The Ministry of Higher Education
and Scientific Research
University of Babylon–College of Medicine
Department of Microbiology

Study of The Co-existing Effect of Parasitic Infections with Micronutrients Deficiencies Among Pregnant Women in Babylon Province.

A Thesis
Submitted to council of the college of medicine , University of Babylon, as a partial fulfillment of the requirements for the degree of master in science/ Medical microbiology .

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صدق الله العظيم

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Dedication

To all my family members, especially my brothers

To all my friends who have helped me

To all study Kind patients

To all people who have helped me to complete this study

Researcher

Acknowledgment

First of all, all thanks to Allah for his kindness and mercy, who have gave me the health, strength and facility to accomplished this work. Full blessing to Mohammed "prophet of Allah" as a guide for believe.

I would like to thank my supervisors Prof. Dr. Hadi Fadhil Alyasari and Assist.prof.Dr. Hanan Khudhair Alkadhim for their unlimited help to complete study work.

I would like to thank all staffs in the hospitals and health care centers for their kind help. I would like to appreciate all people and laboratory workers who have helped me.

Researcher

Summary

The present study was accomplished during the period of October 2021 to March 2022 in order to determine the effect of co-existing of parasitic infection and micronutrients deficiency in pregnant women of Babylon province. Precisely, 100 sample were collected from pregnant women(87stool sample and 13swab samples) who have visited and attending hospitals(Maternity and children Hospital, AL-Amam AL-Sadiq education Hospital) and primary health care centers(Abu-Gharaq health center, AL-Kafal health center, AL-Mohandessin health center) in Babylon province.

Furthermore, a blood sample was taken from each pregnant women. Blood sample put in a gel tube and separated by centrifuge to obtain serum, and then used to know the levels of micronutrients (zinc, VitaminB12, iron) and C- reactive protein that used as an immunological/inflammatory marker. The reminder of blood put in ethylene diamin tetra acetic acid(EDTA) tube to determine the hemoglobin level.

Fresh fecal specimens were collected in a clean container and immediately examined by wet preparation and iodine stain to detect intestinal parasites. Vaginal swabs were taken by use a sterile swab from pregnant women to detect *Trichomonas vaginalis* parasite by use a direct wet mount. Most of pregnant women were asymptomatic or with mild symptoms. Demographical information of pregnant women which concern the age, stage of pregnancy, resident, education level, living conditions.

The study results revealed that a height prevalence of *Blastocyst hominis* 27(27%), *Entamoeba coil* 36(36%) and other nonpathogenic

parasites in a low rate as *Chilomastix mesnili* 3(3%) *and Iodamoeba butschlii* 1(1%) and it was found four species of pathogenic parasites in the study-pregnant women which are *Trichomonas vaginalis* 2(15.3), *Entamoeba histolytica* 2(28.5), *Giardia lamblia* 2(28.5) and *Hymenolips nana*1(14.2). Practically, the present study showed a significant association between the existence of iron deficiency and hemoglobin with parasitic infection pregnant women while that with other micronutrient weren't. Also the present study showed there is no significant association found between CRP and parasitic infection.

The Parasitic infections were with height occurrence in the limited or in the low education level of the pregnant women. Parasitic infection have a higher prevalence in rural area than in urban area. There was an insignificant association between the age of pregnant women and parasitic infection. Parasitic infection with a prevalence in the first and second trimesters than in third trimester of whole gestational period. Virtually, the study has revealed that there was a changes in the studied hematological profiles such as white blood cell(WB), granulocyte, mean corpuscular volume(MCV), HCT(hematocrit), MCH(mean corpuscular hemoglobin), MCHC(mean corpuscular hemoglobin concentration) but not included all pregnant women.

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Abbreviations

Abbreviation	Meaning
ANOVA	Analysis of Variance
CDC	Central disease control
CBC	Complete blood cell count
CRP	C-reactive protein
ELISA	Enzyme linked immunosorbent assay
EDTA	Ethylene Diamin Tetra acetic acid
HCT	hematocrit
Hb	Hemoglobin
HRP	Horse radish peroxidase
HIV	Human immunodeficiency viruses
IgA	Immunoglobin A
IDA	Iron deficiency anemia
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean corpuscular volume
OD	Optical density
PCR	Polymerase chain reaction
RBC	Red Blood Corpuscular
SPSS	Statistical Package for The Social Sciences
UTI	Urinary tract infection
VB12	VitaminB12
WBC	White blood cell
WHO	World Health Organization

Chapter one

Introduction

1-Introduction

Parasite is the term use to describe both a multicellular organisms (helminthes) and a unicellular organisms (protozoa) that depend on other living organisms to obtain its requirement of nutrition and to be survived within or on other organism which called host(Paniker, 2017).

Generally, In developing countries the parasite infection consider as a common cause of morbidity and mortality. Side effects of intestinal parasite infection as vitamin A deficiency, iron deficiency anemia and growth disorders. Intestinal parasite can cause severe complication in patient with hemodialysis, transplant recipient and immunocompromised patient such as HIV patient(Kiani *et al.,* 2016).

Many factors associated with infection as climate, level of education and personal hygiene (Kiani *et al*., 2016). Protozoal parasite with a worldwide distribution and consider as eukaryotic unicellular microorganisms which have wide variety of structure. The parasite use its body structure for obtaining food, movement and for other physiological process and the parasite can be classified according to this structure. Flagellate parasite have whip like structure called flagella, amoebas parasite have finger like structure called pseudopodia which surrounding the food debris and other foreign particles for digestion purposes(Issa,2014). Protozoal parasite have a life cycle alternation between cyst that consider as protective /resist stage and trophozoite form that consider as a reproductive stage. Vacuole "stomach like compartment" be used to digest the food in Protozoal parasite(Issa, 2014). Pregnant women are very susceptible to infection because the suppression in the immune system, physiological and immunological changes in the gestation period (Abu-Raya *et al*., 2020). The

Lowering in vitaminB12 during pregnancy can caused severe effect on mother and her infants like low birth weight, macrocytic anemia and neurological dysfunction(Finkelstein *et al*., 2014). Zinc as an essential micronutrient factor for pregnant women and her infants . Its role range from physiological growth and incorporation in DNA synthesis (Mehata *et al*., 2021).

C-reactive protein(CRP) is a protein found in blood with concentration less than 10mg/l, and its elevation can be occurred during infection and inflammation. CRP can be used as an indicator of acute infection (WHO, 2014).

pregnancy make the pregnant women more susceptible to urinary tract infection because the pregnancy can bring to induce structural, functional, and physiological changes in urinary tract. Therefore pregnant women more susceptible to urinary tract infection(UTI) than non-pregnant women (Kaduma *et al*., 2019).

Many studies show antagonism of co-infection with the parasite sharing the same host like helminthes which can alter the immune system as effect the balance of Th1,Th2 and regulatory T cells this change will give protection against other condition as sever malaria (Blackwell *et al*., 2013).

Giardia lamblia is an intestinal pathogenic flagellated parasite also called *G.duodenalis* or *G.intestinalis*. It is a parasite that found in the duodenum of human being and can Caused acute or chronic diarrhea called giardiasis *(*Carroll *et al.,* 2015*)*.

Entamoeba histolytica is an intestinal pathogenic parasite especially in warm climate that causing amebiasis. *Entamoeba* can caused intestinal and extraintestinal disease/infection as liver abscess. It is a Common reason of death after malaria and schistosomiasis(Paniker, 2017).

Hymenolips nana is a worm belong to tapeworm that caused a disease called hymenolepiasis and can infected human and rat most infection be asymptomatic and recover by itself (Kandi *et al*., 2019).

Trichomonas vaginalis is a pathogenic flagellate parasite that dwell human urogenital tract and causing trichomoniasis. It can be detected from vaginal and urethral discharge. Trichomoniasis as a sexual transmitted disease (Mor *et al*., 2016).

Aim of study:

The study aimed to evaluate the co-existing effects of parasitic infections and micronutrients deficiencies in health status of pregnant women in Babylon province.

Objectives :

The aim was achieved by following objectives :

1-Collection of clinical sample from pregnant women (stool and swab) to detection pathogenic intestinal parasites and urogenital parasites.

2-Collection of blood samples from pregnant women to detection micronutrient deficiency(iron, zinc) detection of hematological factor(Hb) and immunological factor (CRP).

3-Study of demographical factor (age, period of pregnancy, living condition, education level, resident).

Chapter Two

Literature Reviews

2- Literature Review

2-1 : Pregnancy and infection

Many anatomical and physiological changes occur during pregnancy in order to nurture and accommodate the developing foetus and this change resolved after pregnancy. During pregnancy, requirement of certain factors have a height increase as iron and Vitamin B12 because these factors essential for fetus, hemoglobin synthesis and production of certain enzymes. Decline in the hemoglobin concentration, hematocrit and red blood cell. There is no change in mean corpuscular volume(MCV)and mean corpuscular hemoglobin concentration(MCHC) (Soma-Pillay *et al*., 2016) .

The change make pregnant women more exposure to parasitic infection around the world and causing life-threatening condition for pregnant women and fetus. Height prevalence of parasitic infection due to low hygiene, overcrowding conditions, limit clean water sources, socioeconomic and environment conditions. As the result of infection with intestinal parasites like worm the patient will suffer from anemia, low birth weight, fragility in milk production and other worm can cause lower food intake and weight loss (Abaka-Yawson *et al*., 2020).

During pregnancy many changes in immune system occur as alteration in Th1 (decrease in Th1) and their cytokines and increased in Th2 and their cytokines that have necessary changes for successful pregnancy to prevent active inflammatory cytokines from damage of placental cell. The pregnancy and chronic helminthes infection have synergistic effect and that make pregnant women more susceptible to infection and increased severity of this infection. Pregnant women infected with human immunodeficiency

viruses(HIV)with chronic helminthes infection in this can cause increase possibility of HIV transmission to fetus and this called synergistic effect (Abdoli and Pirestani, 2014).

2-2: Malnutrition

The name of malnutrition include both under nutrition and over nutrition statuses. Under nutrition can be measured by observed wasting, stunting, weight loss, weak body and other deficiency criteria in the human being body.

Infection can play an important role in malnutrition beside to insufficient food taken. Infection "especially repeated infection" by parasites can bring to cause malnutrition. Alimentary canal where food digestion with its absorption can occur and it has been considered as a main route of parasites entry to cause both malabsorption and dysfunction of intestinal tract tissues, Anemia, nutrient deficiencies by causing diarrhea, vomiting and blood loss (Yoseph and Beyene, 2020).

malnutrition include acute, chronic and micronutrient deficiencies. The inadequate intake and inadequate absorption of essential micronutrients can cause chronic malnutrition that extended over a long time of lifespan. Stunting consider as an indicator of chronic malnutrition. One of the most common form of chronic malnutrition is micronutrient deficiency which have a sever effect on health and development over life duration(Lenters *et al*., 2016).

Acute malnutrition (wasting) which result from unexpected reduction of food intake or diet quality. Hidden hunger is a common term used when

visible clinical signs cannot give vision about nutrient status. Many risk factors be associated with sever acute malnutrition and moderate acute malnutrition (Lenters *et al*., 2016) as follow:

- Inadequate dietary intake
- Inappropriate feeding
- Family size
- Lack of parental education
- Fetal growth restriction
- Poverty
- Environmental instability and emergency situation

2-3: Anemia

The term anemia use to describe the level of hemoglobin if less than normal value. Most of intestinal parasites considered the main reasons of causing anemia especially by helminthes as a hookworm. Because it bring to cause a severe blood loss. Parasitic infection during pregnancy lead to cause anemia. Furthermore, the parasitic infection and weak nutrient make anemia with very high incidences among developing countries. Anemia during pregnancy can cause severe complications. According to World Health Organization (WHO) the hemoglobin level if less than 11g/ dL consider anemia (Mengist *et al*., 2017).

Anemia classified by WHO to mild anemia if hemoglobin level range is 10-10.9g/dL, moderate anemia if hemoglobin level range is 7.0-9.9g/dL and severe anemia if hemoglobin level less than 7.0g/dL (Mengist *et al*., 2017) . Anemia is a common complication of inflammatory bowel disease. Patient with inflammatory bowel disease suffer from iron deficiency anemia

(IDA) because the inflammation can mediate a defect in iron absorption and chronic or secondary blood loss. Iron deficiency anemia either absolute or functional iron deficiency anemia. Functional iron deficiency occur when insufficient iron available to incorporation in increased iron stores or share in manufacturing of erythrocyte, while the absolute iron deficiency can yielded from poor dietary of iron, defect in iron absorption or increased blood loos . Absolute iron deficiency can occur when the iron stores in bone marrow and the monocyte –macrophage system in liver and spleen become depleted(Kaitha *et al*., 2015).

2-4: Iron

According to WHO, the iron deficiency anemia is with more common occurrence and depend on the socioeconomic status and health condition that reflect nutrient deficiency in women. Iron required for various cellular function and oxygen transport. Nutrional iron have two form haem and non-haem. Haem iron fast absorbed and came from hemoglobin and myoglobin. Animal meat and fish as sources for it. Non-haem iron came from plant food and difficult to be absorbed. (Kumar *et al*., 2022).

Infection with intestinal parasites during pregnancy increase possibility the of iron deficiency anemia. Inflammation and infection as hookworm can cause iron deficiency anemia due to the loss of a height amount of blood (Mengist *et al*., 2017).

In many cases the iron deficiency occur before patient become anemic marked by red cell microcytic and hypochromic. Iron deficiency anemia can be prevented early by diagnosis but not always possible because of not all patients have symptoms and of the delayed interpretation in laboratory

diagnosis. Serum iron , serum ferritin , total iron binding capacity which are common tests have used to diagnose iron deficiency (Sezgin *et al*., 2021). Absorption of iron from the diet always less than sufficient requirements of the body can contribute to cause an iron deficiency. In fact, there are many factors contribute in iron deficiency such as:

1. Physiological (as pregnancy), pathological factor (parasite, inflammatory bowel)
2. Drugs which can caused iron deficiency by increasing blood loss (anti-inflammatory drugs) or decreased iron absorption
3. Chronic disease
4. Genetic defect also caused iron deficiency
5. Gastric surgery, coeliac disease, colonization of *Helicobacter pylori* can cause malabsorption (Lopez *et al*., 2016)

2-5: Vitamin B12

Cobalamin or Vitamin B12 is an important soluble vitamin. Vitamin B12 found in animal products as egg and meat. This vitamin can be absorbed in ileum. Glycoprotein production from stomach cell play an important role in absorption of the vitamin.

Importance of vitamin B12 came from action as it act as a coenzyme and share in DNA synthesis. Many factors causing vitamin B12 deficiency as infection with tape worm as *Diphyllothbothrium latum* (Ankar and Kumar, 2021).

One of the most important effects of vitaminB12 deficiency is macrocytic anemia that distinguished by decline in red blood corpuscular (RBC) count. Always change in RBC can observed by blood film that show alternation in shape and size of red blood cell. Folate intake can be hidden VB12 deficiency. VB12 deficiency came from insufficient dietary, malabsorption and malnutrition. Also this deficiency follow gastric surgery and congenital disorder (Nagao and Hirokawa, 2017).

VitaminB12 deficiency effect on many systems in body and this effect is varied in its severity from mild fatigue to sever neurological weakness. Because of the large amount of VitaminB12 is storage in liver so clinical manifestation, take 5-10 years to appear. Serum Vit B12 elevated in many cases as alcoholism, liver disease and cancer. Many clinical symptoms involved as:

1-Cutaneou as hyperpigmentation, Jaundice and vitiligo.

2. Gastrointestinal as glossitis.

3. Hematologic as anemia (macrocytic anemia and megaloplastic anemia).

4. Neuropsychiatric cognitive impairment and irritability (Langan and Goodbred, 2017).

2-6: Zinc

Zinc deficiency influence on the immune response and debilitated it. Zinc control the immune response that aim to decline the progressive and severity of infection. Elevated or lowered level of this mineral can change the susceptible of intestinal tract against parasitic infection (Fancony *et al*., 2022).

The zinc from important micronutrients that not formed in the body and take from outside environment to maintain its normal level in the body. In developing countries zinc deficiency associated with malnutrition while in developed countries zinc deficiency associated with chronic diseases and aging. The need for zinc increase during pregnancy and lactation. Loss of height amount of zinc occur in many cases like burns, hemodialysis, diarrhea and in case of loss of urine when take diuretics (Maxfield *et al*., 2021).

Manifestation of zinc deficiency is by:

1. Weight loss
2. Diarrhea
3. Open sores on skin
4. More infection
5. Loss appetite
6. Decreased of smell and taste
7. Wound taken long time to heal
8. Hair loss (Hagmeyer *et al*., 2014).

Zinc deficiency either inherited or acquired. Acquired zinc deficiency associated with infection, inflammation, gastrointestinal and cutaneous disease. Much treatment as penicillamine, various diuretics and certain antibiotic inhibited zinc absorption. To management zinc deficiency received zinc supplement which also help in reduce diarrhea duration (Maxfield *et al*., 2021).

2-7: C-reactive protein

It is a protein that produced by liver cell in response to acute and chronic infection or inflammation. Play important role in defense as a first line in innate immunity. CRP give an imagination about the infection that related with morbidity and complication during pregnancy. CRP can help in the diagnosis of asymptomatic and subclinical infection. Precipitation as positive result occur in severe infection but not tell you about the type of pathogen(Kumari *et al*., 2020).

In addition to hepatocyte, the CRP produced by many cell as smooth muscles cell, liver macrophage and adipocytes. When the reason that caused elevated of CRP disappear, CRP decline during 28-20 hour. Interleukin 6 stimulate CRP gene expression while interleukin 1 stimulate CRP effects. CRP can recognized pathogen and infected cell by binding with them and stimulate the complement and phagocytes cell to remove them. CRP have protective and anti-inflammatory function and use as non-specific immunological biomarker.

CRP elevated in acute diarrhea caused by intestinal protozoa as *G.lamblia* and *E. histolytica* but this elevated influenced by many factors as site of parasite in intestinal mucosa, numbers of parasite and long period of infection. Slightly raise in CRP protected parasite from IgM, IgA and phagocytosis. Tissue damage result from attachment of parasite have a role in stimulate hepatic cell to produce CRP. There is no elevated record of CRP in soil transmitted helminthes(Saheb *et al*., 2020).

2-8 Intestinal parasites

Intestinal parasites regarded as a big health problem in tropical and sub-tropical countries such as *E. histolytica* that infect approximately 48 million persons, beside that a height distribution of giardiasis as well as other intestinal helminthes. Parasitic infection associated with many factors that increase its rate of infection such as bad sanitation, environment conditions, way of wastes disposal, walk or play on contaminated soil in which an increase rate of soil transmitted helminthes infection as ascariasis. Many effects associated with intestinal parasitic infection like a poor development, protein malnutrition and iron deficiency anemia (Damtie *et al*., 2021).

Human can infected by pathogenic parasites through several routes such as contaminated water and food, through the skin, eat under cooked products or fecal oral routes (Feleke *et al*., 2019).

Intestinal parasite as a common cause of gastrointestinal disorder and as a faster of height spread of communicable disease. The rate of incidence and frequency of these parasites are different according to different countries, family income, environmental conditions, location and education levels(Kiani *et al*., 2016).

Most intestinal parasitic infection with symptoms and signs can bring to cause stomach pain, diarrhea, weight loss, abdominal pain , rash or itching around the rectum, feeling tired and worm passing in stool (Kiani *et al*., 2016). Parasites have many pathogenic mechanism that occur during infection such as parasite have the ability to produce enzymes to cause lytic necrosis, worms attachment to villi produce a traumatic damage and

bleeding in site of attachment and parasites can cause intestinal obstruction in case of present a large masses of worms during parasitic infection (Paniker, 2017).

2-8-1 *Giardia lamblia*

One of the first described protozoan parasite that inhabit the small intestinal tract of human being. Leeuwenhoek (1681) discovered it in his feces. It is with a cosmopolitan distribution as zoonotic disease. This parasite implicated in traveler's diarrhea as water borne diseases (Paniker, 2017).

2-8- 2 classification

The protozoan parasite *Giardia lamblia* is classified as the following,(Abdalameer, 2018).

Kingdom: Protista

Phylum: Sarcomastigophora

Subphylum: Mastigophora

Class: Zoomastigophora

Order: Diplomonadina

Family: Hexamitidae

Genus: *Giardia*

Species: *lamblia*

2-8-3 Morphology and life cycle

Giardia parasite has two forms cyst and trophozoite. Trophozoite is tears like shape with four pairs of flagella and have two nuclei in the anterior part of trophozoite body to give appearance of face when you look to it. Trophozoite with large adhesive disk used to attachment to intestinal epithelial cells.

Trophozoite stage can be distinguished by falling leaves movement. Cyst is oval shaped with two nuclei in immature cyst and four nuclei in mature cyst. Transmission can occur through ingest of food or contamination of water with cyst. Cyst pass from stomach to intestine, and the change with pH can help it to excystation within intestinal lumen and then encystation accrue with a thick wall after that cyst pass in stool and then to infect another person to form infection (Bogitsh *et al*., 2018).

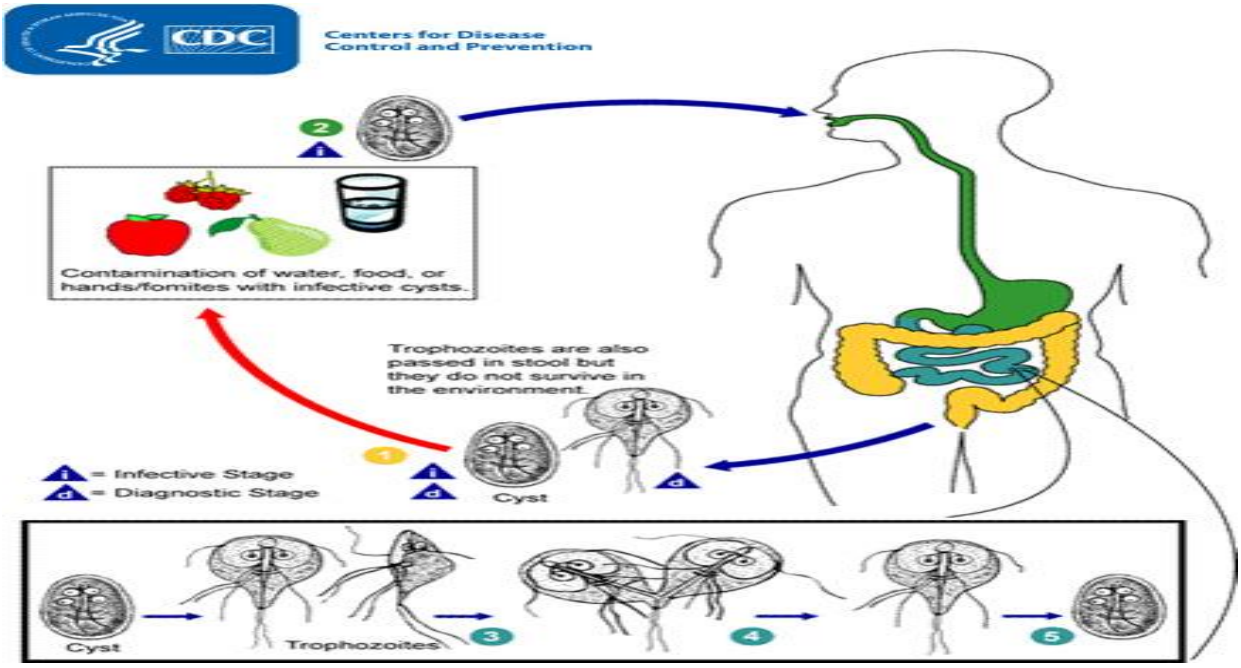


Figure 2-1: life cycle of *G. lamblia* (CDC, 2021).

2-8-4 pathogenesis

Giardia parasite is the etiological agent of giardiasis. After ingest of cyst excystation will occur in duodenum. Trophozoite attachment to villi by adhesive disk. This attachment can caused villi flatting, damage of epithelial cell, inflammation of duodenum lead to malabsorption of fat and protein(Levinson, 2014).

Defect in IgA increase severity of infection as an abdominal pain, weight loss and flatulence. Stool from patients with giardiasis appear as foul smelling, greasy, semisolid or watery stool. *Giardia* parasite never invade and not reach to blood stream(Levinson, 2014).

2-8-5 Treatment of Giardiasis

Infection with this parasite either self-limiting with mild symptoms or chronic ongoing for month to years. *Giardia* associated with post infection complication as chronic fatigue, of gastrointestinal tract or extraintestial complication as arthritis and allergy. Infection by *G.lamblia* related with malnutrition, IgA deficiencies (which is immunoglobin mediate the resolved of parasite infection) and immunosuppression. The treatment use a 5-nitroimidazol, which consist of benzimidazoles, quinacrine, furazolidone, paromomycin and nitazoxanide (Lalle and Hanevik, 2018).

The Aim of the treatment is to short the duration of *Giardia* infection and prevent extraintestial complications. One of the most common reason of treatment failure is repetition of infection (Lalle and Hanevik, 2018).

2-9 *Entamoeba histolytica*

A protozoan parasite was first discovered in Russian people who undergo from sever dysentery by Fedor Losch (1875). The name histolytica came from the ability of this parasite to destroy the tissue cell. All species of *Entamoeba* nonpathogenic except *E.histolytica*. The *E. histolytica* may be invasive and caused extra intestinal abscess like liver abscess or asymptomatic colonization (El-Dib, 2017).

The *E.histolytica* clinical symptoms have come from the ability to damage tissue by three routes direct death of host cell, inflammation and invasion. The destruction of tissue extend to include blood vessels therefore caused bloody diarrhea and reach to blood stream and dissemination to other organ. Parasite have many virulent factors that enable parasite to success and caused infection as lectin that use to bind to galactose and N-acetylgalactosamine sugar of the epithelial cell and establishment of parasite (Leon-Coria *et al*., 2020).

2-9-1 Classification

E. histolytica can be classified as follows, (Sastry and Bhat, 2018).

Kingdom: Protozoa

Subkingdom: Neozoa

Phylum: Amoebozoa

Class: Entamobidea

Order: Euamoebida

Genus: *Entamoeba*

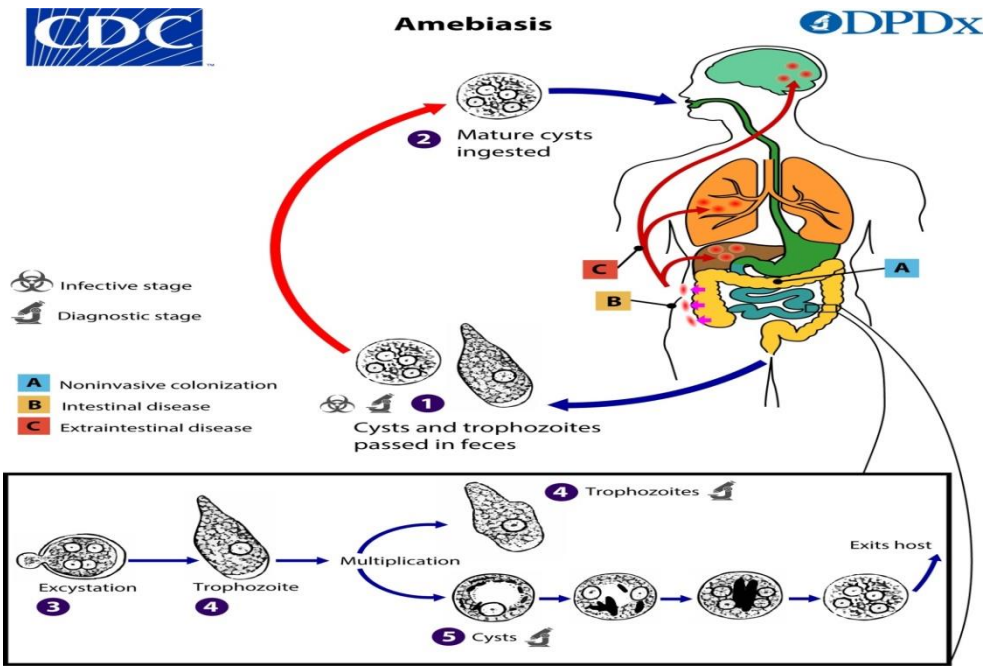
Species: *histolytica*

2-9-2 Morphology and life cycle

Human is the main host of *E.histolytica* and consider as carrier of this parasite. Two forms of *E. histolytica* cyst and Trophozoite stage. Infection occur through fecal oral route by contamination food and water with cyst (Ray and Ryan, 2014).

Cyst reach to colon after ingestation, excyst will occur to produce four nucleated parasite, which then divided to give eight trophozoites. Mature cyst with four nuclei while immature cyst contain 1 to 2 nuclei with chromatoid (aggregation of ribosome) body.

Trophozoite move by finger like pseudopodium in one direction and can distinguish the ectoplasm from endoplasm by appearance as ectoplasm with clear appearance while endoplasm with granular appearance. Invasive strain is a large than that of non-invasive and contain ingested erythrocyte (Ray and Ryan, 2014).



Figure(2-2) : life cycle of *E.histolytica*(CDC,2019).

2-9-3 Pathogenesis

Entamoeba infection as a big health problem in developing countries because the parasite regarded as the main cause of diarrhea and dysentery. The severity of infection come from the ability of the parasite to penetration the epithelial layer and destroyed epithelial cell.

Multiplying of parasite in mucosa of colon result in ulcer and this ulcer resemble flask shape called flask ulcer. This parasite can reaches blood stream and cause extra intestinal lesion. Pregnant women among the most effected group in community and more susceptible to infection than others because pregnancy induce IgA deficiency. Loss of fluid during diarrhea caused by this parasite lead to electrolyte imbalance (Abubakar et al., 2020).

Virtually, less than 10% of infected people with *E. histolytica* can developed to invasive amebiasis. The factor that determine invasive potential

of *E.histolytica* is the ability of parasite to adaptive with intestinal environment and response to acetylcholine that act as neurotransmitter in the site of infection which may help in develop the infection to invasive amebiasis (Medina-Rosales *et al*., 2020).

Trophozoite remain on mucosal surface feed on bacteria and other food particles and start pathological process. parasite have several enzyme as pore forming protein , lipase and cysteine proteases which use to digest food particles in vacuoles and destruction of epithelial cell (Leon-Coria et al., 2020).

2-9-4 Treatment of Amebiasis

A parasite that can reach blood stream is *Entamoeba histolytica*. Its infection range from sever to mild. Get better sanitation and avoid contamination water can help in control of infection. One of the most important factors that help in height spread of this parasite than other is height resistant of cyst which can survive for a long time in environment. Mild anemia, elevated inWBC may absorbed. The parasite can cause infection even in low dose. Severity of disease increase in younger ages than older ages. Increase risk of infection in pregnant women, neonates, person with malignancies, person who received corticosteroids, malnourished individuals and postpartum women. Individuals with amebiasis exposure to secondary bacterial infection, rupture of abscess and extend of infection to pericardium, pleura and gastrointestinal bleeding.

First step of treatment is hydration. Use of metronidazole for 7-14 day (500mg) every 6-8 hour or use of tinidazol for 3day (2g) each day

while treatment of liver abscess by aspiration combination with medicine as metronidazole. Sever gastrointestinal bleeding required surgical intervention (Zulfiqar *et al*., 2021).

2-10 *Hymenolepis nana*

It is one of the most common intestinal helminthes with a worldwide dissemination. *H. nana* as the smallest tapeworm endemic in many regions around the world also called *Rodentolepis nana***.** Human and rats are the definitive host while the arthropod(beetles and fleas) as intermediate host

that ingest by human or rodent and contain the infective stage that develop in small intestine to adult worm. This parasite common in moderate weather than extreme weather because egg cannot survive in hard weather (Kandi *et al*., 2019).

2-10-1Classification

Hymenolepis nana classified as follows, (Al-Bayati, 2013).

Kingdom: Animalia

Subkingdom: Eumetazoa

Phylum: Platyhelminthes

Class: Cestoda

Subclass: Eucestoda

Order: Cyclophyllidea

Family: Hymenolepididae

Genus: *Hymenolepis*

Species: *nana*

2-10-2 morphology and Life cycle

It is the only cestodal worm that can complete live in one host, which is human without need to intermediated host (Shahnazi *et al*., 2019). The adult worm very small in size, white when alive, flattened and life span of adult worm 4-6 week but internal autoinfection allow the infection persists for years. Egg as infective stage that contain hexacanth embryo, which pass in stool of infected person and transport to another person by fecal oral route. Scolex of this enteric parasite like knob with four suckers. Scolex equipped with a row of hooks. Segments of worm body attachment to the neck and these segments are wider than elongate. Uterus of this worm sac like and surrounded with three tested. Egg hatching in lamina propria of villi to release cysticercoid larva (Mahmud *et al*., 2017).

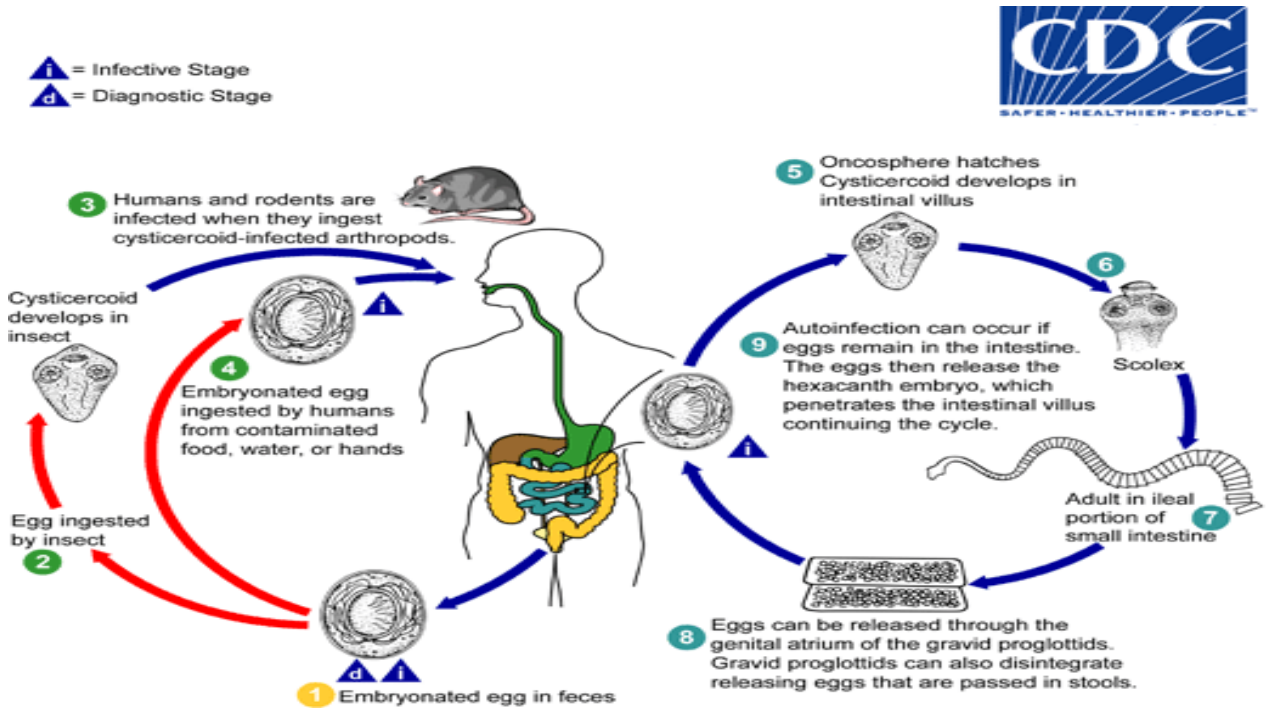


Figure (2-3) : life cycle of *H.nana* (CDC,2012).

2-10-3 Pathogenesis

Hymenolepsis described as the disease that caused by both *H. nana* and *H. diminuta***,** which infect both human and rodents. The distinguished feature of this parasitic infection is autoinfection. Internal autoinfection when egg hatching in villi of small intestinal to larva and development to adult worm while external autoinfection when egg ingested by fecal oral routes.

Symptomatic infection is ranged from mild to severe symptoms, asymptomatic infection depend on worm burden. The *H. nana* parasitic worm rare caused extra intestinal disease but this accrue in immunocompromised patients. Extra intestinal dissemination may lead to death. Symptomatic infection associated with diarrhea, headache, abdominal pain, nausea and vomiting, weight loss, dizziness, pruritus and allergic reaction may occur as urticarial and skin eruption. Height distribution of infection associated with poor sanitation, bad water supply and other non-healthy conditions (Al-Mekhlafi, 2020).

2-10-4 Treatment of Hymenolepsis

One of the most important problems of this worm infection is autoinfection. Density of parasite determine the severity of infection may only cause abdominal pain or suffering from diarrhea, headache, abdominal gas, appetite loss and anal pruritus. Prevalence of infection associated with crowded community, Handlers in public places (Goudarzi *et al*., 2021).

Praziquantel is a common drug use as antihelmenthes with activity against both cestodes and trematodes except Fascioliasis, *Echinococcus granulosus* and *E. multiocularis* infection. The dosage depend on the type of parasite and the site of infection. The dosage use to treatment of

tapeworm 10-25mg/kg as a single dosage. This drug is safety and active with mild side effects as headache abdominal pain therefore should be administered in night to avoid these effects (Chai, 2013).

2-11 Diagnosis of intestinal parasite

2-11-1 Microscopic method

It is one of the most important conventional diagnostic method. Direct examination of patients feces to detect the presence of trophozoite or cyst of parasite by microscopic.

Stool mixing with sodium chloride and examined or mixed with dye to distinguish cyst of parasite or egg of worm named as (O&P) which mean ova and parasite. Usually in direct examination by microscope, we can find cyst in formed stool while trophozoit and cyst in diarrheal stool (Singh et al., 2009).

Microscopic examination requires or bind with clinical symptoms as watery diarrhea, bloody diarrhea and others while in case of amebiasis extraintestial infection not associated with colitis (Saidin *et al*., 2019). In case of giardiasis the wet preparation very important in distinguish of trophozoite in loss and diarrheal stool or use fixation method with sodium acetate –acetic acid formalin to observed trophozoit motility (Hooshyar, 2019).

2- 11-2 Serological method :

To detect the antibody in patient's serum and antigen in patient's stool it can by use the ELISA technique (enzyme-linked immunosorbent assay). In case of extraintestial amebiasis, use of radio clinical method or by use of *E* .

histolytica IgG ELISA which record successful in the diagnosis (Beyls *et al*., 2018).

Virulent factor as GalNAc that mediate adhere of parasite to intestinal mucosa useful in diagnosis. Immunoassays for acetyl -D-galactosamine as important assay in diagnosis to this parasite (Soares *et al*., 2019).

In case of *G. lamella* also use of ELISA to detect of antigen in stool. In many cases, the parasite cannot be diagnosed from stool sample but patient still have symptoms and in such conditions the use of other methods like string test, smear or biopsy form intestinal or duodenal aspiration will be favored for parasitic diagnosis(Jahan, 2014).

2-11-3 molecular method

Molecular method mean extraction and amplification of DNA of parasite as *E .histolytica* in tissue ,stool or abscess. Molecular method include polymerase chain reaction technique(PCR) as real time PCR, nested PCR and multiplex PCR (Saidin *et al.*, 2019). Molecular diagnosis of *Giardia* by PCR not routinely use in medical laboratory only use in research laboratories(Hooshyar, 2019). PCR as rapid procured for detect of *H. nana* and study molecular characteristic of this worm (Shahnazi *et al*., 2019).

2-12 Urogenital parasite :-

A wide variety of causative agents of urinary tract infection especially the parasites of schistosomiasis(bilharziasis), trichomoniasis while echinocoocosis and filariasis are less common. Trichomoniasis is the most common than other parasitic infection in urinary tract. *Trichomonas* infection accompanied with vaginal discharge and more prevalence in

women with the age ranged between 20-40 years and cause severe complication in lower urinary tract.

Donne first descried *Trichomonas* in 1836. Only direct infection accrue from person to person but rare by swimming pools, toilet papers, toilet seats. Human is regarded the only host to this parasite (Mor *et al*, 2016). Urinary tract infection (UTI) can be either with complications or without complications. Occurrence of complication of UTI is associated with many factors that cause compromised urinary tract or defect in host defense as in pregnancy, renal failure and foreign body in urogenital tract as catheter that enable pathogenic microorganism to adhere and to colonize in the urinary tract to cause infection (Flores-Mireles *et al*., 2015).

2-12-1 *Trichomonas vaginalis*

Trichomonas is an extracellular flagellated protozoan parasite and it cause lower urinary tract diseases"known as one of the sexual transmitted diseases". Mistake in diagnosis may accure with bacterial vaginitis. Many factors can make this disease with height prevalance in certain population as in develped countries like poor hygiene, multiple partners and bad socio-economic conditions (Asmah *et al*., 2018).

Infection with this parasite lead to several conditions in women including cervix disorders, pelvic infection, infertility and preterm birth. Transmitted only between sexual parnter so called pink-pong diseae. Women as a reservoir of disease wihle men as carrier. Trichomoniasis found in all climates with little variability betweem seasons. Incidence rate depend on age, sexual activity, number of partners and drugs addiction (Salimi-khorashad *et al*., 2021).

2-12-2 Classification

The following classification was adopted of the *T. vaginalis* parasite by the (Bedair and Ali, 2020).

Kingdom : Protozoa

Subkingdom : Archezoa

Phylum : Parabasalia

Class : Trichomonadea

Genus : *Trichomonas*

Species : *vaginalis*

2-12-3 Morphology and life cycle :

T. vaginalis as a flagellate protozoal parasite that have five flagella, four of which in anterior side of parasite body while the fifth flagellium extended in association with the undulating membrane that reaches to the middleof the body. There is only one stage in the life cycle of this parasite which is the trophozoite stage . Trophozoite form with pear or ovoid shape.

Transmission of this parasite can occur only during sexual intercourse and distinguished by quivering or shaking motility (Mor *et al*., 2016).The protozoan parasite inhabit the cervical or vagina of women and urthera of men, trophozoite multiplication by binary fission. *Trichomonas* as predisposing factor for the infection with HIV viruses. The presence of this parasite is associated with other sexual transmitted diseases such as candidiasis, syphillis and gonorrhoea (Mahmud *et al*., 2017).

Incubation period range from few days to months and the symptoms of infection can appear after six months or more, with ability of the patient to infect other individuals during this period. This parasite can use the red

blood corpuscular (RBC) as a source of fatty acids and iron because the parasite cannot synthesis the lipids and parasite can make pore in plasma membrane of RBC by cytotoxic molecules (Bedair and Ali, 2020).

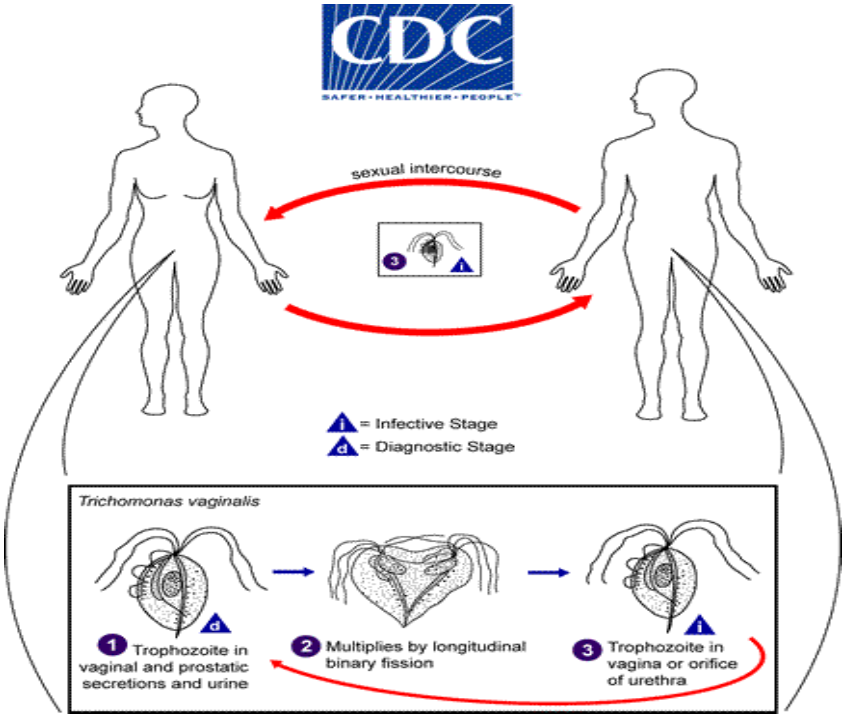


Figure (2-4) :life cycle of *T .vaginalis* (CDC,2017)

2-12-4 Pathogenesis

Trichomoniasis is non viral sexual transmitted infection, and caused by the protozoan *T.vaginalis* parasite. Women are more susceptible to infection than men. Success of parasite to cause disease is due to its ability to change the nature of vagina environment, pH, micribiota, vaginal epithalium and cervical mucosa (Beri *et al*., 2019). To make its influence this parasite adhere to epithalial cells because it has a height affinity to these cells than other cells. *Trichomonas* evasion from the host immune system by produce enzyme as proteinase which act as an important virulent factor that help parasite to overcome the complement in mucosa.

Many studies had proved that this parasite have the ability to hemolysis RBC to obtain its essential factors for surviving by use of perforin like protien(Arab-Mazar and Niyyati, 2015). Symptoms ranged from asymptomatic to acute inflammation. Side effects of infection in pregnant women are premature labor, low weight of baby (Beri *et al*., 2019).

2-12-5 Trichomoniais and treatment

One of the most health problem is urogential tract infection espically of that caused by this parasite which is obligatory extracellular parasite and feeding on bacteria, vaginl epithelial cells and erythrocytes. Use carbohydrate as an energy source in aerobic and anaerobic conditions by fermentation process. The peroid of survive to this parasite is differ btween women and men, and that peroid is longer in women than in men. It may lasts from months to years in women while it can persist for10 days in men(Kissinger, 2015)

Many studies has proved that the relationship between trichomoniasis and vaginitis, candidiasis, pelvic inflamontary disease, gonorrhea, syphilis, low birth weight and preterm delivery. *Trichomonas* infection increase vaginal PH >5. Metronidazole is the first picked treatment for tretment of this parasite.

The drug consider safety to pregnant women in all stage of pregnancy. Some time use of tinidazole which less safety to pregnant women, both drugs belong to the same class but metrondizole cheaper than tinidazole single dose of both treatment be considered as the first line of treatment (2g). The repeated infection may occure due to many factors as drugs resistant or new acquired of infection (Kissinger, 2015).

2-12-6 Labrotary diagnos

2-12-6-1-Microscopic examination

Parasite found in urine and discharge therfore its trophozoit motility can be observed by direct microscope examination via wet prepration from these fluids of infected persons. To confirm the diagnosis stain use along with wet mount as giemsa or acridine stain (Khatoon *et al*., 2014). Microscopic exmination associated with clinical feature as irritation, forthy malodorous discharge. The discharge may yellow or green. Strawberry appearance of cervical mucosa may be observed (Bansal *et al*., 2016).

2-12-6-2-Culture Methode

Among the useful methods for *trichomonas* diagnosis, the sensivity reach to 81-94 % but the problem of this method is the contamination with bacteria to overcome this problem, developed many methods work to reduce bacterial contamination. Diamond's culture is the media use in isolating of this parasitr (Van Gerwen and Muzny, 2019).

2-11-6-3 Serological Methode

The use of ELISA technique to detect human immunoglobin IgM,IgG in patients sera whom have been infected with this parasite, and this method with height sensitivity but not routinely use in a common civilian laboratories and for that may be limited in researcher field only (Bedair and Ali, 2020).

2-12-6-4 Molecular Methode

Molecular method use PCR techniqe to detect DNA sequence of parasite that have be extracted from specimen as urine, swab or discharge. This method with a height senstive but not routinely use (Bandea *et al*., 2013).

Chapter Three

Materials and Methods

3-1 Materials

3-1-1 Equipment and apparatuses

Many different apparatuses and equipment's were be used in this study in different method, laboratories and from different origin in table below(3-1).

Table (3-1):Equipment and apparatuses use in this study

Equipment and Apparatuses	Origin	Company
Capillary tube	China	Vitrex
CBC	Germany	Zybio
Centrifuge	Japan	Hitachi
Disposable syringes	Jordan	Afco
EDTA tube	China	ALS
Gel tube	China	ALS
Gloves	China	Home care
Hematocrit centrifuge	Japan	Hitachi
Incubator	Italy	Paramedical
Khan tube	Jordan	Afco
Macro and Micropipette	Germeny	EMC
Microscope	Japan	Olympus
Plane tube	Jordan	Afco
Plate reader	Italy	Paramedical
Slide and cover	China	ALS
Spectrophotometer	Italy	Paramedical
Sterile container	China	ALS
Sterile Swab	Jordan	Afco
tips	China	Vitrex
Human ELISA reader	Italy	Paramedical
Human ELISA washer	Italy	Paramedical

3-1-2 The solutions

Table (3-2): The solutions used

Fluid	Origin
Lugo's iodine	Himedea, Indian
Normal saline	local market
Distilled water	Local market

3-1-3 The kits

Table (3-3) Kits used

Kits	Content	Origin
CRP kits	Positive control , negative control ,CRP latex	BIOTEC, UK
ELISA kits(Vitamin B12)	Standard, standard diluent , HRP-conjugate reagent , chromogen solution A ,chromogen solution B, Stop solution , wash solution	Sunlong, china
Iron kits	R1, R2 ,R3 ,standard	Taytec, UK
Zinc kits	Standard , R1	CENTRNIC GmbH, Germany

3-1-4: Preparation of stains and solutions

3-1-4-1 Normal saline

Prepared by dissolving 9 gram of Nacl(sodium chloride) in 100ml (1litter) of distilled water (Tonog and Lakhkar, 2021).

3-1-4-2 Lugol's iodine

Dissolved of iodine (5%) with potassium iodine KI (10%) in 100 ml of distilled water(Calissendorff and Falhammar, 2017).

3-2 Methods

3-2- 1 The study design:

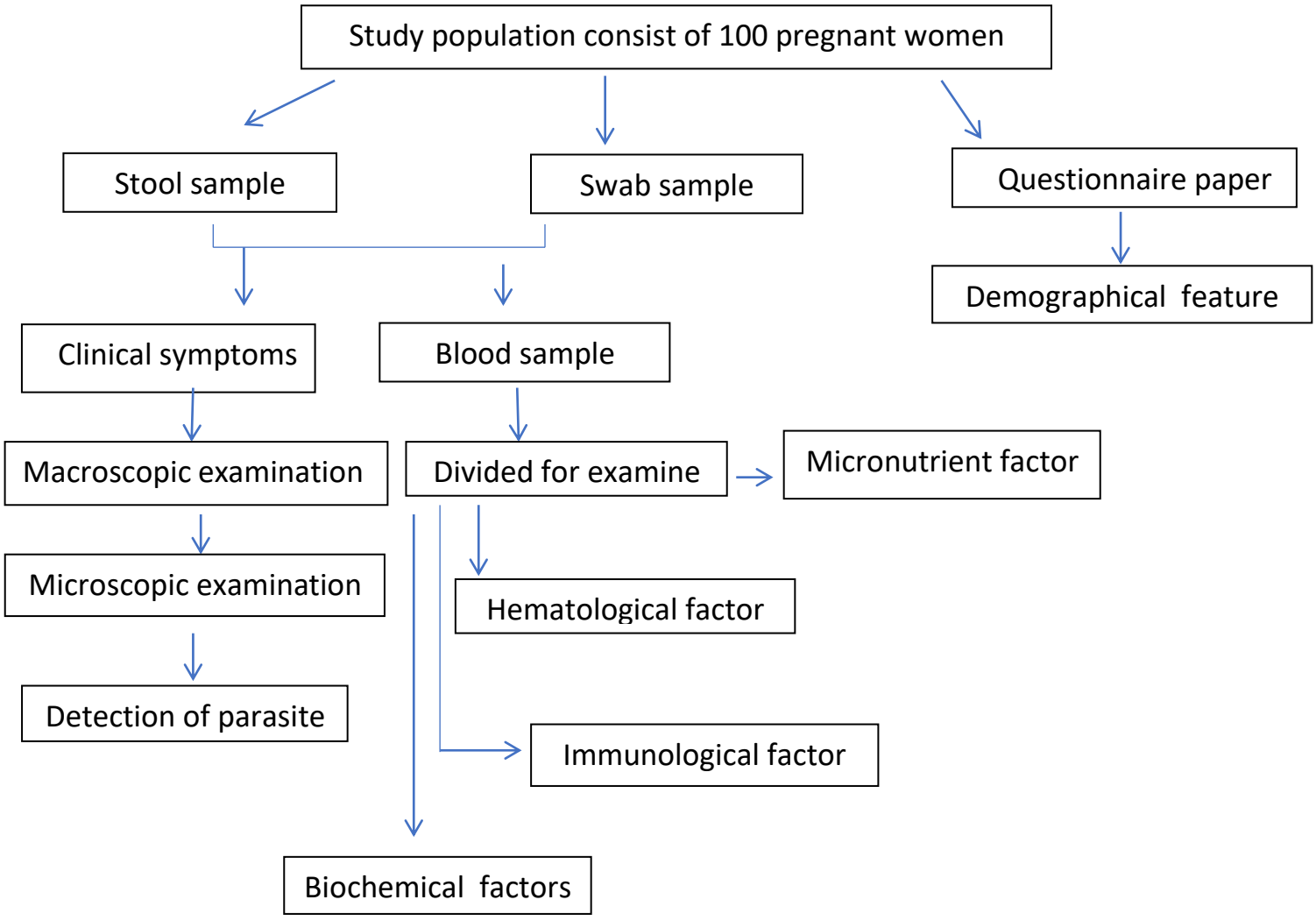


Figure 3-1 Schematic representation of present study samples of pregnant women

3-2-2 Sample Collection

3-2-2-1 Stool and Swab Collection

Collecting of 100 samples of a stool and swab sample from pregnant women who had visit or attending hospital((Maternity and children Hospital, AL-Amam AL-Sadiq education Hospital) and health care center(Abu-Gharaq health center, AL-Kafal health center, AL-Mohandessin health center)in Babylon province from October 2021 to March 2022. Stool samples were collected in a sterile clean container. Record information of patient as age, resident, level of education, period of pregnancy, living condition (questionnaire paper) from each patient.

Stool sample examined during 30minute if liquid or semi liquid to see trophozoit and during one hour if stool formed or semi-solid to diagnose of parasite cyst stages in the stool samples. Formed stool may contain cyst and trophozoite (Sankar Sastry, 2014). Sterile cotton swabs were used to obtain vaginal swab or discharge from pregnant women by added 1ml of normal saline mixed kindly and then examined, even the detection of one motile trophozoite of *Trichomonas* can be considered a positive result of infection (Adjei *et al*., 2019).

3-2-2-2 Blood Collection

Blood sample has to be collected from each patient (5ml)by disposable syringe and then put in a gel tube (3ml) to separate it by centrifuge to obtain serum which then be used to detect the studied nutrient factors values (stored in freeze) Iron, Zinc and VitminB12, and immune protein (CRP). The remainder of blood sample was put in EDTA tube (2ml) for detecting of hemoglobin(Hb) level either by use hematocrit method (use

of capillary tube) or complete blood count(CBC) device to account differential cell count and Hb levels.

3-3 Sample Examination

3-3-1 Stool Examination

3-3-1-1 Macroscopic Examination

Stool appearance can give a good thought about the type of gastrointestinal disease. Color, consistency, quantity, form, odor, presence of mucus. Virtually, the present of small amount of mucus be considered as a normal while the bloody mucus, copious mucus will be regarded abnormal. Consistency may be watery or pasty. Normal color is tawny, color can be differed according to the type of diet. Black stool may refer to bleeding from upper gastrointestinal tract and certain medicines can cause black stool as iron or bismuth. Red color can give an indicator of bleeding from lower gastrointestinal tract, green color of stool is due to bile or bilirubin, clay or putty color due to biliary obstruction (Kasirga, 2019).

3-3-1-2 Microscopic Examination

Microscopic examination is an important diagnostic tool to detect protozoa, helminthes and fecal leucocytes in specimen. Leucocyte and erythrocyte not observed in normal stool. In order to see Leucocyte, examination should be performed in stool sample obtained from the area with mucus. Always, should avoid contaminated of stool with urine because it can effect on the motile stage of parasite. Three stool samples be required for definitive diagnosis but in the case of giardial infection a single sample of 50-70% of cases can be considered as a definitive diagnosis. In the case of

invasive amebiasis the blood in the stool may be found. In some times the ingestion of erythrocytes by *E. histolytica* may be seen but not all time because it may be disintegrated by this parasite (Kasirga, 2019).

3-3-1-2-1Procedure of saline and iodine wet mount Preparation

1- Placed one drop of normal saline on one side of slide and iodine drop on another edge of slide.

2- With wooden stick took 2mg of stool and mixed with normal saline and then covered with coverslip, also took 2mg of stool with wooden stick and mixed with iodine stain on another side of slide and then covered with slide coverslip.

3- Slide should be examined on 10x and then on 40x more than one slide make from different place of stool to confirmation the infection. If doubt the stool contain motile stage in case of diarrhea stool or soft stool don't used iodine stain because may be killed trophozoit (WHO, 2019).

3-3-1-2-2 Procedure of swab wet preparation:

1- Added 1ml of normal saline to vaginal swab, mixed well and then put one drop of the mixture on slide and then covered with slide cover.

2- Sample Examined on 10x objective lens and then on 40x objective lens sample should be examined during 30 minute because parasite may delicate out the vaginal environment. More than one slide should be examined to make sure from diagnosis (Adjei *et al*., 2019).

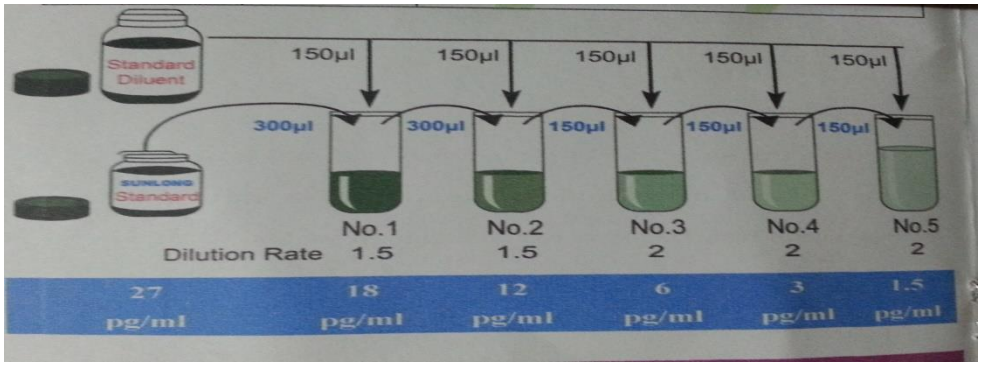
3-4 Biochemocal tests and other tests (hemoglobin and CRP)

3-4-1Vitamin B12

Use of ELISA (sandwich ELIAS) to detect Vitamin B12 deficiency. In sandwich ELISA the microelsia well coated with antidoby specific to human VB12 when added human serum or standard combined with specifific antibody that coated wells. Then added of HRP (horse radish peroxidase) which is conjugated antibody that specific for Vitmin B12 to each microelisa wells and inocubate. Only wells contain Vitamin B12 and HRP conjugate the VB12 antibody appear in blue color and can measured the optical density (OD)by spectrophotometer with wave length 450nm which proportional with VB12 concentration.VB12 concentration can be calculate by comparing OD of the sample with standard curve.

1- Dilution of standard:

By small tubes made serial of dilution for standard and then transport 50µl from each tube to microelisa well each tube use two well from the total 10wells.



Stander dilution

2- two wells should be Left empty as blank control .In sample well put 40µl of sample diluted buffer and then added 10µl of sample mixed well by gentle shaking.
3- Incubation: at 37C° for 30 minute after closed surface of microplate with sealing membrane.
4- Washing: after diluted of washing buffer the microplate should be washed for 5 times.
5- 50µl of HRP-conjugate reagent should be added to all well except blank well
6- Incubation at 37C° for 30 minute .
7- After incubation period washed with washing buffer.
8- added 50µl of chromogen solution A and 50µl from chromogen solution B to each well mixed with shaking and then incubation for 15 minute at 37C°in this step must avoid light.
9- Termination: added 50µl of stop solution to each well to stop the reaction change in color occur from blue to yellow.

10- read absorption at 450nm by use of microtiter plate reader. the OD value of blank Serum Iron control as zero.

11- The factor can be obtained from plot the OD on y-axis and VB12 concentration on x- axis enter to certain system in Exile to obtain factor which multiply with absorption to get concentration of Vitamin B12for each sample.

3-4-2 Iron

Iron is necessary for hemoglobin production and molecules that transport oxygen inside red blood corpuscle.

1- working reagent shoud be preperd by dissolived content of R2vial in bottle of R2 buffer and then mixed weel for us

2-added 1ml of working reagent to tube of standard , blank and sample (100 sample).

3- added 50 µl of R3 to all tubes.

A-added 200 µl of standard to tube of standard instead of that added 200 ml of distilled water to blank tube.

B-added 200 ml of patient serun to serum tube.

C-mixed and inocubation for 5min at at 37C°or 10 minute at room temperature.

4-Absorpation of standard and sample should be read by spectophotomerter at wave length 562nm.

5-Factor can be obtained from divided the concentration of standard on obsorpation which multiplay with absorpation of each sample to obtained concentration .

3-4-3 Zinc

1- 1000µl (1ml) from reagent added to all tube (standard ,sample and blank).
2- added 50µl of standard to tube of standard and 50µl of sample to tube of sample (all tube of sample).
3- mixed well and inocubate at 37C° for 5 minute or at room temperture for 8 minute.
4- Then read absorbation at 560nm in spectrophotometer.

5- When read absorpation of standard divided the concentration on absorpation to obtained facter that multiplay with absoption of all sample to obtain concentration of serum zinc.

3-4-4 Immunological indicator (CRP)

CRP acute phase reactive protein increased in inflammatory diseases CRP test should associated with clinical and laboratory data.

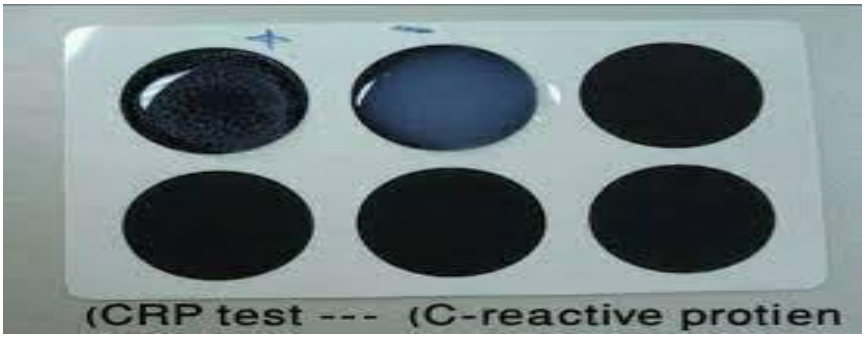
1- One drop of the negative control shoud be placed on one circle of agglutination slide of CRP.

2- One drop of postive control shoud be placed on another circle of agglutination slide of CRP.

3- placed one drop of patient serum on the third circle of the agglutination slide.

4- added one drop of CRP latex reagent on all three circle mixed well with reagent.

5-The slide should be rocked for 2 minute and then absorved the agglutination which mean postive result.



3-4-5 Hematological factor

The measurement of heamoglobn level either by CBC (complet blood cell counit device) or by PCV (packed cell volume) method by take blood from finger or EDTA tube by cappilary tube and then centerfiguation with micocenterfigue then measure pcv by specific ruler and then can be measure Hb by the(HB=pcv-1/3) (Omuse *et al*., 2018).

3-4-6 Data analysis

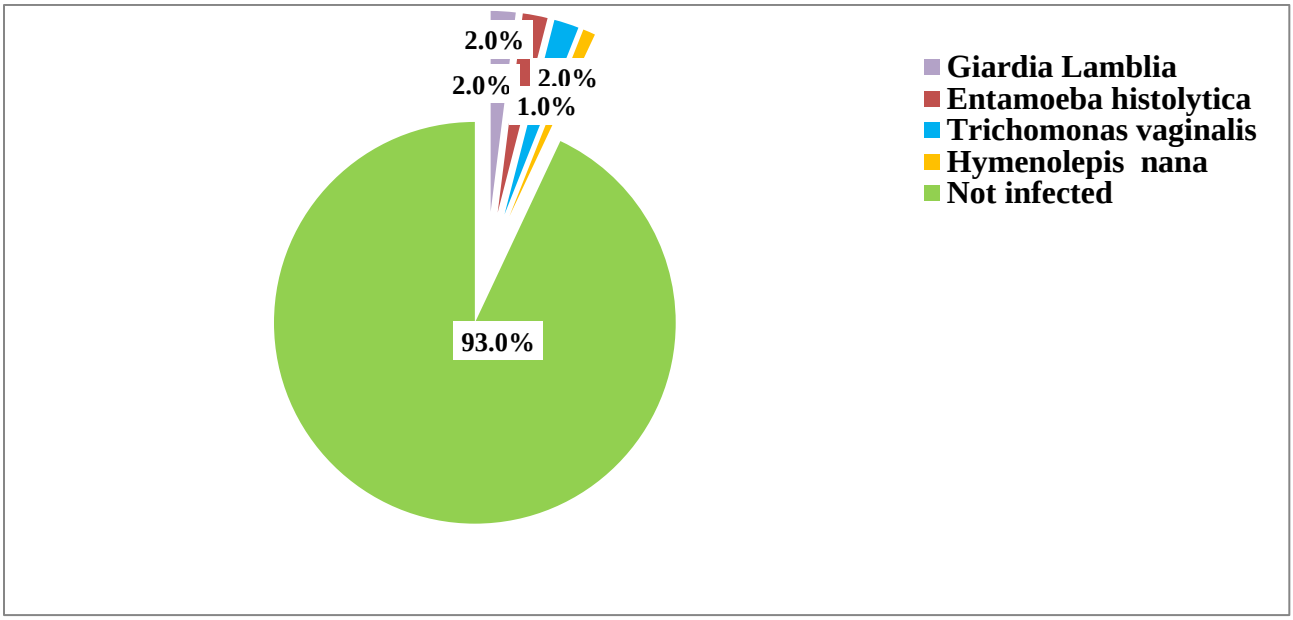
Statistical analysis was carried out using SPSS(Statistical Package for The Social Sciences) version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). ANOVA(Analysis of Variance) test was used to compare means between three groups. Pearson Chi-square and Fisher-Exact Test were used to find the association between categorical variables. A *p*-value of ≤ 0.05 was considered as significant (Daniel, 2018).

Chapter Four

Results and Discussion

Figure(4-1) Type of Parasitic infection in all study groups of pregnant women.

The figure show the percent of parasitic infection which is 7%(*G.lamblia* 2%, *E.histolytica* 2%,*H.nana*1% and *T.vaginalis* 2%while percent of non-infected pregnant women is 93%



The results of present study showed that the percent of parasitic infection in pregnant women is 7% from total number of sample which is 100 while percent of non-infected pregnant women is 93% as in figure(4-1).

The present study near from the previous study that showed there are many factors make parasitic infections with less frequency than other microbial infections "especially within developed countries "such as bacterial or viruses infections. In fact the severity of parasitic infection depend on the following item as dose of etiological parasite , host's infected tissue or organ, multi parasitic infection and other accompanied

complication, duration of infection as acute or chronic status, tolerance and immunity level of targeted host and may be related to other unknown reason (Bogitsh et al., 2018).

The current study showed low parasitic infections in pregnant women this may due to certain limits of the present study such as time of study ,patients refuse to give samples and the history of infection, geographical area of study, educational levels and also may be related to the behavioral nature of pregnant women for providing of the intended study sample that were with more difficult than other patients .

4-2 **Assortment of the parasitic infections in pregnant women according to age group .**

In this study, show there is no significant associated between age group of pregnant women and parasitic infection as in table 4-2.

Table 4-2: Assortment of the parasitic infections in pregnant women according to age group .

Parasitic infection	Age (years)			Total	P-value
	15-24years	25-34 years	35-44 years		
G. Lamblia	0 (0.0)	2 (40.0)	0 (0.0)	2 (28.6)	0.429
E. histolytica	1 (50.0)	1 (20.0)	0 (0.0)	2 (28.6)	
T. vaginalis	0 (0.0)	2 (40.0)	0 (0.0)	2 (28.6)	
H. nana	1 (50.0)	0 (0.0)	0 (0.0)	1 (14.3)	
Total	2 (100.0)	5 (100.0)	0 (0.0)	7 (100.0)	

4-3 Assortment of parasitic infection among pregnant women according to residence.

The present show the parasitic infection in pregnant women who resident in rural area more than women resident in urban area as in table 4-3.

Table4- 3 :-Assortment of parasitic infection among pregnant women according to residence

Parasitic infection	Residence		Total
	Urban	Rural	
G. Lamblia	0 (0.0)	2 (40.0)	2 (28.6)
E. histolytica	0 (0.0)	2 (40.0)	2 (28.6)
T. vaginalis	2 (100.0)	0 (0.0)	2 (28.6)
H. nana	0 (0.0)	1 (20.0)	1 (14.3)
Total	2 (100.0)	5 (100.0)	7 (100.0)

4-4 Assortment of parasitic infection among pregnant women according to level of education .

The current study show the parasitic infection in pregnant women concentrated in women with primary and secondary education than in higher education as in table 4-4 .

Table4- 4 :- Assortment of parasitic infection among pregnant women according to level of education .

Age groups	Infected number	Level of education					
		Primary	%	Secondary	%	Height	%
15-24	2 (28.6)	1	25.0%	1	33.3%	0	0.0%
25-34	5 (71.4)	3	75.0%	2	66.7%	0	0.0%
35-44	0 (0.0)	0	0.0%	0	0.0%	0	0.0%
Total	7 (100.0)	4	100.0%	3	100.0%	0	0.0%

4-5 Parasitic infection among pregnant women according to living condition.

The present study show there is no significant associated between parasitic infection and living condition in pregnant women as in table 4-5.

Table 4- 5: Parasitic infection among pregnant women according to living condition.

Age groups in year	**Infected number**	**Living condition**						**P-value**
		Low	**%**	**intermediate**	**%**	**Height**	**%**	
15-24	2 (28.6)	1	50.0%	1	50.0%	0	0.0%	0.429
25-34	5 (71.4)	1	50.0%	1	50.0%	3	100.0%	
35 -44	0 (0.0)	0	0.0%	0	0.0%	0	0.0%	
Total	7 (100.0)	2	100.0%	2	100.0%	3	100.0%	

The current study showed the age of pregnant women involved in this study ranged from 15 to 44(most of infection in age 25-34) . Most parasitic infections were concentrated in peripheries residences than that in the center residences and most of pregnant women were with limited education or with no height education . Also current study showed that no relation between living conditions or family income and parasitic infection among pregnant women.

The current study is near with previous study that showed the age group of pregnant women was ranged from 15 to 45(most of infection in age less than or equal to 35), the possibility of parasitic infection in pregnant women was almost two time higher in age between 30-40 years than those of age of 40-44 years because there is many factor that caused height infection in age groups than others as habit of hand washing before meals or after toilet, eating of raw unwashed vegetables and habit of soil eating by some of pregnant women(Derso *et al*.,2016).

the prevalence of parasitic infection in rural area was higher than that in urban resident, with limited monthly income of family and also most of infected pregnant women were with no formal education (Hailu *et al.*, 2020). Pregnant women who don't have formal education with more likely to exposure to parasitic infection than those women whom with higher education because the lack of health information about parasitic transmission, symptoms and preventation of parasitic infection.

The low or limited family income may play an important role in infected pregnant women to be with more exposure to parasitic infection, because the limited level of income can be related with nutrients and health status of individuals (Wachamo *et al*., 2021).While current study showed

that there was no relationship between income or living condition and parasitic infection because many factors have a strong effect such as methods of food preparation, hand washing ,uncooked vegetables, health education or even share the toilet with others, domestic animals, all these factors can bring to make persons with more susceptible to infection. The height prevalence of parasitic infections in rural area than in urban area is limited by many factors that make that possible such as walking barefoot, poor hygiene, lack of safe drinking water, working in farmer, animal breeding and unclean environment (Hailu *et al*., 2020).

The current study is in agreement with(Mpairwe *et al*., 2014) that showed pregnant women with helminthes infection more likely to be younger, less education and with low socioeconomic status than uninfected pregnant women. As for trichomoniasis the previous study done in Iran showed the height rate infection of pregnant women with *T.vaginalis* was limited in age group 26- 30 years and also height rate of infection observed in pregnant women with no higher education, while pregnant women whom live in urban resident with height infection than rural area resident (Salimi-khorashad *et al.,* 2021).

4-6 assortment of Parasitic infection among pregnant women according to period of pregnancy.

The present study show the parasitic infection occur in any trimester of pregnancy and the infection with parasite concentrated in first and second trimester as in the table 4-6.

Table 4-6: assortment of Parasitic infection among pregnant women according to period of pregnancy

Parasitic infection	Period of pregnancy			Total
	First trimester	Second trimester	Third trimester	
G. Lamblia	2 (50.0)	0 (0.0)	0 (0.0)	28.6
E. histolytica	0 (0.0)	1 (50.0)	1 (100.0)	28.6
T. vaginalis	2 (50.0)	0 (0.0)	0 (0.0)	28.6
H. nana	0 (0.0)	1 (50.0)	0 (0.0)	14.3
Total	4 (100.0)	2 (100.0)	1 (100.0)	100.0

The current study showed the parasitic infection occur in any trimester but more prevalence in first and second trimester. The current study agree with (Buchala *et al*., 2022) which clear up that parasitic infection can occur in any stage of trimester of gestation and the infection that occur in the first trimester is more sever on fetal and can cause placental complication than those occurring in late trimester . Infection occur in first trimester in pregnant women more severe than other stages of pregnancy . Another study show pregnancy and gestational age associated with parasitic infection and clear up the possibility of infection increased in late trimester (second and third trimester)than in first trimester. Also it show there is a relationship between parasitic infection and gravity because multigravida less possibility exposure to infection than primigravida because multigravida

have previous pregnancy experience and benefits from health education and may have expertise to avoid infection unlike primigravida which don't have any previous expertise in pregnancy (Abaka-Yawson *et al*., 2020) .

About trichomoniasis, a previous study done in Iran show that infection with *T. vaginalis* can occur in the second and third trimester but no infection observed in first trimester and this infection not associated with previous sexual transmitted disease or abortion (Salimi-khorashad *et al*., 2021) .

4-7 Parasitic infection among pregnant women according to CRP results.

The current study show no relationship between parasitic infection and CRP in pregnant women as in table 4-7.

Table 4- 7: Parasitic infection among pregnant women according to CRP results

CRP result / Parasitic infection	Parasitic infection				Total
	G. lamblia (No=2)	*E.histolytica* (No=2)	*T. vaginalis* (No=2)	*H. nana* (No=1)	
Positive	0(0.00)	0(0.0)	0(0.0)	0(0.0)	0.0
Negative	2(100.0)	2(100.0)	2(100.0)	1(100.0)	100.0
Total	2(100.0)	2(100.0)	2(100.0)	1(100.0)	100.0

The current study show there is no relationship between parasitic infection and C- reactive protein . *E.histolytica and G.lamblia* that be considered as

the main cause of diarrhea, and hence the C-reactive protein will be elevated after acute phase of infection as diarrhea caused by these parasites. The immune response play important role in negative relationship between diarrhea and CRP that may due to small number of parasite that swallowed and site of this parasite in mucosal layer of host gut that lead to low level of CRP. An detected lower level of CRP in worm infected pregnant women (Jabbar Saheb *et al*., 2020) .

Regarding Trichomoniasis, the current study is in agree with (Alsakee and Nouraddin,2019)which clear that CRP as a marker of systemic inflammation and no significant alteration in CRP of infected women when compare with control .

4-8Parasitic infection among pregnant women according to clinical symptoms .

The table 4-8 show the most common symptoms appear in pregnant women with parasitic infection, 1(14.3)of pregnant women with *H.nana* infection suffering from abdominal pain, 1(14.3) of pregnant women with *E.histolytica* infection suffering from epigastric pain, 1(14.3)of pregnant women with *E.histolytica* suffering from nausea and vomiting, 2(28.6) of pregnant women with *G.lamblia* infection suffering from greasy stool, 3(42.9)of pregnant women infected with *H.nana* and *G.lamblia* suffering from weight loss and 2(28.6) of pregnant women infected with *T.vaginalis* suffering from yellow discharge.

Table 4-8: Parasitic infection among pregnant women according to clinical symptoms .

Clinical symptoms / Parasitic infection	Parasitic infection				Total
	G.lamblia (No=2)	*E.histolytic a* (No=2)	*T.vaginalis* (No=2)	*H. nana* (No=1)	
abdominal pain Yes No Total	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 1 (100.0) 0 (0.0) 1 (100.0)	 (14.3) (85.7) (100.0)
Epigastric pain Yes No Total	 0 (0.0) 2 (100.0) 2 (100.0)	 1 (50.0) 1 (50.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 1 (100.0) 1 (100.0)	 14.3 85.7 100.0
Nausea and vomiting Yes No Total	 0 (0.0) 2 (100.0) 2 (100.0)	 1 (50.0) 1 (50.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 1 (100.0) 1 (100.0)	 (14.3) (85.7) (100.0)
Greasy stool Yes No Total	 2 (100.0) 0 (0.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 1 (100.0) 1 (100.0)	 28.6 71.4 100.0
Weight loss Yes No Total	 2 (100.0) 0 (0.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 1 (100.0) 0 (0.0) 1 (100.0)	 42.9 57.1 100.0
Yellow discharge Yes No Total	 0 (0.0) 2 (100.0) 2 (100.0)	 0 (0.0) 2 (100.0) 2 (100.0)	 2 (100.0) 0 (0.0) 2 (100.0)	 0 (0.0) 1 (100.0) 1 (100.0)	 28.6 71.4 100.0

The Clinical symptoms in the present study were differed according to type of intestinal parasites and severity of infection as abdominal pain ,epigastric pain, nausea and vomiting ,greasy stool and weigh loss.

Current study near from the previous study (Kiani *et al*., 2016) that show parasitic infection causing gastrointestinal disorders, parasitic infection associated with diarrhea and other clinical symptoms like abdominal pain ,nausea and vomiting ,stomach pain and bloating. The *G.lamblia* responsible of diarrhea, steatorrhea, malabsorption and show the chance to increase infection associated with lower level of education ,poor environmental sanitation and contact with animals.

Previous study (Price *et al*., 2018) show pregnant women with trichomoniasis have one or more of symptoms as abnormal discharge(yellow or green) with foul smelling, urinary frequency, itching, dysuria and dyspareunia even there are some patients were be asymptomatic.

4-9 Parasitic infections among pregnant women according to study's haemo-biochemical markers used .

The table 4-9 show the relationship between study markers and pathogenic parasitic infection in pregnant women and significant association between iron deficiency and parasitic infection .

Table 4- 9 :-Parasitic infections among pregnant women according to study's haemo-biochemical markers used .

Study markers	Parasitic infection				P-value
	G. lamblia (No=2)	E. histolytica (No=2)	T. vaginalis No=2	H. nana No=1	
Serum iron (mcg/dl)	65.50 ± 0.00	92.60 ± 5.79	67.10 ± 0.00	108.10	**0.004***
Hb (g/dl)	11.60 ± 0.00	13.00 ± 0.85	12.10 ± 0.71	12.3	0.345
Zn (mcg/dl)	172.80 ± 21.21	135.40 ± 65.05	102.50 ± 0.00	86.7	0.37
Vitamin B12 (pg/ml)	7.48 ± 0.00	8.96 ± 3.02	7.88 ± 0.00	8.06	0.854

$P \leq 0.05$ was significant.

The present study showed that a mild drop in hemoglobin levels in pregnant women with parasitic infection. The present study similar to the study which show the hemoglobin level in pregnant women with parasitic infection is 12.8g/dl, whereas the hemoglobin level in pregnant women free from parasitic infection 14.4g/dl, also show pregnant women with no parasitic infection have moderate anemia and some parasitic infected women have mild anemia and other have moderate anemia (Demeke *et al*., 2021).

Previous study that carried out in AL-Najaf hospitals was showed decrease in hemoglobin concentration in women infected with *T. vaginalis* because this parasite can cause hemolysis of red blood corpuscular(RBC), phagocytizing of RBC and cause bleeding, and for all these reasons the *T.vaginalis* can also cause iron deficiency anemia (Al-Hadraawy and Al-

fatlway,2013). Other study done on intestinal parasites and showed intestinal parasitic infection during pregnancy associated with iron deficiency and other complications such as impaired nutritional status and maternal anemia (Demeke *et al*., 2021).

The present study showed a negative association between zinc deficiency and parasitic infection and this may agree with previous study that showed a negative association between zinc and parasitic infection but the pregnant women whom have one or more parasitic infection can be with more likely to zinc deficiency than pregnant women-free from parasitic infection and this may due to parasite effect on absorption of essential nutrient factors by blocking mucosa surfaces by bleeding or diarrhea and there is a positive association between zinc deficiency and health education that enhancing nutrient status by learning pregnant women (Kumera *et al*., 2015).

In addition, the present study showed that there is no association between parasitic infection and VB12 deficiency and the reason for that may due to sample size, pregnant women received Folate or to the nutrient status of pregnant women.

Many studies have shown that VitminB12 deficiency may associated with chronic intestinal parasitic infection, increased of intestinal motility due to diarrhea, damage in receptors of Vitamin B12 because destruction of epithelial cell. Virtually, the vitaminB12 deficiency more likely occur with helminthes infection because the use of human nutrients and to the damage of mucosal lining by this helminthes and even the drugs used to treat helminthes that may cause Vitamin B12 deficiency (Layden *et al*., 2018).

4-10 The association between all studied variables and study groups of pregnant women .

The table 4-10show the association between study variables including (educational level, living condition, residence, period of pregnancy, type of sample and C-reactive protein) and study group including (pathogenic infection, non-pathogenic infection and control group). There was significant association between study group and residence, period of pregnancy and type of sample.

Table 4-10:The association between all studied variables and study groups of pregnant women .

Study variable	Study group			Total	P-value
	Pathogenic (N=7)	Non-pathogenic (N=51)	Control group (No=42)		
Educational level					
Primary and Secondary	7 (100.0)	45 (88.2)	41 (97.6)	93 (93.0)	0.22
Higher education	0 (0.0)	6 (11.8)	1 (2.4)	7 (7.0)	
Total	7 (100.0)	51 (100.0)	42 (100.0)	100(100.0)	
Living condition					
Low	2 (28.6)	2 (3.9)	1 (2.4)	5 (5.0)	0.055
Intermediate	2 (28.6)	34 (66.7)	31 (73.8)	67 (67.0)	
Higher	3 (42.8)	15 (29.4)	10 (23.8)	28 (28.0)	
Total	7 (100.0)	51 (100.0)	42 (100.0)	100(100.0)	
Residence					
Center	2 (28.6)	6 (11.8)	15 (35.7)	23 (23.0)	**0.022***
Peripheries	5 (71.4)	45 (88.2)	27 (64.3)	77 (77.0)	
Total	7 (100.0)	51 (100.0)	42 (100.0)	100(100.0)	
Period of pregnancy					
First trimester	4 (57.1)	14 (27.5)	11 (26.2)	29 (29.0)	**0.041***
Second trimester	2 (28.6)	31 (60.8)	17 (40.5)	50 (50.0)	

Third trimester Total	1 (14.3) 7 (100.0)	6 (11.7) 51 (100.0)	14 (33.3) 42 (100.0)	21 (21.0) 100(100.0)	
Type of sample Stool Swab Total	 5 (71.4) 2 (28.6) 7 (100.0)	 51 (100.0) 0 (0.0) 51 (100.0)	 31 (73.8) 11 (26.2) 42 (100.0)	 87 (87.0) 13 (13.0) 100(100.0)	**<0.001***
C-reactive protein Positive Negative Total	 0 (0.0) 7 (100.0) 7 (100.0)	 7 (13.7) 44 (86.3) 51 (100.0)	 6 (14.3) 36 (85.7) 42 (100.0)	 13 (13.0) 87 (87.0) 100(100.0)	0.81

$P \leq 0.05$ was significant.

The obtained results of current study showed a significant associated between parasitic infection in pregnant women and between the type of the collected sample, resident and period of pregnancy. The parasitic infection was with height prevalence in rural resident-women than in urban resident ones, also the parasitic infection was with height prevalence in the first and second trimester and it was with height prevalence of intestinal parasites than in urogenital tract dwelled parasites.

The current study is in nearly to another study which has showed the increase of parasitic infection was found in the second and third trimester and also the same study showed the parasitic infections in rural resident was height when compared with urban resident of pregnant women and the height prevalence is due to poor personal hygiene, low income status, method of waste disposal and hand washing after defecation(Kebede *et al*., 2022).

Another study has shown there is no significant association between intestinal parasitic infection and the area that be resided by pregnant women (Sangare *et al*., 2021).

In spite of the difficulty to obtain two kinds of samples from studied patients but the stool sample is easier when compared with swab sample of pregnant women. In addition, there are many pregnant women had refused to supply their vaginal swabs, and this may be due to other reasons as proved by the study of (Houso *et al*., 2011) which showed that most of the physicians were only depended on the clinical features for diagnosis of trichomoniasis as irritation, discharge, pelvic pain, burning sensation and vaginal pH level.

A study has done in Iran which showed a height prevalence of intestinal parasites and the reasons for such increase were related to the presence of many factors that make it domains as suitable environment, use of human or animal fertilizers in agriculture, height population density, lack of proper disposal of wastes, lack of adequate washing of vegetables, lack of well cooked food, transmission of parasitic infection by food handlers to customers, presence of height resisted cysts and to the ability of larva to penetrate skin. All these factors can make intestinal parasites with a height prevalence (Balarak *et al.*, 2016).

4-11: Assortment of parasitic infection among pregnant women in relation to symptoms and hospitalization.

The present study show that the number of hospitalization symptomatic pregnant women is 1(33.3%),outpatient symptomatic pregnant women 2(66.6%)and outpatient asymptomatic pregnant women is 4(100%).

Table 4-11: : Assortment of parasitic infection among pregnant women in relation to symptoms and hospitalization.

Hospitalization	Number of pregnant women (%)				Total
	Symptomatic	%	Asymptomatic	%	
Inpatient	1	33.3%	0	0.0%	14.3
Outpatient	2	66.6%	4	100.0%	85.7
Total	3	100.0%	4	100.0%	100.0

The present study showed the parasitic infections were rarely cause hospitalization admission for pregnant women and this may due to the infective dose of pathogen, level of immunity status, presence of another co-infection, patient's response to treatment, location of infection and because of many pregnant women were attending/visiting private clinics without visited any hospital.

In the note of worth, a previous study was done in England, which showed the parasitic disease among intestinal infectious diseases could lead to hospital admission. The patient wishing for hospital admission related with their ages. Practically, the patient age below 15 years was with height hospital admission than other ages (Sweiss *et al*., 2022).

Another study has done by (van Seventer and Hochberg, 2017) that showed a significant differences between parasitic infection and hospitalization and this can be related to many factors and the first factor with most important that if patient is a carrier and have been diseased without appearance of any symptoms and enable to transport the infection to others, duration of inflectional stage that differed for each type of infection and depend on environmental factor, susceptibility of host, route of exposure, virulence and infectivity of the pathogenic microbial agent and also depend on patient immune status.

Other factor such as age, stress, coexisting disease, nutrient status . Patient with latent infection that have positive result of investigation without any signs. In addition, environmental influences that increase of vulnerability to infectious disease, social behavior, political, physical and economic facto. According to all these reasons, most of patient infections remain and seemed asymptomatic(van Seventer and Hochberg, 2017).

4-12 The mean differences of hemoglobin concentration according to the type of study groups of pregnant women.

The present study show significant association between parasitic infection and haemoglobin level in pregnant women with parasitic infection as in the table 4-12.

Table4-12:The mean differences of hemoglobin concentration according to the type of study groups of pregnant women

Study variable	Study groups	N	Mean ± SD	F	P-value
Hemoglobin (g/dl)	Pathogenic infection	7	12.24 ± 0.73	**3.562**	**0.032***
	Non-pathogenic infection	51	11.48 ± 1.13		
	Control group	42	11.93 ± 0.75		

*P value ≤ 0.05 was significant.

The obtained result of Present study showed there is relation btween parasitic infection and hemoglobin levels (was significant).

In previous study(Bolka and Gebremedhin, 2019) which show that a higher level of anemia among infected pregnant women than those non infected pregnant women(strong associated between parasitic infection and anemia in pregnant women). Another study show intestinal parasitic infection pregnant women had higher odds of developing anemia than free parastic infected pregnant women and the hemoglobin level in parasitic infection pregnant women was12.8g/dl while the hemoglobin level in free parasitic infected pregnant women was 14.4g/dl (Demeke *et al*., 2021) .

4-13 The mean differences of serum iron concentrations according to the type of study groups of pregnant women.

According to present study there were significant associated between parasitic infection in pregnant women and iron deficiency as in the table 4-13.

Table4-13: The mean differences of serum iron concentrations according to the type of study groups of pregnant women.

Study variable	Study groups	N	Mean ± SD	F	P-value
Serum iron (mcg/dl)	Pathogenic infection	7	79.79 ± 17.77	**3.252**	**0.043***
	Non-pathogenic infection	51	88.24 ± 49.61		
	Control group	42	116.79 ± 69.72		

*P value ≤ 0.05 was significant.

The Current study showed there is a realtionship between iron levels and parasitic infection in pregnant women(significant)that mean parasitic infection can caused iron deficiency and this study in agreement with prior. Study that show parasitic infection aggravates anemia in pregnant women, infection by helminthes lead to malnutrtion, iron deficiency anemin and increase vulnerability to other infection in infected pregnant women(Tegegne, 2021).

Moreover, intestinal parasitic infections might cause anemia; induce deficiencies of iron, protein, folate and zinc(Animaw *et al*., 2021).

Furtheremore, other facter can increase the probaility of iron deficiency anemia in pregnant women as prior aboration, multiparty, increas of maternal age, timely treatment of illness, health education and status of nutrient. Practically the Ages of pregnant women more than 30 years old will increaser probaility of anemia (Rezk *et al*., 2015) .

4-14 The mean differences of Zinc concentrations according to the type of study groups of pregnant women.

The present study show there were no significant associated between means of Zinc and parasitic infection in pregnant women as in the table4-14.

Table4- 14: The mean differences of Zinc concentrations according to the type of study groups of pregnant women.

Study variable	Study groups	N	Mean ± SD	F	P-value
Zinc (mcg/dl)	Pathogenic infection	7	129.73 ± 44.33	0.789	0.457
	Non-pathogenic infection	51	132.80 ± 54.99		
	Control group	42	118.25 ± 58.80		

*P value ≤ 0.05 was significant.

The Current study showed there is no significat associated between zinc deficiency and parsitic infection in pregnant women. Anther study had showed heigh significant association between parasitic infection and zinc deficiency and revealed that pregnant women with parsitic infection had more likely to have zinc deficiency than these pregnant women with no parasitic infection (Kumera *et a*l., 2015). In fact, the different between both studies may due to the fact that pregnant women may recived zinc supplement during pregnancy, zinc rich food and patient nutritional education.

4-15 The mean differences of Vitamin B12 concentrations according to the type of study groups of pregnant women.

The current study show there were no significant differences between means of Vitamin B12 according to study group as in the table 4-15.

Table 4- 15: The mean differences of Vitamin B12 concentrations according to the type of study groups of pregnant women.

Study variable	Study groups	N	Mean ± SD	F	P-value
Vitamin B12 (pg/ml)	Pathogenic infection	7	8.10 ± 1.38	0.051	0.95
	Non-pathogenic infection	51	8.01 ± 0.89		
	Control group	42	7.98 ± 0.98		

*P value ≤ 0.05 was significant.

The current study showed no significat association between VitaminB12 deficiency and parasitic infection in pregnant women and this study approximately near to another study which act on many neglected disease include helminthus and protozoa disease and explain that some heminthes can cause VB12 deficiency and other can not caused. Only one person who has infected with *Fasciolopsis buski* that can damage the intestinal villi physiological function suffer from Vitamin B12 deficiency

while showing an increased in VB12 value after three month of antiparasitic treatment of protozoal parasite (Layden *et al.*, 2018) .

Conclusions and Recommendations

Conclusions:

According to the obtained results of the present study, it can concludes the following items:

1. There is a association between parasitic infection and micronutrients deficiency among pregnant women.
2. There is a significant association between hemoglobin and iron deficiency with parasitic infections among pregnant women, while there is no relationship between parasitic infection and other micronutients deficiency.
3. Height prevalence of parasitic infections among pregnant women whom were in rural area and low educational levels.
4. Changes in blood profiles in some parasitic infections were observed among studied pregnant women.

Recommendations:

1. Further studies are in need to study the effects of all types of micronutrients deficiencies among pregnant women.
2. The study recommend to involve and to study other different diagnostic markers(biochemical, physiological, immunological, microbial, gynaecological) among pregnant women.
3. It is recommend to extend the scope of time, sample size, region of research and the medication use in other further studies.

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Appendixes

The Questionnaire paper which concerns the pregnant women under study

Patients Criteria	**Data**
Age	
Period of pregnancy	
Level of education	
resident	
Living condition	

The exclusion criteria which concerns the pregnant women under study

Patients Criteria	**Data**
Thalassemia	
Thyroid diseases	
diabetes	

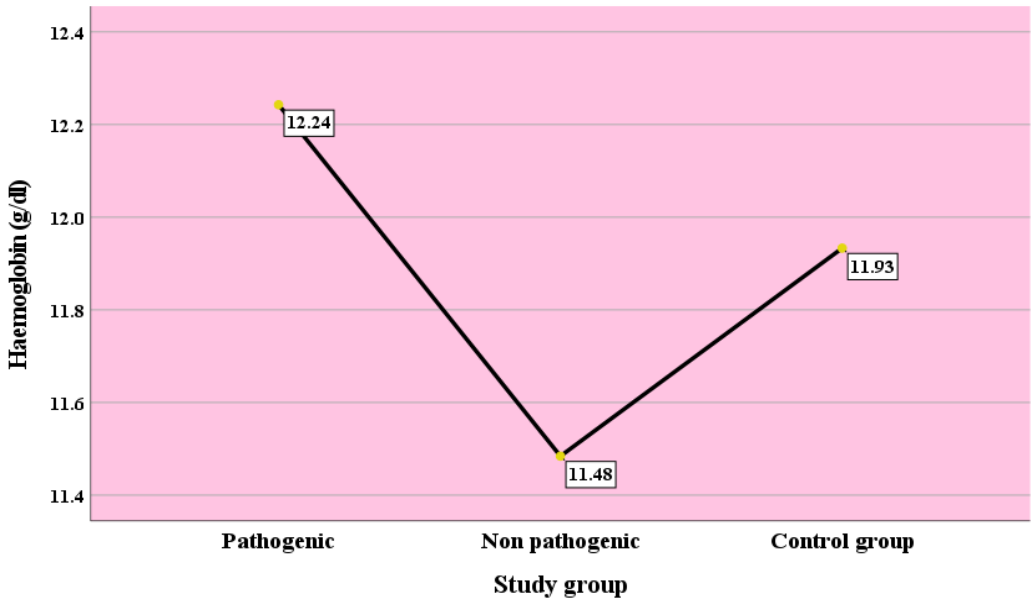

Figure-1- The mean differences of hemoglobin concentration (g/dl) according to the type of study groups of pregnant women

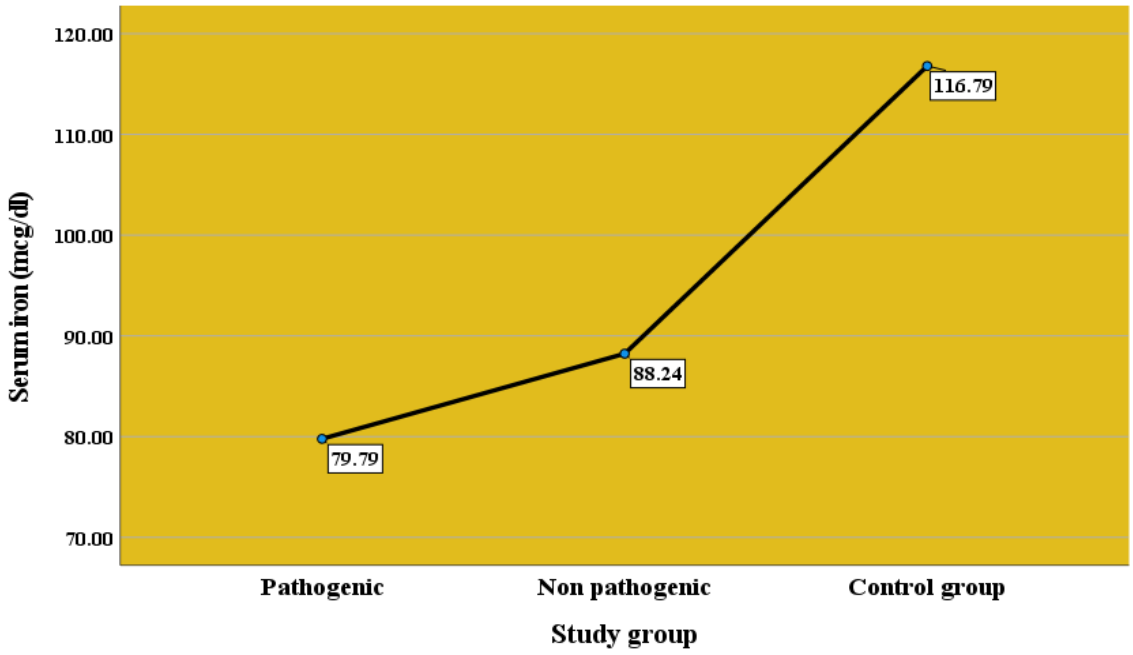

Figure -2- The mean differences of serum iron concentrations according to the type of study groups of pregnant women.

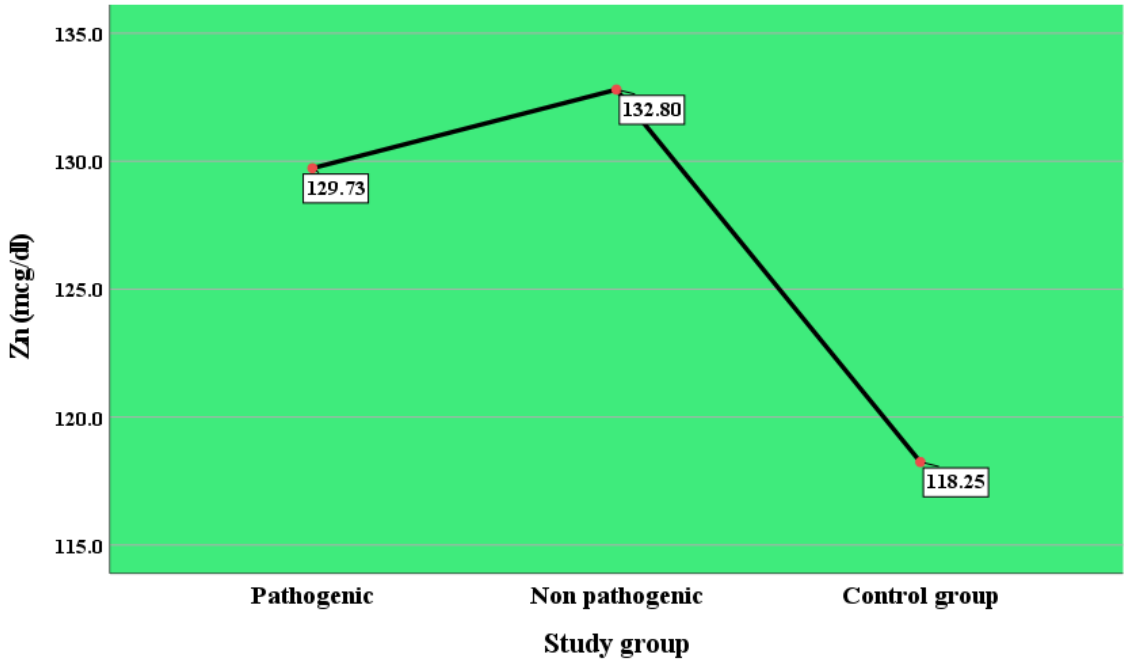

Figure-3- The mean differences of Zinc concentrations according to the type of study groups of pregnant women.

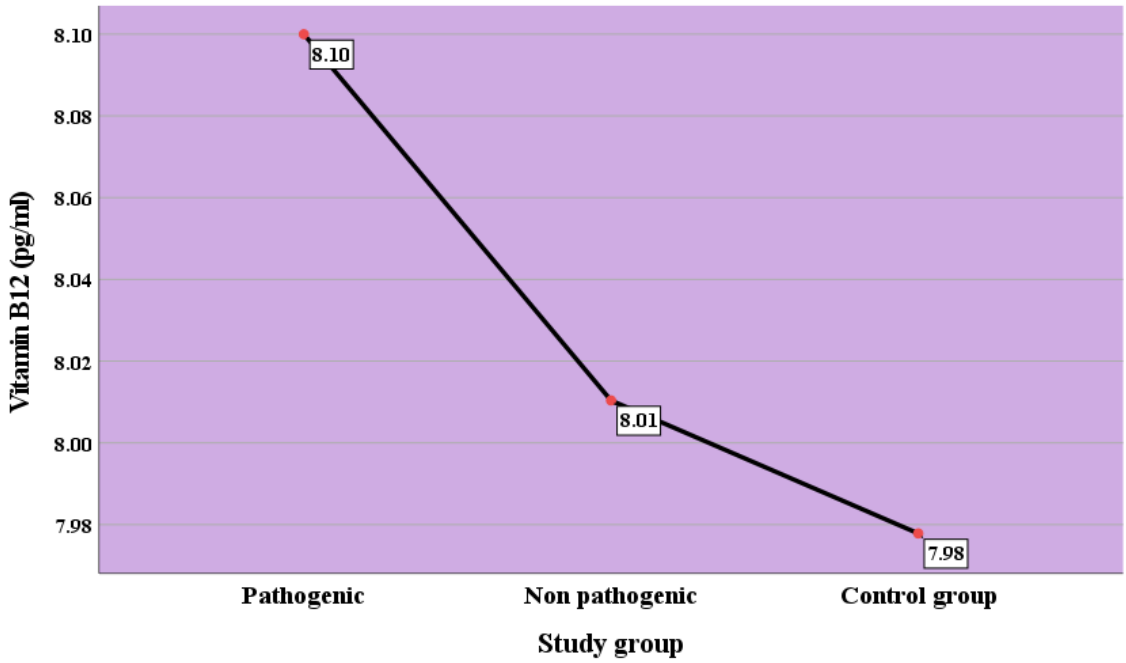

Figure-4- The mean differences of Vitamin B12 concentrations according to the type of study groups of pregnant women.

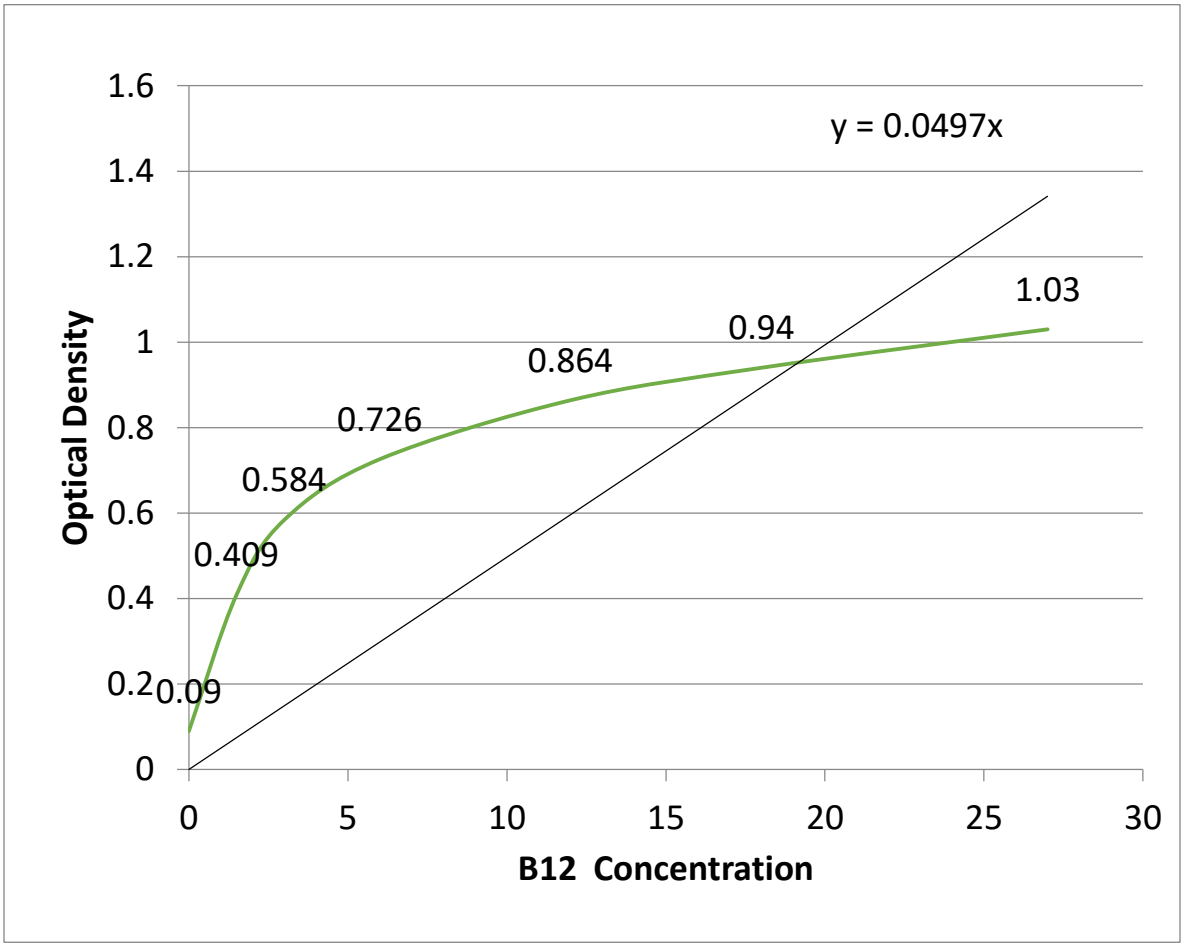

Figure 4: Calibrations curve of vitamin B12

الخلاصة:

أجريت الدراسة الحالية خلال الفترة من تشرين الاول 2021 إلى أذار 2022 لغرض الكشف عن التأثير المشترك للإصابات الطفيلية ونقص المواد التغذوية الدقيقة لدى النساء الحوامل في محافظة بابل. على وجه التحديد، أخذت 100 عينة جمعت من النساء الحوامل(87 من عينات البراز و13 من المسحات) اللواتي قمن بزيارة او مراجعة المستشفيات(مستشفى الولادة والاطفال، مستشفى الامام الصادق التعليمي) ومراكز الرعاية الصحية الأولية(مركز صحي ابو غرق، مركز صحي الكفل، مركز صحي حي المهندسين) في محافظة بابل.

كذلك تم أخذ عينة دم من كل امرأة حامل. تم وضع عينة الدم في أنبوب اختبار مزود بالجل وفصلت بواسطة أجهزة الطرد المركزي للحصول على المصل المستخدم لمعرفة مستوى المواد التغذوية الدقيقة (الزنك، فيتامين B12، الحديد) والبروتين التفاعلي C الذي يستخدم كعلامة مناعية / التهابية. بالنسبة للمتبقي من عينة الدم فقد وضع في انبوب EDTA (ثنائي امين رباعي حمض الاسيتيك) لمعرفة مستوى الهيموغلوبين. تم جمع عينات البراز الطازجة بواسطة حاوية نظيفة وفحصها على الفور عن طريق التحضير الرطب وصبغة اليود للكشف عن الطفيليات المعوية. أخذت المسحات المهبلية من النساء الحوامل بطريقة معقمة للكشف عن طفيلي المشعرات المهبلية. تبين أغلب النساء الحوامل ذات الاعراض المصاحبة للإصابة كانت خفيفة والبعض الاخر من النساء الحوامل كانت الإصابة بدون أعراض.

أخذت المعلومات السكانية والمعيشية للنساء الحوامل المتمثلة بالعمر، مرحلة الحمل، الإقامة، المستوى التعليمي والحالة المعيشية. كشفت نتائج الدراسة عن ارتفاع في معدل انتشار طفيليات المتبرعمة الكيسية البشرية 27(27%)، المتحولة القولونية 36(36%) بشكل كبيرو غيرها من الطفيليات الاخرى غير مرضية ولكن بمعدل منخفض مثل شفوية السياط المنسيلية 3(3%) الأميبا البوتشيلية (1%) . وجدت أربعة أنواع من الطفيليات المسببة للأمراض في الدراسة الحالية للنساء الحوامل وهي المشعرات المهبلية2(15.3)، الأميبا الحالة للنسيج 2(28.5) ، الجيارديا اللمبلية 2(28.5) و المحرشفة القزمة 1(14.2).

عمليا، أظهرت الدراسة الحالية ارتباطا كبيرا بين نقص الحديد والهيموغلوبين والاصابة الطفيلية لدى النساء الحوامل بينما لا يوجد هذا الارتباط مع بقية المواد التغذوية الدقيقة. كما أظهرت الدراسة الحالية أنه لا توجد علاقة ذو دلالة إحصائية على الارتباط بين CRP والاصابة الطفيلية. كانت الإصابة الطفيلية ذات حدوث مرتفع في المستوى التعليمي المحدود والمنخفض للنساء الحوامل. اظهرت الاصابة الطفيلية انتشاراً مرتفعاً في المناطق الريفية عنها في المناطق الحضرية، هناك ارتباط ضئيل بين عمر النساء الحوامل والاصابة الطفيلية.

وجدت الاصابة الطفيلية بانها ذات انتشار كبير في المرحلة الأولى والثانية من الحمل مقارنة مع المرحلة الثالثة من فترات الحمل بأكملها. فضلاً عن ذلك، أظهرت الدراسة وجود تغيرات في صورة الدم الكاملة مثل خلايا الدم البيضاء، الخلايا المحببة، متوسط حجم الكرية، الهيماتوكريت ،متوسط الهيموغلوبين الجسمي، متوسط تركيز الهيموغلوبين الجسمي ولكن لا تشمل هذه التغيرات جميع النساء الحوامل.

وزارة التعليم العالي و البحث العلمي
جامعة بابل- كلية الطب
قسم الاحياء المجهرية

دراسة التأثير المشترك للإصابات الطفيلية ونقص العناصر الدقيقة المغذية في النساء الحوامل في محافظة بابل

رسالة

مقدمة الى مجلس كلية الطب –جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير

في العلوم/ الاحياء المجهرية الطبية

من قبل

سرى سلمان سرحان كاظم

(بكالوريوس علوم حياة /جامعة بابل/ ٢٠٠٨)

بإشراف

الأستاذ الدكتور
هادي فاضل اليساري

الأستاذ المساعد الدكتورة
حنان خضير حسين

أب/٢٠٢٢م

محرم/١٤٤٤هـ