Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Misan

College of Science

Department of Biology

Epidemiological and Molecular Study of *Trichomonas vaginalis* Parasite among Women in Maysan Province, South of Iraq

A thesis

Submitted to Council of the College of Science / University of Misan as Partial Fulfillment of the Requirements for the Master Degree in Biology

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October 2020 A.D. **Rabi` al-Awwal 1442 A.H.**

بِسْـــــمِ اللَّهِ الرَّحْمَـنِ الرَّحِيـمِ

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

سورة المجادلة

(الآية 11)

صدق الله العليّ العظيم

Supervisor 's Certificate

We certify that this thesis entitled " Epidemiological and Molecular Study of *Trichomonas vaginalis* Parasite among Women in Maysan Province, South of Iraq "

" has been prepared under my supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology "

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DEDICATION

To my grandfather

The Prophet of mercy and the last of messengers "Muhammad"

To my Brother's soul

The martyr Ahmed khudair saad al-majidii

To my parents who inspired strength

despite many obstacles in my life

To my brothers who supported morally

To my children who have always been a source of my happiness

With my eternal gratitude

Noor

Thanks and appreciation

My first thanks go to our Almighty God who gave me patience and knowledge to complete my research.

To my supervisor Assis. Prof. Dr. Hussain Ali Mhouse Alsaady and Dr. Aswan Al-Abboodi for their continued encouragement, unlimited assistance and valuable advice in all stages of the research work.

To the staff of the Faculty of Science at the University of Misan to give me the opportunity to join graduate studies.

To M.Sc. Shaima Rabeea Banoon College of Science, University of Misan, to help her me.

To M.Sc. Sadeq Sabeeh College of Science, University of Misan, for his help me.

To M.Sc. Karwan Azeez Mohammad Shekhany, kirkuk province, for his help me in submitting the genes into the Genbank.

To MSc. Hussein A. al-Quzweeni College of Science, University of Misan, for his help me.

To the staff of Al-Kahla General Hospital, Especially Dr. Abdul Amir Al-Maliky to his help me.

To the staff of the health house in Awfiyah, to help me collect samples.

To Mr. Mounir Shkairat, to help me bring work materials.

Finally, I would like to thank all persons whom they help me in any way.

Noor

Summary

Trichomonas vaginalis is one of the most widespread parasite, it which causes trichomoniasis. It cause some serious health consequences for both genders in all world's regions, especially in poor regions. The current study has been conducted to investigate some epidemiological factors and molecular characteristics of *T. vaginalis* among women in some regions of Maysan province in southern Iraq.

The present study data was collected during the period from November 2019 to February 2020, two hundred twenty six samples of high vaginal swabs were taken from women at ages ranged from (5 to 60) years who visited the gynecological clinics in hospitals, health centers and medical clinics in several regions of Maysan Province, including the districts (Al-Amara / Al-Kahla / Al-Maymouna / Al-Majar Al-Kabir).

Data including some sociodemographic factors were taken in a questionnaire sheet , such as age, marital status, educational level, profession, residence, marital status of the husband, ...etc., and some clinical manifestations such as pH vaginal secretion, itching, burning micturition and color of the urine, etc. Samples of high vaginal swabs were examined under light microscope (40X) in two ways: the first is direct microscopic examination by preparing six slides for each sample, three slides was stained with Giemsa stain and three slides without stain, the second swab was cultured in amies transport media, and all culture media were incubated at a temperature of 37°C and examined every two days to confirm the microscopic examination results.

The results were analyzed statistically analysis by using SPSS version (24). Chi-square test (χ^2) for identifying the relationships between infection, sociodemographic and clinical factors among examined women. The results of the microscopic examination of the study population showed that the overall infection rate (IR%) was 75.22% (170/226). AL-Kahla district show the highest IR (96.15%), and age group (34- 40) years has a highest IR (86.95%). The IR (80.92%) among the married women (80.92%) is higher

than that of unmarried women were (40.62%), Non-pregnant women were (81.11%) is higher than that of pregnant women (78.57%). In another hand , it shows the infection is related with education level, for this shows the illiterate level of participates women had the highest IR (77.67%) than others levels, and the housewives women had the highest IR (78.00%) than others occupations status. The women of polygamous husbands have a higher IR (80.67%) than women who have monogamous husband. The group of women with (4-6) births have the highest infection rate (83.82%) and the rural s women had higher IR (81.53%) than the urban ´s women. Women have husbands sexual disease with trichomoniasis were recorded IR (87.50%) compared with women that have husband s without sexual disease. The infection rate is highest among women who have aborted three fetus (100%), than others. The results show the women who using other's tool have a high IR (77.27%). The IR among women don't use a treatment (73.84%) is high than women have treatment.

Regarding to clinical manifestation was used as an indicator for infection. Vaginal pH has a significant role to protect vaginal women from trichomoniasis, highest rate of infection was recorded (96.63%) with a pH(6), while no infection at pH (4). Vaginal discharge associated with *T. vaginalis* was recorded (89.40%) , compare with no vaginal secretions (46.66%). Itching is one of clinical manifestation of trichomoniasis was reported a high IR (77.72%), compared with no itching symptoms (60.60%). Burning micturition was recorded a high rate (76.27%) while women with lower abdominal pain were reported (80.00%). The color of urine was dark yellow and yellow urine (83.56% and 81.10%) respectively.

Finally DNA extracted and the amplified with PCR technique, using BUTB1/2 of seven pure isolates of *T. vaginalis* were taken from different regions of Maysan province and matched with data bases of NCBI bank showed Maysan isolates matched the (L05468.1, XM-001321203.1, XM-001311949.1 , XM-001582993.1, JF513200.1, XM-001284521.1 and JX399872.1) strains of what registered in gene bank with identifying ranged between 90.43% to 100% these consider a first record in Iraq and in Maysan Province.

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List of Abbreviations	
Meaning of	**Term**
AIDS	Acquired immune deficiency syndrome
AP	Adhesion proteins
ANOVA	Analysis of Variance
Et al	And other
CDC	Centers for Disease Control and Prevention
CPLM	Cysteine Peptose Liver Maltose
DNA	Deoxyribonucleic acid
ePKs	eukaryotic protein kinases
HIV	Human immunodeficiency virus
HVEC	Human Vaginal Epithelial Cells
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IR	Infection Rate
IFN-δ	Interferon – gamma
IL-8	Interleukin 8
LPG	Lipophosphoglycan
MN	Machery-Nagel
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Centre for Biotechnology Information
NAAT	Nucleic acid amplification test
PEM	Plastic Envelope Medium
PCR	polymerase chain reaction
PMNs	Polymorphonuclear leukocytes

RBCs	Red Blood Cells
RNA	Ribonucleic acid
STD	Sexually transmitted disease
STI	Sexually transmitted infections
SPSS	Statistical Package for Social Science
Th1	T helper cells type 1
TVCP3	*T.vaginalis* Carbamoyl phosphate synthetase
UV	Ultra violate
VECs	Vaginal epithelial cells
WHO	World Health Organization

CHAPTER ONE

Introduction

1. Introduction

Vagina has a naturally balanced environment with an acidic environment, where the pH is ranging from (3.8 to 4.5), this may due to presence of natural bacteria of *Lactobacillus* spp. Which was a positive role for protecting the vagina from pathogens such as *Trichomonas vaginalis* and vaginitis bacteria (Adnan and Marjani, 2020). *Trichomonas vaginalis* (*T. vaginalis*) is an obligated parasite and human the only host of *T.vaginalis* by transfer from person to person by sexual intercourse or sexual route. the only host to this parasite is the human body (Riestra *et al.*, 2019). It can be recognized from its shape which is found as an oval or pear shape and sometimes, it has the shape as amoeba when attached to the epithelial cells of the vagina **(**Mahmud *et al*., 2018). The human body is occasionally exposed to many pathogens which can infect different organs and tissues. The human reproductive system is one of these system that may be infected with many pathogens like *T. vaginalis* that causing many sexually transmitted diseases (STD) (Brunham and Paavonen, 2020).

Trichomoiasis is a very common non-viral sexually transmitted disease (STD) (Trein *et al*., 2019). It infected both sexes, male and female of all age groups, adults or children in different regions around the world either rich or poor, places that cannot make a difference in distributing this species of parasite (Morris *et al*., 2019). *T. vaginalis* is first recognized as causing trichomoniasis in 1916 (Stephen and Richard, 2001). *T. vaginalis* can be lived in vagina, urethra, endocervix or prostatic tissues (Kučerová, 2019). It can be transmitted between male and female by vaginal sexually intercourse and sometimes by using comminuted

fomites (Ferré *et al*., 2019). Several studies have reported that the infection rate (IR) of *T. vaginalis* increased with the infection of bacterial vaginitis and causes genital inflammation that may be increased the risk of infections with either HIV or (HSV-2), thus increasing the risk of transmission to the sexual partner (Deivam *et al*., 2014; Rostami *et al*., 2017; Shipitsyna *et al*., 2020), *T. vaginalis* is responsible for some medical complications in both sexes, but the infection rate is more in females compered to males (Korich *et al*., 2020).

The world infection rate (IR) of *T. vaginalis* is estimated between 60-80% (Klavs *et al*., 2019) with an annual incidence reached to 284 million cases (Chemaitelly *et al*., 2019). The most vulnerable organs to be exposed to the infection in females with *T. vaginalis* are the lower organs of the genital tract such as the vulva, vagina, cervix or urethra (Abdool Karim *et al*., 2019).The main symptoms of Trichomoiasis in female is abnormal vaginal discharge , which was a frothy yellow-green in colour (Rao & Mahmood, 2020), with an unpleasant odor like fishy smell, soreness, swelling and itchiness around the vulva or the vagina, with pain or discomfort in urination or sexual intercourse (Itriyev, 2020). There are many techniques to culture the *T.vaginalis* like InPouch media is a selective media , the PCR and gene sequences are a best choice techniques for diagnosing and classified *T. vaginalis* (Paxton *et al*., 2019). A wet amount technique of vaginal swabs is the most method easiest that used in diagnosis the vaginal trichomoniasis (Al-Mamoori *et al*., 2020). The culture media of vaginal discharge on culture the media had a sensitivity reached to 63.0–98.2% and specificity reached to 99.4 – 100% (Smith *et al*., 2005).

Metronidazole (Flagyl) is an effective antibiotic and the best choice to treat the trichomoniasis in all the around of the world, especially in the

USA (Workowski & Berman, 2010). It was taken as a single dosage of 2gm per day for 7days, or 500mg twice daily for 7days (Workowski and Bolan, 2015). It is estimated that 2.5–5% of all treated trichomoniasis cases were exhibited resistance to metronidazole (Tien *et al*., 2020). When a treatment is not taken in an accurate dose, this may lead to some other complications including chronic urethritis, epididymitis or infertility (Dutta *et al*., 2020).

Describing the genome of the *T.vaginalis* parasite, which is considered a human pathogen through sexual contact, repetitive elements make up about two-thirds of the base genome of 160-megabase genome, which indicates the high breadth of the genetic material for this parasite, therefore, there is an urgent need to use the molecular techniques to diagnose the parasite more accurately (Carlton *et al*., 2007).

1.2: The aim of study:

Despite the importance of the study of *T. vaginalis*, Iraq lacks in-depth studies that deal with many aspects of the life of this parasite and the extent of its spread, however, in Maysan province studies are almost non-existent, there is only one study in the province in which the parasite was not given much attention because the study was related to the other pathological factor such as bacteria, fungi, …ect.

It is important to make it clear that Maysan province lacks a focused study on this parasite, therefore, the present study is the first study in this province that deals with many aspects related to *T. vaginalis* especially in regards to study the parasite within a molecular level, and thus the current study aims to:

1- Determining Infection Rate (IR) of *T. vaginalis* parasite among women using a wet mount technique of vaginal discharge and endocervical specimen and using swap culture media to incubate and culture the specimens especially the negative cases.

2- To obtain pure culture of *T. vaginalis* use a selective media like InPouch ^{TV} broth .

3- PCR and gene sequences was used to identify a distribution of *T. vaginalis* strains in Maysan province from symptomatic and asymptomatic women by with use a special primers like BTUB1/2 gene.

CHAPTER TWO

Literature Review

2. Literature Review

2.1: History of *Trichomonas vaginalis*

Trichomonas vaginalis (T.vaginalis Alfred Donne) is from flagellated parasite family: Trichomonadidae, Order: Trichomonadida, Class: Parabasaila. It is considered as a commended species with the human vagina (Squire, 2018). Subsequently, the idea of *T. vaginalis* is a major pathogen and it is responsible for causing vaginitis and cervicitis in woman and urethritis in females & males (Jarrett *et al*., 2019).

The history of *T.vaginalis* from its discovery to identify its genome can be summarized in table (2-1).

Table (2-1): The history of *T.vaginalis*.

The history of *T. vaginalis*	
1836	**Alfred Donne** is a first scientist was identified this parasite as a moving organism under a microscope when examining vaginal secretions of women suffering from genital irritation, (Donne, 1836).
1916	**O. Hoehne** is a first scientist give a term trichomoniasis when he was identified the clinical symptoms caused by the *T. vaginalis* parasite, when it colonized the mucous membrane of the vagina, **(**Hoehne, 1916).
(1934-1939)	**L. Procaccini** is the first classify the *T. vaginalis*, cause a sexually transmitted disease (STD), (Woike *et al*., 2015).
1940	**R.E. Trussell** is a first scientist use a culture medium method to diagnose *T. vaginalis* from secretions of women, (Asmah *et al*., 2018).
1959	**D.H. Clark and E. Solomos** were developing and promote the culturing of vaginal specimens for diagnosis, (Edwards *et al*., 2016).

1960	The first scientist was used a nitroimidazole as medication for treatment the trichomoniasis, (Jaloob Al- Janaby *et al*., 2018).
(1980-2000)	The use of molecular biological techniques and immunological methods in the diagnosis of *T. vaginalis*, (Jewarethnam, 2014).
2007	The project of the *T. vaginalis* genome sequencing used whole-genome shotgun methodology found the G3 strain of *T. vaginalis* genome consisted of 160Mbp (Harp & Chowdhury, 2011).

2.2: General morphology of *T. vaginalis:-*

T. vaginalis is flagellated protozoan extracellular parasites that lives in anaerobic conditions, *T. vaginalis* caused a vaginal infection or trichomonasis (Hinderfeld & Simoes-Barbosa, 2020). It is one of the main causes of STD (Margarita *et al*., 2020). *T. vaginalis* is infecting the urogenital tract such as the vagina, urethra, endocervix of the females and in prostate tissues, seminal vesicles, and urethra of males (Pekmezovic *et al*., 2019). Some morphological, genetic, molecular, pathogenicity and metabolism features are summarized in table (2-2).

Table (2-2): Features of *T. vaginalis.*

Features of *T. vaginalis*	
Morphology	**Shape:-** Amoeboid or pyriform (pear shaped). **Size:-** 9-23x7µm average 13µm **Flagella:-** Anterior four and recurrent one. **Internal organelles**:- Nucleus, axostyle, costa, pelta, cytoskeleton and hydrogenosome (Arbabi *et al*., 2018).
	176,441,227bp (strain G3), Six chromosomes. **65% Repetitive genome.** **32.7% Guanine and cytosine** (G + T) rich regions. **65 Genes with short introns.**

Genetics	**60,000 Protein** coding genes**.** **74 Functional** core histones. **5-Spliceosomal small nuclear RNA** (snRNA-U1, U2, U4, U5 and U6). **250 Ribosomal DNA** (rDNA). **Transfer RNA** (tRNAs) for all 20 amino acids. **927 Protein kinase** coding genes (ePKs), (Puente-Rivera *et al*., 2017).
Pathogenicity	**Surface proteins**:- 3000 grouped into three major categories (BspA-like proteins, GP63-like proteins, and adhesions) (Hernandez *et al*., 2014). **Adhesion proteins**:- Adhesion with host vaginal epithelial cells (VECs) through adhesion molecules (AP65, AP51, AP33, AP23), fibronectin binding protein, laminin binding protein and glycolipids Lipophosphoglycan (LPG) (Figueroa-Angulo *et al*., 2012).
Metabolism	**Energy sources**:- Carbohydrate by fermentative metabolism, and amino acids through arginine dihydrolase metabolism; enzyme amino-transferases and glutamate dehydrogenase synthesizes glutamate, aspartate, alanine, glutamine and glycine; enzyme cysteine synthase synthesizes cysteine; synthesize proline from arginine; synthesize phospholipids; metabolize threonine (Ugbede, 2019).
The stage	**Only a trophozoite stage**:- No cystic stage was known (Narayankhedkar *et al*., 2015).

T. vaginalis takes an oval or pear shape and sometimes this parasite is like shape amoeba when attached to the epithelial cells of the vagina (Mahmud *et al*., 2018).

T. vaginalis (figure 2-1) size was ranging from 7-32 x 5-12μm (long x wide), it has 4 free anterior flagella and the 5th flagellum return along the

edge of the undulating membrane and the end posterior of 5th flagellum located in the middle of the body (Roberts and Janovy, 2009).

Axostyle of *T. vaginalis* is very clear and its length is about 3-14μm. The undulating membrane extends about (2/3) of the parasite's body length with no free whip. The flagella and axostyle form a bundle of fine motor tubes interconnected to support the parasite's body. The axostyle is used by the parasite to penetrate the host cells and damage of the host's tissues. This is observed in acute trichomoniasis infections. The flagella and axostyle are the main features of diagnosis *T. vaginalis* (Owino, 2020).

In the front of *T. vaginalis* a present a nucleus is enclosed in a container covering on the follicle and contains rod-like structures and also has a large number of hydrogenosomes that are known, which appear clearly around the axis (Schneider *et al*., 2011).

This parasite does not have mitochondria in the cytoplasm, Therefore, hydrogenosomes replace the mitochondria and appear quite clearly in the important processes of metabolism of cells where they participate in energy production and drug activation. These bodies have a round oval shape that produces the hydrogen molecule as a final product of the metabolism in the parasite's body (Tachezy, 2019).

The cytoskeleton of this parasite consists of tubulin and actin fibers, there are different types of tubulin found in the cytoskeleton composition of the trichomonad cell. The actin fibers which isolated from protozoan parasites were different from the actin of vertebrates like human, sheep and pigs (Rath and Gourinath, 2020).

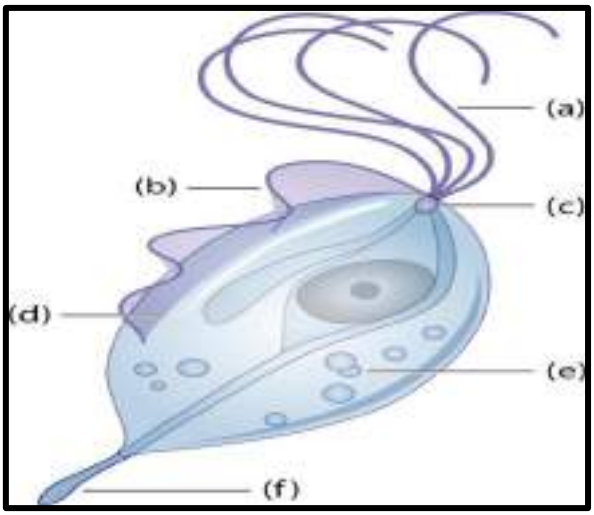


Figure (2-1): Diagram of *T. vaginalis:* (a) Anterior flagellum, (b) undulating membrane, (c) pelto, (d) costa, (e) hydrogenosomes, (f) axostyle (Bouchemal *et al.*, 2017).

2.2.1: Structures and functions of microtubules

The microtubule is a filamentous intracellular structure that is responsible for many types of movement in the cell. The real nucleus participates in the process of cell division and nuclear division. It organizes and arranges the internal cell structure and internal cellular transport and the movement in both cilia and flagella (Van Hooff *et al*., 2019).

This noble combination of the alpha and beta-tubulin units, which are basic structures in the cytoskeleton, flagella, and mitotic spindle, as well as being a necessary target against benzimidazole (Atherton *et al.*, 2017). The role of microtubules in this parasite, its expressive regulation, and basis for its sensitivity to benzimidazole and these are characteristics of the transmission of beta-tubulin genes (Trein *et al.*, 2019).

Which consists of genome DNA encoded by bacteriophage and the polymerase chain reaction PCR of DNA amplified by conserved beta-tubulin gene primers. The DNA stains indicate the presence of two different strains, as there are 6-7 types of gene copies of beta-tubulin, where the sequence has identified three distinct genes (Tan and Phyo, 2020).

2.3: Classification of *T. vaginalis*

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

Domain: Eukarya
Kingdom: Protista
Phylum: Metamonda
Sub phylum: Trichozoa
Class: Parabasaila
Order: Trichomondida
Family: Trichomonadidae
Genus: *Trichomonas*
Species: *Trichomonas vaginalis*

2.4: Life cycle of *T. vaginalis*.

This parasite has a simple life cycle, no cysts stage in its life cycle, the trophozoite stage is the only stage of this parasite and it is responsible for the transmission and infection, and it the diagnostic stage for this parasite (Beri *et al*., 2020). This parasite cannot survive for a long time outside the human body. Human being is the only host of this parasite, where the infection is transmitted through coitus and rarely by the fomites (Valenti *et al*., 2018). The life cycle of this parasite is missing the reservoir and vectors hosts, despite it is the process of cell division was clearly described by using the microscopic method, the life cycle of this parasite is still not well defined, same as many other protozoa because it lacks the cyst stage and has only the trophozoite stage (Kusdian, & Gould, 2014).

This species lives in the urinary tract and prostate in males and in the lower genital tract in females and is the most common in terms of injuries where bilateral longitudinal fission is a method of sexual reproduction and thus transmitted from the affected person to the healthy person through sexual coitus (D'Ancona *et al*., 2019).

Some studies show that *T. vaginalis* parasite can be survived for about 20 hours at temperatures of -10°C and it remains for 7 hours at room temperature. It can also remain in seminal fluid for 6 hours and in urine for 24 hours as they can live in river water for five days, in a bathtub, wet towels, and sitting places on the toilet for 12 hours. From this, the parasite can be spreading outside the human body. The incubation period for this parasite ranges from (5-28) days, and symptoms of the disease may appear on the affected person, but some are without any symptoms (Tompkins *et al*., 2020).

T. vaginalis sometimes take some abnormal shapes such as around shape, un flagellated shape or multiple nucleic, the shapes are abnormal and occur as a result of a favorable condition and these shapes fail to complete life cycle (Bogitsh *et al.,* 2018), but others showed in life cycle *T. vaginalis* takes these shape in mitosis division to give the oval shape (Paniker and Ghosh, 2017). In general, the flagellated small oval shape parasite is cloned by a longitudinal fission process without the disappearance of their nuclear membrane (Hoffman, 2019).

The beginning of this process is occur by selective motor organelles duplication, which follows the development of two accompanying attractophores on the side of the nucleus, followed by the development of microscopic chromosome tubes that move to nucleus, which are connected to the center of the chromosomes, and also extends between the attractophores, which is a spindle outside the nucleus called paradesmose and structural cells separating, each structural cell then produces the lost organelles (Graves *et al*., 2019).

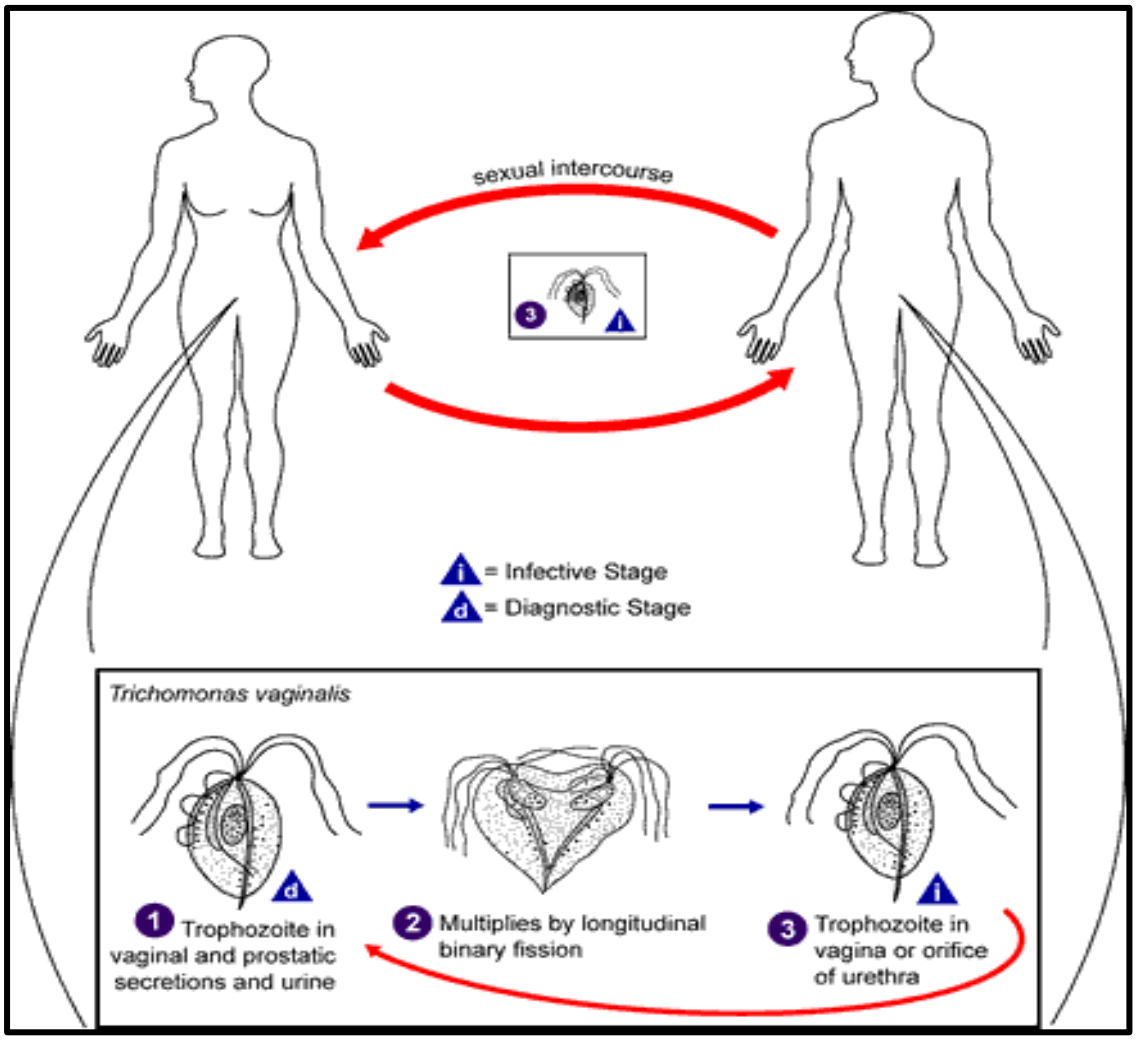


Figure (2-2): Life cycle of *T. vaginalis* (http://www.dpd.cdc.gov/dpdx)

2.5: Epidemiology of *T. vaginalis*

2.5.1: In the World

T.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 *T. 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 *T. 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 *T. 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 *T. 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 appropriate treatment for both sexes in order to prevent the how, and the reasons for the widespread spread of this disease are due to several reasons, including the lack of a male condom, not increasing cleaning and washing, and not seeing a doctor or not using the treatment for both partners (Yang *et al*., 2018).

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 *T.vaginalis* 73.3% (Arbabi *et al*., 2018), In Palestine, Gaza city the IR of *T. 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 *T. 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).

2.5.2: 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 urban 30.2% and primary education and low economic status of the participants in the study recorded high IR with *T. vaginalis* (44.9% & 44.6%) respectively.

In Babylon province show high IR with *T. vaginalis* among women in Al-Hila region, where the rural population recorded the highest IR (96.29%) than urban (79.36%) (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).

2.6: The pathogenicity of *T. vaginalis*

The pathogenicity and virulence of trichomoniasis, especially when it affects humans, to be completely incomprehensible, but the material

secreted by the parasite was identified that causes significant damage to the host cells and tissues (Friedman *et al*., 2020).

The pathogenic mechanism of this parasite was not fully clarified or is still being not clarified, where many reports showed multiple interactions between host and these parasites, which led to the emergence of clinical symptoms of different spectra, but the most concentrated factor is the adhesion of the parasite's surface to the host cells, its hemolytic activity, and its secretion of proteins and cell separation factors proteins with pores an important role in signs of pathogenicity (Dessì e*t al*., 2019).

The role of host factors on *lactobacillus spp.*, the value of pH and the hormonal component, as well as cytokine response and generation of free radicals (Edwards *et al.*, 2016). The interaction of the *T.vaginalis* with protozoan membranes that established natural flora in the vaginal tract is generating important factors for infection. This parasite has many mechanisms that it uses as a mask to avoid the host's immune system (Mercer and Johnson, 2018).

There is a very complicated relationship between *T. vaginalis* and its host, A wide range and clinical symptoms cannot be considered a pathogenic mechanism for this parasite since all clinical isolates material can cause infection and produce disease (Baer, 2020). The cell surface of *T. vaginalis* parasite has an effective role in the interaction and adhesion with the host cells, Thus, the parasite is equipped with nutrients, proteins, and glycoproteins that play an important role in surface functions (Saeidi *et al*., 2019) . From here, molecular analysis of membranes, large molecules and ferocity factors has begun (Hinderfeld, 2018).

2.6.1: Adhesion factors of *T. vaginalis*

Adhesion process is the first important steps in pathogenicity of *T. vaginalis*. *T. vaginalis* parasite tends to adhere to vaginal epithelial cells, which largely depend on temperature, time, and pH, so the high and low pH effect on parasite's survival and its movement (Phukan *et al.*, 2018).

There are four special proteins are responsible for *T. vaginalis* adhesion to the host cells, it is (AP65,AP23,AP33 and AP51) and it operates a special receptor system and ligand reactors. The gene expression of these four proteins is coordinated at the transcription level

by iron atoms (Zhang *et al*., 2020) when AP65 protein is a protein that is encoded by three genes within a multi-gene family (232-18) which is very similar to Malic's enzyme coding (Li *et al*., 2020).

There are many researches study adhesion process of the *T. vaginalis* parasite with the epithelial cells of the vagina, and some several factors that effect on adhesion process, including microtubules, microfilms, four adhesion proteins and cysteine. The electronic microscopic investigation showed significant change occurred in *T. vaginalis* body after its adhesive to human vaginal epithelial cells (HVEC) (Dorkenoo & Ekouevi, 2020).

In one study, the mixing of HVEC of non-infected women with *T. vaginalis* and incubated for half an hour, the parasite's body became more flatted and changed to amoebic form with pseudopodia, which showed strong cohesion and adhesion on vaginal epithelial cells (Espiritu *et al*., 2018).

Adhesions proteins are bound on the membranes of *T. vaginalis* and centered on the opposite side of the undulating membrane, while the binding proteins are laminin present in all parts of the entire parasite's surface. This parasite is associated with many large molecules in the host's body and uses them for feeding purpose and a few of them are used by the parasite to protect it from the host's body defenses and thus is considered an aid to the parasite to avoid the immune cells of the host (Phukan, 2016).

Lamine protein is a glycoprotein in the basement membrane of epithelial cells and a promoter of cellular adhesion differentiation as well as movement and shape of normal cells and has many chemical properties. It has been observed that the *T. vaginalis* strongly adheres to the laminin – coated plastic surface and inner laminin in cells and the molecule polystyrene covered (Daugherty *et al.*, 2019).

There is another part that has a role in the adhesion process of the *T. vaginalis* called Fibronectin which is a glycoprotein matrix extracellular adhesion which is used for feeding and adhesion and these last molecules have a role in haemolysis of red blood cells as surface sugars (Gavinho *et al*., 2019).

The adhesion factors that have been mentioned previously were not associated with the virulence of the parasite, as the virulent strains that were isolated from patients had different symptoms in adhesion and this indicates a very complex relationship between this parasite and its host (Dias-Lopes *et al*., 2018).

2.6.2: Haemolysis activity of *T. vaginalis*

One of the special features of *T. vaginalis* is its ability to hemolysis and the use of haemoglobin as a source of iron, and many experiments have proven that the low intensity of the parasite is due to the low amount of iron (Motlagh *et al*., 2020).

It is possible that the cause of the haemolysis of the red blood cells of the vaginitis patient caused by this parasite, depending on the contact between the parasite's body and RBCs, this process is correlated with specific factors , it starts with fats, iron, temperature, and calcium ions, and these factors of dissolving depending on the role of the proteins in the pores, which are characterized by their ability to insert themselves in the fat layer of the target cells and create trans membranous channels that lead to cell death by osmotic decomposition (Simpson & Criss, 2020 ; Silva *et al*., 2020).

The pores formed on the target cell membrane have a size ranging between (1.34-1.14) nm and thus a change in the permeability of the cell membrane and then the protection was a failure, which leads to cell degradation and loss of hemoglobin proteins that formed the pores are effective by the pH less than (6.5) at a temperature of 37^{O}C. The loss of the spectrin, it causes changes in the shape of the target cell (Zeng *et al.*, 2020).

In addition to fats, iron is an important nutrient for *T.vaginalis*, which the parasite prefers through the process of decomposing RBCs. This decomposition of RBCs is carried out by protein receptors that are present on the surfaces of both the parasite and red blood cells (Abou Gamra *et al*., 2019).

Numerous experiments indicate that five adhesion molecules match with two protein-like proteins, three proteins identical which was cause adhesion to epithelial cells. Decomposition occurs in three main steps:

1- Interacting with a specific receptor that allows the parasite to bind to red blood cell.

2- Liberate protein-like proteins from the pores present in the membrane of red blood cells.

3- *T. vaginalis* separate from the cell and its decomposition occurs contrary to its behavior with epithelial cells.

The intensity of the virulence of this parasite is due to the activity of the parasite on hemolysis and phagocytosis ability of many red blood cells.

2.6.3: Cytotoxicity of *T. vaginalis*

According to many indications, *T. vaginalis* can produce molecules that are delivered to the target cell, which is a means in the cellular toxicity of the host and the destruction of its plasma membrane. There is a special antidote called (CP65) that decreases antibodies levels and increases the cytotoxicity of adenocarcinoma cervical cells (Hella) single-layer cell to more than (64%). It also possesses (CP39) proteins, it was found in the parasites that have special antibodies to (CP39) proteins can destroy adenocarcinoma cells of the cervix (Hella) and not depending on the method of concentration or cellular adhesion. Therefore, this protein was considered as a vital indicator of trichomonas (Miranda- Ozuna *et al*., 2019).

The presence of the iron component leads to increase the cellular toxicity of the trichomonad cell, and consequently the occurrence of adenocarcinoma hella cell cancer, in contrast to the increase in the effectiveness of CP65 in the *T. vaginalis*, which leads to the secretion of CPs in the 30-Kda region (TVCP2 / TVCP3 / TVCP4 / TVCPT) and their ability to infect the vaginal epithelial cells (Rivera-Rivas *et al*., 2020).

Through the semi-quantitative reverse transcription, the polymerase chain reaction (PCR) using mRNA for parasites with different iron concentrations is observed that the gene expression of some CP genes is different, some of which are rich in iron such as (TVCP4) and the other have more than one iron-bound site such as (TVCP12 / TVCP65) These proteins differ although they are similar to molecular weight, they differ

with differentiation between them by iron, and therefore they differ with virulence factors such as cellular toxicity, cellular adhesion, and programmed death of cells, and many of the characteristics are not yet known (Puente-Rivera *et al.*, 2017).

Some studies indicate that treating parasites with a drug leads to a significant reduction in the toxic effect of cells, and therefore it is concluded that *T. vaginalis* is the main cause of causing cellular damage to the target cell (Kim *et al*., 2017).

2.7: Mode of the transmission of *T. vaginalis*

Trophozoite cannot survive outside the host and so the infection has to be transmitted directly from person to person by several routes such as:-
* Sexual transmission is the usual mode of transmission.
* Trichomoniasis often coexists with other sexually transmitted diseases, like candidiasis gonorrhea, syphilis, human immunodeficiency virus (HIV).
*Babies may get infected during birth.
* Fomites such as towels have been implicated in transmission. (Squire *et al*., 2019).

2.8: Interaction *T. vaginalis* with vaginal flora

In the pathology of this parasite that must be mentioned which was the interaction between the vaginal flora that is found naturally in vagina and the *T.vaginalis*. It is known that the natural pH in the vagina is acidic and the ideal pH of *T. vaginalis* is between (5-6.5). It has been observed that the vagina has a high pH with low lactobacilli, or its loss is final, which leads to an increase in anaerobic bacteria. In laboratory conditions and pH control, it was found that lactobacilli did not show an effect on the *T.vaginalis* parasite, while the parasite harmed lactobacilli (Green Baum *et al*., 2019).

In order to cause injury, *T.vaginalis* is crossing with the barrier in vagina. *Lactobacillus*, which is a natural barrier against microbes, because it prevents the parasite adherence to the target cell or inhibits its adhesion, so the parasite must overcome it in several ways, including the

production of special materials such as proteinase, which destroy lactobacillus (Hinderfeld *et al*., 2019).

2.9: Immune system avoidance of *T. vaginalis*

Vagina is a variable and anti-microbial environment, *T. vaginalis* must synthesize many CPs that enable it to avoid the human immune system and thus activate and flourish the parasite, which is more important on the part of pathogenesis (Pekmezovic *et al*., 2019).

One of the most important supplements available in the vagina is menstrual blood, whose activity is half the blood activity in the vein, and according to experiments about a third of samples from menstrual blood is not a complementary activity at all and there is cellular toxicity towards *T. vaginalis*, but even with a low concentration of the parasite during the menstrual period, there is continuity post - menstrual infection while other organisms decrease their number in the vagina during menstruation (Leka and Moran, 2020).

Increased iron levels are due to increased CP and erythrocyte degradation, which makes the iron environment high and increases expression for CP (Moonah *et al*., 2019).

Another method of disguising this parasite is the mechanism of appearance variation by expression or non-expression of protein 270 KDa at the specific region. Where *T. vaginalis* is described as negative or positive for protein 270KDa and is positive but the expression does not occur and notify the adhesion characteristic (Al-Hadraawy *et al*., 2018).

2.10: Host immune response of *T. vaginalis*

Human infection with leads to immune response and formation of specific antibodies against parasite in the reproductive system, In most cases, these antibodies are transmitted in human blood serum. Therefore, vaginal infections lead to the formation of an acquired immune response, which leads to release of cytokines from the T helper cells type 1(Th1) such as interleukin 2 (IL- 2) and interferon – gamma (IFN-δ) which are the most prevalent of diseases caused by other protozoa, according to special studies on cytokines. In this case, Th1 helper cells respond to the

disease and prevent clinical symptoms and signs from appearing, but do not prevent infection (de Aquino *et al*., 2020).

The acquired immunity aims at the presence of special globules against parasite, which is immunoglobulin A (IgA) and immunoglobulin G (IgG), as well as the presence of T helper cells. Therefore, the effect of antigens and antibodies is unknown to the parasite (Nemati *et al*., 2018).

One of the important aims of body-protective antibodies be attack the adhesion molecules that act in perfect contact between the parasite and host cells and this process leads to the destruction of the host cells (Al-Mamoori *et al.,* 2020). The primary adhesion molecules of *T. vaginalis* are four auxiliary molecules located on the surface of parasite that link it with the epithelial cells of the vagina. The genetic expression of these molecules begins when the parasite is bound to its host. The role of the antibodies is protection the target cell from the cellular toxicity that the parasite carries (Riestra *et al*., 2019).

In vivo, the immune response against adhesion of *T. vaginalis* is a very important process to protect from vaginal parasites. Vaginal secretions in trichomoniasis women contain neutrophils that respond to IL- 8 (Lee *et al*., 2017).

Generally, infection with this parasite occurs in the human urogenital system, the majority due to a defect in the host's immune system, so many infections was associated with an increase in HIV infection in both males and females (Vaca, 2020).

In HIV patient the immune response fails to remove this parasite and unable to have long-term immune memory in the occurrence of infections. Also, lymphocytes attack by the immune response to trichomoniasis that fights HIV infection in humans (Barajas-Mendiola *et al*., 2019).

2.11: Symptomatic and clinical signs of *T. vaginalis*

2.11.1: In women

About a third of women in the world and most men don’t appear symptoms of trichomoniasis, but they infected with *T. vaginalis*. The incubation period (5-28 days) was need to appear a symptoms the patient (CDC). In women, the symptoms of trichomoniasis vary from a severe

and severe inflammatory condition to none. The classic signs of this disease include dysuria, a foul-smelling vaginal discharge accompanied by burning urine, itching, dyspareunia, and lower abdominal pain (Rein, 2020).

Clinically examining women infected with *T. vaginalis* , mucous vaginal secretions appear greenish-yellow (Schwebke *et al*., 2020).This infection leads to serious complications such as cervicitis, premature birth of fetuses and infertility in women as well as men. Recent studies have shown that there is a link with this parasite and increased infection with HIV and genital epithelium ulcers and may develop into bleeding or necrosis as well as acute prostate cancer and cervical cancer uterus (Shaw *et al*., 2019).

In pregnant women the infection with *T. vaginalis* leads to more serious complications, including low fetal weight at birth, rupture of the membranes in the uterus prematurely and premature births (Thompson *et al*., 2020). Rarely, in 2% women, a strawberry cervix (areas with limited exudation or macular colitis) or on the vagina when examining patients (Van Gerwen and Muzny, 2019).

2.11.2: In men

T. vaginalis infection in men causes urethritis without other causes, and it is mostly without symptoms. If the patient does not take treatment, it leads to complications, including urethritis, chronic prostatitis, epididymitis, infertility and prostate cancer (Schwebke *et al*., 2018).

Trichomoniasis in men causes urethritis without other causes, and it is mostly without symptoms. (15-50%) The percentage of men who reported cases of *T. vaginalis* infection because they were husbands of infected women, and their infection was often without symptoms. The most common symptoms in men with this parasite are dysuria, urinary tract irritation, frequent urination, and urethral secretions. The rare symptom appearing is prostatitis with profuse purulent secretions (Daugherty *et al*., 2019).

2.12: Complication of *T. vaginalis*

Some studies have shown that cases of this disease were recorded in the urinary tract, pelvis, and fallopian tubes, as well as bronchitis,

pneumonia, and stomatitis. The condom is the most effective in reducing the transmission of infection, but it is not complete prevention (Ifeanyi *et al.*, 2018).

The shattering of the vaginal epithelium caused by this parasite increases the risk of contracting HIV. In addition to the aforementioned, this parasite analyzes the epithelial cells in the vagina and the red blood cells in the affected area, which causes breaches of the barrier formed by the epithelial cells, which increases the appearance of infections due to other causes HIV, and thus it is transmitted to couples (Stewart *et al.*, 2020).

The natural history of this parasite such as the length of infected in pregnancy, as well as the absence of symptoms. It often has a dynamic role in the transmission of HIV, as these parasites stimulate the human immune system and what causes an intense presence of white blood cells in the area of infection even with infected patients without symptoms and 50% of the affected women suffer from spotted bleeding or the appearance of bleeding drops (Mwatelah *et al*., 2019).

In the case of a person not infected with HIV, the targeting of cells and access to the bloodstream, but in the case of the person with HIV, it amplifies the exit gate of the virus and thus causes an increase in the secretion of HIV type 1 in the genital area specifically, and from here we conclude that the presence of trichomoniasis It increases the risk of infection with HIV, so by reducing the spread of sexually transmitted diseases, the most important of which is trichomoniasis, an important role for the prevention of AIDS (Saeidi *et al*., 2019).

2.13: Diagnosis of *T. vaginalis*

Samples which are used to diagnose *T. vaginalis* are clinical samples such as urine, semen, vaginal fluid, and a vaginal swab from the cervix. Each sample treat with special method for diagnosing trichomoniasis by a physician (Bruni *et al*., 2019). Some the following method used for diagnosis as:-

2.13.1: Saline wet mount evaluation

Under microscopic examination a wet swab was taken from the vagina to diagnose trichomoniasis, it is done by using saline solution, where a small amount of it is placed on a clean glass slide and mixed with a small amount of vaginal secretions from the infected person and then examined under a microscope lens with different enlargement forces, with this method, many motile vaginal parasites, epithelial cells, as well as white blood cells are seen (Rayan *et al.,* 2019).

The positive result of the examination is recorded by watching the pear-shaped, or the so-called trichomonad, this parasite has an oval shape slightly larger than the polymorphonuclear leukocytes (PMNs), which are a type of white blood cell. The trichomonad can also be recognized by its amoebic movement, as it causes an inflammatory reaction, and the presence of many white blood cells (Leli *et al*., 2016).

When examining a group of samples and the results are positive for trichomonad in the first reading, it is read again every 10 minutes. The first reading (20%) of the samples become negative and after 30 minutes, (35%) of the samples become negative, after two hours after the first reading it becomes (78%) of samples are negative. Therefore, The method wet amount under microscopy examination has a low sensitivity for detection of *T.vaginalis*, estimated at 50-70%. Therefore, it is not considered the best method or standard criterion for diagnosing trichomoniasis because it depends on the direct reading of the test strip of trichomonad, and it is mostly positive in women carrying large numbers of these parasites (Yazısız *et al*., 2020).

Not seeing the parasite in the slide when the microscopic examination is not considered the absence of vaginal trichomoniasis because the sensitivity is weak for this method, but sensitivity can be increased by using saline to wash the cervix. The sensitivity rate for this method ranges from (54.7%) to (74%). The wet amount technique method under microscopy is not considered the best and effective way to diagnose this parasite in men (Menezes *et al*., 2016).

2.13.1.1: Giemsa stain

German chemist Gustav Giemsa 1902 who invented the dye solution and name Giemsa stain . This dye has a range of applications in pathology and microbiology. Giemsa stain was used for detection malaria in blood scans, and later in histology and for routine blood testing. It is a mixture of Azure, eosin dye and methylene blue. They are specific to the phosphate group in DNA. Eosin and Azure are acidic dye that stains basic components in the cell, such as granules and cytoplasm. Methylene blue is a basic dye that stains acidic components in a cell, such as a nucleus. Methanol acts as a stabilizer that attaches cells to the glass slide and does not allow for alteration. (Lillie *et al*., 1978).

2.13.2: The culture

This method is more sensitive than the microscopic examination method for diagnosing trichomoniasis in human (Divakaruni *et al*., 2018). Commercial culture systems (such as InPouch ^{TV}, Biomed Diagnostics, USA) provide a greater ability to diagnose this parasite than other culture media, including diamonds. *T. vaginalis* can be grown in liquid, solid, egg and tissue media, and the usual medium for use is cysteine Peptose liver maltose (CPLM) as well as plastic envelope medium (PEM). After the completion of the culture process, the shocked media are transferred with the samples to the incubator and then the results are read after five days using microscopic examination (Hassan *et al.,* 2019).

T. vaginalis growth inside the incubator at temperature ranging between (35-37°C) and anaerobic conditions and a pH of about (5.5-6.0). This culture method confirms the results of examination and diagnosis, especially in case of diagnosing samples that have given a negative result in microscopic examination, where the degree of sensitivity to them is about (95%) (Adjei *et al.,* 2019).

2.13.2.1: Transport medium swabs

Transport medium swabs comes sterile with a single swab in a tube and kept with a peel. The polystyrene shaft is in the shape of one mid-tip plastic stick of medical cotton with a viscose bud at the top of the other end. It is used to transporting microbial samples to laboratory for study

(Islam *et al*.,2019). These swabs are used to transport aerobic and anaerobic microbes to culture it in laboratory (Mohammed *et al*., 2019).

It is of several types, including amies transport swab, which is one of the most common transport swabs, has a wide range of microbial examination of surfaces, and has a semi-solid texture to reduce the diffusion of oxygen and is considered to be temporary rather than permanent storage to reduce microbial overgrowth (Selim *et al*., 2020).

2.13.2.2: InPouch ^{TV} media

InPouch is a highly sensitive medium with an effective diagnosis of *T.vaginalis* in various samples of urine, vaginal swabs and others for patients with trichomoniasis. This medium is called the gold standard for diagnosing sexual transmitted infection by microorganisms. In Pouch is considered a special medium for this parasite, as it allows *T. vaginalis* to grow and inhibits the growth of others microorganisms, such as bacteria, yeasts and molds (Patel & Sheth, 2020).

The effectiveness of this medium in isolating and diagnosing the *T.vaginalis* responsible for the infection of the most famous sexually transmitted disease, which is Trichomoniasis, and what it saves in reducing effort, money, time, and the number of samples collected upon diagnosis (Vieira-Baptista & Bornstein, 2019).

InPouch consists of a high-system barrier resistant to entry of oxygen to provide anaerobic growth conditions for this parasite, and it is a plastic bag consisting of two chambers connected by a narrow passageway, where this installation provides a V-shaped system, the lower part for the culture of the sample and the upper part for monitoring i.e. examination and thus provides This fully closed system is growing and controlled simultaneously, thereby reducing the cost as well as medical waste, whose danger is not overlooked (Khurana and Singh, 2018).

One of the advantages of this medium it is ease to storage, and kept at a room temperature of 18-25°C. Also, the parasite can be kept inside a media for two days before incubation (Seña *et al.*, 2014).

It is a soft plastic box with two lounges (upper and lower) as shown in the figure (2-3). The sample is placed in the lower hallway when it is taken

from the examined person and after that, it is examined microscopically and the upper hall is not examined because its contents have descended to the lower hall before entering the sample and then it is placed in the incubator at a temperature of 37°C, Then continue examination periodically after (24, 48 ,96) hours to see the movement of the parasite and its phenotype (Muthini, 2016).

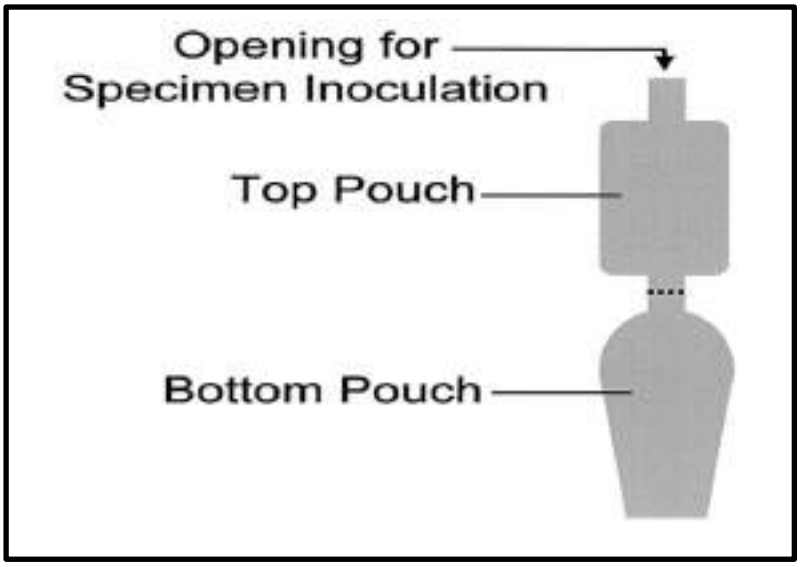


Figure (2-3): Diagram of InPouch ^{TV} Medium (sood, *et al*.,2007).

2.13.3: Polymerase chain reaction (PCR) technique

This method is considered better than previous one and contributes to the knowledge of dead organisms, as well as the detection of the molecular sequence of samples in clinical examination of patients, as well as identification of cells that are fixed or that have been destroyed (Ahady *et al.*, 2016). Many special tests for the diagnosis trichomoniasis have been evaluated and developed, including this technique (Jamshidi, 2016).

Using the (PCR) technique that determines the beta-tubulin genes to diagnose the presence of this parasite in urine and vaginal swab samples, this gene encodes by a specific sequence of amino acids of the beta-tubulin protein, which is the main component of the cytoskeleton of this parasite. DNA amplification techniques, especially PCR, have become the most used in diagnosing STDs likewise for diagnosing trichomoniasis (Tipple *et al*., 2018).

The most popular method of using DNA replication sequencing as a diagnostic goal. This technique allows the production of non-specialized pieces due to the presence of additional jammed pieces as a result of mutations in some strains, so the migration of the panda produced by different strains varies. Sometimes this type of diagnosis fails to detect the beta-tubulin gene. In identifying some strains because of their different virulence (Nwuba *et al.*, 2019).

The specificity and sensitivity of this test for vaginal swab samples is (100%) and (98%), respectively. As for urine samples, their specificity and sensitivity for this test was (100%) and (99.7%) compared with vaginal swab samples, so this type is considered the most sensitive and specific for diagnosing this parasite, but its low use is due to its cost, which limits its widespread use in diagnostics (Grad *et al*., 2020).

2.13.4: DNA sequencing

The DNA sequencing refers to method used to determine the arrangement of nitrogenous bases, which includes adenine, guanine, cytosine, and thymine in the genetic material molecules. This technology has witnessed a fundamental development in terms of cost and accuracy concerning scientific progress in various fields, the most important of which is the molecular genetics aspect (Munshi, 2012).

2.14: Treatment of *T. vaginalis*

Metronidazole is a treatment of choice and effective antibiotic for trichomoniasis in the United States. The recommended dose to take orally is 2mg once per day. The cure rate for this disease was about (97%) (Sherrard, 2020). One of the important things that must be emphasized is that the treatment is comprehensive for both sexes, meaning the patient and his sexual partner. Despite the emergence of some controversy about the danger of this treatment for pregnant women, no relationship proves the occurrence of a malformation of the fetus attributed to metronidazole even when used in the first three months of pregnancy (Buggio *et al*., 2019).

In recent, studies have been published showing the relationship of this treatment during pregnancy with premature birth of the fetus. Two recently published studies suggested that taking this treatment during

pregnancy may increase the risk of premature labor rather than reduce its risks (Faught and Reyes, 2019). Some patients suffer from an allergy to Allergy to metronidazole occur due to the lack of an alternative treatment that is effective against this parasite, so the only solution is desensitization (Beyaz *et al*., 2020, Bouchemal *et al*., 2017). Some estimates indicate that equivalent to 2.5–5% of all trichomoniasis treated cases with metronidazole show resistance to this treatment (Tien *et al*., 2020).

Tinidazoles are 5-nitromidazole compounds that are chemically linked with metronidazole and are widely used outside the United States but more recently it has been licensed to treat trichomoniasis in the United States. Tinidazole has a plasma half-life twice that of metronidazole. For example, if there are about 6 to 7 hours in metronidazole, tinidazole is 12 to 14 hours (Mukherjee *et al*., 2016, Cortez-Maya *et al.*, 2020). Some side effects of trichomoniasis were treated with oral metronidazole 500mg twice a day for a week, but when they were treated with tinidazole at a higher oral dose of 2-3g and a sedative of 1-1.5g, the cure rate was 92% and no treatment was discontinued due to the emergence of side effects of this treatment (Argüello-García *et al*., 2020).

CHAPTER THREE

Materials & Methods

3. Materials & Methods

3.1: Materials

3.1.1: The apparatus and the tools: -

Table (3-1): The apparatus and the tools that were used in the current study with the manufacturing companies and the country of the origin are as following:

NO.	The apparatus and the tools	The company	The country
1-	Centrifuge	HETTICH	Japan
2-	Centrifuge refrigerated (5415 R)	EPPENDORF	Japan
3-	Distillation apparatus	LAB TEACH	Korea
4-	DNA sequencer	The test done by MACROGEN	Korea
5-	E-Box UV Filter system	VILBER	China
6-	Electrophoreses apparatus	BIOCOM DIRECTCOM	China
7-	Freezer	DEKO	Turkey
8-	Gel Documentation System	BIOMETRA	Germany
9-	Hood	TIPS HOW	USA
10-	Incubator	MEMMERT	Germany
11-	Light microscopic	OLYMPUS	Japan
12-	Microscope camera system	S-EYE	China
13-	Microwave	SHONIC	China
14-	Prime	BIO-TECHNE	USA
15-	Refrigerator	CONCORD	Lebanon
16-	Sensitive balance	SARTORIUS	Japan

17-	Speculum	MAX	China
18-	Thermal-cycler	EPPENDORF	Japan
19-	Trans illuminator (UVIFOR)	ELETTROFOR	China
20-	UPS (MAX3300)	MAXIMA	China
21-	Vortex	HEIDOLPH	Germany
22-	Water bath	BINDER	Germany
23-	Collection Tubes (2 ml)	STERILE EO	China
24-	Cylinder (10ml, 250ml, 500ml)	MEMMERT	China
25-	Conical flask (250ml, 500ml)	MEMMERT	China
26-	Cover slide	HIRSHMAN	China
27-	Disposable syringe 5ml	KILANIMEDICAL	Jordan
28-	EDTA tubes	AFCO	Jordan
29-	Eppendrof tube	BIONEER	Korea
30-	Flask (50ml, 500ml)	MEMMERT	China
31-	GS Columns (1.5ml)	STERILE EO	China
32-	Glass slide	SUPERTEK	India
33-	Marking pen	DOMS	India
34-	Medical cloves	OMEGA	China
35-	Micropipettes(1-10µL, 5-50µL, 20-200µL, 100-1000µL)	WATSON	Japan
36-	Micropipettes Tips(100µL, 1000µL)	STERELLIN Ltd	UK

37-	Nano drop	THERMO	USA
38-	Rack	AFCO	Jordan
39-	Slide case	HUIDA	China
40-	Vaginal swab	CITOSWAB	China

3.1.2: The Biological and chemical materials:-

The biological and chemical materials used in this study are listed in the table below (3-2).

Table (3-2): The biological and chemical materials.

NO.	Type of material	Manufacturer	Origin
1-	Agarose	DIFCO	USA
2-	BTUB-1 and BTUB-2 primers	MACROGEN	South Korea
3-	Distil water	LAB TEACH	Korea
4-	Ethanol Absolute (70%)	BDH	UK
5-	Ethidium bromide solution	BDH	UK
6-	Giemsa stain	SOLARBIO	china
7-	Hand sanitizer	SAFETY	china
8-	InPouch ^{TV} media	BIOMED	France
9-	Loading dye	BIONEER	Korea
10-	Marker ladder (L100) (100bp)	BIONEER	Korea

11-	Nail polish	MIRROR	China
12-	Normal saline 0.9% NaCl	PIONEER	Iraq
13-	Nuclease-free water	PROMEGA	USA
14-	PCR premix	BIONEER	Korea
15-	Phosphate buffer saline	TISSUEPRO	US
16-	Proteinase K	GENEAID BIOTEACH LTD	New Taiwan dollar
17-	Sterile Transport medium swap	BIOZEK MEDICAL	Netherlands
18-	Strips of paper to measure the PH	MACHERY-NAGEL	Germany
19-	TE buffer 10x solution	BDH	UK

3.1.3: The software programs: that have been applied in the present results which are mentioned in the table (3-3).

Table (3-3): The software programs used in this study.

The software programs	
Epidemiological study	**Molecular study**
Scope image dynamic pro	Bio edit
	Basic local alignment search tool
IBM SPSS statistics version 24	CLC sequence viewer 8.0
	Multiple sequence alignment

3.2: Methods

3.2.1: Regions of the study:-

Maysan province is located in the south-eastern part of Iraq beside the border with Iran. Its area is about (16.072) km^2 and the population is about (922.072) peoples. It consists of six districts (Ali-Algarbi, Al-Amara, Al-Maymouna, Al-Kahla, Qulat Saleh and Al-Majar Al-Kabir). Misan province has a dry climate and high average of temperature in summer of 42^OC. This leads to an increasing in evaporation rates, rainfall is concentrated in the winter months at annual rate about (177) mm per year (https://www.ncciraq.org). In this study four districts randomly selected : Al-Amara, Al-Kahla, Al-Maymouna, Al-Majar Al-Kabir (Figure 3-1).

Al-Amara city is located in the center of the province it is a capital city of Maysan province, with 6474Km^2 which consists about 40.30% of Maysan province area and a population of 420,000 people, and the area and population of Al-Maymouna1500 km^2, 150.678 people; 2717km^2, 85000 people; 33km^2, 215.000 people for Al-Kahla; Al-Majar Al-Kabir respectively (https://ar.m.wikipedia.org ; Kjeilen, 2006).

3.2.1.1: The Study Population

There is some information that were interviewed from the study population such as: The Regions (Al-Amara, Al-Kahla, Al-Maymouna and Al-Majar Al-Kabir), Age (<15, 15-19, 20-26, 27-33, 34-40, 41-47, 48-54 and ≥55) years age group, Social Status (Married and Unmarried), Education level (illiteracy, primary, Secondary and A graduate), Occupation (Housewife, Employee, Student, Baby girl), Marital Status of the Husband (Monogamous, Polygamous), Number of Birth (0, 1-3, 4-6, and 7-9), Residency (Urban and Rural), Status of Women (Pregnant and Non- pregnant), Does the Husband have Genital Diseases (Yes or No), Childbirth Type (Normal Births and Caesarean Births), Number of Abortions (0,1,2,3,4 and 5), Others Tools (Used and Not used), Treatment (Used and Not used), pH values (4, 5.5, 6 and 6.5), Secretions (Yes or No), Itching (Yes or No), Burning Micturition (Yes or No), Lower Abdominal Pain (Yes or No), Urine Color (Dark yellow, Yellow and Normal), This information is saved in the questionnaire sheet in Appendix (A).

3.2.2: Samples:-

The research has been conducted under the agreement of the Maysan health statement and all samples were taken under the direct supervision of the gynecologist physician in all visited hospitals, health care centers and medical clinics after obtaining the agreement of participants. Using the vaginal speculum, the vaginal wet amount samples of 226 females from different ages ranged between (5-60) years were collected from women who visited hospitals, health care centers and medical clinics from different regions of Maysan province are distributed as: Al-Amara city (161), Al-Kahla (26), Al-Maymouna (25) and Al-Majar Al-Kabir (14) from the period Nov.10, 2019 to Feb.10, 2020. The steps of methodology were followed the scheme which described in figure (3-2)

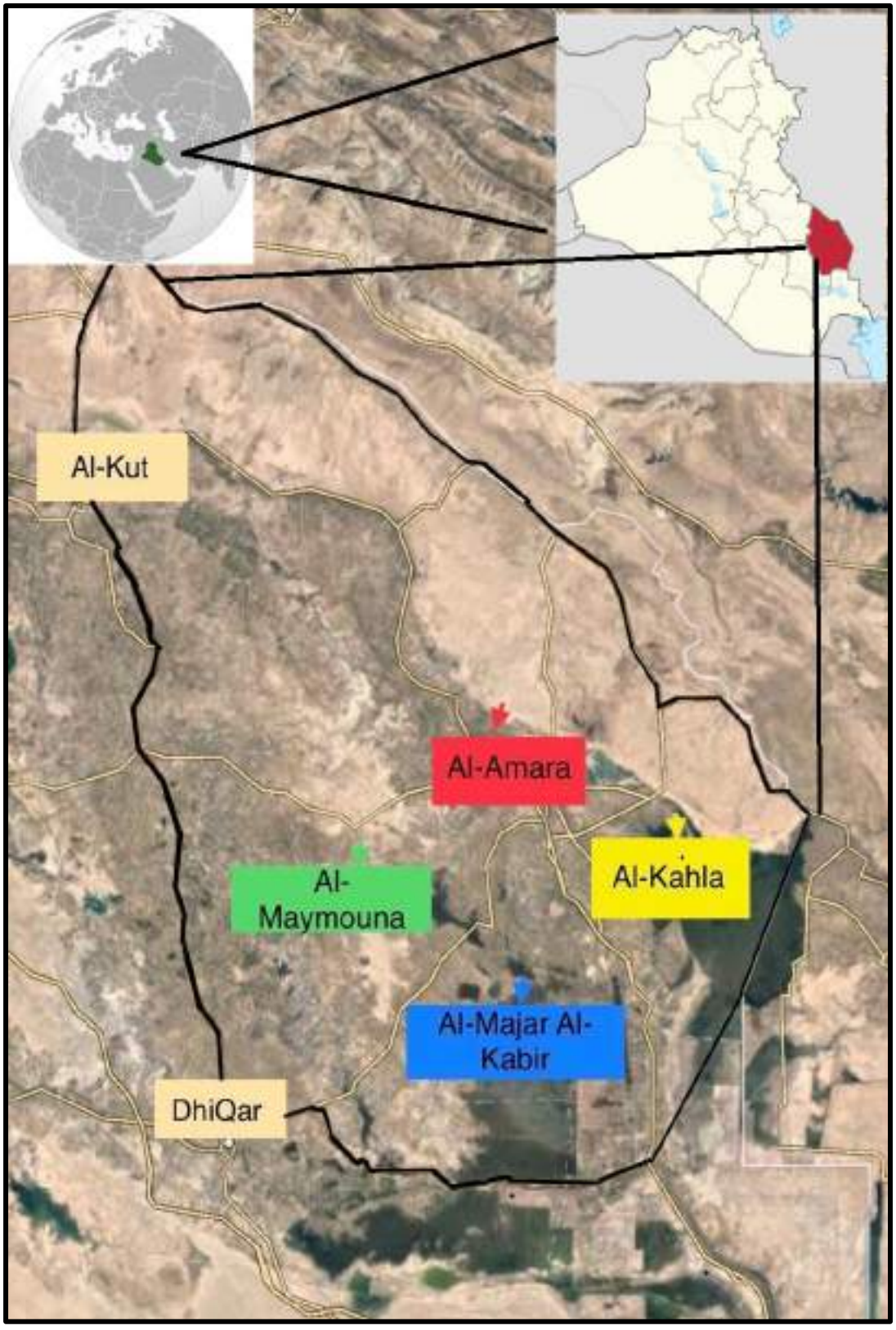


Figure (3-1): Regions of the study in Maysan province , ▼Al-Amara ▼Al-Kahla ▼ Al- Majar Al-Kabir ▼ Al- Maymouna (https://en.wikipedia.org).

3.2.3: Scheme of the plan of study : was summarized as following figure (3-2)

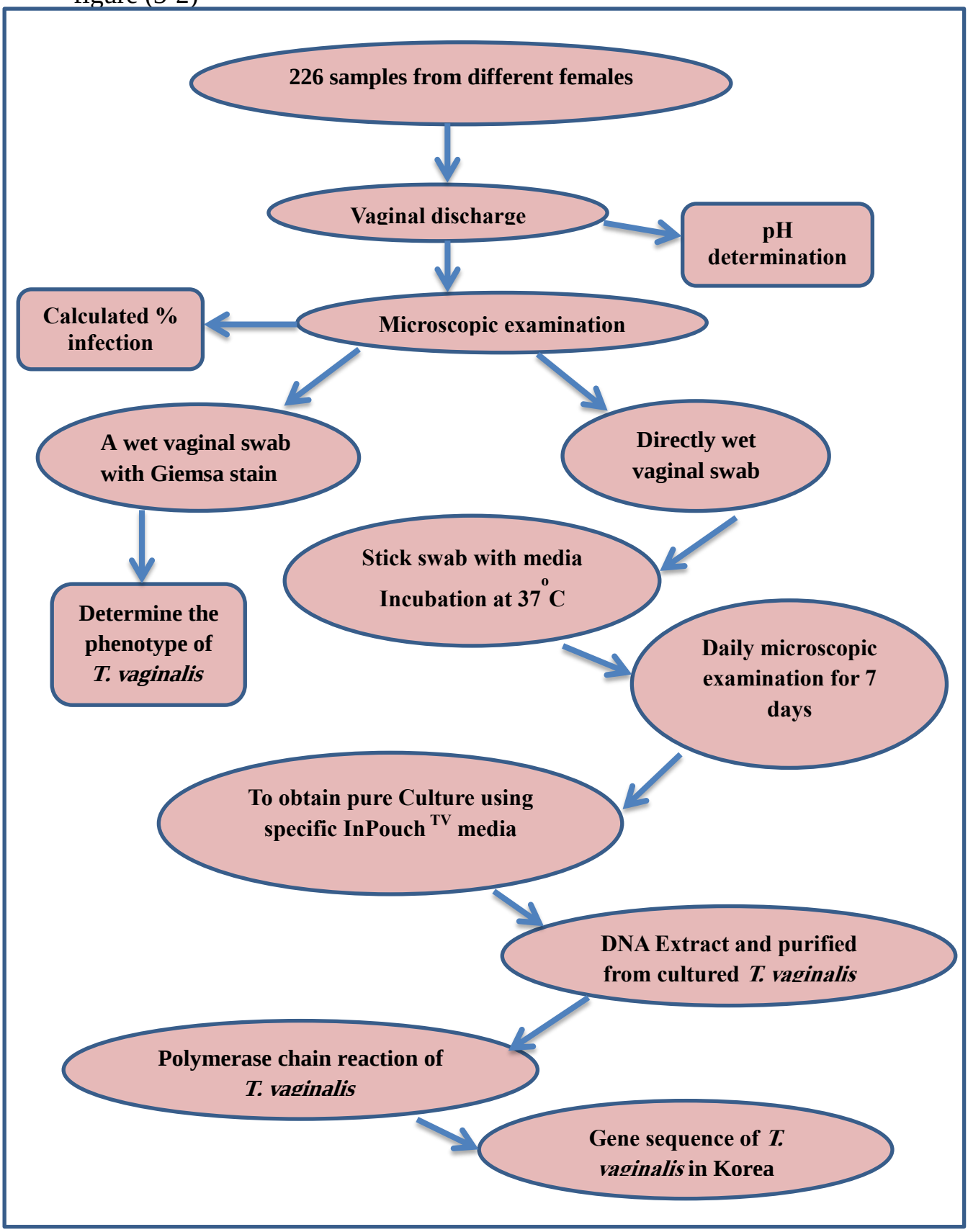


Figure (3-2): The plan of study.

3.2.4: Epidemiological study:-

The study has been conducted under the agreement of the Maysan health statement. All samples were taken under direct supervision of the gynecologist physician in all visited hospitals, health care centers and medical clinics. Using the vaginal speculum and by means of vaginal swabs, samples were taken from different women by inserting the swab into the high vagina position of vagina for married women and the front part for unmarried women and rotating it 180 degrees to make sure that an adequate amount of vaginal secretion was collected. Later, it has been withdrawn very carefully to avoid occurrence of smear contamination, then the swab has been passed over the slides for microscopic examination method of direct wet amount with and without Giemsa stain, which was prepared according to the ingredients mentioned in the table (3-4). Droplets of vaginal secretion for pH measurement of the vaginal secretion. The collection samples was cultured in amies transport media and kept in cool box and transferred to the laboratory of parasitology, biology department, science college, university of Misan and then Incubator at 37°C.

Table (3-4): Components of giemsa stain.

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

3.2.4.1: pH Measurement :-

The pH of vagina was measured by taking a drop of vaginal secretions and placed it on a paper tape (pH test strips) (Figure3-3) (Machery-Nagel (MN), Germany) (Sgibnev and Kremleva. 2020).

Figure (3-3): pH strip that used to determine pH vaginal secretion

3.2.4.2: Microscopic examination of vaginal discharge samples:-

Two vaginal swab were taken from each woman, one for cultivation and the second for direct examination. One drop of normal saline was mixed with vaginal secretion and three 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 vagina 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.

3.2.4.3: Cultivation of vaginal discharge:-

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

Figure (3-4): The culture of samples on amies transport medium.

3.2.4.4: Culturing vaginal specimens on InPouch ^{TV} medium:-

There are many liquid culture media available for diagnosis. InPouch ^{TV} medium is considered as the best and it is called the gold standard that was used to be the culture system to diagnose **25** samples of *T. vaginalis* that were collected in this study (Figure 3-5). In addition, each patient had a questionnaire and one related number.

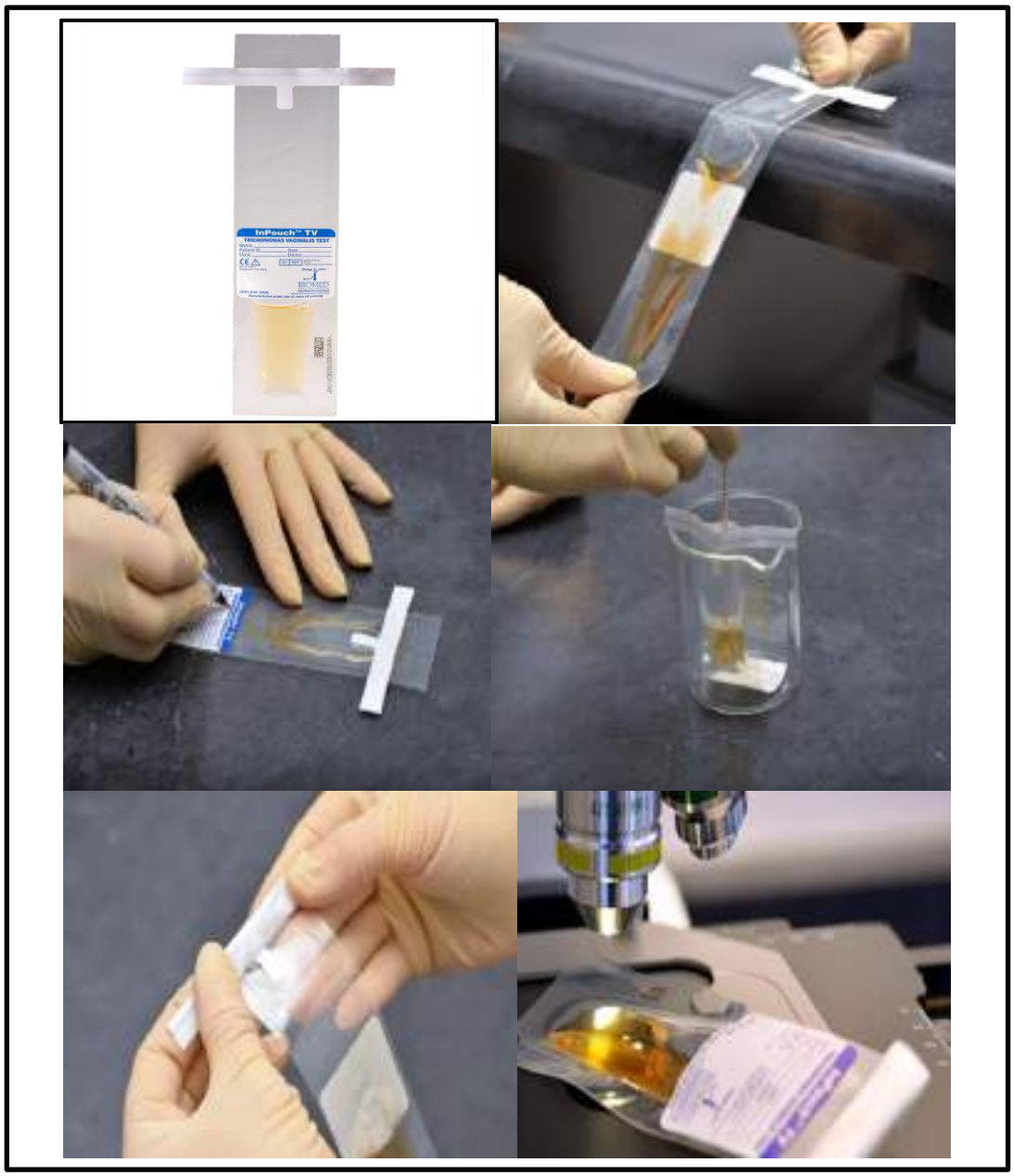


Figure (3-5): Images illustrate the steps for culturing samples on InPouch ^{TV} medium.

3.2.5: Molecular study:

3.2.5.1: Preparing vaginal swab samples for DNA extraction:-

Vaginal swabs samples of *T. vaginalis* have been cultured on the InPouch ^{TV} medium of incubator and withdrew the appropriate amount according to instructions of the kit used for DNA extraction.

3.2.5.2: Extraction of *T. vaginalis* DNA:-

Samples of *T. vaginalis* on InPouch ^{TV} medium were prepared for DNA extraction as the following protocol for DNA extraction kit (gSYNC ^{TM} DNA Extraction Kit, geneaid, Korea) with some modification for some samples.

3.2.5.2.1: Protocol steps for cervical and vaginal swabs

A. Cultured sample preparation

1-Transfer (1ml) from fresh culture to a 1.5ml microcentrifuge tube.

2- Add 200µl of Phosphate buffer saline and 20µl of proteinase K then mixed thoroughly with vortex.

3-Incubator the samples at 60°C until the sample lysate become clear.

B. Cell Lysis

1-Insoluble material retain to incubation then centrifuge for 2minutes at 13000rpm then that transfer the supernatant to 1.5ml microcentrifuge tube.

2-Add (200µl) of GSB Buffer and shake vigorously for 10 seconds.

C. DNA Binding

1- Add 200µl of absolute ethanol to the sample and mixed immediately by shaking vigorously for10 seconds.

2- Place a GS column in a (2ml) collection tube. Transfer all of the mixture (including insoluble precipitate) to the GS column.

3- Centrifuge all samples at 13000rpm for one minute or until mixture passed completely.

4- Discard (2ml) collection tube containing the flow-through then transfer the GS column to a new 2ml collection tube.

D. Wash

1-Add (400µl) of W1 Buffer to the GS column.

2- All samples was centrifuged at 13000rpm for 30 seconds then discard the flow-through, place the GS column down (2 mL) collection tube.

3- Add (600µl) of Wash Buffer to the GS column. Centrifuge at 13000rpm for 30seconds then discard the flow-through.

4-Place the GS column back in the 2ml collection tube. Centrifuge again 3minutes at 13000rpm to dry the column matrix.

E. Elution

1-Transfer the dried GS column to a clean (1.5 ml) centrifuge tube.

2- Add (100µl) of pre-heated Elution to the center of the column matrix.

3- Let stand for at least 3minutes to allow elution buffer to be completely absorbed.

4-Centrifuge at 13000rpm for half a minute to extract the purified DNA.

To avoid degradation of the DNA Eluted with water it should be stored at -20 ° C.

3.2.5.2.2: Imaging of DNA extracted by gel electrophoresis:-

The steps for making the electrophoresis and a garose gel are as follows:

a-Method for preparing a garose gel electrophoresis tray

A garose gel was prepared by dissolving 1.5 g of a garose powder in 100 ml of 1X TBE buffer (TBE Buffer (5X):108g Tris, 55g Boric acid, 40ml 0.5M EDTA, 2L H_2O) at 100°C until the solution was completely clear. After that, a garose solution is cooled to 50° C, it is mixed with a dye of ethidium bromide by 3µl and then poured into electrophoresis sealed with rubber and then put the comb and leave aside to harden at room temperature or at a temperature of 5°C.

b-Preparation of DNA samples for the gel electrophoresis

Add 8µl purified DNA to a clean glass slide and mix with a loading dye of 4µl.

c-To Extract DNA by gel electrophoresis

1-The a garose gel and the electrophoresis tray were prepared.

2- Remove the comb very carefully from the Agarose gel.

3- Load the DNA samples with the running buffer into the clean wells and then into the electrophoresis chamber and a tray fitted with the separator device filled with buffer 1 for half an hour.

4-Connect to the power source at 80mV for half an hour.

5-Under ultraviolet (UV) trans illumination the gel is examined for the presence of DNA bands in the dark.

6-The gel was photographed directly by the camera located in the (E-Box UV Filter system).

7-Storing DNA tubes at -20°C for preservation.

3.2.5.3: Polymerase chain reaction (PCR) of *T. vaginalis*:-

Polymerase chain reaction (PCR) of pure DNA samples was performed with a Master Mix (premix), the component of the premix PCR kit shown in table (3-5). Then a sufficient cumulative volume of the DNA of *T. vaginalis* was dissolved with amplification mixture shown in the table (3-6).

Table (3-5): The master mix (Premix) ingredients.

The component	Reaction size 20µl
Top DNA Polymerase	1U
Each: dNTP (dATP, dCTP, dGTP, dTTP)	250µM
Tris-HCl (pH 9.0)	10mM
KCl	30mM
MgCl2	1.5mM
Stabilizer and Tracking dye	0

Table (3-6): Components of the PCR amplification mixture for a reaction volume of 20µl.

Component of Sample	Volume
PCR premix	Dried pellet
Forward primer	2µl(10pmol)
Reverse primer	2µl(10pmol)
Template DNA	7µl
Nuclease-free water	9µl
Total Reaction Volume	20µl

Then, about 20µl of DNA *T. vaginalis* was added to each tube and all the tubes were closed and mixed by a vortex apparatus, then with a centrifuge at maximum speed for 10seconds.

3.2.5.3.1: Primers used for PCR amplification:-

Primer used to detect microbial vaginitis is based on an amplification of the beta-tubulin gene. This primer used in this study was designed using the NCBI Gene Bank online database. The specialized gene beta-tubulin is amplified using polymerase chain reaction (PCR) by the primer mentioned in the following table (3-7).

Table (3-7): Primers used for PCR amplification of specific gene for *T. vaginalis*.

Primer	The Sequence	Primer Length
BTUB1/2	(Dwivedi *et al*., 2012)	
F:	′5 -GGACAGTGCGGT AACCAAATT- 3′	21pb Tm=58.9
R:	′5 –GCACTCACTTGTGCCTGGTT- 3′	20pb Tm=59.9

***F (**forward primer**); R**(reverse primer**).**

3.2.5.3.2: Polymerase chain reaction (PCR) amplification cycles:-

Beta-tubulin gene amplification cycle is shown in table (3-8) that started on a thermal cycling apparatus.

Table (3-8): PCR amplification cycles condition for BTUB1/2 gene.

Steps	Temperature °C	Time	
Pre-Denaturation	94	4min	
Denaturation	94	45sec	30 Cycle
Annealing	48	45sec	
Extension	72	45sec	
Final Extension	72	10min	

After completing the PCR cycles, sample tubes are kept in the refrigerator until electrophoresis is performed.

3.2.5.3.3: Gel electrophoresis for PCR products:-

1- Collect electrophoresis device.

2- Add 1.5g of a garose powder in (100 ml) of 1x TBE in a sterile glass beaker.

3- Heat a garose suspension to a boil until it becomes completely homogeneous with TBE, then leave a little to cool and then add 3µl Of ethidium bromide with care and caution and mix gently.

4- Pour a mixture on electrophoresis tray after applying the comb, and then leave it to cool and stick.

5- Remove the comb quietly and then load the PCR product into each well with 2µl and the first well of each row in which we place 3µl of a ladder mark (100 bps ladder).

6- Put the tray with the gel into an electrophoresis device and cover the gel with a 1 x TBE buffer.

7- Connect electrophoresis cell to the power source and operate it at (80) millivolts for half an hour.

8- After completion, then turn off the power source and put the gel under UV light for examination.

9- Transfer the gel to the imaging system (E-Box UV Filter system) and the results are photographed.

3.2.5.4: DNA Sequencing:-

3.2.5.4.1: The sequence of PCR products of BTUB 1/2 gene identification with NCBI databases and Genbank reference genes.

The gene sequencing analyses were carrying out for PCR products of seven isolations of *T. vaginalis* The sequencing results of BTUB1gene were identified with a genomic database using bioinformatics technology as the Blast search on NCBI gene bank to determine the similarity and identify with other available genotypes that recorded in other regions of the world**,** the seven isolations of *T. vaginalis* were sent to NCBI Genbank for recording. (Bricheux & Brugerolle, 1997).

3.2.5.4.2: Assembly and alignment of PCR products of BTUB1

The sequences were aligned and using Boiedit (Hall .1999). The results of the Btub1 gene sequence were compared with standard sequences published on the National Center for Biotechnology information on the Internet, Statistically analyzed by CLC Sequence viewer (8.0) program.

3.2.5.4.3: Neighbor-joining tree

A neighbor-joining tree (NJT) using Multiple sequence alignment software (Kumar *et al*., 2018). NJT trees are used to illustrate the relationships between haplotypes or populations from genetic distance estimation (Saitou and Nei. 1987).

3.2.6: Statistical analysis:-

The statistical analysis was done by using statistical package for social science program (SPSS version $_{24}$), Chi-Square test (χ^2) was used to determine relationship between the infection rate and variables that used in the current study and percentage, with a probability (p) value of 0.05 or less were considered as statistically significant (Giolo-Ruiz . 2004).

CHAPTER FOUR

Results &Discussion

4. The results

4.1: Epidemiological study

4.1.1: The main characters of population study

The population included in a current study consists of 226 wet amount vaginal discharge swab specimens were collected from specific regions that determined previously named as Al-Amara 161 specimens, Al-Kahla 26 specimens, Al-Maymona 25 specimens, Al-Majar Al-Kabir 14 specimens (Figure 4-1).

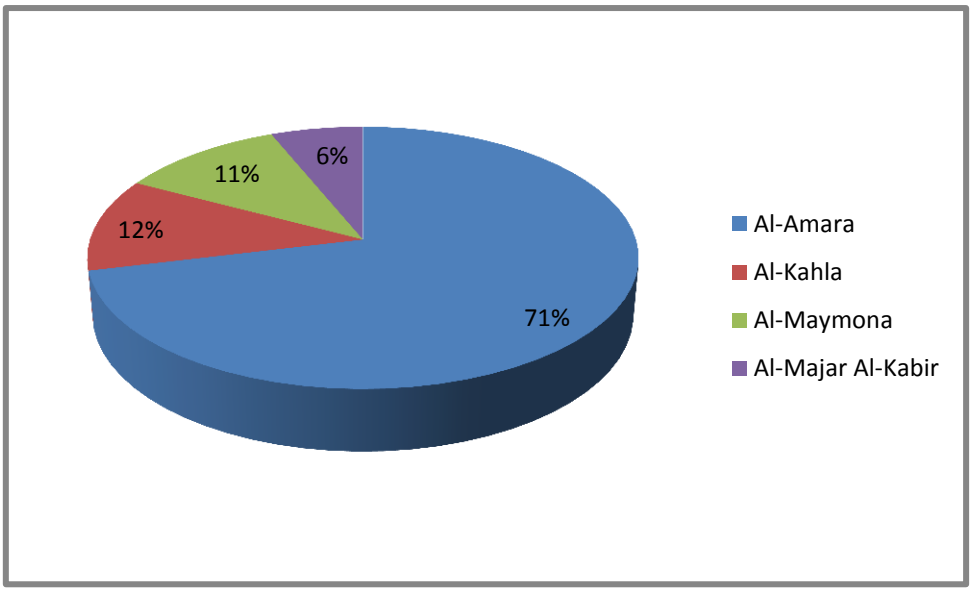


Figure (4-1): Distribution of the study population among regions of Maysan province.

The study population was distributed among the age groups <15 (8), 15-19 (22), 20-26 (49), 27-33 (42), 34-40 (46), 41-47 (27), 48-54 (26), ≥ 55 (6) (Figure 4-2).

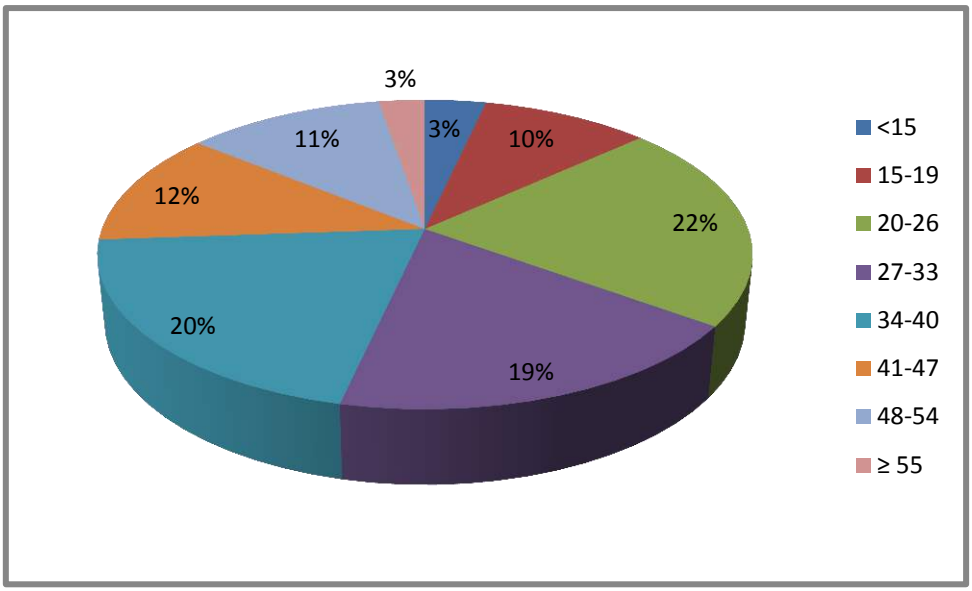


Figure (4-2): Distribution of the study population among age groups of the women.

Marital status of 194 married women and 32 unmarried women (Figure 4-3).

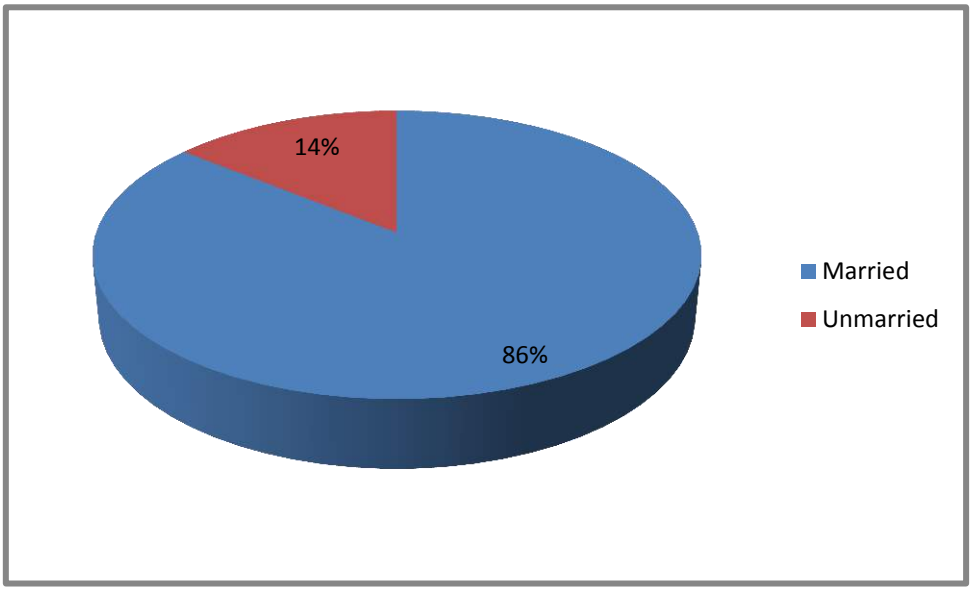


Figure (4-3): Distribution of the female specimens infect according to marital status.

It was noticed that the study population is distributed at the level of education as follows: the illiterate level (112), the primary level (85), the secondary level (16) and the female graduates (13) (Figure 4-4).

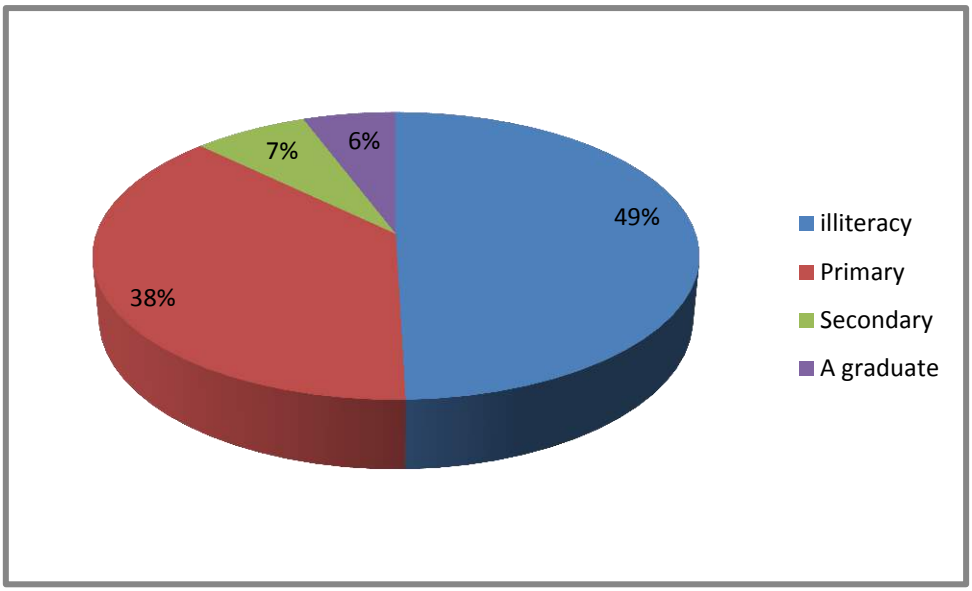


Figure (4-4): Distribution of the female specimens according to education level.

As for the pregnancy status of women, the study population consists of 14 pregnant women and 180 Non-pregnant women (Figure 4-5).

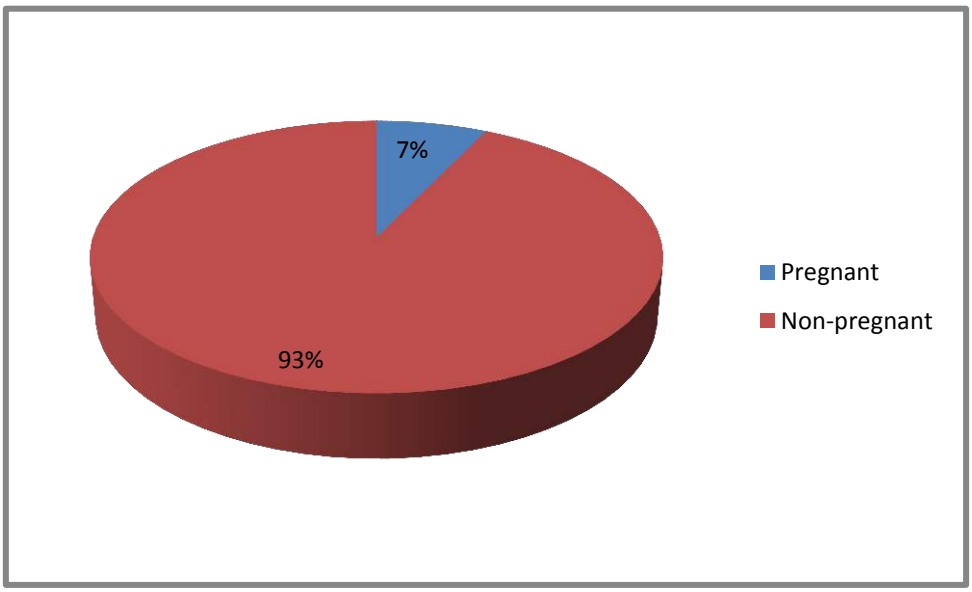


Figure (4-5): Distribution of the infect female specimens according to the pregnancy status.

It consists of 161 women residing in urban areas and 65 women residing in rural areas (Figure 4-6).

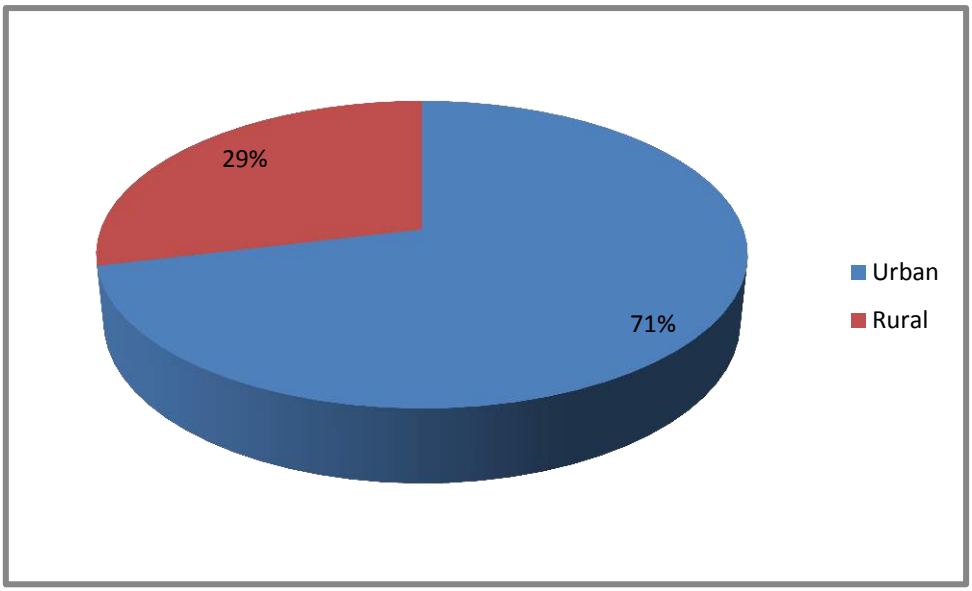


Figure (4-6): Distribution of the female specimens according to the residency.

The results showed that the vaginal discharge of women population in this study was identified with different pH levels (Figure 4-7).

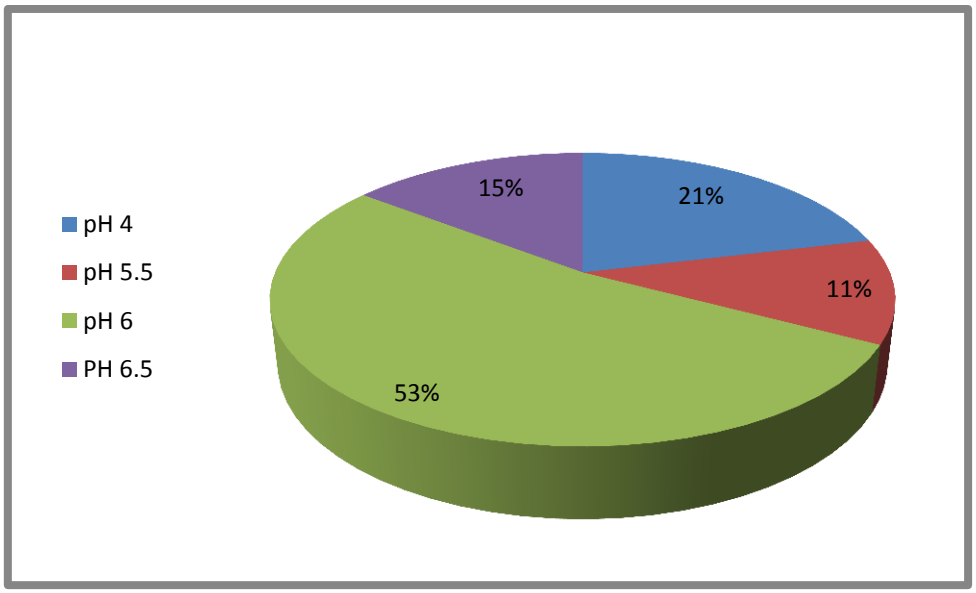
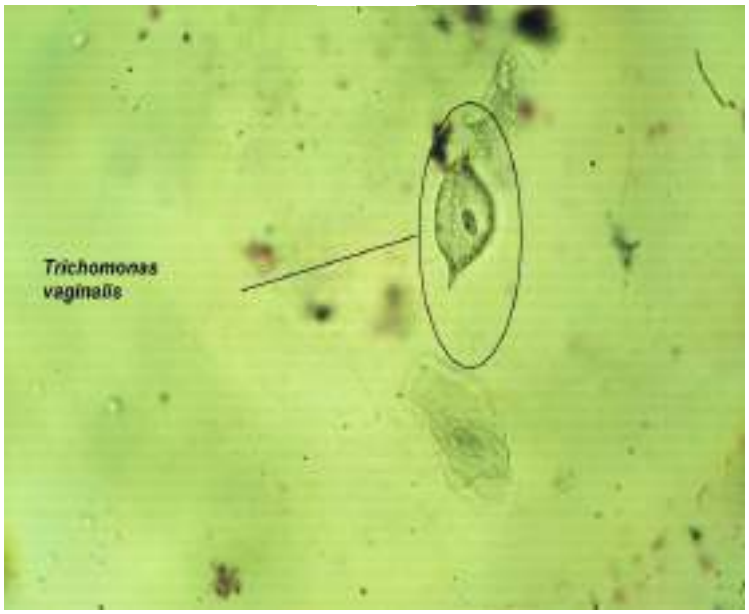


Figure (4-7): Distribution of the female specimens according to pH levels.

The results of the direct microscopic examination of the wet amount or stained specimens were indicted based on seeing *T. vaginalis* under microscopic field with magnification 40X (Figure 4-8).

(a)



(b)

Figure (4-8): The picture of *T. vaginalis* under microscope 40X wet amount by microscopic digital camera (S-EYE, China) (5 mega pixel), (a) without staining (b) with giemsa stain.

4.1.2: Sociodemographic factor

The results of microscopic examination of *T. vaginalis* of the present study showed that the overall IR of *T. vaginalis* among women was (75.22%). This finding was higher that have been recorded in some previous studies that conducted in various countries such as: USA women (38.0%) (Schwebke & Burgess, 2004), Saudi Arabia (28%) (Madani, 2006), Turkey (3.2%) (Kassem & Majoud, 2006), Iran (1.7%) (Matini *et al*., 2012), and in Iraq with (25.86%) in Mosul (Al-Malah, 1981) and (22.60%) in Baghdad (Al-Kaisi, 1994), (24.60%) in Diyala (Al-Hussuny, 2015), (26.00%) in Al-Muthana (Al-Abodi *et al*., 2019).

4.1.2.1: Distributed of infection among regions:-

Table (4-1) shows that the high IR of *T. vaginalis* among women was recorded in Al-Kahla region (96.15%) and the low IR was in Al-Maymouna region (60.00%).

Table (4-1): Distributed of infection among regions.

Regions	No. Exam	No. Infection (%)	χ 2	P -value
Al-Amara	161	117(72.67)	12.118	0.007**
Al-Kahla	26	25(96.15)		
Al-Maymona	25	15(60.00)		
Al-Majar Al-Kabir	14	13(92.85)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This results are in line with Saheb *et al*. (2016) was (85.50%), and does not agree with Al-Muqdadi *et al*., 2017was recorded (19.10%), the highest IR in Al-Kahla may due to the lack of personal hygiene, or due to asymptomatic infection which may extend to six months, or lack of female doctors in primary health care center, specialized in gynecology, or a result of not taking treatment correctly, There are significant differences (χ^2=12.118, p=0.007) among the regions in IR values.

4.1.2.2: Distribution of infection among women's age:-

Table (4-2) shows that highest IR (86.95%) was showed in (34-40) years age groups and lowest IR (37.50%) was in (<15) years age groups.

Table (4-2): Distribution of infection among women's age.

Age group	No. Exam	No. Infection (%)	χ 2	P -value
<15	8	3(37.5)	13.334	0.064(NS)
15-19	22	13(59.09)		
20-26	49	38(77.55)		
27-33	42	32(76.19)		
34-40	46	40(86.95)		
41-47	27	19(70.37)		
48-54	26	20(76.92)		
≥ 55	6	5(83.33)		
Total	226	170(75.22)		

It is shown that the age group (34-40) years has the highest IR (86.95%) and lowest was (37.50%) at (<15) years age group, this findings are in agreement with Fattah & Kadir (2010) and disagreement with Sutton *et al*. (2007) who found the group (14-19) years, was the most affected, IR was increasing at these ages because of the greater sexual activity, either at age ≥55 due to the high level of estrogen that makes the vaginal environment suitable to grow of the *T. vaginalis* (Nwokah *et al*., 2019), non-significant differences (χ^2 = 13.334, p=0.064).

4.1.2.3: Relation between trichomonasis infection rate and women social status:-

Table (4-3) shows that the highest *T. vaginalis* IR was recorded among married women (80.92%) and the lowest IR was recorded among unmarried (40.62%).

Table (4-3): Relation between trichomonasis infection rate and women social status.

Social Status	No. Exam	No. Infection (%)	χ 2	P -value
Married	194	157(80.92)	23.938	<0.001***
Unmarried	32	13(40.62)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This results are in line with the findings of Al-Kahfaji (2020) which found IR was (81.90%), This may due to the transmitted of the infection that occurred through sexual contact, as well as the use of contraception,

which may have increased the IR of trichomoniasis among married women (Paniker and Ghosh, 2017). There are significant relationship between social status and trichomoniasis IR (χ^2 =23.938, p<0.001).

4.1.2.4: Relationship between the trichomoniasis infection rate and the education level of the women:-

In table (4-4) shows there result no significant relationship between the trichomoniasis IR and education levels of women (χ^2 =6.342, p=0.096).

Table (4-4): Relationship between the trichomoniasis infection rate and the education level of the women.

Education level	No. Exam	No. Infection (%)	χ 2	P -value
illiteracy	112	87(77.67)	6.342	0.096(NS)
Primary	85	66(77.64)		
Secondary	16	8(50.00)		
A graduate	13	9(69.23)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

Despite that the illiterate women have the highest IR (77.67%) and the lowest IR was (50.00%) at women with secondary level, these findings agree with Jarallah (2013) and did not agree with Salman and Kareem (2013), which found the primary education was the highest IR. This may due to poor health care and lack of women's awareness programs which put the women at the risk of infection (Yeh *et al*., 2013).

4.1.2.5: Relationship between the women's occupation and trichomoniasis infection rate:-

In table (4-5) found a significant relationship between the women's occupation and vaginal trichomoniasis IR (χ^2 =10.598, p=0.014) it is showed that the highest infection rate is recorded among housewives (78.00%) and no infection was showed among baby girls (0.00%).

Table (4-5): Relationship between the women's occupation and trichomoniasis infection rate.

Occupation	No. Exam	No. Infection (%)	χ 2	P -value
Housewife	200	156(78.00)	10.598	0.014*
Employee	14	8(57.14)		
Student	10	6(60.00)		
Baby girl	2	0(0.00)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

These findings agree with Nas *et al*. (2020), Where they found that the highest IR was among unemployed women, and are not in agreement with Mahdi *et al*. (2001), this may return to the poor economic status, which caused a lack in immunity as a result of malnutrition, lack of awareness, and neglect of taking appropriate treatment (Wiesenfeld *et al*., 2001).

4.1.2.6: Relation of women's husband merited status and trichomoniasis infection rate:-

Table (4-6) shows that the marital status of husband is one of the matters that shows a direct effect on the rates of infection with *T. vaginalis*, where the infection rate is high among women with polygamous husbands (80.67%) compared with the women have monogamous husbands (65.33%).

Table (4-6): Relationship of women's husband merited status and trichomoniasis infection rate.

Husband marital status	No. Exam	No. Infection (%)	χ 2	P -value
Monogamous	75	49(65.33)	5.734	0.017*
Polygamous	119	96(80.67)		
Total	194	145(74.74)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

The current study shows a significant effect of women's husband marital status on IR (χ^2=5.734, p=0.017). It is shown the women of polygamous husband had high IR (80.67%) than women have monogamous husband. This finding may be returned to poor sexual behavior or to multi-sexual of women husband with his wives which

increased the chance of sexual transmitted infection especially trichomonasis (Thurman and Doncel, 2011).

4.1.2.7: Relationship between the number of births and trichomoniasis infection rate:-

Table (4-7) shows that the group of women with (4-6) births had the highest infection rate (83.82%) and the lowest infection rate was among women who had (7-9) births (64.70%), it was found that there is a significant relationship between the trichomoniasis IR and number of births.

Table (4-7): Relationship between the number of births and trichomoniasis infection rate.

No. Birth	No. Exam	No. Infection (%)	χ 2	P -value
0	15	11(73.33)	5.566	0.135(NS)
1 – 3	77	54(70.12)		
4 – 6	68	57(83.82)		
7 – 9	34	22(64.70)		
Total	194	144(74.22%)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This finding agrees with Al-Hussuny (2015) and Al-Muk (1992) and dis agrees with Nouraddin and Alsakee (2015), this may due to increase of the period of pregnancy which caused the weakening of the body's immunity and the physiological changes that occur in woman's body during pregnancy (Poole and McClelland, 2013).

4.1.2.8: Relationship between the women's residency and the infection rate:-

Table (4-8) shows that highest infection rate was among rural women (81.53%), and the lowest infection rate was among women residing in urban areas (72.67%).

Table (4-8): Relationship between the women's residency and the infection rate.

Residence	No. Exam	No. Infection (%)	χ 2	P -value
Urban	161	117(72.67)	1.954	0.162(NS)
Rural	65	53(81.53)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

The present study shows a high trichomoniasis IR among women living in rural areas (81.53%) compared with urban´ s women (72.67%) but there is no significant relationship between the residency and vaginal trichomoniasis (χ^2=1.954, p=0.162). This finding is agreed with Taher and shaker (2018), and does not agree with Ali *et al*. (2017), the high IR in rural s women may be returned to lack of health services, poor or delay of treatment, neglect of hygiene (Al Saeed, 2011).

4.1.2.9: Relationship between pregnancy status of women and the infection rate:-

The results of this study show that there is a high significant relationship between vaginal trichomoniasis IR and the women pregnancy status (χ^2 =0.054, p=0.816). The high IR is recorded among non-pregnant women (81.11%), while the IR is lowest among pregnant women (78.57%).

Table (4-9): Relationship between pregnancy status of women and the infection rate.

Status of women	No. Exam	No. Infection (%)	χ 2	P -value
Pregnant	14	11(78.57%)	0.054	0.816(NS)
Non-pregnant	180	146(81.11%)		
Total	194	157(80.92%)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

It was shown that the non-pregnant women have a high IR (81.11%) compared to IR of pregnant women (78.57%). This finding is in line with Abdul-Aziz *et al*. (2019) and disagree with Kadhum (2012). The vaginal infections are greatly affecting the sperms activity and hinder their access to the eggs and thus result in inhabitation the fertilization (Yasmeen *et al*., 2011).

4.1.2.10: Relationship between the women with husband's genital diseases:-

The present study shows in table (4-10) a significant relationship (χ^2 =2.595, p=0.107) between woman's husbands with sexual disease and trichomoniasis IR. It shows that women whom husbands have sexual disease, have high vaginal trichomoniasis IR (87.50%) compared to women that have husbands without sexual disease (72.04%).

Table (4-10): Relationship between the women with husband's genital diseases.

Husband genital diseases	No. Exam	No. Infection (%)	χ 2	P -value
No	161	116(72.04)	2.595	0.107(NS)
Yes	24	21(87.50)		
Total	185	137(74.05)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This result is in line with the findings of Joesoef *et al*.(2001), This is because of the husband's infection with genital diseases, leads to increase the IR of vaginal trichomoniasis and then transmitted to the wife, as well as weakening the immunity and increasing the possibility of infection rates (Habib *et al*. 2005).

4.1.2.11: Relationship between vaginal trichomoniasis and the women type of childbirth:-

The result table (4-11) was observed that there is a statically significant relationship between birth status of women with vaginal trichomoniasis (χ^2=0.038, p=0.845). It was observed that the lower IR (70.00%) was among women who had not given birth regardless of the nature of the birth status whether it is normal or caesarean delivery and at the same time it was observed the IR among women with normal childbirth (72.83%) is higher than with caesarean delivery.

Table (4-11): Relationship between vaginal trichomoniasis and the women type of childbirth.

Childbirth type	No. Exam	No. Infection (%)	χ 2	P -value
Normal births	162	118(72.83)	0.038	0.845(NS)
Caesarean births	10	7(70.00)		
Total	172	125(72.67)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This result agrees with Al-Abbas and Al- Radhi (2019) and disagree with Al-Daheen *et al*.(2005), It was showed that chances of contamination of vagina during normal delivery was higher than that of caesarean delivery (Abduluahab *et al*., 2011).

4.1.2.12: Relationship between the abortions number and vaginal trichomoniasis infection rate:-

Table (4-12) shows that the infection rate is highest among women who have aborted 3 children (100%), and no infection among women who have aborted 5 children (0.00%).

Table (4-12): Relationship between the abortions number and vaginal trichomoniasis infection rate.

No. Abortions	No. Exam	No. Infection (%)	χ 2	P -value
0	163	127(77.91)	11.703	0.039*
1	18	10(55.55)		
2	7	3(42.85)		
3	2	2(100)		
4	3	2(66.66)		
5	1	0(0.00)		
Total	194	144(74.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

The number of abortions recorded the highest IR among women with abortions 3 children (100%) and the lowest IR among women with abortions 5 (0%) and this result is in agreement with Nouraddin and Alsakee (2015); Xueqiang *et al*. (2007) and disagree with Azambakhtair *et al*. (2018) and the reason is due to leaving the disease without treatment, which causes complications and birth of low-weight babies and

increases the chances of miscarriages in pregnant women. The decreasing in the IR of women with 5 miscarriages is due to the lack of samples from this group, which are less than the size of other groups (Al-Hussuny, 2015), significant differences between number of abortions in IR (χ^2 =11.703, p=0.093).

4.1.2.13: Effect of women using other's tools on the infection rate of vaginal trichomoniasis:-

The result shows there is not statistically significant effect of using other's tools from women on vaginal trichomoniasis IR (Table 4-13).

Table (4-13): Effect of women using other's tools on the infection rate of vaginal trichomoniasis.

Others tools	No. Exam	No. Infection (%)	χ 2	P -value
Used	132	102(77.27)	0.717	0.397(NS)
Not used	94	68(72.34)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This finding agrees with Ahmad (2006). This result disagree with (Sulyman, 2008) who study the role of using other's tool in transmitted vaginal trichomoniasis infection. This is may be returned to the hot-dry conditions that may kill the parasite which had poor ability to survival outside the vaginal and the moisture is essential for survival of *T. vaginalis* which die immediately if dried (Whittington , 1951). It was known the non-sexual transmission is rare (Petrin *et al*., 1998).

4.1.2.14: Effect of treatment usage on infection of women with *T. vaginalis*:-

It showed that the vaginal trichomoniasis IR among untreated women (83.07%) was high compared to women who use treatment (72.04%) but there is no significant differences (Table 4-14).

Table (4-14): Effect of treatment usage on infected women with *T. vaginalis.*

Treatment	No. Exam	No. Infection (%)	χ 2	P -value
Used	161	116(72.04)	3.021	0.082(NS)
Not used	65	54(83.07)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

The highest IR among women who did not use the treatment (83.07%) and lowest IR among women who use treatment (72.04%) and this result is in agreement with Kissinger *et al*. (2010); Swygard *et al*. (2004). The reason is leaving the disease without treatment may lead to complications as a result of health work, the low level of health care, and the higher infection among women who have a remedy is used because some strains are resistant to the treatment (Al-Jamaly, 2005), non-significant differences were found (χ^2 =3.021, p=0.082).

4.1.3: Clinical Manifestation:-

4.1.3.1: Relationship between vaginal pH and vaginal trichomoniasis infection rate:-

A current result in table (4-15) shown a highest significant statistically effect (χ^2 =185.276, p<0.001) of women's vagina on vaginal trichomoniasis IR. It shows that women's vagina which had pH (5.5,6.0,6.5) has the highest vaginal trichomoniasis IR (92.30,96.63,93.93) % respectively compared with IR (0.00%) among women's vagina with low pH at (4).

Table (4-15): Relationship between vaginal pH and vaginal trichomoniasis infection rate.

pH Value	No. exam	No. Infection(%)	χ 2	P -value
4	48	0(0.00)	185.276	<0.001***
5.5	26	24(92.30)		
6	119	115(96.63)		
6.5	33	31(93.93)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This result agrees with finding of Hawel and Alasadiy (2018), when they show that all women infected with *T. vaginalis* parasite has a pH of (6), and also in line with Glenn *et al*. (2016), which showed the highest IR at (pH >4.5), this may due to the ability of this parasite to change the pH of the vagina in order to maintain its survival (Korosh *et al*., 2017). No infection had been recorded at vaginal (pH 4).

4.1.3.2: Relationship between the vaginal trichomoniasis infection rate and vaginal secretions:-

The result (Table 4-16) is shown a highest significant statistically (χ^2 =49.105, p<0.001) relationship between *T. vaginalis* infection and secretion status of women's vagina. It shows there is (89.40%) of women who had vaginal secretions had *T. vaginalis* infection compare with (46.66%) of women without discharge.

Table (4-16): Relationship between the vaginal trichomoniasis infection rate and vaginal secretions.

Secretions	No. exam	No. Infection(%)	χ 2	P -value
Yes	151	135(89.40)	49.105	<0.001***
No	75	35(46.66)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

These results are in agreement with Asiegbu *et al*. (2018) and disagreement with Ranjit *et al*. (2018) who found the highest IR was with light secretions (Maeus *et al*., 2016).

4.1.3.3: Relationship between *T. vaginalis* infection and itching of lower genital organs of women:-

The result found in this study (Table 4-17) is shown a significant statistically relationship between itching of lower genital organs & *T. vaginalis* infection (χ^2 =4.428, p=0.035). it was shown that the women with itching had high IR (77.72%) compared with women without itching (60.60%).

Table (4-17): Relationship between *T. vaginalis* infection and itching of lower genital organs of women.

Itching	No. exam	No. Infection(%)	χ 2	P -value
Yes	193	150(77.72)	4.428	0.035*
No	33	20(60.60)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

The infection related with presence of vaginal itching, so the highest IR is recorded among women with lower genital organs itching (77.72%) compared to women without lower genital organs itching (60.60%), this result agrees with findings of Ajayi *et al*. (2016), while it disagree with Nzomo *et al*. (2013) where itching may be caused by the combination with other some pathogenic microorganisms such as bacteria, fungi and yeasts which may enhance the effect of the infection with this parasite, and it is attributed to the cause of the abundance of vaginal secretions, as well as skin itching, and also accompanied by other clinical manifestations such as unpleasant odors that smell like fish (Al-Marsomy, 2020).

4.1.3.4: Relationship between the women's urogenital tract infection with *T. vaginalis* and the burring micturition:-

This study is found Table (4-18) no significant statistically (χ^2 =2.652, p=0.103) between urine burring and women vaginal trichomoniasis, shows that the infection rate is higher among women who suffer from burning micturition (76.27%) and the infection rate is lower among women without burning micturition (54.54%).

Table (4-18): Relationship between the women's urogenital tract infection with *T. vaginalis* and the burning micturition.

Burning micturition	No. exam	No. Infection(%)	χ 2	P -value
Yes	215	164(76.27)	2.652	0.103(NS)
No	11	6(54.54)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

As for burning micturition, the highest IR was recorded among women who suffer from burning micturition (76.27%) and the lowest IR among

women who did not suffer from burning micturition (54.54%), and this result agrees with von Glehn *et al*. (2017); Mavedzenge *et al*. (2010) and disagree with Heine *et al*. (1994), the reason may due to the pH changes in the vaginal environment that the parasite plays a vital role in making these changes, that may lead to a burning micturition (Al-Ani , 2005).

4.1.3.5: Relationship between the women's urogenital tract infection with *T. vaginalis* and the lower abdominal pain:-

The result of the current study finds that there is no significant statistically Table (4-19) between *T. vaginalis* infected women and the lower abdominal pain shows that the infection rate was high among women suffering from lower abdominal pain (80.00%) and the lower infection rate among women without low abdominal pain (73.68%).

Table (4-19): Relationship between the women's urogenital tract infection with *T. vaginalis* and the lower abdominal pain.

Lower abdominal pain	No. exam	No. Infection(%)	χ 2	P -value
Yes	55	44(80.00)	0.891	0.345(NS)
No	171	126(73.68)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

The highest IR was recorded among the women who are suffering from lower abdominal pain (80.00%) and the lowest IR was recorded among the women without lower abdominal pain (73.68%). This result agrees with Schwebke *et al*. (2020); Sena *et al*. (2007), because the parasite can infect the urogenital tract such as cervix, vagina, urethra which usually leads to tissue damage occurs in the affected area, especially when complications of the disease occur, all that may lead to pain in the lower abdomen (Al- Mahdawy, 2006). Non-significant differences were found (χ^2 =0.891, p=0.345).

4.1.3.6: Relationship of women's urogenital tract infection and color of urine:-

The result (Table 4-20) shows a significant statistically relationship (χ^2 =43.01, p<0.001) between *T. vaginalis* infection and urine color, it shows that the women who had dark yellow and yellow urine, normally had a

high *T. vaginalis* IR (83.56% & 81.10%) respectively compare to the women who had normal urine (23.07%).

Table (4-20): Relationship of women's urogenital tract infection and color of urine.

Urine color	No. exam	No. Infection(%)	χ 2	P -value
Dark yellow	73	61(83.56)	43.01	<0.001***
Yellow	127	103(81.10)		
Normal	26	6(23.07)		
Total	226	170(75.22)		
* P<0.05, ** P<0.01, *** P<0.005, NS= No Significance, P>0.05				

This result agrees with Asmah *et al*. (2019) and disagree with Al-Samarraie (2002), the reason may due to the changes caused by the parasite in the environment of the urinary tract and thus changes the color of urine and may be accompanied by blood drops (Al-Somaeday, 2006).

4.2: Molecular Study of *T. vaginalis*

In different regions, seven *T.vaginalis* pure cultured samples in "InPouch ^{TV} selective media (BIOMED) were randomly chosen for the molecular study. The 1.5% Agarose gel electrophoresis of PCR products found the BTUB1/2 gene products had 198bp (Figure 4-9).

The results of gene sequencing of seven PCR products of BTUB 1/2 gene were identifying with available data base of *T. vaginalis* isolates in NCBI gene bank. It showed the identification of the seven isolates of our study (isolates of Maysan) with that of gene bank ranged from 90.43% to 100% (Table 4-21).

The first sample matches with each (L05468.1) and (XM-001321203.1) isolates of (98.86%) and (97.73%) respectively. The second sample matches with each (L05468.1, XM-001321203.1 and XM-001311949.1) isolates of 100% 98.86% and 94.32%) respectively.

The third sample matches with each (L05468.1, XM-001321203.1 and XM-001311949.1) isolates of (100%, 98.86% and 94.32%) respectively. The fourth sample matches with each (L05468.1, XM-001321203.1, JF513200.1 and XM-001284521.1) isolates of (98.86%, 97.73%, 94.26% and 94.92%) respectively. The fifth sample matches with each (L05468.1,

XM-001321203.1, XM-001579619.1 and JX399872.1) isolates of (99.43%, 98.30%, 94.32% and 90.34%) respectively. The sixth sample matches with each of (L05468.1, XM-001321203.1and XM-001582993.1) isolates of (98.86%, 97.73% and 93.18%) respectively. The seventh sample matches with each (L05468.1, XM-001321203.1) isolates of (99.43%, 98.30%) respectively.

4.2.1: Nucleotide sequence statistics

Statistical analysis of nucleotides was performed by CLC Sequence viewer 8.0 program, and the sequence type, length (base pair) and weight were identified. The results of the contents of each nucleotide sequence from each of the nitrogenous bases (Adenine, Thymine, Cytosine and Guanine) and the base pair A + T and C + G and their frequencies are shown in the Tables (4-22), (4-23) and (4-24).

4.2.2: Neighbor joining relationship analysis:-

Similarity and differences among strains can be used to infer evolutionary relationship (Phylogeny). This is because; if two strains are very similar they are likely to have shared a common ancestor. In the present study, beta-tubulin gene of *T. vaginalis* has been analyzed, to detect out the conservation among DNA nucleotides using multiple sequence Alignment technique. Resulting alignment indicates the Samples (2 and 3) have similar reference sequence at 100% , regions of similar sequences at least 93.18 % in all the sequences that define a conserved conscience pattern or domain. In this case alignment is particularly strong, used to align position to try and drive the possible evolutionary relationship among the sequences. The presence of similar domain in several similar sequences implies a similar biochemical function or structure fold that become the basis of further experimental investigations. A group of similar sequences may define a protein family, which may share a common biochemical function or evolutionary origin. A neighbor joining distance was generated by BLAST N result indicating the distance from related species of *T. vaginalis*, interpreting the neighbor joining relationship (Figure 4-10). This results is in agreement with Dwivedi *et al*., (2012).

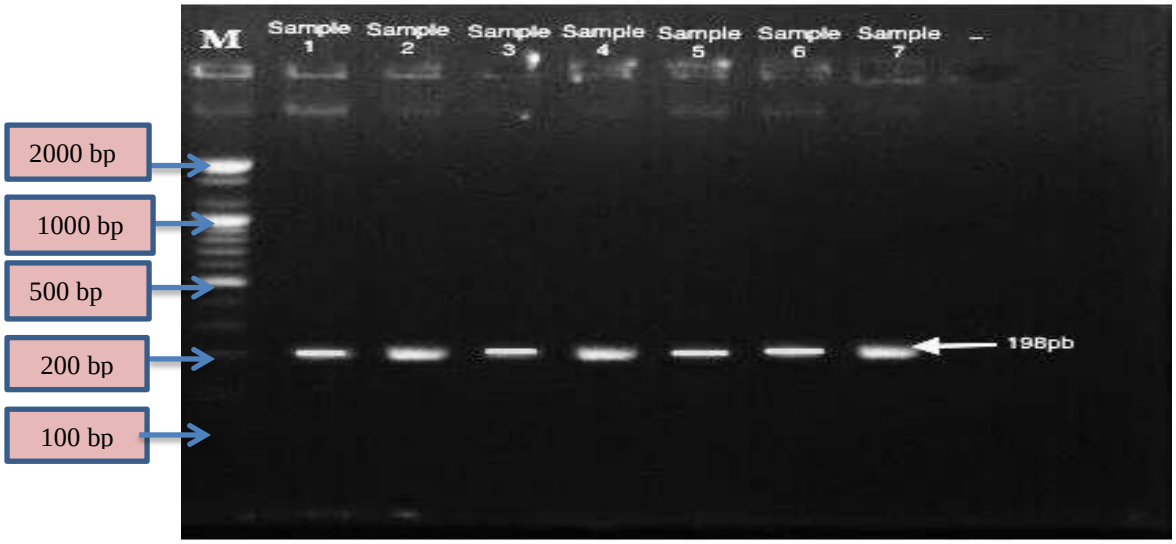


Figure (4-9): Electrophoreses design of PCR product for gene BTUB 1/2 198bp, 1.5% Agarose , 80mV, 60min, M: DNA Marker Ladder 100bp.Sample1, Sample2, Sample3, Sample4, Sample5, Sample6, Sample7.

Table (4-21): Sequences of PCR products of BTUB 1gene identification with NCBI databases and the reference genes in gene Bank.

Sample	Query coverage	Identity %	Accession in Genbank
Sample 1	88%	98.86%	L05468.1
	88%	97.73%	XM-001321203.1
Sample 2	88%	100%	L05468.1
	88%	98.86%	XM-001321203.1
	88%	94.32%	XM-001311949.1
Sample 3	88%	100%	L05468.1
	88%	98.86%	XM-001321203.1
	88%	94.32%	XM-001311949.1
Sample 4	88%	98.86%	L05468.1
	88%	97.73%	XM-001321203.1
	61%	94.26%	JF513200.1
	59%	94.92%	XM-001284521.1
Sample 5	88%	99.43%	L05468.1
	88%	98.30%	XM-001321203.1
	88%	94.32%	XM-001579619.1
	88%	90.34%	JX399872.1
Sample 6	88%	98.86%	L05468.1
	88%	97.73%	XM-001321203.1
	88%	93.18%	XM-001582993.1
Sample 7	88%	99.43%	L05468.1
	88%	98.30%	XM-001321203.1

Table (4-22): Sequence information of PCR products of BTUB 1gene identification with nucleotide sequence statistics

Information	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Sequence type	DNA	DNA	DNA	DNA	DNA	DNA	DNA
Length	198bp	198bp	198bp	197bp	198bp	197bp	198bp
Organism	Not available	Not available	Not available	Not available	Not available	Not available	Not available
Description	1	2	3	4	5	6	7
Modification Date	Not available	Not available	Not available	Not available	Not available	Not available	Not available
Weight (Single stranded)	60.816 kDa	60.806 kDa	60.806 kDa	60.502 kDa	60.821 kDa	60.532 kDa	60.791 kDa
Weight (double stranded)	122.391 kDa	122.389 kDa	122.389 kDa	121.772 kDa	122.388 kDa	121.77 kDa	122.39 kDa

Table (4-23): Counts of nucleotides of PCR products of BTUB 1gene identification with nucleotide sequence statistics

Nucleotide	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Adenine (A)	48	48	48	48	48	48	48
Cytosine (C)	66	65	65	65	64	63	66
Guanine (G)	45	44	44	44	44	44	44
Thymine (T)	39	41	41	40	42	42	40
C + G	111	109	109	109	108	107	110
A + T	87	89	89	88	90	90	88

Table (4-24): Frequencies of nucleotides of PCR products of BTUB 1gene identification with nucleotide sequence statistics

Nucleotide	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Adenine (A)	0.242	0.242	0.242	0.244	0.242	0.244	0.242
Cytosine (C)	0.333	0.328	0.328	0.33	0.323	0.32	0.333
Guanine (G)	0.227	0.222	0.222	0.223	0.222	0.223	0.222
Thymine (T)	0.197	0.207	0.207	0.203	0.212	0.213	0.202
C + G	0.561	0.551	0.551	0.553	0.545	0.543	0.556
A + T	0.439	0.449	0.449	0.447	0.455	0.457	0.444

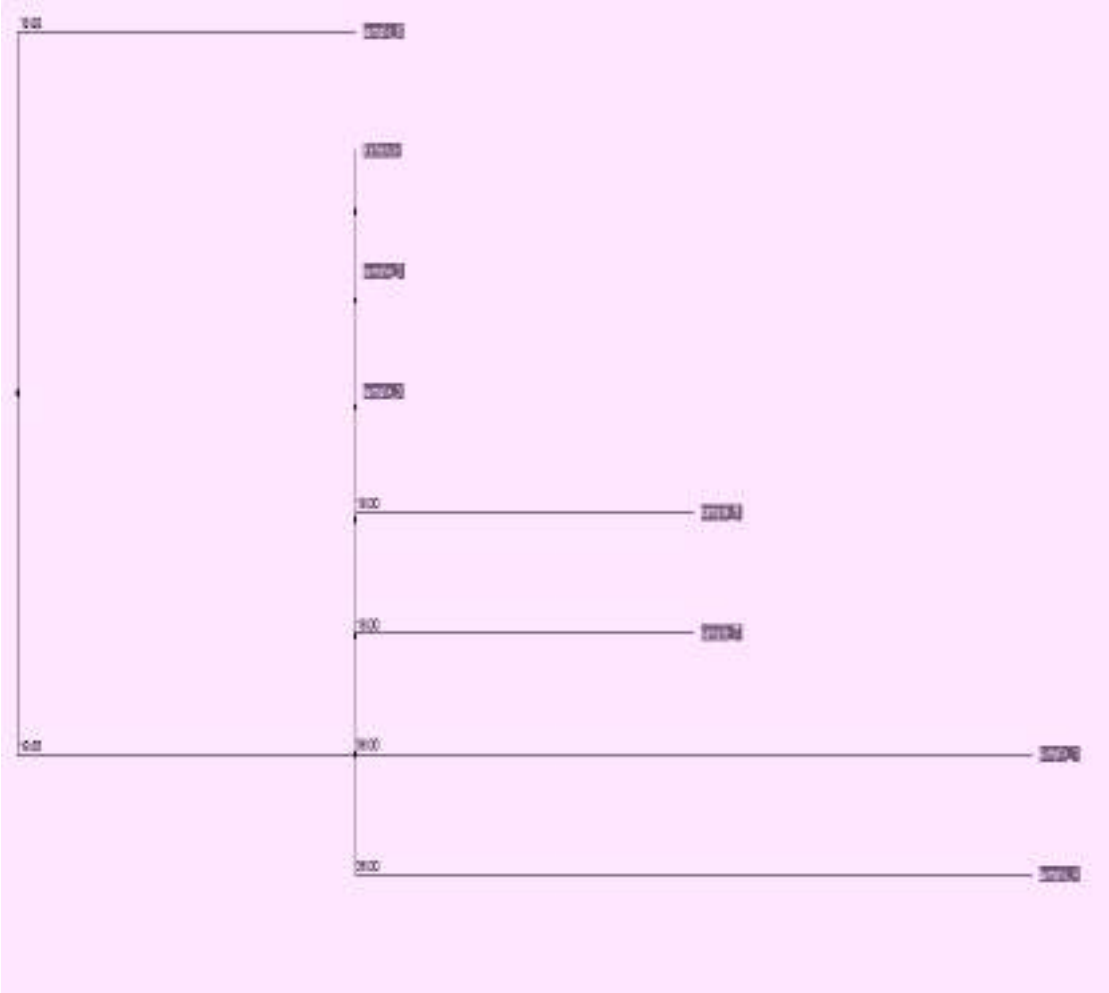


Figure (4-10): Neighbor joining showing relationship between trichomonads.

4.2.3: Recording the strains of *T. vaginalis* in NCBI Genbank

The NCBI Genbank was recording the seven isolations of *T.vaginalis* (Isolations of Maysan) under accession numbers as:

Banklt2409417 Seq1 MW375675
Banklt2409417 Seq2 MW375676
Banklt2409417 Seq3 MW375677
Banklt2409417 Seq4 MW375678
Banklt2409417 Seq5 MW375679
Banklt2409417 Seq6 MW375680
Banklt2409417 Seq7 MW375681

These isolations are the first recorded in Iraq.

Rapid and sensitive diagnosis of *T. vaginalis* infections is important for appropriate treatment and to reduce the spread of the disease. It is important to reduce the prevalence of this disease. The infection of *T. vaginalis* was detected by PCR with primer set BTUB1/2 was observed 100% (7 of 7) sensitive and specific for long term culture maintained strains. It was found more sensitive for Maysan population.

All the axenic strains of *T. vaginalis* were 100% PCR positive from designed primer set. PCR with designed primer set BTUB 1/2 had good analytical sensitivity and was able to amplify even one pictogram of DNA per PCR. The predicted DNA product (198 bp) in the targeted beta-tubulin gene was amplified with all *T. vaginalis* strains tested and agenized (7 of 7). The analytical specificity of primer set BTUB 1/2 was optimal, since no targeted DNA products were detected with other protozoa or vaginal pathogens. As no such targeted product was amplified with DNA from *E. histolytica*, *G. lamblia*, *L. donovani*, *N. gonorrhoeae* and *T. gondii* (Simpson *et al*., 2007). In our study, the results of sequencing showed that two samples (2 and 3) match 100% with the reference sequences, as for the rest of the: Sample1, Sample4, Sample5, Sample6, Sample7. It showed that there are differences in the sequencing of nucleotides due to the occurrence of point mutations, including deletion mutations and replacement mutations. The process of recording these samples in the Genbank as new strains of the *T. vaginalis* parasite in Maysan province. The differences between the present Maysan isolates and other recorded strains from different regions of the world might due to differences in the environment nature (Krashin *et al*., 2010), which depended on the status of women in terms of race, marital status, level of education, nature of treatment as well as the environmental conditions that surrounding the study community (Johnston and Mabey, 2008). The present study records several isolates, this may due to vary in the region, nature of treatment, environment and ethnicity (Chapin and Andrea, 2011) but disagree with what Van Der Pol (2007) reported that no difference in strains in closed communities. These differences may due to genetic mutations that may occur due to the wrong habits in the use of treatment (Alessio & Nyirjesy, 2019).

CONCLUSION

&

RECOMMENDATIONS

Conclusion

1- Trichomoniasis is a common widespread disease in Maysan province/ southern Iraq. The infection with trichomoniasis is linked to many health problems and social customs , as well as the personal hygiene of the patient.

2- Infection rate (IR) of women with *T. vaginalis* is influenced by socio-demographic factors such as age, marital status, occupation, region, residence, pregnancy, number of births, level of education, reproductive health status, marital status of a polygamous husband, among others.

3-The IR is affecting by the pH level of vagina. It has been found that clinical features such as the nature of vaginal discharge, burning and itching are related to PH value, and therefore it is important to consider this value as an evidence of the degree of vagina infection with this parasite.

4- Direct microscopic examination of a wet amount of vaginal discharge showed high sensitivity to detect *T. vaginalis* . It was concluded that the increasing in infection rates among illiterates is due to the absence of culture and education among this group of women.

5-The infection is more common among married women because the main factor in transmission by sexual intercourse. Women and couples with genital diseases are more susceptible to be infected because of the increasing the risk of higher rates of infection and the occurrence of infection.

6- Result of PCR and gene sequences, we found seven strains or isolates of *T.vaginalis* in Maysan province. These isolates sent to register in Gene Bank, these strains, it can be considered that the first record in Iraq and Maysan province.

Recommendations

1- Focusing on some issues, an accurate diagnosis and overall health monitoring of the patients, also pay attention to the person's immunity and serious complications.

2- IR recommend non-use of special patient needs, especially clothing and towels.

3-Spreading health awareness and holding educational seminars on sexually transmitted diseases.

4-Giving appropriate treatment to both spouses while someone is injured to prevent and control infection.

5-An accurate study of parasite complications in infected women.

6-Finding other appropriate methods to administer treatment other than oral metronidazole because some strains are resistant to this treatment

7-Use a molecular study to identify to differentiated the varied strain of *T. vaginalis* in Iraq especially in Maysan province.

8- Conducting extensive future studies on this parasite aimed at trying to limit its spread or treat and eliminate it.

9- Governmental institutions, especially the Ministry of Health and the satellite channels, take their role in educating society, especially women, about the necessity of taking care of public hygiene, especially the cleanliness of the genitals, and avoiding forbidden mixing, especially as we live in an Islamic society committed to values.

REFERNCES

Reference

Abdool Karim, S. S., Baxter, C., Passmore, J. A. S., McKinnon, L. R., & Williams, B. L. (2019). The genital tract and rectal microbiomes: their role in HIV susceptibility and prevention in women. Journal of the International AIDS Society, 22(5), e25300.

Abdul-Aziz, M., Mahdy, M. A., Abdul-Ghani, R., Alhilali, N. A., Al-Mujahed, L. K., Alabsi, S. A., ... & Al Karawani, M. (2019). Bacterial vaginosis, vulvovaginal candidiasis and trichomonal vaginitis among reproductive-aged women seeking primary healthcare in Sana'a city, Yemen. *BMC infectious diseases*, *19*(1), 879.

Abou Gamra, M. M., Kamel, H. H., Eldin, H. M. E., & Zahran, F. M. T. (2019). Efficacy of netropsin dihydrochloride against the viability, cytopathogenicity and hemolytic activity of Trichomonas vaginalis clinical isolates. *Journal of Infection and Chemotherapy*, *25*(12), 955-964.

Adjei, C., Boateng, R., Dompreh, A., Okyere, B., & Owiredu, E. W. (2019). Prevalence and the evaluation of culture, wet mount, and ELISA methods for the diagnosis of Trichomonas vaginalis infection among Ghanaian women using urine and vaginal specimens. *Tropical medicine and health*, *47*(1), 33.

Adnan, Z., Dhaher, H., & Marjani, M. F. (2020). Prevalence of Aerobic Bacterial Vaginosis and Trichomonas Vaginalis Associated with Socioeconomic Factors among Women in Misan Governorate.. Indian Journal of Forensic Medicine & Toxicology, 14(1), 745-752

Ahady, M. T., Safavi, N., Jafari, A., Mohammadi, Z., Abed, S., & Pourasgar, S. (2016). Prevalence of Trichomoniasis among 18–48 year-old women in northwest of Iran. *Iranian Journal of Parasitology*, *11*(4), 580.

Ahmad A. 2006. Commonly encountered reproductive tract infection women. JK-Practitioner, 13(1): 52-55.

Ajayi, V. D., Sadauki, H. M., & Randawa, A. (2016). Bacterial vaginosis is a common vaginal infection among first-time antenatal clinic attendees: evidence from a tertiary health facility in North-West Nigeria. *J Prev Inf Cntrl*, *2*, 2.

Al- Mahdawy H.S.M (2006): Laboratory diagnosis of T vagi11C!lis in patients with vaginal discharge. M Sc thesis, College of Health and Medical Technology, Baghdad Univ.

Al Saeed, W. M. (2011). Detection of Trichomonas vaginalis by different methods in women from Dohok province, Iraq. EMHJ-Eastern Mediterranean Health Journal, 17 (9), 706-709, 2011

References

Al- Samarra'ie H.F. (2002) Comparative study of Trichomonas vagina/is and bacteria coexistence in vaginal infection in pregnant and non- pregnant women. MSC thesis, College of Medicine, Baghdad University.

Al-Abbas, W. D. S., & RADHI, O. A. (2019). Incidence of Chlamydia trachomatis and Trichomonas Vaginalis Genital Infections among Non-Pregnant Women in Al-Najaf Province. Kufa Journal for Nursing sciences, 9(1), 1-8

Al-Abodi, H. R. J., Al-Shaibani, K. T. M., & Shaker, E. M. (2019). Molecular investigation of Trichomoniasis in women in Al-Muthana province/Iraq. In Journal of Physics: Conference Series (Vol. 1234, No. 1, p. 012078). IOP Publishing.

Al-Ani , Zeanah H.S. (2005). Seasonal Epidemiology study for vaginal infection in Baghdad . M. Sc. Thesis College of Education (Ibn Al-Haitham), Baghdad University

Al-Assadi, A. F., Yassin, Z., & Abood, H. S. (2020). The Prevalence of Chlamydia Trachomatis Infection Among Gynecological Outpatients Attendees at Central Basra Hospitals Using One-Step Chlamydia Test. *J Obst Gynecol Surg*, *1*(2), 1-6.

Al-Daheen, G., Y Al-Dabbagh, N., & M Al-Habib, H. (2005). The prevalence of trichomonas vaginalis in association with other micro-organisms among women with vaginal discharge in Mosul. Annals of the college of Medicine, Mosul, 31(1), 37-44

Al-Dahmoshi, H. O. M. (2017). Rapid Detection of Microbial Profile among Women with Vaginitis in Hilla City, Iraq. *Journal of Applied Pharmaceutical Science*, *7*(02), 228-232.

Alessio, C., & Nyirjesy, P. (2019). Management of Resistant Trichomoniasis. *Current infectious disease reports*, *21*(9), 31.

Al-Hadraawy, S. K., Al-Katib, S. R., & Al-Awady, H. M. (2018). Immunological study for infected women with T. vaginalis in AL-Najaf AL-Ashraf province. *Al-Kufa University Journal for Biology*, *10*(1), 132-137.

Al-Hussuny, E. M. (2015). An epidemiological study of Trichomonas vaginalis in among women living in Baquba City, Diyala Province, Iraq. *Diyala Journal for Pure Science*, *11*(3).

Ali, M. K., Hathal, H. D., & Almoayed, H. A. (2017). 7. prevalence and diagnosis of sexually transmitted pathogens in a sample of Iraqi women: a molecular study. Iraqi Journal of Medical Sciences, 15(4), 364-376

Al-Jamaly, M.M.H. (2005) Urinary tract infections among female les in Mosul City. MSC Thesis, College of Science, Mosul University.

References

Al-Jawabreh, A., Ereqat, S., Dumaidi, K., Al-Jawabreh, H., Abdeen, Z., & Nasereddin, A. (2019). Prevalence of selected intestinal protozoan infections in marginalized rural communities in Palestine. *BMC Public Health*, *19*(1), 1667.

Al-Kahfaji, M. S. A. (2020). Infection Rate of Trichomoniasis among Women in AL Hilla City. *Indian Journal of Public Health Research & Development*, *11*(2), 2358-2362.

Al-Kaisi, A.A. 1994. The incidence of Trichomonas vaginalis among female with vaginal discharge, MSc. Thesis, College of Medicine, University of Baghdad, Iraq.

AL-Khalidy, K. A. H., & Al-abodi, H. R. (2020). Effect of Vaginal Trichomoniasis on Women who Suffer from Delayed Pregnancy. *European Journal of Molecular & Clinical Medicine*, *7*(1), 45-50.

Al-Malah, O. A. 1981. Studies on Trichomonas vaginalis infection in Mosul. Thesis MSc. College of Medicine, University of Mosul, Iraq.

Al-Mamoori, Z. Z. M., Alhisnawi, A. A. A., & Yousif, J. J. (2020). prediction of trichomoniasis in women complaining vaginal discharge by different methods and determine some immunological markers. *plant archives*, *20*(1), 3653-3658.

Al-Marsomy, H. D. (2020). Association between Trichomonas vaginalis and vaginal bacterial community composition in Human vagina. Research Journal of Pharmacy and Technology, 13(6), 2925-2931.

Al-Muk, L.M.(1992). Micro flora of the vagina during pregnancy with special emphasis on Gardenrella vaginalis. M.Sc. Thesis in Microbiology. College of Medicine, Basra University

Al-Muqdadi, S. F., Mhaisen, F. T., & Al-Tae, A. A. (2017). Distribution of the Infection with Trichomonas vaginalis and Associated Microorganisms in Women Attending Two Hospitals in Al-Sader City, Baghdad. Ibn AL-Haitham Journal For Pure and Applied Science, 23(1), 19-25.

Al-Somaeday, E.G.A. (2006) Study on the prevalence of trichomoniasis in married women attended Tikret Teaching Hospital and the effect of some plant extracts on the parasite. MSC Thesis, College of Education for Women, Tikret Univ.,.

Arbabi, M., Delavari, M., Fakhrieh-Kashan, Z., & Hooshyar, H. (2018). Review of trichomonas vaginalis in Iran, based on epidemiological situation. *Journal of reproduction & infertility*, *19*(2), 82.

Argüello-García, R., Leitsch, D., Skinner-Adams, T., & Ortega-Pierres, M. G. (2020). Drug resistance in Giardia: Mechanisms and alternative treatments for Giardiasis. In *Advances in Parasitology* (Vol. 107, pp. 201-282). Academic Press.

References

Asiegbu, O. G., Asiegbu, U. V., Onwe, B., & Iwe, A. B. C. (2018). Prevalence of bacterial vaginosis among antenatal patients at federal teaching hospital Abakaliki, South East Nigeria. *Open Journal of Obstetrics and Gynecology*, *8*(1), 75-83.

Asmah, R. H., Agyeman, R. O., Obeng-Nkrumah, N., Blankson, H., Awuah-Mensah, G., Cham, M., ... & Ayeh-Kumi, P. F. (2018). Trichomonas vaginalis infection and the diagnostic significance of detection tests among Ghanaian outpatients. BMC women's health, 18(1), 1-10.

Asmah, R. H., Squire, D. S., Lymbery, A. J., Walters, J., Ahmed, H., & Thompson, R. C. A. (2019). Trichomonas vaginalis infection in southern Ghana: clinical signs associated with the infection., 113(7), 359-369.

Atherton, J., Jiang, K., Stangier, M. M., Luo, Y., Hua, S., Houben, K., ... & Roberts, A. J. (2017). A structural model for microtubule minus-end recognition and protection by CAMSAP proteins. *Nature structural & molecular biology*, *24*(11), 931.

Azambakhtiar, A., Nikmanesh, B., Rezaeian, M., Dashti, N., Safari, F., & Zarebavani, M. (2018). The Prevalence of Trichomoniasis in Women Referred to Clinical Centers in South of Tehran, Iran during 2015–2016. Iranian Journal of Parasitology, 13(1), 108.

Baer, A. K. (2020). An investigation into host-defensive mechanisms by Lactobacillus species against pathogenic Trichomonas vaginalis (Doctoral dissertation, Research Space@ Auckland).

Barajas-Mendiola, M. A., Castellano, L. E., Barrios-Rodiles, M., Deveze-Alvarez, M. A., Avila, E. E., & Cuéllar-Mata, P. (2019). Reduced Trichomonas vaginalis viability in mice pretreated with parasite DNA. *Parasitology*, *146*(13), 1636-1645.

Beri, D., Yadav, P., Devi, H. R., Narayana, C., Gadara, D., & Tatu, U. (2020). Demonstration and Characterization of Cyst-Like Structures in the Life Cycle of Trichomonas vaginalis. *Frontiers in Cellular and Infection Microbiology*, *9*, 430.

Beyaz, Ş., Akdeniz, N., Yılmaz, A., Demir, S., Öztop, N., Çolakoğlu, B., ... & Gelincik, A. 2020 Diagnostic work-up including CD203c-based basophil activation test in immediate hypersensitivity due to metronidazole and ornidazole and evaluation of cross-reactivity in between. *Allergy*.

Bogitsh, B. J., Carter, C. E., & Oeltmann, T. N. (2018). *Human parasitology*. Academic Press, (385).

Bolumburu, C., Zamora, V., Muñoz-Algarra, M., Portero-Azorín, F., Escario, J. A., & Ibáñez-Escribano, A. (2020). Trichomoniasis in a tertiary hospital of Madrid,

Spain (2013–2017): prevalence and pregnancy rate, confections, metronidazole resistance, and endosymbiosis. *Parasitology Research*, 1-9.

Bouchemal, K., Bories, C., & Loiseau, P. M. (2017). Strategies for prevention and treatment of Trichomonas vaginalis infections. *Clinical microbiology reviews*, *30*(3), 811-825.

Bricheux, G., & Brugerolle, G. (1997). Molecular cloning of actin genes in Trichomonas vaginalis and phylogeny inferred from sequences. FEMS microbiology letters, 153(1), 205-213

Brunham, R. C., & Paavonen, J. (2020). Reproductive system infections in women: lower genital tract syndromes. *Pathogens and Disease*, 78(5), ftaa022.

Bruni, M. P., da Silveira, M. F., Stauffer, D., de Oliveira Bicca, G. L., dos Santos, C. C., da Rosa Farias, N. A., ... & Unemo, M. (2019). Aptima Trichomonas vaginalis assay elucidates significant under diagnosis of trichomoniasis among women in Brazil according to an observational study. *Sexually transmitted infections*, *95*(2), 129-132.

Buggio, L., Somigliana, E., Borghi, A., & Vercellini, P. (2019). Probiotics and vaginal microecology: fact or fancy?. *BMC women's health*, *19*(1), 1-6.

Carlton, J. M., Hirt, R. P., Silva, J. C., Delcher, A. L., Schatz, M., Zhao, Q., ... & Sicheritz-Ponten, T. (2007). Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science, 315(5809), 207-212.

Chapin, K., & Andrea, S. (2011). APTIMA® Trichomonas vaginalis, a transcription-mediated amplification assay for detection of Trichomonas vaginalis in urogenital specimens. *Expert review of molecular diagnostics*, *11*(7), 679-688.

Chemaitelly, H., Weiss, H. A., Smolak, A., Majed, E., & Abu-Raddad, L. J. (2019). Epidemiology of Treponema pallidum, Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, and herpes simplex virus type 2 among female sex workers in the Middle East and North Africa: systematic review and meta-analytics. *Journal of global health*, *9*(2), 1-20.

Chetty, R., Mabaso, N., & Abbai, N. (2020). Genotypic Variation in Trichomonas vaginalis Detected in South African Pregnant Women. *Infectious Diseases in Obstetrics and Gynecology*, *2020*.

Cortez-Maya, S., Moreno-Herrera, A., Palos, I., & Rivera, G. (2020). Old Antiprotozoal Drugs: Still viable Options for Parasitic Diseases or New Options for Other Diseases?. *Current Medicinal Chemistry*.

D'Ancona, C., Haylen, B., Oelke, M., Abranches-Monteiro, L., Arnold, E., Goldman, H., ... & Schizas, A. (2019). The International Continence Society (ICS)

report on the terminology for adult male lower urinary tract and pelvic floor symptoms and dysfunction. *Neurourology and urodynamic*, *38*(2), 433-477.

Daugherty, M., Glynn, K., & Byler, T. (2019). Prevalence of Trichomonas vaginalis Infection Among US Males, 2013–2016. *Clinical Infectious Diseases*, *68*(3), 460-465.

De Aquino, M. F. K., Hinderfeld, A. S., & Simoes-Barbosa, A. (2020). Trichomonas vaginalis. *Trends in Parasitology*, *36*(7), 646-647.

Deivam, S., Rajalakshmi, R., Priyadharshini, S., Seethalaksmi, R. S., Balasubramanian, N., Brindha, T., ... & Prabhu, N. (2014). Prevalence of Trichomonas vaginalis infection among patients that presented to rural tertiary care hospital in Tiruchirapalli, India in 2011 and 2013. *Int J Pharm Res Health Sci*, *2*(3), 255-260.

Dessì, D., Margarita, V., Cocco, A. R., Marongiu, A., Fiori, P. L., & Rappelli, P. (2019). Trichomonas vaginalis and Mycoplasma hominis: new tales of two old friends. *Parasitology*, *146*(9), 1150-1155.

Dias-Lopes, G., Wiśniewski, J. R., de Souza, N. P., Vidal, V. E., Padrón, G., Britto, C., ... & De Jesus, J. B. (2018). In-depth quantitative proteomic analysis of trophozoites and pseudo cysts of Trichomonas vaginalis. *Journal of proteome research*, *17*(11), 3704-3718.

Divakaruni, A. K., Mahabir, B., Orrett, F. A., Rao, A. S., Srikanth, A., Chattu, V. K., & Rao, A. V. C. (2018). Prevalence, clinical features, and diagnosis of Trichomonas vaginalis among female STI clinic attendees in Trinidad. *Journal of Family Medicine and Primary Care*, *7*(5), 1054.

Donné, A. (1836). Animalcules observes dans les matieres purulentes et le product des secretions des organs genitaux de l'homme et de la femme. *CR Acad Sci*, *3*, 385-386.

Dutta, S., Sengupta, P., Izuka, E., Menuba, I., Jegasothy, R., & Nwagha, U. (2020). Staphylococcal infections and infertility: mechanisms and management. *Molecular and Cellular Biochemistry*, 1-16.

Dwivedi, S. P., Husain, N., Singh, R. B., & Malla, N. (2012). PCR based diagnostic assay targeting the beta tubulin gene for the detection of Trichomonas vaginalis infection in vaginal swab samples of symptomatic and asymptomatic women in India. Asian Pacific Journal of Tropical Disease, 2(5), 352-357

Edwards, T., Burke, P., Smalley, H., & Hobbs, G. (2016). Trichomonas vaginalis: Clinical relevance, pathogenicity and diagnosis. Critical reviews in microbiology, 42(3), 406-417.

References

Entesar G.Abduluahab1 , Tawfeek I.alalosy2 , Enas M.Yaseen3 (2011) : Study on The prevalence of trichomoniasis in married women intended Tikret teaching hospital, vol.16, No. 1, 143-146.

Entsar M. Al-Hussuny (2015) : An epidemiological study of Trichomonas vaginalis in among women living in Baquba City, Diyala Province, Iraq. Diyala Journal for pure science Vol.11 No. 3, 13-25.

Espiritu, C. A. L., Justo, C. A. C., Rubio, M. J., Svobodova, M., Bashammakh, A. S., Alyoubi, A. O., ... & O'Sullivan, C. K. (2018). Aptamer selection against a Trichomonas vaginalis adhesion protein for diagnostic applications. *ACS infectious diseases*, *4*(9), 1306-1315.

Fattah, C. O., & Kadir, M. A. (2010). Trichomonas vaginalis Among Women in Sulaimania Governorate-Iraq. *Tikret Journal of Pharmaceutical Sciences*, *6*(1), 1-9.

Faught, B. M., & Reyes, S. (2019). Characterization and treatment of recurrent bacterial vaginosis. *Journal of Women's Health*, *28*(9), 1218-1226.

Ferré, V. M., Ekouevi, D. K., Gbeasor-Komlanvi, F. A., Collin, G., Le Hingrat, Q., Tchounga, B., ... & Dagnra, A. C. (2019). Prevalence of human papillomavirus, human immunodeficiency virus and other sexually transmitted infections among female sex workers in Togo: a national cross-sectional survey. *Clinical Microbiology and Infection*, *25*(12), 1560-e1.

Figueroa-Angulo, E. E., Rendón-Gandarilla, F. J., Puente-Rivera, J., Calla-Choque, J. S., Cárdenas-Guerra, R. E., Ortega-López, J., ... & Arroyo, R. (2012). The effects of environmental factors on the virulence of Trichomonas vaginalis. *Microbes and infection*, *14*(15), 1411-1427.

Friedman, M., Xu, A., Lee, R., N Nguyen, D., A Phan, T., M Hamada, S., ... & M Land, K. (2020). The Inhibitory Activity of Anthraquinones against Pathogenic Protozoa, Bacteria, and Fungi and the Relationship to Structure. *Molecules*, *25*(13), 3101.

Gavinho, B., Rossi, I. V., Evans-Osses, I., Lange, S., & Ramirez, M. I. (2019). Peptidylarginine deaminize inhibition abolishes the production of large extracellular vesicles from Giardia intestinalis, affecting host-pathogen interactions by hindering adhesion to host cells. *Biorxiv*, 586438.

Giolo, S. R. (2004). Turnbull's nonparametric estimator for interval-censored data. *Department of Statistics, Federal University of Paraná*, 1-10.

Glehn, M. D. P., Ferreira, L. C. E. S., Da Silva, H. D. F., & Machado, E. R. (2016). Prevalence of Trichomonas vaginalis and Candida albicans among Brazilian

Women of reproductive age. *Journal of Clinical and Diagnostic Research: JCDR*, *10*(11), LC24.

Grad, A. I., Vica, M. L., Ungureanu, L., Siserman, C. V., Tătaru, A. D., & Matei, H. V. (2020). Assessment of STI screening in Romania using a multiplex PCR technique. *The Journal of Infection in Developing Countries*, *14*(04), 341-348.

Graves, K. J., Ghosh, A. P., Kissinger, P. J., & Muzny, C. A. (2019). Trichomonas vaginalis virus: a review of the literature. *International journal of STD & AIDS*, *30*(5), 496-504.

Greenbaum, S., Greenbaum, G., Moran-Gilad, J., & Weintraub, A. Y. (2019). Ecological dynamics of the vaginal microbiome in relation to health and disease. *American Journal of Obstetrics and Gynecology*, *220*(4), 324-335.

Habib, H.M.; Al-Dabbagh, N.Y. and Al-Daheen, G.A. (2005) The prevalence of Trichomonas vaginalis in association with otl1er 111icro-organi s111s a1nong women with vaginal discharge in Mosul. Annals College Medicine, Mosul, 31(1), 37-44.

Hall, T. A. (1999). Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Sump., 41: 95-98.

Hanna, J., Yassine, R., El-Bikai, R., Curran, M. D., Azar, M., Yeretzian, J., ... & Hubeish, M. (2020). Molecular epidemiology and socio-demographic risk factors of sexually transmitted infections among women in Lebanon. *BMC Infectious Diseases*, *20*, 1-11.

Harp, D. F., & Chowdhury, I. (2011). Trichomoniasis: evaluation to execution. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *157*(1), 3-9.

Hassan, F. A., Al-Marsomy, H. D., & Mustafa, S. A. (2019). Diagnosis of Trichomonas vaginalis infection by detection of glutaminase (Glut) gene by nested PCR. *scopus ijphrd citation score*, *10*(7), 478.

Hawel, N. H., & Alasadiy, Y. D. K. (2018). Immunological Diagnosis for Trichomoniasis in Women in Al-Muthana Province. *Almuthanna Journal of Pure Science (MJPS)*, *5*(1), 1-9.

Hegazi, M. M., Makhlouf, L. M., Elbahey, M. A., El-Hamshary, E. M., Dawoud, H. A., & El-Gayar, E. K. (2009). Polymerase chain reaction versus conventional methods in the diagnosis of vaginal trichomoniasis. Journal of the Egyptian Society of Parasitology, 39(1), 11-21.

References

Heine, R. P., McGregor, J. A., Patterson, E., Draper, D., French, J., & Jones, W. (1994). Trichomonas vaginalis: diagnosis and clinical characteristics in pregnancy. Infectious diseases in obstetrics and gynecology, 1.

Helms, D. J., Mosure, D. J., Metcalf, C. A., Douglas Jr, J. M., Malotte, C. K., Paul, S. M., & Peterman, T. A. (2008). Risk factors for prevalent and incident Trichomonas vaginalis among women attending three sexually transmitted disease clinics. *Sexually transmitted diseases*, *35*(5), 484-488.

Hernández, H. M., Marcet, R., & Sarracent, J. (2014). Biological roles of cysteine proteinases in the pathogenesis of Trichomonas vaginalis. *Parasite*, *21*.

Hinderfeld, A. (2018). *When our microbiota goes rogue: A possible inter-domain microbial interaction between Trichomonas vaginalis and bacteria of the human vaginal microbiota* (Doctoral dissertation, Research Space@ Auckland).

Hinderfeld, A. S., & Simoes-Barbosa, A. (2020). Vaginal dysbiotic bacteria act as pathobionts of the protozoal pathogen Trichomonas vaginalis. *Microbial Pathogenesis*, *138*, 103820.

Hinderfeld, A. S., Phukan, N., Bär, A. K., Roberton, A. M., & Simoes-Barbosa, A. (2019). Cooperative interactions between Trichomonas vaginalis and associated bacteria enhance Para cellular permeability of the cervicovaginal epithelium by deregulating tight junctions. *Infection and immunity*, *87*(5).

Hoehne, O. (1916). Trichomonas vaginalis als häufiger Erreger einer typischen Colpitis purulent. *Zentralbl Gynäkol*, *40*, 4-15.

Hoffman, G. L. (2019). *Parasites of North American freshwater fishes*. Cornell University Press.

http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Trichomoniasis-il.htm

https://en.wikipedia.org/wiki/Maysan_Governorate#Demographics

https://www.ncciraq.org

Huh, S. (2019). Status of common parasitic diseases in Korea in 2019. *Journal of the Korean Medical Association*, *62*(8), 437-456.

Ifeanyi, O. E., Chinedum, O. K., & Chijioke, U. O. (2018). Trichomonas vaginalis: Complications and Treatment. *Int. J. Curr. Res. Med. Sci*, *4*(5), 76-89.

References

Islam, M. Z., Larsen, J., Skov, R., & Angen, Ø. (2019). Carry-over of host nutrients during sampling enhances undesired growth of Staphylococcus aurous in liquid Amies transport medium. Diagnostic microbiology and infectious disease, 93(1), 5-8

Itriyeva, K. (2020). Evaluation of vulvovaginitis in the adolescent patient. *Current Problems in Pediatric and Adolescent Health Care*, 100836.

Jaloob ALJanaby, A. A., AlHadraawy, S., & Mushattat, S. (2018). Immune Response in Pregnant Women Infected with Acute Vaginal Abscess Caused by Staphylococcus Aureus and Trichomonas Vaginalis. *Journal of Clinical & Diagnostic Research*, *12*(6).

Jamshidi, A. (2016). Comparison of three methods of clinical diagnosis, microscopic and PCR techniques for detection of trichomoniasis in women in the Yasuj City. *Science*, *5*(1), 12-15.

Jarallah, H. M. (2013). Trichomonas vaginalis infection among women in Basra marshes villages south Iraq. Egyptian Journal of Experimental Biology, 9(1), 71-74

Jarrett, O. D., Srinivasan, S., Richardson, B. A., Fiedler, T., Wallis, J. M., Kinuthia, J., ... & McClelland, R. S. (2019). Specific vaginal bacteria are associated with an increased risk of trichomonas vaginalis acquisition in women. *The Journal of infectious diseases*, *220*(9), 1503-1510.

Jewarethnam, K. (2014). *Phenotypic characterization of amoeboid forms of trichomonas vaginalis/Kasthuri A/P Jewarethnam* (Doctoral dissertation, University of Malaya).

Joesoef, M. R., Karundeng, A., Runtupalit, C., Moran, J. S., Lewis, J. S., & Ryan, C. A. (2001). High rate of bacterial vaginosis among women with intrauterine devices in Manado, Indonesia. Contraception, 64(3), 169-172.

Johnston, V. J., & Mabey, D. C. (2008). Global epidemiology and control of Trichomonas vaginalis. *Current opinion in infectious diseases*, *21*(1), 56-64.

Kadhum, S. A. (2012). Epidemiological Study of Trichomonas Vaginalis in married females. The Iraqi Journal of Veterinary Medicine (ISSN-P: 1609-5693 ISSN-E: 2410-7409), 36(0E), 293-298

Kassem, H. H., & Majoud, O. A. K. (2006). Trichomoniasis among women with vaginal discharge in Benghazi city, Libya. Journal of the Egyptian Society of Parasitology, 36(3), 1007-1016.

Khurana, S., & Singh, S. (2018). Human Trichomoniasis. In *Infectious Diseases and Your Health* (pp. 99-111). Springer, Singapore.

Kim, J. H., Han, I. H., Kim, S. S., Park, S. J., Min, D. Y., Ahn, M. H., & Ryu, J. S. (2017). Interaction between Trichomonas vaginalis and the Prostate Epithelium. *The Korean journal of parasitology*, *55*(2), 213.

Kissinger, P., Mena, L., Levison, J., Clark, R. A., Gatski, M., Henderson, H., ... & Martin, D. H. (2010). A randomized treatment trial: single versus 7 day dose of metronidazole for the treatment of Trichomonas vaginalis among HIV-infected women. Journal of acquired immune deficiency syndromes (1999), 55(5), 565.

Kjeilen, Tore (2006). "Al Amarah". Encyclopaedia of the Orient. Archived from the original on 21 October 2006. Retrieved 2006-10-20.

Klavs, I., Milavec, M., Kustec, T., Vitek, M. G., Lavtar, D., Zaletel, M., ... & Čretnik, T. Ž. (2019). P370 Prevalence of chlamydia, gonorrhoea, M. genitalium and T. vaginalis in the general population of slovenia, 2016–2017. *Sexually Transmitted Infections*, *95*(Suppl 1), A187.

Korich, F., Reddy, N. G., & Trent, M. (2020). Mycoplasma genitalium and Trichomonas vaginalis: addressing disparities and promoting public health control of two emerging sexually transmitted infections. *Current Opinion in Pediatrics*, *32*(4), 482-488.

Korosh, T., Bujans, E., Morada, M., Karaalioglu, C., Vanden Eynde, J. J., Mayence, A., ... & Yarlett, N. (2017). Potential of bisbenzimidazole-analogs toward metronidazole-resistant Trichomonas vaginalis isolates. Chemical Biology & Drug Design, 90(4), 489-495.

Krashin, J. W., Koumans, E. H., Bradshaw-Sydnor, A. C., Braxton, J. R., Secor, W. E., Sawyer, M. K., & Markowitz, L. E. (2010). Trichomonas vaginalis prevalence, incidence, risk factors and antibiotic-resistance in an adolescent population. *Sexually transmitted diseases*, *37*(7), 440-444.

Kučerová, J. (2019). Interaction between hydrogenosomes and endoplasmic reticulum in Trichomonas vaginalis.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6), 1547-1549.

Kusdian, G., & Gould, S. B. (2014). The biology of Trichomonas vaginalis in the light of urogenital tract infection. *Molecular and biochemical parasitology*, *198*(2), 92-99.

Lee, H. Y., Kim, J., Ryu, J. S., & Park, S. J. (2017). Trichomonas vaginalis α-actinin 2 modulates host immune responses by inducing tolerogenic dendritic cells via

IL-10 production from regulatory T cells. *The Korean journal of parasitology*, *55*(4), 375.

Lee, J. Y., & Yang, J. S. (2020). Prevalence and antimicrobial susceptibility of Mycoplasma hominis and Urea plasma species in no pregnant female patients in Korea: Increasing trend of pristinamycin-resistant isolates. *Antimicrobial Agents and Chemotherapy*.

Leka, A., & Moran, J. (2020). Investigating the role of CR3 in trogocytosis of Trichomonas vaginalis cells by Neutrophil-like cells.

Leli, C., Castronari, R., Levorato, L., Luciano, E., Pistoni, E., Perito, S., ... & Mencacci, A. (2016). Molecular sensitivity threshold of wet mount and an immunochromatographic assay evaluated by quantitative real-time PCR for diagnosis of Trichomonas vaginalis infection in a low-risk population of childbearing women. *Le infezioni in medicina*, *24*(2), 112-116.

Li, Y., Wang, S., Li, H., Song, X., Zhang, H., Duan, Y., ... & Zhang, Z. (2020). Development of a convenient detection method for Trichomonas vaginalis based on loop-mediated isothermal amplification targeting adhesion protein 65. *BMC Infectious Diseases*, *20*, 1-8.

Lillie, R. D., Donaldson, P. T., Pizzolato, P., Russo, A., & Schefstad, F. (1978). Preparation of Eosinates and Giemsa Stains of Low Azure B Content from Hot Acid Dichromate Oxidized Commercial Medicinal Methylene Blue. *Stain technology*, *53*(6), 337-343.

Madani, T. A. (2006). Sexually transmitted infections in Saudi Arabia. BMC infectious diseases, 6(1), 3.

Maeus De ,Paul AG, Lana C,Hian D., Eleuza R.M (2016): Prevalence of Trichomonas vaginalis and Candida albicans among Brazilian women of reproduction age .Journal of clinical and diagnostics Research , 2016;Vol.10(11):LC24-LC27.

Mahdi, N. K., Gany, Z. H., & Sharief, M. (2001). Risk factors for vaginal trichomoniasis among women in Basra, Iraq. EMHJ-Eastern Mediterranean Health Journal, 7 (6), 918-924, 2001

Mahmud, R., Lim, Y. A. L., & Amir, A. (2017). Medical Parasitology. Springer International Publishing: Cham, Switzerland.

Mantini, C., Souppart, L., Noël, C., Duong, T. H., Mornet, M., Carroger, G., ... & Duboucher, C. (2009). Molecular characterization of a new Tetratrichomonas species in a patient with empyema. *Journal of clinical microbiology*, *47*(7), 2336-2339.

References

Margarita, V., Fiori, P. L., & Rappelli, P. (2020). Impact of symbiosis between Trichomonas vaginalis and Mycoplasma hominis on vaginal dysbiosis: a mini review. *Frontiers in Cellular and Infection Microbiology*, *10*.

Masha, S. C., Cools, P., Sanders, E. J., Vaneechoutte, M., & Crucitti, T. (2019). Trichomonas vaginalis and HIV infection acquisition: a systematic review and meta-analysis. *Sexually transmitted infections*, *95*(1), 36-42.

Matini, M., Rezaie, S., Mohebali, M., Maghsood, A. H., Rabiee, S., Fallah, M., & Rezaeian, M. (2012). Prevalence of Trichomonas vaginalis Infection in Hamadan City, Western Iran. Iranian journal of parasitology, 7(2), 67.

Mavedzenge, S. N., Van Der Pol, B., Cheng, H., Montgomery, E. T., Blanchard, K., de Bruyn, G., ... & van der Straten, A. (2010). Epidemiological synergy of Trichomonas vaginalis and HIV in Zimbabwean and South African women. Sexually transmitted diseases, 37(7), 460-466.

Menezes, C. B., Mello, M. D. S., & Tasca, T. (2016). Comparison of permanent staining methods for the laboratory diagnosis of Trichomoniasis. *Revista do instituto de medicina tropical de são Paulo*, *58*.

Mercer, F., & Johnson, P. J. (2018). Trichomonas vaginalis: Pathogenesis, symbiotic interactions, and host cell immune responses. *Trends in parasitology*, *34*(8), 683-693.

Miranda-Ozuna, J. F., Rivera-Rivas, L. A., Cárdenas-Guerra, R. E., Hernández-García, M. S., Rodríguez-Cruz, S., González-Robles, A., ... & Arroyo, R. (2019). Glucose-restriction increases Trichomonas vaginalis cellular damage towards HeLa cells and proteolytic activity of cysteine proteinases (CPs), such as TvCP2. *Parasitology*, *146*(9), 1156-1166.

Moonah, S., Ghosh, S., Jiang, N., Farr, L., & Ngobeni, R. (2019). Parasite-produced MIF cytokine: role in immune evasion, invasion, and pathogenesis. *Frontiers in immunology*, *10*, 1995.

Morris, B. J., Hankins, C. A., Banerjee, J., Lumbers, E. R., Mindel, A., Klausner, J. D., & Krieger, J. N. (2019). Does Male Circumcision Reduce Women's Risk of Sexually Transmitted Infections, Cervical Cancer, and Associated Conditions?. *Frontiers in public health*, *7*, 4.

Motlagh, A. A., Dolatian, M., Mojab, F., Nasiri, M., Ezatpour, B., Sharifi, N., ... & Mahmoodi, Z. (2020). Comparative examination of the therapeutic deficiency of oral metronidazole plus prangos ferulacea vaginal cream versus oral metronidazole

plus placebo vaginal cream in accelerating trichomonas vaginalis infection recovery: A triple-blind clinical trial. *International Journal of Women's Health and Reproduction Sciences*, *8*(2), 174-183.

Mukherjee, S., Mukherjee, N., Gayen, P., Roy, P., & P Sinha Babu, S. (2016). Metabolic inhibitors as ant parasitic drugs: pharmacological, biochemical and molecular perspectives. *Current drug metabolism*, *17*(10), 937-970

Munshi, A. (Ed.). (2012). DNA Sequencing: Methods and Applications. BoD–Books on Demand.

Muthini, F. (2016). *Prevalence of Trichomonas Vaginalis in Relation to Sexual Behavior Among Female Sex Workers Visiting Sex Workers Outreach Program Clinics in Nairobi, Kenya* (Doctoral dissertation, University of Nairobi).

Mwatelah, R., McKinnon, L. R., Baxter, C., Abdool Karim, Q., & Abdool Karim, S. S. (2019). Mechanisms of sexually transmitted infection-induced inflammation in women: implications for HIV risk. *Journal of the International AIDS Society*, *22*, e25346.

Narayankhedkar, A., Hodiwala, A., & Mane, A. (2015). Clinic etiological characterization of infectious vaginitis amongst women of reproductive age group from Navi Mumbai, India. *Journal of sexually transmitted diseases*, *2015*.

Nas, F. S., Yahaya, A., Muazu, L., Halliru, S. A. N., & Ali, M. (2020). Prevalence of Trichomonas vaginalis among pregnant women attending ante-natal care in Kano, Nigeria. *Eur. J. Med. Health Sci*, *2*(2), 39-45.

Nemati, M., Malla, N., Yadav, M., Khorramdelazad, H., & Jafarzadeh, A. (2018). Humoral and T cell–mediated immune response against trichomoniasis. *Parasite Immunology*, *40*(3), e12510.

Nouraddin, A. S., & Alsakee, H. M. (2015). Prevalence of Trichomonas vaginalis infection among women in Erbil governorate, Northern Iraq: An epidemiological approach. European Scientific Journal, 11(24)

Nwokah, E. G., Monday, Z. M., & Azike, C. A. (2019). Improving Detection of Trichomonas vaginalis Infection among Adult Females in a Resource-poor Setting in Ogoniland, Niger Delta, Nigeria. *Asian Journal of Research in Infectious Diseases*, 1-5.

Nwuba, R. I., Evbuomwan, I. O., Olasunkanmi, B., & Odukogbe, A. A. (2019). Comparative Evaluation of Microscopy and Polymerase Chain Reaction Sensitivity in the Diagnosis of Trichomonas vaginalis Using Vaginal Swab Samples from Women in Ibadan, Oyo State, Nigeria. *NISEB Journal*, *17*(2).

References

Nzomo, J., Waiyaki, P., & Waihenya, R. (2013). Bacterial vaginosis and correlates in women of reproductive age in Thika, Kenya.

Owino, E. A. (2020). *Trends in Parasitology: Protozoology*. Excelled Books.

Paniker, C. J., & Ghosh, S. (2017). *Paniker's textbook of medical parasitology*. JP Medical Ltd.

Patel, A. S., & Sheth, A. N. (2020). Vaginitis and Cervicitis. In Sexually Transmitted Infections in Adolescence and Young Adulthood (pp. 53-68). Springer, Cham.

Paxton, R., Munson, E., & Barta, K. (2019). Update in the Molecular Diagnostics of Sexually Transmitted Infections. *Physician Assistant Clinics*, *4*(3), 501-518.

Pekmezovic, M., Mogavero, S., Naglik, J. R., & Hube, B. (2019). Host–Pathogen Interactions during Female Genital Tract Infections. *Trends in microbiology*, *27*(12), 982-996.

Petrin, D., Delgaty, K., Bhatt, R., & Garber, G. (1998). Clinical and microbiological aspects of Trichomonas vaginalis. *Clinical microbiology reviews*, *11*(2), 300-317.

Phukan, N. (2016). *Host cell adhesion of Trichomonas vaginalis is down-modulated by Lactobacillus gasseri with the contribution of an aggregation-promoting factor-like protein* (Doctoral dissertation, Research Space@ Auckland).

Phukan, N., Brooks, A. E., & Simoes-Barbosa, A. (2018). A cell surface aggregation-promoting factor from Lactobacillus gasseri contributes to inhibition of Trichomonas vaginalis adhesion to human vaginal ectocervical cells. *Infection and Immunity*, *86*(8).

Pillay, A., Liu, H., Chen, C. Y., Holloway, B., STURM, W. A., Steiner, B., & Morse, S. A. (1998). Molecular Subtyping of Treponema pallidum Subspecies pallidum. Sexually transmitted diseases, 25(8), 408-414.

Poole, D. N., & McClelland, R. S. (2013). Global epidemiology of Trichomonas vaginalis. *Sexually transmitted infections*, *89*(6), 418-422.

Puente-Rivera, J., Villalpando, J. L., Villalobos-Osnaya, A., Vázquez-Carrillo, L. I., León-Ávila, G., Ponce-Regalado, M. D., ... & Alvarez-Sánchez, M. E. (2017). The 50 kDa metalloproteinase TvMP50 is a zinc-mediated Trichomonas vaginalis virulence factor. *Molecular and biochemical parasitology*, *217*, 32-41.

References

Rahi, A. A., Ali, M. A., & Khamesipour, A. (2014). Comparison of Real time PCR with traditional tools for diagnosis of Trichomonas vaginalis in patient with vaginal discharge at Kut city, Iraq.

Ranjit, E., Raghubanshi, B. R., Maskey, S., & Parajuli, P. (2018). Prevalence of bacterial vaginosis and its association with risk factors among non-pregnant women: A hospital based study. International journal of microbiology, 2018(8349601),1-9.

Rao, V. L., & Mahmood, T. (2020). Vaginal discharge. *Obstetrics, Gynaecology & Reproductive Medicine*, *30*(1), 11-18.

Rath, P. P., & Gourinath, S. (2020). The Actin cytoskeleton orchestra in Endameba histolytic. *Proteins: Structure, Function, and Bioinformatics*.

Rayan, H. Z., Zaki, W. M., & Madkhali, A. M. (2019). Evaluation of staining methods for diagnosis of trichomoniasis in clinically suspected women in jazan, kea. *journal of the Egyptian society of parasitology*, *49*(1), 11-16.

Rein, M. F. (2020). Trichomoniasis. In *Hunter's tropical medicine and emerging infectious diseases* (pp. 731-733). Content Repository Only!.

Riestra, A. M., Valderrama, J. A., Patras, K. A., Booth, S. D., Quek, X. Y., Tsai, C. M., & Nizet, V. (2019). Trichomonas vaginalis induces NLRP3 inflammasome activation and pyroptotic cell death in human macrophages. *Journal of innate immunity*, *11*(1), 86-98.

Rivera-Rivas, L. A., Lorenzo-Benito, S., Sánchez-Rodríguez, D. B., Miranda-Ozuna, J. F., Euceda-Padilla, E. A., Ortega-López, J., ... & Arroyo, R. (2020). The effect of iron on Trichomonas vaginalis TvCP2: a cysteine proteinase found in vaginal secretions of trichomoniasis patients. *Parasitology*, *147*(7), 760-774.

Roberts, L. S., & Janovy, J. (2009). *Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology* (No. 574.5249 R6/2009).

Rostami, M. N., Rashidi, B. H., Habibi, A., Nazari, R., & Dolati, M. (2017). Genital infections and reproductive complications associated with Trichomonas vaginalis, Neisseria gonorrhoeae, and Streptococcus agalactiae in women of Qom, central Iran. *International journal of reproductive biomedicine*, *15*(6), 357.

Saeidi, S., Forgani, F., Javadian, F., & Javadian, E. (2019). Effects of Rosmarinus officinalis plant extract on Trichomonas vaginalis parasites and Candida albicans under laboratory conditions: An Experimental Study. *Gene Cell Tissue*, *6*(3), 1-6.

Saheb, E. J., Kuba, R. H., Zghair, K. H., & Mosa, I. S. (2016). A comparison between trichomoniasis Infection and other vaginal infection among females in Baghdad governorate-Iraq. Iraqi Journal of Science, 57(1C), 545-551.

References

Said, R. I., Mohammad, N. F., Ahmed, H. M., & Salih, D. J. (2020). Isolation and identification of pathogens in patients with Pelvic inflammatory disease in Dohok, Kurdistan of Iraq. *Iraq Medical Journal*, *3*(4).

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406-425.

Salman, Y. J., & Kareem, E. A. (2013). Detection of Trichomonas vaginalis among females attending private gynecological clinics in Kirkuk province using different laboratory methods. J Kirkuk Med Coll, 1(2), 1-8

Schneider, R. E., Brown, M. T., Shiflett, A. M., Dyall, S. D., Hayes, R. D., Xie, Y., ... & Johnson, P. J. (2011). The Trichomonas vaginalis hydrogenosome proteome is highly reduced relative to mitochondria, yet complex compared with mitosomes. International journal for parasitology, 41(13-14), 1421-1434.

Schwebke, J. R., & Burgess, D. (2004). Trichomoniasis. Clinical microbiology reviews, 17(4), 794-803.

Schwebke, J. R., Gaydos, C. A., Davis, T., Marrazzo, J., Furgerson, D., Taylor, S. N., ... & Ferris, D. (2018). Clinical evaluation of the Cepheid Xpert TV assay for detection of Trichomonas vaginalis with prospectively collected specimens from men and women. *Journal of clinical microbiology*, *56*(2).

Schwebke, J. R., Taylor, S. N., Ackerman, R., Schlaberg, R., Quigley, N. B., Gaydos, C. A., ... & McKinney, B. (2020). Clinical validation of the Aptima bacterial vaginosis and Aptima Candida/Trichomonas vaginitis assays: results from a prospective multicenter clinical study. *Journal of clinical microbiology*, *58*(2).

Selim, M. A., Fawzy, E. M., Abd El-Rahman, E. M., Hady, R. S. A., Badr, M. S., & Hamed, E. F. A. (2020). Evaluation of the effect of some medicinal plants on cultured Trichomonas Vaginalis. The Journal of Infection in Developing Countries, 14(07), 793-799

Seña, A. C., Bachmann, L. H., & Hobbs, M. M. (2014). Persistent and recurrent Trichomonas vaginalis infections: epidemiology, treatment and management considerations. *Expert review of anti-infective therapy*, *12*(6), 673-685.

Sena, A. C., Miller, W. C., Hobbs, M. M., Schwebke, J. R., Leone, P. A., Swygard, H., ... & Cohen, M. S. (2007). Trichomonas vaginalis infection in male sexual partners: implications for diagnosis, treatment, and prevention. Clinical infectious diseases, 13-22

Sgibnev, A., & Kremleva, E. (2020). Probiotics in addition to metronidazole for treatment Trichomonas vaginalis in the presence of BV: a randomized, placebo-

controlled, double-blind study. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(2), 345-351.

Shahraki, F., Fouladi, B., Salimi-Khorashad, A., Sepehri-Rad, N., & Dabirzadeh, M. (2020). Epidemiology and Identification of Actin Gene of Trichomonas vaginalis Genotypes in Women of Southeast of Iran Using PCR-RFLP. *Crescent Journal of Medical and Biological Sciences*, *7*(1), 82-90.

Shaw, M. K., Porterfield, H. S., Favaloro, S., Dehon, P. M., Van Der Pol, B., Quayle, A. J., & McGowin, C. L. (2019). Prevalence and cervical organism burden among Louisiana women with Trichomonas vaginalis infections. *PloS one*, *14*(6), e0217041.

Sherrard, J. (2020). How to diagnose and manage Trichomonas vaginalis. *Evaluation*, *14*(47), 19..

Shipitsyna, E., Khusnutdinova, T., Budilovskaya, O., Krysanova, A., Shalepo, K., Savicheva, A., & Unemo, M. (2020). Bacterial vaginosis-associated vaginal microbiota is an age-independent risk factor for Chlamydia trachomatis, Mycoplasma genitalium and Trichomonas vaginalis infections in low-risk women, St. Petersburg, Russia. *European Journal of Clinical Microbiology & Infectious Diseases*, 1-10.

Silva, L. N., Rigo, G. V., Silva, D. B., Carollo, C. A., Trentin, D. S., Silva, M. V., ... & Macedo, A. J. (2020). Hydrolysable tannins from Poincianella (Caesalpinia) microphylla fruits: metabolite profiling and anti-Trichomonas vaginalis activity. *Food Research International*, 109236.

Simpson, K., & Criss, B. (2020). Urethritis. In *Sexually Transmitted Infections in Adolescence and Young Adulthood* (pp. 87-96). Springer, Cham.

Simpson, P., Higgins, G., Qiao, M., Waddell, R., & Kok, T. (2007). Real-time PCRs for detection of Trichomonas vaginalisβ-tubulin and 18S rRNA genes in female genital specimens. Journal of medical microbiology, 56(6), 772-777.

Singh, B. (1997). Molecular methods for diagnosis and epidemiological studies of parasitic infections. International Journal for Parasitology, 27(10), 1135-1145.

Smith KS, Tabrizi SN, Fethers KA *et al.* (2005). Comparison of conventional testing to polymerase chain reaction in detection of Trichomonas vaginalis in indigenous women living in remote areas. *Int J SD AIDS* 16(12): 811–15.

Sood, S., Mohanty, S., Kapil, A., Tolosa, J., & Mittal, S. (2007). In Pouch TV (TM) culture for detection of Trichomonas vaginalis. *Indian J Med Res*, *125*, 567.

Squire, D. (2018). *Genetic and phenotypic variations of Trichomonas SPP in humans* (Doctoral dissertation, Murdoch University).

References

Squire, D. S., Lymbery, A. J., Walters, J., Ahmed, H., Asmah, R. H., & Thompson, R. A. (2019). Trichomonas vaginalis infection in southern Ghana: clinical signs associated with the infection. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, *113*(7), 359-369.

Stephen, H. G., & Richard, D. (2001). Principles and practice of clinical parasitology. John Willey & Sons, Ltd.

Stewart, J., Bukusi, E., Celum, C., Delany-Moretlwe, S., & Baeten, J. M. (2020). Sexually transmitted infections among African women: an opportunity for combination sexually transmitted infection/HIV prevention. *Aids*, *34*(5), 651-658.

Sulyman, M.A. (2008) Epidemiological study on Trichomonas vaginalis and some associated bacteria that causing sexual transmitted diseases and effect of some herbal extraction on the parasite in vitro in Kirkuk city. MSc Thesis, College of Science, Tihit Univ.

Sutton, M., Sternberg, M., Koumans, E. H., McQuillan, G., Berman, S., & Markowitz, L. (2007). The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001–2004. *Clinical infectious diseases*, *45*(10), 1319-1326.

Swygard, H., Sena, A. C., Hobbs, M. M., & Cohen, M. S. (2004). Trichomoniasis: clinical manifestations, diagnosis and management. Sexually transmitted infections, 80(2), 91-95.

Tachezy, J. (Ed.). (2019). *Hydrogenosomes and mitosomes: mitochondria of anaerobic eukaryotes* (Vol. 9). Springer (second edition).

Taher, J. H., & Shaker, M. A. (2018). Epidemiological Study of Trichomonas vaginalis and Other Microorganisms Isolated from Genital Tract of Women in Najaf Province–Iraq. Al-Kufa University Journal for Biology, 10(2), 1-9

Tan, L. T., & Phyo, M. Y. (2020). Marine Cyanobacteria: A Source of Lead Compounds and their Clinically-Relevant Molecular Targets. *Molecules*, *25*(9), 2197.

Tchankoni, M. K., Sadio, A. J., Gbeasor-Komlanvi, F. A., Ferré, V. M., Zida-Compaore, W. I. C., Dorkenoo, A. M., ... & Ekouevi, D. K. (2020). Prevalence and factors associated with Trichomonas vaginalis infection among men who have sex with men and female sex workers in Togo, 2017.

Thompson, A., Timm, K., Borders, N., Montoya, L., & Culbreath, K. (2020). Diagnostic performance of two molecular assays for the detection of vaginitis in symptomatic women. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(1), 39-44.

References

Thurman, A. R., & Doncel, G. F. (2011). Innate immunity and inflammatory response to Trichomonas vaginalis and bacterial vaginosis: relationship to HIV acquisition. *American journal of reproductive immunology*, *65*(2), 89-98.

Tien, V., Punjabi, C., & Holubar, M. K. (2020). Antimicrobial resistance in sexually transmitted infections. *Journal of Travel Medicine*, *27*(1), taz101.

Tipple, C., Rayment, M., Mandalia, S., Walton, L., O'Neill, S., Murray, J., ... & Wilkinson, D. (2018). An evaluation study of the Becton-Dickinson ProbeTec Qx (BDQx) Trichomonas vaginalis trichomoniasis molecular diagnostic test in two large, urban STD services. *Sexually transmitted infections*, *94*(5), 334-336.

Tompkins, E. L., Beltran, T. A., Gelner, E. J., & Farmer, A. R. (2020). Prevalence and risk factors for Trichomonas vaginalis infection among adults in the US, 2013–2014. *PloS one*, *15*(6), e0234704.

Trein, M. R., e Oliveira, L. R., Rigo, G. V., Garcia, M. A. R., Petro-Silveira, B., da Silva Trentin, D., ... & Tasca, T. (2019). Anti-Trichomonas vaginalis activity of chalcone and amino-analogues. *Parasitology research*, *118*(2), 607-615.

Ugbede, S. B. (2019). Trichomonas Vaginalis: A Review of Its Epidemiologic, Clinical and Treatment Challenges in Nigeria, 1(4), 100-104.

Vaca, G. B. B. (2020). Topical Use of Monoclonal Antibodies as a Multipurpose Prevention Technology Offering Contraception and Decreased Transmission of HIV-1 and Trichomonas vaginalis (Doctoral dissertation, Boston University).

Valenti, P., Rosa, L., Capobianco, D., Lepanto, M. S., Schiavi, E., Cutone, A., ... & Mastromarino, P. (2018). Role of lactobacilli and lactoferrin in the mucosal cervicovaginal defense. *Frontiers in immunology*, *9*, 376.

Van Der Pol, B. (2007). Trichomonas vaginalis infection: the most prevalent non-viral sexually transmitted infection receives the least public health attention. *Clinical Infectious Diseases*, *44*(1), 23-25.

Van Gerwen, O. T., & Muzny, C. A. (2019). Recent advances in the epidemiology, diagnosis, and management of Trichomonas vaginalis infection. F1000Research, 8. ISO 690

van Hooff, J. J., Tromer, E., van Dam, T. J., Kops, G. J., & Snel, B. (2019). Inferring the Evolutionary History of Your Favorite Protein: A Guide for Molecular Biologists. *BioEssays*, *41*(5), 1900006.

Vieira-Baptista, P., & Bornstein, J. (2019). Candidiasis, bacterial vaginosis, trichomoniasis and other vaginal conditions affecting the vulva. In *Vulvar Disease* (pp. 167-205). Springer, Cham.

References

Von Glehn, M. D. P., Sá, L. C. E. F., da Silva, H. D. F., & Machado, E. R. (2017). Prevalence of Trichomonas vaginalis in women of reproductive age at a family health clinic. The Journal of Infection in Developing Countries, 11(03), 269-276.

Whittington, M. J. (1951). The survival of Trichomonas vaginalis at temperatures below+ 37° C. *Epidemiology & Infection*, *49*(4), 400-409.

Wiesenfeld, H. C., Lowry, D. L., Heine, R. P., Krohn, M. A., Bittner, H., Kellinger, K., ... & Sweet, R. L. (2001). Self-collection of vaginal swabs for the detection of Chlamydia, gonorrhea, and trichomoniasis: opportunity to encourage sexually transmitted disease testing among adolescents. *Sexually transmitted diseases*, *28*(6), 321-325.

Woike, P., Gaur, R., Sharma, R., & Gaur, Y. (2015). Trichomoniasis-An Overlooked Sexually Transmitted Disease.

Workowski, K. A., & Berman, S. M. (2010). Sexually transmitted diseases treatment guidelines, 2010.

Workowski, K. A., & Bolan, G. A. (2015). Sexually transmitted diseases treatment guidelines, 2015. MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports, 64(RR-03), 1.

Xueqiang, F., Yingzhi, Z., Yanfang, Y., Yutao, D., & Huiqing, L. (2007). Prevalence and risk factors of trichomoniasis, bacterial vaginosis, and candidiasis for married women of child-bearing age in rural Shandong. Japanese Journal of Infectious Diseases, 60(5), 257.

Yang, S., Zhao, W., Wang, H., Wang, Y., Li, J., & Wu, X. (2018). Trichomonas vaginalis infection-associated risk of cervical cancer: A meta-analysis. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *228*, 166-173.

Yasmeen H, Mohammed AF, Asad R and Tamer E (2011): Detection of trichomonas vaginalis in vaginal swab clinical samples from Palestinian women by culture. International scholar Research Net Work. ISRN Microbiology . 2011., Volume 2011,Article ID 872358,4 page.

Yazısız, H., Özhak, B., Öngüt, G., Özekinci, M., Dönmez, L., Çolak, D., ... & Öğünç, D. (2020). Evaluation of Microscopic Examination, Culture and Polymerase Chain Reaction Tests in the Diagnosis of Trichomonas vaginalis Infection. Microbiology bulletin, 54(1), 135-143

Yeh, Y. M., Huang, K. Y., Gan, R. C. R., Huang, H. D., Wang, T. C. V., & Tang, P. (2013). Phosphoproteome profiling of the sexually transmitted pathogen Trichomonas vaginalis. *Journal of Microbiology, Immunology and Infection*, *46*(5), 366-373.

References

Zeng, W., Ma, H., Fan, W., Yang, Y., Zhang, C., Kombe, J. A. K., ... & Zhou, Y. (2020). Structure determination of CAMP factor of Mobiluncus curtisii and insights into structural dynamics. *International journal of biological macromolecules*, *150*, 1027-1036.

Zhang, Z., Li, Y., Wang, S., Hao, L., Zhu, Y., Li, H., ... & Li, X. (2020). The Molecular Characterization and Immunity Identification of Trichomonas vaginalis Adhesion Protein 33 (AP33). *Frontiers in Microbiology*, *11*.

APPENDICES

Appendix (A): The Questionnaire paper used in this study in English language:-

A questionnaire form to study the prevalence of the *Trichomonas vaginalis* parasite among women in different areas of Maysan province, south of Iraq

Sample code

Name:- **Age:-**

Region:- **Education level:-**

Residence:- Urban:- **Rural:-**

Social status:- **Occupation:-**

Is the husband polygamous? **Number of Children:-**

Childbirth type:- **Date of the last birth:-** **No. Abortions:-**

History of the disease:- **Treatment history:-** **Type of treatment:-**

Clinical symptoms:- Burning:- **Itching:-** **Secretions:-**

Urine color:- **Is there pain in the lower abdomen?**

Does the husband suffer from genital disease? **Does the patient have a hysterectomy?**

Type of washing water:- **Extent of using others' tools:-**

pH value:-

Notes:-

Result

( )

Research student

Noor K. S. Al- Majidii

Patient s signature

Date

(/ /)

Appendix (B): The Questionnaire paper used in this study in Arabic language:-

استمارة استبيان لدراسة معدل انتشار طفيلي ***Trichomonas vaginalis***
بين النساء في مناطق مختلفة من محافظة ميسان جنوب العراق

رمز العينة

الاسم / العمر/

السكن / مستوى التعليم /

الحالة الاجتماعية/ المهنة/

هل الزوج متعدد الزوجات/ عدد الاولاد/

عدد الاجهاضات/ نوع الولادة/ تاريخ اخر ولادة/

تاريخ المرض/ تاريخ العلاج/ نوع العلاج/

وجود اعراض مثل / حرقة/ حكة/ إفرازات/

لون الادرار/ هل يوجد ألم اسفل البطن/

هل يعاني الزوج من امراض الاعضاء التناسلية / هل تعاني المريضة من استئصال الرحم/

نوع مياه الغسل/ مدى استخدام المريض لأدوات الغير/

قيمة درجة الحموضة /

الملاحظات/

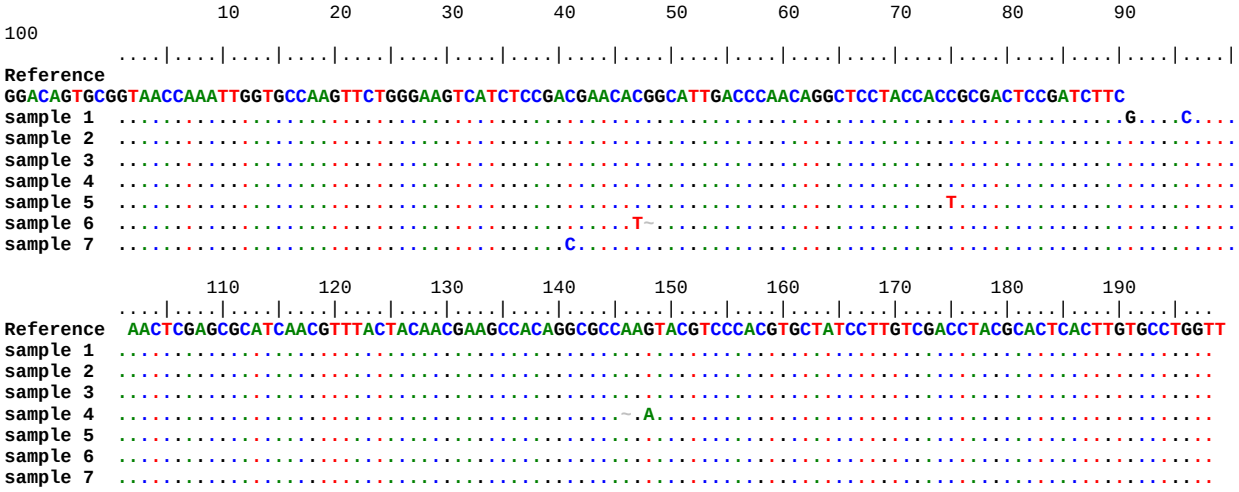
نتيجة الفحص
( )

توقيع المريضة

طالبة البحث/ نور خضير سعد الماجدي

تاريخ اخذ العينة
(/ /)

Appendices (C): sequences alignment of PCR products for specific BTUB 1/2 gene with NCBI reference gene sequences of *T. vaginalis* varied genotypes.



الخلاصة

أن المشعر المهبلي *Trichomonas vaginalis* هو واحد من أكثر الطفيليات انتشارا. فهو يسبب داء المشعرات ، والذي يتسبب في حدوث مخاطر صحية لكلا الجنسين في مختلف أنحاء العالم ، وخاصة المناطق الفقيرة. أجريت الدراسة الحالية لتقصي بعض العوامل الوبائية والخصائص الجزيئية لطفيلي المشعر المهبلي *Trichomonas vaginalis* فيما بين النساء في بعض المناطق من محافظة ميسان جنوبي العراق.

جمعت بيانات الدراسة الحالية خلال الفترة شهر تشرين الثاني 2019 إلى نهاية شهر شباط عام 2020 ، أخذت خلالها 226 عينة من المسحات المهبلية من النساء المشتركات بالدراسة حيث تراوحت أعمارهن من (5 - 60) سنة من اللواتي قدمن لمراجعة العيادات النسائية في المستشفيات والمراكز الصحية والعيادات الطبية والتي شملت أقضية : (العمارة / الكحلاء / الميمونة / المجر الكبير) .

سجلت المعلومات الخاصة بالعوامل السكانية –الاجتماعية للمشاركات في الدراسة في ورقة استبيان معُدة لهذا الغرض مثل العمر والحالة الاجتماعية ومستوى التعليم والمهنة والاقامة والحالة الاجتماعية للزوج ...الخ وبعض المظاهر السريرية مثل درجة حموضة المهبل والحكة وحرقة التبول و لون البول ...الخ . عينات المسحات المهبلية فحصت تحت المجهر الضوئي (قوة تكبير 40) بطريقتين : الاولى بالفحص المجهري المباشر بواسطة تحضير ست شرائح زجاجية لكل عينة ، ثلاثة منها صبغت بصبغة الكيمزا وثلاث بدون صبغة ، العينة الثانية زرعت في وسط Amies transport media وحضنت عند درجة حرارة 37مْ وفحصت كل يومين ولمدة اسبوع لتأكيد نتائج الفحص المجهري .

تم تحليل نتائج الدراسة الحالية احصائيا باستخدام نظام SPSS اصدار24 واستعمل اختبار مربع كاي (χ^2) لتحديد العلاقة بين معدل الاصابة وبعض العوامل السكانية – الاجتماعية والعوامل السريرية للنساء المفحوصات واظهرت نتائج الفحص المجهري لمجتمع الدراسة ان المعدل العام للإصابة قد بلغ (75.22%) وبينت النتائج ان قضاء الكحلاء امتلك اعلى معدل اصابة (96.15%) والفئة العمرية (40-34) سنة لديها اعلى معدل اصابة (86.95%) ومعدل الاصابة (80.92%) لدى النساء المتزوجات كان اعلى من النساء غير المتزوجات (40.62%) وفيما بين النساء الغير حوامل (81.11%) اعلى من النساء الحوامل (78.57%). من جانب اخر تبين ان الاصابة ترتبط بمستوى التعليم ولهذا نرى ان النساء المشاركات الاميات امتلكن اعلى معدل للإصابة (77.67%) مقارنة بغيرهن.

النساء ربات البيوت لديهن اعلى معدل اصابة (78.00 %) من المهن الاخرى ، النساء ذوات الازواج متعددي الزوجات تكون اصابتهن (80.67 %) اعلى من النساء ذوات الازواج احادي الزوجة ، والمجموعة النسوية ذات (6-4) ولادة لديها اعلى معدل اصابة (83.82%) والنساء الريفيات اعلى معدل اصابة (81.53%) من نساء المدينة. واظهرت النتائج ان النساء ذوات الازواج الذين لديهم امراض جنسية امتلكوا أعلى معدل إصابة بداء المشعرات (87.50%)

مقارنة باللواتي ذوات الازواج بدون امراض جنسية. معدل الاصابة يكون أعلى عند النساء اللواتي اجهضن ثلاث اجنة (100%) من الاخريات. واظهرت النتائج ان النساء اللواتي يستخدمن ادوات الغير امتلكن اعلى معدل اصابة (77.27%). ومعدل الاصابة لدى النساء اللواتي لا يستخدمن العلاج (83.07%) هو اعلى من النساء اللواتي يستعملن العلاج.

فيما يتعلق بالمظاهر السريرية كدليل على الاصابة ،تلعب درجة الحموضة pH دورا مهما لحماية مهبل النساء من الاصابة بداء المشعرات حيث لوحظ ان اعلى معدل للإصابة (96.63%) في المهبل ذي درجة حموضة PH يساوي (6) بينما لم تسجل اي اية اصابة لدى النساء اللواتي تكون قيمة ال PH للمهبل لديهن تساوي (4) وهنالك حوالي (89.40%) من النساء اللواتي لديهن افرازات مهبلية مقارنة مع (46.66%) عند النساء اللواتي ليست لديهن افرازات مهبلية ،الحكة هي احد المظاهر السريرية لداء المشعرات حيث كان معدل الاصابة الاعلى بين النساء اللواتي لديهن اعراض الحكة (77.72%) مقارنة مع عدم وجود اعراض الحكة (60.60%). يكون معدل الاصابة عالي (76.27%) فيما بين النساء اللواتي تعاني من حرقة عند التبول ، وان معدل الاصابة يكون عاليا بين النساء اللواتي تعاني من الم اسفل البطن (80.00%). النساء اللواتي يكون لون بولهن اصفر داكن او اصفر يملكن معدل اصابة بالمشعرات المهبلية بلغت (83.56% و 81.10%) على التوالي .

أخيرا استخلاص الDNA وتفاعل السلسلة المتعدد ال PCR والتسلسل الجيني DNA Sequence والمطابقة مع البنك الدولي للجينيات Gene Bank لسبعة عزلات نقية مأخوذة من مناطق مختلفة من محافظة ميسان بوجود تطابق مع العينات الموجودة في قاعدة بيانات البنك الدولي للجينات NCBI بنسبة تراوحت من (90.43% الى 100%) والعزلات تعتبر سلالات تسجل لأول مرة في العراق او على الاقل في محافظة ميسان.

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

دراسة وبائية وجزيئية لطفيلي المشعرات المهبلية للنساء في محافظة ميسان جنوب العراق

رسالة مقدمة

الى مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

نور خضير سعد الماجدي

بكالوريوس علوم الحياة /جامعة المثنى (2009)

بأشراف

أ.م. د. حسين علي مهوس الساعدي

م . د. أسوان كاظم جبر العبودي

تشرين الاول 2020 م

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