavailable, diagnostic by seroconverting single is rare accomplished in practice.

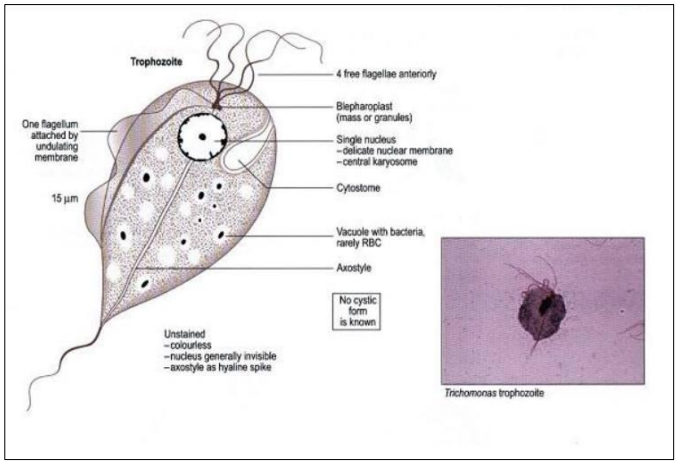
However, so were stored before conception or early childbearing serum is availability, a comparison with it is optimal ,when cmv immune status prior to pregnancy is uncertain, independent identification of viral IgG avidity to detect how advanced or qualitative IgM antibody are insufficient singular steps for diagnosing *Cytomegalovirus* case(Lazzaroto., 2000; Mace *et al*., 2004; Munro and Hall, 2005).

Antibodies to (IgM) and lower middle avidity to (IgG) provide a strong indications of acute primary infection (Lazzaroto *et al*., 2011)higher hazard of symptomatic congenital infectious before (12-16) weeks of pregnancy (Euders *et al*., 2013) mostly prevalent of the TORCH infection, that were infections disorders classed altogether because whether there might be harmful influence on fetal, is *Cytomegalovirus* , toxoplasma, rubella, and the *Herpes* simplex virus(HSV) are among them(Nelson and Demmler,1997**).**

2.8 *Trichomonas vaginalis*

Trichomonas vaginalis is a parasite protozoan which causes trichomoniasis, and related with sexually transmitted infection (STI) on the worldwide (Newman *et* al., 2015) it is pear-shaped single cell parasite with a variety of sizes and shapes(Arroyo *et al*., 1993) It`s appended to epithelia cell it takes on an amoeboid appearance is a flagellum protozoal with four anterior and one later flagella protozoan has a longitudinally axial, stick like arrangement named axostyle, which

extends beyond parasite's posterior end, the posterior flagellum is inserted into the axostyle's middle an undulating membrane covers half of the cell body, allowing nutrients to be swept through the pathogen's 'mouth-like' structure the cytosome is a type of cell the nucleus of *Trichomonas vaginalis* is establish for front segment of the organism, it surrounding by atomic sheath(Petrin *et al*.,1998), as in figure(2-4) .



Figure(2-4):Trophozoites of *Trichomonas vaginalis*.

They have five flagella: four anteriorly directed flagella and one posteriorly along the outer membrane of the undulating membrane.

The large nucleus is usually located at the wider, anterior end and contains many chromatin granules and a small karyosome.

2.8.1 Classification of *Trichomonas vaginalis*

Trichomonas vaginalis is classified as follows (De Aquino *et al*., 2020)

Domain: Eukarya

Kingdom: Protista

Phylum: Metamonada

Sub Phylum: Trichozoa

Class: Parabasilia

Order: Trichomonadida

Family: Trichomonadidae

Genus: Trichomonas

Species: *Trichomonas vaginalis*

Human trichomoniasis is a sexually transmitted infection widely around the world, and causes sexually transmitted diseases pervasiveness of parasite world-wide range of 2-50 % depending on the country, gender, and populated criteria of the research population as well as the diagnostic procedures (Ginocchoi *et al*., 2012), It almost ordinary neither-virology sexually transmissible illness global(Rowely *et al*., 2019).

Fifty percent from whole woman kind with it do not have symptoms sign of infectious in characteristic woman involve smell vagina discharge, oedema or erythema, strawberry cervical and bleeding lesions, vulvar irritant flammability, other complaint include dysuria, Pruritus, dysmenorrhea and smaller intestinal hurt (Johnston and DC, 2008).

A serious complications linked with trichomoniasis including preterm tear of placenta membrane, pre-term birth and lowly delivery mass in pregnant woman (Johnson, 2009) injuries were reported rate for male and female are equivalent, rather lady are normally symptoms, whereas infection in men are usually non symptoms, transport Usually take place through immediate grit to skin touching with an affected person, most of the time in vaginal coitus (Harp and Chowdhuary, 2011).

Trichomoniasis depend at several parameters inclusive old, sexuality act, enumerate of sexes partner, existence of other sexually transmitted diseases, sex habits, stage of the menstruation, screening technique and sample compiling, and lab technology trichomoniasis, along toward change pathology, cysticercosis toxocariasis, toxoplasmosis, belong to cluster of neglected parasitic infections (NPIs),whom the centres for illness controlling and prevent (CDC)have targeted as a priority in public health action (Secor *et al.,* 2014).

Some studies have shown associated between *Trichomonas vaginalis* infection and increases danger of premature parturition ,early laceration of the membrane (Swadpanich *et al*., 2008).

2.8.2 Life cycle and transmission:

Trichomonas vaginalis has a life cycle, humans are the parasite's natural hosts, and it locate in the women lower genital-urinary paths as well as urethral man urethra and prostate place where it reproduce a sexually via Mitotic dividing and bilateral fusion, *Trichomonas vaginalis* has never been found to reproduce sexually. It`s is a facultative anaerobic with no phase and only a trophozoite stage (Petrin *et al*.,1998) it`s miss cyst, flagella get killed external the human bodies protégé from dry and greatest heat, transmission requires a wet environmental *Trichomonas vaginalis* can survive in urine up to an hour (Shafir and Sorvillo, 2006) for anumber of hourly in the waters of rosaries (Pereira *et al*., 2003) no sexually relocation has been communicating a sex transport is most common style of move (Crucitti *et al*., 2011) many cases of *Trichomonas vaginalis* go undetected due to the use of culture tests in rectal detection, connection at infected bathroom seat, waterproof, speculum, sextoys,

towel, socks or sheet has been suggested as another method of nonsexual transmission, demonstrated that trichomonads remained viable at 45 minute and glazed places and 30 minutes on an un coated (Whittington *et al*.,1957).

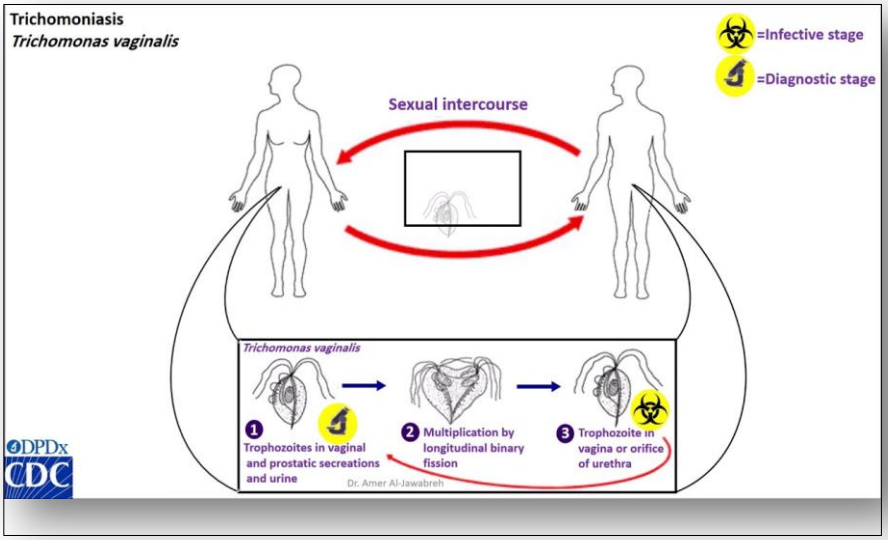


Figure (2-5): Life cycle of *Trichomonas vaginalis*

(http:WWW.dpd.cdc.gov/dpdx)

2.8.3 Epidemiology of *Trichomonas Vaginalis*

2-8-3-1:In World

Trichomonas vaginalis is widespread in all regions of the world (Masha *et al*., 2019) it is considered as one of the most common types of infectious diseases, that are sexually transmitted and being treatable (Sherrard, 2020)the incidence of new cases as estimated (174 million) most of occur in resource-poor settings at an average of (154 million) new cases per year (Alessio and Nyirjesy, 2019) the *Trichomonas vaginalis* prevalence and epidemiology are significantly affected by the fact that they are often asymptomatic and can be transmitted through

sexual intercourse without the knowledge of both partners (Chemaitelly *et al*., 2019)the trichomonal infection has appeared on all continents, all climates, and seasonal changes, this parasite has a global distribution in all ethnic and social strata in the USA (Rayan *et al*., 2019)the number of *Trichomonas vaginalis* infections is estimated at (3.7 million) men and women, women between the ages of (14-49) years had a highest rates of infection with vaginal signs (Shahraki *et al*., 2020)

The infection rates of *Trichomonas vaginalis* affected by many different factors such as economic and social status and personal health care. Infection with this parasite has been linked to the history of sexually transmitted diseases, aging, pregnancy, and treatment (Hanna *et al*., 2020) may be related with reproductive hormones(Tchankoni *et al*., 2020).

In African women, vaginal infections that include vaginal trichomonad are highly correlated with the appearance of sex at ages less than 20 years, as well as with more frequent of *Trichomonas vaginalis* infections in that the partner because of frequent traveler, trichomoniasis IR among black women is higher than that of white women, this may be affected the low level of education and socioeconomic factors that effect on the prevalence of trichomoniasis (Chetty *et al*., 2020).

There is a relationship between the low level of infection and the use of oral contraceptive treatments, the age of the most vulnerable group is between 20-45 years old, the oldest of most sexually transmitted diseases is controlling this infection, the oldest age group had been controlling the infection of STD and this depends on the examination of both women with symptoms and their partners(Stewart *et al*., 2020)

The prevalence of infection among pregnant women in some countries of the world according to WHO estimates is as follows: in Brazil, about 2.11% in Chile, about 5-2.7%, in Central Africa, it is about 9.9%, and in South Africa, about 41.4% (Bolumburu *et al*., 2020)in Iran, the IR of *Trichomonas vaginalis* 73.3% (Arbai *et al*., 2018), in Palestine, Gaza city the IR of *Trichomonas vaginalis* was 5.8%(Al_Jawabreh *et al*.,2019).

Due to, the different prevalence rates of infection of this disease and according to many research and topics in the republic of Korea, the first case of *Trichomonas vaginalis* was recorded by Lee and Yang (2020), and according to one of the studies there, the infection rate among women attending women's clinics in the city of Seoul was 17.3% (Huh, 2019).

2-8-3-2: In Iraq

In Iraq, the trichomoniasis neglected disease and had poor studies:

There were some studies among women IR in some province such as, in Al-Najaf, Al-Abbas and Radhi (2019) reported that the (34-25) years age group had the highest IR 49.01% and the rural had higher IR 41.37% than city 30.2% and primary education and low economic status of the participants in the study recorded high IR with *Trichomonas vaginalis* respectively (44.6% & 44.9%).

In Babylon province show high IR with *Trichomonas vaginalis* among women in Al- Hila region, where the rural population recorded the highest IR 96.29% than city79.36% (Al-Dahmoshi, 2017) in Wasit province, Al-Kut city showed (14-43) years age had the highest IR (Rahi *et al*., 2014).

In Basra province, the overall of IR is 5.7-8.5%, the highest IR was 61.58% among married women and the lowest IR was 31.81% unmarried women, the highest IR was 69.72% non-pregnant women, the highest IR was 25.71% at (36-40) year age group (Al-Assadi *et al*., 2020)

In Baghdad, the highest IR (17.2%) among pregnant women, the lowest IR (8.8%) among non-pregnant women, while the highest IR (63.07%) among women who live in rural areas and the lowest IR (36.93%) among women who live in urban areas (AL-Khalidy and Al-abodi, 2020). In Dohok province, the highest IR (7.6%) at (20-25) age group and the lowest IR (2.2%) at (36-40) age group (Said *et al*., 2020).

2.8.4. Pathogenesis and clinical manifestation

Pathogenesis of *Trichomonas Vaginalis* is thought to involve a number of processes, including cell-to-cell adhesion (Alderete and Garza, 1988) it has been observed asymptomatic individuals; possible multi center studying find there (72 %) of man sexual partner of feminization with sick she`s hurt with *Trichomona vaginalis*, with (77)% of no symptoms (Sena *et al*., 2007), It`s detected with asymptomatic individuals, signs of trichomoniasis in women as pus vagina trigger, irregular vaginal smelling, scratch, dysury, lower abdominal pain and strawberry cervix in shape (Pastorek *et al*., 1996). Infertility has been linked to trichomoniasis (Petrin *et al*.,1998). Preemies was born , premature rupture of membrane, and low birth weight are all possible complications of the illness (Margrita *et al*., 2020).

Trichomonas infection has been linked to an increased incidence of high-grade cervical cancer in human papillomavirus (HPV16) patients, suggesting that the protist may influence virus carcinogenicity (Yung *et*

al., 2006)women who have trichomoniasis have a higher risk of pelvic inflammatory disorder than women who do not have trichomoniasis (Moodley *et al*., 2002).

Pregnant women who are experiencing symptoms, regardless of their pregnancy stage, should be checked and treated.

Chapter Three

Material and Methods

3. Material and Methods:

3.1. Materials

3.1.1.Subjects

The study has been conducted under the agreement of the maysan health department, all samples were taken under direct supervision of the gynecologist physician in Al-Sadr Teaching Hospital (339) samples were collected from aborted women, with serial number for every patient, medical history was also reviewed, also clinical signs and symptoms (fever, beelly pain, dysury, bleeding, premature birth).

3.1.2 Apparatus ,Equipment

The apparatus, equipment using in this Study summarized at tabulation 3-1

Table 3-1: the apparatus and equipment that used with their producing companies and countries.

Apparatus and Equipment	Company/Origin
Anaerogen	Thermo Scientific/USA
Anerobic jar	Thermo Scientific Oxoid/USA
Autoclave	Hirayama/Japan
Beakers	Iso Lab/Germnay
Biosafety Cabinet	Lab Tech/France
Brown glass(Vials)	Indiamart/India
Burner	Indiamart/India
Cooling centerfuge	Eppendroff/Germnay
Cover slides	Superestar/India
Cylinder	Iso Lab/Germnay
Disposable Petri dishes	Al-malak company/Iraq
Distillator	Gfr/Germnay
Flask(250,500)	Iso Lab/Germnay
Funnel glass	Iso Lab/Germnay
Gloves	Broche/Malaysia
Incubator	Human Lab/Korea
Jel tube(8ml)	DiseRA A.S/izmir-Turkey
Light Microscope	Nikon/Japan
Loop	John Blotn/England
Micropipettes	DragonMED/china

Microwave	Shownic/Korea
Minividas	BioMeriexu/France
Oven	Memmert/Germnay
pH Paper	DF/China
Plane tubes(10ml)	AFCO/Dispo/JORDAN
Screw-Cap	Citoglas/china
Sensitive Balance	Sartorius/Germnay
Slides	Superestar/India
Standard Wire Loop	John Bolten/England
Swaps	AFCO/Jordan
Syringes	Sterileo/China
Thermal Cycler	Prime/UK
Tips	Sterelline Ltd/UK
Vitek-2-Compact System	Biomerieux/France
Vortex Mixture	Medilab/Korea
Water path	Memmert/Germnay
Wood stick	Supteme/china

3.1.3 Chemistry and Biology articles

Table (3.2): Chemistry and Biology materials used.

NO	Materials	Corporation
1	Absolute Ethanol	Schariauiau/Spain
2	Blood	Blood bank/Mysan city
3	KoH	Oxford labchem/India
4	Normal saline	Iraq
5	Oil Immersion	Jourilabs

3.1.4 Culture media

Agriculture media are clarify In the table(3-3).

Table (3.3):Culture Media use at this study

No	media	Company
1	Amies Transport medium	Sterile transport medium swab/jordan
2	Blood agar	Neogen/USA
3	Brain Heart infusion broth	Neogen/USA
4	Brain-Heart agar agar	Neogen/USA
5	Inpouch TV	GenobleCedx 2/France
6	MacConkey agar	Neogen/USA
7	Nutrient agar	NEogen/USA
8	Nutrient Both	himedia Laboratories/india
9	Transport media	Neogen/USA

3.1.5.Diagnostic Kit

kit use In this search are contained in the Table (3-4).

Table(3-4): kit use in this Study.

No	Kit type	company	Purpose		
1	CMV IgG/IgM Rapid test	Biochrome Scientific	Detection of IgG and IgM antibodies to cytomegalovirus		
2	Giemsa stain	Volu-Sol/USA	Diagnosis Parasites		
3	Gram stain	Himedia/India	Differention of microorganism		
4	Vidas cmv G	Biomerienx France	To detect IgG specific for cytomegalovirus		
5	Vidas CMV M	Biomerienx France	To detect IgM specific for Cytomegalovirus		
6	Vitek2 GN Kit	Biomerienx France	Identification of Gram-Negative bacteria		
7	Vitek2 GP Kit	Biomerienx France	Identification of Gram Positive bacteria		

Methods:

The general steps for research are shown in this figure:

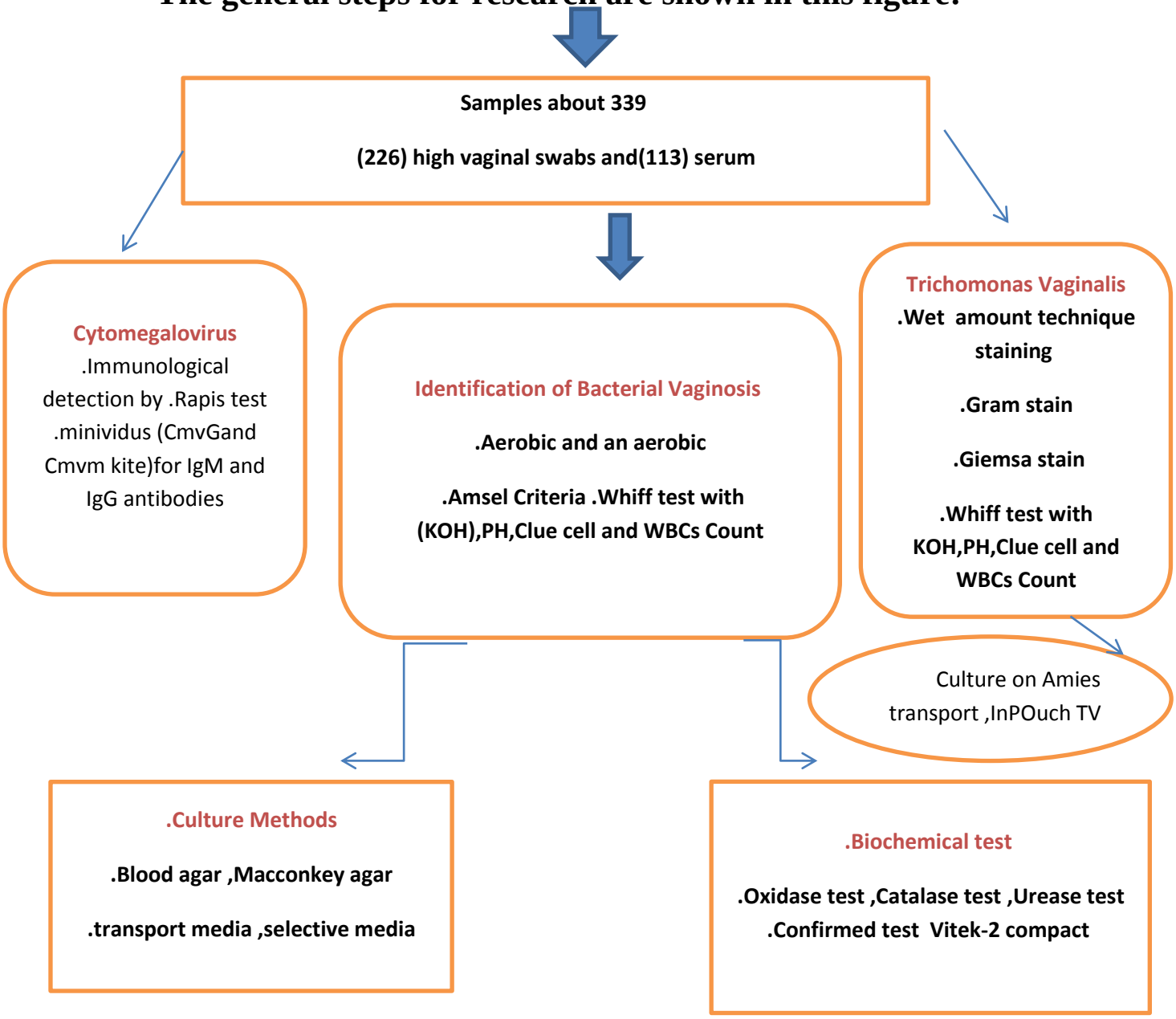


Figure (3-1):Study design.

3.2 Methods:

3.2.1 Sample collection:

Total of (339) samples (226) high vaginal swab and(113) serum collected from aborted women in obstetrics and gynecology department in AL-Sadder Teaching Hospital in maysan governorate and under the supervision of the competent staff during the period from November 2020-May 2021, high vaginal swabs were taken from aborted women in age (14-45) years.

3.2.2 Questionnaire sheet:

Clinically history from each case, complete data were taken direct from patient, information were arranged in clear detail formulation paper as shown in (Appendix1).

3.2.3. Collection of vaginal swabs :

3.2.3.1. High vaginal swabs (HVS)

- The speculum has been used to separate the vaginal walls
- Wipe away any excess cervical mucus with a cotton swab
- Carefully insert swab into vagina about 2 inches (5 cm) past the introitus and gently rotate the swab for 10 to 30 seconds make sure the swab touches the vagina walls

3.2.3.2 Specimen Transport and Storage:

- Vaginal swab specimens must be transported to the laboratory in the provided swab specimen transport medium and tube

- Vaginal swab specimens must be transported to the laboratory at 2oC to 30^{o}C and tested within 60 days of collection
- If longer storage is needed, freeze at -20 ºC to -70 C for up to 12 months after collection.

In this study the samples were transferred and cultured immediately after collection.

3.2.4. Preparation of Culture media :

3.2.4.1. Preparation of MacConcy agar Media:

1. Attended this medium according to the instructions of the manufacturer
2. Sterilization in the autoclave with a temperature of 121°C for 15minute

3. This medium for the purpose of diagnosing lactose-fermented bacteria (Negative Gram) and non-fermenting lactose (Positive Gram).

3.2.4.2. Preparation of Blood agar Media :

1. Attended this medium according to the instructions of the manufacturer

2. Sanitizes in autoclaving at a temperature of 121°C for 15min, and left to cool down

3. Human blood was added to it by 5% Mix well

3.2.4.3. Preparing of Nutrient Agar :

1 . Dissolve 28 g of nutrient Agar in a liter of dripped Water

2. Sterilized in the Autoclave at a temperature of 121 C. at15 minutes

3.2.4.4. Preparation Brain Heart Infusion Agar Media :

1. Attended this media in conformity with the direction of the maker

2. Sterilizer in the autoclave at temp of 121° C at 15minutes

3.2.5 Sterilization Methods :

3.2.5.1 Sterilization by Dry Heat :

The glassware and was sterilized by oven at 150 C for 2 hours

3.2.5.2 Sterilization by Autoclaving

The culture Medium had been sterilized by Autoclave at 121C at 15 min under pressurize (15) PSI.

3.2.6. Diagnosis bacteria isolation:

3.2.6.1. Identification of Bacterial vaginosis:

The morphological characteristics of the growing colonies of bacterial vaginosis include color, size, elevation and margin of the colonies on enrichment, selective and differential media (Nutrient agar, Blood agar and MacConcy agar) (Collius *et al*., 2004)

3.2.6.2 Staining :

3.2.6.2.1.Gram stain:

1. Prepare the smear of suspension on the clean slide with a loopful of sample, air dry and heat fix

2. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water

3 . Flood the gram's iodine for 1 minute and wash with water.

4. Wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water

5. Add safranin for about 1 minute and wash with water

6. Air dry, Blot dry and Observe under microscope.

3.2.6.2.2. Wet amount Preparation:

1. One or two drops of normal saline was put on the prepared smear with cover slide

2. Examined under microscopic(Collius *et al*., 2004)

3.2.6.3 Biochemical Examination:

3.2.6.3.1 Oxidase test :

1. On a piece of filter paper

2.Two to three drops of tetramethyl phenylenediamine dihydrochloride (oxidase reagent) (1 percent) were inserted.

3. A part of the culture was scraped off with the edge of a slide and smeared across the impregnated paper's surface.

4. Within ten seconds, a dark purple color was produced, indicating a favorable reaction (Cown ST and Steel,1974).

3.2.6.3.2 Catalase Test :

1.Transfer little quantity Of colonization the growth at Surface of a cleanly dried glass slip use a bacteriological loopful or antiseptic woody rod.

2. Drop of 3 percent hydrogen peroxide should be placed at the an inoculum (Cruckshank *et al*.,1975)

3.2.6.3.3 Urease test:

1. By streaking the surface of the agar in a zigzag pattern, heavily inoculate the slope (from an 18 - 24 hour pure culture) throughout the whole surface. Stabbing the butt interest like a colour controlling.

2. Keep of the infected slope for loosened seals for 24-48 hours at 35-37°C .

3. After 6 hours and overnight incubation, check the slopes for color change. Longer amounts of time may be required (Christensen.,1946; Macfaddin., 2000).

3.2.6.4. Confirmatory Diagnosis :

3.2.6.4.1. VITEK 2 Diagnostic System :

3.2.6.2.4.1.1 PRINCIPLE

The Vitek-2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number. Test kits available include ID-GN (gram negative bacillus identification), ID-GP (gram positive cocci identification), AST-GN(gram negative susceptibility) and AST-GP (gram positive) susceptibility.

The Vitek 2 ID-GN card identifies 154 species of enterobacteriaceae and a select group of glucose non- fermenting gram negative organisms

within 10 hours.theVitek-2 ID-GP card identifies 124 species of *staphylococci*, *streptococci*, *enterococci* and a select group of gram positive organisms within 8 hours or less.

The Vitek-2 Antimicrobial susceptibility tests (AST) are for most clinically significant aerobic gram negative bacilli, *Staphylococcus* spp., *Enterococcus* spp., and *Streptococcus agalactiae* susceptibility results are available for bacteria in less than 18 hours.

3.2.6.2.4.1.2.Procedure:

The Vitek-2 device was been used to diagnosis of bacteria isolates from culture media sterile swabs or wooden is utilize to transport one to two colony of pure culture then suspended bacteria in (0.3) ml of pasteurized brine in clear Polystyrene test tube turbidity is adjust according to mcfarland turbidly rang from in gram negative and gram positive (0.5-0.63)then the cassette bear with 10 Card and suspense, tube and bar code to input data , then loading in to automatri transport system (Pincus ., 2000).

3.2.7. *Cytomegalovirus* (CMV)Samples :

3.2.7.1.Rapid Test:

3.2.7.1.1.Principle:

CMV IgG-IgM Rapid Test contains two test strips (left panel: CMV IgM test; right panel: CMV IgG)test

The CMV IgM Rapid Test in the left panel is a lateral flow chromatographic immunoassay the test cassette consists of 1) a burgundy colored conjugate pad containing mouse anti-human IgM conjugated with

colloidal gold (IgM conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line) the T line is pre-coated with recombinant CMV antigen, and the C line is pre-coated with a control line antibody. The CMV IgG Rapid Test in the right panel is a lateral flow chromatographic immunoassay, the test cassette consists of 1) a burgundy colored conjugate pad containing CMV antigens conjugated with colloidal gold (CMV conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with anti-human IgG antibody, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well (S well) of the cassette, the specimen migrates by capillary action across the cassette. IgM anti-CM, if present in the specimen, will bind to the IgM conjugates the immunocomplex is then captured on the membrane by the pre-coated CMV antigen forming a burgundy colored T line in the left panel, indicating a CMV IgM positive test result.

IgG anti-CMV, if present in the specimen, will bind to the CMV conjugates the immunocomplex is then captured on the membrane by the pre-coated anti-human IgG forming a burgundy colored T line in the right panel, indicating a CMV IgG positive test result.

Absence of color development on both T lines suggests a negative result. The test contains an internal control (C line) which should exhibit burgundy colored lines of the immunocomplex of the control antibodies in both the left and right panels regardless of the color development on any of the T lines. If the C line does not develop in a panel, the test result is invalid and the specimen must be retested with another device an

invalid result in one panel does not invalidate the test result in the other panel.

3.2.7.1.2. ASSAY PROCEDURE

1. Bring the specimen and test components to room temperature if refrigerated or frozen. once thawed, mix the specimen well prior to assay

2. When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface

3. Be sure to label the device with the specimen's ID number

4. Fill the plastic dropper with the specimen

5. Set up timer and Results can be read in 15 minutes

3.2.7.2.Principle of minividas

The VIDAS® principle is based on the interaction of two elements: the coated SPR® receptacle, containing antigens or antibodies, and the Strip, made up of a series of wells containing the correct amount of reagent necessary for the test. All reactions occur within the SPR in two key phases: immunological reaction.

3.2.7.2.1.PRINCIPLE OF THE PROCEDURE(IgM)

The VIDAS CMV IgM (CMVM) assay is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument all assay steps and assay temperature are controlled by the instrument a pipette tip-like disposable device, the Solid Phase Receptacle (SPR®), serves as the solid phase as well as the pipettor for the assay the SPR is coated at the time of manufacture with CMV antigen (strain AD169), the VIDAS

CMV IgM (CMVM) assay configuration prevents nonspecific reactions with the SPR reagents for the assay are in the sealed reagent strips.

After an IgG and rheumatoid factor absorption step, the sample is cycled in and out of the SPR for a specified length of time, Anti-CMV IgM antibodies present in the specimen will bind to the CMV antigen coating the interior of the SPR. Unbound sample components are washed away. Mouse monoclonal anti-human IgM antibodies conjugated with alkaline phosphatase are cycled in and out of the SPR and will attach to the human anti-CMV IgM bound to the SPR wall. A final wash step removes unbound conjugate.

A fluorescent substrate, 4-methylumbelliferyl phosphate, is introduced into the SPR. Enzyme remaining on the wall of the SPR will catalyze the conversion of the substrate to the fluorescent product, 4-methylumbelliferone. The intensity of the fluorescence is measured by the optical scanner in the instrument.

When the VIDAS CMV IgM (CMVM) assay is completed, the results are analyzed automatically by the instrument, a test value is generated, and a report is printed for each sample.

3.2.7.2.2.Assay procedure:

1-Remove necessary components from the kit and return all unused components to storage at 2-8oC.

2-Allow components to reach room temperature (approximately 30 minutes).

3-Use one "CMVM" strip and one "CMVM" SPR for each sample, control or standard to be tested make sure the storage pouch has been carefully resealed after the required SPRs have been removed.

4-The test is identified by the "CMVM" code on the instrument. The standard must be identified by "S1", and tested in duplicate. If the positive control is to be tested, it should be identified by C1. If the negative control is to be tested, it should be identified by C2

5-If needed, label the "CMVM" Reagent Label strips with the appropriate sample identification number.

6-Mix the standard, controls, and samples using a vortex- type mixer (for serum separated from the pellet).

7- For this test, the standard, control, and sample test portion is 100 µL.

8-Insert the "CMVM" Reagent Strips and SPRs into the appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match

9-Initiate the assay processing as directed in the User Manual. All the assay steps are performed automatically by the instrument

10-Reclose the vials and return them to 2–8°C after pipettin.

11-The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and strips from the instrument.

12-Dispose of the used SPRs and strips into an appropriate receptacle.

3.2.7.2.3. PRINCIPLE OF THE PROCEDURE(IgG)

The VIDAS CMV IgG (CMVG) Assay is an enzyme-linked fluorescent immunoassay (ELFA) that is performed in an automated instrument, all

assay steps and assay temperature are controlled by the instrument. a pipette tip-like disposable device, the solid phase receptacle (SPR®), serves as the solid phase as well as the pipettor for the assay, reagents for the assay are available in the sealed reagent strips.

After a sample dilution step, the sample is cycled in and out of the SPR for a specified length of time. Anti-CMV IgG antibodies present in the specimen will bind to the purified CMV antigen coating the interior of the SPR unbound sample components are washed away.

A monoclonal anti-human IgG conjugated with alkaline phosphatase is cycled in and out of the SPR and will attach to any human IgG bound to the SPR wall. a final wash step removes unbound conjugate.

A fluorescent substrate, 4-methylumbelliferyl phosphate, is introduced into the SPR. enzyme remaining on the wall of the SPR will catalyze the conversion of the substrate to the fluorescent product, 4-methylumbelliferone (450 nm) the intensity of the fluorescence is measured by the optical scanner in the instrument it is proportional to the quantity of CMV IgG found in the sample.

When the VIDAS CMV IgG (CMVG) Assay is completed, the results are analyzed automatically by the computer. The quantity of anti-CMV IgG present in the sample is calculated in reference to a calibration curve stored in the instrument. A report is printed for each sample.

3.2.7.2.4. Assay procedure

1-Remove necessary components from the kit and return all unused components to storage at 2 – 8 °C.

2-Allow components to reach room temperature (approximately 30 minutes).

3-Use one "CMVG" strip and one "CMVG" SPR for each sample, control or calibrator to be tested. Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.

4-The test is identified by the code "CMVG" on the instrument (to do so, refer to the Instrument User Manual). The calibrator must be identified by "S1", and tested in duplicate. If the positive control is to be tested, it should be identified by C1. If the negative control is to be tested, it should be identified by C2.

5-If needed, label the "CMVG" Reagent Strips with the appropriate sample identification numbers.

6-Mix the calibrator, control, and sera using a vortex- type mixer (for serum separated from the pellet).

7-For this test, the calibrator, control, and sample test portion is 100 µL.

8-Insert the "CMVG" Reagent Strips and SPRs into the appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.

9-Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.

10-Reclose the vials and return them to 2–8°C after pipetting.

11-The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument.

12-Dispose of the used SPRs and strips into an appropriate recipient.

3.2.7.2.5. Results:

Table(3-5):Cutoff minividas CMV (IgM)

Signal/cutoff Ratio	Interpretation
<**0.7**	**Negative**
≥0.7 to <0.90	**Equivocal**
≥0.90	**Positive**

Table(3-6): Cutoffs of the minividas CMV (IgG)

Signal/cutoff Ratio	Interpretation
<4.0	**Negative**
4.0 ≤ to <6.0	**Equivocal**
≥6.0	**Positive**

3.2.8 *Trichomonas vaginalis* :

3.2.8.1 Microscopically examination

Two vaginal swab were taken from each woman, one for cultivation and direct examination, one drop of normal saline was mixed with vaginal discharge and two slides of each stained with Giemsa stain (Solarbio, China) and non-stain of wet amount of vaginal discharge were prepared and examined under compound microscope with 40X magnification (Nwokah *et al*., 2019).

The most common test is a wet amount method under microscopy examination where it is done by taken a swab from the cervix of vaginal and mixed with 3ml of normal saline, then one drop of the mixture was taken on a glass slide and put it a cover of slide and examined under a light microscope with a magnification of 40X.

3.2.8.2. Giemsa Stain

The prepared smear was fixed by immersion in methanol for one minute and allowed to dry. It was then stained with Giemsa stain(Hi Media Laboratories, India), diluted 1 part to 19 parts of 1/15M phosphate buffer, pH 7.2 for 10 min and scanned for Trichomonas vaginalis at 100X magnification(Mason *et al*., 1976) both the internal and external structures of the organism were clearly visualized, the former stained dark blue with a red nucleus and the latter was sharply outlined, showing clearly the flagella and the undulating membrane.

Table (3-7) :Component of Giemsa stain.

Ingredients	Amount Gm/L
Giemsa powder	7.6
Glycerol	500ml
Methanol	500ml

3.2.8.3: Cultivation of vaginal discharge

The cultivation samples with sterile collection swab amies transport medium (Biozek Medical, Netherlands) were incubated for 5 days at a temperature of 37 ^{o}C, and the samples were examined periodically every day to confirm the final results

3.2.8.4: Culturing vaginal specimens on InPouch.

There are many liquid culture media available for diagnosis, InPouch *Trichomonas vaginalis* medium is considered as the best and it is called the gold standard that was used to be the culture system to confirm diagnose *Trichomonas vaginalis* that were collected in this study.

3.2.8.5:Component of InPouch medium:

The InPouch medium contains the following: peptones, maltose and other sugars, amino acids, salts and antimicrobial agents in a phosphate buffered saline base. An unopened pouch should contain a clear, amber liquid. Final pH of media is 6.1 ± 0.05.

3.2.8.6:Description and Principle:

Human trichomoniasis is a sexually transmitted infection (STI) caused by the flagellated protozoan *Trichomonas vaginalis*, it is recognized as one of the most prevalent sexually transmitted infections world-wide, in both males and females (Krieger *et al*.,1988; Krieger, 1995) the CDC estimates five million new cases occur in the U.S. annually.

The pouch is designed for user-friendly and convenient early microscopic detection by culture confirmation of *Trichomonas vaginalis*(Borchardt *et al*.,1992; Draper *et al*., 1993) the Inpouch consists of a high-barrier, oxygen-resistant, plastic with two V-shaped-chambers connected by a narrow passage that, together, provide a variety of benefits. The pouch allows users to easily inoculate a specimen, immediately observe (wet mount) the specimen, store and/or transport (optional) before transfer to the lab for incubation and recording.

3.2.9. Statistical Analysis:

SPSS program version (23) was used in statistical by effect of different factors or parameters, Chi-Square test was used for comparing significant between percentages in this study. The lower level of accepted statistical significant difference is bellow or equal to ($p < 0.05$), and the high significant difference is bellow or equal to ($p < 0.001$).

Chapter Four

Results

4 Results

4.1. Age groups with miscarriage women:

Age is very important factor, which associated with recurrent miscarriage, In the table (4-1) was describes the age groups of miscarriage Women`s in this study, high percent was recorded in age (24-33) years (48) 42%, low rate at groups >44 was(7)6.2% , In the table (4-2) describe the number of miscarriage with age one miscarriage was a high percentage and recorded in age group (24-33) years 20 (40%) and low Percentage in age more than(44) year 3(6%),twice miscarriage the age (24-33) year also recorded high rate 25 (50%), and low rate in age >44 was 1(2%), in more than twice high rate in age (14-23) year 5 (38.5%), and low percentage in age groups (34-43)year 2(15.4%) as in table (4-1) and (4-2).

Table (4-1): Age of Miscarriage women

Age	Frequency	%
14-23	34	(30.10)
24-33	48	(42.5)
34-43	24	(21.2)
≥44	7	(6.2)
Total	113	100%

Table (4-2): Age associated with Number of Miscarriage:

Age(group)	Once Miscarriage No(%)	Twice Miscarriage No(%)	More Than Twice(%)	P-value	Chi-square	
14-23	17(34.0)	12(24)	5(38.5)	0.045*	10.89	
24-33	20(40)	25(50)	3(23.1)			
34-43	10(20)	12(24)	2(15.4)			
≥44	3(6)	1(2)	3(23.1)			
Total	50(100)	50(100)	13(100)	113%		

(p-valu≤0.05)

4.2 Residence associated with Miscarriages:

Regarding the residence associated with miscarriage as in table (4-3),It was seen that the women live in city recorded high percentage in (once miscarriage 40(46%) and low rate in rural10(38.5%) 11%, In twice miscarriage the high rate 36(41.4) in city, low rate in rural 14(53.8%),In more than twice miscarriage 11(2.6%) in city and 2(7.7) in rural.

Table (4-3): Residence associated with miscarriage

Miscarriage	City No(%)	Rural No(%)	Total	P-value	Chi-square
Once	40(46)	10(38.5)	50(44.2%)	0.25	1.83
Twice	36(41.4)	14(53.8)	50(44.2%)		
More Than Twice	11(12.6)	2(7.7)	13(11.5%)		
Total	87	26	113%		

(p-valu≤0.05)*

4.3. Education levels with recurrent miscarriages:

High percentage of aborted women with primary education had been documented a high percentage rate of infection, In primary education the high rate was recorded in once miscarriage 20(40%) and the low percent in more than twice miscarriage 5(38.5%), In secondary education the high rate was in once miscarriage 16(32%) while, the low rate in more than miscarriage 6(46.2) and the higher education high rate in once miscarriage 14(28%) low rate in more than twice miscarriage 2(15.4%).

Table (4-4): Education associated with miscarriage

Education	Once No(%)	Twice No(%)	More Than Twice	P-value	Chi-Sqaure
Primary	20(40)	31(62)	5(38.5)	0.03*	9.051
Secondary	16(32)	7(14)	6(46.2)		
Higher	14(28)	12(24)	2(15.4)		
Total	50(100%)	50(100%)	13(100%)	113(100%)	

(p-valu≤0.05)*

4.4. using contraceptive drug in women miscarriage :

In this table(4-5) show using the contraceptive associated with miscarriage, most of them did not use contraceptive in this study, in once miscarriage was recorded high rate 32(46.4%) wich was not use and low rate18(40.9) using contraceptive, in twice miscarriage also the high rate was not use28(40.6) and low rate 22(50%)using the drug, in more than twice miscarriage was 9(13.6%) not use contraceptive and low rate using contraceptive 4(9.1%), with no significance differences.

Table (4-5): Contraceptive use associated with Miscarriage

Miscarriage	Contraceptive use		Total	P-value	Chi-square
	Yes(%)	No(%)			
Once	18(40.9)	32(46.4)	50(44.2%)	0.25	1.058
Twice	22(50)	28(40.6)	50(44.2%)		
More Than Twice	4(9.1)	9(13)	13(11.5%)		
Total	44(100%)	69(100%)	113(100%)		

(p-valu≤0.05)*

4.5. Type of Contraceptive associated with Miscarriage :

In this table (4-6) show the types of contraceptives was been used by miscarriage women who have abortions are explained, most of them were injection, in Once miscarriage the highest percentage was using injection 9(60%) and the lowest rate was using oral and condom 0(0%), twice miscarriage the highest rate was also injection 5(33%), while the lowest was intrauterine contraceptive device (IUD) 0(0%),and more than twice miscarriage high percentage injection 1(6.7%), low rate who use oral ,condom and IUD 0(0%) and in case of don't had non contraceptive was40(43.5%).

Table (4-6): Type of Contraceptive associated with Miscarriage

		Type of contraceptive						
Miscarriage	No Contraceptive No(%)	Oral No(%)	Injection No(%)	Condom No(%)	IUD No(%)	Total	P-value	Chi-Sqaure
Once	40(43.5)	0	9(60)	0	1(2)	50(100%)	0.15	7.711
Twice	40(43.5)	1(2)	5(33.3)	4(100)	0	50(100%)		
More Than Twice	12(13)	0	1(6.7)	0	0	13(100%)	113(100 %)	
Total	**92(100%)**	**1(100%)**	**15(100%)**	**4(100%)**	**1(100%)**	113(100 %)		

4.6.Vaginal discharge consistency associated with miscarriage:

Table (4-7) show and explained the consistency of vaginal secretions with miscarriage ,where in all abortions the highest percentage was the crud secretions and low rate was thick ,in once miscarriage the high rate crud 33(51.6) and the low rate was thick 7(33.3), in twice miscarriage highest crud 23 (35.9%) and lowest percentage thick 12(24%), in more than twice miscarriage the highest percentage crud 8(12.8%) and low rate thick 2(9.5%) with no significance different .

Table (4-7): Vaginal discharge consistency associated with miscarriage

Miscarriage	Consistency of discharge			Total	P-value	Chi-sqaure
	Thick% No(%)	Thin% No(%)	Crud% NO(%)			
Once	7(33.3)	10(35.7)	33(51.6)	50(44.2)	0.15	4.280
Twice	12(57.1)	15(53.6)	23(35.9)	50(44.2)		
More Than	2(9.5)	3(10.7)	8(12.8)	13(11.5%)		
Total	21(100%)	28(100%)	64(100%)	113(100%)		

(p-valu≤0.05)*

4.7 Color of discharge associated with Miscarriage:

In the table(4-8) was reported the color of discharge which was high rate colorless and low rate red color, in once miscarriage a high percentage in colorless discharge 24(40%) and low rate red(1%), in twice the highest colorless 31(51.7%) and lowest percentage red color was reported 0(0%), In more than twice miscarriage the colorless discharge was recorded a high rate 5(8.3%) and low rate brown color discharge 0(0%), with significance difference

Table (4-8): Color of discharge associated with Miscarriage.

Color Discharge	Once No(%)	Twice No(%)	More Than Twice No(%)	Total No(%)	P-value	Chi-sqaure
Colorless	24(40)	31 (51.7)	5(8.3)	60(100%)	0.1	11.7
White	5(33.3)	8(53.3)	2(13.5)	15(100%)		
Yellow	14(56)	8(32)	3(12)	25(100%)		
Brown	3(60)	2(40)	0(0)	5(100%)		
Green to Yellow	3(50)	1(16.7)	2(3.33)	6(100%)		
Red	1(50)	0(0)	1(50)	2(100%)		
Total	50(100%)	50(100%)	13(100%)	100(100%)		

(P-valu≤0.05)*

4.8. Bacterial vaginosis isolated in Miscarriage women:

Many bacterial vaginosis was been isolated from miscarriage women, in the table (4-9), was discommended the types, number and percentage of bacterial vaginosis, it was diagnosed from aborted women The highest percentage was *Klebsiella* spp. with 26(23%), while the lowest was *Acinetobacter* spp. *Enterobacter cloacae*, *Staphylococcus sciuri* and *Proteus* spp. 1(0.9%) with respectively.

Table (4-9): Bacterial Vaginosis isolates in Miscarriage women

NO	Bacterial vaginosis	Frequency	Percent %
1	*Neisseria* spp.	4	3.6
2	*Acientobacter* spp.	1	0.9
3	*Bacillus* spp.	4	3.6
4	*Klebsiella* spp.	26	23.6

5	*Escherichia coli*	25	22.7
6	*Staphylococcus sciuri*	1	0.9
7	*Micrococcus luteus*	5	4.5
8	*Enterobacter cloacae*	1	0.9
9	*Kocuria kristinae*	4	3.6
10	*Staphylococcus haemolyticus*	7	6.3
11	*Enterococcus faecalis*	9	8.1
12	*Staphylococcus aureus*	10	9.09
13	*Burkholderia cepacia*	3	2.7
14	*Pseudomonas* spp.	2	1.8
15	Staphylococcus *warneri*	2	1.8
16	*Enterococcus* spp.	2	2.7
17	*Streptococcus* spp.	3	2.7
18	*Proteus* spp.	1	0.9
Total		110	100%

4.9 Different pathogenic associated with miscarriage:

In the table (4-10) and the figure (4-6) explain the different pathogenic in aborted women, most them were mix infection between bacterial vaginosis, *cytomegalovirus* and *Trichomonas vaginalis*, in once miscarriage the high rate was mix infection 46(45.19) and low rate *cytomegalovirus* 1(50%), *Trichomonas Vaginalis* 1(50%), in twice miscarriage highest percentage mix infection 43(42.2%) and lowest percentage *cytomegalovirus* 1(50%), *Trichomonas Vaginalis* 1(50%), in more than twice mix infection recorded high rate 13(12.7) and bacterial vaginosis 0(0%), *cytomegalovirus* 0(0%) and *Trichomonas Vaginalis* low rate, with no statistical significance.

Table (4-10): Pathogen associated with Miscarriage

Pathogen	Once No(%)	Twice No(%)	More Than Twice No(%)	Total	p-value	Chi-sqaure
Bacterial vaginosis	2(28.6%)	5(71.4%)	0	7(100%)	0.35	3.137
Cytomegalovirus	1(50%)	1(50%)	0	2(100%)		
Trichomonas vaginalis	1(50%)	1(50%)	0	2(100%)		
Mix infection	46(45.19)	43(42.2)	13(12.7)	102(100%)		
Total	50(44.2)	50(44.2)	13(11.5)	113(100%)		

(p-value ≤0.05)*

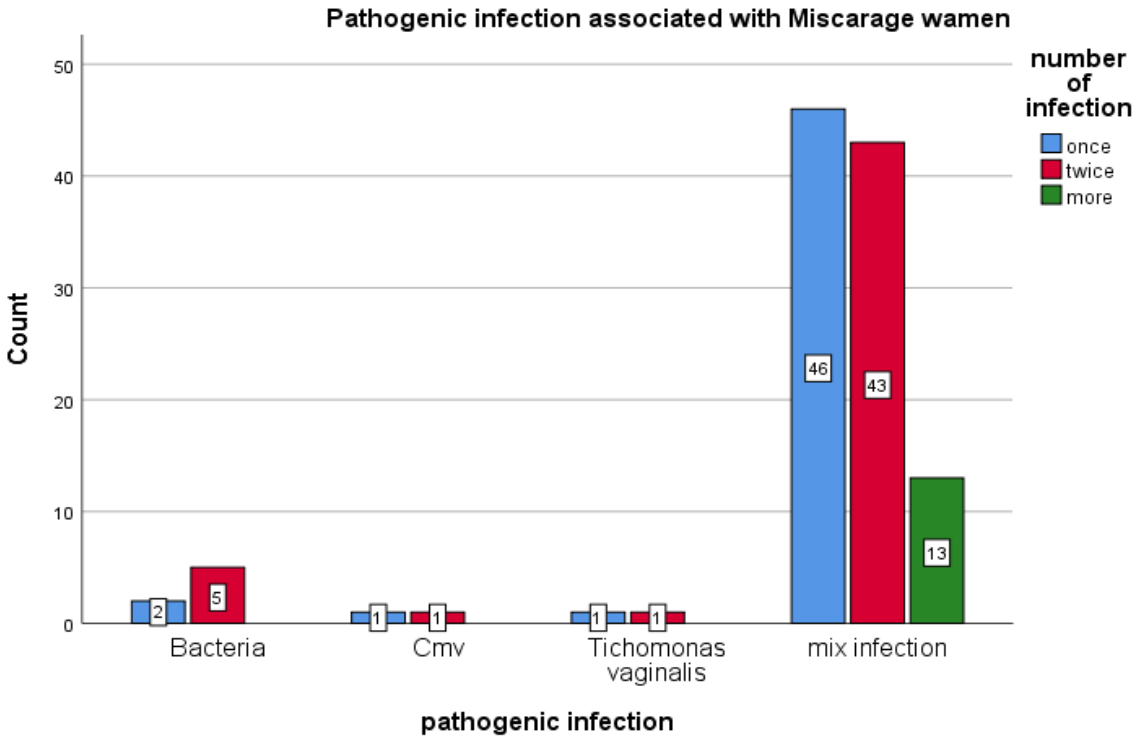


Figure (4-1): Pathogenic infection associated wih Miscarriage women.

4.10. Immunoglobuline (IgM ,IgG) of *Cytomegalovirus* in miscarage women :

In relation to immunoglobuline of this virus in aborted women. woman with immunoglobulin M positive indcates to acute infectious with viral,while Immunoglobuline G Positive stand for an chronic infection or re activation, mixture IgM positive, IgG positive mension to reactivation or new infected virus ,in once miscarriage the high rate was IgG 21(36.8%) low rate mix IgG-IgM10(50%), in twice miscarriage highest percentage was IgG 28(49.1%)while, lowest percentage IgG-IgM 7(35%), more than twice miscarriage the high rate was IgG 8(14%) low rate IgM 2(5.6%) as in table(4-11) and figure(4-2).

Table (4-11): Immunoglobulin types associated with of Miscarriage

Immunoglobulin	Once Miscarriage No(%)	Twice Miscarriage No(%)	More Than Twice No(%)	Total No(%)	p-value	Chi-sqaure
IgM	19(52.8%)	15(41.7%)	2(5.6%)	36(31.9%)	0.21	3.83
IgG	21(36.8%)	28(49.1%)	8(14%)	57(50.4%)		
IgG & IGM	10(50%)	7(35%)	3(15)	20(17.7%)		
Total	50(44.2%)	50(44.2%)	13(11.5%)	113(100%)		

(P-value≤ 0.05)*

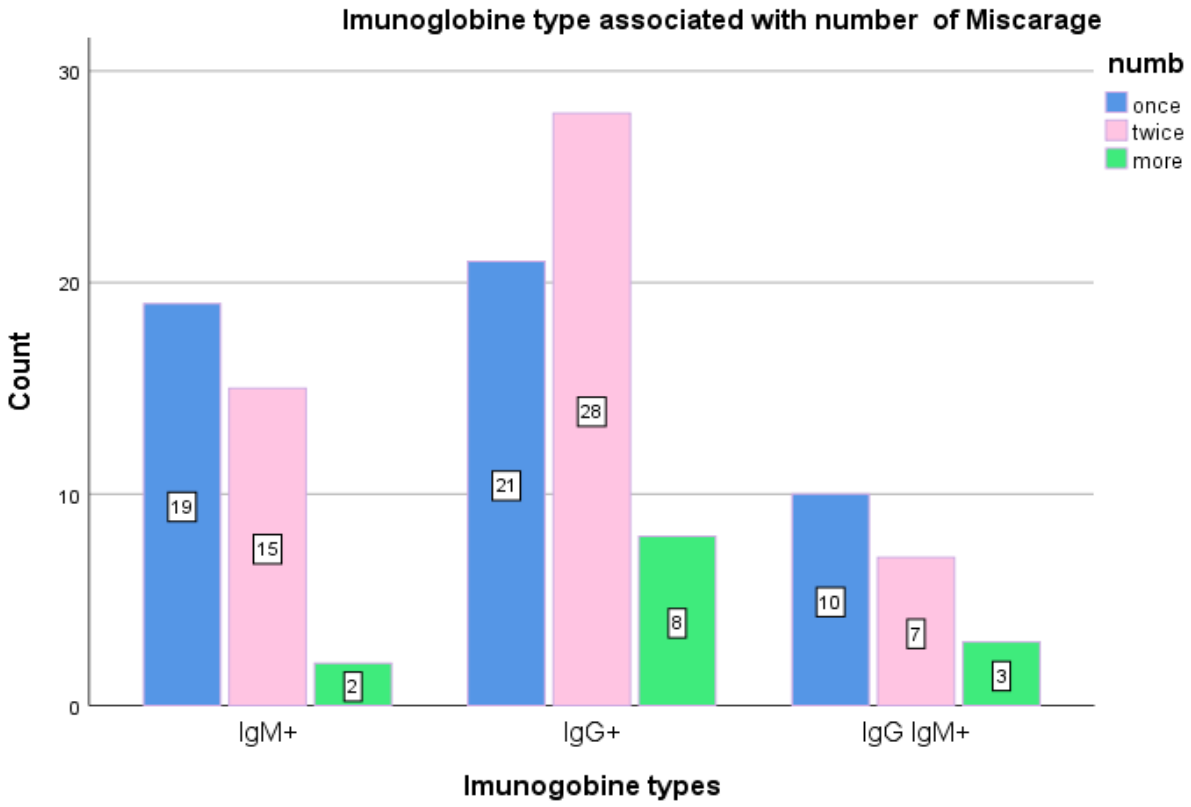


Figure (4-2): Immunoglobulin types of *CMV* in miscarriage women

4.11. PH level and type of pathogenic causing Recurrent Miscarriage :

PH is very important factors for growth of pathogenic table (4-12) and figure (4-3) was been reported in acidity PH level as a high rate, which most them more than 4.5,in once miscarriage acidity recorded high rate 36(40.9%) low rate alkaline 14(56%), in twice miscarriage highest percentage acidity 41(46.6%) lowest percentage alkaline 9(36%), in more than twice miscarriage high rate acidity 11(12.5%) while, low rate alkaline 2(8%).

Table (4-12):PH Levels associated with number Miscarriage

PH Level	Once	Twice	More Than Twice	Total	p-value	Chi-sqaure
Acidity	36(40.9%)	41 (46.6)	11(12.5%)	88(100%)	0.15	1.83
Alkaline	14(56%)	9(36%)	2(8%)	25(100%)		
Total	50(44.20%)	50(44.20%)	13(11.5)	113(100%)		

(P-value≤0.05)*

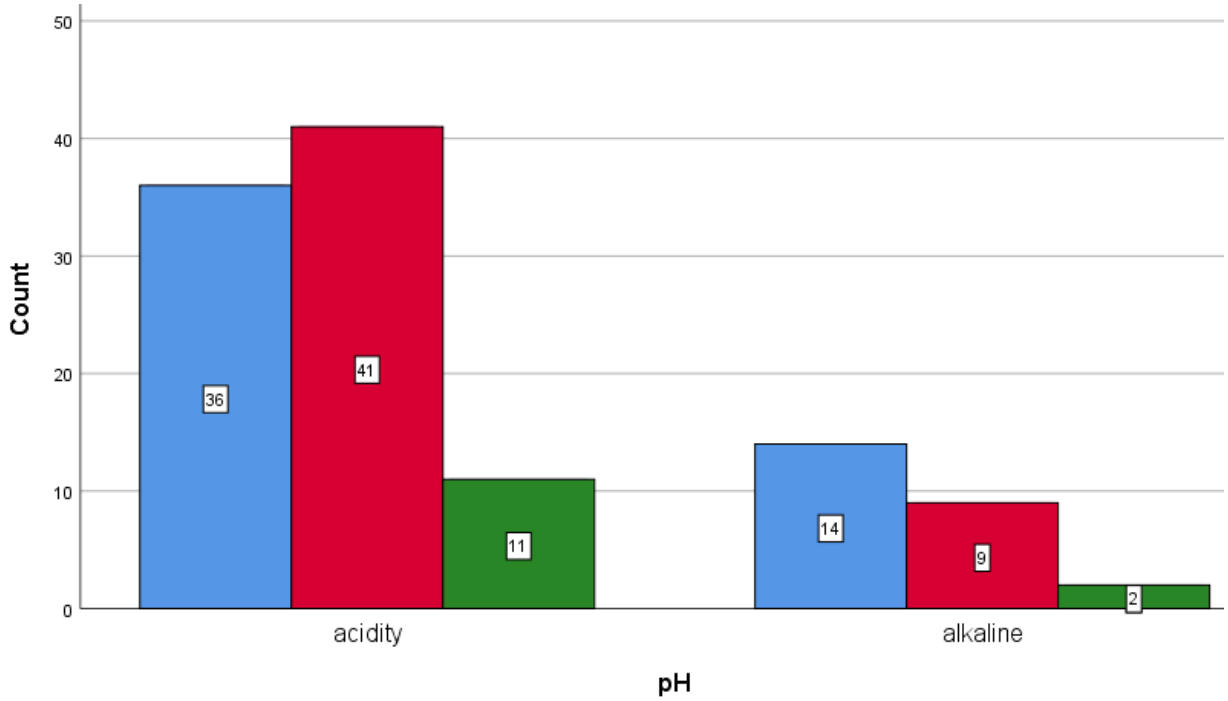


Figure (4-3): Percentage PH level in miscarriage women

4.12. Leukocytes count and whiff test and its relation with pathogen:

White blood cells refers as an immune cell for protect the body from any infectious diseases, in table (4-13) was reported a high rate with WBCs≥5, in Once miscarriage high rate recorded 44(43.1%) low rate when leukocytes less than five 6(54.5%), in twice miscarriage highest percentage when leukocytes more than five 46(45.1%) low rate 4(36.40)when leukocytes less than five, in more than twice miscarriage the high rate 12(11.8%) ≥5 and lowest percentage 1(9.1%)<5.

According to whiff test as in table (4-14), which recorded a high results of positive cases, in once miscarriages high rate was 45(45.9%) positive test while low rate 5(33.3%)negative test, in twice miscarriage highest percentage 41(41.8%) and low rate 9(60%)negative test ,in more than twice high rate 12(12.2%) positive test and lowest percentage 1(9.1%) negative test with no significance .

Table (4-13): White Blood Cells Count (WBCs) in discharge Miscarriage women

WBCs Count	Once No(%)	Twice No(%)	More Than Twice No(%)	Total No(%)	P-value	Chi Sqaure
WBCs<5	6(54.5%)	4(36.40%)	1(9.1%)	11(100%)	0.35	2.32
WBCs≥5	44(43.1%	46(45.1%)	12(11.8%)	102(100%)		
Total	50(44.21%)	50(44.2)	13(11.50%)	113(100%)		

(p-value≤0.05)*

Table (4-14):Whiff Test associated in discharge of miscarriage women

Whiff Test	Once No(%)	Twice No(%)	More Than Twice No(%)	Total No(%)	P-value	Chi-sqaure
Positive test	45(45.9%)	41(41.8%)	12(12.2%)	98(100%)	0.20	1.787
Negative test	5(33.3%)	9(60%)	1(6.7%)	15(100%)		
Total	50(44.2%)	50(44.2%)	13(14.5%)	113(100%)		

(P-value≤ 0.05)*

4.13 Clue cell associated with pathogenic in miscarriage women:

In the table (4-15) show the number of clue cell in wet amount and using Gram stain the number (0) main one to five(1) the number of clue cell between five to ten and (2)more than ten, the high perecent wae less than five 64(56.6%) and low percent was more than five 21(18%).

Table: (4-15) Clue Cell count in smear

Clue cell	Frequency	Valid Percent
0	64	56.6
1	28	24.8
2	21	18.6
Total	113	100%

Chapter five

Discussion

5 Discussion

5.1. Age groups with Miscarriage women

In the present study, different age group range from (14-45) years were reported in table (4-1) and the table (4-2)miscarriage women in the age group (24-33)year was reported a high rate of the miscarriage 48(42.5%) this a high percentage occur due to high level of estrogen hormone, elevated of pH level and change in secretion of glycogen in vaginal miscarriage women which lead to change of growth of pathogen miscarriage (Maria C *et al*., 2019).

In women, the age-related . decline in reproductive capacity is explained by a gradual decrease in . ovarian reserve and oocyte integrity (te Velde and Pearson, 2002). More frequent chromosome segregation errors result in oocyte ane- . uploidy, and this is thought to be primarily responsible for maternal . age-related miscarriage.

5.2. Education associated with Miscarriage women

Education is define a majority of them had low levels of education, such as read and write, primary, and intermediate schools, this finding is consistent with (Norsker *et al*., 2012) who found that women with low levels of education have a higher risk of spontaneous abortion (Nohr *et al*., 2006) study was based on a large population and a large number of spontaneous abortions the data show that women with less than ten years of education had a higher chance of spontaneous abortion than women with more than 12 years of education.

Women's education is one of the most important investments that can be made since it empowers women to postpone marriage and childbirth and provides them with the information they need to make informed decisions about contemporary contraception use(Tekelab *et al*., 2015).

Many governments now fund women's education in order to promote economic growth as well as smaller family sizes, better child health, and women's sexual reproductive health (modern family planning)(Rutaremwa *et al*., 2015).

The most common causes of miscarriage among women with primary education and poor hygiene include a shortage health conscious initiatives whereas, mothers who are educated are more concerned about their health.

5.3. Residence associated with Miscarriage women :

In accurate study in the city women with one miscarriage was recorded 40(46%) and the rural region 10(38.5%), and in twice miscarriage women in city was 36(41.4%)and in rural 14(53.8%) as in table(4-3) Some findings were made possible by the fact that city areas were becoming increasingly populated, and transportation was becoming more readily available, making it easier for people to obtain health care and this is agree with present study in rural areas, women who had spontaneous abortions were stigmatized, thus a huge percentage of them preferred to go to a midwife to get to their facilities care, this is in disagree with a study by (Carlson and Mourgova, 2003) who found that where you live affects your risk of miscarriage (David, 2000) found that women in cities reported a higher rate of miscarriage than women in towns and the rural and this agree with present study.

5.4. Type of Contraceptive associated with Miscarriage

In the table (4-6) was reported the effect of correlation between type of contraceptive and miscarriage in recurrent miscarriage. This study disagree with (David, 2000) who recorded the use of oral contraceptive don't effect on normal flora which permit another microorganisms as *Trichomonas vaginalis*, bacterial vaginosis and *Cytomegalovirus* therefore this disagree with present study which recorded injection contraceptive 9/113(18%) (Gupta *et al*., 2000) who suggested that oral contraceptive and it alter vaginal flora and his founded the count of *E. coli* was increased five times in women using oral contraceptives and these permit to initiate infection and lead to miscarriage this study agree with (Fiscina *et al*., 1973; Gupta *et al*., 2000) study associated between IUD and normal flora of vaginal is made from copper which toxic to vagina and change of bacterial vaginosis also these study disagree with this study, this study disagree with (Alkaisi *et al*., 1994) had seen a high ratio of *Trichomonas vaginalis* when IUDs were used as (34.61%),

contraceptive is a hormone which effect on normal flora and increased pathogenic microorganisms and lead to growth microorganisms in uterus.

5.5. Vaginal discharge consistency and color associated with Miscarriage :

In the table (4-7) vaginal mucosa, secretion vaginal discharge because of inflammation in epithelium mucosa, bacterial vaginosis, *Trichomonas vaginalis* and CMV reported to most common abnormal vaginal discharge or vaginal sign and symptoms in vagina (Pabich *et al*., 2003) the current results which roved a crud discharge was reported 33(51.6%) in one mischarge and thin 15(53.6%) in twice miscarriage in women was disagree with (Ranjit *et al*.,2018) the discharge differ in color and consistency this related with degree of inflammation , type of causative agents, hormones, contraceptive use, chronic diseases such as diabetes mellitus, douching ,smoking and others factors as douching perfumed and soaps the color of vaginal discharge in miscarriage related to causative agent, which causes vaginosis in the table (4-8) was recorded a high rate in colorless discharge color 31(51.7%) in twice miscarried (Walke *et al*.,1990)study the color and consistency of vaginal discharge haunted with *Trichomonas vaginalis* was receded vaginal discharge was (67%) as green-yellow and frothy this study disagree with present study. The color of discharge related with some causes as hemolysis of RBCs and releasing of Iron and gray color discharge related with mixed infection of *Trichomonas vaginalis* and bacterial vaginosis(Kadir and Fattah, 2010) were reported a related between discharge color and consistency with *Trichomonas vaginalis* with a great percenrtage was 6 (60%) in white to gray color discharge this is agree with this study.

5.6. Miscarriage with different pathogenic :

In first trimester of abortion about (50%) because some factors as Chromosomal abnormalities in fetus, urinary tract infection (UTI), genital tract infection and other factors as cervical abnormalities (1 pam IC), In this study bacterial vaginosis isolation from aborted women was 2/50(28.6%) in once miscarriage and 5/50(71.4%) in twice miscarriage

but a high rate was recorded in mix infection 46(45.19%) in one miscarriage women and 43 (42.2%) in twice misarrange women (Ranaldo *et al*., 2017) informed un natural birth as high rates 49.2 % and death foetal through delivery lower ratio 2.9 % percent, this linked with affect *Cytomegalovirus* on grow of embryo in uterus And causative moral virus inflectional, or weak immunology, have contraception, changing of natural flora return in uterine and vaginal drive to encourage viral to the increasing this results agree with (Natacha *et al*., 2014) was recorded in her study a high rate in premature delivery or abortion in 21 week from pregnancy.

This present results agree with (Omer and Ahmed.,1992), who found that *Staphylococcus aureus* are the popular etiological agent with sins of underside reproductive system Infection tracked by *Escherichi*a *coli* miscarriage women, in Iraq (Al-Mousawi *et al*., 2006), was record *Staphylococcus aureus* was the dominanting what colonizing membrane vagina and caused poisonous trauma Syndrome (Razzak *et al*., 2011)، report highest rates of bacterial pathogenic cause via gram negative bacterial as 12 16.2% and *Staphylococcus aureus* was 14 18.9%, this Study aligned at present result *Escherichia coli* was a reasoned factor of vaginitis because, *Escherichia coli* is normal flora at gastrointestinal tract and moved to venereal canal and creat bacteria vaginal this bacteria fermenting lactose to produce lactic acid, therefore it may be effected by the quantified of glycogen also, cleans underwear with strength detergents might be affect normal flora, poor body hygiene especially when using the toilet, finally antibiotics may conducive to vaginosis microorganism in vaginal and promote opportunism bacteria.

Regarding to *Trichomonas vaginalis* was reported 1/50(50%) in once miscarriage case and twice miscarriage *Traichomonas vaginalis* infection was been related an increased danger of preterm delivery and low delivery weight infants(Cotch *et al*., 1997) metronidazole successful treat *Trichomonas vaginalis*, but its use through conception is polemical due to, illustrated mutagenicity and cancerous in laboratories model(Burtin *et al*., 1995) reporte an analyzing of seven study, that propose metronidazole not increases the risks for childbirth defective in an

embryo through the first three months of conceive, metronidazole using currently advice just during the two and three trimester .

5.7. Immunoglobuline (IgM &IgG) of CMV in miscarage women :

Regarding to immunoglobuline of CMV in miscarage women .Women with IgM positive was report to recent infectious with *Cytomegalovirus* while, with IgG Positive indicating past infection or re-activation ,mixing immunoglobulins positive(IgM)and(IgG)indcate to reactivation or acute infection with virus, in this study higher level was record 19(52.8%) of IgM in once miscarrge but IgG was reporeted 28(49%) as in IgG in twice miscarrage and IgG and IgM was reported 10(50%) as in table(4-11).this study agree with (Gandhoke, 2009) was recordrd great rate with cytomegalovirus –IgG, 27 (21.6%) this study Disagree with this study , he reported larger rate with CMV-IgG 27(21%) (Salih and Kazhal, 2013) they studied human antibodies IgM and IgG seropositivly with pregenent woman and their correlation at aborting in 185 female experienced in Salaimani town, they observed 17 (9.18) % were positive anti *Cytomegalovirus* IgG, age related at Imnuogobuline because of woman was incfected in reprotective years group, variations hormones and others agent as in reccurent infection.

5.8 Bacterial Vaginosis associated with Miscarriage women :

Recurrent abortion was linked with bacterial vaginosis in women(Xia *et al*., 2015) percentage of this disease at this present study was 103(93.7%)as in table (4-10) some study show the percentage of other organisms (non-bacterial vaginosis) was (76.3 %)this present study agree with the conclusions reached by (Isik *et al*., 2016) who spotted related between bacterial vaginosis and recurrent spontaneous abortion according to, the findings of this study proportion of Gram positive bacteria isolation was 47 (43%) and the percentage of Gram negative bacteria isolates was 63 (57%) which contrasts with the findings of a study to

identify bacteria vaginal in ladies with repeating spontaneity abort, in which (Llahf *et al*., 2017) found a considerably larger frequent of bacterial vaginosis in female with recurring auto miscarriage (21 %) this data is agree with this study Also, (Ralph *et al*., 2009) was found that mothers at had history at the very least one delayed miscarriage had a highly greater epidemic p =0.001 of bacterial vaginosis (21.%)

The following were the total microorganisms recovered from women's miscarriages : *Neisseria spp*. 3.6%, *Acinetobacter spp.* 0.9%, *Bacillus spp*. 23.6%, *Escherichia coli* 22.7%, *Staphylococcus sciuri* 0.9%, *Micrococcus luteus* 4.5%, *Enterobacter cloacae* 0.9%, *Kocuria kristinae* 3.6%, *Staph haemolyticus* 6.3%, *Enterococcus faecalis* 8.1% *Staphylococcus aureus* 9.09%, *Burkholderia cepacia* 2.7%, *Pseudomonas* spp. 1.8%, *Staphylococcus warneri* 1.8%, *Enterococcus facium* 1.8 %, *Streptococcus* spp. 2.7%, *Proteus* spp. 0.9%.

Several authors have suggested that abnormal genital tract colonization can lead to an in-utero inflammatory, and that pathological bacterial in vagina at expectant mothers can result with complications like pre-mature tears of membrane, preterm birth, respiratory distress syndrome, or necrotizing enterocolitis (NEC) in preterm infant as *Enterococcus facealis*.

Escherichia coli, *Staphylococcus aureus*, and *Enterococcus faecalis* are common microbes found in an inflammatory vaginitis(Sobel *et al*., 2011) (Giovanini *et al*., 2019)

A significant incidence of *Escherichia coli* was been discovered in this study, which can colonize the vagina but also contribute to the replacement of native microflora by removing *Lactobacilli*. When compared to *Enterococcus spp*., aerobic vaginosis it less frequently identified, yet, it might cause frequent miscarriages, chorioamnionitis, and premature birth during pregnancy (Chmielarczyk *et al*., 2013) (Saez-Lopez *et al*., 2016;Numanovic *et al*., 2017)

This is disagree with this study was recovered *Klebsiella* spp. is more infection than a other bacteria in miscarriage women 26(23.6%).

5.9 PH associated with miscarriage women :

Vaginal pH is range between (3.8-4.2), it can fluctuate depending on vaginal microbial activity (Ma *et al*., 2012)additionally to bacterial vaginosis, vaginal pH can be raised by *Trichomonas vaginalis,* cervical secretions, contact with sperm, and the use of lubrication gels as a result, combining pH tests with other symptoms can improve results(Gutman *et al*., 2005;Simoes *et al*., 2006) when the pH of the discharge is low amines such as, trimetylamine (TMA) are rapidly released and dissolved as an acid in the discharge. In the current study vaginal discharge in one miscarriage 36(40.9%) and in twice miscarriage 41(46.6%)most of the samples were acidity with a pH of less than seven and samples that indicate bacterial infection with a pH of more than 4.5 by 67%, while the samples with a pH of more than 7 (23%) and the rest of the samples were with a pH of less than 4.5, which is the normal number for the natural environment of the vagina.

5.10. Leukocytes count , Clue cell and its relation with pathogen

White blood cells and vaginal epithelial cells or clue cell appear in wet amount technique have been accepted as a sign and symptoms these are useful for diagnosis of miscarriage depending on the absence or presence of infection and the ratio of leukocytes to vaginal epithelial cells in clue cells in three a high power field were examine at least WBCs $\leq$ 5 44(43.1%) in women with one miscarriage and 46(45.1%)in twice miscarriage women and more than miscarriage 13(11.50%). In this study is agree with(Geisler *et al*., 2004) who observed a high proportion of *Trichomonas vaginalis* in vaginal secretion and this agree with accurate study also, aerobic bacterial vaginosis associated with leukocytes count was documented as a high percentage with bacterial vaginosis when count leukocytes less than five was 204/78(5%) and this disagree with accurate study.

(Goran Lasson and Jens, 1991) found a high percentage of bacteria with more leukocytes than five was 19(36.5%) and count leukocytes less than five was recorded 33(63.5%) these results agree with this study the cases of increase of lead leukocytes and related to aerobic bacterial and *Trichomonas vaginalis* associated with humoral mediated immune

response could attenuate the inflammatory response which lead to increase the leukocytes count with miscarriage women.

5.11. Whiff test associated with miscarriage women

The test are release of a fish-like smell subsequent insert one drop of (10%) Potassium hydroxid is other standard for bacterial vaginosis prognosis, in this study was recorded a positive test 45(45.9%) associated with once miscarriage and 41(41.8%) in twice mischarge (Hallen *et al*.,1987) was found positive outcome for screening in 95 % of sick people, this study agree with this study was determined 45(45.9%) Positive test in one miscarriage women and 5(33.3%) in negative result .

A strong fishy odor is refer of a positive test result,this coclusion might suggest whether *Trichomonas vaginalis* or bacterial vaginosis. It`s doesn`t have used accurate evaluation mean of diagnostic *Trichomonas vaginalis* or bacterial vaginosis. It`s 1 of the 4 part from amsel criterion(Amsel *et al*.,1983)

odor mold fish is caused by TMA, which might be found in vagina specimen of bacteria vagina patients whiff test, which involves smelling directly from the speculum, was proposed by (Gardner and Dukes, 1955).

The sensitivity was 91.1 percent and the specificity was 61.2 percent in a research by (Thomason *et al*.,1990).

Chapter Six

Conclusion and Recommendation

Conclusion:

1. Injection contraceptive use before pregnancy is more effect on miscarriage.
2. The most common bacterial vaginosis were isolated from miscarriages women, *Klebsiella* spp. was documented 26(23.6%) .
3. Mix infection of bacterial vaginosis, *Trichomonas vaginalis* and *Cytomegalovirus* was recorded a high rate in once miscarriage 46(45.17%).
4. Immunoglobulin IgG was been reported a high rate in twice miscarriage 28(49.1%).

Recommendation :

1. Identification of bacterial vaginosis isolated from vagina by molecular and phylogenetic level .
2. Determination genes, which responsible on a virulence factors of bacterial vaginosis associated with miscarriage.
3. Study the relation between *Trichomonas vaginalis*, bacterial vaginosis and *herpes* virus .
4. Determination virulence factors of *Trichomonas vaginalis* which related with miscarriage .
5. Study the relation between bacterial vaginosis associated and opportunistic fungi .
6. Detection of genotypes of bacterial vaginosis in recurrent miscarriage women .

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Appendixes

Questionnaire paper(Appendix(1))

The Name: **Age**
Academic Achievement:

Primary Secondary High

Diseases:

Diabetes Blood Pressure other diseases

Contraceptive Use: yes No

The Type of means used:

The Number of miscarriage:

Once **Twice** **More than**

Symptoms and signs: Fever Pelvic inflammation
Belly Pain

Arthritis Premature birth dysury bleeding

Infected Case: Pregnant Miscarriage
Other than that

Immunological examination:

IgG IgM & IgG-IgM

Whiff Test:

PH KOH Clue cell WBCs Discharge Color
Discharge

Microscopy:

Wet amount Gram stain Bacteria culture

Biochemical tests:

Catalase test: Urease test:
Oxidase test:

الخلاصة

يعتبر الاجهاض من أكثر المشاكل شيوعا عند النساء الحوامل وهو فقدان تلقائي للحمل قبل(12) أسبوعا ويسمى الإجهاض المبكر أو خلال (12–24) أسبوعا ويسمى بالإجهاض المتأخر. ويحدث في واحده من كل خمس حالات من النساء يعرف الإجهاض المتكرر بأنه حاله مرضيه نتيجة إجهاضين متتاليين أو أكثر قبل الأسبوع العشرين من الحمل وتحدث حالات الإجهاض المتكرر في حوالي (5%) من النساء الذين يحاولن الحمل .

تم جمع حوالي 339 عينه من مستشفى الصدر التعليمي محافظة ميسان / العراق226 مسحه من عنق الرحم و 113عينة دم من النساء المجهضات في قسم النسائية والتوليد و وفقآ لحجم العينة التي كانت مسحات من عنق الرحم ودم خلال شهر تشرين الثاني 2020 الى شهر أيار 2021 وفي هذه الدراسة تمت دراسة مجموعه من المتغيرات التي ترتبط ارتباطا وثيقا منها العمر و التعليم ومكان السكن والامراض المشتركة مع الإجهاض .

تم إجراء التحليل الإحصائي باستخدام برنامج ال SPSS الإصدار(23) وأستخدم أختبار مربع كاي لتحديد العلاقة بين معدل الأصابة وبعض العوامل واعتبرت ايضا قيمة $P < 0.05$ ذات دلاله إحصائية . تم تحديد العلاقة بين الفئات العمرية (14_45)سنه مع الإجهاض حيث سجلت أعلى نسبة للفئة العمرية (24–33) بمعدل 48 حالة وبنسبة 42.5% أما أقل نسبة كانت للفئات العمريه الأكبر سنا (45) في هذه الدراسة حيث بلغت 7% سجل التعليم الابتدائي اعلى نسبه حيث بلغ 59% واقل نسب في التعليم العالي 12% في ما سجل الإجهاض المتكرر نسبة اعلى بلغت 55% مقارنة مع الإجهاض لمره واحده 44%.

تعتبر الالتهابات المهبلية البكتيرية مشاكل صحيه تؤدي الى مضاعفات ونتائج طبيه خطيره وتحدث الالتهابات بسبب زيادة انواع معينة من البكتريا الممرضة على حساب البكتريا غير الممرضة و من بين الالتهابات البكتيرية *Ureaplasma urealyticum* و *Mycoplasma hominis* . تم الحصول على مسحات من عنق الرحم لتشخيص البكتريا المرضية الموجودة عند النساء المجهضات النساء وباستخدام طرق الفحص المجهري وطرق التصبيغ بصبغه غرام والفحص المجهري المباشر كما تم زراعة المسحات على اوساط زراعية مختلفة وتم أجراء بعض الاختبارات البيوكيماوية و تم تشخيص النهائي بأستخدام جهاز الفايتك حيث كانت أعلى نسبة من الأنواع البكتيري المرضية هي بكتريا الكلبسيلا *Klebsiella* spp. (23) وأقل نسبه هي الأنواع*Acinetobacter* spp. ، *Staphylococcus sciuri*، *Enterobacter cloaca*و *Proteus* spp. بنسبة 0.9%.و تم توثيق الالتهاب البكتيري

ببكتريا العنقودية الذهبية *Staphylococcus aureus* بنسبة(9%) كذلك الالتهاب البكتيري اللاهوائي ببكتريا النايسيرا .Neisseria spp بنسبة(3.6 %) . يجب أخذ كل من العمر وطريقة منع الحمل في الاعتبار عند تشخيص الجراثيم المهبلية غير الطبيعيه حيث كانت نتائج النمو ايجابيه اكثر للنساء اللواتي أستخدمن موانع الحمل.

فيما يتعلق بالغلوبيولين المناعي للفيروس المضخم للخلايا *Cytomegalovirus* سجلت أعلى نسبة للغلوبيولين المناعي IgG بنسبة(86%) وهو يشير إلى إصابة قديمة (سابقة) وقد يتمكن الفيروس من أعادة التنشيط بينما سجلت أقل نسية للغلوبيولين المناعيIgM بنسبة(3.7%)وهو يشير الى الأصابة الحديثة.

تم تشخيص الفيروس بأستخدام الفحص السريع كفحص أولي بعد ذلك أستخدام جهاز الميني فيداس Minividas كفحص تأكيدي حيث تم فصل عينات الدم باستخدام جهاز الطرد المركزي وقياس نسبة الغلوبيولينات المناعية ال IgG والأجسام المناعية المضادة ال IgM التي تشير الى الإصابة الحديثة والتي كانت نسبتها(4%)في هذه الدراسه . كما يمكن إجراء فحص ال Avidity بالاضافه الى فحص الأجسام المضادة من اجل قياس قوة الأجسام المضادة فهذه القوة تزداد كلما مر وقت أكبر من وقوع الاصابه.

فيما يخص داء المشعرات المهبلية وهو من اكثر الأمراض المنقوله بالاتصال الجنسي في جميع أنحاء العالم تم تشخيص (49) أمرأة مُصابه بهذا الطفيلي من بين 113 من النساء المُجهضات عن طريق الفحص المجهري المباشر ومشاهدة حركة الطفيلي تحت المجهر الضوئي (قوة تكبير40x بواسطة تحضير عدة شرائح لكل عينه, كذلك تم أستخدام صبغتي غرام وكيمزا ومشاهدة الطفيلي كذلك تم أستخدام وسط Amies transport media ووسط الInPouch وحضنت عند درجة حرارة 37م وفحصت يوميا لمدة 5 أيام لمراقبة نمو الطفيلي ولتأكيد نتائج الفحص المجهري.

كشف الألتهاب المهبلي البكتيري و الفيروس المضخم للخلايا البشري وداء المشعرات المهبلية في النساء المجهضات في محافظة ميسان

رسالة مقدمة إلى

مجلس كلية العلوم – جامعة ميسان

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل الطالبة

حوراء ضمد حميدي

بكالوريوس علوم الحياة / جامعة ميسان 2012

بإشراف

أ.م.د. زهره عدنان داخل

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