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Nod Like Receptor p3 inflammasome and some proinflammatory cytokines production during Giardiasis

A thesis

Submitted to the council of college of Medicine , University of Babylon, as a partial fulfillment of the requirements for degree of Master in science /medical Microbiology

By

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

نَرْفَعُ دَرَجَاتٍ مَّن نَّشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ

صدق اللَّهُ العظيم

سورة يوسف(٧٦)

Supervisor certification

I ,certify that this thesis was prepared under my supervision at the department of microbiology–college of Medicine/ University of Babylon, as a partial fulfillment of requirements for degree of Master of science in Medical Microbiology.

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We ,the examiner committee , certify that we have read the thesis entitled (**Nod Like Receptor p3 inflammasome and some proinflammatory cytokines production during Giardiasis)** and have examined the student **Zainab Mohamed jawad Jasim** in its contents , and that in our opinion it is accepted as a thesis for degree in Master in Microbiology with excellent estimation .

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Dedication

To my kind father....my role model, and my ideal in life; He taught me how to live with dignity and dignity.

To my loving mother... I cannot find words that can give her her due, as she is the epic of love and the joy of life, and an example of dedication and giving.

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To my brothers.... my support, my joys and my sorrows.

To my son..... the pleasure of my liver.

And to my second family (my in-laws).

To all those who supported me in completing the science achievement.

Zainab

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Summary

This study include 150 stool sample were collected from patient with diarrhea attended to hospitals in Hilla city (Imam Al_Sadiq Hospital, Babylon Hospital for Maternity and Children , Hospital Al-noor for Children and Ali Obeiss Hospital in Al Midhatiya)from September 2022 to january 2023 the results of this study showed that the total percentage of infection with positive *G.lamblia* was 15.3% the percentage of infection with *G.lamblia* was higher in female 52.2% than male 47.8%,in which showed the results that the infections with *G. lamblia* was the highest percentage 39.1 % for age group 1-5 year,the percentage of infection with *G.lamblia* was higher in rural area 65.2% than urban area 34.8%.

The current results showed high levels of Nod Like Receptor p3 in age group 21-30 years compared to healthy control,this increasing was significantly $p \leq 0.05$,also high level of NFKB-P65 in age group(6-10/ 21-30)years compared to healthy controls ,the increasing significantly $p \leq 0.05$,while the level of NFKB-65 was decreased in age group 16-20 year compared to controls and the result were not significantly.

The result showed increasing in the level of IL-1β in patient with *G.lamblia* and high levels of IL-1β in age group 21-30 years compared to healthy controls.the increasing was significantly ($p \leq 0.05$).in addition there were high level of IL18 in age group 6-10 year compared to controls and the results were significantly ($p \leq 0.05$).

The results of the present study showd there was positive correlation between IL-18 and NLRP3 (N= 23, r = 0.263 , p = 0.226)

and there was positive correlation between IL-18 and NFKB-P65) (N= 23, r = 0.358 , p = 0.093). but this correlation was not significant also there was positive correlation between IL-1β and NLRP3(N= 23, r = 0.205 , p = 0.347) and there was positive correlation between IL-1β and NFKB-P65 (N= 23 , r0.209 , p=0.338).

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Chapter One

Introduction and Literature Review

1.1:Introduction:

The protozoan parasite *Giardia* can cause a gastrointestinal disease known as giardiasis, which occurs through the ingestion of cyst-contaminated food and water. It is estimated to cause 280 million cases of intestinal diseases annually worldwide, with a higher prevalence in areas with low sanitation and hygiene standards (Hemphill *et al* ,2019). Giardia infection, is characterized by small intestine malabsorption and fatty diarrhea, this occurs due to direct damage to the intestinal lining or mechanical obstruction of the intestinal villi and absorption (AL-kahfaji & Alsaadi , 2019).

G. lamblia has a simple life cycle, consisting of disease-causing trophozoites and infectious cysts. Infection begins when the host ingests the cysts, which then enter the digestive tract, where they ultimately develop into motile trophozoites in the small intestine through a process called excystation. The trophozoites can then transform back into their cystic form through a process called encystation.*Giardia* can be maintained in the laboratory and induced to complete its life cycle by using laboratory methods (Fink *et al*, 2020)Metronidazole is a common first-line treatment for giardiasis (Krakovka *et al* ,2022).

Disease initiation and progression during *Giardia* pathogenicity is a multifactorial process that exhibits intestinal barrier dysfunction, increased enterocyte apoptosis, host lymphocyte activation, a shortage of brush-border microvilli, and atrophy of the intestinal villi, which ultimately resulted in malabsorption (Buret *et al*., 2015; Allain and Buret., 2020).

In the small intestine, environmental conditions are generally harsh due to rapid transit times and the periodic influx of digestive enzymes, bile, and stomach contents. As a result, bacterial populations tend to be more dynamic, but less diverse, and with lower overall biomass than in the large intestine(Kastl *et al*., 2020).

Laboratory diagnoses of *Giardia* spp. rely on the observation of cysts or trophozoites in stool samples, with limited options for immunological-based assays and molecular methods for the diagnosis of giardiasis (Hooshyar *et al*.,2019).

Giardia intestinalis has a highly developed cytoskeletal system, consisting of microtubules, microfilaments, and cytoskeletal proteins. Recent studies have confirmed that the cytoskeletal proteins of *Giardia* are closely related to its pathogenicity.(Zhou *et al*.,2022).

Giardia intestinalis infection leads to intestinal cell damage and loss of the brush border of the intestinal epithelium, resulting in shortened microvilli and impaired epithelial barrier function. Watery diarrhoea, diarrhoea, nausea, abdominal pain, vomiting, and weight loss are all symptoms of this pathological alteration. Most infections are asymptomatic. its pathogenesis, including structural proteins and excretion of *Giardia* intestinalis, surface antigen variants, and the role of *G.lamblia* in the small intestine, has been extensively studied.(Willenborg *et al*.,2022).

The virulence factors involved in *Giardia* pathogenesis have been described, including the cysteine proteases, which have been well-studied. These are involved in intestinal epithelial junctional complex disruption and

degradation of host immune factors, e.g., cytokines (Bartelt and Sartor, 2015; Cotton *et al*., 2015).

The innate immune system is the primary mechanism by which most organisms respond immediately to infections or injury. Pattern-recognition receptors (PRRs) in the host are activated, which recognize molecules released by pathogens or damaged cells. These molecular signals are known as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Gong *et al* .,2020).

Inflammasomes are multimeric cytosolic complexes that sense the presence of pathogens or changes in cellular homeostasis and activate commensurate innate immune responses. The inflammasome complex is defined by the responding sensor protein, which has characteristic structural domains and includes the nucleotide-binding oligomerization domain-like receptors (NLRs) NLRP1, NLRP3, and NLRC4, as well as the AIM2 and pyrin proteins (Place and Kanneganti,2018). Inflammasome assembly is initiated by NLR recognition of pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns (DAMPs) (Place and Kanneganti, 2018).

Aim of study:

The aim of this research to show that effect of NLRP3 inflammasomes on *G. lamblia* infection which leading to bioactive IL-1β production and other proinflammatory response against this parasite,. This goal can be achieved by the following objectives:

Objective:

1- Detection of *Giardia lamblia* in stool sample by direct examination.
2- Using Rapid test chromatography to confirn Diagnosis of *G. lamblia* infection.
3- Estimation of NLRP3, IL-1β , IL-18 and NF-κB by using ELISA technique in giardiasis patients and control.
4- Study the Associations between immunological marker and giardiasis.

1.2.Literature Review:

1.2.1.Historical review

The first observation of *Giardia* within 330years ago , *Giardia lamblia* discovery in 1681 by Antonie Van Leeuwenhoek , when he examined asample of his own diarrheal stool. But the real study of this parasite whose in 1859 by Vilem Dusan Lambel who ranked the Giardia which isolated from human and called it by cercomonas intestesinal.(Adam,2001).

Raphael Blanchard proposed the name lamblia intestinalis in 1888 so as to honor lamble ,it was not unit 1915 that the name *Giardia lambilia* was introduced, the name was selected so as to commemoeate the work of the Franch *Giardia lamblia*, as well as (Ford,2005).

Giardia lamblia (syn., *G.intestinalis, G. duodenalis*) is a flagellated protozoan that inhabits the upper small intestine of many vertebrates. Infections are initiated by ingestion of cysts, followed by excystation and colonization of small intestine by the trophozoites. When trophozoites descend through the intestine, they differentiate into cysts, which are shed with the feces (Tilahun *et al* .,2022) .

Intestinal parasitic disease (IPD) is the most common public health problem around the world which imposes economic and mortality burdens. WHO declared that about one-third of the world's population are infected with IPD the majority of whom are children. (*giardia, D. fragilis, B. coli*) cryptosporidium spp: and *Giardia* spp : are the most important intestinal parasites (Gholipoor *et al*., 2019).

1.2.2.Epidemiology:

The etiological agent of Giardiasis, *Giardia duodenalis*(syn.*G. intestinalis G. lamblia*) is one of the most prevalent intestinal protozoan flagellate of the human. The life cycle of *Giardia* species is simple and it is included of two active trophozoite and cystic forms. This parasite transmits via fecal-oral route through direct or indirect ingestion of infectious cysts. The incubation period varies from 9 to 15 days after ingestion of cysts. Symptoms of infection are varied from the absence of symptoms to acute watery diarrhea, nausea epigastric pain and weight loss (Ryan *et al*.,2013).

In fact, it is estimated that, for example, in the United States, more than 1 million people are infected with this parasite every year, with children being at higher risk than adults . Untreated, it can have long-term complications, such as reactive arthritis, irritable bowel syndrome, and recurring diarrhea (Einarsson *et al*.,2016) and it can jeopardize a child's development. Importantly, *G.intestinalis* resistance to metronidazole, a clinically important drug of choice against protozoa, has been reported (Krakovka *et al*.,2022).

Giardiasis has a global distribution and it is common in both children and adults. The prevalence of *Giardia* infection is higher in developing countries. More than 200 million cases of giardiasis are annually diagnosed worldwide. Since 2004, *Giardia* has been included in the "neglected diseases initiative" by World Health Organization (Savioli *et a.l*,2006).

The infection rate in asymptomatic children has been reported from 8% to 30% in developing countries and 1-8% in industrialized regions (Smith *et al* .,2011). The occurrence of giardiasis is probably higher in individuals with

diarrhea.The Protozoan *Giardia* duodenalis is one of the most common human parasitic enteropathogens worldwide, infecting on average 2% of adults and 8% of children in developed countries and up to 33% of individuals in the developing world (Dunn and Juergens, 2020) .

The ingestion of water or food contaminated with *G. intestinalis* cysts by susceptible mammalian hosts leads to giardiasis, especially in young children, with clinical presentations that range from no symptoms at all to severe diarrhea (Leung *et al* .,2019).

Four characteristics of *G. lamblia* that boost its prevalence are as follows: a) the low infective dose of 10 to 100 cysts in humans; b) rapid spread among mammals after stool excretion; c) cysts can survive for weeks to months and; consequently d) presence in drinking water and food Fecal-oral and polluted water/food are direct and indirect transmission routes of giardiasis from person to person. Nevertheless, pets cats and dogs are also the major zoonotic transmission routes to humans. (Aw *et al*., 2019).

The global estimation reported that more than 200 million people of all age groups have giardiasis related diarrhea yearly, whereas children especially those who live in poor sanitation areas, are at higher risk. In this respect, the infection ranges of 10-50% and 0.1-5% were dedicated to *Giardia* in developing and developed countries, respectively (Masangkay *et al*., 2020).

1.2.3 Classification:

kingdom : Protista

Subkingdom : Protozoa

Phylum : Sarcomastigophora

Subphylum : Masigophora

Class : Zoomastigophora

Order : Diplomonadida

Family : Hexamitidae

Genus : *Giardia*

Species *G. lamblia*

(AL-kahfaji and Alsaadi ,2019).

1.2.4:Morphology:

G. lamblia exists in two morphological form- trophozoite and cyst: the trophozoite active feeding stage of parasite which is responsible for colonization in intestine. The shape of trophozoite is pear shape or tennis racket shape with broad round anterior end and a tapering posterior end.It measures 9-21 µm in length and 5-5µm in width. The dorsal surface is convex while ventral surface is concave with a sucking disc (adhesive disc) which acts as an organ for attachment Behind the adhesive disc lies a pair of large curved and transverse median bodies, unique to It is bilaterally symmetrical and all organs of body are paired. They have two median bodies, two axostyle, two nuclei and

four pairs of flagella. Each nucleus consists of large central karyosome giving a characteristic face like appearance to the parasite in stained preparation. Cytoplasm is uniform and finely granulated. Motility shown typical ‘fallling leaf type’motility.(Müller *et al*.,2016).

The Trophozoite live attached on dudodenal and jejunal epithelial cells and thrive on nutrients from the intestinal fluid with amino acids, especially arginine as their preferred fuel(Vermathen *et al* .,2018).

The Cyst is an infective stage of parasite. A fully mature cyst is oval or ellipsoidal in shape and measures 8-12µm in length and 7-10µm in width . Cyst is surrounded by a thick cyst wall. Cytoplasm is granulated and is separated from the cyst wall by clear space. The axostyle lies more or less diagonally. A cyst contains 4 nuclei The remaining of flagella and the margins of sucking disc may be seen inside the cytoplasm. The cysts are excreted in the feces and constitute the infectious stage. Thus, giardiasis is caused by fecal contaminations of drinking water food or direct contact with feces waterborne transmission being regarded as a major source (Efstratiou *et al* .,2017).

1.2.5. Life cycle and transmission routs:

Infection with *G.lamblia* occurs through ingestion of contaminated food and water, The life cycle of *Giardia lamblia* (Figure 1) consists of three parts. The cyst becomes a trophozoite, the trophozoite multiplies asexually and the trophozoite returns to the cyst form. *Giardia* cysts are an infectious stage. The hard wall of the cyst allows it to resist decomposition in an acidic environment. When a cyst is swallowed, it migrates to the small intestine where it undergoes the process of exocytosis, i.e., the transformation of the cyst into a trophozoite.

This process is initiated when the environment changes from a low pH to a natural pH. The optimum pH for the efflux process is pH 4.0. Once released, the trophozoite undergoes asexual multiplication, also called binary longitudinal fission. After multiplication, the trophozoites begin to absorb nutrients from the host. During this period, not all people show symptoms. When a *Giardia* cyst moves into the large intestine, it undergoes encystation, which is the opposite of excystation. Encystation is the process of transformation of trophozoites into *Giardia* cysts and takes place in the rectum. During this process, metabolism begins to slow down as the cells close and enter a resting state. Eventually, the cysts are eliminated with the host's feces (Tilahun *et al* .,2022).

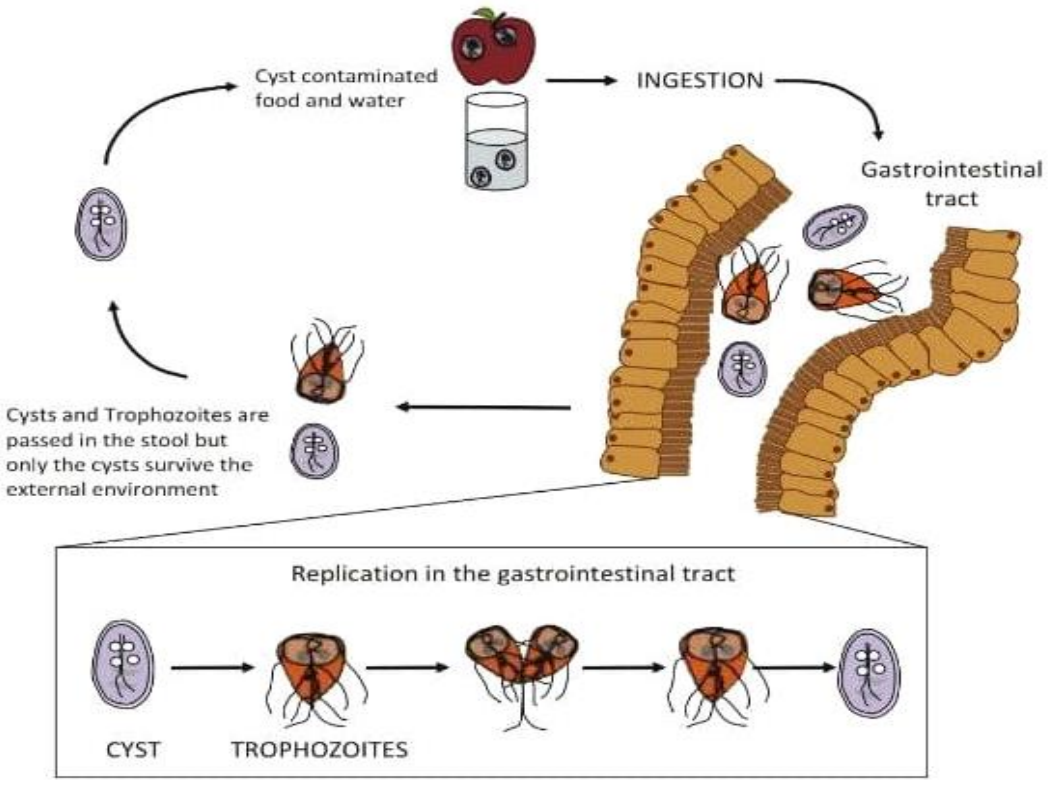


Figure.(1-1) Life cycle of *G.lamblia* (Tilahun *et al* .,2022).

1.2.6: Pathogenesis and Clinical signs of *Giardia Lamblia*

G. lamblia is a noninvasive pathogen of the small intestine and produces a wide range of clinical presentations, including chronic diarrhea with weight loss, postinfectious complications of irritable bowel and chronic fatigue, growth stunting, and asymptomatic infections. These varied manifestations may result from host, parasite, or microbiota differences and make it particularly difficult to elucidate the mechanisms resulting in this range of illness. Nevertheless, there has been substantial progress over the past 2 decades in elucidating some of the mechanisms. Recent in-depth reviews of these advances are also available (Singer *et al*., 2019).

So the possibility of direct pathogenesis from the mechanical attachment has been raised. However, there is currently no evidence to support this possibility. Rather, current data suggest that a combination of secreted proteases and other *Giardia* factors, the host immune response, and the interaction of these factors with the intestinal microbiota contribute to the various manifestations (Fink *et al*., 2017). Through the entire immune response, it is actually remarkable that in patients who have biopsies for symptomatic giardiasis, the findings consist of flattening of the villi but no obvious inflammatory changes. However, an inflammatory picture can be seen and may actually be separate from the location of the trophozoites. A series of cases was reported in which trophozoites were seen in ileal biopsy specimens from ileocolonoscopy of symptomatic patients but inflammatory changes were found in the duodenal biopsy specimens of these patients (Oberhuber *et al*., 2016).

The role of excretory secretory products (ESPs), most notably the cysteine proteases, has received substantial attention over the last decade (Allain *et al*., 2019).

Around about 200 million of people in the world are with clinically manifested giardiasis, With a global illness burden of around 280 million cases each year (Rahman *et al*.,2020). Predisposing factors are coincident in both groups of these pathogens, such as low age, immune suppression status, low socioeconomic and educational status, and consumption of contaminated water sources, and they are upper gastrointestinal complaints such as upper abdominal pain, abdominal bloating, nausea, vomiting, and epigastric bleeding.(Krzyżek *et al*.,2017).

Giardia may even have protective effects against diarrheal disease in some settings, particularly in children exhibiting polymicrobial infections. In contrast, *Giardia* infection has also been associated with development of post-infectious complications including irritable bowel syndrome (IBS) and chronic fatigue and has been associated with cognitive defects and stunted growth in children (Halliez and Buret,2013; Allain and Buret, 2020).

The intestinal microbiota plays a critical role in homeostasis of the gut and overall health and is frequently found to be altered during gastrointestinal disease. *Giardia* interacts both directly and indirectly with the microbiome, and through these interactions can modulate host metabolism, immune responses, pain signaling, and the mucus barrier, all of which may have systemic effects that potentially persist even after parasite clearance. Functional and compositional changes to the intestinal microbiota have been demonstrated during. the course of *Giardia* infections, including disruption of the microbial

biofilm structure, altered virulence in commensal species, and altered species abundance and diversity. (Carding *et al*., 2015; Shreiner *et al*., 2015; Nagao *et al*., 2016; Buret *et al*., 2019).

Important functions of the microbiome include digestion and absorption of nutrients, immune maturation and modulation, regulation of host cell proliferation and function, modulation of intestinal permeability transit, and neurotransmission, and defense against opportunistic pathogens via niche exclusion (Lynch and Pedersen ,2016).

Giardia intestinalis has a highly developed cytoskeletal system, consisting of microtubules, microfilaments, and cytoskeletal proteins. Recent studies have confirmed that the cytoskeletal proteins of *Giardia* are closely related to its pathogenicity. Among the many proteins involved in the composition of the cytoskeleton, the cytoskeletal proteins specific to *Giardia* intestinalis are one of the main components. In 1983, the concept of Giardin was first introduced by Crossley (JZhou *et al*.,2022).

Several *Giardia* excretions may be involved in host pathogenesis, including metabolic enzymes released by *Giardia*, soluble substances of different sizes and unknown cysteine proteases. *Giardia* has been found to release arginine deaminase (ADI) and ornithine carboxyltransferase (OTC), both of which may be involved in the metabolism of L-arginine, a nutrient that is the preferred energy source of the growth and reproductive stages of *Giardia*(Liu *et al*.,2021).

The mechanisms responsible for pathophysiology in giardiasis include both parasite and host immune factors. *G.lamblia* virulence factors have been suggested on the basis of their targeted effects.(Minetti *et al*.,2016).

Clinical signs of giardiasis often occur 1-2 weeks after infection. Giardiasis illness outcomes are modulated by a range of host and environmental variables, like other infectious diseases. These include gut flora, age, immunological variables, nutrition, and concomitant illnesses (Barash *et al.*, 2017).

Diarrheal symptoms mostly occur during the acute phase of the infection, and at least some of the effects of the infection appear to be isolate-dependent (Allain *et al*., 2017).

Catalytic proteins, metabolic enzymes, surface molecules, and soluble mediators that bind to host cell receptors and extracellular matrix components are among the secreted molecules that play a virulent role in the cytotoxic effect on epithelial cells of *Giardia duodenalis* excretory/secretory products (ESP)/secretome. These parts are divided into five major categories: enzymes involved in energy metabolism, tenascins, toxins-like compounds, cysteine-rich surface proteins, and cysteine proteases (Jex *et al*., 2020;Ortega-Pierres and Argüello-García, 2019).

After being ingested, cysts are digested in the stomach, where they grow into trophozoites that colonize the duodenal and proximal jejunal epithelium. As a first line of defense, the host may cause epithelial tight junctions to be broken, stimulate CD8+ lymphocytes, and cause apoptosis in cells in contact with the trophozoites. due to the shortening of brush-border microvilli, disaccharidases, and other enzymes are deficient. In the following stage, CD4+

T-cells that produce IL-17 and TNF-α and dendritic and mast cells that produce IL-6 are used to trigger adaptive immune responses. Tuft cells are another possible source of IL-17. These cells may be triggered by trophozoite metabolites, which would then heighten pro-inflammatory responses in the intestinal epithelium, as has been shown with closely similar protists and helminths (Schneider *et al*., 2018).

Trophozoites are eliminated from the gastrointestinal surface as a result of the cytotoxic secretory IgA and defensins that are produced. The production of nitric oxide (NO), which is also produced by immune and epithelium cells, prevents trophozoites from growing. In addition, mast cell degranulation and NO production in neurons stimulate intestinal peristalsis, which helps in the ejection of trophozoites. The efficacy of the immune response against *Giardia* may be enhanced by the gut microbiota. Trophozoites may cause the microbiota to become dysbiotic, which would have an immunological impact that would favor infection (Fink and Singer, 2017).

1.2.7:Virulence factor of *Giardia Lamblia*:

G. lamblia is a non-invasive Parasite that reproduces by attaching itself to the intestinal epithelium. Giardiasis is a disease with varying degrees of severity; it can be acute or chronic, and asymptomatic carriers are prevalent.. *Giardia lamblia* is an anaerobic, but to some extent also aerotolerant, eukaryote with several prokaryotic properties belonging to the phylum Diplomonadida, super-group Excavata(Paniker *et al*.,2017).

The virulence factors involved in *Giardia* pathogenesis, including the cysteine proteases,These are involved in intestinal epithelial junctional

complex disruption and degradation of host immune factors, e.g,cytokines The virulence of an intestinal pathogen results from its own genetic background, the competence of the host immune system, its nutritional status (e.g., the acquisition of iron and the interaction with other intestinal microorganisms (Bartelt and Sartor, 2015; Cotton *et al*., 2015; Liu *et al*., 2018).

Pathogenicity and virulence are the result of interactions between the genotype of the pathogen, the immune response, the nutritional status, and the intestinal microbiome of the host figure 1-2 (Buret *et al* .,2019).

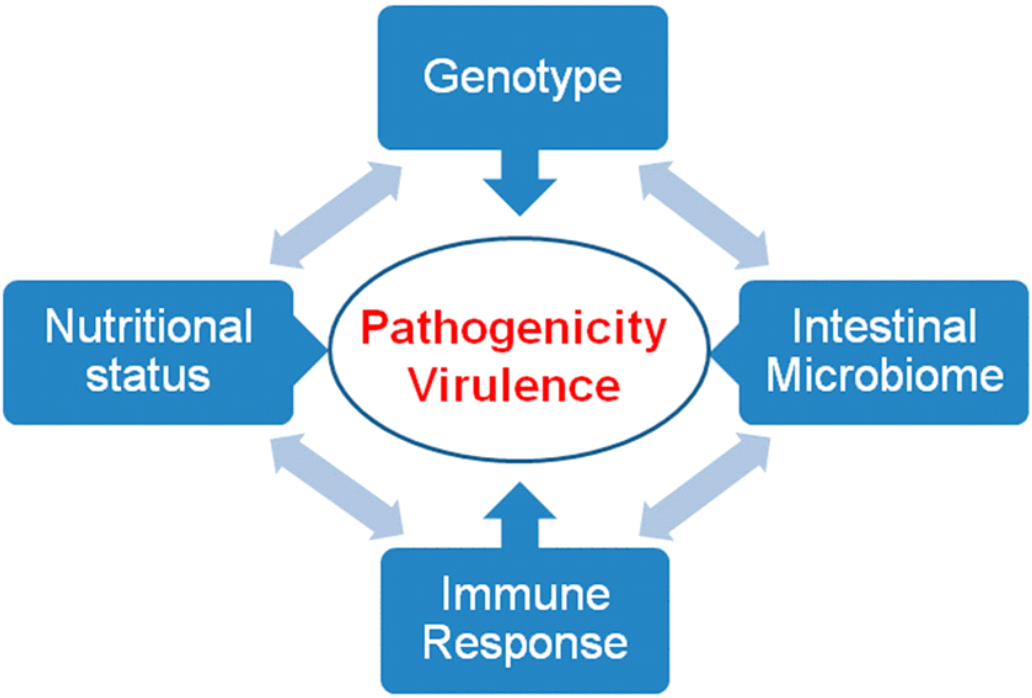


Figure (1-2) Pathogenicity of *G.lamblia (Buret et al .,2019)*

The normal pattern of infection is a pre-patent period of 5–7 days. This is followed by 1–2 weeks of acute symptoms and then elimination of the parasite As noted above, acute symptoms like diarrhoea and cramps are often not present during this ‘acute’ phase. In most individuals, the parasite is

eliminated within 3–4 weeks, but chronic infections can also occur. Chronic infections are common in individuals with certain immunodeficiencies, in particular common variable immune deficiency (CVID) and X-linked immunodeficiency (XID). Both of these syndromes are characterised by reduced production of IgG, suggesting that antibody responses may play a pivotal role in preventing chronic infection (Robertson *et al*.,2010).

When combined, these immune-evasive effects will aid in the spread of infection and are hence crucial to pathogenesis. The impacts of this parasite's proteolytic activity may thus be used to simulate a large portion of the pathophysiological reactions that it causes; the role of Giardia cysteine proteases in the pathogenesis of giardiasis has lately been thoroughly studied. The pathogenicity and trophozoite adhesion to host epithelial cells of *Giardia* is thought to be aided by the secretion of tiny extracellular, membrane-bound vesicles by the parasite. (Gavinho *et al*., 2020).

1.2.8: Immune response :

Giardia lamblia is a non-invasive enteropathogen that does not trigger an overt inflammatory response. However, both innate and adaptive immunity are required for control and clearance of *Giardia*. Critical roles have been identified for the cytokines IL-6 and IL-17, as well as for secretory IgA (Zhou and Shine,2003); (Dreesen *et al*., 2014; Dann *et al*., 2015). CD4+ and CD8+ T cells play important roles in parasite clearance and pathogenesis, respectively (Keselman *et al*., 2016).

Combined innate and adaptive immune responses are needed to control *Giardia* infection and replication(Singer *et al*.2019)*Giardia* excretorysecretory

products and variant-specific surface proteins (VSPs) have been known to cause high levels of serum and salivary IgA in infected patients (Serradell *et al* .,2018). Dendritic cells (DCs), mast cells IECs, and macrophages have been shown to contribute to host defense responses in *Giardia* infection (Singer *et al*.,2019). Mature DCs can produce proinflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin IL-6 and IL-12 when stimulated by *Giardia* binding immunoglobulin protein (Lee *et al*.,2014). Our recent studies have indicated that the apoptotic program can be initiated in IECs upon *Giardia* trophozoite treatment (Liu *et al*.,2020). It has been shown that macrophages are involved in the immune response to *Giardia* infection via the activation of AKT/MAPK signaling (Li *et al*.,2017).

In addition, mucosal mast cells and macrophages appear to play a role in parasite clearance, while nitric oxide and antimicrobial peptides produced by intestinal cells may have direct or indirect cytostatic effects against *Giardia* trophozoites (Stadelmann *et al*., 2013; Fink *et al*., 2019). However, recent findings have demonstrated that *Giardia* is capable of modulating host immunity. *Giardia* may directly cleave host-produced chemokines and cytokines, resulting in dampened immune responses (Cotton *et al*., 2014; Liu *et al*.,2018;Ortega-Pierres *et al*., 2018; Allain *et al*., 2019). Furthermore, Giardia may avoid host adaptive immunity through the expression of variant specific surface proteins (Hjollo *et al*., 2018).

The Immune Response to *G.*lamblia aboth humoral and cellular immune responses are induced by *G.lamblia*. IgA, IgM, and IgG isotypes have

been demonstrated in both local and systemic antibody responses *G.lamblia* infection also induces the activation of innate and acquired response from the host immune system (Ieni *et al* .,2016).

Giardia lamblia infection elicits both innate and adaptive immune responses in humans and animals. The innate immune system is responsible for the first identification of *Giardia* by the host. Antimicrobial peptides like as defensins are released by Paneth cells in the Lieberkühn crypts in addition to pancreatic secretions. Lactoferrin, which has been demonstrated to be harmful to *Giardia* trophozoites in vitro, is released continually by the gallbladder.(Brenchley *et al*.,2012).

Giardia lamblia does not generally infect healthy mucosal tissue, yet new research suggests that this could happen in the lab. Because of the parasite's robust anti-inflammatory activity, which appears to be caused by its cysteine protease, this infection displays no symptoms of local inflammation. Significant progress has been made in our understanding of *Giardia lamblia* innate and adaptive immunity in recent years, particularly the role of variant-specific surface proteins (VSPs) and antigenic variations in chronic infection in complicated animal models like the Mongolian gerbil. *Giardia intestinalis* can elicit Th1, Th2, and Th17 immune responses in mature hosts infected with *G.lamblia*, and new findings reveal that pancreatic rennin mast cell protease-4 modulates intestinal cytokine production in *Giardia* intestinalis-infected mice. Infection triggers a powerful protective and adaptive immunological response in humans and animals, with CD4+ T lymphocyte-mediated IgA synthesis playing a key role (Allain *et al*.,2020)., the altered duodenal mucosal lymphocytes were maintained for several months after infection in human

patients. Consistent with previous observations, these reports also reveal that CD8+ T cells are not involved in immune protection; instead, in *G. lamblia*, CD8+ T cells are responsible for microbial and dysaccharidase epithelial damage; serum gamma interferon levels are also elevated in infected patients or mice, an observation consistent with a possible role for macrophages in mediating, rather than directly controlling, infection. *Giardia* intestinalis has also been demonstrated to modify macrophage pro inflammatory signaling by cleaving the p65RelA component of the nuclear transcription factor kappa b. (Allain *et al* .,2019).

The microbiota can also influence the expression of cytokines during *Giardia* infection. In germ-free mice reconstituted with human colonic microbial communities, mice that received microbes previously exposed to *G. lamblia* trophozoites (isolate NF) showed increased production of proinflammatory cytokines, as well as proliferation and enlargement of lymphocyte aggregations within the follicles, compared to mice receiving control bacteria. Mice receiving *Giardia* modified microbial communities showed increased production of IL-6 IL-1β, TNF-α, and IFN-ɣ suggesting Giardia may alter microbial communities in such a way as to promote a pro inflammatory response that is driven by the microbes themselves (Beatty *et al*., 2017).

1.2.8.1:Innate immune system :

The initial recognition of *Giardia* by the host occurs through the innate immune System Biochemical and genetic studies of *Giardia* have shown that the parasite is able to glycosylate numerous proteins with the addition of N-Acetyl-Glucosamine (GlcNAc). GlcNAc is a known ligand for the mannose

binding lectin (MBL), and trophozoite lysis by na¨ıve complement-containing sera in vitro can occur following MBL binding. The cyst walls of *Giardia* also contain a large amount of carbohydrate polymer-containing GlcNAc. The ability of this cyst wall material to stimulate any type of immune response however, has not been investigated. (Evans *et al* ., 2010).

Dendritic cells (DCs) are also able to recognise and respond to *Giardia* trophozoites. DCs produce small amounts of cytokine, including IL-6 and TNF-, in response to *Giardia*. Addition of *Giardia* extracts to these TLR agonists also increases IL-10 production by the DCs. Thus, *Giardia* infection is likely to promote an anti-inflammatory environment within the intestinal tract. Dendritic cells (Kamda and singer .,2009) .

The innate immune system is the first defense line of organism to resist multiple pathogens invasion through triggering non-specific immune response in immune cells. Therefore, an in-depth study of the immune mechanisms that mediate host resist to *G. duodenalis* would help to develop new approaches to control giardiasis. Pattern recognition receptors (PRRs), termed Toll-like receptors (TLRs) and nucleotide oligomerization domain (Nod)-like receptors (NLRs), in innate immune cells, such as macrophages, dendritic cells, could recognize the pathogen-associated molecular patterns (PAMPs) of pathogens (Franchi *et al* .,2009).

TLRs are transmembrane signaling receptors and activation of TLRs not only involves in inflammatory responses but also regulates adaptive immunity. Previous reports indicated that TLR2 involved in the initial recognition of *G.lamblia* trophozoites, influenced the production of proinflammatory cytokines IL-6, IL-12 and TNF-α in WT mouse peritoneal macrophages,

increased the parasite burden in hosts and aggravated giardiasis when comparing with the TLR2 knockout mice However, the interaction of PAMPs in G. duodenalis with the PRRs on innate immune cells has not yet been fully elucidated (Li *et al* .,2017).

A discrepancy between in vitro and in vivo effects was also found in studies of the effect of nitric oxide (NO) on *Giardia*. Initial in vitro studies showed that NO could inhibit trophozoite replication as well as differentiation into cysts. In vivo studies were then conducted using mice lacking the enzyme inducible NO synthase (NOS2), which showed no deficit in control of the infection (Allain *et al* .,2019)

The PRR family has many members, including Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors, retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), as well as several intracel- lular DNA sensors (Cao *et al*.,2016). Innate immune cells play critical roles in PRR-initiated innate inflammatory response, but the effect of nonimmune cells, such as endothelial cells (ECs), is also a force to be reckoned with The tran- scriptional upregulation of pro-inflammatory genes by PRRs triggers a cascade of inflammatory responses. Although inflammation has the beneficial effect of limit- ing cellular and organ damage, disruption in its regulation may result in a sustained inflammatory response and ultimately, local or systemic inflammation (Takeuchi and Akira,2010).

The inflammasome, a type of PRR, constitutes an essential component of the innate immunity. Abnormal activation of inflammasomes is the pathogenesis of var- ious inflammatory diseases. The inflammasome is a high-molecular-weight protein complex that acts as a cytosolic innate immune

signaling receptor that senses PAMPs or DAMPs and mediates a highly inflammatory state. The first inflammasome was discovered in 2002 following which, various inflammasomes have been identified, including NLR-family pyrin domain-containing protein (NLRP) 1, NLRP3, NLRP6 NLR-family caspase recruit- ment domain (CARD)-containing protein 4 (NLRC4), absent in melanoma 2 (AIM2), and pyrin inflamma-somes(Prochnicki and Latz.,2017).

Microvascular ECs at a site of inflammation are both active participants and regulators of inflammatory processes. During the transition from acute inflammation to chronic inflammation or from innate immunity to adaptive immunity, the characteristics of ECs change through EC activation, rapid recruitment of neutrophils, and increased vascular leakage of plasma proteins. These changes eventually lead to endothelial dysfunction.(Pober *et al*.,2007)

Interleukin (IL-1β is an important pro-inflammatory cytokine released during the endothelial inflammatory response, Interestingly activation of NLRP3 inflammasome can produce high numbers of IL-1β.(Godo and Shimokawa.,2017). Additionally, high-quality research has shown that NLRP3 inflammasome activation is correlated with multiple chronic inflammatory diseases and metabolic disorders, including obesity hypertension, diabetes, atherosclerosis, neuroinflammation, retinopathy stroke, and cancer.(Moossavi *et al*.,2018).

1.2.8.2: Adaptive immunity against *Giardia*:

B cells and antibodies Infection with *Giardia* typically results in a strong antibody response against the parasite. Incubation of trophozoites with specific antibody:*Giardia* inhibition of parasite replication as well as parasite death, depending on the particular antibodies and the presence of complement. While IgG is made in significant amounts, IgA is believed to be more important in parasite control (Mackey,2016)

IgA is transported across epithelial surfaces by the poly-Immunoglobulin receptor (pIgR) in a process called transcytosis. Thus, IgA is the most abundant isotype in intestinal secretions, and it is also the dominant isotype in mother's milk. Studies have shown that passive transfer of anti- *Giardia* antibodies in milk can help protect newborn humans, as well as neonatal mice. IgA in adults has also been shown to be protective in studies using mice lacking the pIgR As noted earlier, humans with CVID or XID are prone to developing chronic giardiasis (Inamine and Schnabl,2018).

suggesting that antibodies may be important for preventing chronic infection. Interestingly, infection of mice unable to produce any antibodies with the GS strain of *G. intestinalis* resulted in parasite elimination with kinetics identical to wild-type control mice. This experiment shows that antibody independent mechanisms can contribute to parasite elimination, but it does not mean that antibodies are unimportant in normal infections. (Davids *et al* .,2006).

Several studies have shown that T cells are essential for proper control of *Giardia* infections. Given that *Giardia* is an extracellular pathogen, it is not

surprising that CD4+ helper T cells are primarily responsible for this protective effect. One role of helper T cells is to promote antibody production and isotype switching. Other roles include cytokine production to help recruit other effector cells of the immune response. (Matowicka *et al* .,2009).

The adaptive system establishes specific response and memory towards a pathogen and is responsible for antibody production. Infection with *G. lamblia* typically results in strong antibody response against the parasite (Fink and Singer ,2017).

The parasite is also capable of evading the adaptive immune defense through antigenic variation (Prucca *et al*., 2008; Serradell *et al*., 2018). Yet, pathogenesis. remains to be fully understood. Finally, chronic infection is a significant concern in giardiasis, as it represents nutritional implications for children and immunocompromised individuals(Halliez and Buret., 2013; Bartelt and Sartor, 2015). Adaptions by protozoans for survival in the host involve sophisticated forms of host-pathogen communication (Cipriano and Hajduk, 2018). Importantly, many reports have described the release of extracellular vesicles (EVs) from pathogens to be relevant to the disease status (Cwiklinski *et al*., 2015).

1.2.8.3:Nod Like recepter p3 (NLRP3):

NLRs are intracellular innate immune receptors containing NOD1 NOD2, and NLRP3, etc. NOD1 and NOD2 mediate the activation of NF-κB signal pathway (Newton and Dixit.,2012). Other NLRs could recognize the intracellular pathogens or danger-associated molecular patterns (DAMPs), induce the assembly of the inflammasome. Inflammasomes are multiprotein complexes composed of NLR family members and/or apoptosis-associated speck-like protein (ASC). They could mediate immune response to resist or promote pathogens infection. NLRP3 inflammasome is most well-investigated for it could be widely activated by various particles, uric acid crystals, toxins, bacterials, viruses as well as parasites through caspase-1 dependent canonical pathway or caspase-11 dependent non-canonical pathway (Jia *et al* .2020).

Inflammasomes are multimeric cytosolic complexes that sense the presence of pathogens or changes in cellular homeostasis and activate commensurate innate immune responses. The inflammasome complex is defined by the responding sensor protein, which has characteristic structural domains and includes the nucleotide-binding oligomerization domain-like receptors (NLRs) NLRP1, NLRP3, and NLRC4, as well as the AIM2 and pyrin proteins (Place and Kanneganti,2018). Inflammasome assembly is initiated by NLR recognition of pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns (DAMPs) (Place and Kanneganti ,2018).

Mature protease caspase-1 then cleaves the pro-forms of inflammatory cytokines IL-1β and IL-18 to generate the mature bioactive forms capable of

binding their cognate receptors and initiating cell signaling (Afonina *et al*., 2015).

NRLP3 inflammasome activation requires two steps. The first step known as the priming step, is induced by PRRs or TNFR activation. This leads to the activation of NF-kB and promotes the expression of NLRP3 pro-IL-1b, and pro-IL-18. Additionally, IFNAR also activates the priming stage of NLRP3 inflammasome activation (Swanson *et al*.,2019).

The second step, also called the activation step, is triggered by a range of stimuli that emerge during infections, tissue damage, or metabolic imbalances. Such stimuli include ATP, pore-forming toxins, crystalline substances, nucleic acids, and invading pathogens (Lamkanfi, Dixit .,2014). NLRP3 recruits ASC through its N-terminal pyrin domain (PYD) by homophilic interactions, resulting in the formation of ASC prion-like oligomerizes (Lu *et al* .,2014).

As an effect factor, caspase-1 mediates the proteolytic processing of pro IL-1β, pro- IL-18, and the propyroptotic factor gasdermin D (GSDMD) (Shi *et al*.,2015). GSDMD forms pores in the membrane of infected cells facilitating the secretion of IL-1β/IL-18 and inducing the inflammation associated cell death known as pyroptosis (He *et al*.,2015). The secretion of IL-1β subsequently recruits neutrophils to the inflammatory site to aid in the elimination of invading viruses (Niu *et al*.,2019). Moreover, both IL-1β and IL-18 are responsible for the subsequent induction of the adaptive immune response (Joosten *et al*.,2013).

Activation of the NLRP3 inflammasome requires two signals. The first signal is the stimuli triggered NF-κB signal pathway activation leading to the up-regulation of NLRP3, pro-IL-1β and pro-IL-18 mRNA level .The second

signal is the stimuli mediated NLRP3 oligomerization with ASC and pro-caspase-1 or pro-caspase-11/4, the activated caspase-1 or caspase-11/4 would cleave pro-IL1β/pro-IL-18 into the mature IL-1β/IL-18 (Sutterwala *et al*.,2014).

A newly research found that *G.lamblia* could promote the production of antimicrobial peptides and attenuate disease severity induced by attaching and effacing enteropathogens via the induction of the NLRP3 inflammasome. However, the unequivocal role of NLRP3 inflammasome in the *G. duodenalis* triggered host innate immunity are poorly understood. (Manko *et al*.,2020).

As an extracellular pathogen, limited research reports the immune mechanisms mediated by the intracellular NLRs in *G.lamblia*. Communications between cells is mediated by biological molecules including proteins, lipids, and nucleic acids, which are widely existed in extracellular vesicles (EVs) and pyrin domain (PYD)-containing protein 3 (NLRP3) inflammasome has been studied extensively and was found to be activated by a wide spectrum of stimuli. It is generally accepted that NLRP3 inflammasome activation is regulated through a two-step process with priming at the transcriptional and posttranslational levels (Signal 1) and assembly by multiple pathways in response to a variety of exogenous pathogen-derived or endogenous danger molecules(Signal2). (Cronemberger *et al* .,2014 ,, Douanne *et al* ., 2020).

Recently, there has been a renaissance in our understanding of the posttranslational modification (PTM) and protein-protein interactions of NLRP3 inflammasome components that license cells for full activation of inflammasome assembly.7–10 The breadth of our current understanding

extends to the regulation of the priming that is involved in NLRP3 inflammasome complex assembly including accumulating evidence indicating a number of molecular mechanisms underlying the positive or negative regulation of NLRP3 inflammasome activation. Indeed, inflammasome and IL-1β activity are important for host defense against numerous bacterial, viral, and fungal infections. However, excessive or altered regulation of NLRP3 inflammasome activity is related to the pathogenesis of a wide variety of inflammatory, autoimmune, and degenerative diseases.

Pyroptosis, a form of programmed cell death mediated by inflammatory caspases, involves both tissue homeostasis and an immune response (Yuan *et al*.,2018) Macrophage pyroptosis participates in direct parasite killing inside the host cells by causing inflammation (de Carvalho *et al*.,2020) Gasdermin D (GSDMD), a common executor of pyroptosis, can be cleaved by inflammasome-activated caspase (CASP)-1 into the N- and C-termini N-GSDMD then oligomerizes and forms pores in the plasma membrane and increases membrane permeability, leading to pyroptosis and IL-1β and IL-18 release (He *et al*.2015) .

PAMPs can also be recognized by membrane-associated innate immune sensors, such as toll-like receptors (TLRs) 2 and 4 (Vijay .,2018) . An elevated inflammatory response mediated by TLR2/MAPK signaling plays a role in giardiasis severity (Li *et al*.,2017) TLR4 is a vital mediator of the proinflammatory response via regulating multiple signaling pathways including NLRP3 inflammasome signaling (Chen *et al*.,2019). The VSPs of Giardia were reported to activate TLR2 and TLR4 in HEK293 cells (Serradell *et al*.,2019) . The latest research has shown that *Giardia* extracellular vesicles

(EVs) activate NLRP3 inflammasome signaling via TLR2 (Zhao *et al*.,2021). Growing evidence shows that activation of the NLRP3 inflammasome involves its deubiquitination or phosphorylation (Song *et al*.,2017). It is, therefore, worthwhile to examine the correlation between noninvasive *Giardia* infection, TLR-mediated recognition of parasite PAMPs, NLRP3 deubiquitination, and the activation and regulation of inflammasomes and pyroptosis.

The potential function of these proteins in *Giardia*–host cell interactions needs further in-depth investigation. To date, there has been no report on the involvement of macrophage pyroptosis in Giardia infection. Here we provide the first evidence that *G. lamblia* and its secreted PPIB can induce macrophage pyroptosis, and the Activation of the NLRP3 inflammasome requires two signals. The first signal is the stimuli triggered NF-κB signal pathway activation leading to the up-regulation of NLRP3 pro-IL-1β and pro-IL-18 mRNA level (Sutterwala *et al*.,2014) The second signal is the stimuli mediated NLRP3 oligomerization with ASC and pro-caspase-1 or pro-caspase-11/4, the activated caspase-1 or caspase-11/4 would cleave pro-IL1β/pro-IL-18 into the mature IL-1β/IL-18 .A newly research found that *G. duodenalis* could promote the production of antimicrobial peptides and attenuate disease severity induced by attaching and effacing enteropathogens via the induction of the NLRP3 inflammasome(Manko *et al*.,2020) . However, the unequivocal role of NLRP3 inflammasome in the *G. lamblia* triggered host innate immunity are poorly understood.

As an extracellular pathogen, limited research reports the immune mechanisms mediated by the intracellular NLRs in *G.lamblia*. Communications between cells is mediated by biological molecules including

proteins, lipids, and n ucleic acids, which are widely existed in extracellular vesicles (EVs) (Cronemberger *et al* .,2014; Douanne *et al* .,2020).

1.2.8.4: Nuclear factor kappa- p65 (NF-κB)

NF-κB is considered to be a critical molecule in inflammation due to its regulates proinflammatory transcriptional processes that coordinate and execute an inflammatory response. Recent evidence also indicates that NF-κB and the signaling pathways associated in its activation are essential for the development of a tumor (Khiong *et al*., 2010). This theory is based on a wide body of circumstantial evidence such as the excellently-established capacity of NF-KB to regulate the generation of essential proinflammatory cytokines and enzymes, including IL1, IL6, TNF-a, and COX-2(Karin., 2009).NF-KB also influences the expression of several genes whose products can suppress the death of tumor cells, promote epithelial to mesenchymal transfer, which plays a significant role in tumor invasiveness, and induce the progression of the tumor cell cycle(Karin and Lin., 2002; Khiong *et al*., 2010).

Nuclear factor-Kappa B is a type of transcription factors that found in every cell's cytoplasm and when activated; translocate to the nucleus of cells. its activation by a broad range of factors such as cigarette smoke, stress, parasites, viruses, bacteria, free radicals, carcinogens, cytokines, and endotoxins. Upon activation, NF-κB controls and regulates the expression of approximately 400 various genes enzymes(iNOS and COX-2) cytokines (IL-1, IL-6, IL-8, TNF, and chemokines), cell cycle regulatory molecules, and adhesion molecule. NF-κB has been associated with a wide range of human illnesses including atherosclerosis, asthma, diabetes, and cancer. NF-κB and signaling pathways that activate it; are essential coordinators of innate and

adaptive immune responses.in more recent times, it has become apparent that NF-KB signaling already plays a vital role in the development and progression of cancer (Serasanambati and Chilakapati,2016) Nuclear Factor –Kappa B(NF-κB)

NF-KB is a transcription factor that functions as a major switch for some immune and inflammatory. In several ways, NF-κB changes cell behavior, it inhibits apoptosis; increases immune and inflammatory responses, increases cell proliferation(Karin and Lin, 2002).

TNF is one of the most important cytokines that can be synthesized and secreted by several other types of cells; including eosinophils neutrophils, and mast cells, the primary source of Tumer necrosis factor is activated macrophages. TNF has a pleiotropic influence on cells of mammalian; it promote apoptosis or ensure its survival by induced their proliferation and NF-κB (Ankri, 2015).

One of the defense mechanisms of the infected cell to defeat the invasion pathogen is the activation of apoptosis; a mechanism of cellular self-devastation that performs essential roles naturally through immune selection, development and regeneration of tissue (Molestina *et al*., 2003).

Giardia secreted giardipain-1 is a likely contributor to the barrier damage and IEC apoptosis (Ortega *et al*.,2018). Three *Giardia* proteases instigate the macrophage-mediated inflammatory response via cleavage of the NF-κB p65 subunit (Faria *et al*.,2020).Via secretome analysis, several proteins were found to be highly expressed in the culture supernatant of *G. lamblia* assemblage

A,such as pyridoxamine 5-phosphate oxidase (PNPO) peptidyl-prolyl cis-trans isomerase B (PPIB), and three tenascins.

1.2.8.5: Interleukin 1 beta (IL-1β)

IL-1β is mainly produced by innate immune cells (such as monocytes DCs, and macrophages), and the major source of IL-1β in colon is macrophages located in the lamina propria (Zhu *et al* .,2017) During infection, mucosal injury and stress, the activation of IL-1β can trigger local mucosal immune responses, by stimulating T cell proliferation, and direct neutrophils to injury or infection site through the combination of IL-1β and IL- 1R complexes (Lang *et al*.,2018) and further activate NF-κB and MAPK pathways, leading to the upregulation of other pro-inflammatory cytokines and chemokines (such as IL-6, IL-8, and TNF). Meanwhile, IL-1β can upregulate IL-2 receptor expression, prolonging survival of T cells, and enhance antibody production by B cell proliferation. Early reports showed an overproduction of IL-1β in patients with IBD and mice models indicating that the function of IL-1β in the development of mucosal inflammation (Cominelli *et al*.,2005). However, recently, many researches in the chemical-induced model have reported that IL-1β can protect mice from intestinal infection of Citrobacter rodentium and Clostridium difficile, by promoting phagocytosis and eradication of bacteria in mononuclear Phagocytes (Alipour *et al*.,2013). Besides, a study by (Fan *et al*.,2015) also showed that the transplantation of mesenchymal stem cells (MSC) primed by IL-1β could alleviate the chemical-induced colitis. Taken together, the rather ambiguous results concerning the role of IL-1β in mucosal immune response and IBD demand a further investigation with careful consideration.

1.2.8.6: Interleukin 18 (IL-18)

IL-18 is a multifunctional cytokine, which is mainly expressed in the gut epithelium in both mice and Humans (Zhu *et al*.,2017), recent findings suggested that the epithelium IL-18 secretion was not dependent on NLRP3 (Song *et al*.,2017), but on caspase-1 (Yao *et al*.,2017).

However, we know NLRP3 plays a central role in the activation of caspase-1, and then NLRP3 may also contribute to IL-18 production in intestine. IL-18 is functionally found to induce interferon (IFN)- γ and promote Th1 response (Bazan *et al*.,2012). but these studies failed to tell whether the increased IL-18 level in patients was a consequence or causing factor for IBD. Later, polymorphisms in IL-18 genomic locus were showed to be a risk factor for IBD (Aizawa *et al*.,2005). Recently, a series of studies have pointed out that IL-18 can provide protection against colitis and/or colitis associated cancer (Oficjalska *et al*.,2015), and IL-18 deficiency may predispose the host to chemically induced colitis. Moreover, IL-18 can induce Th1 cells and NK cell to secrete IFN-γ, which can regulate a proliferation and repairment response in the intestinal tract when the epithelium is injured. And recent studies have also shown that the signals of IFN-γ and downstream STAT-1 were decreased in mice deficient in NLRP3, which were dependent on IL-18 (Zaki *et al*.,2010). These data indicate that IL- 18 may be involved in repair of the epithelial layer of the gut by maintaining proper levels of epithelial cell proliferation during acute experimental colitis. Further, IL-18 also has the function of immunomodulation, for instance, it can enhance proliferation of Th1 cells and host defense against pathogens, inhibit IgE production, and has antitumor effects.

The IL-1β and IL-18 are crucial mediators of inflammation, and a defective control of their release may cause serious diseases. Yet, the mechanisms regulating IL-1β and IL-18 secretion are partially undefined. Both cytokines are produced as inactive cytoplasmic precursors. Processing to the active form is mediated by caspase-1, which is in turn activated by the multiprotein complex inflammasome(Chakraborty *et al*.,2010). Unlike IL-1β, IL-18 is constitutively produced by monocytes but, like IL-1β, requires cleavage by caspase-1 to be secreted in its active form(Boraschi and Dinarello,2006).

However, whether IL-18 processing and secretion are regulated by the same mechanisms that control IL-1β is unclear, Previous studies have reported that interleukin (IL)-1β is released during invasion, which involves sequential disruption of the colonic epithelial barrier leading to direct contact of ameba with the underlying lamina propria, where resident and recruited myeloid cells are present that are the major producers of IL-1β.(Martinon *et al*., 2009).

Inflammasomes are cytosolic sensors of pathogens and various cellular stresses that upon activation form high molecular weight complexes that mediate recruitment and activation of caspase-1. Active caspase-1 in turn cleaves the precursors of IL-1β and into bioactive fragments and mediates their release along with several other leaderless proteins via an undefined casaspe-1-dependent secretion event. *G.lambilia* induces inflammasome activation through a contact-dependent event involving binding of surface associated Gal-lectin to the macrophage membrane, leading to release of IL-1β, *G.lambilia* induces inflammasome activation through a contact dependent event involving binding of surface-associated Gallectin.the macrophage membrane, leading to release of IL-1b (Kissoon *et al*.,2013).

1.2.9.Laboratory Diagnosis:

Giardiasis is diagnosed in the laboratory by detection of parasite in specimens or immunologic techniques(Singh *et al*.,2009). There are multiple ways to evaluate patients with suspected Giardiasis, including microscopy, antigen detection,serology, endoscopic procedures, and imaging modalities.

1.2.9.1.Microscopy:

Giardiasis has been diagnosed based on the demonstration of cyst and/or trophozoite stages of *G. lamblia*. Microscopic examination of stool/ pus specimen in saline wet mount is a less sensitive technique (sensitivity < 10%) even when viewed by an expert microscopist and needs to be examined within a short period of collection time (usually within half an hour) for motile trophozoites (Parija *et al*.,2014).

Microscopic techniques employed in a diagnostic clinical laboratory include wet preparation, concentration, and permanently stained smears for the identification of *G.lamblia* in feces. Microscopic examination of direct saline (wet) mount is a very insensitive method (10%) which is performed on a fresh specimen.to further improve the sensitivity of the microscopic examination, it is indicated to examine two or three stool specimens on different days, or to do microscopic examination of the second motion after a saline purge. However, microscopy cannot distinguish the pathogenic from the non- pathogenic strains of *Giardia*. (El-Dib, 2017).

1.2.9.2.Culture:

Culture is a method of diagnosis, however it is not easy to perform and less sensitive than microscopic examination with a success rate of 50–70%(Fotedar *et al.*,2007).

Culture media include xenic (diphasic and monophasic) and axenic systems. Xenic cultivation is defined as the growth of the parasite in the presence of an undefined flora(Clark and Diamond,2002).

1.2.9.3.Antigen Detection:

Detection of *G.lamblia* by ELISA is a sensitive and specific test that depends on the use of monoclonal antibodies kits(Den *et al*.,2013).

Some very important tests are the immune chromatographic assays. They are practical and easy to perform and could be applied in the laboratories with limited facilities or in the field. A test can detect *G. lamblia* separately or may combine three intestinal pathogens *G.lamblia*, and Cryptosporidium) in one step to be detected in fecal samples .It has the disadvantage of inability to differentiate between pathogenic and non-pathogenic strains. Unfortunately, though these tests are rapid and easy to perform, they require fresh, not fixative-preserved, stool for analysis. (Shirley *et al*., 2020).

1.2.9.4.Antibody Detection:

Cases with pathogenic intestinal, showing symptoms, possess antibodies against E. histolytica in their sera (Fotedar *et al*.,2007). These antibodies appear in blood after 1 week and stay for years after acute infection that is why detection of antibodies does not differentiate between new and past infection.

However, in areas where infection is endemic and people have been exposed to *G. lamblia*, the inability of serological tests to distinguish past from current infection makes a definitive diagnosis difficult (Stanley,2006).

Serum IgG antibodies persist for years after *G. lamblia* infection, whereas the presence of IgM antibodies is short lived and can be detected during the present or current infection. An ELISA for detection of serum IgM antibodies to amebic adherence lectin was successfully used with patients suffering from acute colitis for less than 1 week, as 45% had detectable anti lectin IgM antibodies, The recommended serological tests such as ELISA, those that demonstrate the presence of serum anti-lectin antibodies are the most frequently used for diagnosis of patients (Misra and Srivastava, 2020).

Chapter two

Materials and Methods

2.1:Materials:

2.1.1:Instruments and Equipment's:

The Instruments and Equipment's which used in the present study show in the table (2-1).

Table(2-1): instrument and equipment's .

Instrument and Equipment	Company/Origin
Centrifuge	Fison (England).
Compound light microscope	Olympus(Japan).
Cooling box	Shanghai Blopak Co. Limited, (China)
Deep freezer	Concord(Italy).
Disposible Loop	Hi media(India).
ELISA reader	Diatek(Indonesia).
Light microscope	Olympus(Japan).
Micropipette and Tips	Slamed(Germany).

2.1.2: Disposable and Chemical Materials:

The disposable and Chemical Materials which used in the present study show in table(2-2) .

Table (2-2): The disposable and Chemical Materials.

Material	Company/Origin
Clean container	Sail brand(China).
Cotton & Gauze	Medical ject, (Syria)
Disposable syringes	Abu Dhabi Medical Devices Co. L.L.C(USA).
Eppendorf tube	Biobasic (Canada).
Ethanol(80%).	CHROM agar(France).
Ethyl alcohol absolute(99%)	Local market-Iraq
Gel tube	AFCO(Jordan).
Glass slides	Sail brand(China).
Lugol's iodine	BDH-England
Medical gloves	Broche(PRC).
Microscopic cover slide	Gitoglas (China).
Normal Saline	Pharmaline(Egypt).
Plane tube	AFCO(Jordan).
Sterile swabs	Lab .service(S.P.A).
Wooden sticks	Supreme (China).

2.1.3 :Kits:

The kits which used in the present study show in table(2-3).

Table(2-3): The ELISA kit:

Kits	Company/Origin
Human NFKB-p65(Nuclear factor NF-kappa-B p65 subunit) ELISA Kit	Bioassay technology laboratory (Usa).
Human IL-18(Interleukin 18) ELISA Kit	Bioassay technology laboratory (Usa).
Human IL-1β(Interleukin 1 Beta) ELISA Kit	Bioassay technology laboratory (Usa).
Human NLRP3(NACHT, LRR and PYD domains-containing protein 3) ELISA Kit	Bioassay technology laboratory (Usa).

2.1.4:Ethical approval :

The necessary ethical approval was obtained by verbal consent from patients. This study was approved by the committee of publication ethics at college of medicine , Babylon university ,Iraq.

2.2:Methods:

2.2.1:Samples:

2.2.1.1:stool samples:

Stool specimens were collected from patients with diarrhea during period of September 2022 to January 2023, from patient who attended into four hospitals in Hilla city (Imam Al_Sadiq Hospital, Babylon Hospital for Maternity and Children , Hospital Al_Noor for Children, and Ali Obeiss Hospital in Al Midhatiya).

One handred fifty stool specimens were collected from patients between (1-30) years. All collected samples were separately labeled with stickers having date, name of sample, name of collecting area. Stool samples were collected using a wide opened stool container and transported to the laboratory . The specimens were transported to the laboratory within 1h of passing of the stool, since *Giardia* trophozoites die and become unrecognizable after longer periods of time. Precautions were taken to prevent the samples from being contaminated with urine or dirt particles(Samie *et al*., 2020).

2.2.1.2:Examination of stool samples:

The examination were done with direct smear method by taking a drop of Lugol's iodin solution and put on one end of the glass slide, and a drop of normal saline put on the other end, took a small amount of stool trace the head of stick and mixed well the smears were covered with cover slides and examined under the microscope using 40× objective lens to detecting protozoan trophozoites and cysts (Lee *et al*., 2009).

2.2.1.3:Blood Samples Collection:

Blood samples were collected about (5ml) by disposable syringe from each patient diagnosed with *Giardia lamblia* .the blood was put in sterilize tube free from coagulant (Gel tubes) ,the blood was left in room temperature allow serum to clot for 10-20 minutes. centrifuge at 2000-3000 rpm for 20 minutes to obtain serum for immunological tests (ELISA). The serum was put in sterile tube (Eppendorf tube), then frozen in -20C^0 until required.

2.2.1.4: The Steps of the Present Study's Design

The study steps of the present work are illustrated in Figure (2-1).

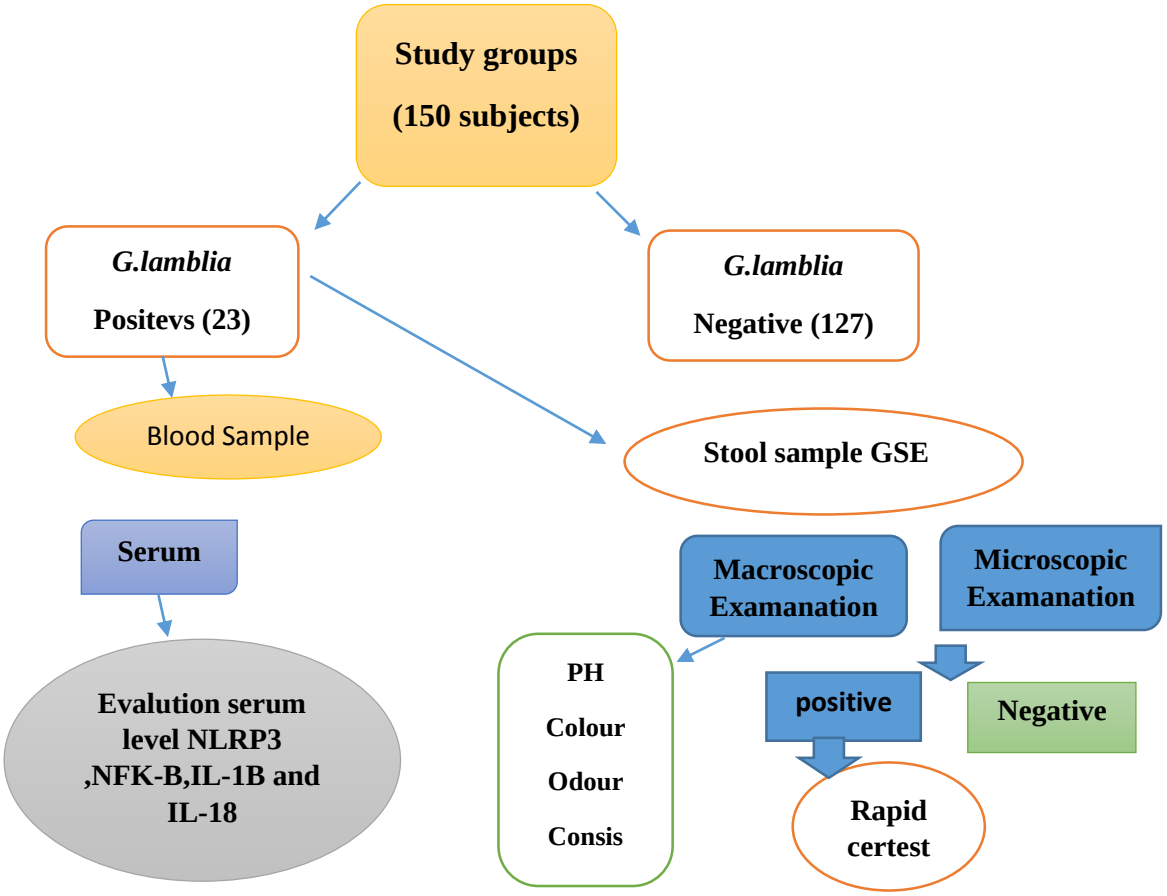


Figure (2-1).The steps of the present studys Design

2.2.3. Diagnostic Study Tests

2.2.3.1.CERTESTCrypto+Giardia+EntamoebaCOMBO CARD TEST, Zaragoza(Spain) Immuno Chromatography (IC).

The Triage parasite panel is a new qualitative enzyme immunoassay (EIA) panel for the detection of *Giardia lamblia* (α1-giardin), in fresh or frozen, unfixed human fecal specimens. to examine the parasite, a triage immunochromatography assay was used to identify the three types of parasite.

A. Assay Principle :

This technique is called A lateral flow assay (LFA) and is composed of a chromatographic system (separation of components of a mixture based on differences in their movement through reaction membrane) and immunochemical reaction (between antibody-antigen It is based on the movement of the sample across the membrane via capillary force.is composed of four parts: a sample pad, which is the area on which the sample is dropped; a conjugate pad, on which labeled tags are combined with biorecognition elements; a reaction membrane (usually nitrocellulose membrane pre-coated with monoclonal antibodies on the test line T) containing test line and control line for target antigen-antibody interaction; and absorbent pad, which reserves waste (Singh *et al*., 2015, Hu *et al*., 2014). For the construction of LFAs gold nanoparticles, colored latex beads, carbon nanoparticles, quantum dots, and enzymes are used as a label for increasing the sensitivity.(Bahadır and Sezgintürk, 2016؛ Mao *et al*., 2013).

B. Specimen preparation :

• Use the stick to pick up a sufficient sample quantity.

• Add into stool collection tube (for liquid samples, add 125 µL in the stool collection tube using a micropipette).

• Close the tube with the diluent and stool sample. Shake the tube to assure good sample dispersion

C. Test Procedure:

1-Proceed to shake the stool collection tube to assure good sample dispersion.

2- Remove the sealed bag before using it.

3- Take the stool collection tube, cut the end of the cap, and pour three drops into the circular window with the letter A, three drops into the window with the letter B, and three drops into the window with the letter C. Avoid mixing solids with the liquid.

4-Read the result for 10 minutes.

D. Interpretation of the results:

Strip A consist of a nitrocellulose membrane pre-coated with monoclonal antibodies against Cryptosporidium.

Strip B consists of a nitrocellulose membrane pre-coated with monoclonal antibodies against Giardia.

Strip C consists of a nitrocellulose membrane pre-coated with monoclonal antibodies against Entamoeba .

2.2.4:Immunological Examination:

2.2.4.1:HumanNLRP3(NACHT, LRR and PYD domains-containing protein 3) ELISA Kit:

A.Principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human NLRP3. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human NLRP3 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human NLRP3, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human NLRP3. You can calculate the concentration of Human NLRP3 in the samples by comparing the OD of the samples to the standard curve.

B.Reagent:

Table (2-4) Components of Human NLRP3 Reagent

Components
Micro ELISA Plate (Dismountable)
Reference Standard
Concentrated Biotinylated Detection Ab (100×)
Concentrated HRP Conjugate (100×)
Reference Standard & Sample Diluent
Biotinylated Detection Ab Diluent
HRP Conjugate Diluent
Concentrated Wash Buffer (25×)
Substrate Reagent
Stop Solution
Plate Sealer
Product Description

C.Assay Procedure:

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 uL for each well). Add the samples to the other wells (100 uL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Remove the liquid out of each well, do not wash. Immediately add 100 μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.

3. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.

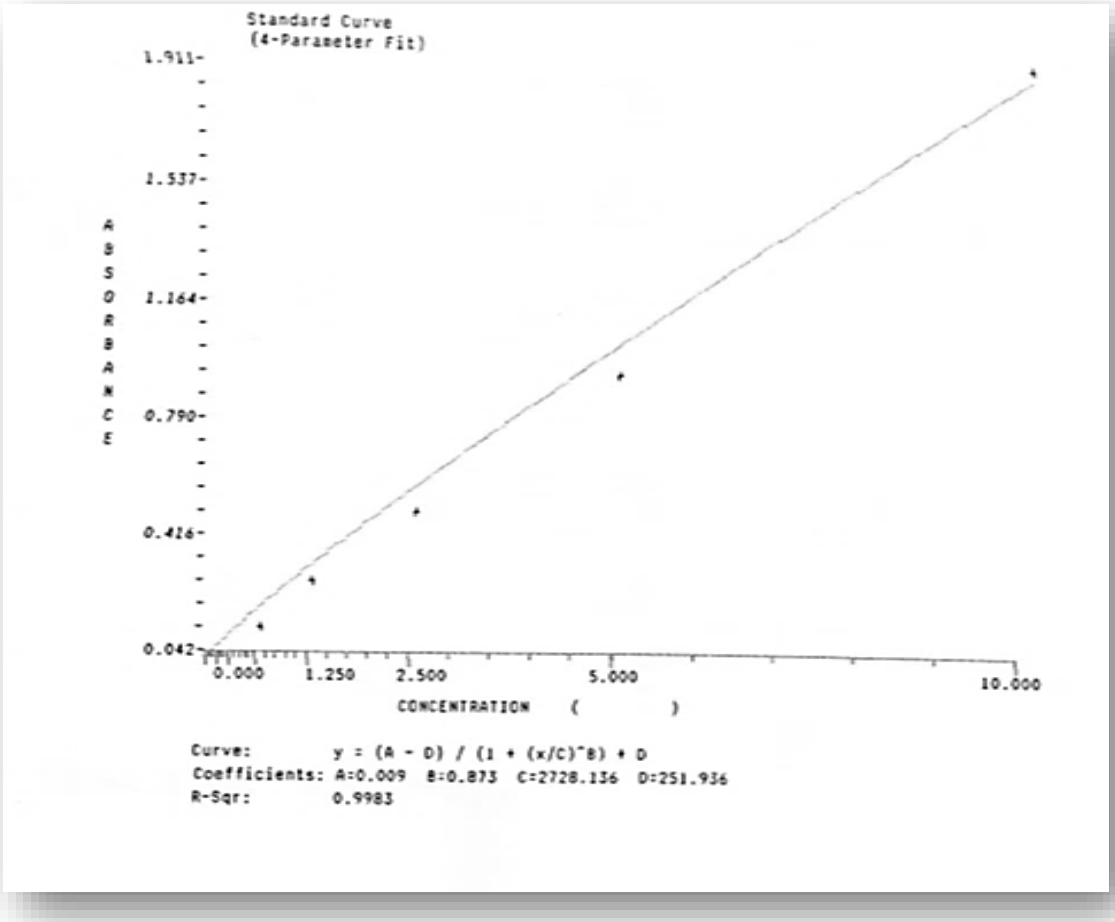
4. Add 100 μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.

6. Add 90 μL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.

7. Add 50 μL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.



Figure(2-2) :Standard curve of NLRP3.

2.2.4.2: Human NFKB-p65(Nuclear factor NF-kappa-B p65 subunit) ELISA Kit:

A.Principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human NFKB-p65. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human NFKB-p65 and Avidin- Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human NFKB-p65, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human NFKB-p65. You can calculate the concentration of Human NFKB-p65 in the samples by comparing the OD of the samples to the standard curve.

B.Reagent:

Table (2-5) Components of Human NFKB Reagent

Components
Micro ELISA Plate (Dismountable)
Reference Standard
Concentrated Biotinylated Detection Ab (100×)
Concentrated HRP Conjugate (100×)
Reference Standard & Sample Diluent
Biotinylated Detection Ab Diluent
HRP Conjugate Diluent
Concentrated Wash Buffer (25×)
Substrate Reagent
Stop Solution
Plate Sealer
Product Description
Certificate of Analysis

C.Assay Procedure:

1. Determine wells for diluted standard, blank and sample. Add 100 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Decant the liquid from each well, do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

3. Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

4. Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.

6. Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.

7. Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to450nm.

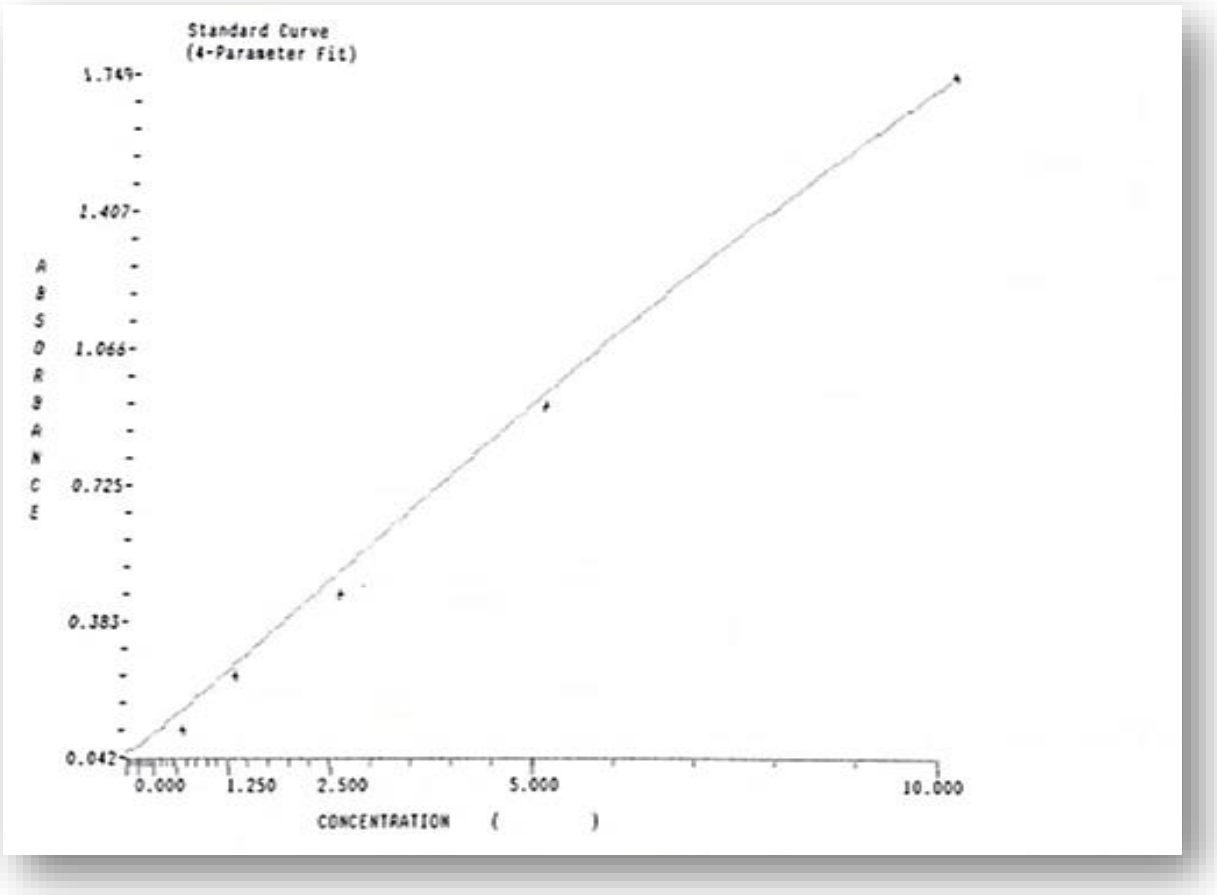


Figure (2-3):Standard curve of NF-KB.

2.2.4.3:Human Interleukin 1 Beta (IL-1β):

A:Principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-1β. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-1β and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-1β biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL-1β. You can calculate the concentration of Human IL-1β in the samples by comparing the OD of the samples to the standard curve.

B.Reagent:

Table (2-6) Components of Human (IL-1β) Reagent

Components
Micro ELISA Plate (Dismountable)
Reference Standard
Concentrated Biotinylated Detection Ab (100×)
Concentrated HRP Conjugate (100×)
Reference Standard & Sample Diluent
Biotinylated Detection Ab Diluent
HRP Conjugate Diluent
Concentrated Wash Buffer (25×)
Substrate Reagent
Stop Solution
Plate Sealer
Product Description
Certificate of Analysis

A:Assay Procedure:

1. Determine wells for diluted standard, blank and sample. Add 100 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note:

solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Decant the liquid from each well, do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

3. Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

4. Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

5.Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.

6. Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more

than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.

7.Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

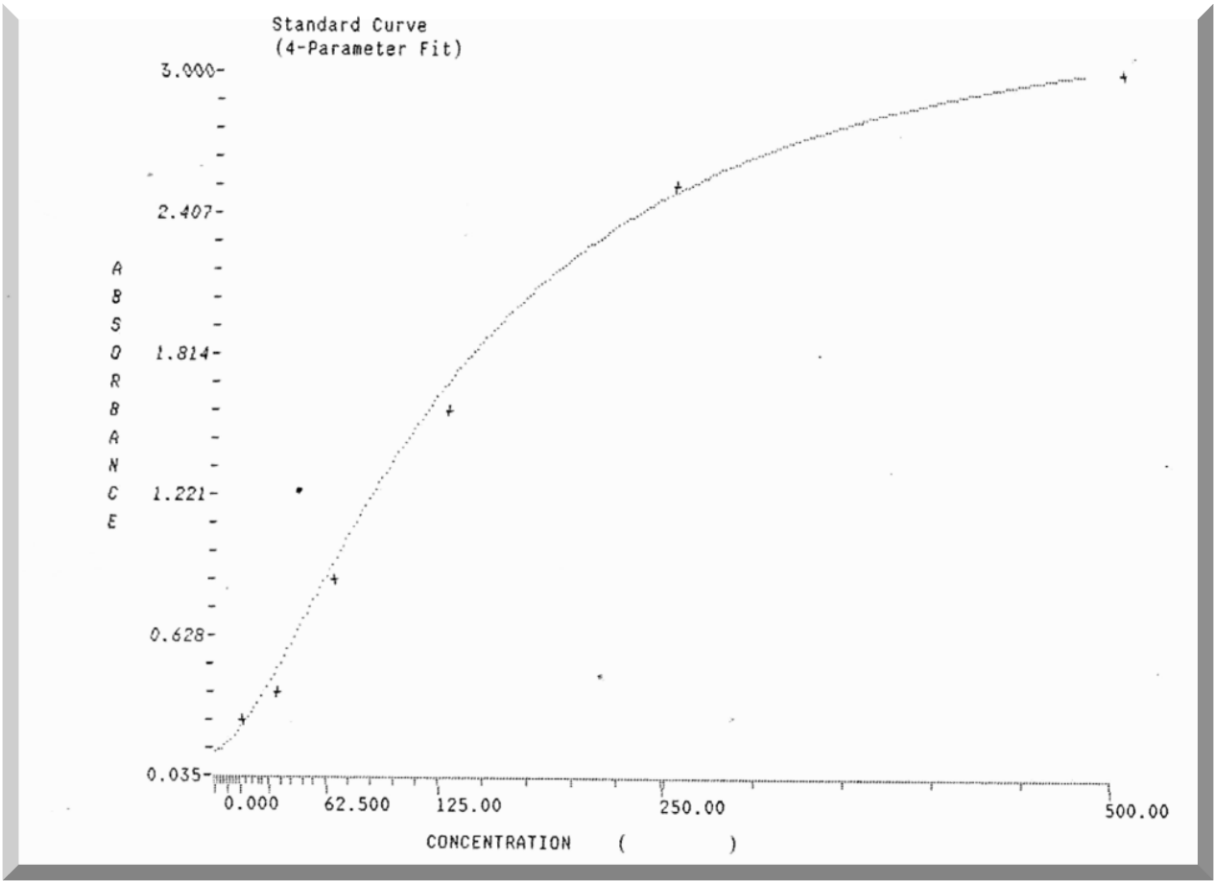


Figure (2-4):Standard curve of IL-1β.

2.2.4.4:Human IL-18(Interleukin 18) ELISA Kit:

A.Principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-18. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-18 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-18, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL-18. You can calculate the concentration of Human IL-18 in the samples by comparing the OD of the samples to the standard curve.

B.Reagent:

Table (2-7) Components of Human (IL-18) Reagent

Components
Micro ELISA Plate (Dismountable)
Reference Standard
Concentrated Biotinylated Detection Ab (100×)
Concentrated HRP Conjugate (100×)
Reference Standard & Sample Diluent
Biotinylated Detection Ab Diluent
HRP Conjugate Diluent
Concentrated Wash Buffer (25×)
Substrate Reagent
Stop Solution
Plate Sealer
Product Description
Certificate of Analysis

C.Assay Procedure:

1. Determine wells for diluted standard, blank and sample. Add 100 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Decant the liquid from each well, do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

3. Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

4. Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.

6. Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more

than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.

7. Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to450nm .

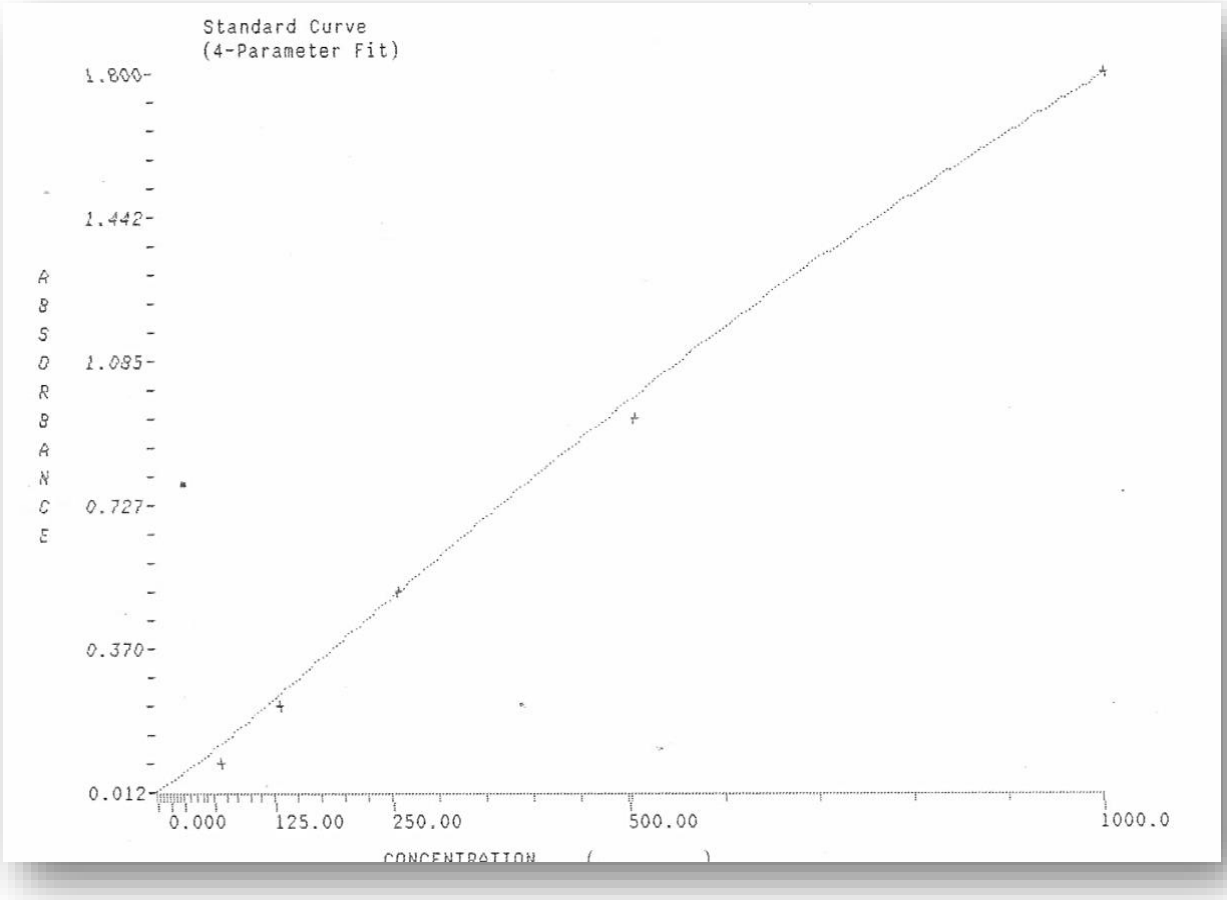


Figure (2-5): Standard curve of IL-18

2.2.5.Statistical Analysis:

Statistical analysis was carried out using SPSS version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means and standard deviations. Student t-test was used to compare means between two groups. Pearson correlation coefficient was used to assess relationship between two continuous variables. P-value of ≤ 0.05 was considered as significant.

Chapter three

Results
And
Discussion

3.1. Results and Discussion

3.1.1 Detection of *G. lamblia* infection

This study include 150 stool specimens were collected from patient with diarrhea attended to hospitals in Hilla city (Babylon Hospital for Maternity and Children , Hospital Al_Noor for Children, and Ali Obeiss Hospital in Al Midhatiya) the results of this study showed that out of 150 sample , only 23 was positive for *Giardia lamblia* and the total percentage of infection with positive *G.lamblia* was 23(15.3%) as show in figure (3-1) .

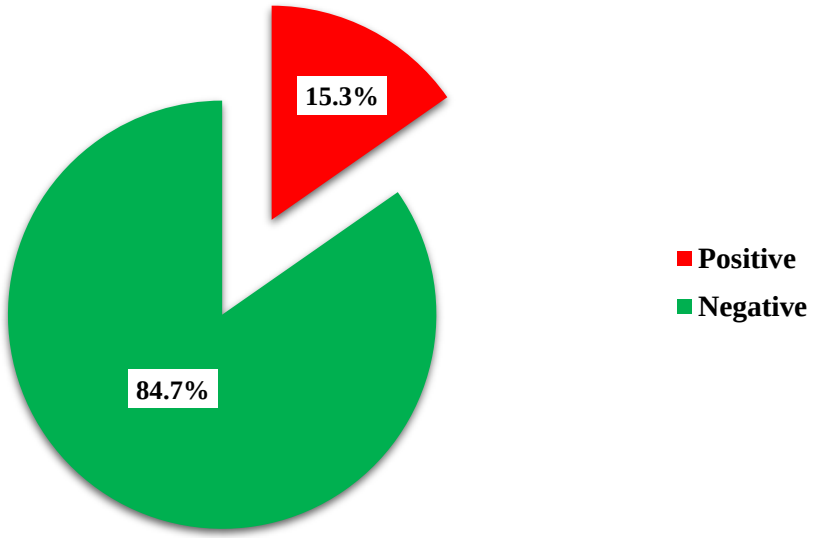


Figure (3-1): Distribution of total samples examined according *Giardia lamblia* infection (N=150).

Giardia lamblia infected people at all ages, while it is more common in children. The increased incidence in children suggests that adults have acquired some level of infection resistance **(**Al-Yousofi *et., al* 2022).

The results of the present study was agree with another studies who Showed positive and negative Percentage of *Giardia* infection. the presence of *Giardia lamblia* in Al-Nahda city with (13.33%) done by AL-Khalidy and Jabbar(2020).Also Haque *et al* .(2006) confirm that 20% of diarrhea cases associated with *G.lamblia.* study of khudair (2010) show the prevalence of *G.lamblia* infection in diarrheic children.

3.1.2:distribution of *G.lamblia*

3.1.2.1:distributionof *G.lamblia* accourding to the age:

The distribution of *G.lamblia* according to age group show there were high percentage of infection with *G.lamblia* was 9(39.1%) in 1-5 years old , while the lowest percentage of infection with Giardsais reported in (11-15 and 16-20)years old ,it was 2(8.7),2(8.7) respectively as show table(3-1).

Table (3-1): Distribution of total patients examined and total patients infected according to age .

Age (years)	Samples Examined(%)	Samples infected(%)
1-5	29 (19.3)	9 (39.1)
6-10	29 (19.3)	5 (21.8)
11-15	24 (16.0)	2 (8.7)
16-20	17 (11.4)	2 (8.7)
21-30	51 (34.0)	5 (21.7)
Total	150 (100.0)	23 (100.0)

The high prevalence rate was obtained in age groups 1-5 years, this is maybe due to they have very active playing habits in the soil, which harbors this parasite and are less mindful of some very important personal hygiene practices such as the washing of hands with soap and water before eating, after playing in soil and after toilets use (Al-taie and Ali,2009).

In addition, another source of infection is the in leafy vegetables such as Leek, Celery, Cress and Green onion that sold in Buying a lot of food from street vendors some of whom do not have a proper personal hygiene practice, may be regarded as carriers of the infective parasites (Takaoka *et al* .,2016). The age range of 1-5 years was shown to have the highest prevalence of giardiasis in the current study, This might be explained by these children's increased activity, which includes spending more time outside the house and at playgrounds than younger children, as well as a lack of personal cleanliness compared to older children. Haile *et al.* (2017) which found that Early children were found to be at the greatest danger, whereas older children and adults were shown to be at a lower risk.

Also other study agree with current results mentioned that the infection rate was higher in (1-5) years and lower in (11 -15and 16-20) years (Latif *et al*,2020).as well as another study that showed the most parasitic infections were in the age groups (6-10) years, and the number of parasitic infections decreased in the Elderly as done by Alatbee *et al.* (2020).

3.1.2.2: distribution of *G.lamblia* according to the sex.

The Distribution of *G.lamblia* accourding to six show there were high percentage of infaction was in female12(52.2)than male 11(47.8)as show in table(3-2).

Table (3-2): Distribution of total patients examined and total patients infected according to sex.

Sex	Samples Examined(%)	Samples infected(%)
Male	67 (44.7)	11 (47.8)
Female	83 (55.3)	12 (52.2)
Total	150 (100.0)	23 (100.0)

This result was in agreement with other studies by Amer *et al* (2018),also in agreement with results of Bazzaz *et al.* (2017) ,which founded that female is most frequent infection with *G. lamblia* , in which the prevalence of intestinal parasitic was higher in females than males, the higher infection in females may be justified by taking into account that females are more susceptible to the infective stages of parasitic infection due to the nature of the job they perform at home and their lifestyle .

The results was inagreement with Mohammed *et al*. (2019) who reported a higher prevalence in males than in females for a parasitic intestinal infection found in Nigeria. Occupational status, low education levels, inadequate water supply are among significant risk factors for these infections . This might be due to the female gender as a risk factor for giardiasis could be related to the women's role as caretakers of children and direct contact with infected children and

changing diapers. Nursing has been identified as a risk factor for giardiasis in the communities where the infection rate is high in children.

These results agree with finding in Brazil, who recording infection with *G. lamblia* in females more than males (New man *et al*.,2001).

3.1.2.3: distribution of *G.lamblia* accourding to the residence.

The result of the precent study show that the percentag of *G.lamblia* was high prevelance in rural (65.2%) than in urban , it was (34.8%) table (3-3).

Our study agree with study done by Fetensa *et al*. (2020), who showed that people residing in urban settings had less infection when compared with those people living in rural residences. Also another studies agree with current results done by Al-Masoudi (2009)and Al- Ebrahimi (2013), who showed the same results of that the people in rural areas are more susceptible to infection than urban areas the combined infections were more common in rural areas.

Table (3-3): Distribution of total patients examined and total patients infected according to residence.

Residence	Samples Examined (%)	Samples infected (%)
Urban	54 (36.0)	8 (34.8)
Rural	96 (64.0)	15 (65.2)
Total	150 (100.0)	23 (100.0)

The difference may be attributed to the variation in population distribution, urban characteristics and availability of infrastructures in different countries. In reality, urban residents have access to clean water which might not be possible in rural as most of the diarrheal cases arise from drinking water source, also This

variance could be due to the population living in urban areas having better access to an improved water source, sanitation facility, health care facility and better knowledge about the prevention and control of diarrheal disease in comparison to rural populations. Another possible reason could be that people living in rural areas tend to be poorer than their urban counterparts are, a factor known to have an impact on the level of hygienic practice. it is indicated that children living in rural areas were more vulnerable to diarrhea than their urban counterparts. People from rural households were 1.9 times more likely to have diarrhea as compared to their counterparts (Alebel *et al*.,2018).

3.1.3:immunological parameters:

3.1.3.1: Nod like receptors p3(NLRP3):

The mean differences of NLRP3according to study group including (giardiasis and control group). There were significant elevation in level of NLRP3 among patients with giardia group in comparison to control group as show in table (3-4)

Table (3-4): The mean differences of NLRP3 (ng/ml) according to study group.

Study variable	Study group	N	Mean ± SD	t-test	P-value
NLRP3 (ng/ml)	Giardia group	23	0.933 ± 0.429	**2.994**	**0.005***
	Control group	17	0.519 ± 0.437		

NLRs are intracellular innate immune receptors containing NOD1, NOD2, and NLRP3, etc. NOD1 and NOD2 mediate the activation of NF-KB signal pathway (Newton K, Dixit VM.2012).

NRLP3 inflammasome activation requires two steps. The first step known as the priming step, is induced by PRRs or TNFR activation. This leads to the activation of NF-kB and promotes the expression of NLRP3, pro-IL-1β, and pro-IL-18. Additionally, IFNAR also activates the priming stage of NLRP3 inflammasome activation Swanson *et al*.(2019).

The second step, also called the activation step, is triggered by a range of stimuli that emerge during infections, tissue damage, or metabolic imbalances. Such stimuli include ATP, pore-forming toxins, crystalline substances, nucleic acids, and invading pathogens (Lamkanfi and Dixit, 2014).

Table (3-5): The mean differences of NLRP3 (ng/ml) according to study group among different age group.

Age (years)	Study group	N	Mean ± SD	t-test	P- value
1 – 5	Giardiasis	9	0.87 ± 0.34	1.498	0.158
	Control group	6	0.55 ± 0.51		
6 – 10	Giardiasis	5	0.82 ± 0.28	2.116	0.072
	Control group	4	0.50 ± 0.48		
11 – 15	Giardiasis	2	0.53 ± 0.03	2.251	0.11
	Control group	3	0.36 ± 0.10		
16 – 20	Giardiasis	2	0.92 ± 0.50	0.036	0.975
	Control group	2	0.89 ± 1.03		
21 – 30	Giardiasis	5	1.32 ± 0.59	**2.774**	**0.039***
	Control group	2	0.51 ± 0.18		

The mean differences of NLRP3 (ng/ml) according to study group including (giardiasis and control group) among males show There were significant elevation in level of NLRP3 (ng/ml) among males patients with giardiasis in comparison to males control group show Table(3-6).

Table (3-6): Mean differences of NLRP3 (ng/ml) according to study group among males (N=21).

Study variable	Study group	N	Mean ± SD	t-test	P-value
NLRP3 (ng/ml)	Giardiasis	11	0.88 ± 0.37	2.487	0.022*
	Control group	10	0.46 ± 0.41		

While The mean differences of NLRP3 (ng/ml) among females confirm There were no significant elevation in level of NLRP3 (ng/ml) among females patients with giardiasis in comparison to females control group Table (3-7).

Table (3-7): Mean differences of NLRP3 (ng/ml) according to study group among females (N=19).

Study variable	Study group	N	Mean ± SD	t-test	P-value
NLRP3 (ng/ml)	Giardiasis	12	0.98 ± 0.49	1.619	0.124
	Control group	7	0.60 ± 0.50		

other previous studies showed that *G. lamblia*, a noninvasive extracellular parasite, activated the NLRP3 inflammasome signaling pathways of murine macrophages to regulate the host inflammatory response and that this process was enhanced by released GEVs (Zhao *et al* ., 2021)؛(Argüello *et al* .,2021) .

However, the exact PAMPs in GEVs that are involved in NLRP3 inflammasome-regulated inflammation and the role of the NLRP3 inflammasome in giardiasis remain to be elucidated.

The NLRP3 inflammasome is located in the cytoplasm of immune cells and can be activated by various particles, such as uric acid crystals, toxins, bacteria, viruses and parasites. In studies on bacteria, toxins have been identified as key PAMPs that activate inflammasome sensors, leading to inflammation and cell death (Greaney *et al* ., 2015).

NLRP3 interacts with ASC, recruits pro-caspase and generates active caspase, which cleaves pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18, respectively. Inflammatorycaspases (caspase1,4,5 and -11), a family of conserved cysteine proteases, are critical for innate defenses and are involved in inflammation and programmed cell death (Kesavardhana *et al* ., 2020).

3.1.3.2: Nuclear factor kappa (NF-κB)

The mean differences of NF-κB in patients and control confirm there were significant elevation in level of NF-κB among patients with giardiasis in comparison to control group as show Table(3-16) and figure (3-8).

Table (3-8): The mean differences of NF-κB (ng/ml) according to study group .

Study variable	Study group	N	Mean ± SD	t-test	P-value
NF-κB (ng/ml)	Giardiasis	23	0.999 ± 0.504	**3.029**	**0.004***
	Control group	17	0.505 ± 0.518		

The results agree with the studies of Naeem and Samaafaik (2015) that showed the elevated level of NF-κB after *G.lamblia* parasite exposure.

Other result demonstrated that *G. lamblia* does not trigger in macrophage-like cells the activation of canonical pro-inflammatory signaling cascades such as NF-κB,These results correlate well with data showing limited intestinal inflammation during giardiasis. (Fisher *et al*., 2013).

In most *Giardia* infections, clinical manifestations are self-limiting, with transient intestinal complications. However, infections may also last for several months/years as chronic. (Fink and Singer *et al* ., 2017). or as asymptomatic infections (the majority in endemic areas). In these cases, the parasite is likely to subvert host's immune system.

The expression and production of inflammatory mediators by immune cells is under tight control of a complex network of intracellular signaling pathways and transcription factors. As part of their pathogenic strategies, several microorganisms manipulate these signaling cascades in host cells in order to inflammation and to evade the activities of effector immune cells (Finlay *et al* .,2006).

Therefore other study investigate the effects of Giardia parasites on the levels of activation NF-κB subunit p65, three central players in inflammatory signaling pathways. Mechanisms aiming the manipulation of NF-κB activity are also common in other pathogens. (Christian *et al* .,2010).

The result in the table (3-9) showed the level of NFk-B was increasing significantly ($p \leq 0.05$) and showed high level of NF-κB in age group(6-10/ 21-30)years compared to healthy controls,the increasing significantly ($p \leq 0.05$) .while the level of NF-κB was decreased in age group (16-20) year compared to controls and the result were not significantly.

Table (3-9): The mean differences of NF-κB (ng/ml) according to study group among different age group.

Age (years)	Study group	N	Mean ± SD	t-test	P- value
1 – 5	Giardiasis	9	0.91 ± 0.35	1.485	0.161
	Control group	6	0.54 ± 0.62		
6 – 10	Giardiasis	5	0.97 ± 0.36	2.365	0.05*
	Control group	4	0.43 ± 0.31		
11 – 15	Giardiasis	2	0.49 ± 0.03	2.053	0.132
	Control group	3	0.30 ± 0.12		
16 – 20	Giardiasis	2	0.94 ± 0.53	0.01	0.993
	Control group	2	0.93 ± 1.21		
21 – 30	Giardiasis	5	1.42 ± 0.75	2.541	0.05*
	Control group	2	0.45 ± 0.26		

Indeed, besides the highly variable clinical manifestation of giardiasis between individuals, it is well-known that infection with *G. lamblia* does not cause overt inflammation of the intestinal mucosa and most of the infected individuals had asymptomatic giardiasis Reviewed in Cotton *et al* .(2015) .

There are several mechanisms through which the microorganism proteases could enter into the cytosol in order to cleave NF-kB. Recent studies suggest that extracellular vesicles (EVs) are used by parasites to deliver contents into the host cells, constituting a mechanism by which they manipulate immune response Nawaz *et al* .(2019).

Other study demonstrated for the first time, that *Giardia lamblia* can differentially target NF-κB in order to modulate the inflammatory functions of macrophage cells. this results evidence that the impairment of NF-κB transcription factor is a crucial strategy through which parasites limit COX-2 and iNOS expression and downstream NO production. The knowledge of the intracellular signaling profile modulated by Giardia parasites in host immune cells highlight putative molecular targets for further development of new therapeutic strategies against giardiasis.

Also, results confirm the mean differences of NF-κB according to study group was elevated among males. Statistical analysis show significant elevation in level of NF-κB among male patients with giardiasis in comparison to male control group as show Table(3-18). In addition, The results of the study showed a significant increase in the serum level of (NF-κB) in female patients infected with *G.lamblia* parasite compared with the control group table(3- 10).

Table (3-10):Mean differences of NFK-B (ng/ml) according to study group among males.

Study variable	Study group	N	Mean ± SD	t-test	P-value
NF-κB (ng/ml)	Giardiasis	11	0.91 ± 0.37	2.372	0.028*
	Control group	10	0.46 ± 0.49		

The mean differences of NF-κB according to study group including (giardiasis and control group) among females show There were no significant elevation in level of NF-κB among female patients with giardiasis in comparison to female control group as show Table(3-11).

Table(3-11):Mean differences of NF-κB (ng/ml) according to study group among females.

Study variable	Study group	N	Mean ± SD	t-test	P-value
NF-κB (ng/ml)	Giardiasis	12	1.08 ± 0.60	1.796	0.09
	Control group	7	0.57 ± 0.58		

The results of the study showed a significant increase in the serum level of (NF-κB) in female patients infected with *G.lamblia* parasite compared with the control group, also in total patients with *G.lamblia* compared to the total control group; this increase may be due to the presence a large number of parasite trophozoite, which leads to stimulation of NF-κB, the parasite depends on bacteria in its feed and pathogenicity are directly linked to bacterial microbiota exposure. The parasite-bacterium relationship is very selective since it ingests only bacteria with the appropriate recognition molecule (Varet *et al*., 2018). Bacterial stimulated NF-κB activated in the intestine, the Toll-like receptors (TLRs), recognize extracellular microbial-associated molecular patterns (MAMPs) also MAMPs consumed via endocytosis, while NOD-like receptors (NLRs) recognize cytosol; MAMPs. Both TLRs and NLRs activate signalling pathways leading to NF-κB activation and can, therefore, ultimately lead to an inflammatory response (Atreya *et al*., 2008).

Human data indicate that antibody response is essential in preventing infection, and though functions in cellular response have not been exempted. *G. lamblia* induces NF-KB activation through infection for the control and elimination of disease, so this NF-KB as the increase in patients with amoebiasis influences amoeba pathogenesis .(Naeem and Samaafaik, 2015).

In the healthy intestine; the mucosal immune system maintains an equilibrium between pro-and anti-inflammatory mediators that provides the effective defence

against the luminal pathogen, at the same time; however, an overwhelming immune reaction directed against an enormous amount of harmless luminal antigens is prevented. NF-KB has been described as one of the major regulators in this immunological situation in IBD patients. NF-KB significantly affects the course of mucosal inflammation through its ability to promote the activities of various pro-inflammatory genes (Atreya *et al*., 2008).

Various pro-inflammatory mediators such as IL-1β, TNFα and IL6, activate NF-KB, which means that NF-KB acts as a pro-inflammatory factor and contributes to intestinal inflammatory illnesses (Karrasch and Jobin, 2008; Zaidi and Wine, 2018). In colonic epithelial cells, it was found that NF-KB was induced via IL-6 and produced an increase in adhesion molecule expression in epithelial cells, which is essential for recruiting neutrophil to the site inflammation (Zaidi & Wine, 2018). Correlation between both NF-KB and inflammation indicates that NF-KB regulates the expression of several producing genes, chemokines, cytokines, and adhesion molecules associated with the inflammation cascade (Malaponte *et al.*, 2015).

3.1.3.3:interleukin 1beta (IL-1β)Levels .

The mean differences of IL- 1β according to study group including (giardia group and control group) finding That were significant elevation in level of IL-1β among patients with giardiasis in comparison to control group (Table 3-12) .

Table(3-12): The mean differences of IL- 1β (pg/ml) according to study group among different age group.

Study variable	Study group	N	Mean ± SD	t-test	P-value
IL- 1β (pg/ml)	Gairdia group	23	1.063 ± 0.843	2.711	0.01*
	Control group	17	0.505± 0.442		

This result was constand with Mohsen(2020) confirm that IL-1β increase in children infected with intestinal parasite .

Intestinal epithelial cell production of interleukin(IL-1β)and IL-18 causes an influx of inflammatory cells into the intestinal mucosa with resultant tissue damage Zhang *et al* .(2000).

IL-1β is a pro-inflammatory cytokine that plays a critical role in host defense and innate immunity. The production of IL-1β is given its potent inflammatory properties, The production of IL-1β can also trigger immune pathology and tissue damage, reinforcing the importance of IL-1β regulation for maintaining innate immune function without excessive inflammation. IL-1β plays a vital role in protective immunity against parasitic infection Silver *et al*.(2010) study of Silver and Hunter (2010).demonstrate that monocytes are critical for controlling *G. lamblia* infection by inducing the cleavage of pro-IL-1β and the release of mature IL-1β.

The result in the table (3-13) showed that the high levels of (IL- 1β) in age group (21-30)compared to healthy controls, it was significant elevation in level of IL- 1β (pg/ml) among patients with giardiasis in comparison to control group.

Table(3-13): The mean differences of IL- 1β (pg/ml) according to study group among different age group .

Age (years)	Study group	N	Mean ± SD	t-test	P- value
1 – 5	Giardiasis	9	1.12 ± 1.07	0.944	0.362
	Control group	6	0.67 ± 0.54		
6 – 10	Giardiasis	5	0.94 ± 0.79	1.295	0.237
	Control group	4	0.38 ± 0.34		
11 – 15	Giardiasis	2	1.01 ± 0.18	1.943	0.147
	Control group	3	0.43 ± 0.38		
16 – 20	Giardiasis	2	0.08 ± 0.09	-0.856	0.545
	Control group	2	0.52 ± 0.73		
21 – 30	Giardiasis	5	1.50 ± 0.46	**2.962**	**0.031***
	Control group	2	0.34 ± 0.47		

Also in age (6-10) and (11-15) years was increase IL1β but the statistical analysis she not significantly (p > 0.031)and this result similar to finding of Lyke *et al* .(2009). No significant differences were noted in cytokine levels between children(Lyke *et al.*,2009) .

Infection with *G.lamblia* may causes malnourished in children , so the study of Kumari *et al* .(2022) is covered that malnourished children had significantly higher levels of interleuin 1(IL-1) than healthy control children . the elevated concentration pro-inflammatory in malnourished children could be linked to the fact that kumari *et al* .(2022). Micrountrient dificiencies in such children affect various immunity elemints,including cell – nediated immune response and production (Tan *et al* ., 2015).

From the results of the current syudy The mean differences of IL-1β (pg/ml) show There were no significant elevation in level of IL-1β (pg/ml) among male patients with giardiasis in comparison to male control group as show in Table(3-14).

Table (3-14): The mean differences of IL-1β (pg/ml) according to study group among males.

Study variable	Study group	N	Mean ± SD	t-test	P-value
IL- 1β (pg/ml)	Giardiasis	11	0.84 ± 0.50	1.463	0.16
	Control group	10	0.53 ± 0.45		

The mean differences of IL-1β (pg/ml) according to study group including (giardiasis and control group) among females show There were significant elevation in level of IL- 1β (pg/ml) among female patients with giardiasis in comparison to female control group Table (3-15).

Table (3-15): The mean differences of IL-1β (pg/ml) according to study group among females.

Study variable	Study group	N	Mean ± SD	t-test	P-value
IL- 1β (pg/ml)	Giardiasis	12	1.27 ± 1.05	**2.304**	**0.035***
	Control group	7	0.47 ± 0.46		

Had no Significant effect on serum IL-1β in the study groups.Other results mentioned that pro-inflammatory cytokines (IL-1β) were significantly and specifically secreted in the presence of G.lamblia (Bansal *et al* .,2009).

also studies mentioned increase in the release of IL-1β done by Mortimer *et al*. (2010) and Quach *et al*. (2019). also Study of Wang (2014) seen enhanced IL-1β release in response to *G.lamblia*.

The current study concluded that the levels of interleukin-1β are increased in female patients with giardiasis when compared to healthy controls, that the increased level of interleukin-1β is due to immune response during infection**.**

3.3.1.4.Interleukin 18 (IL-18) Levels.

The mean differences of IL-18 in patients and control There were significant elevation in level of IL-18 among patients with giardiasis in comparison to control group as show in Table(3-16) and figure(3-16).

Table (3-16): The mean differences of IL-18 (pg/ml) according to study group.

Study variable	Study group	N	Mean ± SD	t-test	P-value
IL- 18 (pg/ml)	Giardiasis	23	587.114 ± 497.818	**3.702**	**0.001***
	Control group	17	185.332 ± 130.459		

Previous studies have shown that infection with other protozoa results in increased production of IL-12 that is necessary for NK cell production of IFN-γ required for innate resistance to this pathogen. The data presented here demonstrate that, although infection resulted in an increase in serum levels of IL-18.Sher *et al* .(2007).

Macrophages are widely distributed innate immune cells throughout the body, which play vital roles in immunity as early effectors, initiating inflammatory response, modulating adaptive immune response, defending against microbial infections, and restoring tissue homeostasis after infection clearance

Macrophages normally cooperate with neutrophils in combating microbial infections and maintaining inflammation via producing enough pro-inflammatory cytokines like TNF-α, IL-1β and IL-18 are elevated during Giardia infection, their deficiency facilitates delayed elimination of the parasite He *et al*. (2021) .

The result showed in tables (3-17,3-18 and 3-19) investigated high level of IL18 in age group 6-10 year compared to controls in both sex, and the results were significantly ($p \leq 0.05$).

Table (3-17): The mean differences of IL- 18 (pg/ml) according to study group among different age group .

Age (years)	Study group	N	Mean ± SD	t-test	P- value
1 – 5	Giardiasis	9	449.28 ± 508.24	1.784	0.106
	Control group	6	130.53 ± 138.94		
6 – 10	Giardiasis	5	878.72 ± 489.95	**2.571**	**0.05***
	Control group	4	293.60 ± 122.93		
11 – 15	Giardiasis	2	238.60 ± 141.48	0.487	0.66
	Control group	3	174.38 ± 145.88		
16 – 20	Giardiasis	2	420.44 ± 289.71	0.925	0.453
	Control group	2	220.56 ± 97.56		
21 – 30	Giardiasis	5	749.69 ± 568.92	1.492	0.196
	Control group	2	114.39 ± 23.62		

Herjan *et al.* (2018) suggest that pro-inflammatory is a key element in inflammation and is involved in the immune responses to microbial and parasite infection and autoimmune disease.

The present study was in agreement with the finding of Mohsen (2020). which showed that high levels of IL-18 were increasing in patients infected with intestinal parasite compared with healthy controls .

The mean differences of IL-18 according to study group including (giardiasis and control group) among males show There were significant elevation in level of IL-18 among male patients with giardiasis in comparison to male control group as show table(3-18)

Table (3-18): The mean differences of IL-18 (pg/ml) according to study group among males .

Study variable	Study group	N	Mean ± SD	t-test	P-value
IL- 18 (pg/ml)	Giardiasis	11	617.73 ± 471.32	**2.81**	**0.015***
	Control group	10	194.64± 157.28		

The mean differences of IL-18 among females. There were significant elevation in level of IL-18 among female patients with giardiasis in comparison to female control group as show table (3-19).

Table (3-19): The mean differences of IL- 18 (pg/ml) according to study group among females (N=19).

Study variable	Study group	N	Mean ± SD	t-test	P-value
IL- 18 (pg/ml)	Giardiasis	12	559.05 ± 540.28	**2.426**	**0.032***
	Control group	7	172.04± 89.05		

Addition,IL-18 may play apotent role in activating CD8 which have against Micropial infection , IL-18 suppressed IgE production following infection , the weakened IgE production was restored by stration of anti-IL-12 and IL-18 signifying active suppression that secrete IL-18 (Ihim *et al* .,2022)

3.1.3.5 : The correlation among the study immunology markers.

The correlation between IL-18 (pg/ml) and NLRP3 (ng/ml) among patients with giardiasis. There was positive correlation between IL-18 and NLRP3 of that correlation was no significant correlation between two study markers among patients with Giardiasis. (N=23, r = 0.263, P=0.226) show figure(3-6) also The correlation between IL-18 (pg/ml) and NF-κB (ng/ml) among patients with giardiasis. Positive correlation between IL-18 and NFKP-65 of that correlation There was no significant correlation between two study markers among patients with giardiasis. (N=23, r = 0.358, P=0.093). show figure(3-7) .

The correlation between IL-1β (pg/ml) and NLRP3 (ng/ml) among patients with giardiasis. Positive correlation between IL-1β and NLRP3 of There was no significant correlation between two study markers among patients with giardiasis. (N=23, r = 0.205, P=0.347).show figure (3-8). Also The correlation between IL-1B (pg/ml) and NF-κB (ng/ml) among patients with giardiasis. There was positive correlation between IL-1β and NF-κB of that correlation was no significant correlation between two study markers among patients with giardiasis. (N=23, r = 0.209, P=0.338).show figure(3-9).

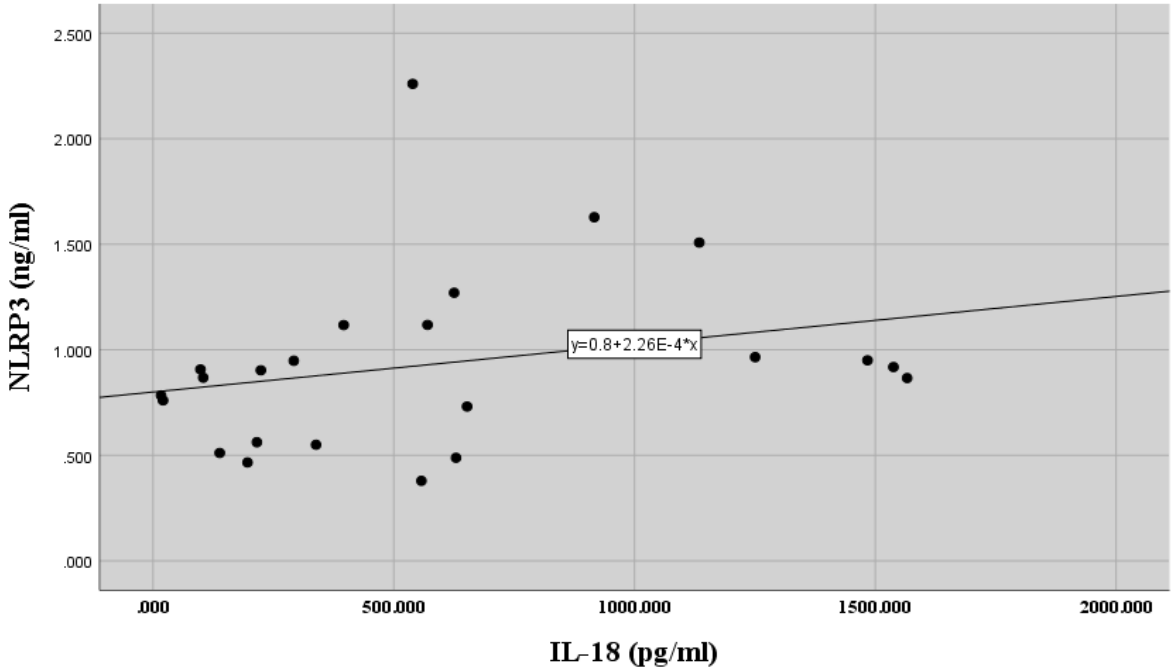


Figure (3-2): The correlation between IL- 18 (pg/ml) and NLRP3 (ng/ml) among patients with giardiasis .

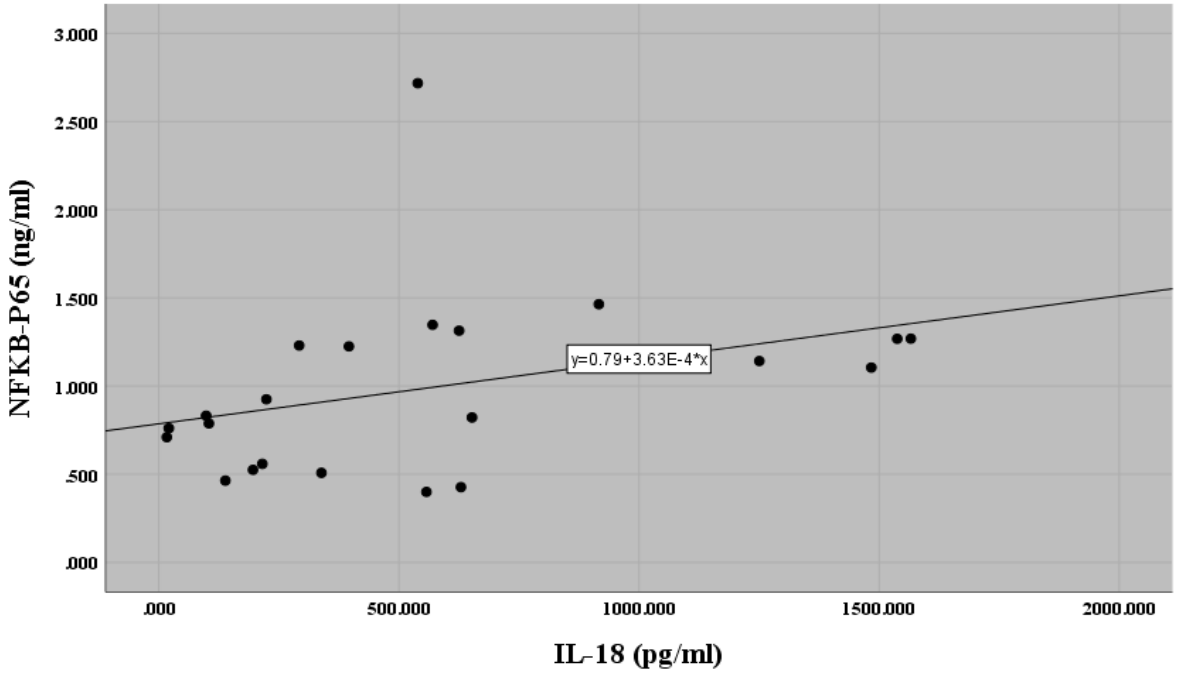


Figure (3-3): The correlation between IL- 18 (pg/ml) and NF-κB (ng/ml) among patients with giardiasis.

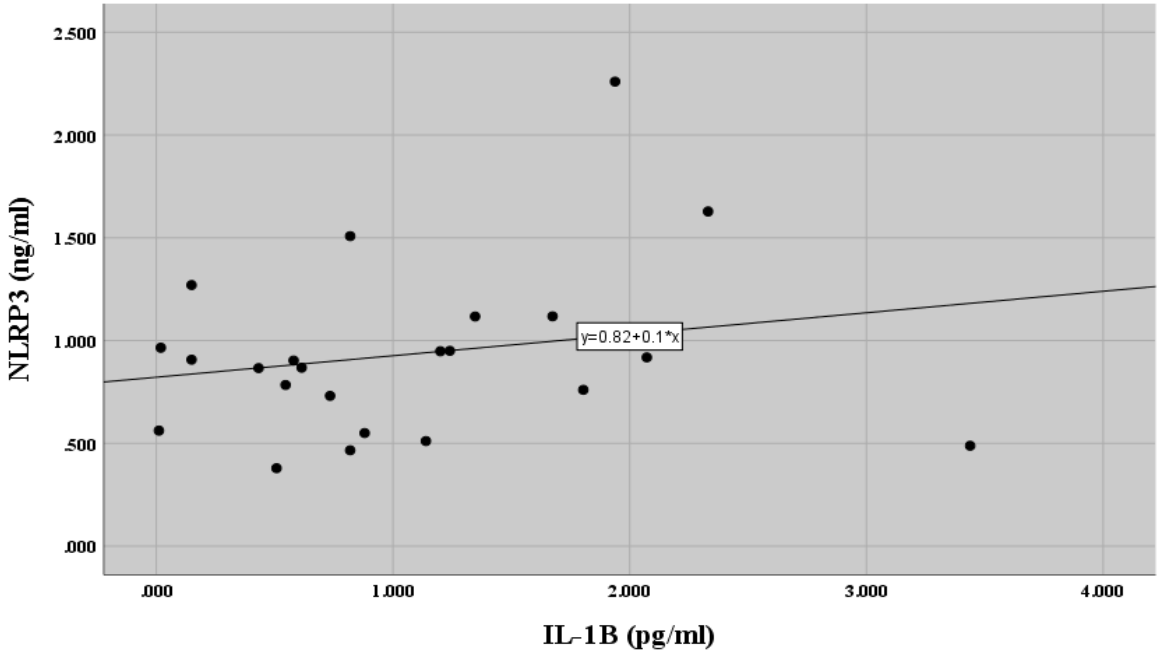


Figure (3-4): The correlation between IL- 1β (pg/ml) and NLRP3 (ng/ml) among patients with giardiasis.

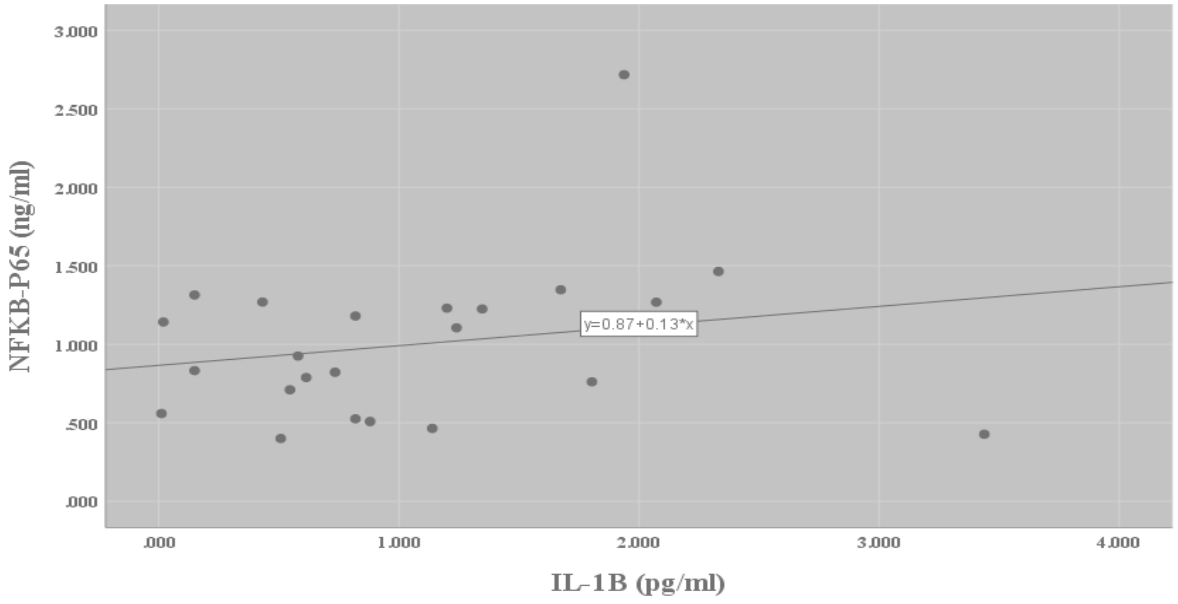


Figure (3-5): The correlation between IL-1β (pg/ml) and NF-κB (ng/ml) among patients with giardiasis.

NLRs are mainly expressed in immune cells and shares the function of NF-KB activation, such as NOD1 and NOD2, or secretion of pro-inflammatory

cytokines IL-1β and IL-18, such as NLRP3 .Zamboni DS *et al* (2015) NLRP3 has been deeply studied since it could recognize various bacteria, viruses, fungi, parasites and nigericin and uric acid crystals, etc. and involves in many intracellular path- ogens induced disease, such as *Staphylococcus aureus* bacteremia. Rasmussen *et al.* (2019).

However, limited research focus on the immune mechanisms mediated by the intracellular NLRs in extracellular pathogen of *G. lamblia* except for a newly research, which reports that *G. lamblias* can attenuate giardiasis *in vivo* via NLRP3 Manko *et al* .(2020).

Moreover, the exact PAMPs that deliver biological information into host cells still need to be determined. In the present study, we detected varie- ties of NLRs expression levels when exposure macrophages to GEVs and *G. lamblia* and found that NOD2, NLRP3, NLRC4 and NLRC5 were obviously up-regulated. Among them, NLRP3 was the most obviously up-regulated receptor. Furthermore, many cytokines were inhibited when NLRP3 was blocked with either Glibenclimide or CA-074 methyl ester . Wei *et al.* (2020).

Changes of NLRP3 and IL-1β levels led us to explore the process of IL-1β secretion and NLRP3 inflammasome activation. We inoculated GEVs into macrophages for different time and with different dose and then detected the expression levels of NLRP3, pro-IL-1β, pro-caspase-1, active IL-1β p17, and active caspase-1 p20. *G.lamblia*-treated group was used as positive control and no treatment group was used as negative control. Results indicated that NLRP3 inflammasome was activated and IL-1β production was in dose-dependent and time dependent manners, which was accordance with Naegleria fowleri or N. caninum in vitro and *G. lamblia* in vivo. Kim *et al.*(2016) found that NOD2,NLRP3,NLRC4 and NLRC5 were obviously up-regulated Among them.

Conclusions

&

Recommendations

Conclusions

According to the results that obtained in present study , the following conclusions have been detected :

1. The frequency of *G.lamblia* from 150 stool samples were about (15.3%) in Hilla city. The percentage of infection with G.lamblia was higher in female than male. with highest percentage for age group (1-5)years

2. The distripution of *G.lamblia* higher in rural area than urban area.

3. The result showed increasing in IL-18 in patient with *G.lamblia* and high levels of IL-18 in age group (6-10) and high level IL-1β in age group (21- 30) compared to healthy controls .

4. The result of present study showed high level of NLRP3 in age group (21- 30) compared to healthy controls , also showed high level of NF-κB in age group (21-30) compared to healthy controls.

5. The study showed there was positive correlation between IL-18 and IL-1β with NLRP3 and NF-κB.

Recommendations:

1- Farthur study determine role of NLRP3 inflammasome in mediatinf IL-1B production in other intestinal protozoa.

2- Study immunological marker in *Giardiasis* children with diabetes.

3- Study the role of NLRP3 indlammasome in mediating other proinflammatory cytokins during Giardiasis .

4- Study the relationship between malnutrition and inflammatory markers in patients with Giardiasis.

References

References:

Adam,R.D.(2001). Biology of *Giardia lamblia*.Clin Microbiol Rev .,14(3);447-475.

Afonina, I. S., Müller, C., Martin, S. J., & Beyaert, R. (2015). Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. Immunity, 42(6), 991-1004.

Aizawa Y, Sutoh S, Matsuoka M, Negishi M, Torii A, Miyakawa Y, et al. (2005). Association of interleukin-18 gene single-nucleotide polymorphisms with susceptibility to inflammatory bowel disease. *Tissue Antigens*. 65:88–92.

Alatbee, Ahmed Hamid Dawood, Shafeeqa Abdusayed Obaid, and Entihaa Abdul-Zahra Abdul-Kareem. (2020). "ISolation and diagnosis parasitic etiology of gastrointestinal infection of patients at the al-mwanee hospital in basra city southern of iraq." Plant Archives 20.2: 996-998.

Alebel, A., Tesema, C., Temesgen, B., Gebrie, A., Petrucka, P., & Kibret, G. D. (2018). Prevalence and determinants of diarrhea among under-five children in Ethiopia: A systematic review and meta- analysis. *PLoS One*, *13*(6), e0199684.

Alipour, A., Hindle, A., & Stroulia, E. (2013, May). A contextual approach towards more accurate duplicate bug report detection. In 2013 10th Working Conference on Mining Software Repositories (MSR) (pp. 183-192). IEEE.

Atreya, I., Atreya, R., & Neurath, M. F. (2008). NF-κB in inflammatory bowel disease. Journal of Internal Medicine, 263(6), 591–596.

Al-Ebrahimi, H. N. M. (2013). Detection of major virulence factors of *Entamoeba histolytica* by using polymerase chain reaction (PCR) Technique (MSc. Thesis, Coll. Med. Univ. Al-Qadisiya).

Al- Kahfaji M.A and Alsaadi Z.H. (2019).*Gardia lamblia* and Gardiasis journal of university of babylon for pure and applied science ,vol 27, no 5.

AL-Khalidy, K. A. H., & Jabbar, H. K. (2020). Detection of *Entamoeba histolytica* and cyptosporidiumparvium in drinking water of Al-diwaniyah City, Iraq. Eurasian Journal of Biosciences, 14(1), 1839-1843.

ALLAIN, T., AMAT, C. B., MOTTA, J.-P., MANKO, A. & BURET, A. G. (2017). Interactions of *Giardia* sp. with the intestinal barrier: Epithelium, mucus, and microbiota. Tissue Barriers, 5, e1274354.

ALLAIN, T., Fekete, E., and Buret, A. G. (2019). *Giardia* cysteine proteases: the teeth behind the smile. Trends Parasitol. 35, 636–648. doi: 10.1016/j.pt.2019.06.003.

ALLAIN, T., and Buret, A. G. (2020). Pathogenesis and post-infectious complications in *giardiasis*. Adv. Parasitol. 107, 173–199. doi: 10.1016/bs.apar.2019. 12.001sometimes never stop? Trends in Parasitology 26(2), 75–82.

Al-taie, Lazem HK, and Fatema M. Ali. (2009). "Epidimiology of giardiasis in Sulaimaniya and Chamchamal with its effect on some biochemical parameters and PCV." Al-Qadisiyah Medical Journal 5.7: 45-53.

Al-Masoudi, H. K. A. (2009). The Effect of Some Plants Extracts and Drugs on Immobilized Cysteine Proteinase of Entamoeba histolytica Isolated from Diarrhea in Al- Exandaria Districts/ Babylon province. PhD Thesis, Faculty of Pure Science, University of Baghdad.

Al-Yousofi A, Yan Y, Al Mekhlafi AM, et al. 2022. Prevalence of Intestinal Parasites among Immunocompromised Patients, Children, and Adults in Sana'a, Yemen. J Trop Med. :5976640.

Amer, O. S. O., Al-Malki, E. S., Waly, M. I., AlAgeel, A., & Lubbad, M. Y. (2018). Prevalence of intestinal parasitic infections among patients of King Fahd Medical city in Riyadh Region, Saudi Arabia: A 5-year retrospective study. Journal of Par .

Ankri, S. (2015). *Entamoeba histolytica*-tumor necrosis factor: a fatal attraction. Microbial Cell, 2(7), 216.

Argüello-García, R.; Ortega-Pierres,M.G.(2021)*Giardia duodenalis* Virulence—"To Be, or Not To Be". Curr. Trop. Med. Rep., 8, 246–256.

Aw, J.Y.H., Clarke, N.E., McCarthy, J.S., Traub, R.J., Amaral, S., Huque, M.H., Andrews, R.M., Gray, D.J., Clements, A.C.A., Vaz Nery, S., (2019). *Giardia duodenalis* infection in the context of a community-based deworming and water, sanitation and hygiene trial in Timor-Leste. Parasites Vectors 12, 491.

BAHADıR, E. B. & SEZGINTÜRK, M. K. (2016). Lateral flow assays: Principles, designs and labels. TrAC Trends in Analytical Chemistry, 82, 286-306.

Bansal, D., Ave, P., Kerneis, S., Frileux, P., Boché, O., Baglin, A. C., ... & Mirelman, D. (2009). An ex-vivo human intestinal model to study *Entamoeba histolytica* pathogenesis. PLoS Negl Trop Dis, 3(11), e551.

Barash NR, Nosala C, Pham JK, McInally SG, Gourguechon S, McCarthy-Sinclair B, Dawson SC. (2017). *Giardia* colonizes and encysts in high-density foci in the murine small intestine. *mSphere* 2:e00343-16.

Bartelt LA, Sartor RB.(2015) Advances in understanding *Giardia*: determinants and mechanisms of chronic sequelae. F1000Prime Rep;7. doi:10.12703/P7- 62.

Bazan, J., Całkosiński, I., & Gamian, A. (2012). Phage display—A powerful technique for immunotherapy: 1. Introduction and potential of therapeutic applications. Human vaccines & immunotherapeutics, 8(12), 1817-1828.

Bazzaz, A. A., Shakir, O. M., & Alabbasy, R. H. (2017). Prevalence of two gastrointestinal parasites Entamoeba histolytica and *Giardia lamblia* within Samarra city, Iraq. *Advances in Bioscience and Biotechnology*, *8*(11), 399-410.

Beatty, J. K., Akierman,S.V., Motta, J. P.,Muise,S., Workentine,M. Harrison, J. J., et al. (2017). *Giardia* duodenalis induces pathogenic dysbiosis of human intestinal microbiota biofilms. Int. J. Parasitol. 47, 311–326. doi: 10.1016/j.ijpara.2016.11.010.

Boraschi, D., & Dinarello, C. A. (2006). IL-18 in autoimmunity. European cytokine network, 17(4), 224-252.

Brenchley JM, Douek DC.(2012) Microbial translocation across the GI tract. Annu Rev Immunol.;30:149–173. doi:10.1146/annurev- immunol-020711-075001.

Buret, M., Uskov, A. V., Dellinger, J., Cazier, N., Mennemanteuil, M. M., Berthelot, J., ... & Bouhelier, A. (2015). Spontaneous hot-electron light emission from electron-fed optical antennas. Nano letters, 15(9), 5811-5818.

Buret, A.G.; Motta, J.P.; Allain, T.; Ferraz, J.;Wallace, J.L.(2019) Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: A role for iron? J. Biomed. Sci.26, 1.

Cao, X. Self.(2016).regulation and cross-regulation of pattern-recognition receptor signalling in health and disease. Nat. Rev. Immunol. 16, 35–50.

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., and Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. Microb. Ecol. Health Dis. 26:26191. doi: 10.3402/mehd.v26.26191.

Chakraborty, S., Kaushik, D. K., Gupta, M., & Basu, A. (2010). Inflammasome signaling at the heart of central nervous system pathology. Journal of neuroscience research, 88(8), 1615-1631.

Chen X., Liu G., Yuan Y., Wu G., Wang S., Yuan L.(2019) NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-κB signaling. Cell Death Dis.;10:906.

Christian, J., Vier, J., Paschen, S. A. & Häcker, G.(2010)

activity factor (CPAF) impairs proinflammatory signaling in cells infected with Chlamydiae. J. Biol. Chem. 285, 41320–41327.

Clark, C. G., & Diamond, L. S. (2002). Methods for cultivation of luminal parasitic protists of clinical importance. *Clinical microbiology reviews*, *15*(3), 329-341.

C.R. de Almeida, R.C. Bezagio, C.M. Colli, L.I. Lopes Romera, A. Ferrari, M.L. Gomes(2022). Elimination of *Giardia duodenalis* BIV in vivo using natural extracts in microbiome and dietary supplements, Parasitology International, 86 .

Cipriano, M. J., and Hajduk, S. L. (2018). Drivers of persistent infection: pathogen-induced extracellular vesicles. Essays Biochem. 62, 135–147.

Cominelli, E., Galbiati, M., Vavasseur, A., Conti, L., Sala, T., Vuylsteke, M., ... & Tonelli, C. (2005). A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. Current biology, 15(13), 1196-1200.

Cotton, J. A., Amat, C. B. & Buret, A. G. (2015). Disruptions of host immunity and inflammation by *Giardia duodenalis*: potential consequences for co-infections in the gastro-intestinal tract. Pathogens 4, 764–792.

Cronemberger-Andrade A, Aragão-França L, Araujo CF, Rocha VJ, Borges-Silva MC, Figueira CP, et al.(2014). Extracellular vesicles from Leishmania-infected macrophages confer an anti-infection cytokine-production profile to naïve macrophages. PLoS Negl Trop Dis.;8(9): e3161.

Cwiklinski, K., de la Torre-Escudero, E., Trelis, M., Bernal, D., Dufresne, P. J., Brennan, G. P., et al. (2015). The extracellular vesicles of the helminth pathogen, Fasciola hepatica: biogenesis pathways and cargo molecules involved in parasite pathogenesis. Mol. Cell. Proteomics 14, 3258–3273.

Dann, S. M., Manthey, C. F., Le, C., Miyamoto, Y., Gima, L., Abrahim, A., et al. (2015). IL-17A promotes protective IgA responses and expression of other potential effectors against the lumen-dwelling enteric parasite *Giardia*. Exp. Parasitol.156, 68–78.

Davids, BJ et al . (2006). Polymeric immunoglobulin receptor in intestinal immune defense against the lumen-dwelling protozoan parasite *Giardia*. Journal of immunology 177(9), 6281–6290.

De Carvalho R.V.H., Zamboni D.S.(2020) Inflammasome activation in response to intracellular protozoan parasites. Trends Parasitol.36:459–472.

Den Hartog, J., Rosenbaum, L., Wood, Z., Burt, D., & Petri Jr, W. A. (2013). Diagnosis of multiple enteric protozoan infections by enzyme- linked immunosorbent assay in the guatemalan highlands. *The American journal of tropical medicine and hygiene*, *88*(1), 167-171.

Douanne N, Dong G, Douanne M, Olivier M, Fernandez-Prada C.(2020). Unravelling the proteomic signature of extracellular vesicles released by drug-resistant Leishmania infantum parasites. PLoS Negl Trop Dis. 2020;14(7): e0008439.

Dreesen, J., Destouni, A., Kourlaba, G., Degn, B., Mette, W. C., Carvalho, F., ... & Traeger-Synodinos, J. (2014). Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study. European Journal of Human Genetics, 22(8), 1012-1018.

Dunn, N., and Juergens, A. L. (2020). "*Giardiasis*" in StatPearls. Treasure Island, FL: StatPearls 2. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchali ngamS, et al.

Efstratiou, A.; Ongerth, J.; Karanis, P.(2017). Evolution of monitoring for *Giardia* and Cryptosporidium in water. Water Res., 123, 96–112.

Einarsson E., Ma'ayeh S., Svärd S.G.(2016) An up-date on *Giardia* and *Giardiasis*. Curr. Opin. Microbiol.34:47–52. doi: 10.1016/j.mib.2016.07.019.

El-Dib, N. A. (2017). *Entamoeba histolytica*: an Overview. *Current Tropical Medicine Reports*, *4*(1), 11-20.

Evans-Osses, I., Ansa-Addo, E. A., Inal, J. M., & Ramirez, M. I. (2010). Involvement of lectin pathway activation in the complement killing of *Giardia intestinalis*. Biochemical and biophysical research communications, 395(3), 382-386.

Fan, F., Turkdogan, S., Liu, Z., Shelhammer, D., & Ning, C. Z. (2015). A monolithic white laser. Nature nanotechnology, 10(9), 796-803.

Faria-Silva, C., Ascenso, A., Costa, A. M., Marto, J., Carvalheiro, M., Ribeiro, H. M., & Simões, S. (2020). Feeding the skin: A new trend in food and cosmetics convergence. Trends in Food Science & Technology, 95, 21-32.

Fetensa, G., Fekadu, G., Tekle, F., Markos, J., Etafa, W., & Hasen, T. (2020). Diarrhea and associated factors among under-5 children in Ethiopia: A secondary data analysis. *SAGE Open Medicine*.

Finlay, B. B. & McFadden, G.(2006). Anti-immunology: Evasion of the host immune system by bacterial and viral pathogens. Cell 124, 767–782 (2006).

Fink MY, Singer SM.(2017). The intersection of immune responses, microbiota, and pathogenesis in *Giardiasis*. Trends Parasitol.;33(11):901–913.

Fink, M. Y., Maloney, J., Keselman, A., Li, E., Menegas, S., Staniorski, C., et al. (2019). Proliferation of resident macrophages is dispensable for protection during *Giardia duodenalis* infections. Immunohorizons 3, 412–421..

Fink, M.Y.; Shapiro, D.; Singer, S.M.(2020). Maintenance, lifecycle induction, and infection of murine models. Curr. Protoc. Microbiol. 57, e102.

Fisher, B. S., Estraño, C. E. & Cole, J. A.(2013). Modeling long-term host cell-*Giardia lamblia* interactions in an *in vitro* co-culture system. *PLoS One* **8**, e81104.

Fotedar, R., Stark, D., Beebe, N., Marriott, D.,Ellis,J.,& Harkness, J.(2007). Laboratory diagnostic techniques for Entamoeba species. Clinical microbiology reviews, 20(3), 511-532.

Ford, E. S. (2005). Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. Diabetes care, 28(7), 1769-1778.

Franchi, L., Eigenbrod, T., Muñoz-Planillo, R., & Nuñez, G. (2009). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nature immunology, 10(3), 241-247.

Gavinho B, Sabatke B, Feijoli V, Rossi IV, Silva JM, Evans-Osses I, et al.(2020) Peptidylarginine deiminase inhibition abolishes the production of large extracellular vesicles from *Giardia intestinalis*, affecting host-pathogen interactions by hindering adhesion to host cells. Front Cell Infect Microbiol.;10: 417.

Gholipoor, Z., Khazan, H., Azargashb, E., Youssefi, M.R., Rostami, A., (2019). Prevalence and risk factors of intestinal parasite infections in Mazandaran province, North of Iran. Clin. Epidemiol. Global Health.

Godo, S. & Shimokawa, H. (2017). Endothelial functions. Arterioscler Thromb. Vasc. Biol. 37, e108–e114.

Gong, T., Liu, L., Jiang, W. & Zhou, R. (2020). DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat. Rev. Immunol. 20, 95–112.Ctries. 2018, 12, 492–498.

Greaney, A. J., Leppla, S. H., & Moayeri, M. (2015). Bacterial exotoxins and the inflammasome. Frontiers in immunology, 6, 570.

Haile A, Abera T, Dana D, Wolkite E.(2017) The prevalence of intestinal parasitic infection and associated factors among primary school children in Gurage Zone, South Ethiopia. Prevalence.;15.

Hajishengallis, G. & Lambris, J. D. (2011). Microbial manipulation of receptor crosstalk in innate immunity. *Nat. Rev. Immunol.* **11**, 187–200

Halliez, M. C. M., and Buret, A. G. (2013). Extra-intestinal and long term consequences of *Giardia duodenalis* infections. World J. Gastroenterol. 19, 8974–8985. doi: 10.3748/wjg.v19.i47.8974.

Haque, R., & Petri Jr, W. A. (2006). Diagnosis of amebiasis in Bangladesh. Archives of medical research, 37(2), 272-275.

He W, Heinz A, Jahn D, Hiller K. (2021). Complexity of macrophage metabolism in infection. Curr Opin Biotechnol.

68:231–9. https://doi.org/10.1016/j.copbio..01.020 PMID: 33610128.

He W.T., Wan H., Hu L., Chen P., Wang X., Huang Z., Yang Z.H., Zhong C.Q., Han J.(2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. Cell Res.;25:1285–1298.

Hemphill, A.; Müller,N.;Müller,J.(2019).Comparative pathobiology of the intestinal protozoan parasites *Giardia lamblia*, *Entamoeba histolytica*, and Cryptosporidium parvum. Pathogens.8, 116.

Herjan, T., Hong, L., Bubenik, J., Bulek, K., Qian, W., Liu, C., ... & Li, X. (2018). IL-17-receptor-associated adaptor Act1 directly stabilizes mRNAs to mediate IL-17 inflammatory signaling. Nature immunology, 19(4), 354-365.

Hjollo, T., Bratland, E., Steinsland, H., Radunovic, M., Langeland, N., and Hanevik, K. (2018). Longitudinal cohort study of serum antibody responses towards *Giardia lamblia* variant-specific surface proteins in a non-endemic area. Exp. Parasitol. 191, 66–72.

Hooshyar, H.; Rostamkhani, P.; Arbabi, M.; Delavari, M.(2019). *Giardia lamblia* infection review of current diagnostic strategies. Gastroenterol. Hepatol. Bed Bench, 12, 3–12.

HU, J., WANG, S., WANG, L., LI, F., PINGGUAN-MURPHY, B., LU, T. J. & XU, F. (2014). Advances in paper-based point-of-care diagnostics. Biosensors and Bioelectronics, 54, 585-597.

Ihim, S. A., Abubakar, S. D., Zian, Z., Sasaki, T., Saffarioun, M., Maleknia, S., & Azizi, G. (2022). Interleukin-18 cytokine in immunity, inflammation, and autoimmunity: Biological role in induction, regulation, and treatment. Frontiers in Immunology, 4470.

Ieni A, Barresi V, Rigoli L, Fedele F, Tuccari G, Caruso R.(2016). Morphological and cellular features of innate immune reaction in Helicobacter pylori gastritis: a brief review. Int J Mol Sci. 17(1):109.

Inamine, T., & Schnabl, B. (2018). Immunoglobulin A and liver diseases. Journal of gastroenterology, 53, 691-700.

JEX, A. R., SVÄRD, S., HAGEN, K. D., STARCEVICH, H., EMERY-CORBIN, S. J., BALAN, B., NOSALA, C. & DAWSON, S. C. (2020). Recent advances in functional research in *Giardia intestinalis*. Advances in parasitology, 107, 97-137.

Jia X, Qiu TM, Yao XF, Jiang LP, Wang NN, Wei S, et al.(2020) Arsenic induces hepatic insulin resistance via mtROS-NLRP3 inflammasome pathway. J Hazard Mater. 2020;399: 123034.

Joosten LAB, Netea MG, Dinarello CA. (2013) Interleukin-1 beta in innate inflammation, autophagy and immunity. Semin Immunol. 25:416.24.

J. Zhou, Y. Miyamoto, S. Ihara, A.V.(2022). Kroll, N. Nieskens, V.N. Tran, E.M. Hanson, R.H. Fang, L. Zhang, L. Eckmann, Codelivery of Antigens and Adjuvant in Polymeric Nanoparticles Coated With Native Parasite Membranes Induces Protective Mucosal Immunity Against *Giardia lamblia*, Journal of Infectious Diseases, DOI: 10.1093/infdis/jiac074.

Kamda, JD & Singer, SM (2009). Phosphoinositide kinase-dependent inhibition of dendritic cell interleukin-12 production by *Giardia lamblia*. Infection and Immunity 77(2), 685–693.

Karrasch, T., & Jobin, C. (2008). NF-κB and the intestine: friend or foe? Inflammatory Bowel Diseases, 14(1), 114–124.

Karin, M. and Lin, A. (2002). NF-KB at the crossroads of life and death. Nat. Immunol. 3, 221-227.

Karin, M. (2009). NF-κB as a critical link between inflammation and cancer. Cold Spring Harbor Perspectives in Biology, 1(5), a000141.

Kastl, A. J. Jr., Terry, N. A., Wu, G. D., and Albenberg, L. G. (2020). The structure and function of the human small intestinal microbiota: current understanding and future directions. Cell. Mol. Gastroenterol. Hepatol. 9, 33–45.

Kesavardhana S, Malireddi RKS, Kanneganti TD.(2020) Caspases in cell death, inflammation, and pyroptosis. Annu Rev Immunol.;38:567–95.

Keselman, A., Li, E., Maloney, J., and Singer, S. M. (2016). The microbiota contributes to CD8+ T cell activation and nutrient malabsorption following intestinal infection with *Giardia duodenalis*. Infect. Immun. 84, 2853–2860.

Khiong, K., Adhika, O. A., & Chakravitha, M. (2010). Inflammation, Immunity, and Cancer: The Role of Transcription Factors NF-kB and STAT3. Majalah Kedokteran Indonesia, 60(8), 369–373.

khudair Hussein, T. (2010). Prevalence and related risk factors for *Giardia lamblia* infection among children with acute diarrhea in thi-qar, southern Iraq. University of Thi-Qar Journal Of Medicine, 4(4), 68-74.

Kim JH, Sohn HJ, Yoo JK, Kang H, Seong GS, Chwae YJ, et al.(2016). NLRP3 inflammasome activation in THP-1 target cells triggered by pathogenic Naegleria fowleri. Infect Immun. 2016; 84(9): 2422–8. https://doi.org/10.1128/IAI.00275-16 PMID: 27297387

Kissoon-Singh, V., Moreau, F., Trusevych, E., & Chadee, K. (2013). *Entamoeba histolytica* exacerbates epithelial tight junction permeability and proinflammatory responses in Muc2−/− mice. The American journal of pathology, 182(3), 852-865.

Krakovka, S.; Ribacke, U.; Miyamoto, Y.; Eckmann, L.; Svärd, S.(2022). Characterization of metronidazole-resistant *Giardia intestinalis* lines by comparative transcriptomics and proteomics. *Front. Microbiol*, *13*, 834008.

Krzyżek P, Gościniak G.(2017). Frequency and immunological consequences of Helicobacter pylori and intestinal parasite co-infections: a brief review. Ann Parasitol;63(4): 255–263.

Kumari, R., Sisodia, A., Sah, A. K., & Dubey, G. P. (2022). Evalution of Positive Relation between ProInflammatory Cytokines and Malnourished Children: Eastern Uttar Pradesh-India. Annals of Medical and Health Sciences Research.

Lamkanfi, M., & Dixit, V. M. (2014). Mechanisms and functions of inflammasomes. Cell, 157(5), 1013-1022.

Lang T, Lee J, Elgass K, Pinar AA, Tate MD, Aitken EH, et al.(2018). Macrophage migration inhibitory factor is required for NLRP3 inflammasome activation. *Nat Commun.* 9:2223.

Latif, H., Junaidi, J., Cahyaningsih, U., Purnawarman, T., Sudarnika, E., Hayati, Z., & Muslina, M. (2020). *Entamoeba histolytica* Neglected Tropical Diseases (NTDs) Agents that Infect Humans and Some Other Mammals: A Review. In *E3S Web of Conferences* (Vol. 151, p. 01019). EDP Sciences.

Lee, L. M., Cui, X., & Yang, C. (2009). The application of on-chip optofluidic microscopy for imaging *Giardia lamblia* trophozoites and cysts. Biomedical microdevices, 11(5), 951.

Lee H.Y., Kim J., Noh H.J., Kim H.P., Park S.J.(2014). *Giardia lamblia* binding immunoglobulin protein triggers maturation of dendritic cells via activation of TLR4-MyD88-p38 and ERK1/2 MAPKs. Parasite Immunol.;36:627–646.

Leung, A.K.C.; Leung, A.A.M.; Wong, A.H.C.; Sergi, C.M.; Kam, J.K.M.(2019). *Giardiasis*: An overview. *Recent Pat. Inflamm. Allergy Drug Discov*, *13*, 134–143.

Liu, J., Ma'ayeh, S., Peirasmaki, D., Lundstrom-Stadelmann, B., Hellman, L., and Svard, S. G. (2018). Secreted Giardia intestinalis cysteine proteases disrupt intestinal epithelial cell junctional complexes and degrade chemokines. Virulence 9, 879–894. doi: 10.1080/21505594.2018.1451284.

Liu, L., De Vel, O., Han, Q. L., Zhang, J., & Xiang, Y. (2018). Detecting and preventing cyber insider threats: A survey. IEEE Communications Surveys & Tutorials, 20(2), 1397-1417.

Liu L., Wei Z., Fang R., Li X., Li W.(2020). *Giardia duodenalis* induces extrinsic pathway of apoptosis in intestinal epithelial cells through activation of TNFR1 and K63 de-ubiquitination of RIP1 in vitro. Microb. Pathog.;149:104315. doi: 10.1016/j.micpath.2020.104315.

Li X., Zhang X., Gong P., Xia F., Li L., Yang Z., Li J.(2017). TLR2-/- mice display decreased severity of *Giardiasis* enhanced proinflammatory cytokines production dependent on AKT signal pathway. Front. Immunol.;8:1186. doi: 10.3389/fimmu.2017.01186.

Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, Vos MR, et al.(2014) .Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell. 156:1193–206. doi: 10.1016/j.cell.2014. 02.008.

Lyke, K. E., Daou, M., Diarra, I., Kone, A., Kouriba, B., Thera, M. A., ... & Plowe, C. V. (2009). Cell-mediated immunity elicited by the blood stage malaria vaccine apical membrane antigen 1 in Malian adults: results of a Phase I randomized trial. Vaccine, 27(15), 2171-2176.

Lynch, S. V., and Pedersen, O. (2016). The human intestinal microbiome in health and disease. N. Engl. J. Med. 375, 2369–2379. during interaction with human intestinal epithelial cells: the impact on host cells. PLoS Negl. Trop. Dis. 11:e0006120.

Mackey, G. (2016). Synthesis, Modification, and Application of Siloxane Materials for Environmental Sensing (Doctoral dissertation).

Malaponte, G., Signorelli, S. S., Bevelacqua, V., Polesel, J., Taborelli, M., Guarneri, C., ... & Libra, M. (2015). Increased levels of NF-kB-dependent markers in cancer-associated deep venous thrombosis. PLoS one, 10(7), e0132496.

Manko-Prykhoda A, Allain T, Motta JP, Cotton JA, Feener T, Oyeyemi A, et al.(2020). *Giardia* spp. promote the production of antimicrobial peptides and attenuate disease severity induced by attaching and effacing enteropathogens via the induction of the NLRP3 inflammasome. Int J Parasitol. 50(4): 263–275. pmid:32184085.

MAO, X., WANG, W. & DU, T.-E. (2013). Rapid quantitative immunochromatographic strip for multiple proteins test. Sensors and Actuators B: Chemical, 186, 315-320.

Martinon, F., Mayor, A., & Tschopp, J. (2009). The inflammasomes: guardians of the body. Annual review of immunology, 27, 229-265.

Masangkay, F.R., Milanez, G.D., Tsiami, A., Hapan, F.Z., Somsak, V., Kotepui, M., Tangpong, J., Karanis, P., (2020). Waterborne protozoan pathogens in environmental aquatic biofilms: Implications for water quality assessment strategies. Environ. Pollut. 259, 113903.

Matowicka-Karna, J et al . (2009). IFN-, IL-5, IL-6 and IgE in patients infected with Giardia intestinalis. Folia Histochemica et Cytobiologica/Polish Academy of Sciences, Polish Histochemical and Cytochemical Society 47(1), 93–97.

Minetti C, Chalmers RM, Beeching NJ, Probert C, Lamden K. *Giardiasis.* BMJ.; Müller, N.; Müller, J.(2016). *Giardia*. In Molecular Parasitology; Walochnik, J., Duchêne, M., Eds.; Springer: Vienna, Austria, pp. 93–114. 355.

Misra, G., & Srivastava, V. K. (Eds.). (2020). Molecular Advancements in Tropical Diseases Drug Discovery. Academic Press.

Mohammed, K., Gulma, M. K., Yahaya, M., Spencer, T. H. I., Nataala, S. U., Garba, M. K., Imam, A. U., Aschroft, O. F., & Micheal, U. (2019). Prevalence of Intestinal Parasitic Infections among Patients Attending Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Asian Journal of Research in Infectious Diseases, 1–9.

Mohsen RH. (2020) Assessment of the role of some immunological parameters in individual infected with *Entamoeba histolytica* in Babylon province. MSc thesis, College of Medicine, University of Babylon,; p. 89.

Molestina, R. E., Payne, T. M., Coppens, I., & Sinai, A. P. (2003). Activation of NF-κB by Toxoplasma gondii correlates with increased expression of antiapoptotic genes and localization of phosphorylated IκB to the parasitophorous vacuole membrane. Journal of Cell Science, 116(21), 4359–4371.

Moossavi, M., Parsamanesh, N., Bahrami, A., Atkin, S. L., & Sahebkar, A. (2018). Role of the NLRP3 inflammasome in cancer. Molecular cancer, 17(1), 1-13.

Mortimer, L., & Chadee, K. (2010). The immunopathogenesis of *Entamoeba histolytica*. Experimental parasitology, 126(3), 366-380.

Müller, V. C., & Bostrom, N. (2016). Future progress in artificial intelligence: A survey of expert opinion. Fundamental issues of artificial intelligence, 555-572.

Murray, M.J.; Murray, A.; Murray, C.J.(2022). The salutary effect of milk on amoebiasis and its reversal by iron. Br. Med. J. 1980, 280, 1351–1352. Pharmaceutics, 14, 1702 25 of 28.

Naeem, A., & Samaafaik, S. &. (2015). Nf-Kb Activation By Infestation With *Entamoeba Histolytica*. International Journal of Research in Applied, Natural and Social Sciences, 3(10), 2321–8851.

Nagao-Kitamoto, H., Kitamoto, S., Kuffa, P., & Kamada, N. (2016). Pathogenic role of the gut microbiota in gastrointestinal diseases. Intestinal research, 14(2), 127.

Nawaz, M., Malik, M. I., Hameed, M. & Zhou, J.(2019). Research progress on the composition and function of parasite-derived exosomes. Acta Trop. 196, 30–36 .

New man, RD.; Moore, SR.; Lima, AM. (2001). A longitudinal study of *Giardia lamblia* in north–east Brazilian children. Trop Med Int Health; 6: 624– 634.

Newton, K., & Dixit, V. M. (2012). Signaling in innate immunity and inflammation. Cold Spring Harbor perspectives in biology, 4(3), a006049.

Niu JL, Wu SX, Chen MK, Xu K, Guo QH, Lu AL, et al. (2019) . Hyperactivation of the NLRP3 inflammasome protects mice against influenza A virus infection via IL-1 beta mediated neutrophil recruitment. Cytokine. 120:115–24. doi: 10.1016/j.cyto.2019.04.019.

Oberhuber G, Mesteri I, Kopf W, Muller H. (2016). Demonstration of trophozoites of G. Lamblia in ileal mucosal biopsy specimens may reveal giardiasis in patients with significantly inflamed parasite-free duodenal mucosa. Am J Surg Pathol 40:1280–1285.

Oficjalska K, Raverdeau M, Aviello G, Wade SC, Hickey A, Sheehan KM, et al. (2015) . Protective role for caspase-11 during acute experimental murine colitis. *J Immunol.* 194:1252–60. doi: 10.4049/jimmunol.1400501.

Ortega-Pierres, G., Arguello-Garcia, R., Laredo-Cisneros, M. S., Fonseca-Linan, R., Gomez-Mondragon, M., Inzunza-Arroyo, R., et al. (2018). Giardipain-1, a protease secreted by *Giardia duodenalis* trophozoites, causes junctional, barrier and apoptotic damage in epithelial cell monolayers. Int. J. Parasitol. 48, 621–639. doi: 10.1016/j.ijpara.2018.01.006.

ORTEGA-PIERRES, M. G. & ARGÜELLO-GARCÍA, R. (2019). *Giardia duodenalis*: Role of secreted molecules as virulent factors in the cytotoxic effect on epithelial cells. Advances in parasitology, 106, 129-169.

Paniker CJ.(2017). Paniker's Textbook of Medical Parasitology. JP Medical Ltd.

Parija, S. C., Mandal, J., & Ponnambath, D. K. (2014). Laboratory methods of identification of *Entamoeba histolytica* and its differentiation from look-alike Entamoeba spp. *Tropical parasitology*, *4*(2), 90.

Place DE, Kanneganti TD(2018). Recent advances in inflammasome biology. Curr Opin Immunol. Feb;50:32–8.

Pober, J. S. & Sessa, W. C.(2007) Evolving functions of endothelial cells in inflammation. Nat. Rev. Immunol. 7, 803–815 .

Prochnicki, T. & Latz, E. (2017). Inflammasomes on the crossroads of innate immune recognition and metabolic control. Cell Metab 26, 71–93.

Prucca, C. G., Slavin, I., Quiroga, R., Elías, E. V., Rivero, F. D., Saura, A., et al. (2008). Antigenic variation in *Giardia lamblia* is regulated by RNA interference. Nature 456, 750–754. doi: 10.1038/nature07585.

Quach, J., Moreau, F., Sandall, C., & Chadee, K. (2019). Entamoeba histolytica-induced IL-1β secretion is dependent on caspase-4 and gasdermin D. *Mucosal immunology*, *12*(2), 323-339.

Rahman, M. A., Zaman, N., Asyhari, A. T., Al-Turjman, F., Bhuiyan, M. Z. A., & Zolkipli, M. F. (2020). Data-driven dynamic clustering framework for mitigating the adverse economic impact of Covid-19 lockdown practices. Sustainable cities and society, 62, 102372.

Rasmussen G, Idosa BA, Ba¨ ckman A, Monecke S, Strålin K, Sa¨ rndahl E, et al.(2019). Caspase-1 inflamma- some activity in patients with Staphylococcus aureus bacteremia. Microbiol Immunol. 63(12): 487–99. https://doi.org/10.1111/1348-0421.12738 PMID: 31403210.

Robertson, D. (2010). The judge as political theorist. In The Judge as Political Theorist. Princeton University Press.

Ryan U, Cacci SM.(2013). Zoonotic potential of *Giardia*. Int J Parasitol 2013;43: 943–56.

Saha, N., Dutta, S., Datta, S. P., and Sarkar, S. (2018). The minimal ESCRT machinery of *Giardia lamblia* has altered inter-subunit interactions within the ESCRT-II and ESCRT-III complexes. Eur. J. Cell Biol. 97, 44–62. doi: 10.1016/j.ejcb.2017.11.004.

Samie, A., Mahlaule, L., Mbati, P., Nozaki, T., & ElBakri, A. (2020). Prevalence and distribution of Entamoeba species in a rural community in northern South Africa. Food and Waterborne Parasitology, 18, e00076.

Savioli L,Smith H,ThompsonA.(2006).*Giardia* and cryptosporidium join the 'neglected diseases initiative'. Trends Parasitol; 22:203-208.

SCHNEIDER, C., O'LEARY, C. E., VON MOLTKE, J., LIANG, H.-E., ANG, QY.,TURNBAUGH,P.J., RADHAKRISHNAN, S., PELLIZZON, M., MA, A. & LOCKSLEY, R. M. (2018).

A metabolite-triggered tuft cell-ILC2 circuit drives small intestinal remodeling. Cell, 174, 271-284. e14.

Serasanambati, M., & Chilakapati, S. R. (2016). Function of nuclear factor kappa B (NF-kB) in human diseases-a review. South Indian Journal of Biological Sciences, 2(4), 368–387.

Serradell M.C., Rupil L.L., Martino R.A., Prucca C.G., Carranza P.G., Saura A., Fernández E.A., Gargantini P.R., Tenaglia A.H., Petiti J.P., et al.(2019). Efficient oral vaccination by bioengineering virus-like particles with protozoan surface proteins. Nat. Commun. 10:361. doi: 10.1038/s41467-018-08265-9.

Serradell M.C., Gargantini P.R., Saura A., Oms S.R., Rupil L.L., Berod L., Sparwasser T., Luján H.D.(2018). Cytokines, antibodies, and histopathological profiles during *Giardia* infection and variant-specific surface protein-based vaccination. Infect. Immun;86:e00773-17. doi: 10.1128/IAI.00773-17.

Sher, A., Denkers, E. Y., & Gazzinelli, R. T. (2007).Induction and regulation of host cell-mediated immunity by Toxoplasma gondii. In Ciba Foundation Symposium 195-T Cell Subsets in Infectious and Autoimmune Diseases: T Cell Subsets in Infectious and Autoimmune Diseases: Ciba Foundation Symposium 195 (pp. 95-109). Chichester, UK: John Wiley & Sons, Ltd..

Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H., ... & Shao, F. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature, 526(7575), 660-665.

SINGH, J., SHARMA, S. & NARA, S. (2015). Evaluation of gold nanoparticle based lateral flow assays for diagnosis of enterobacteriaceae members in food and water. Food Chemistry, 170, 470-483.

Shirley, D. A., Hung, C. C., & Moonah, S. (2020). *Entamoeba histolytica* (Amebiasis). In Hunter's Tropical Medicine and Emerging Infectious Diseases (pp. 699-706). Content Repository Only!.

Shreiner, A. B., Kao, J. Y., and Young, V. B. (2015). The gut microbiome in health and in disease. Curr. Opin. Gastroenterol. 31, 69–75. doi: 10.1097.

Silver J.S., Hunter C.A. (2010). Gp130 at the nexus of inflammation, autoimmunity, and cancer. Journal of Leukocyte Biology 88 (6): 114.

Singer, M., Bulled, N., Ostrach, B., & Mendenhall, E. (2017). Syndemics and the biosocial conception of health. The lancet, 389(10072), 941-950.

Singer S.M., Fink M.Y., Angelova V.V.(2019). Recent insights into innate and adaptive immune responses to *Giardia*. Adv. Parasitol.;106:171–208. doi: 10.1016/bs.apar.2019.07.004.

Singh, A., Houpt, E., & Petri, W. A. (2009). Rapid diagnosis of intestinal parasitic protozoa, with a focus on *Entamoeba histolytica*. *Interdisciplinary perspectives on infectious diseases, 2009*.

Smith HV, Mank TG.(2011). Diagnosis of human *Giardiasis*. In: Lujan HD, Svard S, Eds. *Giardia* a model organism. New York: Springer-Verlag; Pp353-74.

Song N., Liu Z.S., Xue W., Bai Z.F., Wang Q.Y., Dai J., Liu X., Huang Y.J., Cai H., Zhan X.Y., et al.(2017). NLRP3 phosphorylation is an essential priming event for inflammasome activation. Mol. Cell.;68:185–197. doi: 10.1016/j.molcel.2017.08.017.

Stadelmann, B., Hanevik, K., Andersson, M. K., Bruserud, O., and Svard, S. G. (2013). The role of arginine and arginine-metabolizing enzymes during *Giardia*—host cell interactions in vitro. BMC Microbiol. 13:256. doi: 10.1186/1471-2180-13-256

Stanley, S. L. (2006). Vaccines for amoebiasis: barriers and opportunities. Parasitology, 133(S2), S81-S86.

Sutterwala FS, Haasken S, Cassel SL(2014). Mechanism of NLRP3 inflammasome activation. Ann N Y Acad Sci;1319(1): 82–95. pmid:24840700.

Swanson, K. V., Deng, M. & Ting, J. P. (2019). The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat. Rev. Immunol. 19, 477–489 .

T. Allain, E. Fekete, A.G. (2019) . Buret, Giardia Cysteine Proteases: The Teeth behind the Smile, Trends in Parasitology, 35 .636-648. DOI: 10.1016/j.pt.2019.06.003.

Takaoka, Motoko, Kyoko Suzuki, and Dan Norbäck.(2016) "Sick building syndrome among junior high school students in Japan in relation to the home and school environment." *Global Journal of Health Science* 8.2 :165.

Takeuchi, O. & Akira, S. (2010). Pattern recognition receptors and inflammation. Cell 140, 805–820 .

Tan, C. S., Read, J. A., Phan, V. H., Beale, P. J., Peat, J. K., & Clarke, S. J. (2015). The relationship between nutritional status, inflammatory markers and survival in patients with advanced cancer: a prospective cohort study. Supportive Care in Cancer, 23, 385-391.

Tilahun, M., Gedefie, A., Belayhun, C., Sahle, Z., & Abera, A. (2022). Helicobacter pylori pathogenicity islands and *Giardia lamblia* cysteine proteases in role of coinfection and pathogenesis. Infection and Drug Resistance, 21-34.

Varet, H., Shaulov, Y., Sismeiro, O., Trebicz-Geffen, M., Legendre, R., Coppée, J. Y., ... & Guillen, N. (2018). Enteric bacteria boost defences against oxidative stress in *Entamoeba histolytica*. Scientific reports, 8(1), 1-12.

Vermathen, M., Müller, J., Furrer, J., Müller, N., & Vermathen, P. (2018). 1H HR-MAS NMR spectroscopy to study the metabolome of the protozoan parasite *Giardia lamblia*. Talanta, 188, 429-441.

Vijay K.(2018). Toll-like receptors in immunity and inflammatory diseases: Past,present, and future. Int.

Immunopharmacol.;59:391–412.doi: 10.1016/j.intimp.2018.03.002.

Wang, Y. (2014). Innate immune modulation in atherosclerosis-with focus on ApoB100 derived danger associated signal 1 (ApoBDS-1). Inst för medicin, Solna/Dept of Medicine, Solna.

Wei C, Ma L, Chi H, Li L, Zhang S, Yin WH, et al(2020). The NLRP3 inflammasome regulates corneal allograft rejection through enhanced phosphorylation of STAT3. Am J Transplant. https://doi.org/10.1111/ ajt.16071 PMID: 32583615.

Willenborg, C. M., Červená, B., Thompson, P., Rosario, E., Ruaux, C., Vogelnest, L., & Šlapeta, J. (2022). *Giardia duodenalis* in a clinically healthy population of captive zoo chimpanzees: Rapid antigen testing, diagnostic real-time PCR and faecal microbiota profiling. International Journal for Parasitology: Parasites and Wildlife, 17, 308-318.

Yao X, Zhang C, Xing Y, Xue G, Zhang Q, Pan F, et al. (2017) Remodelling of the gut microbiota by hyperactive NLRP3 induces regulatory T cells to maintain homeostasis. *Nat Commun.* 8:1896. doi: 10.1038/s41467-017-01917-2.

Yuan Y.Y., Xie K.X., Wang S.-L., Yuan L.W.(2018). Inflammatory caspase-related pyroptosis: Mechanism, regulation and therapeutic potential for inflammatory bowel disease. Gastroenterol. Rep.;6:167–176. doi: 10.1093/gastro/goy011.

Zaidi, D., & Wine, E. (2018). Regulation of nuclear factor kappa-light-chain-enhancer of activated B Cells (NF-κβ) in inflammatory bowel diseases. Frontiers in Pediatrics, 6, 317.

Zaki, M. H., Boyd, K. L., Vogel, P., Kastan, M. B., Lamkanfi, M., & Kanneganti, T. D. (2010). The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity, 32(3), 379-391.

Zamboni DS, Lima-Junior DS.(2015). Inflammasomes in host response to protozoan parasites. Immunol Rev; 265(1): 156–71. https://doi.org/10.1111/imr.12291 PMID: 25879291.

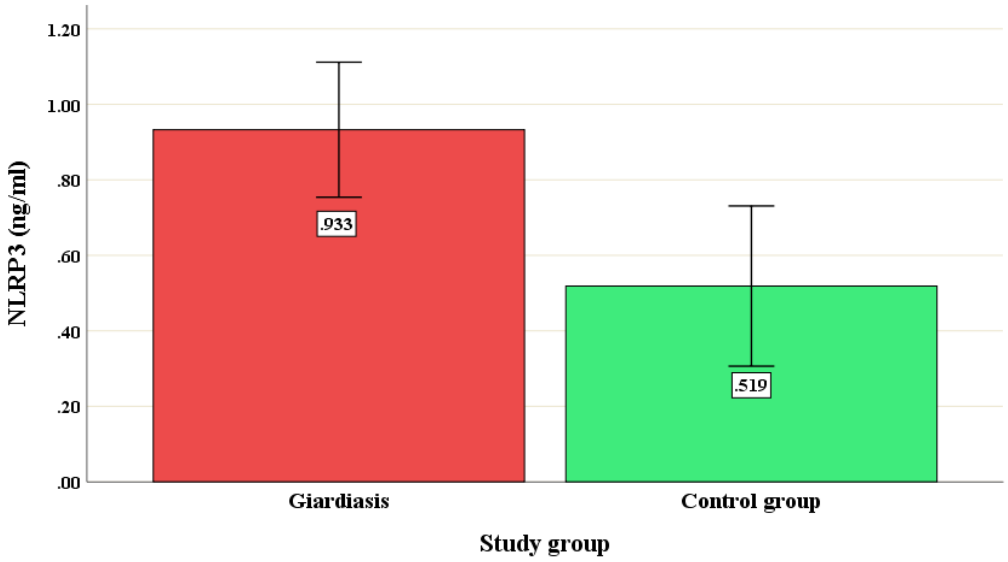
Zhang, Z., Le Yan,, Wang, L., Seydel, K. B., Li, E., Ankri, S., ... & Stanley Jr, S. L. (2000). *Entamoeba histolytica* cysteine proteinases with interleukin-1 beta converting enzyme (ICE) activity cause intestinal inflammation and tissue damage in amoebiasis. Molecular microbiology, 37(3), 542-548.

Zhao PP, Cao LL, Wang XC, Dong JQ, Zhang N, Li X, et al. (2021). Extracellular vesicles secreted by *Giardia duodenalis* regulate host cell innate immunity via TLR2 and NLRP3 inflammasome signaling pathways. PLoS Negl Trop Dis. 2021;15:e0009304.

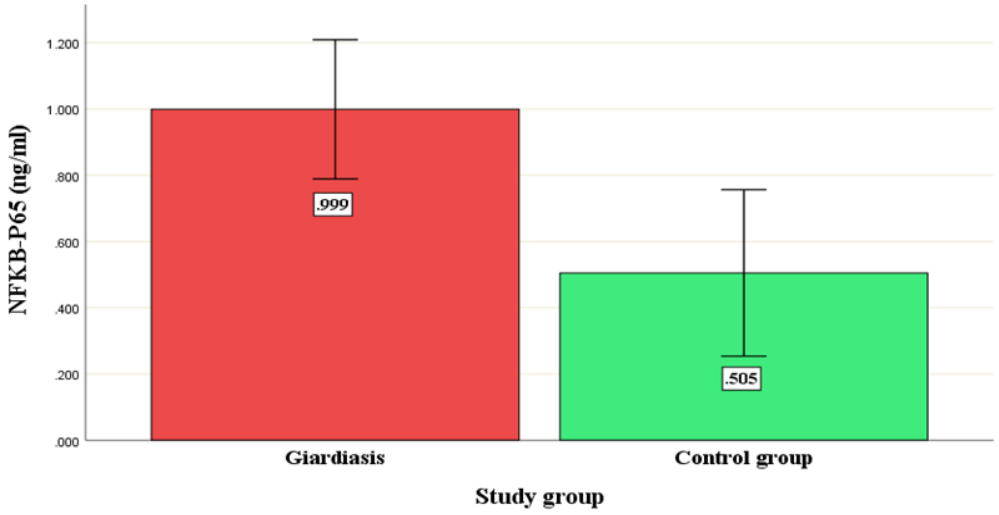
Zhou, L., & Shine, H. D. (2003). Neurotrophic factors expressed in both cortex and spinal cord induce axonal plasticity after spinal cord injury. Journal of neuroscience research, 74(2), 221-226.

Zhu S, Ding S, Wang P, Wei Z, Pan W, Palm NW, et al. (2017) Nlrp9b inflammasome restricts rotavirus infection in intestinal epithelial cells. *Nature*. 546:667–70. doi: 10.1038/nature22967.

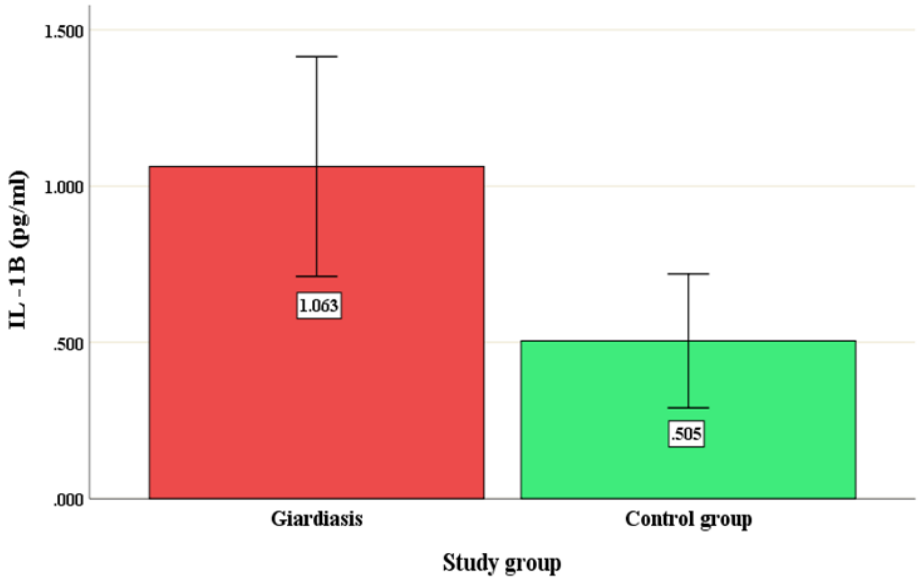
Appendix



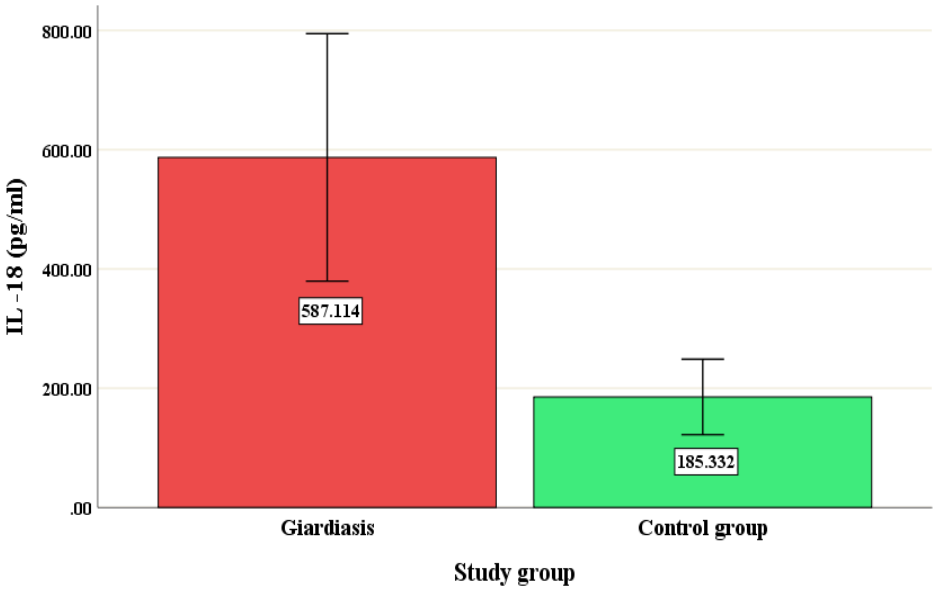
Appendix(1): The mean differences of NLRP3 (ng/ml) according to study group



Appendix(2) :The mean differences of NF-κB (ng/ml) according to study group .



Appendix(3) :The mean differences of IL- 1β (pg/ml) according to study group.



Appendix(4): The mean differences of IL- 18 (pg/ml) according to study group.

الخلاصة :

تضمنت هذه الدراسة 150 عينة براز تم جمعها من مرضى الإسهال الوافدين إلى مستشفيات مدينة الحلة (مستشفى الإمام الصادق ، مستشفى بابل للولادة والأطفال ، مستشفى النور للأطفال ، مستشفى علي عبيس بالمدحتية) ، وأظهرت نتائج هذه الدراسة من ايلول 2022 الى كانون الثاني 2023ان النسبة الإجمالية للإصابة بجرثومة *G.lamblia* *بلغت* 15.3٪ ، كما أظهرت نتائج هذه الدراسة أن نسبة الإصابة بجرثومة *G.lamblia* كانت أعلى عند الإناث 52.2٪ مقارنة بالذكور 47.8٪ ، حيث أظهرت النتائج أن الإصابات بالجيارديا لامبليا أعلى نسبة 39.1٪ للفئة العمرية 1-5 سنوات ، وكانت نسبة الإصابة بجرثومة *اللامبليا* أعلى في الريف 65.2٪ منها في الحضر 34.8٪.

كما أظهرت النتائج ارتفاع مستويات NLRP3 في الفئة العمرية 21-30 سنة مقارنة بمجموعة السيطرة ، وازدادت معنويا $p < 0.05$ وأظهرت ارتفاع مستوى NLRP3 في الفئة العمرية (6-10 (/) 21-30) سنة مقارنة مع مجموعة السيطرة ازدادت معنويا $p \leq 0.05$ بينما انخفض مستوى NFKB في الفئة العمرية 16-20 سنة مقارنة بمجموعة السيطرة ولم تكن النتيجة معنوية.

أظهرت النتائج ارتفاع مستويات IL1B لدى مرضى *G.lamblia* في الفئة العمرية 21-30 سنة مقارنة بمجموعة السيطرة ، وزيادة معنوية $p < 0.05$. وأظهرت مستويات عالية من IL18 في الفئة العمرية 6-10 سنوات مقارنة بمجموعة السيطرة وكانت النتيجة معنويا $p \leq 0.05$.

أظهرت الدراسة الحالية وجود علاقة إيجابية بين IL-18 و NLRP3 (N = 23 ، r = 0.263 ، p = 0.226). وكان هناك ارتباط إيجابي بين IL-18 و NFKB-P65 (N = 23 ، r = 0.358 ، p = 0.093). . وكان هناك ارتباط إيجابي بين IL-1β و NLRP3 (N = 23 ، r = 0.205 ، p = 0.347) وكان هناك ارتباط إيجابي بين IL-1β و NFKB-P65 (N = 23 ، r0.209 ، p = 0.338) .

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية الطب

المستقبل Nod Like Receptor p3 الالتهابية وبعض السيتوكينات المنشطة للالتهابات خلال داء الجيارديا

رسالة

مقدمة الى مجلس كلية الطب /جامعة بابل

كجزء من متطلبات نيل شهادة الماجستير في العلوم/ الاحياء المجهرية الطبية

تقدمت بها

زينب محمد جواد جاسم

(بكالوريوس / علوم الحياة / جامعة بابل (2015)

بإشراف

أ.د. هيام خالص المسعودي

1444 هـ 2023 م