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Epidemiology of Some Intestinal Parasites and Molecular Study of Some Immunological and Physiological Parameters for Peoples Infected With *Entamoeba histolytica*

A thesis

Submitted to the Council of the College of Science, University of Babylon, in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biology/Zoology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Dedication

The sake of Allah, my Creator and my Master, My great teacher and messenger, Mohammed (May Allah bless and grant him), who taught me the purpose of life,

To my family and children

To my sisters

To my friends and colleagues

To all who help me

Aseel

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Summary

This study was carried out for the period from February 2021 till January 2022 to determine some hematological, immunological and physiological parameters and their relationship to intestinal parasitic infection in hospitalized and visiting children in the holy Karbala province of . Additionally, it was investigated the single nucleotide polymorphism in relation to immunological and physiological markers. Children aged (1 – 15) years .Using the direct smear technique and rapid test, a total of 3,748 feces samples were evaluated.

The study indicated that 13% of all intestinal illnesses were caused by parasites. 14% of males were afflicted whereas 11.7% of females were infected. *Entamoeba histolytica* (10.54%), *Giardia intestinalis* (2.64%), *Cryptosporidium* spp (0.4%), *Hymenolepis nana* (0.24%), *Enterobius vermicularis* (0.13%), and *Trichomonas hominis* (0.03%) were identified as intestinal parasites in the present investigation. The result showed the incidence of the individual intestinal parasite was single and double infected and triple intestinal infection.

The largest proportion of intestinal parasite infections were observed in September 2022 (30.1%), while the lowest rate were recorded in February 2021 (3.5%). In addition, the age range (5-10) years had the greatest prevalence of intestinal parasite infections, at 17.2%. To undertake the hematological, immunological, and physiological investigation, 48 *E. histolytica* infected children and 41 healthy children were chosen.

Among children infected with *E. histolytica*, the hematological parameters analysis revealed a substantial rise ($p \leq 0.05$) in WBCs,

Neutrophils, and Lymphocytes, and a significant reduction ($p \leq 0.05$) in Hb, as compared to the healthy group.

For the infected group, the immunological and physiological analysis revealed a substantial ($p \leq 0.05$) increase in the levels of Adiponectin, resistin, and IL-18 in *E. histolytica* infected children . Also there are a significant increase in the level of Adiponectin, and resistin in both males and females .

Depending on the statistical results, no differences were observed between the different age groups for both study groups. Except for IL-18 and resistin, the connection between biomarker levels in infected individuals was not statistically significant. The result showed the distribution of Genotype of IL18 single-nucleotide polymorphisms in intestinal parasites patient in contrast to the control group. This indicated that the mutation in the polymorphism of SNP 1 (rs1866694757), SNP 4 (rs 1946518), SNP 6 (rs1946519), and SNP 7 (rs 1215648807) were demonstrated a risk factor of intestinal parasites in the patient's group than in control group, (OR = 1.333, 1.800, 1.200 and 1.750; 95% confidence interval = (0.321- 5.538), (0.264-12.296), (0.396-7.732) and (0.396-7.732) respectively. While, in the genotype polymorphism of SNP 2 (rs 940255648), SNP 3 (rs 1037707423), SNP 5 (rs1213044637), SNP 8 (rs 1866697972), SNP 9 (rs 1866698066) and SNP 10 (rs1866698286) were showed a protective factor (OR = 0.364, 0.308, 0.955, 0.500, 0.393, 0.073; 95% confidence interval = (0.084- 1.583), (0.052- 1.829), (0.235- 3.878), (0.101-2.477),(0.081-1.909) and (0.008-0.689) respectively. Interestingly, only the genotype of SNP 10 (rs1866698286) AA (Wild type) / AT (Mutant heterozygous) was shown a statistically significant difference for patients group.

The polymorphism of SNP1 (rs1862513), SNP2 (rs567367264), and SNP3 (rs536392382) demonstrated a risk factor of intestinal parasites in patients group than in the control group, (OR = 2.068, 1.360 and 1.360; 95% confidence interval = (0.477- 8.973), (0.344- 5.379) and (0.344-5.379) respectively. While, in the genotype of resistin polymorphism of SNP 4 (rs2032442393) showed a protective factor (Odd Ratio = 0.952; 95% CI = (0.056- 16.279)). There were no significant differences across the Genotype of resistin polymorphisms.

Although IL-18 gens polymorphisms were never shown any statistically significant differences with any of the gene polymorphisms SNPs *(* $p \leq 0.05$). there are an increasing in the level of IL-18 in SNPs (SNP1/CA, SNP2/GC, SNP3/CT, SNP5/GA, SNP7/AC, SNP8/GT, and SNP9/GT) compare with wild type, respectively and (SNP4/TG/GG,SNP6 AC/CC), compare with wild and hetero type respectively, and (SNP10 TT/AC)compare wild and hetero type respectively. Resistin concentration was statistically significantly different between Resistin gene polymorphisms, SNP 1 and SNP5. There were significant increases from SNP 1 (rs1862513) CC to CG , and from SNP 1 (rs1862513) CG to GG , and was a statistically significant difference between resistin level and SNP4 (rs2032442393). LSD post hoc test revealed that there was a difference in the resistin concentration in the SNP 4 (rs2032442393) from CC (Wild type) to CT (Mutant heterozygous), $p = .019$), while no statistically significant difference with the rest of the Resistin gene SNPs ($p \leq 0.05$).

Infection with intestinal parasites, particularly *E. histolytica*, has a substantial effect on the majority of blood, immunological, and physiological parameters in infected children, according to the findings of this research.

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List of abbreviations

Abbreviation	Meaning
A	adenine
Ab	antibody
AMP	adenosine monophosphate
AMPK	adenosine monophosphate kinase
AML	acute myeloid leukemia
APN	Adiponectin
B lymphocytes	Bursa-dependent cells
Ba	Basophil
BAT	brown adipose tissue
BMC	Bugando Medical Centre
BMI	Body mass index
C	cytosine
cAd	collagenous domain
CAD	coronary artery disease
CCL2	c-c motif chemokine ligand 2
CD3	Cluster of differentiation 3
CD4	Cluster of differentiation 4
CD81	Cluster of Differentiation
CRP	C reactive protein
DCs	dendritic cells
DNA	Deoxy ribonucleic acid
DSS	dextran sulfate sodium
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	enzyme-linked immune sorbent test
Es	Eosinophils
FasL	Fas ligand
FcεRI	high-affinity IgE receptor

G	guanine
gAd	carboxy-terminal globular domain
GI	gastrointestinal
GWA	genome-wide association
Hb	Hemoglobin Concentration
HD	hemodialysis
HMW	high-molecular-weight
HrCLM	hookworm-related cutaneous larva migrans
HRP	Horseradish peroxidase
IBD	Inflammatory bowel disease
ICAM1	like intercellular adhesion molecule1
IECs	Intestinal epithelial cells
IFN-γ	interferon-γ
IgE	Immunoglobulin E
IKB	I kappa B kinase
IL- 22	Interleukin-22
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-18	Interleukin-18
IL-18R	Interleukin- 18 receptor
IL-33	Interleukin- 33
IL-36	Interleukin-36
IL-6	Interleukin-6
ILCs	innate lymphoid cells
INF-a	Interferon- a
INF-γ	Interferon - γ
IPIs	intestinal parasite infection
IRF-1	interferon regulatory factor 1
KA	Kala Azar
LPS	lipopolysaccharide

LTA	Lipoteichoic acid
LTi	lymphoid tissue inducer
Ly	Lymphocytes
MAPKs	mitogen-activated protein kinases
MCH	Mean Corpuscual Hemoglobin
MCHC	Mean Corpuscual Hemoglobin Concentration
MCV	Mean Corpuscual Volume
Mo	Monocytes
mRNA	Messenger ribo nucleic acid
Mtb	*Mycobacterium tuberculosis*
NET	neutrophil extracellular traps
Neu	Neutrophil
NF-B	nuclear factor-B
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK cells	natural killer
OD	Optical density
ORs	odds ratios
PBMC	peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PCV	Packed Cell Volume
PLT	Platelets
PMN	Polymorphonuclear leukocyte
PRBCs	packed red blood corpuscles
RA	rheumatoid arthritis
RBCs	Red blood cells
RegIIIg	Regenerating islet-derived protein III-gamma
RELM	resistin-like molecule
RETAN	Resistin
RSM	recurrent spontaneous miscarriage
RT-PCR	reverse transcription-polymerase chain reaction

RV	rotavirus
SD	standard deviation
SLE	systemic lupus erythematosus
SNPs	single nucleotide polymorphisms
STHs	soil-transmitted helminthes
T	thymine
T lymphocytes	Thymus dependent cells
TBE buffer	Tris base,boric acid and EDTA
TBI	Tuberculosis
TG	triglycerides
Th1	T helper1
TH17	T helper 17 cell
TH22	T helper 22 cell
TLC	total leucocytic count
TLR	Toll-like receptor
TNF	Tumer necrosis factor
TNFa	Tumer necrosis factor alfa
TNF-R	Tumer necrosis factor recptor
TREG	Regulatory T cells
TRL	triglyceride-rich lipoprotein
UC	ulcerative colitis
UTR	untranslated region
WAT	white adipose tissue
WBC	White blood cells

Chapter one

Introduction

1-1:Introduction

Intestinal parasitosis continues to be a global public health problem because to its high prevalence in numerous countries and its nutritional repercussions. The contribution of intestinal parasites to morbidity and mortality, as well as the etiology of other infectious diseases, varies by species. Intestinal parasite infection is particularly prevalent in school-aged children and tends to result in severe infections in this age range (Hama, and Rahemo, 2014). Intestinal parasites are divided into two major types, they are helminths and protozoa. Protozoa are unicellular organisms and belong to the Protista kingdom and can reproduce in the human body which can allow forming of serious infection (Steele-Ogus *et al*., 2022).

Ascariasis, trichuriasis, and hookworm infection (necatoriasis, ancylostomiasis) are the most prevalent human infections. Schistosomiasis and lymphatic filariasis are the next most prevalent (Quinzo *et al*., 2022). Human intestinal parasitic infections are amongst the highest common infections in the world and are dependable for substantial morbidity and mortality, Infection with intestinal parasites are related to malnourishment, mental role, verbal capability, physical faintness, inhibiting of lined growth and lower learning attainment in school children with malnourishment. Anorexia, malabsorption, weight loss, malnutrition, and anemia are correlated with intestinal parasites, There exists a synergistic association between diarrhea and malnutrition. Diarrhea may have a negative effect on nutritional status (Hajare *et al*., 2021).

The parasites have many antigens because the body structure is complex and they can evade the immunity of the host by inhibiting the

host's immune response by different ways, such as changing their surface antigen, encysted, and migration (Schmid-Hempel, 2009).

Entamoeba histolytica, Giardia intestinalis, and Cryptosporidium spp. are three of the most prevalent protozoan diseases that cause diarrhea. *E. histolytica* is liable for diarrhea, Amebic colitis, liver abscess, and anemia, and it may have an effect on newborn development, At least 50 million individuals suffer from invasive amebic infection yearly, resulting in 40,000–100,000 fatalities annually, according to estimates (Hegazi *et al*., 2013).

Intestinal mucosal infection by parasites stimulates lymph nodes close to adipose tissue in the mesentery, so once the lymph nodes are active, the adipocytes release the so-called adipocytokines to provide energy for the lymph nodes' activity. Adiponectin has a function in energy homeostasis management, Adiponectin affects the control of glucose and the oxidation of fatty acids, it is prevalent in plasma and is released into the circulation by adipose tissue, Infection with some forms of intestinal parasites (*E. histolytica* and Strongyloides) may deregulate the release of adiponectin and impair the absorption of certain nutrients, which can disrupt the body mass index (BMI) and lead to anorexia. Anorexia caused by parasites is an acute phase reaction to infection (Yahya *et al*., 2018).

Resistin is one of the adipokine, mainly produced by macrophages and detectable in human serum. It is a cysteine-rich peptide with different biological effects and initially proposed to regulate obesity, glucose metabolism and insulin sensitivity. Some researchers reported that human resistin may also play a major role in regulating inflammation. Resistin expression has been identified in the non-adipocyte stromal vascular parts of

white adipose tissue moreover, inflammatory bowel disease (IBD) is typically characterized by malnutrition, body composition changes and mesenteric white adipose tissue hypertrophy(Deng *et al*., 2020; Ali *et al*., 2021).

Intestinal epithelial cells, macrophages, and dendritic cells at infection sites release interleukin-18, a key proinflammatory cytokine in the management of Cryptosporidium infection. Although the cytokine is associated with the pathology of numerous diseases, it has been demonstrated that IL-18 produced by IECs is essential for maintaining epithelial integrity during inflammation. It has been shown that IL-18 inhibits parasite reproduction in human intestinal cell lines and that the cytokine increases IEC production of a -defensin that inactivates *C. parvum* sporozoites (Choudhry *et al*., 2012).

CD4+ T cells, most notably TH17 and TH22 cells, CD8+ T cells, natural killer (NK) cells, lymphoid tissue inducer (LTi) cells, and innate lymphoid cells(ILCs) are capable of producing IL-22. Through the creation of antimicrobial peptides, the promotion of epithelial regeneration, and the control of wound healing, these actions of IL-22 are especially significant in regulating inflammatory responses inside the gut. Several animal models of colitis generated by dextran sulfate sodium (DSS) as well as TH1- and TH2-mediated colitis have been used in recent research to examine potential protective roles for IL-22 in IBD (Leung & Loke, 2013)a.

The fundamental PCR concept is straightforward. It is, as the name suggests, a chain reaction: One DNA molecule gets replicated twice, then four times, then eight times, and so on. Specific proteins known as polymerases, enzymes capable of stringing together individual DNA

building pieces to produce lengthy molecular strands, do this constant doubling. Polymerases need a supply of DNA building blocks, i.e. the nucleotides consisting of the four bases adenine (A), thymine (T), cytosine (C), and guanine (G), in order to perform their function.Additionally, they need a primer, a tiny segment of DNA to which they connect the building blocks, and a longer DNA molecule to act as a template for generating the new strand. If all three components are present, the enzymes will replicate the templates exactly. PCR is a technique used to obtain multiple copies of a certain nucleic acid strand (Joshi, 2010).

Genome-wide patterns of variation between people are the most potent source of information for elucidating the history of human migration, expansion, and adaptation. With the advent of modern technologies that can type millions of single nucleotide polymorphisms (SNPs) in a single experiment, SNP in genome-wide association (GWA) test has become a smart endeavor. SNPs are the most prevalent kind of sequence variation in genomes and are regarded as useful genetic markers for exposing the evolutionary history and common genetic variants that explain the heritable risk for common illness (Fareed & Afzal, 2013).

1.2. The Aim of the Study

The current study aimed to detectction the effect of intestinal parasites infections on some physiological and immunological parameters(Complete blood count, Adiponectin, Resistin, IL-18 and IL-22) This aim has been achieved through the following objective:-

1-Determining the species of intestinal parasites and their prevalence of infection in patients attending health institution in the holy Kerbala province.

2-Studying the effect of the most prevalent intestinal parasites on some physiological and immunological parameters (Compelet blood count, Adiponectin, Resistin, IL-18, IL-22).

3-Finding the correlation between the studied parameters.

4-Investigating of gene polymorphism of the parameters most susceptible to parasitic infection.

Chapter Two

Literatures Review

Literatures Review

2-1:Intestinal parasites

Parasites in the intestines pose a major threat to public health, especially in tropical and subtropical regions, it is believed that 3.5 billion individuals are infected with some form of this disease, the protozoa *E. histolytica*, *G. intestinalis*, and *Cryptosporidium* species are the most frequent human intestinal parasites, although other parasites including *Ascaris lumbricoides*, *Ancylostoma duodenale, Trichuris trichiura, and Schistosoma* spp. were also found responsible to cause diarrhea in human (Turki *et al*., 2017).

The incidence of intestinal parasites varies greatly from one region to another for several reasons, including but not limited to: topography, climate, economics, diet, population density, hygiene practices, and general health. In addition, the availability of clean water is limited in areas where intestinal parasites thrive, and people in poor health also contribute significantly to the spread of these parasites (Al-Hasheme *et al*., 2020).

Infection with even one of these intestinal parasites may significantly lower a child's resistance to illness and increase their vulnerability to further illnesses, mortality due to protozoan intestinal parasite infections is very uncommon, but the associated morbidity and indirect effects may be devastating. Diarrhea, dysentery, and nausea and vomiting are among gastrointestinal symptoms that might arise (Butera *et al*., 2019).

Intestinal parasite infections, which are more common in developing countries, are a major public health problem, infectious parasites can be distributed through skin penetration, water consumption, and soil or food contaminated by feces containing infective stage. The parasites' clinical

features on humans are typically asymptomatic, yet they operate as carriers and sources of infection within their hosts (Derso *et al*., 2021).

The intestinal protozoa live in the human intestine's lumen and are extracellular parasites like *G. intestinalis*, while Sporozoa, which habitate in the cells that line the intestine and are intracellular parasites. Coccidia is a group of parasites that are spread in animals and have particular importance (Al-Taei, 2019).

Iron deficiency anemia, growth retardation, and gastrointestinal problems like diarrhea, nausea, vomiting, and abdominal discomfort can be caused by Intestinal Parasite Infection (IPI) in children and people with compromised immune systems. IPIs can also contribute to numerous co-infections like *Mycobacterium tuberculosis* and *Helicobacter pylori*, and their effects extend beyond morbidity and mortality to include nutritional problems and psychological distress (Al-Abodi, 2018; Assemie *et al*., 2021 Barati *et al*., 2021).

2-1-1: Prevalence of Intestinal Parasites Infections

Malnutrition, linear growth stunting, physical and mental health problems, and iron deficiency anemia are just some of the many health problems that have been linked to intestinal parasitic infections, making them a major public health concern in many countries, especially among children under the age of 12 years (Brown *et al*., 2012).

2-1-1-1: Intestinal Parasites Infection in World

A total of 3152 stool samples from Tanzania were submitted to the Department of Medical Parasitology at BMC for examination (1887 in 2008, 963 in 2009, and 302 in 2010); of these, intestinal parasitic infections were isolated from 57.1% (1799/3152) of the samples. Hookworm eggs were found in 25.2% (793/3125) of the samples, and *S. mansoni* was found in 5.6% (177/3125) of the samples; overall, helminth eggs were discovered in 36.6% (1,153/3,152) of the samples. 25% (646/3152) of the samples had protozoan parasites; 13.6% (428/3152) contained *E. histolytica/E. dispar*, and 6.9% (218/3152) contained *G. intestinalis* (Mazigo *et al*., 2010).

In Ujjain medical facilities. Over the course of five years, a total of 5990 stool samples were obtained (3,580 from Ujjain Charitable Trust Hospital (urban population) and 2,410 from R. D. Gardi Medical College) (Rural population). The among all people, 21.4% had the parasite in their intestines. The most prevalent protozoa were *E. histolytica* (10.5%) and *G. intestinalis* (3.9%). *Ascaris lumbricoides* (2.8% prevalence) was the most common. 70% of samples had multiple infections. The prevalence was lower among the urban (20.2%) than the rural (23.1%) population. The largest incidence of infection was seen in children less than 10 years old, with a prevalence of 27.4% among females compared to 18.2% among males (Yogyata & Binita, 2011).

In Surat, India, 298 children aged from (6 – 12) years were enrolled to determine the prevalence and related variables in hospitalized children intestinal parasites were found to be present in 8.7% of hospitalized

youngsters. The parasite *G. intestinalis* was found to be the most frequently (5.4 %), followed by *A. lumbricoides* (1.3 %) (Brown *et al*., 2012).

At least one intestinal parasite was detected in 342 (or 39.9%) of the patients investigated in the Gamo region of South Ethiopia, There were a total of 98 (11.4%) cases of *E. histolytica/dispar*, followed by 91 (10.6%) cases of *G. intestinalis*, 67 (7.8%) cases of *A. lumbricoides*, 51 (5.7%) cases of *Strongyloides stercoralis*, 42 (4.9%) cases of hookworm, 24 (2.8%) cases of *Taenia* spp. 18 (2.1%) cases of Taenia species, 7 (0.6%) cases of *Hymenolepis nana* (Wegayehu *et al*., 2013).

In Primary School Nepal it was found that 18% of the 163 kids tested were infected with parasites. The most common parasite was *E. histolytica* (61.0%), next *T. trichuria* (22.0%), and finally Hookworm, *Ascaris/Giardia*, *T. trichuria/E. histolytica* (6.0%) (Jaiswal *et al*., 2014).

Intestinal parasite surveys were done in several parts of Ethiopia, with an emphasis on school-aged children. As a result, there is data of the prevalence of intestinal parasites among Kindergarten students. In this study, the overall prevalence of intestinal parasite infection was 29.3 %. *H. nana* was the most common protozoan and helminthic parasite found (8.75%), followed by *G. intestinalis* (5.75%), *E. histolytica* (5%), *Enterobius vermicularis* (5%), and *A. lumbricoides* (4 %). The least common species were hookworms and *S. mansoni* (Alemu *et al*., 2015).

About 385 students, ages (8-14) years representing eight schools in the Plateau Central and Centre-Ouest areas of Burkina Faso, were polled in 2015. Stool samples were processed with the Kato-Katz and a formalin-ether concentration procedure for the diagnosis of helminths and intestinal

protozoa infections, the prevalence of intestinal parasite infections were high; helminthes infections were discovered in 10.7% of children and intestinal protozoa were defected in 84.7% of children (Erismann *et al*., 2016).

To find out common intestinal parasites among Santarem schoolchildren, as well as the risk factors connected with them in the city of Santarém, Pará State, Brazil. Intestinal parasite sample analysis found that 189 (51.5%) of the 367 fecal samples had some protozoan infection and 59 (16%) had helminthes infection, a prevalence of 67.5 % in intestinal parasite sample analysis *Entamoeba coli* was the most prevalent protozoan (20.4%), followed by *E. histolytica* (13.9%), and *G. intestinalis* (13.3%), *A. lumbricoides* was the most common helminths (9.0%), followed by *T. trichura* (2.1 %). Single infections were more common (51.6%) than double mixed infections (4.6%) and multiple mixed infections (2.4 %) (Banhos *et al*., 2017).

During the same time frame (August 2017–October 2017), another research included a total of 205 people from Anakaputhur's Urban Health Training Centre and 185 people from Padappai's Rural Health Training Centre, both located in the Kancheepuram district. *E. histolytica* (40%), *A. lumbricoides* (2%), *Ancylostoma duodenalae* (5%), *G.intestinalis*(1%) and *T. trichura* (3%) were the most common intestinal parasites found in 185 stool samples from the rural community. In contrast, 23.4% of intestinal parasites were found in 205 stool samples from the urban population *E. histolytica* was 18%, *A. lumbricoides* 2%, *A. duodenalae* was 5% (Mareeswaran *et al*., 2018).

Butera *et al*. (2019) did a cross-sectional study in a rural part of Rwanda's Rutsiro district to investigate the prevalence and risk factors of intestinal parasites in children under the age of two; they found that 44.8% of the infants were infected with parasites. *A .lumbricoides* was the most frequent parasite, affecting 28.5% of people, followed by *E. histolytica* (24.95%), and *G. intestinalis* (19.6%). Multiple infectious agents, including *Ascaris* and yeasts (8.9%), were found to be present in the infected individuals.

The incidence of intestinal parasite infections in Bulgarians was studied by Harizanov *et al*.(2020). This research included the years 2015-2017. Those living in Bulgaria at the time of the research were all required to undergo testing for intestinal protozoa and helminths. There were a total of 23,785 illnesses found, with 17,712 (or 74.47%) being caused by helminths and 6,073 (or 25.53%) being caused by protozoa. Those infected with intestinal helminths were more likely to have Enterobiasis (81.75%), whereas patients infected with protozoans were more likely to have Giardiasis (62.05%).

In contrast, a total of 63 children out of 283 tested positive for having parasites in their intestines in a research done in Tehran, Iran. 21.55% (61/283) of the people had intestinal protozoa, while 0.70% (2/283) had helminth parasites. The most common protozoan parasite was *Giardia duodenalis* (20 people, 7.06%). 26 people (7.06%) positive for human (*Blastocystis hominis*), 12 people (4.2%) positive for *Microsporidia,* five people (1.7%) positive for *Cryptosporidium* spp., and four people (1.4%) positive for *Entamoeba*. Both of the two people who positive for helminth infection had *H. nana* (Barati *et al*., 2021).

2-1-1-2:Prevalence of intestinal parasite infections in the Arab World countries.

Intestinal parasite infection was shown to be prevalent in the Jenin province (Northern Palestine) population research, with prevalence ranging from 32.0-41.5 % over 10-year age group. There are at least seven parasites that can be detected, *E. histolytica* (8.2-18.2%), and *E. vermicularis* (15.6-28.9%) were the most prevalent pathogenic parasites found, And other *G. intestinalis*, *A. lumbricoides*, *S. stercoralis*, *Taenia* sp. and *A. duodenale* (Hookworms) are also present (Bdir & Adwan, 2010).

The high frequency of intestinal protozoan infections among patients seeking medical treatment in Sanaa, Yemen, has also been the subject of a cross-sectional research. 39% of patients had protozoan infections in their intestines. Infection rates for *Cryptosporidium*, *G. duodenalis*, and *E. histolytica/dispar* were 1%, 17.1%, and 17.7%, respectively. Parasites such as *S. mansoni* (0.3%), *H. nana* (1.4%), and *E. vermicularis* (0.4%) may also be discovered (Alyousefi *et al*., 2011).

64% of persons in the Elengaz Area of Khartoum, Sudan, were found to have intestinal parasite infections in an epidemiological investigation. The most common parasites in the intestines were *G. intestinalis*, *H. nan*, *T. saginata*, *E. vermicularis*, *S. mansoni*, and *E. histolytica*. Most of the sick children had two parasites (Gabbad & Elawad, 2014).

120 foreign employees, 47.5% male and 52.5% female, participated in a survey research by Imam *et al*.(2015) to investigate the prevalence of parasite infection among international workers in Madina, Kingdom of Saudi Arabia. 53 cases (44.2%) were confirmed to have intestinal parasites.

Many samples had multiple infections from the same parasite, with the overall number of infections reaching 69 (57.5%). *T. trichiura* (5.6%), *S. stercoralis* (5.6%), are the most common parasites, followed by *E. histolytica* (27.5%), *G. intestinalis* (18.8%), hookworm (14.5%), *A. lumbricoides* (11.6%), Moreover, eggs of *E. vermicularis*, *S. mansoni*, and *Taenia* were found in 1.4% of the all cases.

Among the 29,286 persons in Qatar who were sent to Hamad Medical Corporation for a stool analysis between 2005 and 2014, the prevalence of intestinal protozoan infections were analyzed. *Blastocystis hominis* was the most common kind of protozoan found (3.45%), *G. duodenalis*, *Chilomastigo Mesnili, E. coli*, *E. hartmanni*, *E. nana*, *Iodamoeba butschlii*, *E. histolytica/dispar*, *Cryptosporidium sp.* and a single case of *Isospora* were also detected (Abu-Madi *et al*., 2016).

Epidemiological study of the intestinal parasite among selected group of primary school children in Alhag Yousif Area, Khartoum, Sudan in 2017, the result showed that *G. intestinalis* was found to be the most common among schoolchildren (46.4%), followed by *H. nana*(19%).While *A. lumbricoides* and *E. vermicularis* had the lowest prevalence (1.2%) (Siddig *et al*., 2017).

A survey in 2019 on the frequency of intestinal parasites among native Libyans attending various health centers (both public and private) in the Zawia State of Libya has been done. A total of 12,850 patients had their stools examined, 8950 were found to be infected with intestinal parasites (69.6%) of the indigenous population was infected. Females were infected at a higher rate than males(65%) (34.9%) respectively. Overall, protozoa

infections outnumbered helminth infections represented by (92.4 %) (7.6 %). *E. histolytica* (60.70%) and *G. intestinalis* (31.73%) and among the helminths, *A. lumbricoides*, *T. trichiura* and *H. nana* were the frequent ones (Shawesh *et al*.,2019).

Research conducted in Yemen found that among school-aged children, intestinal parasitic infection was prevalent (62.7%), with single infections being more common (85.1% and 14.36%) than multiple infections. Also, Protozoa infected 85.64 % of the people, whereas helminths infected 14.36 %. *E. histolytica* was found in 61.70 % of cases, *G. intestinalis* in 23.94%, *A. lumbricoides* in 7.45 %, *H. nana* in 4.%, and *E.vermicularis* in 2.61 %. Moreover the highest frequency in this area *E. histolytica*, *G. intestinalis A. lumbricoides* were found in a group of 9-12 year olds. *H. nana* was between the ages of 9 - 12, and between the ages of 13-16. *E.vermicularis* was found in children aged 5-8 and 9-12 Furthermore, female were infected at a rate of 69 %, which was much greater than males' 54.55 % infection rate (Qasem *et al*., 2020).

Another study, by Soliman *et al*.(2021) in Benha city, Egypt showed that the frequency of IPIs were 12.7%, with 7.9% of double infections. Protozoan infections accounted for 47.4 % of all infections, compared to 44.7 % for helminthic infections. The most prevalent protozoan was *G. duodenalis*, and the most common worm was *E. vermicularis.*

The prevalence of intestinal parasites, namely *G. intestinalis*, and their clinical manifestations in Algerian children and adults were investigated as part of an epidemiological investigation. Parasite infection rates were found to be 28% overall. Most of the 567 parasite-positive samples included

Blastocystis (57.3%), then *Endolimax nana* (41.0%), *E. histolytica/dispar* (19.6%), *G. intestinalis* (17.1%), *E.coli* (13.9%), *C.mesnili* (1.0%), *I. butschlii* (0.7%), *E. hartmanni*(0.5%),and *Cryptosporidium* spp (Belkessa *et al*., 2021).

From a total of 9,653 analyzed stools in a recent research in Kuwait, 74 stool samples positive for either *G. intestinalis* or *Entamoeba* sp. There was no association between this and the high incidence of intestinal parasite infections in Kuwait, and the rate of infection was just 1%. low rates of intestinal parasitic infection throughout the two governorates of Kuwait reflecting no statistical relevance to the distribution of the examined parasites in Kuwait were found, however, when comparing infection rates between seasons (Alayyar *et al*., 2022).

2-1-1-3: Intestinal Parasite Infection in Iraq

Intestinal parasites are one of the major problems that Iraqi society suffers from, especially children, because of its effect on their health Therefore, many studies have been conducted on this topic, and below is a summary of these researches and studies.

In 2010 the study was part of the routine diagnostic work carried out in Kerbala hospital for children during the period from March to June 2009**.** A total of 277 stool samples were tested, with a total infection percentage of 11.6 %, The age range of 2-4 years had the largest number of cases (38.5 %). There were no significant infections in both males and females. *E. histolytica* and *G. intestinalis* were parasites that have the largest infection percentage (4.0 % and 6.5 %,respectively) *C. mesnili* (0.7%) *Trichomonas*

hominis (1.1%) and only one type of helminths *H. nana* (0.4%) (Hasan, 2010).

While another study was conducted by Ibrahim (2012) to investigate the prevalence of *E. histolytica* and *G. intestinalis* in Kadhmiyah hospital for the period between September to December 2010, In this study 1520 stool sample were collected and examined , the result showed that the number of a patient infected with *E. histolytica* were 149 (9.80%) more than in *G. intestinalis* 27 (1.77%). In addition to that males are more infected with *E. histolytica* and *G. intestinalis* than females.

In Hilla, city of Babylon province, Al-Kahfaji,(2014) detected the prevalence of intestinal parasites in children under the age of five years, it was found that the rate of parasite infection was greater in bottle-fed children58 (52.2 %) than in breast-fed children 32 (31.4 %). *E.histolytica* (13.2%), *G.intestinalis* (8.3%), *H. nana* (7.2%), *E. vermicularis* (6.0%), and *E.coli* (5.2 %) were the most frequent intestinal parasites.

Asurvey study by Al-Saqur *et al*.(2015)includes reported cases of illnesses using an available surveillance database from the Ministry of Health for all provinces in Iraq from the period January to December 2015. The parasites reported to be the most common include *E. histolytica/dispar*, *E. vermicularis*, and *G. intestinalis*. Males were found to be at a higher risk of infection than females, with the majority of instances occurring in the age ranges of 5-14 years and 15-45years. Baghdad, Basrah, Thi-Qar, Najaf, Diyala, Babil, and Qadisiyah each had around 79 % of total cases under ten thousand.

The incidence of intestinal parasitic infections were 19.66% in a recent epidemiological research of intestinal parasites in Kirkuk Province, with 10.31% attributable to *G. intestinalis* and 9.35% to the other 9 intestinal parasites examined. There are 4.17 % of *B. homonis*, 1.67 % of *E. histolytica*, 1.43 % of *C. parvum*, 0.71 %of *E. coli*, and 0.23% of *E. hartmani*, *I. butschili, H. nana*, and *Ancylostoma duodenale* (Salman *et al*., 2016).

In Azadi-Teaching Hospital in Duhok City, Kurdistan Region which were Examined 3,976 stool samples, 1,196 were found to be positive for one or more of the intestinal parasites *E. histolytica*, *G. intestinalis*, *T. hominis*, *E. vermicularis*, and *H. nana*. Males had a higher infection rate than females, with 656 (54.8 %) and 540 (45.2 %) respectively. The parasite *E. histolytica* was found to be the most frequent. other parasites are reported which had minimal prevalence rates, (28.3%) *G. intestinalis* (1.1%), *E.vermicularis* (0.05%)and *H .nana* (0.1%) (Murad *et al*., 2018).

Parasite (protozoan and helminth) infection rates were investigated over the course of seven years (2011-2017) in a separate research that took place in the period from June to September of this year (2018). A total of 2877 stool samples from patients at Al-Hashimyah hospitals in Babylon province, Iraq, ranging in age from less than a year to more than 71 years old, were scored and recorded in the laboratory management's working documents. *E. histolytica, G. intestinalis, H. nana, T. hominis, and E. vermicularis* were found to infect 88.0%, 10.80%, 0.766%, 0.38%, and 0.03% respectively in this investigation (Al-Taei, 2019).

In 2020 the epidemiological study of the prevalence of intestinal parasitic infection in the city of Khanaqin - Diyala Province the total number of specimens examined was 805 stool specimens. These samples were collected from children aged (1 – 10) years. Intestinal parasite infection had a prevalence rate of 19.373 % *E. histolytica* was found to have a parasite percentage of 62.179 %, followed by *G. intestinalis* at 12.820%, *E. coli* at 12.820 %, and *I.butschillii* at 3.205% Furthermore, the study found that one species of Helminthes, *E. vermicularis*, has a 6.410 % occurrence ratio (Abed, 2020).

In a survey study by (Flaih *et al*., 2021) in Thi-Qar Province in southern Iraq Amoebiasis was found in 38,004 (11.1%) of the 341,554 cases of intestinal parasitic infections, the largest proportion of infections were found in 2015 (26.1%) and the lowest in 2020. (8.1 %). Amoebiasis was found in all age ranges, with the 5-14 year olds accounting for the largest percentage (27.3 %).

Epidemiological study of the intestinal parasite among children in Al-Aziziyah Hospital in Wasit province in Iraq, a total of (460) patient samples were infected, with 217 (47.17%) of them being males and 116 (53.5%) of them being females. The numbers and percentages of single (one kind of parasite) and double (two types of parasite) infections were found to be 207 (95.4%) and10(4.6%), respectively. Intestinal parasitic infections have been observed (95.4 %) among the population under investigation (17.9 %1-5 age).Positive samples from intestinal parasite species obtained in this study were more likely to contain *E. histolytica* (Jaffar *et al*., 2021).

In a research conducted at Ibn Al-Atheer Hospital in Mosul, Iraq, 2,296 samples were examind for the period from January 2019 and December 2020. The ages of the patients ranged from far under a year to well more than 12 years. Infections caused by *E.histolytica* were found to have a prevalence of 13.2% in 2019 and 15.7% in 2020, while prevalence rates for *G.intestinalis* were found to be 0.86% in 2019 and 1.04% in 2020. Intestinal parasite infections were shown to have a markedly age and gender distribution, with *E. histolytica* and *G .intestinalis* being more common in men. In addition, under one years old and over12years old age groups had the greatest and lowest prevalence rates of *E.histolytica* and *G.intestinalis* infections, respectively (Zaki, 2022).

2-2 ***Entamoeba histolytica***

Human amebiasis is caused by *E. histolytica*, which is found in many tropical countries. *E. histolytica* infections are known to have a wide range of clinical consequences, the majority of infections are asymptomatic; some cause diarrhea and dysentery, and only a few cause extra-intestinal consequences including liver abscess, the parasite is thought to be responsible for millions of episodes of dysentery and liver abscess each year, with up to 100,000 deaths worldwide (Ali *et al*., 2008), *E. histolytica* is a parasite infection that is second only to malaria as a leading cause of death due to parasitic infection. Diarrhea with cramps, lower abdomen pain, low-grade fever, feces containing blood and mucus, and flask-shaped ulcers are all symptoms(Chowdhury *et al*., 2022). When trophozoites breach the mucosal barrier and enter the underlying tissue, they emit enzymes that break down the extracellular matrix, kill cells, and phagocytoze cellular debris, resulting in disease. Tropozoites may enter portal circulation and

spread to the liver and other soft organs after infecting the mucosa and submucosa. Amebiasis appears in a variety of forms and is classified as either intestinal or extraintestinal based on the site of infection (Mortimer & Chadee, 2010).

2-3: Physiological and Immunological parameter.

2-3-1: The Blood

The life-giving properties of blood make it an essential liquid organ. The components of whole blood include cells, colloids, and crystalloids. By using centrifugal force, various blood components may be isolated based on their individual sedimentation rate, size, and relative density. The greatest specific gravity may be found in plasma, platelets, leucocytes, and packed red blood corpuscles (PRBCs) (Basu & Kulkarni, 2014).

2-3-1-1: The Erythrocytes , Red blood Corpuscles (RBCs)

Red blood corpuscles (RBCs) are micron-sized cells that make up around 45% of the volume of blood. Blood circulates through the body via a network of channels with diameters ranging from a few microns in the microcirculation (e.g. capillaries) to a few millimeters in the macrocirculation (e.g., aorta) (Vlahovska *et al*., 2013).

Under normal physiological settings, the body's red blood corpuscles content is maintained at about five million per liter (4.52-5.90 in males and 4.10-5.10 $\times 10^6$ mm in women) by balancing its production and destruction. Red blood cells are the product of the maturation of erythroid precursors from the hematopoietic stem cell pool, After an average of 120 days, red corpuscles are phagocytosed and eliminated by reticuloendothelial

macrophages because of the damage they have sustained during their lifetime. About five million erythrocytes (the average number per one) are removed from circulation every second (Thiagarajan *et al*., 2021).

Some gastrointestinal (GI) parasites such as *E. histolytica* deplete RBC from circulation by absorbing them directly from the host's blood or by creating intestinal ulcers, Chronic blood loss can alter the profile of the host blood by reducing the volume of RBC in the short term and the number of RBC counts in the long term, so diminishing the renewal of red blood corpuscles results in the production of small, hemoglobin-deficient RBCs (Budischak *et al*., 2012).

2-3-1-2: Hemoglobin (Hb)

Hemoglobin is a protein involved in respiration that is present in the red blood corpuscles of vertebrates. Its physiological functions include delivering oxygen to the body's tissues and allowing the return of carbon dioxide from those tissues to the lungs (Phillips *et al*., 1983).

Anemia is defined as a condition in which the body's hemoglobin (Hb) level is lower than normal, reducing red blood corpuscles ability to transport oxygen to tissues. . Mild, moderate, and severe anemia are the three types of anemia. Anemia is widespread in poorer nations due to poor nutrition and a high prevalence of intestinal parasites (Demeke *et al*., 2021).

Anemia is a blood disease caused by the decrease of normal hemoglobin, which is considered the leading cause of iron deficiency in the body, which in turn is considered the most prevalent nutritional deficiency in the whole world screening for iron deficiency anemia associated with

parasites such as intestinal parasite. some studies could report correlation between parasites and anemia the presence of intestinal parasites with the appearance of anemia deficiency other authors showed a relationship between anemia and intestinal parasites and observed a 26% reduction in cases of anemia in children treated with anti helminthes (Tsuyuoka *et al*., 1999).

Intestinal parasites and their association with haemoglobin concentration in Erbil Province's elementary school pupils were the focus of an epidemiological investigation. *E. vermicularis* and *G. intestinalis* were found in 29.8% and 13.1% of the study's 512 mals and 516 females, respectively. Children with the parasite had significantly lower mean values for hemoglobin (gm/dl) and packed cell volume (PCV) (%) than children without the parasite, at both the 0.01 and 0.05 confidence levels. Haemoglobin concentration is significantly affected by both *G. intestinalis* and *B. Hominus*. The average number of RBCs did not vary significantly between those with the infection and those without (Hama, and Rahemo, 2014).

From December 2017 to February 2019, researchers wanted to see how intestinal parasite infection affected the hematological profiles of pregnant women receiving prenatal care at Debre Markos Referral Hospital in Ethiopia, the result showed 5% of pregnant women (95 percent confidence interval (3.5–6.8) were anemic, having hemoglobin levels < 11 g/dl. Pregnant women who had intestinal parasites were more likely to develop anemia during the first three months of their pregnancies (12.2%). Of those affected by anemia, 8.2% have mild cases and 4% have significant ones. Only 1.2% of pregnant women free of intestinal parasites were found to have

anemia, and its severity was quite mild in most cases. Hemoglobin levels in pregnant women with intestinal parasites were 12.8 g/dl, whereas those without parasites were at 14.4 g/dl. Human intestinal parasites were shown to have a significant correlation with anemia (Demeke *et al*., 2021).

2-3-1-3: Leukocytes or White blood cells (WBC)

Leukocytes are another name for white blood cells, They defend the body from infection and play an important role in the immune system, If there is more WBC in the blood circulation, it indicates that there is an infection present in the body, They can be further classified into two groups based on whether or not their cells contain granules. Granulocytes and agranulocytes are the two types of cells. Granulocytes are further divided into three categories of white blood cells (WBCs). Neutrophils, Eosinophils, and Basophils. Lymphocytes and monocytes are two types of agranulocytes (Yildirim & Çinar, 2019).

By phagocytizing germs in bodies, neutrophils perform an important function in destroying foreign objects, particularly bacteria. The function of eosinophils is to fight parasitic worm infections by producing poisons. During homeostasis, most eosinophils are found in organs where they provide distinct physiological functions, including the thymus, the uterus, adipose tissue, and the lamina propria of the gastrointestinal (GI) tract (Kim & Jung, 2020).

Basophils work by producing two chemicals: histamine (which causes allergic reactions) and heparin (anti- coagulant).Monocytes are responsible for the production of macrophages, which phagocytize foreign material. T lymphocytes and B lymphocytes are two types of lymphocytes, which are

important white blood cells. T lymphocytes (Thymus dependent cells) attack diverse infected cells and tumors directly through cell-mediated immunity. B lymphocytes (Bursa-dependent cells) are responsible for humoral immunity because they create antibodies that target bacteria, viruses, and other foreign elements selectively. Lymphocytes vary from other WBCs in that they can remember invading foreign elements(Gautam & Bhadauria, 2014;Syeda Juveriya and Khanum, 2017).

Some authors have reported that *E. histolytica* affects blood parameters by invading the colon and feeding on red blood corpuscles after active trophozoites penetrate and attack the intestinal muscle wall (Shaker & Hussein, 2016).

2-3-1-4: Platelet (PLT)

Platelets in the blood play a crucial role in coagulation, inflammation, and tissue healing. In a matter of seconds, they respond to the unique signals brought forth by tissue damage or microbial invasion. When an artery wall is injured, the first cellular corpuscles to cluster at the site and in the surrounding tissue are called platelets (Vahidkhah *et al*., 2014).

Platelets respond to stimulus by expressing new receptors for sticky proteins, aggregating, releasing a variety of cytokines and other mediators. Furthermore, platelets that have been activated into close proximity to endothelial cells, granulocytes, monocytes, and lymphocytes. Platelets facilitate transendothelial migration of leukocytes into the tissue by establishing connections with other inflammatory cells, which may either boost or hinder their activities. Several inflammatory illnesses, including rheumatoid arthritis, inflammatory bowel disease, arterial thrombosis,

asthma, and transplant rejection, all include platelet activation. Although it is unclear whether or not platelet activation has a causal role in many disorders, platelets undoubtedly contribute to the manifestation of clinical symptoms (Vahidkhah *et al*., 2014; Harifi & Sibilia, 2016).

A result of the effect of intestinal parasite infection on different blood parameters many research and studies have been conducted on this subject the following is a review of the most important of those studies.

The study was conducted by Sree *et al*. (2015) to determine the most common intestinal parasite infections and their haematological correlations among patients in tertiary care hospitals in Karnataka in India of all ages. The outcome revealed in *G. intestinalis* infestation, the mean value of haematological parameters such as Hb, PCV, MCHC, and platelet count was lower, followed by *E. histolytica* infection.

The results of a study into the connection between *E. histolytica* infection and various blood parameters showed that, when compared to a healthy control group at the Central Teaching Hospital for Pediatric and other hospitals in the city of Baghdad, Iraq, those with *E. histolytica* infection had markedly lower rates of red blood cell count, hemoglobin level, platelet count, circulating blood volume, and mean corpuscular hemoglobin concentration. Also, compared to a healthy control group, patients with *E. histolytica* infections had substantially higher total leucocyte counts and differential types of neutrophils, lymphocytes, and mixed cells (monocytes, eosinophils, and basophils) (Shaker & Hussein, 2016).

In 2018 the study was conducted by Bayoumy *et al*.(2018) the purpose of the study was to find out how common intestinal parasite infections are

among school children in Gharbia Governorate, Egypt, and to research the possible links between these disorders and various hematological parameters and serum ferritin levels. The result showed 42 of the 56 individuals with *E. histolytica/dispar* infection were anemic.

Parasites in the intestines are common among the pediatric patients in Al Muthanna Province, Iraq, and this research looks at how they affect several blood parameters. The research was conducted in Al Muthanna province between June 2017 and May 2018. Stool samples from children under 12 years old were obtained in two different studies (Obiead *et al*.,2020), with results of showing a significant influence of age and sex of occurrence. Twenty patients with *E. histolytica* had a Hb of 9.88 0.455, a PCV of 31.61 1.359, and a WBC count of 9.55 2.81; twenty patients with *G. intestinalis* had a Hb of 9.71 0.519, a PCV of 31.12 1.549, and a WBC count of 7.94 1.117; twenty patients with *E. vermicularis* had a Hb of 9.62

Another research looked at the correlation between intestinal parasite infection and a variety of blood counts and immune system markers including histamine, interleukin-5, and immunoglobulin E in kids who went to the Karbala Children's Hospital in Kerbala, Iraq. shown by the results Red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs), neutrophils (Neu), basophils (Ba), eosinophils (Eos), lymphocytes (Ly), monocytes (Mo), platelets (Plt), mean corpuscular volume (MCV Some hematological parameters were affected by intestinal parasite infection, either increasing them or decreasing them (Al-Hasheme *et al*., 2020).

Recently, researchers at Ain Shams University's Eygept Pediatric Hemodialysis unit set out to learn how often children on hemodialysis (HD) experience common parasite infections and the severity of their symptoms. The results show that the average hemoglobin level was 10.531.99% g/dl, the average platelet count was 191.38 87.2 x103/UL, the average total leucocytic count was 6.552.09 x103/UL, the average neutrophil count was 3.681.63 x103/UL, the average lymphocyte count was 2.11.89 x103/UL, and the average eosinophil count was(0.39 x103/UL). There was no discernible difference between the parasitic infection positive and negative patients across any and all blood picture metrics (Sharaf *et al*., 2021).

2-3-2:**Adiponectin (APN)**

Adiponectin is one of the most intriguing hormone. It was first discovered in 1995 by a group of scientists(Scherer *et al*., 1995). Other names for Adiponectin are Acrp30, Adipo Q, GBP-28, and apM1 (Díez & Iglesias, 2003). Adipose tissue is the primary source of the 244-amino-acid protein known as adiponectin (Lewis *et al*., 2021) Adiponectin consists of a signal sequence at its N-terminus, a variable region, a collagenous domain (cAd), and a globular domain (gAd) at its C-terminus. (Chandran *et al*., 2003).

Adiponectin is a hormone that appears to suppress inflammatory reactions in vitro and is mainly expressed and secreted into the bloodstream by adipose tissue (Ouchi *et al*., 2001). However, later it has proven, by different research groups, that Adiponectin is found in a variety of tissues, including liver parenchyma cells, placental tissue, epithelial cell myocytes human and mouse osteoblasts (Delaigle *et al*., 2004; Caminos *et al*., 2005;

Patel *et al*., 2008; Lewis *et al*., 2021).Adiponectin is found in three primary oligomeric multimers in circulation: a low-molecular-weight trimer, a medium-molecular-weight hexamer, and a high-molecular-weight (HMW) multimer. The biologically most active type of adiponectin is HMW adiponectin. Unlike other adipokines, each form has distinct functions. HMW, for example, is linked to glucose absorption and obesity in the central nervous system. HMW levels rise after losing weight and have a positive correlation with it(Kadowaki *et al*., 2006). Adiponectin levels in the bloodstream are inversely proportional to total fat mass. This is especially true in the case of the adiponectin-leptin interaction. These two adipokines are controlled in different directions under practically all physiological situations. In general, high leptin indicates low adiponectin, whereas low leptin indicates high Adiponectin (Zhao *et al*., 2021). Interestingly, gender affects an adiponectin levels. Several studies have found that women had higher amounts of adiponectin than men, most likely due to higher levels of estrogen hormone, which is known to affect adipose tissue(Takenouchi *et al*., 2009;Cui *et al*., 2010; Aleidi *et al*., 2015; Khoramipour *et al*., 2021).

Adiponectin may improve insulin sensitivity in overweight animals and people, and it also has anti-inflammatory effects. Adiponectin's anti-diabetic and anti-atherosclerotic effects are well-documented (Achari & Jain, 2017).Furthermore, this hormone promotes the fatty acid breakdown and regulates blood sugar levels. Adiponectin promotes fatty acid oxidation in skeletal muscle, which lowers the buildup of triglycerides (TG). Unlike other adipokines, its concentration has been observed to be lower in obese patients, perhaps due to insufficient physical activity and sedentary/unhealthy lifestyles (Khoramipour *et al*., 2021).

Physical activity can help to reverse this situation by stimulating the creation and release of adiponectin, which increases glucose absorption and fatty acid oxidation by activating the 5'-adenosine monophosphate (AMP) kinase (AMPK)(Kelly *et al*., 2016).

Adiponectin (APN) has been proven to suppress pro-inflammatory cytokines and has other anti-inflammatory effects. Adiponectin has been demonstrated to exhibit anti-inflammatory activities on several cell types and tissues, and several studies have shown negative associations between circulating levels of adiponectin and the inflammatory markers C-reactive protein and IL-6. Cell culture experiments demonstrate that adiponectin has multiple effects on macrophage function, including downregulation of adhesion molecule expression, antagonism of toll-like receptors (TLR) and their ligands like lipopolysaccharide (LPS), and suppression of class A scavenger receptor expression (Ohashi *et al*., 2010;Summer *et al*., 2011). By activating ceramidase, APN decreases intracellular levels of pro-inflammatory ceramides while boosting concentrations of sphingosine-1-phosphate, a chemical with essential immunoregulatory and anti-inflammatory properties. One such activity is the influence of APN on the activation of NF-B, the prototypical inflammatory transcription factor (Fantuzzi, 2013).

It also has an essential function in the prevention of obesity and obesity-related illnesses, which has just lately been recognized. In diseases with immunological and inflammatory components, such as cardiovascular disease, type 2 diabetes, and metabolic syndrome, adiponectin has a wide spectrum of effects. Adiponectin has important effects on both innate and adaptive immunity. It disrupts macrophage function by reducing phagocytic

activity as well as the generation of IL-6 and TNF. It also inhibits B-cell lymphopoiesis, lowers T-cell response, and causes human monocytes, macrophages, and dendritic cells to produce essential anti-inflammatory mediators like IL-10 and IL-1RA (Lago *et al*., 2007).

In study conducted by Fantuzzi,(2013) APN's effects were found to extend to its role in immune response modulation. As a result, this study claims that APN stimulates dendritic cells, resulting in increased Th1 and Th17 responses. Other studies show that APN suppresses the expression of costimulatory molecules such as I kappa B kinase (IKB) that involoved in propagating the cellular respone to inflammation (Conde *et al*., 2012).

As for the relationship of Adiponectin with parasitic infection, study in Egypt it was found that Intestinal parasites (*E. histolytica* and *Strongyloides*) can disrupt the secretion of leptin and adiponectin, as well as the absorption of specific nutrients, causing anorexia and a change in body mass index (BMI). Anorexia caused by a parasite is an acute phase response to infection. The result showed increase in serum leptin and a significant drop in serum adiponectin ,negative connection between leptin and adiponectin levels .In *E.histolytica*, *Strongyloides*, and combined *E. histolytica* and *Giardia* infections, there was a large increase in leptin and a significant drop in adiponectin levels. compared to the control group(Yahya *et al*., 2018).

In a study conducted by Santamaría *et al*. (2021) in Argentin to study the relationship between Adiponectin levels and *Trypanosoma cruzi* infection. The findings revealed that *Trypanosoma cruzi* infection had a significant impact on adiponectin levels. After 17 days of infection, plasma adiponectin levels in mice were significantly lower than those of uninfected

mice. White adipose tissue adipocyte-derived adiponectin (AD) protein expression was likewise downregulated during this parasite infection stage.

2-3-3: Resistin RETA

Two kinds of endocrine cells, known as white adipose tissue (WAT) and brown adipose tissue (BAT), make up adipose tissue (BAT). While the brown adipose tissue (BAT) is responsible for thermogenesis and lipid oxidation, the white adipose tissue (WAT) is essential for insulating and supporting the body mechanically and storing energy. Channel efficiency for the removal of triglyceride-rich lipoprotein (TRL) and the prevention of systemic fat buildup are both modulated by BAT activation (Al-Suhaimi & Shehzad, 2013).

Resistin is a 12.5-kDa polypeptide with a 108-amino-acid propeptide that is released as a dimer with a cysteine disulfide bridge. The protein resistin is a member of the family of molecules known as resistin-like molecules (RELM) (Lago *et al*., 2007; Tiaka *et al*., 2011). Resistin is only released by adipocytes in mice, and it is hypothesized to affect glucose homeostasis and obesity-related endocrine resistance (Bełtowski, 2003) . While resistin expression is quite high in human bone marrow, it is also found in various tissues, including the placenta, pancreas, primary cell leukemia, synovial fluid, synovial tissue, and blood. Expression of resistin in white adipose tissue is restricted to monocytes and macrophages. These results indicate that the non-fat stroma-vascular component of human adipose tissue is responsible for resistin production (Filková *et al*., 2009).

Resistin expression is not only found in adipose tissue, but also in the stomach, small and large intestine, adrenal gland, and skeletal muscle,

according to numerous studies. Resistin mRNA expression varies according to white adipose tissue deposition and gender, with the highest levels found in female gonadal fat (Deb *et al*., 2021).

The human version of resistin has a 53 % similar to the mouse protein. There are five other intramolecular disulfide bridges in addition to this intermolecular one (Wolf, 2004; Tripathi *et al*., 2020).

Inflammation is facilitated by a protein called resistin. Cases of severe infection, such as septicemia, and chronic inflammatory processes, such as alcoholic liver disease, viral-induced chronic liver disease, hepatitis C virus, arthritis, and chronic kidney disease, have been linked to significantly high levels of resistin (Vrachnis *et al*., 2013).

Resistin is involved in inflammatory situations in humans because mononuclear cells secrete large amounts of it. Also, in individuals with obstructive sleep apnea, resistin levels are linked to those of cell adhesion molecules like intercellular adhesion molecule1(ICAM1),and in atherosclerotic patients, resistin levels are linked to soluble TNF-R type II and lipoprotein-associated phospholipase (Lago *et al*., 2007).

Juvenile patients with chronic renal failure and end-stage renal illness have also been reported to have elevated serum resistin levels, suggesting that renal functions are involved in regulating systemic resistin levels. TNF- was also associated with resistin, suggesting that resistin contributes to the latent inflammatory state that characterizes chronic kidney disease. High serum resistin levels, as seen in diseases like uremia, may also impair the chemotactic capacity and oxidative burst of polymorphonuclear leukocytes, leading to an abnormal immunological response (Filková *et al*., 2009).

Also serum resistin, an important adipokine, harms bone strength, although body weight has a beneficial effect (Tariq *et al*., 2021).

According to a study, healthy adults had significantly greater plasma resistin levels than type 1 diabetes patients. Pre- and post-islet transplantation, plasma resistin levels were assessed in healthy persons and type 1 diabetic patients. Plasma resistin levels were shown to be considerably greater in type 1 diabetic patient (Pang & Le, 2006). Resistin, like other adipokines, has been linked to breast cancer and has been shown to be significant. Furthermore, the link proved to be significantly stronger in Asian women than in non-Asian women (Chu *et al*., 2019).

Some studies have shown a link between serum resistin expression and higher parasite load or proinflammatory cytokine production in infected schoolchildren; one such research was conducted in the United States in 2015. They found a significant positive correlation between the level of resistin expression in the blood and the quantity of *A. lumbricoides* eggs found in the stool. The subsequent step was to analyze the serum for cytokine concentrations using Luminex assays. Studies showing that resistin directly promotes TNFa expression led the researchers to select proinflammatory cytokines (e.g. TNFa, CCL2, IL-6), and they hypothesized that helminth-induced resistin expression in infected children promoted a pro-inflammatory cytokine environment that was responsible for impaired parasite clearance. Moreover, the researchers analyzed the connection between serum resistin and inflammatory cytokines and found a positive link between resistin and IL-6, CCL2, TNF-, and other cytokines involved in inflammation (Jang *et al*., 2015).

S. haematobium was detected in 14% of 299 patients in another research from the Shamva area of the Mashonaland Central Province of Zimbabwe. According to the results of the serological test, 46% of the people had been exposed to cercarial antigens from *S. haematobium*, whereas only 9% had been exposed to adult worm antigens. Patients infected with *S. haematobium* (egg-positive) had significantly higher P-selectin levels compared to those of healthy controls. Compared to non-exposed patients, individuals who had been exposed to cercarial antigen had significantly greater levels of the protein resistin (Chimponda *et al*., 2019).

2-3-4: Interleukin- 18 (IL-18)

Inflammatory cytokine IL-18 belongs to the IL-1 family Precursor IL18 has 193 amino acids and is released after being processed by caspase 1 from an inactive form (24 kDa) lacking a signal peptide into an active form (18 kDa). The IL18 gene is located on chromosome 9 in mice and chromosome 11 in humans, it has an interferon consensus sequence binding protein, seven exons, and two promoters on exons 1 and 2 (Rex *et al*., 2020). Signals mediated by IL-18 are transmitted via the IL-18 receptor (R), a member of the IL-1 receptor/Toll-like receptor superfamily. Each IL-18R molecule consists of two subunits, IL-1Rrp1 (also known as IL-1Rrp1, IL-1R5, or IL-18R-) and IL-1Rrp2 (Toldo *et al*., 2018).

In the beginning, interferon (IFN)inducing IL18 was discovered. Originally identified as a factor that may cause a surge in IFN production, IL1 later made an unexpected appearance as a possible ally (Vecchié *et al*., 2021).

Kupffer cells were discovered to have the highest levels of the IL-18 precursor (Rex *et al*., 2020). In contrast to IL-1, IL-18 is constitutively expressed in the vast majority of cell types, such as human PBMCs, macrophages, and dendritic cells(DCs), as well as osteoblasts, epithelial cells, chondrocytes, and epidermal keratinocytes (Chen *et al*., 2013; Sanders & Mishra, 2016).

Epithelial cells, especially IECs, have been shown in several studies to produce IL-18, which may contribute in the development of a protective Th1 response to various pathogens including *C. parvum* infection. According to a group of researchers (McDonald *et al*., 2006) It has been suggested from research that IL-18 may have a unique proinflammatory effect (Sansonetti *et al*., 2000). The production of IL-1, TNF-, and IL-8 is increased in macrophages and neutrophils treated with IL-18.(Leung *et al*., 2001). In studies have shown that IL-18 production by macrophages increases in response to direct infection, and that IL-18 expression by epithelial cells may also be changed in these situations (McDonald *et al*., 2006).

Kupffer cells, which produce pro-IL-18 in a constitutive manner, were first shown to be the origin of IL-18. Furthermore, caspase-1 activation results from LPS binding to TLR4, which then triggers the synthesis of IL-18. but in response to LPS, macrophages upregulate transcription of pro-IL-18 mRNA, which leads to increase synthesis of pro-IL-18, which is then processed by caspase-1 into mature IL-18 before being released. Other cells than keratinocytes, intestinal epithelial cells, and osteoblasts generate pro-IL-18, indicating that it plays a significant pathophysiological function in both health and sickness. IL-18, like other cytokines, has pleiotropic activity

that varies with the cytokine environment in which it is present (Yasuda *et al*., 2019).

Loss of function of interleukin 18 (IL-18) may lead to dysbiosis and accelerate disease development because of its crucial involvement in gut microbiota monitoring and homeostasis (Zhang *et al*., 2019) .

Rex *et al*. (2020) performed research demonstrating the importance of IL-18 in activating hematopoietic cell types that drive Th1 and Th2 responses. Since IL-18 activates nuclear factor-B (NF-B) upon binding to the IL-18 receptor complex, a wide array of cytokines, chemokines, and cell adhesion molecules are produced and released as a consequence of this interaction. Furthermore, IL-18 induces the production and secretion of proinflammatory cytokines via stimulating the mitogen-activated protein kinases (MAPKs) and phosphoinositide 3-kinase/AKT serine/threonine kinase (PI3K/AKT) signaling modules in certain cell types. Signaling via IL-18 is crucial to the function of immunomodulatory cytokine networks that regulate host defense, inflammation, and tissue regeneration.

IL18 is a multifunctional cytokine with substantial immunomodulatory biological action that inhibits infection, parasite infection, and tumor growth by generating interferon gamma (Niu *et al*., 2019),which increases perforin and FasL-mediated cytotoxicity, and inhibits and kills malignant tumors directly or indirectly through various routes (Cheng *et al*., 2014). Thus, breast cancer, bladder cancer, and neuroblastoma are all inhibited or prevented by IL18 Tanaka *et al*.(2001) and Ma & Kong, (2021) suggested has been proposed that IL-18 may play a role in activating immune

responses and reflecting disease activity in mild and moderate asthmatic patients.

Defense against helminths and bacteria are two of the areas where IL-18 shines. More recently, however, it has been clear that IL-18 expression is increased in a number of allergic disease manifestations. Pathologically, IL-18 promotes a type II helper T cell (Th2) response by stimulating mast cell and basophil degranulation, recruiting granulocytes to sites of inflammation, increasing the cytotoxic activity of natural killer (NK) and NK-T cells, inducing immunoglobulin (Ig)E production and isotype switching, and impacting a wide variety of T cells (Sanders & Mishra, 2016).

Across Africa, in the country of Togo, researchers examined the region's core Neutralization of IL-18 with specific antibodies greatly boosted IL-10 release in individuals exposed to *Onchocerca volvulus* and *E. histolytica*, while IL-18 blocked parasite antigen-driven IL-10 secretion by peripheral blood mononuclear cells (PBMC). They discovered that the suppressive effects of IL-12 and IL-18 on IL-10 were cumulative, and that the combination of IL-12 and IL-18 with the chemokine IL-10 was not as effective as IL-18 alone in lowering IL-10 levels (Pfaff *et al*., 2003).

Intestinal epithelial cells (IECs) have been shown to express interleukin IL-18, a cytokine that regulates the immune system. IECs are a host cell for *C. parvum*, an intracellular parasitic protozoan. As measured by quantitative reverse transcription-polymerase chain reaction, IL-18 mRNA was shown to be upregulated after *C. parvum* infection of the human enterocyte cell line HCT-8 (RT-PCR). By using an enzyme-linked immune sorbent assay, we were able to determine that the IL-18 protein was present in control cells that

had not been infected, and that its expression increased in infected cells (ELISA). Gene expression studies of cell lines and freshly isolated IECs revealed the presence of IL-18 receptor subunits, suggesting that IL-18mediated signaling events may contribute to epithelial host defense during infection (McDonald *et al*., 2006).

Between August and December 2018, researchers analyzed data from 249 patients at the Kirkuk General Hospital and the Kirkuk Children's Hospital in Kirkuk, Iraq, who were being treated for gastrointestinal diseases and some healthy individuals. They want to learn how *B. hominis* infection affects blood counts and cytokines by measuring IL-10 and IL-18 levels (IL-18),. Consequences It was located the concentration of IL-18 in the serum of *B. hominis* patients was significantly higher than that of the control group, whereas the concentration of IL-10 was similar (Mutlag *et al*., 2019).

Both in vivo and in vitro studies have shown that a mechanism mediated by interleukin IL-18 cascades plays a significant role in the host's defense against parasite infections. A Pattaya, Thailand, woman with hookworm-related cutaneous larva migrans (HrCLM) was studied to determine whether or not she had had a shift in blood Th2 cytokines. A healthy 5-year-old child was seen walking around barefoot on the beach in Pattaya, Thailand. Once she got back to Japan, she started getting these red, linear lumps on the bottom and top of her feet. Even though the parasite was not found during surgery, she was treated with oral ivermectin twice, each time waiting a week between doses. Her eosinophil count, immunoglobulin E, and Th2 cytokine (IL-3, IL-4, IL-5, IL-13, IL-18, and IL-33) levels in blood were all measured simultaneously. It was shown that the IL-18 cascade is critically involved in Hr- CLM. Anti-inflammatory effects on

helminthic activity and increased eosinophil levels are mediated by interleukin IL-18 and IL-13 (Makoto & Keiichi, 2019).

At the meanwhile, researchers in Erbil's Raparin Pediatric Hospital conducted their own study. Children aged 1 month to 13 years of both sexes had stool samples taken (n=548). The researchers wanted to see how the immunological characteristics of the Cryptosporidium spp. positive group stacked up against those of the control group, so they used molecular characterisation based on sequence analysis to do so. Results showed the IL-18 levels were substantially greater in the cryptosporidiosis-positive group (84.11 6.098 ng/L) than in the control group (32.37 4.668 mg/L) (Kanabe & Darogha, 2020).

2-3-5: Interleukin-22(IL- 22)

Dumoutier *et al.* (2000) originally discovered IL-22 in IL9-treated murine lymphoma cells in 2000. At first, researchers thought it belonged to the IL-10 cytokine family and could be induced in T cells. The 179-amino-acid protein known as IL-10-related Tcell-derived inducible factor (IL-TIF) has been shown to have about 20% sequence similar to both murine and human IL-10 cytokines. Secreted IL-22 from humans is 146 amino acids long (Nagem *et al*., 2002; Foxall *et al*., 2016; Saxton *et al*., 2021). Although most IL-10 family members are only active when bound in pairs (dimers), this one may activate its receptor by binding to it alone. IL-22 may form higher-order self-complexes, such as dimers and tetramers, although this is not guaranteed (Perusina Lanfranca *et al*., 2016). Approximately 100,000 bp upstream of the IFN- locus on chromosome 12p15, the human IL-22 gene consists of 5 exons for a total of 5,200 bp in length (Wolk & Sabat, 2006).

Numerous cell types, including CD4+ T cells (especially TH17 and TH22 cells), CD8+ T cells, natural killer (NK) cells, cd T cells, lymphoid tissue inducer (LTi) cells, and innate lymphoid cells(ILCs), may produce IL-22 (Zenewicz & Flavell, 2011; Stange *et al*., 2012;Voigt *et al*., 2017).

Signals are sent through the IL-22 receptor complex, which consists of two subunits, IL-22R1 and IL-10R2. However, IL-22R1 is exclusively localized on the surfaces of non-hematopoietic cells such epithelial cells, hepatocytes, and keratinocytes, while IL-10R2 is located on almost all cell types (Mizoguchi, 2012). Due to the low expression of IL-22R1 on most cell types, IL-22 may specifically target innate cell populations in organs such the gut, liver, skin, kidneys, and lungs. And myeloid cells including monocytes (Mo), dendritic cells (DCs), and macrophages (M) create IL-22, particularly in inflammatory situations (Leung & Loke, 2013; Kulkarni *et al*., 2014).

The main functions of IL-22 in the intestine are the stimulation of epithelial cells to produce a wide variety of antibacterial proteins, the reinforcement of the mucus barrier by stimulating the production of mucin 1 under intestinal inflammatory conditions, and the improvement of epithelial regeneration with goblet cell restitution. Positive benefits of IL-22 include its capacity to improve intestinal wound healing after acute intestinal damage and its role in the treatment of numerous kinds of experimental chronic colitis that are mediated by Th1 or Th2 responses (Mizoguchi, 2012; Li *et al*., 2014).

Pan *et al*.(2014) indicated IL-22 responsibilities which include maintaining tissue integrity and barrier functions, as well as preventing

damage from invading pathogens or the inflammatory response itself. In this way, IL-22 can boost tissue cells intrinsic immunity, protect them from injury, and help them regenerate. IL-22 is a well-known antioxidant factor for hepatocytes, as it causes anti-oxidative genes to be upregulated (e.g., Metallo- thioneins) Finally, because of its antiapoptotic, antisteatotic, antifungal, and antibacterial properties, IL-22 medication could be used as an adjuvant therapy in the treatment of severe disorders such as viral hepatitis and other liver diseases as well as the potential added benefit of few side effects.

One way in which IL-22 increases inflammation is by inducing chemokines in the skin, liver, and intestines that draw neutrophils, such as CXCL1. Another way is by the production of acute-phase responses, such as serum amyloid A (SAA)-1/2, which helps activate inflammatory Th17 cells (Lee *et al*., 2020).

In studies suggest that interleukin-22 (IL-22) has a role in the pathogenesis of autoimmune diseases such systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome (SS), and psoriasis. Therefore, IL-22-targeting therapies have potential in treating a range of autoimmune diseases (Pan *et al*., 2013).

The capacity of IL-22 to aid in wound healing and tissue repair is due to its anti-apoptotic properties, as well as its ability to stimulate regeneration and proliferation. These actions, in combination with the activation of acute-phase response proteins in the colon and skin, help to maintain barrier integrity following injury or infection. In addition, IL-22 promotes the development of various antimicrobial peptides such as CXCL2, which can

help to inhibit bacterial invasion in the intestine and lungs (Perusina Lanfranca *et al*., 2016).

An increase in epithelial barrier function is not the only effect of the cytokine interleukin-22; a recent research shows that this cytokine also restricts bacterial systemic expansion by decreasing iron availability to the pathogen. Researchers employed an unbiased proteomic approach to investigate the molecular basis of IL-22-dependent iron retention in the host, and they found that IL-22 triggers the production of the plasma hemoglobin scavenger haptoglobin and the heme scavenger hemopexin (Sakamoto *et al*., 2017).

It has been shown that IL-22 levels rise in the gut after an infection with some worms. described a case in which the sufferer of ulcerative colitis intentionally infected himself with the whipworm *T. trichuria* in an attempt to alleviate his condition. *T. trichuria* infection suppressed the patient's disease activity, which was associated with elevated IL-22 and Th2 cytokine production in the gut. By stimulating mucus hypersecretion and goblet cell hyperplasia, as well as activating a TH2 and IL-22 response, *T.trichuria* was supposed to facilitate its own expulsion from the host (Sonnenberg *et al*., 2011). More evidence that helminths infection generates IL-22 in the human intestinal mucosa comes from a study with *Necator americanus* in people with Celiac disease in Queensland, Australia (McSorley *et al*., 2011; Gaze *et al*., 2012). The study showed that restimulation with *N. americanus* excretory/secretory proteins in vitro led to elevation of IL-22 mRNA levels in intestinal biopsies from patients infected with *N. americanus* larvae. Hookworm infection did not lead to enhanced IL-22 expression in intestinal biopsies stimulated with it.

In experimental animals, IL-17 and IL-22 have been demonstrated to boost resistance to certain bacterial and fungal diseases. However, IL-17 and IL-22 critical involvement in immunity against infections has not been shown by human investigations. The devastating visceral illness Kala Azar (KA) is caused by *Leishmania donovani*, and research conducted in Barbar el Fugarra, in the eastern portion of Sudan (Pitta *et al*., 2009) has shown that *Leishmania donovani* drives the formation of Th17 cells, which produce IL-17, IL-22, and IFN-. Analysis of Th1, Th2, and Th17 cytokine responses by cultured PBMCs from persons in a cohort of people who got KA or were protected from KA during a severe outbreak found that IL-17 and IL-22 were highly and independently related with protection against KA. In addition to Th1 cytokines, the results revealed that IL-17 and IL-22 play similar roles in the human immune response to KA.

2-4: Molecular study

In molecular biology, the polymerase chain reaction (PCR) is used to make hundreds or millions of copies of a certain DNA sequence from only a few,The polymerase chain reaction (PCR) is a widely used and frequently essential method in medical and biological research laboratories. Denaturation, annealing, and extension are the three main phases of the PCR process. There are an increasing number of disorders for which polymerase chain reaction (PCR) is valuable in both research and diagnosis. Human and microbial gene detection are both possible using qualitative PCR. Since just a little quantity of the original DNA is needed, PCR is also used in forensics labs and proves to be quite beneficial in these settings. Genes that have been linked to cancer may be found using PCR (Ishmael & Stellato, 2008).

Genome-wide data sets are increasingly being employed in the drug development process to find biological pathways and networks underlying complicated disorders. Analyzing genomic data through sets defined by functional pathways, in particular, has the potential to increase the power of discovery and reveal natural linkages to biological mechanisms. SNPs, which are the consequence of single base-pair changes (substitutions or deletions) among chromosome sequences, account for a large portion of genetic variation in the human genome. There are a variety of laboratory and computational procedures for discovering single nucleotide polymorphisms (SNPs) in a genome, but they all entail some sort of comparison of the same DNA sequence from various individuals or haplotypes (Fareed & Afzal, 2013).

The pro-inflammatory IL-1 family member IL-18 is encoded by the IL-18 gene on chromosome 11q23.1. Dendritic cells (DCs), macrophages, Kupffer cells, keratinocytes, osteoblasts, astrocytes, and astrocytes all express IL-18 (Alruwaisan *et al*., 2021). When coupled with IL-12, IL-18 has been reported to generate Th1 cells through acting as an IFN-G-inducing factor. In addition to promoting IFN-G production, IL-18 may activate CD81 T cells and natural killer (NK) cells, which both play important roles in viral clearance (Hirankarn *et al*., 2007; Harada *et al*., 2009).

Polymorphisms in cytokine genes are among the factors that influence the cytokine response. Polymorphisms in cytokine genes may affect gene transcription, resulting in cytokine production differences between individuals. Polymorphisms in the cytokine gene have been linked to disease susceptibility, severity, and clinical outcome in a variety of diseases, including infectious disorders. Single nucleotide polymorphisms (SNPs)

have been shown to affect transcription factor binding and alter the transcriptional activity of the IL-18 gene. Because IL-18 plays a key role in the Th1 response as an IFN-inducing factor (Harishankar *et al*., 2007).

These days, immunotherapies need to know the ahead of time how a patient's immune system would react. Patients' varying reactions to immunotherapy may be explained by the fact that single nucleotide polymorphisms (SNPs), which are single base pair variations in the human genome, can impact the function of certain immune genes, especially those implicated in response to cancer and other disorders. SNPs (single nucleotide polymorphisms) may alter biological processes by affecting the expression or alteration of proteins encoded by essential immune genes (Ramos *et al*., 2021).

IL-18 is a gene that has been extensively studied and is known to consist of a 5'- untranslated region (UTR) promoter, 6 exons, and a 3'UTR region, for a total of 20.9 kb. Genotyping of the SNPs was performed using TaqMan assays designed specifically for the SNPs and polymerase chain reaction (PCR),Polymorphisms in the IL-18 gene have been associated with atherosclerosis, rheumatoid arthritis, systemic lupus erythematosus, allergic asthma, and allergic dermatitis (Alruwaisan *et al*., 2021).

One such adipokine is resistin, which was shown to be a gene in 3T3-L1cells and is upregulated during adipocyte differentiation and downregulated by peroxisome proliferators activated receptor ligands. A chromosomal gene located at 19p13 codes for resistin (RETN). Variants in the RETN gene have been the subject of extensive study, and it has been shown that genetics account for 70% of the observed variation in blood

levels of resistin. Several studies have shown associations between RETN SNPs and measures of insulin resistance. Two examples of such SNPs are rs10401670 and rs1862513 (Antonio de Luis *et al*., 2016).

In research has linked RETN gene variations to differences in RETN gene expression and resistin levels in the blood (Hashemi *et al*., 2018).

Chapter Three

Materials and Methods

3: Materials and Methods

3-1:Materials

3-1-1:Laboratory equipment and tools

The laboratory equipments and instruments used in the present study are listed in the Table (3-1).

Table (3-1): Laboratory Equipments and Instruments

NO.	Equipment& instrument	Company	Origin
1	Bench centrifuge	Memmert	Germany
2	Centrifuge	Hettich	Germany
3	Digital camera	Sony	Japan
4	Disposable syringe 5ml and 3ml	Sterile EO.	China
5	EDTA tube	Xinel	China
6	Electric sensitive balance	Denver	USA
7	ELISA	Bio tech	U.S.A
8	Eppendorf centrifuge	Hettich	Germany
9	Eppendorff tube	Bioneer	Korea
11	Gel electrophoresis system	Cleaver Scientific	UK
12	Gel tubes	ALS	China
13	Glass slides and cover slides	Superestar	India
14	Hematology Analyzer	Sysmex (XN-350)	Japan
15	Incubator	Binder	Germany
16	Light microscope	Olympus	Japan
17	Micropipettes	Capp (Denmark)	Denmark
18	Oven	Mammert	Germany

19	PCR machine (Thermocycler)	Techne (UK)	UK
20	Plastic containers	Sterile EO.	China
21	Refrigerator	Concord	Lebanon
22	Slides and cover slides	Superstar	India
23	UV-trans illuminator	Syngene	England
24	UV-Vis spectrophotometer	Analytic Jena	Germany
25	Vortex	Gemmy	Taiwan

3-1-2: Chemical materials

Chemical materials used in this study are listed in the Table (3-2).

Table (3-2) Chemical Materials

NO.	Chemical Materials	Company	Origin
1	6X DNA Loading buffer Blue	Eurx	Poland
2	Acid fast stain	Al Hanoof factory	Jordan
3	Agarose, TBE buffer	Condalab	spain
4	Ethanol	Hayman	UK
5	Ethylene diamine tetracetic acid (EDTA)	Rideal	UK
6	Iodine stain	sidra	Turkey
7	Nuclease Free Water	Bioneer	Korea
8	Simply Safe	Eurx	Poland

3-1-3: Kits

The kits that were used throughout the study will be mentioned and their origins in the following Table (3-3):

Table (3-3): The kits used in present study.

	Kits	Company	Origin
Immunological and Physiological kits	Human ADP/Acrp30 (Adiponectin) ELISA Kit	Elab science	China
	Human RETN (Resistin) ELISA KIT	Elab science	China
	Human IL18 (Interleukin 18) ELISA Kit	Elab science	China
	Human IL22(Interleukin 22) ELISA Kit	Elab science	China
Diagnosed kit	Kit of Rapid test	Certest	France
Kits for genetic study	DNA extraction Kit	Favorgen	Taiwan
	DNA ladder	Bioner	Korea
	Green master mix	Bioner	Korea
	Primers	Macrogen	Korea
	protein cockatiel inhibitor Proteinase K	Bioner	Korea

3-1-4: Study design

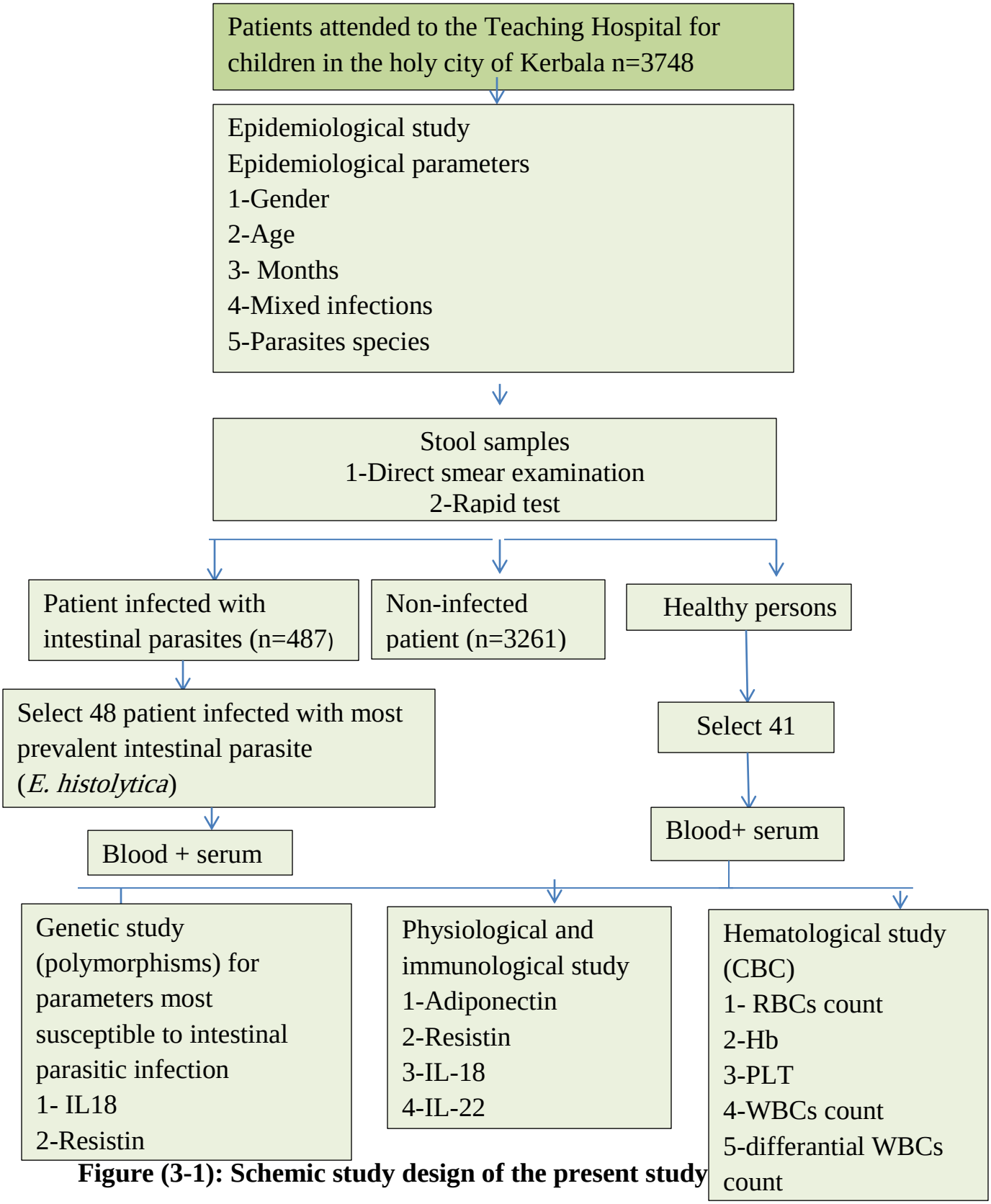


Figure (3-1): Schemic study design of the present study

3-2: Method

3-2-1: Parasitological Study

3-2-1-1: Stool sample collection

Stool samples were collected from patients who attending to the (Kerbala Teaching Hospital for children in holy Kerbala city),during the period from February 2021 till January 2022.where collecting about 3748 stool samples. A questionnaire sheet was obtained that covered kind of infection from patients and age group, residence area, gender, and the months, as well as sterilized plastic containers with a tight lid that are used to retain stool samples to prevent dryness and moisture.

3-3-1-2: Stool Sample Examination

3-3-1-2-1: Macroscopic Examination

The volume of feces, form, consistency, and color of stool samples are all factors to consider when examining them, Trophozoites frequently appear in liquid or soft specimens, whereas cyst stages appear in semisoft samples, because the stool may contain blood or mucus, these parts should be examined separately and carefully; they may contain the trophozoites stage of the parasite. If brought more than one sample to the lab, the liquid sample and mucus should be examined first (Al-Ammash, 2015).

3-3-1-2-2: Microscopic Examination

Stool samples were examined using direct smear methods, which involved putting a small drop of normal saline (0.9 %) or iodine stain on the sliding glass, mixing well with a small portion of feces using a wooden stick,

covering the slides, and examining the sample under power enlarge 40X and 100X (Tanyuksel *et al*., 2005; Hussien *et al*., 2017).

3-3-1-2-3: Staining by hot modified Ziehl–Neelsen Stain

This method is used for parasites such as *C.parvum*

1- A sterile pipette or microbiological loop was used to collect a tiny amount of stool sample on a clean glass slide (which was then combined with normal saline (if necessary) and allowed to dry for 10 minutes at room temperature.

2- Methanol was used to fix the smear for five minutes.

3- Carbol- fuchsin was sprayed for 10-15 minutes while the oven was heated to 60C°. (modified method) .

4- After washing with tap water, put in acidic alcohol for 30 seconds.

5- The smears were then saturated for two minutes with a methylene blue stain.

6- The stains were rinsed for 10 seconds in tap water.

7- For 10 minutes, the smears were allowed to dry.

8- The smear was lubricated with oil immersion using a wooden stick.

9- Light microscopes with 40X and 100X oil objectives were used to examine the smears(Baxby *et al*., 1984).

3-3-1-2-4: Method of Rapid test (Chromatographic immunoassay)

CerTest is composed of :-

- CerTest *C. parvum +G.lamblia +E. histolytica* combo card tests

- Manual Procedure:

1- The stool samples and controls were left at room temperature (15-30C°) and do not open pouches until the assay is completed.

2- The sample was checked to assure good sample dispersion.

3- Cer Test *E. histolytica+ G. lamblia + C. parvum* Just before using the combo card test, it was taken out of its sealed cyst.

4- A small amount of feces was collected from the loop of the collecting tube, and the end of the cap was cut.

5- 4 drops in the circular window marked were dispensed with (*E. histolytica*), were used in the same tube and the same amount in the circular window marked with (*G. lamblia*), which was used the same tube in the circular window marked with (*C. parvum*)

6- If the result window has two lines (T red, C green), the kit is working and the result is positive. If only one color (C green) is present, the kit is producing negative results. If there are no colors, that means the kit is not working. The results were read after 10 minutes (the test results should not be reviewed after 10 minutes) (Mariscal & Mora, 2012).

3-3-2:Physiological & Immunological Study

3-3-2-1:Blood Sample Collection and Analysis

Five milliliters of blood were obtained from (48) patients infected with *E. histolytica*, as well as five milliliters of blood from (41) healthy youngsters (sample control) put 2.5 ml of blood in a gel tube with a sterile medical needle (G23) that is usable once disposable. For use once and

without clotting substance, leave for 10 minutes without anticoagulant, then place in a centrifuge type (T30) fast 3000 r / min period 10 minutes, serum placed in apendroof tubes and stored for further use. To calculate, the following parameters that were: WBCs count, WBC differentiated, RBCs count, platelets (PLT), Hb concentration.

place 2.5 mL of blood in a special tube containing Ethylene diamine tetra acetic acid (EDTA), a blood clotting inhibitor, by hematological analyzer called Sysmex (XN-350) and used for genetic study.

3-3-2-2:Human Adiopnectin (ADP/Acrp30) ELISA Kit procedure

1-Add sample: 100µl of standard, blank, or sample were added per well. The blank well was added with reference standard and sample diluent. The solution was added to the bottom of micro ELISA microplates to avoid inside wall touching and foaming as possible. Mix it gently, the plate was covered with the sealer provided, incubate for 90 minutes at 37c°.

2-Biotinylated detection Ab: the liquid was removed from each well, do not wash. Immediately,after100µl of biotinylated detection, Ab has added working solution to each well, covered with the plate sealer, gently tap the plate to ensure thorough mixing, incubate for 1 hour at 37c°.

3-Decant the solution from each well, wash buffer 350 µl was added to each well soaked for 1-2 min and the solution was aspirated or decanted from each well and put it to dry, the process was repeated three times .

4-HRP Conjugate: 100 µl of HRP conjugate working solution was added to each well. The plate was covered with a new sealer, and incubated for 30 minutes at 37c°.

5-Wash: the wash process was repeated five times as conducted in step 3.

6-Substrate reagent: 90 µl of substrate solution was added to each well. It covered with a new plate sealer. Incubated for about 15 minutes at 37c°.

The plate was protected from light, the reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. The microplate was preheated for about 15 min before OD measurement.

7- Stop solution: 50 µl of stop solution was added to each well. The order to add the solution should be the same as the substrate solution.

8-OD measurement: optical density (OD value) was determined for each well at once, using a microplate reader set to 450 nm.

3-3-2-3: Human resistin (RETN) ELISA Kit procedure.

The same procedure in 3-3-2-2.

3-3-2-4: Human IL-18 ELISA Kit procedure.

The same procedure in 3-3-2-2.

3-3-2-5: Human IL22 ELISA Kit procedure.

The same procedure in 3-3-2-2.

3-4: Genetic study

3-4-1:Genomic DNA extraction

Favor PrepTM Genomic DNA Mini Kit was used to extract genomic DNA from (50) blood samples following the manufacturer's protocol. Transfer up to 200 µl sample whole blood to a microcentrifuge tube, 20 µl

Proteinase K and 200 µl FABG Buffer was added to the sample. Mix thoroughly by pulse-vortexing, Incubate at 60 ºC for 15 minutes to lyse the sample. During incubation, vortex the sample every 3-5 minutes. Briefly spin the tube to remove drops from the inside of the lid, and 200 µl ethanol (96- 100 %) was added to the sample. Mix thoroughly by pulse-vortexing for 10 sec., Briefly spin the tube to remove drops from the inside of the lid, Place a FABG Mini Column to a Collection Tube. Transfer the mixture (including any precipitate) carefully to the FABG Mini Column.

Centrifuge at 6,000 rpm for one min then place FABG Mini Column to a new Collection Tube, 400 µl W1 Buffer was added to the FABG Mini Column and centrifuge at full speed (18,000 rpm) for 30 sec. then discard the flow-through, 750 µl Wash Buffer was added to the FABG Mini Column and centrifuge at full speed for 30 sec. then discard the flow-through, Centrifuge at full speed for an additional three minutes to dry the column, Place the FABG Mini Column to an Elution Tube, 50 µl of Elution Buffer was added to the membrane center of FABG Mini Column. Stand FABG Mini Column for three minutes. Centrifuge at full speed for one minute to elute total DNA and then stored at -20Cº until use.

3-4-2:Conventional Polymerase Chain Reaction

3-4-2-1:Primer Pairs Preparation

The primer pair used in this study(Macrogen/south Korea) in Table(3-4) was dissolved using TE Buffer, (pH 8.0) composed of 10mM Tris-HCl containing 1mM EDTA-Na2. Firstly the primer stock tube prepared and then the working solution would be prepared from primer stock tube.

According to the instruction provided by the primer manufacturer (Bioneer /south Korea), the TE buffer was added to get 100

picomole/microliter concentration of primer stock solution. The working solution was prepared from stock by dilution with TE buffer to get 10 picomole/microliter.

Table(3-4): Primer Sequence of IL1-8 and Resistin

Primer name	Sequence	Product size(pb)	Reference
IL 18 F	CCGTAAAAGTTGGGGCTCTG	701	This study
IL18R	CACTCTGCTCTTCAAACGTTAC		
Resistin F	GTTTGCATCAGCCACCCTTG	454	This study
Resistin R	GAGTAGGATCTGCCCCTGGA		

3-4-2-2: Preparation of 1X TBE (TRIS -Borate - EDTA) buffer

1X TBE buffer was prepared by dilution of concentrated 10X TBE buffer. This solution was used to dissolve agarose and in the electrophoresis process. Each 100ml of 10X TBE was added to 900ml of sterile distal water to give a final concentration,1X TBE (Green and Sambrook, 2012).

3-4-2-3: 6X DNA Loading buffer blue

It is provided in a premixed, ready to use form containing 100 mMTris-HCl (pH 8.0), a mix of dyes (Xylene Cyanol and Bromophenol Blue), and 30% glycerol,100 mM EDTA (pH 8.0). 6 x Loading Buffer BLUE is designated for the preparation of DNA samples for loading on agarose and polyacrylamide gels. The glycerol content increases the density of the sample and ensures that DNA forms a layer at the bottom of the well. EDTA is known for the inhibition of metal dependent nucleases. Tracking dyes permit monitoring of the progress of electrophoresis. Only 1µl of loading dye was mixed with a 5 µl DNA sample to check DNA after extraction (Green and Sambrook, 2012).

3-4-2-4 Agarose Gel Electrophoresis

The agarose sheet was prepared by dissolving agarose powder in100 ml 1X TBE buffer (pH=8). The amount of agarose which can be dissolved depends upon the purpose for which the agarose sheet is used. 0.7% agarose sheet was used for visualization the DNA after extraction while 1.5%-2% agarose sheet visualization of PCR product (amplicon).

Simply safe (alternative for ethidium bromide) stock solution concentration was 10 mg/ml. Only 5µl of simply safe stock solution was added to 100ml of melting agarose gel to get the final concentration of 0.5µg/ml. The comb was fixed at the end of the tray for making wells used for PCR product loading, the agarose was gently poured into the tray and waited to solidify at room temperature for 30 min, the comb was gently removed from the tray and then the tray was fixed in an electrophoresis chamber and filled with a TBE buffer, PCR product was loaded into the wells directly.

The electrophoresis instruments voltage was set to ensure an electrical field of 5 v.cm-1 for the distance between the cathode and anode, at the end of the run which is about 90 min and an ultraviolet transilluminator was used at 320-336 nm for the band detection at the end the gel was photographed using a digital camera(Green & Sambrook, 2018).

3-4-2-5: Reaction Mixture

Amplification of DNA has been carried out in a final volume of 50 µl reaction mixture as mentioned in Table (3-5):

Table (3-5): Contents of the Reaction Mixture

No.	Contents of reaction mixture	Volume
1.	Green master mix	25 µl
2.	Upstream primer (10pmol/µl)	3µl
3.	Downstream primer (10pmol/µl)	3µl
4.	DNA template	5 µl
5.	Nuclease free water	14 µl
Total volume		50 µl

3-4-2-6: Polymerase Chain Reaction (PCR)

Conventional PCR was used to amplify the target DNA using specific primer pairs(IL18,Resitin). It includes three consecutive steps that are repeated for a specific number of cycles to get PCR product (amplicon) which can be finally visualized after agarose gel electrophoresis. The thermal cycling conditions mentioned in Table (3-6).

Table(3-6): Polymerase Chain Reactions for genotypying of (IL18,Resistin)

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Reference
IL-18	95C° 2min.	95C° 30sec.	58.7C° 30sec.	72C° 80sec.	72C° 5min.	This study
	1 cycle	29 cycle			1 cycle	
Resistin	95C° 2min.	95C° 30sec.	60.3C° 30sec.	72C° 50sec.	72C° 5min.	This study
	1 cycle	29 cycle			1 cycle	

3-4-2-7: Sequencing of PCR products

40 µl of PCR product were sent to Macrogene/south Korea for Sanger sequencing. After trimming each sequence, the results of the trimmed sequence were blasted in NCBI to check the similarities and differences with database. Mega5 software was used to check the similarities and differences.

3-4-2-8:SNPs position

All ABI file of sequence were opened with Bioedit (version 7.2.5.) (Hall, 1990), trimmed and only correct normal sequence were compared to seek the SNPs.

Table (3-7): SNPs position of IL-18 gene (NCBI Reference Sequence: NC_000011.10)

SNP	Position (GRCh38.p13)
rs1866694757	112164707
rs940255648	112164712
rs1037707423	112164718
rs1946518	112164735
rs1213044637	112164782
rs1946519	112164784
rs1215648807	112164792
rs1866697972	112164797
rs1866698066	112164798
rs1866698286	112164822

Table (3-8): SNPs position of resistin gene (CBI Reference Sequence: NC_000019.10)

SNP	Position (GRCh38.p13)
rs1862513	7668907
rs567367264	7668970
rs536392382	7668971
rs2032442393	7668974

3-5: Statistical analysis

Information from the questionnaire from all participants were entered a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020) (1). Descriptive statistics was performed on the participants' data of each group. Values were illustrated by n (%) for categorical. The distribution of the data was checked using Shapiro-Wilk test as numerical means of assessing normality.

The association between the analyzed factors and presence of intestinal pathogenic parasites was estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a non-conditional logistic regression.

Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with $p \leq 0.05$ (two-side) were considered to be statistically significant.

Chapter Four

Results

4 :The Results

4-1: The Epidemiological Study

4-1-1:The Total Percentage Rates of Infection with Intestinal Parasites.

Table (4-1) illustrated the total infected and non-infected percentages with the intestinal parasites in patients attending to Kerbala Teaching Hospital for children, where the number of patients infected with intestinal parasites were 487 from 3784 person examined.

Table (4-1): The total infection percentages with intestinal parasites

Examined No.	Infected patients		Non-infected	
	No.	%	No.	%
3748	487	13	3261	87.00

4-1-2: The Percentage rates of intestinal parasite infection according to gender

The result in table (4-2) shows the percentage intestinal parasites in both gender. The total infected males were about 14.1% while the total infected females were 11.7%.The statistical analysis showed a significant difference between percentages of infection in both gender.

Table (4-2): Relationship between intestinal parasites infection with gender.

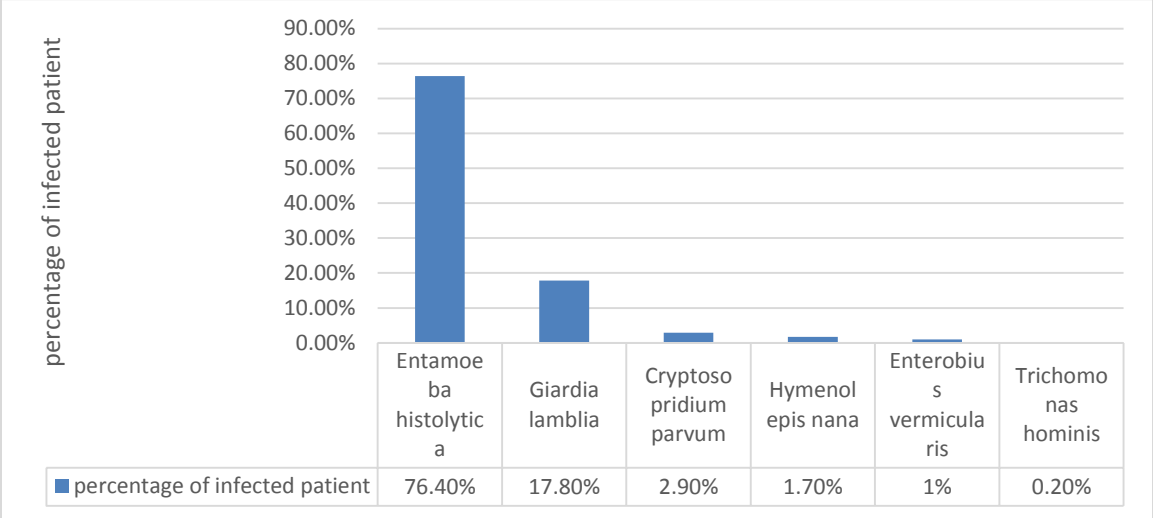
Gender	No .of the tested patients	Infected patients		Non-infected	
		No.	%	No.	%
Male	1966	278	14.1	1688	85.9
Female	1782	209	11.7	1573	88.3
Total	3748	487	13.0	3261	87.0
x^2= 4.810 calculated **x^2= 3.841 tabled** **df=1**					

4-1-3: Percentage rates of intestinal parasitic infections according to the species of parasites.

The results indicated that the highest percentage of the intestinal parasites species were *E. histolytica*, while the lowest infected species were *T. hominis* (Table 4-3 and figure 4-1). There are an important difference between the infection percentages of the parasitic species recorded in current study ($p \leq 0.05$).

Table(4-3): percentages infection rates with different intestinal parasites species(n=3748)

Species of parasites	Number of infected patient	%
E. histolytica	395	76.40
G. lamblia	92	17.80
C. parvum	15	2.90
H. nana	9	1.70
E. vermicularis	5	1.00
T. hominis	1	0.20
χ^2= 139 calculated χ^2= 3.841 tabled df= 1 p ≤ 0.05		



Figure(4-1): Percentages infection rates of intestinal parasites according to the species of parasites

4-1-4: Percentages infection rates with intestinal parasites according to infection type

Table(4-4) and figure (4-2), illustrated that the most patients(487) infected with intestinal parasites were infected with one type of parasites (12.65%),while (12) patients were infected with two types of intestinal parasites (3.2%), and only (1) patient was infected with more than two types of parasites (0.03%) .Statiscal analysis showed a significant differences between the single percentage of infection and mixed percentage of infection.

Table (4-4): The percentages of single and mixed infections with intestinal parasites (n=3748)

Type of infection	No. of infected patients	%
Single infection	474*	12.65 %
Double infection	12	3.2 %
Multiple infection	1	0.03 %
x^2= 89.7 calculated x^2= 5.991 tabled df= 2 p ≤0.05		

*Means significant results

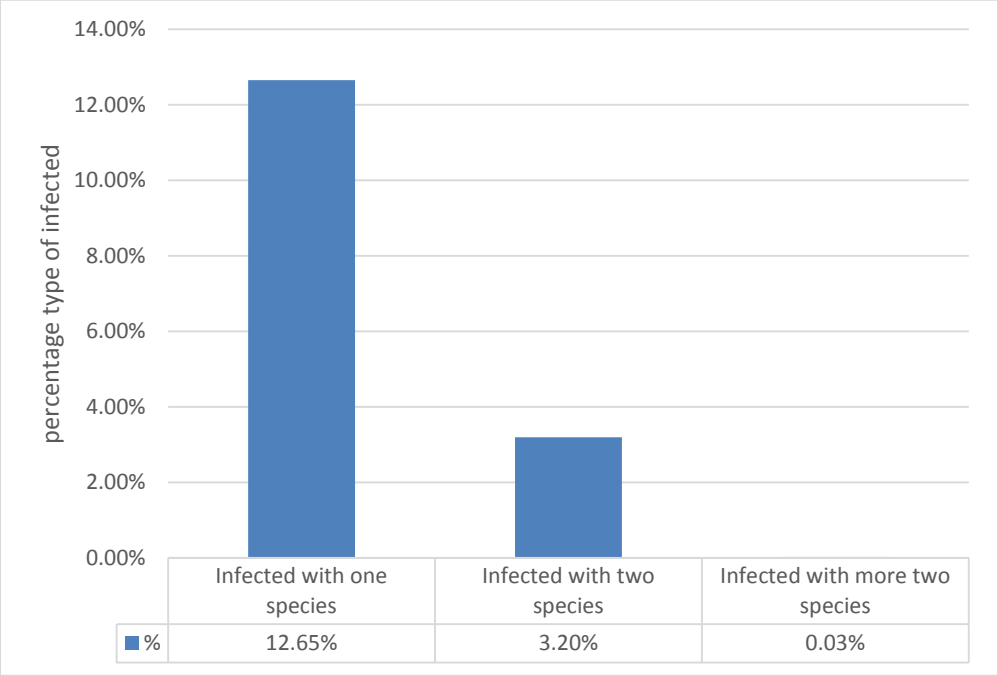


Figure (4-2): Percentages rates of single and mixed infections with intestinal parasites.

4-1-5: Percentage rates infection with intestinal parasites according to the months of study.

In Table (4-5)figure(4-3) results were shown the percentages rates of infection of intestinal parasites according to the months of the study. September of 2021 illustrated the highest infected percentage 30.1% while February of 2021 has shown the lowest percent (3.5%) ($p \leq 0.05$).

Table (4-5):The infection percentages of intestinal parasites according to the months of the study.

Month	Number of tested patients	Infected patients		Non-Infected	
		No.	%	No.	%
February(2021)	315	11	3.5	304	96.5
March	273	30	11.0	243	89.0
April	332	46	13.9	286	86.1
May	367	62	16.9	305	83.1
June	346	64	18.5	282	81.5
July	212	32	15.1	180	84.9
August	135	20	14.8	115	85.2
September	183	55	30.1	128	69.9
October	450	61	13.6	389	86.4
November	518	62	12.0	456	88.0
December	347	29	8.4	318	91.6
January(2022)	270	15	5.6	255	94.4
Total	3748	487	13.0	3261	87.0
χ^2= 108.24 calculated χ^2= 19.675 tabled Df=11 p ≤0.05					

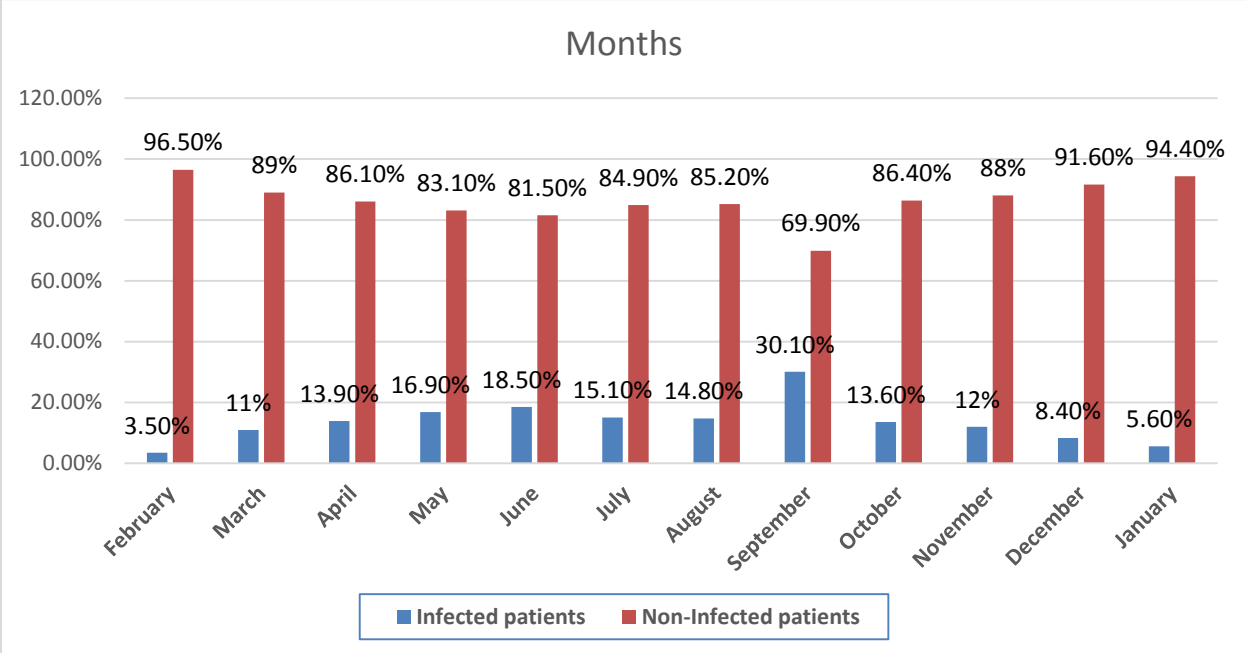


Figure (4-3) Percentages rates infection of intestinal parasites according to the months of the study

4-1-6: Percentages rates infection with intestinal parasite according to age groups.

Table (4-6) and figure (4-4) illustrated the prevalance of intestinal parasite infection percentages according to the age groups of patients. Since the age range of the participants were divided into subgroups, results indicated that the highest infected age groups were the children who were from five to ten years old, and the lowest infected was for the age groups who were more than 10 years old ($P \leq 0.05$).

Table (4-6): Percentage rate infection with intestinal parasite according to Age groups.

Age group	Number of tested patients	Infected patients		Non-Infected	
		No.	%	No.	%
<5	1937	253*	13.1%	1684	86.9%
5-10	1150	198	17.2%	952	82.8%
>10	661	36	5.4%	625	94.6%
Total	3748	487	13%	3261	87%
x^2= 51.46 calculated **x^2= 5.991 tabled** **df= 2** **p ≤0.05**					

*Means significant results

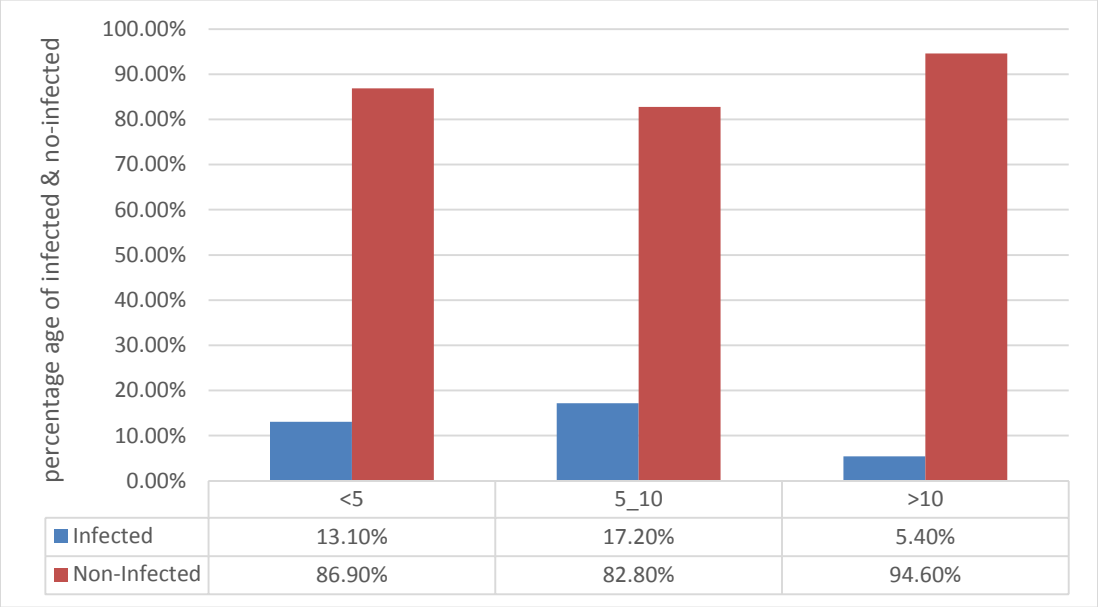


Figure (4 -4): percentages rates infection of intestinal parasite according to age groups.

4-2: The Physiological and Immunological Study.

The result of the epidemiological study showed that the parasite that recorded the highest prevalence percentage was *E. histolytica* .Therefore ,the physiological and immunological parameters were studied ,in addition to the molecular study in patient infected with this parasite only.

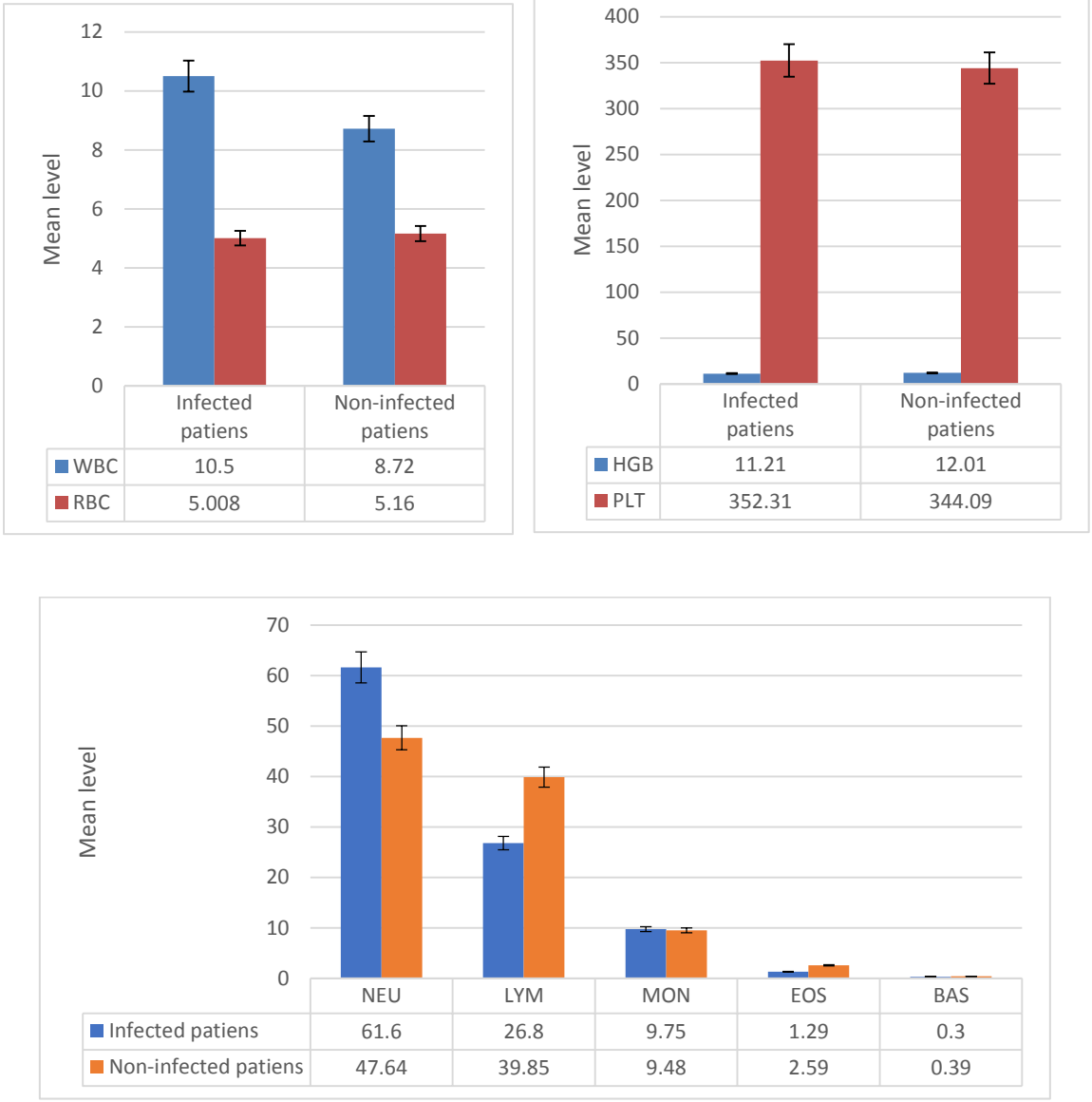
4-2-1: Effect of *E. histolytica* infection on blood parameters of infected patients and non-infected .

Hematological parameters in the Infected and Non-infected patients were also examined,and results indecated the WBC., NEU., LYM., and Hb. were statistically significantly different among infected patients compared to non-infected, ($p \leq 0.05$), as shown in Table (4-7 and figure 4-5) .

Table(4-7): Hematological parameters of the study groups.

Hematological parameters	Infected patients (Mean± SD)	Non-infected (Mean± SD)	P value
WBCs.(cells×10^3/mm^2)	10.5 ± 4.6	8.72 ± 4.11	0.05*[S]
NEU.%	61.6 ± 18.5	47.64 ± 11.54	0.00065*[S]
LYM.%	39.85 ± 8.90	26.8 ± 14.2	0.00021*[S]
MON.%	9.75 ± 4.6	9.48 ± 3.18	0.75
EOS.%	1.29 ± 3.1	2.59 ± 2.99	0.064
BAS.%	0.30 ± 0.2	0.39 ± 0.25	0.11
RBCs. (X10^6/mm^3)	5.008 ± 0.6	5.16 ± 0.60	0.25
Hb.(g/dl)	11.21 ± 1.7	12.01± 1.50	0.020*[S]
PLT.	352.31 ± 141.2	344.09 ± 125.64	0.77

*[S]: Means significant difference results.



Figure(4-5): Hematological parameters in patient infected with *E. histolytica* and non-infected group

4-2-2:The effect of *E. histolytica* infection on some physiological immunological parameter.

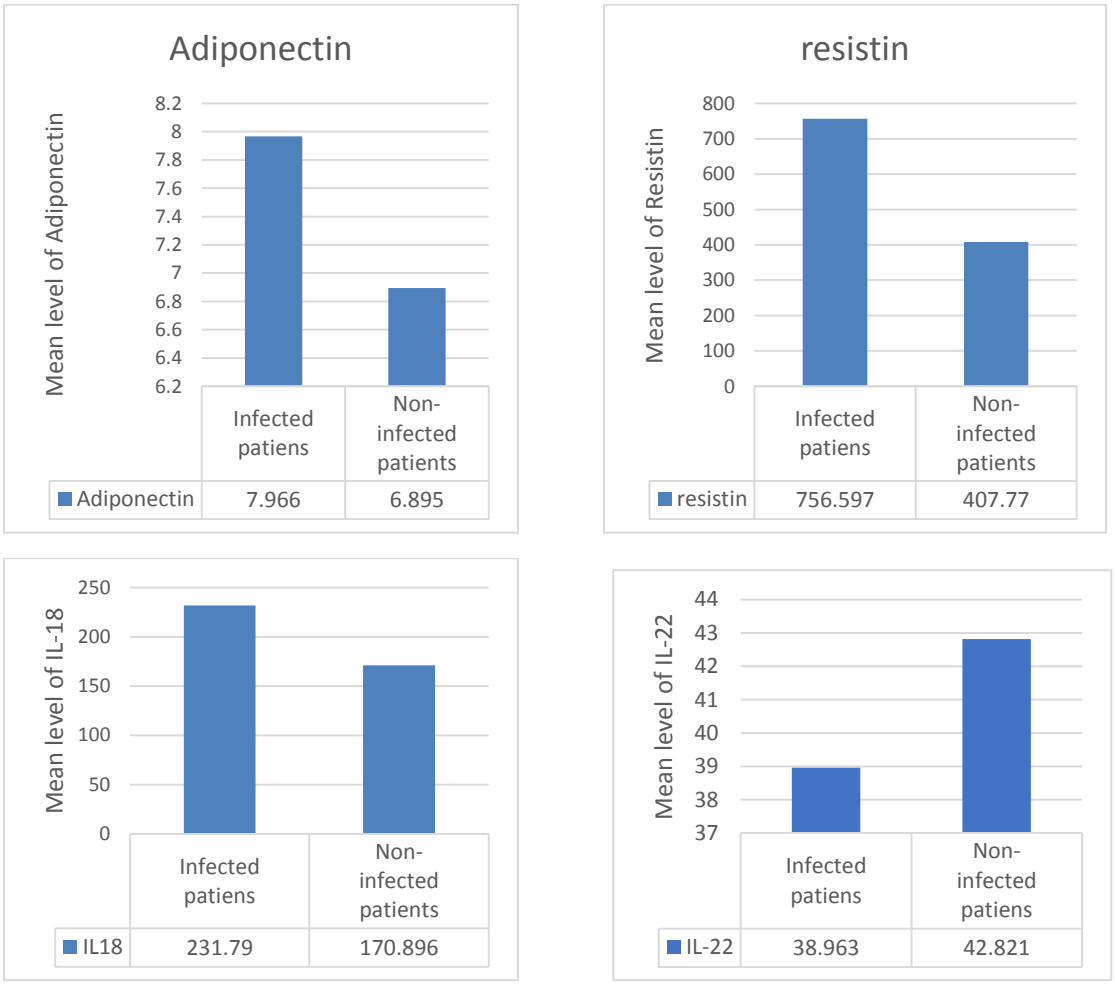
Table (4-8) and figure (4-6) illustreate the effect of *E. histolytica* infected on some physiological and Immunological parameters in Infected and Non-infected patients. Results were shown statistically significantly difference

among infected patients in the levels of Adiponectin, resistin, and IL18 compared to healthy.

Table(4-8): **The effect of *E. histolytica* infected on some physiological and Immunological parameters in the study groups.**

Variables	Infected NO.	Non-infected NO.	P value
Adiponectin(ng/ml)	7.966 ± 0.749	6.895 ± 0.585	0.0005*[S]
Resistin (pg/ml)	756.597 ± 319.553	407.770 ± 122.987	0.00027*[S]
IL18(pg/ml)	231.790 ± 115.018	170.896 ± 90.326	0.05*[S]
IL22(pg/ml)	38.963 ± 17.249	42.821 ± 30.904	0.44

*[S]:Means significant results



Figure(4-6): The effect of *E. histolytica* infected on Phsiological and Immunolgical parameters in the Infected and Non-infected group.

4-2-3: The effect of *E. histolytica* infected on some physiological and Immunological parameters according to gender.

Table (4-9) and figure (4-7) show the effect of *E. histolytica* infected on some physiological and Immunological parameters according to gender. Results show that the levels of Adiponectin and resistin were statistically significantly differenece among infected male & female patients compared to non-infected.

Table(4-9): The effect of *E. histolytica* infected on some physiological and Immunological parameters according to gender.

Variables Mean ±SD	Infected Female	Non-Infected Female	P value	P value between infected male & female	Infected male	Non-Infected male	P value	P value between non-infected male &female
Adiponectin (ng/ml)	7.90 ± 0.74	6.87± 0.42	0.00021*[S]	0.37 [NS]	8.02 ± 0.76	6.91 ± 0.71	0.00018* [S]	0.41[NS]
Resistin (pg/ml)	757.6 ± 310.9	393.3 ±112.9	0.00052*[S]	0.49 [NS]	755.6 ±334.6	421.5 ± 151.7	0.00021* [S]	0.24[NS]
IL-18 (pg/ml)	222.9 ± 113.8	181.5 ± 60.1	0.33	0.38 [NS]	240.6 ± 118.0	160.7± 124.6	0.081	0.23[NS]
IL-22 (pg/ml)	41.1 ± 18.9	55.4± 43.1	0.16	0.29 [NS]	36.8 ± 15.4	30.88 ± 13.2	0.41	0.016[S]

*[S]: Means significant results

[NS]: Means non significant results

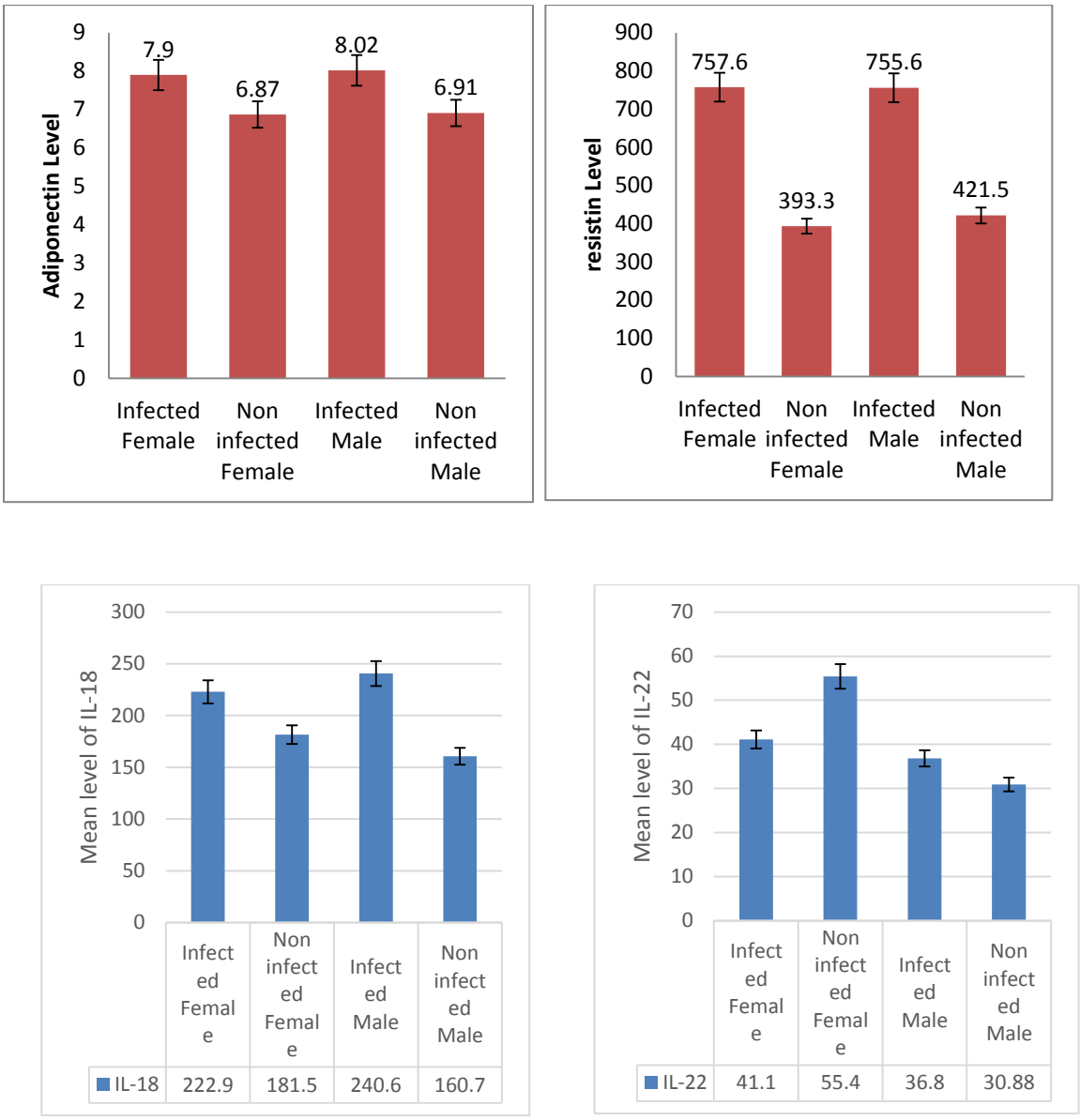


Figure (4-7): The effect of *E. histolytica* infected on Physiological and Immunological parameters concentration according to gender.

4-2-4: Effect of *E. histolytica* on physiological and Immunological parameters according to age groups.

Table (4-10) illustreates the effect of *E. histolytica* on physiological and Immunological parameters according to the age groups which were not statistically significantly differenece among study groups.

Table(4-10):Effect of *E. histolytica* on physiological and Immunological parameters in Infected & Non-Infected according to age groups.

Parameters	Age range >5 year			Age range (5-10) year			Age range > 10		
	Infected Patients	**Non-Infected**	**P value**	**Infected Patients**	**Non-Infected**	**P value**	**Infected Patients**	**Non-Infected**	**P value**
Adiponectin(ng/ml)	8.28 ± 0.74	6.73 ± 0.65	0.95 [NS]	8.04 ± 0.71	6.75 ± 0.66	0.25 [NS]	7.536 ± 0.606	7.16 ± 0.27	0.34 [NS]
Resistin (pg/ml)	739.8 ±386.8	442.5 ± 132.1	0.60 [NS]	739.3± 293.1	399.8±156.5	0.61 [NS]	790.5±272.1	375.6 ± 108.0	0.94 [NS]
IL-18 (pg/ml)	211.9 ±101.7	157.1 ± 73.2	0.72 [NS]	237.6 ±101.9	222.2 ±137.8	0.72 [NS]	248.9 ±141.1	135.4 ± 47.3	0.87 [NS]
IL-22 (pg/ml)	41.59 ±18.95	27.16 ± 11.99	0.26 [NS]	34.46 ±18.39	60.8 ± 52.1	0.61 [NS]	39.94 ±14.27	42.8 ± 13.9	0.51 [NS]

[NS]: Means non significant result

4-2-5:Correlation between the physiological and Immunological parameters in the Infected patients.

The Correlation was used for determining linear relationships between each marker in infected patients. There was a non-significant connection between all biomarkers levels in infected patients except IL-18 and resistin ($p \leq 0.05$, Table 4.11 and fig. 4-8).

Table(4-11): Correlation between physiological and Immunological parameters in infected patients

Variables	Correlation coefficient (r)	P-value
	Interleukin -18	
IL-22	0. 1	0.61
Resistin	-0.4	0.05*[S]
Adiponectin	0.1	0.83
Interleukin- 22		
Resistin	-0.2	0.84
Adiponectin	-0.1	0.43
Adiponectin		
Resistin	-0.1	0.53

*[S]: Mean significant results

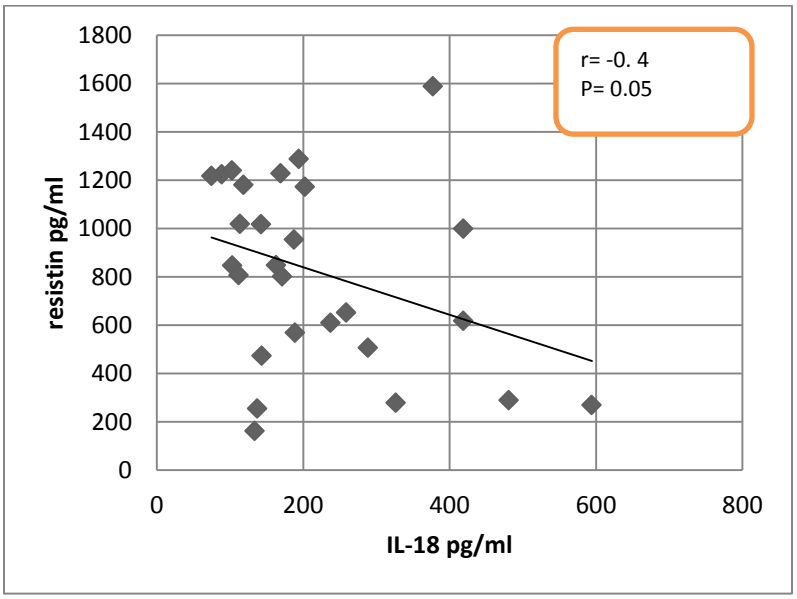


Figure (4-8): Correlation between the IL-18 and Resistin concentration in infected patient.

4-3:The Molecular study.

4-3-1: Study of Associated factors of Genotypes of IL-18 (single-nucleotide polymorphisms) in intestinal parasites Patients compared to control group

The results showed the distribution of Genotype of IL8 single-nucleotide polymorphisms in *E. histolytica* patient compared to the control group. Results of the genotype (Table 4-12 and fig. 4-9 A,B,C,D). which were showed that the mutation in the polymorphism of SNP 1 (rs1866694757), SNP 4 (rs 1946518), SNP 6 (rs1946519), and SNP 7 (rs 1215648807) were demonstrated a risk factor of intestinal parasites in patients group than in control group, (OR = 1.333, 1.800, 1.200 and 1.750; 95% confidence interval = (0.321- 5.538) , (0.264- 12.296), (0.396-7.732) and (0.396-7.732) respectively. No significant difference were found. While, in the genotype polymorphism of SNP 2 (rs 940255648), SNP 3 (rs 1037707423), SNP 5 (rs1213044637), SNP 8 (rs 1866697972), SNP 9 (rs 1866698066) and SNP 10 (rs1866698286) were showed a protective factor (OR = 0.364, 0.308, 0.955, 0.500, 0.393, 0.073 ; 95% confidence interval = (0.084- 1.583), (0.052- 1.829), (0.235- 3.878), (0.101-2.477),(0.081-1.909) and (0.008-0.689) respectively.

Interestingly, only the genotype of SNP 10 (rs1866698286) AA (Wild type) / AT (Mutant heterozygous) was shown a statistically significant difference among study groups.

Table(4-12):Associated factors of Genotype of IL18 (single-nucleotide polymorphisms) in *E. histolytica* Patients compared to control group.

Genotype of IL-18	Odds Ratio (OR)	Relative Risk (RR)	95% CI (Lower-Upper)	P value P≤0.05	Patient(%)	Control(%)
SNP 1(rs1866694757) CC (Wild type)/CA (Mutant heterozygous)	1.333	1.167	0.321- 5.58	(0.731)	C (0.88) A (0.12)	C (0.88) A (0.12)
SNP 2 (rs 940255648) GG (Wild type) / GC (Mutant heterozygous)	0.364	0.559	0.084- 1.583	(0.284)	G (0.92) C (0.08)	G (0.82) C (0.18)
SNP 3 (rs 1037707423) CC (Wild type)/ CT (Mutant heterozygous)	0.308	0.481	0.052- 1.829	(0.242)	C (0.96) T (0.04)	C (0.88) T (0.12)
SNP 4 (rs 1946518) TT (Wild type)/ TG (Mutant heterozygous)	1.800	1.421	0.264-12.296	(0.661)	T (0.66) G (0.34)	T (0.64) G (0.36)
SNP 4 (rs 1946518) TT (Wild type)/GG (Mutant homozygous)	1.02	1.306	0.564- 12.80	(0.91)	T(0.94) G(0.06)	T(0.86) G(0.14)
SNP 5 (rs1213044637) GG (Wild type)/ GA (Mutant heterozygous)	0.955	0.975	0.235- 3.87	(0.614)	G (0.82) A (0.18)	G (0.78) A (0.22)
SNP 6 (rs1946519) CC (Wild type) /AC (Mutant heterozygous)	1.200	1.111	0.160- 9.01	(0.633)	A (0.64) C (0.36)	A (0.6) C (0.4)
SNP 6 (rs1946519) CC (Wild type) / AA (Mutant homozygous)	0.25	0.25	0.24-0.43	(0.15)	A (0.76) T (0.14)	A (0.66) T (0.04)
SNP 7 (rs1215648807) AA (Wild type) / AC (Mutant heterozygous)	1.750	1.375	0.396- 7.73	(0.712)	A (0.78) C (0.22)	A (0.78) C (0.22)
SNP 8 (rs1866697972) GG (Wild type) / GT (Mutant heterozygous)	0.500	0.667	0.101- 2.47	(0.458)	G (0.94) T (0.06)	G (0.88) T (0.12)
SNP 9 (rs1866698066) GG (Wild type) / GT (Mutant heterozygous)	0.393	0.575	0.081- 1.909	(0.283)	G (0.94) T (0.06)	G (0.86) T (0.14)
SNP 10(rs1866698286) AA (Wild type) / AT (Mutant heterozygous)	0.073	0.175	0.008- 0.68	(0.018)*	A (0.86) T (0.14)	A (0.6) T (0.4)
SNP 10(rs1866698286) AA (Wild type) / AT (Mutant heterozygous)	0.65	0.235	0.264-0.43	(0.3)	A (0.56) T (0.24)	A (0.86) T (0.14)

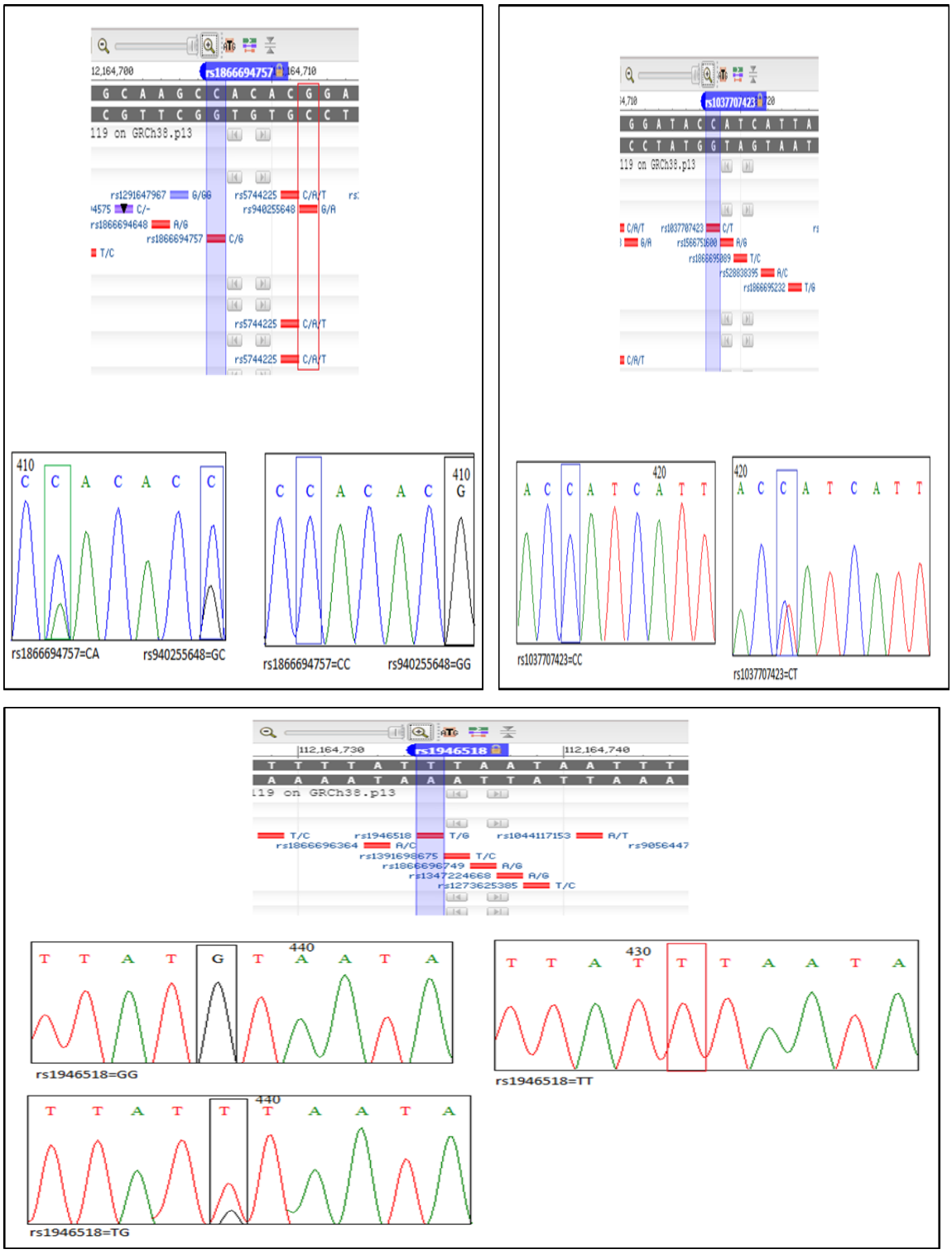


Figure (4-9)A : The genotypes of IL-18 (SNP1 , SNP2,SNP3 and SNP4) in *E .histolytica* patient compared to the control group

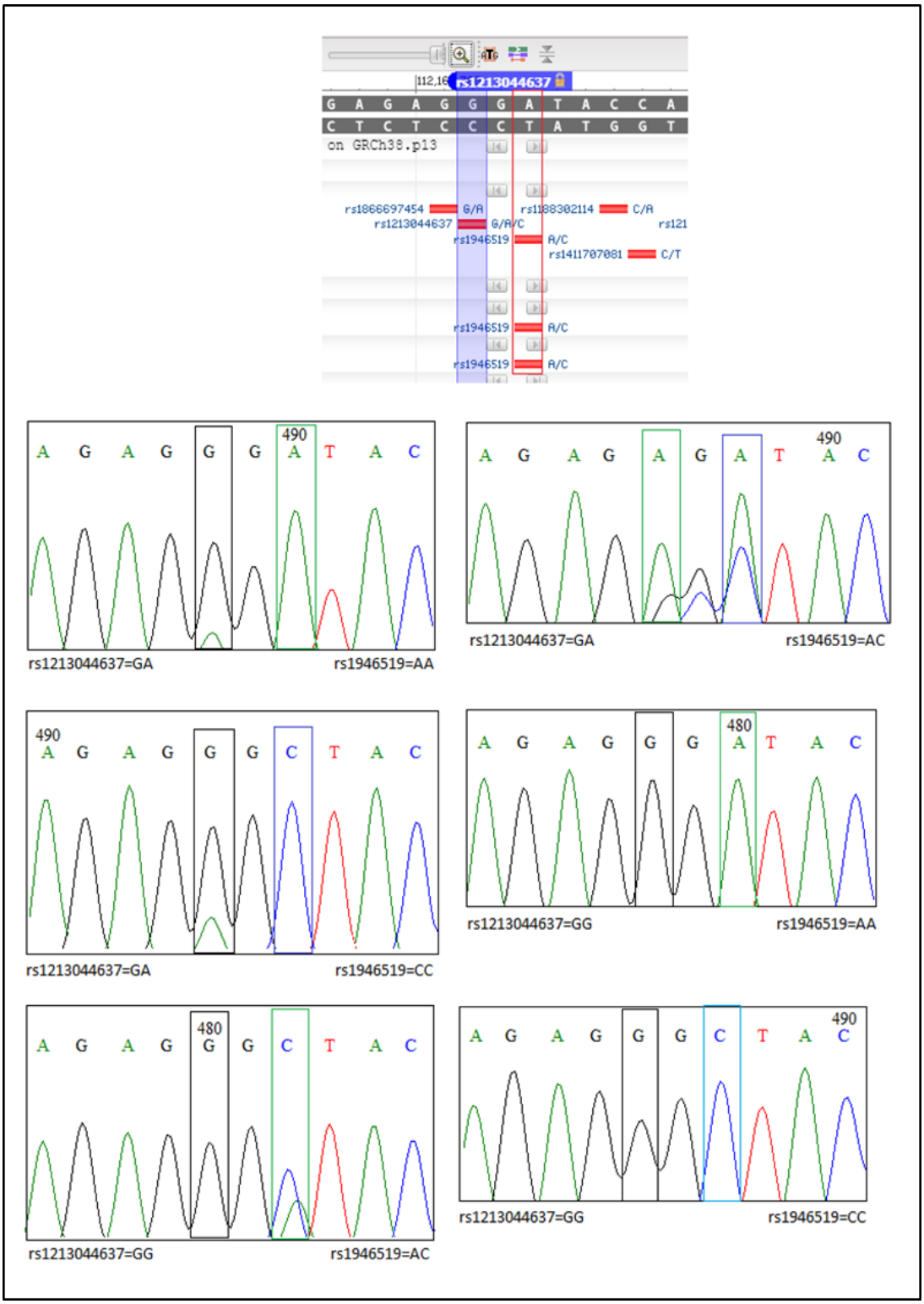


Figure (4-9)B: The genotype of IL-18 (SNP5 and SNP6) in *E.histolytica* patients compared to the control group.

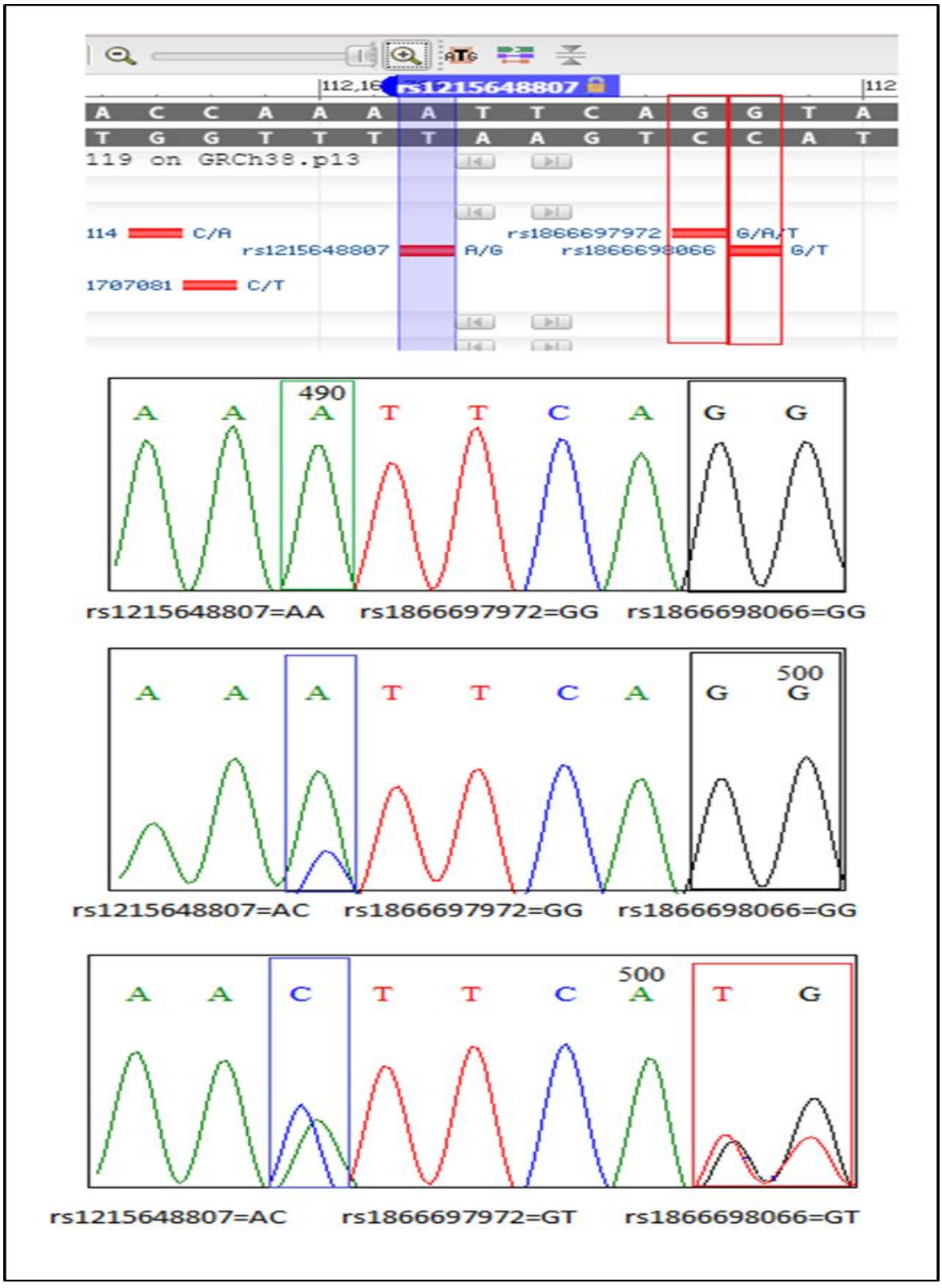


Figure (4-9) C: The genotype of IL-18 (SNP7 , SNP8 and SNP9) in *E.histolytica* patients compared to the control group

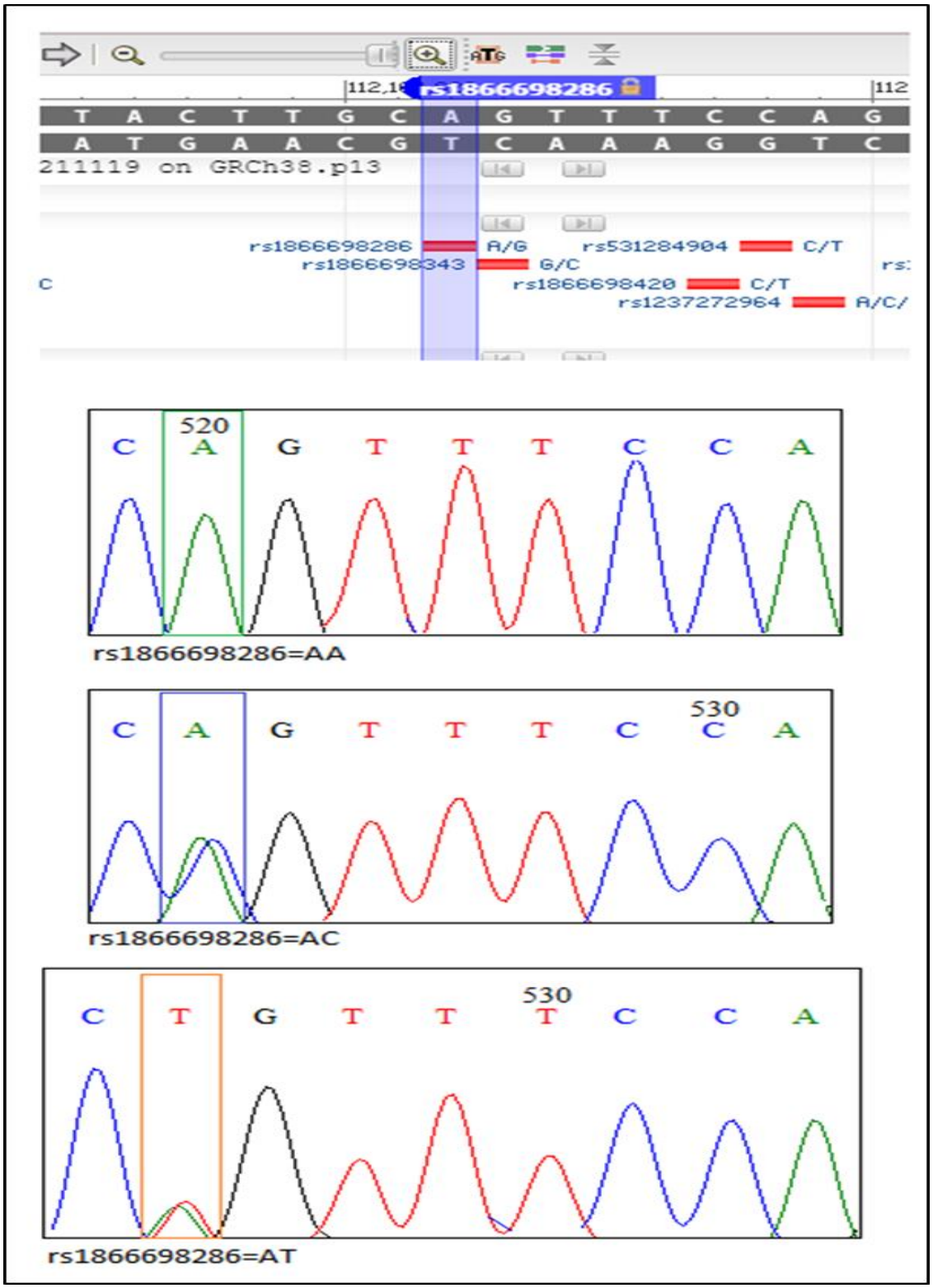


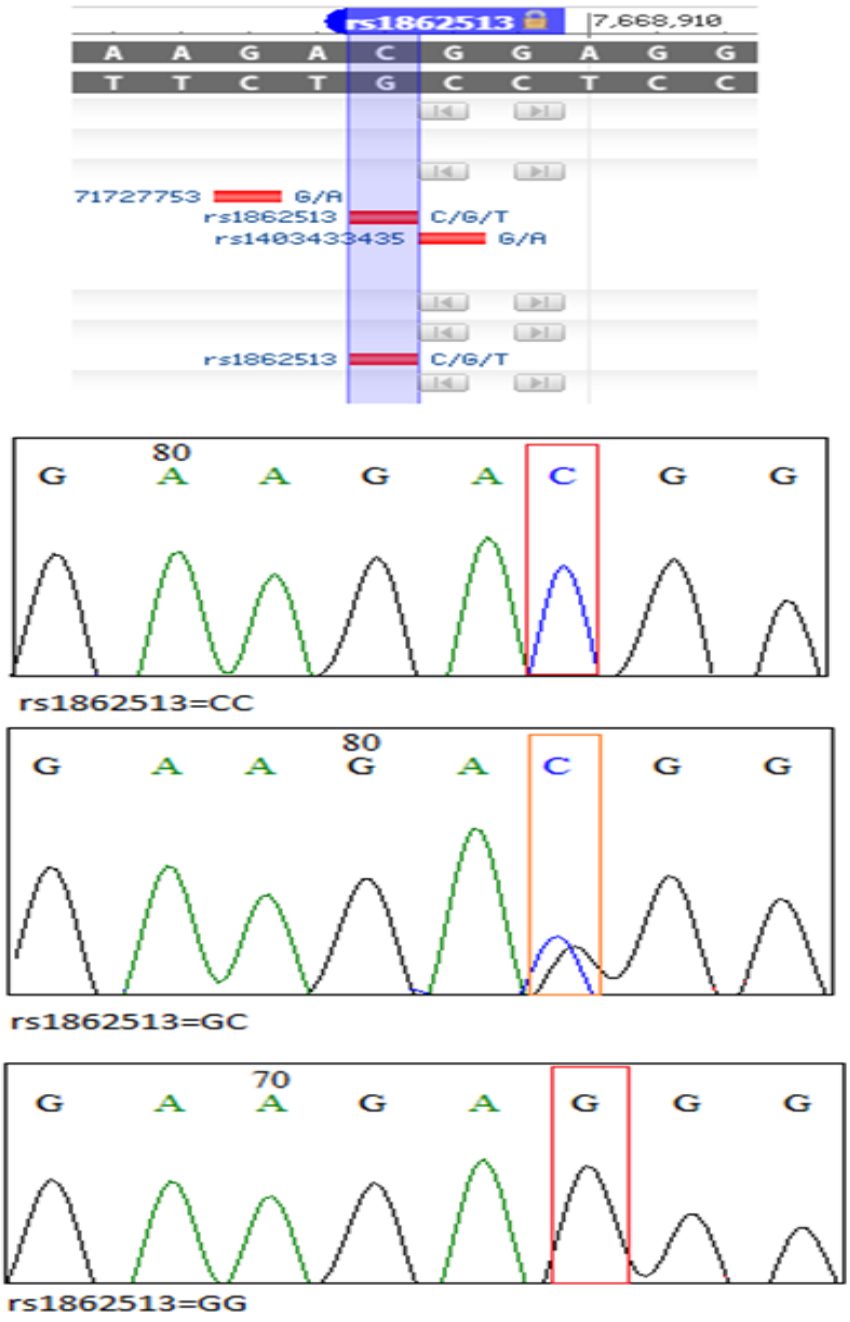
Figure (4-9) D: The genotypes of IL-18(SNP10) in *E. histolytica* patients compared to the control group.

4-3-2:Study the Associated factors of Genotypes of resistin (single-nucleotide polymorphisms) in *E. histolytica* patients compared to the control group.

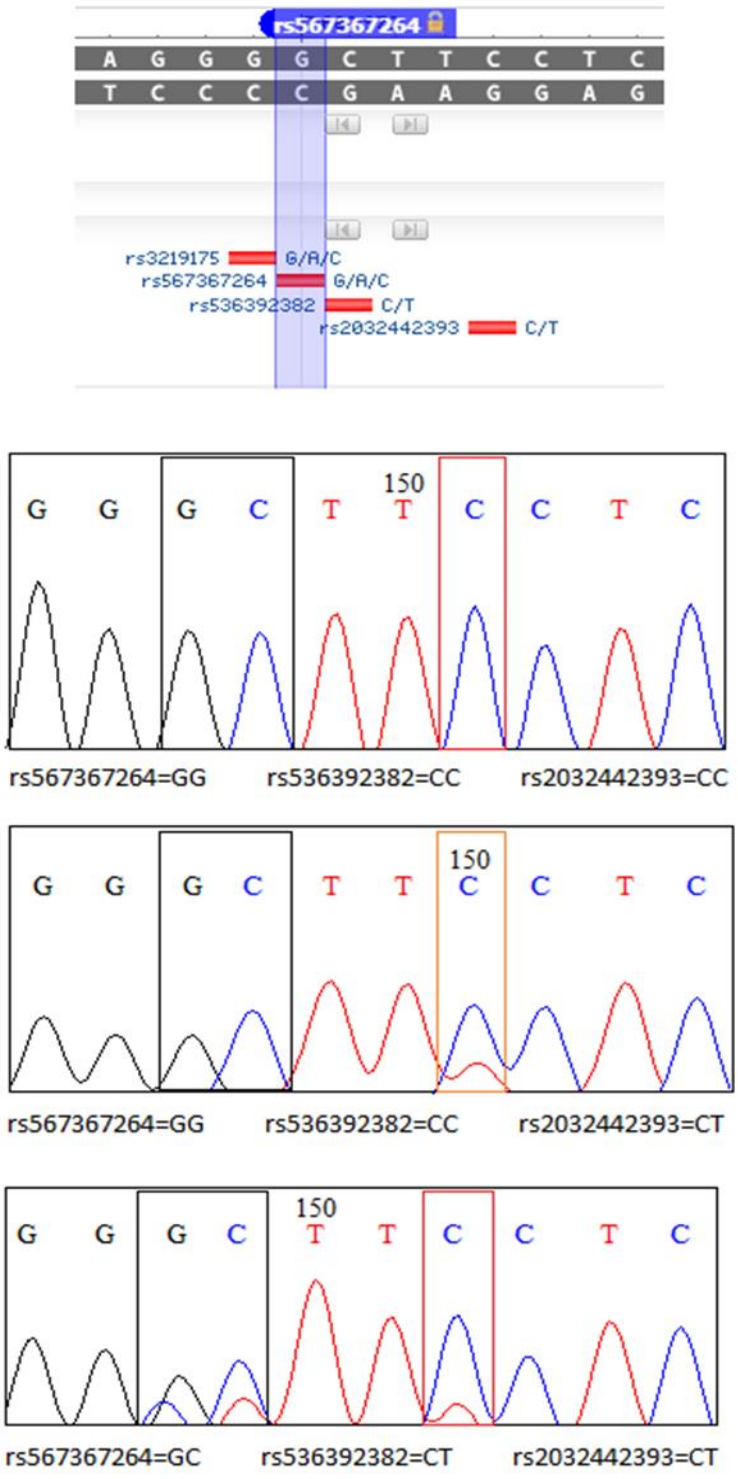
Figure(4-10A and B) demonstrated the distribution of Genotype of resistin single-nucleotide polymorphisms in *E. histolytica* patients compared to control group. Results of the genotype of resistin showing in Table (4-13) was indicated that the mutation in the polymorphism of SNP1(rs1862513),SNP 2(rs567367264) and SNP3(rs536392382) were demonstrated a risk factor of intestinal parasites in patients group than in control group, (OR = 2.068, 1.360 and 1.360 ; 95% confidence interval = (0.477- 8.973) , (0.344- 5.379) and (0.344-5.379) respectively. While, in the genotype of resistin polymorphism of SNP 4 (rs2032442393) was showed a protective factor (OR = 0.952; 95% confidence interval = (0.056- 16.279)). No significant differences were found in all Genotype of resistin polymorphisms.

Table(4-13):Associated factors of Genotype of resistin (single-nucleotide polymorphisms) in *E. histolytica* Patients Compared to control group.

Genotype of resistin	Odds Ratio (OR)	Relative Risk (RR)	95% CI (Lower-Upper)	P value P≤0.05	Patient (%)	Control (%)
SNP 1 (rs1862513) CC (Wild type)/ CG (Mutant heterozygous)	2.068	0.720	(0.477- 8.973)	(0.471)	C (0.58) G (0.42)	C (0.66) G (0.34)
SNP 1 (rs1862513) CC (Wild type) / GG (Mutant homozygous)	1.750	1.375	(0.275-11.152)	(0.658)		
SNP 2 (rs567367264) GG (Wild type)/ GC (Mutant heterozygous)	1.360	1.164	(0.344- 5.379)	(0.736)	G (0.88) C (0.12)	G (0.9) C (0.1)
SNP 3 (rs536392382) CC (Wild type)/ CT (Mutant heterozygous)	1.360	1.164	(0.344-5.379)	(0.736)	C (0.88) T (0.12)	C (0.88) T (0.12)
SNP 4 (rs2032442393) CC (Wild type) / CT (Mutant heterozygous)	0.952	0.976	(0.056-16.279)	(0.774)	C (0.6) T (0.4)	C (0.58) T (0.42)



Figure(4-10)A: The genotypes of resistin (SNP1) in *E. histolytica* patients compared to the control group



Figure(4-10)B: The genotypes of resistin (SNP2,SNP3 and SNP4) in *E. histolytica* patients compared to the control group.

4-3-3:Association between IL-18 , Resistin parameters and gene polymorphisms.

4-3-3-1 Association between IL-18 concentration and polymorphisms of IL-18 Gene.

Although IL-18 gens polymorphisms were never shown any statistically significant differences with any of the gene polymorphisms SNPs ($p \leq 0.05$). There are a increase in the level of IL-18 in SNPs(SNP1/CA, SNP2/GC, SNP3/CT, SNP5/GA, SNP7/AC, SNP8/GT, SNP9/GT) compare with wild type respectively and (SNP4/TG/GG,SNP6 AC/CC),compare with wild and hetero type respectively and (SNP10 TT/AC)compare wild and hetero type respectively (Table 4.14).

Table (4-14):Multiple Comparisons of Dependent Variable and Least Significant Difference-Post Hoc Test for IL18 concentration with gene IL18 polymorphisms

Dependent Variable: IL18 LSD		Mean Difference	P value
SNP1/ (rs1866694757)			
CC	CA	-128.51900	0.138
	AA	/	/
SNP2/ (rs940255648)			
GG	GC	-107.79806	0.258
	CC	/	/
SNP3 / (rs1037707423)			
CC	CT	-14.01779	0.815
	TT	/	/
SNP4/ (rs1946518)			
TT	TG	-71.72067	0.317
	GG	-119.06442	0.135
TG	TT	71.72067	0.317
	GG	-47.34375	0.595
SNP5/ (rs1213044637)			
GG	GA	-7.66439	0.911
	AA	/	/
SNP6/ (rs1946519)			

AA	↑ AC	-71.72067	0.317
	↑ CC	-119.06442	0.135
AC	AA	71.72067	0.317
	↑ CC	-47.34375	0.595
SNP7/ (rs1215648807)			
AA	↑ AC	-88.45599	0.153
	CC	/	/
SNP8/ (rs1866697972)			
GG	↑ GT	-91.05894	0.378
	TT	/	/
SNP9/ (rs1866698066)			
GG	↑ GT	-120.23421	0.110
	TT	/	/
SNP10/ (rs1866698286)			
AA	AT	44.20238	0.501
	↑ TT	-69.30383	0.295
AT	↑ AA	-44.20238	0.501
	↑ TT	-113.50621	0.199

↑: Means increas in concentration

4-3-3-2:Association between Resistin concentrationand polymorphisms of Resistin Gene.

Resistin concentration was statistically significantly differenece between Resistin gene polymorphisms, SNP 1 and SNP 4. There are a significant increases from SNP 1 (rs1862513) CC to CG (p = 0.005), and from SNP 1 (rs1862513) CG (Mutant heterozygous) to GG (Mutant homozygous),p= 0.009).

Moreover, there was a statistically significant difference between resistin level and SNP4 (rs2032442393) and the resistin concentration in the SNP 4 (rs2032442393) from CC (Wild type) to CT (Mutant heterozygous), p = .019), while no statistically significant difference with the rest of the Resistin gene SNPs (Table 4.15).

Table (4.15):Multiple Comparisons of Dependent Variable and Least significant difference-post hoc test for Resistin level with Resistin gene polymorphisms.

Reference Group	Comparison Group	Mean Difference	P value
SNP1/ (rs1862513)			
CC	↑ CG	-432.88830*	0.005 *[S]
	GG	196.97970	0.298
CG	CC	432.88830*	0.005 *[S]
	↑ GG	629.86800*	0.009 * [S]
SNP**2**/ (rs567367264)			
GG	↑ GC	-136.79157	0.293
	CC	/	/
SNP**3** / (rs536392382)			
CC	↑ CT	-128.36423	0.322
	TT	/	/
SNP**4**/ (rs2032442393)			
CC	CT	427.77945*	0.019 *[S]
	TT	/	/

↑: Means increase in concentration

*[S]: Means significant results

Chapter Five

Discussion

5:Discussion

5-1:Epidemiological Study

5-1-1:Overall percentage rates of intestinal parasitic infections

Parasitic infection is widespread throughout the world, especially in tropical and subtropical regions and particularly in developing countries, as a result of the involvement of numerous complex and interrelated factors, including host factors, environmental behaviors, economic state, genetic factors, and immunological response (Al-Ammash, 2015).

Table (4-1) shows that 13 % of Kerbala province residents examined positive for having intestinal parasites as part of this investigation. The present percentage rates of total infection nears many studies involved: Jaeffer (2011) in Al-Shulaa and Al-Khadimya Baghdad-Iraq, with the percentage of infection being (13.64%), Khalil (2018) in Erbil City the percentage of infection is (14%), Alemu *et al*.(2019) in Chagni Town, Northwest Ethiopia, the prevalence of infection with at least one of the intestinal parasites infection was 14.8% and Nayyef *et al*.(2022) in Al-Furat general hospital in Baghdad/Iraq the prevalence of *E. histolytica* was 15.89% among 497 patients.

However, Al-Kahfaji (2014) research in Al-Hilla city, Babylon Province, showed a rate of infection with parasites of about (39.5%), therefore the current proportion of overall infection is lower. A total prevalence of intestinal parasite infection of 19.66% was found in the Kirkuk Province conducted by (Salman *et al*., 2016). Al-Hasheme *et al*.(2020) in holy city of Kerbala, Iraq showed that the total infection percentage of intestinal parasites was (18.93%) and the study of prevalence of intestinal parasites

among 405 children (1-12 years old) from both gender in Al- Diwaniyah province was investigated by Al-Waaly (2020) the rate of infection was 61.23%, (Alsadoon *et al*., 2021) in Baghdad province the total rate of infection was 17.8%, also in French (Aboikoni *et al*., 2021).

While the current total of infection rate was higher than it was recorded by Hasan (2010) in Kerbala, Iraq, (11.6%), Ibrahim (2012) in Kadhmiyah Hospital with the rate of infection of *E. histolytica*(9.80%), and *G. lamblia* was (1.77%), and also Flaih *et al*., (2021) in Thi-Qar province when the rate of infection is (11.1%).

According to this study, intestinal parasite infections may occur in different geographical areas for a variety of reasons, including environmental factors, poor personal hygiene, the socioeconomic status of the community, social customs and traditions, unsanitary conditions, lack of access to clean drinking water sources, and climatic factors (Sitotaw & Shiferaw, 2020).

Different habitats and geographies, sample sizes, research durations, weather and economic conditions, and other variables may all have an impact on the overall prevalence of intestinal parasite infection, as shown by the data. Intestinal parasite detection varies among age groups, kinds, and study environments; some of these factors include relying only on the direct method and excluding any sort of concentration from feces studies, sedimentation or flotation, and so on (Jarallah, 2012; Al-Hasheme *et al*., 2020).

5-1-2:**The percentage of intestinal parasite infection according to the gender**

Table (4-2) shows that in Kerbala province, the rate of infection with the intestinal parasite were highest in males (14.1%), and lowest in females (11.7%). The current study agreed with study Siddig *et al*., (2017) in Khartoum, Sudan when recorded the highest rate of infection among males 80% and the lowest rate of infection among females60%. Also agreeing with the study of Dayoub (2020) in Lattakia/Syria when recorded a higher rate of infection in the males 2.15% than in the females 1.60%. As well as agreed with the study Zaki (2022) in Mosul, Iraq when recorded the highest rate were in the males (9.59%)while the lowest rate of infection were in the females (6.19%).

This study disagreed with the study Mazigo *et al*. (2010) in Tanzania when recorded the highest rate of females (55.7%)while the lowest rate of infection in males (44.3%) as well as with study Mezeid *et al*. (2014) in Palestine demonstrated a higher prevalence of intestinal parasitic infections in females 42.7% than males 39.0%.Also the study by Barati *et al*. (2021) in Tehran, Iran, recorded the highest rate of females 24.64% while the lowest rate of infection in the males 19.71%.

Also the current study disagree with Belete *et al*. (2021) in Ethiopia when scored the higher rate of infection were in the females 68% while the lower rate of infection were in the males (32%).

The weaker immunity of men, who often demonstrate the increased severity of illness, may explain why males have a higher infection rate compared to females. This is because females are more resistant to parasite infections than males (Klein, 2000). In humans, these variations are often

thought to result from a combination of hormonal and environmental variables, When it comes to the environment, variables like sex-based differences in vulnerability to parasites may play a role (Abioye *et al*., 2019).Variations in endocrine-immune interactions can also be invoked to explain these differences in infection between gender. Additionally, sex steroids, particularly androgens in males and estrogen in femals, alter a variety of aspects of host immunity by regulating the expression of Toll-like receptors, cytokines, and antibodies. Androgens also lower immune competence. The steroid hormones have an impact on the genes and behaviors that increase male susceptibility to infection and disease. The lowest incidence in females may also be related to their greater attention to personal cleanliness (Callixte *et al*., 2019).

5-1-3:**The percentage of intestinal parasite infection according to the species of parasites**

Table 4-3 shows that in the province of Kerbala, the infection rate was greatest for *E. histolytica* (10.54%), followed by *G. lamblia* (2.46%), *C. parvum* (0.4%), *H. nana* (0.24%), *E. vermicularis* (0.13%), and finally *T. hominis* (0.03%). This study agreed with Chala (2013) in Ethiopia when recorded 8.3% for *E. histolytica* /*E. dispar* and 6.5% for *G. lamblia* in addition to the least prevalent intestinal parasite was *T. trichiura* 0.001%, as well as agreed with Turki *et al*. (2017) .It also agree with Al-Daoody *et al*. (2021) in Erbil city, Iraq where found *E. histolytica* was the most prevalent parasite (80.1%), followed by *G. lamblia* (19.8%) and *H. nana* (0.1%).

While Salman *et al*.(2016) found an intestinal parasitic infection rate of 19.66% in Kirkuk province, this study found a rate of 10.31% for *G. lamblia*

and a rate of 9.35% for the other nine intestinal parasites, including *B. homonis* (4.17%), *E.histolytica* (1.67%),*C. parvum* (1.43%), *E. coli* (0.71%), *Cyclospora cayetanensis*, (0.49%). While Jaran (2016) found that *G. lamblia* was the most common parasite in northern Jordan (41%), he found that *E. histolytica* and *E. coli* were the next most common (31% each), and that *A. lumbricoides* (1%), *H. nana*, *Taenia sp*., and *Chilomastia mesnili* (all 1%) were the least common.

It also disagree with Al-Waaly (2020) in Al-Diwaniyah City, Middle of Iraq, the highest rate (41.93%) was reported for *E. vermicularis*, followed by *G. lamblia* (23.46%), *E. histolytica* (15.06%), *E. coli* (3.70%), *H. nana* (4.44%), *Dientamoeba fragilis* (1.73%), *A. lumbricoides* (1.48%) and *T. saginata* (0.49%).

Iraq is one of the countries with the highest frequency of *E. histolytica* in the world, (Al-mozan *et al*., 2017). Additionally, the humid climate creates ideal conditions for Entamoeba's mature cyst (Egwunyenga & Ataikiru, 2005). Also attribute the high prevalence of *E. histolytica* to the direct mode of transmission because it does not require an intermediary host on this side. On the other side, there is less focus on aseptic water treatment, and a shortage of materials required for water purification (Hadi, 2011). Also represents flies carrier mechanical for cysts of parasites can participate in increasing the rate of prevalence of this parasite (Jabar, 2017).

This study found that more people had protozoan infections than helminth infections. This finding may be related to the fact that protozoa parasites are easier to transfer than helminth eggs or larvae. The eggs or larvae of parasitic worms, for example, continue to grow and develop in the soil or

intermediary host long after the worms themselves have died off (Jacobsen *et al*., 2007; Arani *et al*., 2008). The single-stage life cycle of *T. hominis*, during which infection takes place, and the fact that the infecting stage only feeds during the quiescent phase may both contribute to the parasite's relatively low prevalence (Al-Hasheme *et al*., 2020).

5-1-4:Percentage rates infection with intestinal parasites according to types of infection(single or mixed)

Table (4-4), the results showed that a single infection pattern is the most common representing (12.65%) while the rates of double, triple, and infections were 3.2%, and 0.03% respectively. The Chi-square (X^2) test revealed a statistically significant difference in the ratios of parasite types in children at the $p \leq 0.05$ level. The result of the current study agrees with the result of Abdullah & Al-Abbadie (2005) in Mosul City recorded the percentage of single, double, triple and more than triple infections were 7.48%, 4.27%, 1.92% and 0.43%, respectively.

In accordance with the findings of Abossie & Seid (2014) Southern Ethiopia, at the town of Chencha, in this analysis, the most common types of infections were single infections (59.5%), double infections (19.0%), and multiple infections (2.5%). The frequency of this one infection was much greater than that of any other.

Gabbad & Elawad (2014) in Khartoum, Sudan illustrated that most of the infected children were suffering from a single infection (47.6%), double infection (15.8%), and triple infection(1%).

The researcher concur with the findings of Al-Taei (2019) in the Babylon province of Iraq, who found that the prevalence of single parasite infection was highest (99.51%) and the prevalence of double infection was lowest (0.49%).

Al-Waaly (2020) in Al-Diwaniyah City, Middle Iraq recorded that a single infection pattern is the most common representing 38.27%, while the rates of double, triple, and quadruplicate infections were 25.40%, 11.29%,and 0.81%, respectively.

Dayoub (2020) in in Lattakia/Syria found the prevalence of infection with intestinal helminths of patients attending the microbiological laboratory of Tishreen University Hospital in Lattakia from of 2016-2019 was 1976%, the majority of them were infected with only one species of intestinal helminths, while only two cases of double infection were recorded.

It also agree with Jaffar *et al*. (2021) in Wasit province/Iraq who found that the numbers and percentages of a single and double (two types of parasites) infections were (95.4 %), and (4.6 %), respectively.

Due to the existence of favorable conditions for the growth and development of several parasites, children are more likely to contract multiple types of intestinal parasites(Al-Bayati & Al-Hassany, 2014), in addition to socio-environmental elements that regulate the occurrence and spread of intestinal parasite diseases, such as climatic elements, drinking water, sanitation, and personal hygiene practices (Erismann *et al*., 2016).

5-1-5: **Percentage rates infection with intestinal parasites according to the months of study.**

Tables (4-5) show that there is a seasonal variation in infection rates. During the month of September of 2021, the infection rate peaked at 30.1%. In contrast, the incidence of infection was 3.5% in February of 2022.Several studies had shown differences in infection rates according to the months of the year such as the study of Hussein *et al*. (2011) in Sebha City, Libya showed that the highest infection rate was in February (22.8%), and the lowest rate was in June by 8.0%, with significant differences in the prevalence of infection between the year months. According to research conducted by Al-Garawi (2015) in Karbala, the month of November had the greatest infection rate (47.88%) while the month of February which was the lowest (36.36%). As well as the study of Al-Ammash (2015) in Samarra the percentage of total infection in January (54.1%) was higher than in other months of this study, while it was lowest in March (16.9%). The conclusion of the research Jaran (2016) in northern Jordan indicated that the frequency of various parasites varied according to season, on average the summer months showed the greatest incidence of parasitic infection (62%) compared with the winter months (16%). Al-Waaly (2020) in Al-Diwaniyah City, also showed the highest incidence of 92.5% in July, while the minimum was 36.36% in January. Al-Hasheme *et al*. (2020) found that intestinal parasite infection rates increased in March (23.41%) and droping to July (15.11%). It was shown in a recent research conducted by Al-Daoody *et al*.(2021) in Erbil City, that September had the greatest infection rate (10.3%), while October and November had the lowest infection rate (6.2%).

Parasitic disease seasonality may be affected by a number of factors, including differences in agriculture, water availability, high temperatures, lack of rain and moisture, and the presence and spread of insects and other animals that serve as intermediate or reservoir hosts for many parasites (Al-Garawi, 2015).

And this might be because Iraq is one of the countries with long, hot, dry summers and short, chilly, rainy winters, the population characteristics, such as swimming in rivers and lakes increase susceptibility to infections and have a part in the promotion of parasite infection in hot and dry weather(Stuart *et al*., 2003). An important part of the Iraqi diet is fresh vegetables like lettuce, leeks, and celery, but if they are not properly cleaned, they may cause serious diseases (Al-Daoody *et al*., 2021).

5-1-6 Percentage rates infection with intestinal parasites according to age groups.

The result in Table (4-6) showed the prevalence of intestinal parasite infection in different age groups in the present study, which shows that the highest rate of infection (17.2%) was at the age group of 5-10 years, while the lowest rate of infection (5.4%) was at the age >10 years. This study agrees with the study of Gelaw *et al*. (2013) when they recorded a higher rate of infection at the age group ≤9 years, while a lower rate of infection at the age group ≤13 years. Also it agrees with Al-Garawi (2015) in Kerbala who showed the highest infection was recorded in children >3-6 years representing (52.38%), and the lowest infection was recorded in children 9-12 year representing (24.33%).

Similarly, Siddig *et al*. (2017) in Sudan reported that intestinal parasites were more common among children age group(5-7) years (55%), and less common among children age group (12–14) years (33.3%). A recent research conducted in Madagascar by Habib *et al*., (2021) found that children age group (4-5) years were more likely to be infected with intestinal parasites than children aged 2-3 (53.9% vs. 44.9%).

Also it agrees with Alsadoon *et al*. (2021) in Baghdad they recorded the prevalence of intestinal parasites between the younger ages group (1-10)years which was 9.7% that were more affected than the older patients (15-60) (2.9%), respectively. Findings from the modren research are at odds with those from other studies, such as the one conducted by Abossie & Seid (2014) in Ethiopia, which found that parasite infection was more common among children ages 12-15 than among those ages 9-11 or 5-8 (85.5%, 80.1%, and 78.1%, respectively). Contrary to the findings of Hussein & Meerkhan (2019) ,the infection rate was greatest in children under the age of three and lowest in those who were nine or older in Duhok, Iraq. The rates were 57.85% and 4.98%, respectively. Also Mohammed *et al*.(2019) in Nigeria recorded the age range of (11-15) years which had the highest prevalence of parasitic infection at (36.8%) and none was recorded among the age group 31years and above. The proportion of children infected with intestinal parasites varies with age group, and Al-Hasheme *et al*.(2020) research in Kerbala explains this variation. The incidence of intestinal parasite infection is greatest in children aged (1-5) years (20.34%), next in children aged (5 – 10) years (19.18%), and lowest in children aged (10 – 15) years (13.99%). Children between the ages of (5 – 10) were shown to have a higher rate of IPI infection than children of any age not included in the

research. This finding might be the consequence of unsupervised play and eating near the muck and slack water where human waste is dumped after being removed from latrines. The second probable explanation is that this is the age when kids start venturing out of the house and into less hygienic situations (Habib *et al*., 2021).

Children are more likely to be exposed to soil-transmitted parasites when they play outdoors, and they are also more likely to develop unhealthy behaviors like putting their fingers in their mouth, among other things. poor sanitation and an inadequate education system. Additionally, they interact with pet cats and birds, which are intermediate or reservoir hosts for several parasites (Al-Ammash, 2015). The tendency of this age group to eat unwashed fruits, which may be contaminated with protozoan cysts, might have also contributed to the higher prevalence of *E. histolytica* in this age group (Alemu *et al*., 2019).

5-2: The Physiological and Immuniological study

5-2-1:**TheBlood parameters between patients infected with *E. histolytica* and control group**

Tables (4-7) reveal that compared between infected to non-infected individuals, *E. histolytica* patients have considerably higher white blood cell counts and a more positive white blood cell differential (NEU, LYM). This finding is consistent with others showing a reduction in RBCs count, Hb level,in *E. histolytica* infected patients compared to healthy control group**(**Shaker & Hussein 2016). Also, compared to healthy controls, *E. histolytica*-infected patients had substantially higher total leukocyte counts and differential types of neutrophils, lymphocytes, and mixed cells

(monocytes, eosinophils, and basophils). Also it agrees with Sree *et al*. (2015) in Bengaluru, Karnataka, who found the value of the hematological parameters like Hb and platelet count was less in *E.histolytica* than in control group.

Samarra city's anemia prevalence was determined by hemoglobin percentages (Hb%) in red blood corpuscles (RBCs) and protein insufficiency, as shown by Bazzaz *et al.*(2017). It was assumed in this research that both anemia and protein deficit patients were infected with parasites, only 16% of the 780 individuals screened were found to have anemia; of those, 12% were infected with *E. histolytica* and 4% were infected with *G. lamblia*. Yet only 36% of anemia patients had *E. histolytica* infections and 25% had *G. lamblia* infections.

According to study conducted by Abdullah *et al*.(2017) in the Kashmir valley of India, the average hemoglobin level of infected children increased from 7.960.33g/dl to 9.380.61g/dl, while that of uninfected children decreased from 11.151.23g/dl to 10.052.04g/dl and 11.361.15g/dl to 10.080.80g/dl, respectively. Differential leukocyte count revealed a statistically significant increase in eosinophils (5.00.71%) among infected people compared to uninfected persons (3.20.44%), whereas differences were seen for other white blood cells but were not significant. Also Al-Abodi (2018) in Maysan governorate the results of the study showed a decrease in the level of hemoglobin Hb in children with parasites and the rise in the count of WBC.

They also demonstrated the means of hematological parameters in infants infected and uninfected with intestinal parasites Kerbala province,

which is consistent with the findings of Al-Hasheme *et al*. (2020). Children infected with intestinal parasites had significantly lower levels of red blood corpuscles (RBCs), hemoglobin (Hb), and platelet count compared to children without parasites ($p \leq 0.005$), but higher levels of white blood cells (WBCs), neutrophils (NEUs), basophils (BASs), eosinophils (EOSs), and platelet-like thrombocytopenia (PLTs).

Hemoglobin (Hb), and white blood cell (WBC) counts for *E. histolytica* (20) patients and (20) control were as follows: *E. histolytica* (Hb 9.88g/dl 0.455g/dl, WBC 9.55% 2.81%), and control group (Hb 11.01g/dl 2.597g/dl, and WBC 7.41% 1.179%). Parasites had a statistically significant impact on hemoglobin (Hb) concentration, and white blood cell count (WBC) at the $p \leq 0.05$ level (Obiead *et al*. 2020).

Researchers in Najaf City looked at how the cyst and trophozoite stages of the *E. histolytica* parasite affected a number of blood values (PCV, Lymphocyte, Granulocyte, and Platelets count), Additionally, findings indicated a significant difference only in the cyst stage in (MCV, MCH, and Monocyte), while the other blood parameters (Hb, WBC, RBC, and MCHC) showed no significant changes between the infected and non-infected individual with *E.histolytica* parasite (Al-Shaibani , 2020).

Intestinal parasite *E. histolytica* causes digestive disturbance, vitaminosis, and also it releases the trophozoite motile feeding stage and adheres to villi of the large intestine and sucks the chime from villi, this parasite sucks nearly (50 ml) of blood per day, this is why the current study is concerned with a decrease in Hb concentration in patients with *E. histolytica* compared to a healthy group (Adday, 2009). In addition to the bleeding that

accompanies this process, *E. histolytica* causes necrosis of the intestinal mucosa, which damages and degenerates the absorption sites of necessary substances and also serves as a barrier, blocking the transmission of these substances from the lumen of the intestine to the bloodstream (Al-Mosa & Al-Taie, 2007). Intestinal parasites populate the gastrointestinal tract, particularly iron absorption sites, where they impede the body's ability to absorb carbohydrates, lipids, vitamins (particularly D and B12), folate, iron, and zinc. Anemia is brought on by iron deficiency, which is caused by inadequate absorption (Levy *et al*., 2005).

It is possible that the rise in the frequency of certain kinds of white blood cells is attributable to their function in the immune system's reaction to the successful removal of intestinal parasites, Neutrophils are crucial in inhibiting the immune system's ability to fight off parasites of all kinds (both internal and external). Despite their brief lifespan, they generate a great deal of immunosuppressive chemicals such histamine, cytokines, chemokines, and active lipids that trigger an immune response of type II (cellular immune response to toxicity). The high binding of IgE antibodies to the FcRI receptor on the cell surface is responsible for the activation of these cells, either directly by chemicals produced by the parasite or indirectly by identifying parasite antigens. The number of these cells in tissues is often raised due to several parasite diseases (Eberle & Voehringer, 2016).

Protective functions of neutrophils are shown in amoebiasis. By coming into close touch with neutrophils, amoebas cause the leukocytes to lyse, releasing their lytic enzymes and causing tissue infection. Thus, neutrophils' function in this parasite infection is still up for debate, migrating from the bloodstream to sites of infection, neutrophils engage in a number of

antimicrobial functions once there. These include phagocytosis, degranulation, and the formation of neutrophil extracellular traps (NET). NETosis is a unique kind of cell death that is distinct from apoptosis. Recent studies suggest that autophagy is linked to NETosis (Neeli & Radic, 2012). There has been a recent discovery that trophozoites may trigger the creation of NETs. When neutrophils came into contact with amoebas, they released NET in an explosive fashion, surrounding the parasites and covering them in a cloud of DNA and cell aggregates where they were stuck and eventually destroyed. Furthermore, the microbiome may alter the phenotype of neutrophils, providing immunity against amoebas (Rosales, 2021).

Neutrophils, tissue macrophages, monocytes, dendritic cells, and eosinophils may all be activated in response to some of these reactive cells that appear early in the process and are crucial to the protective response and to creating lifelong immunity to the parasite, neutrophil chemotaxis and activation rely heavily on chemokine induction, namely IL-8. As it is shown in amebic infection, the recruitment and accumulation of polymorphonuclear leukocytes (PMN) may play a role in the development of the intestinal lesion, intestinal lesions may be avoided by reducing PMN levels or blocking them from entering an infected gut (Kasper & Buzoni-Gatel, 2001).

The presence of significant differences between parasites in the percentage of lymphocytes, neutrophils, monocytes, and eosinophils except basophils may be attributable to differences in the nature of the damage caused by each parasite (with worms typically being more effective than protozoa on blood criteria) as well as differences in the importance and work of each cell of white bloods cells and the immunity of the patient (Al-Mozan *et al*., 2017).

5-2-2: The effect of *E. histolytica* infection on some physiological and immunological parameter

The result in Table (4-8) and figure (4-5) showed significant differences in Adiponectin, resistin, and IL18 concentration between infected and non-infected patient and the result showed that present difference in IL-22 concentration was not significant. This result disagrees with the result of Yahya *et al*. (2018) at Mansoura University Children Hospital/Egypt, which showed significant decrease of adiponectin level that whene observed in *E. histolytica*, Strongyloides and both *E. histolytica* and *Giardia* infections compared to the control group. This is because intestinal parasites may cause damage to the intestinal mucosa, such as ulceration, shortening of intestinal villi, and dilatation of crypts, which in turn can activate mesenteric lymph nodes and, in turn, stimulate surrounding adipose tissues to release adiponectin (Desreumaux *et al*., 1999).

Adiponectin has been found to reduce TNF-alpha–mediated inflammatory responses in human aortic endothelial cells and to have a role in the control of inflammatory responses by decreasing the proliferation of myelomonocytic cells (Yokota *et al*., 2000; Yamaguchi *et al*., 2005). The phagocytic activity of lipopolysaccharide (LPS)-stimulated TNF-a generation in macrophages can be blocked by adiponectin. Altogether, these findings point to adiponectin role as a powerful anti-inflammatory cytokine. Critically, several investigations, including (Hemmi *et al*., 2000; Aderem & Ulevitch, 2000), have shown that intracellular signaling generated by microbial cell components is triggered by their binding to members of the Toll-like receptor (TLR) family.

Multiple clinical investigations show a negative correlation between plasma adiponectin levels and C-reactive protein and other inflammatory indicators. By regulating signaling pathways in several cell types, adiponectin dampens inflammation in response to a wide range of stressors, like other members of the collectin family of proteins, adiponectin functions to suppress inflammation by encouraging the removal of early apoptotic cells by macrophages, which may be a key component of its beneficial effects on cardiovascular and metabolic disorders such as atherosclerosis and insulin resistance (Ouchi & Walsh, 2008).

Additionally, adiponectin ability to regulate glucose levels in the blood is well documented. Refeeding increases adipose tissue glucose metabolism and decreases adiponectin, whereas the opposite is true during weight loss (Eizadi *et al*., 2012; Ayyappan *et al*. 2018)

The inflammatory response to microorganisms is kept in check in equilibrium by resistin. The innate immune response is refined and amplified by resistin, and in the event of helminthes (nematode) infection, the inflammatory response of the host is also increased. However, resistin may suppress inflammation in response to a microbial challenge when there is a simultaneous infection with bacteria and helminths or when bacterial compounds (such as LPS or LTA) are used as a stimulant. Overall, resistin's ability to modulate inflammation in varied situations suggests that it does more than just limit unchecked microbial development; it also regulates inflammation, so preventing microbial products from inflicting excessive inflammatory harm to the host (Li *et al*., 2021).

There has been some research on the role of human resistin in helminth and viral infections. Elevated plasma resistin levels have been linked to increased parasite load and inflammatory cytokines such TNF, CCL2, and IL-6 in both chronic filarial nematode infection and intestinal *Ascaris* infection. Here, elevated TNF and CCL2 gene expression in humans were associated with higher Nippostrongylus brasiliensis parasite loads (Gabrielle *et al*., 2018).

Further studies showed that in humans, resistin is mainly produced by leukocytes, especially macrophages, and its contribution to the development of insulin resistance remains unclear. The role of resistin as an inflammatory factor started to be unraveled by the early finding that injection of bacterial lipopolysaccharide (LPS) into healthy volunteers caused a rise in circulating resistin levels. Specific receptors for resistin have not been identified, but it belongs to the endogenous ligands of the inflammation triggering toll-like receptor 4 (TLR-4).Accordingly, resistin is associated with an array of inflammatory diseases including sepsis, inflammatory bowel disease, arthritis, and asthma. Have previously found resistin as a contributing factor and biomarker involved in osteoarthritis, rheumatoid arthritis, and inflammatory lung diseases (Mantula *et al*., 2018).

IL-18 is a potent proinflammatory cytokine that enhances T cell and natural killer cell maturation, as well as the production of cytokines, chemokines and cell adhesion molecules, in CD4+ lymphocytes IL-18 can stimulate both type 1 helper T (Th1) and Th2 responses depending on its cytokine milieu: IL18 may stimulate a Th2 response in combination with IL-2 and may act synergistically with IL-12 to stimulate a Th1 response with production of IFN-$^{\gamma}$ (Troseid et al., 2010;Mantula *et al*., 2018).

Cytokine interleukin IL-18 is a strong proinflammatory cytokine that promotes T cell and natural killer cell maturation and the production of cytokines, chemokines, and cell adhesion molecules. It is important to note that IL-18 may induce both type 1 helper T (Th1) and Th2 responses in CD4+ cells, depending on the cytokine environment which is exposed to. When combined with IL-2, IL18 may promote a Th2 response, while when combined with IL-12, it may stimulate a Th1 response and the generation of IFN$^{\gamma}$ (Troseid *et al.*, 2010)

Also pointed by McDonald *et al.* (2006) the IL-18 may used not only in inhibiting *C. parvum* development but in enhancing mucosal immunity in general against enteropathogens during infections of the gastrointestinal tract. Also the results of the present study are in consistent with the result of a study by Mutlag *et al*., (2019) who showed a significant increase in the concentration of IL-18 in the serum of patients with *B. hominis* compared with the control group. The study concluded the infection with *B. hominis* affects the hematological parameters, such as (WBCs), neutrophils, monocytes, lymphocytes and hemoglobin ratio, and immunological parameters, especially Pro-inflammatory cytokines such as (IL-10) and interleukin-18(IL-18).

The antigen for the parasite, stimulates the expression of pro-inflammatory cytokine such as IL-2 and IL-18, that IL-18 is a member of the family IL-1 (IL-33,IL-36,IL-18) that involved in the process of innate and adaptive immunity (Wawrocki *et al*., 2016).

IL-18 was first identified as a factor that stimulates the production of IFN- by Th1 cells. Inducing IFN- production by Th1 cells is IL-18 primary

function in the immune system. A strong Th1 response may be triggered by the combination of IL-18 and IL-12 (Tominaga *et al*., 2000). An essential part of the host's immune response to infections from microorganisms including bacteria, viruses, fungus, and protozoa, IL-18 is a cytokine that helps the body fight off invaders (Nakanishi *et al*., 2001). Along with IL12, IL-18 stimulates IFN- production by natural killer (NK) cells, B cells, and macrophages. Co-stimulation with IL-12 causes IL-18 to stimulate IFN production as a Th1 cytokine, however when IL-18 is present alone, it behaves as a Th2 cytokine (Hirooka & Nozaki, 2021).

It has been shown that T-cell-derived IL-22 has a role in host defense at environmental interfaces like the skin, where it controls wound healing and helps maintain the skin's barrier function, When protecting against Gram-negative bacteria like *Klebsiella pneumoniae*, IL-22 is essential for the mucosal lining of the airways. Human primary bronchial epithelial cells were shown to retain their through capacity and establish transepithelial integrity after injury *in vitro* when treated with interleukin IL-22 (Zheng *et al*., 2008). The results confirmed a function for IL-22 at mucosal surfaces and showed that IL-22 produced from cells other than T cells produces antimicrobial proteins like RegIIIg that are crucial for early mucosal defense against some microorganism in the intestine. The results of these study point to the importance of IL-22 in resistance to germs that enter the body via the lungs and digestive tract. Yet, in noninfectious models of IBD, IL-22 has been shown to have protective effects (Wilson *et al*., 2010).

These IL-22 effects, which include the creation of antimicrobial peptides, the stimulation of epithelial regeneration, and the regulation of wound healing, play a crucial role in controlling inflammatory responses

inside the gut. Therefore, many study has investigated IL-22 potential protective functions (Leung & Loke, 2013).

Regenerating broken epithelium monolayers and stimulating the production of antimicrobial peptides are two of IL-22 most important functions. Recent advances in genome wide association studies have led to results suggesting that the IL-22 pathway is closely related to some major inflammatory bowel disease (IBD) susceptibility genes, and independent groups using different experimental methods have demonstrated IL-22 ability to promote intestinal wound healing and proliferation of intestinal epithelial cells in mice and humans (Li *et al*., 2014).

5-2-3:The effect of *E. histolytica* infected on some physiological and Immunological parameters according to the gender groups.

The result in Table (4-9) and figure (4-6) showed a significant increase in the adiponectin level in infected females compare to the non-infected females (control group) ($p \leq 0.001$),and the result showed an increased level in infected males compared to the non-infected males (control group).

Also, the level of resistin significant increases in infected females compare to non-infected as well as the result showed an increase in the level of resistin in infected males compare to non-infected while the level of IL18 and IL22 although there are differences, but they are not significant.

The reason of that the close proximity of adipose tissue to the gonads and the presence of steroid hormone receptors in adipose tissue suggest that sex hormones may have some role in modulating adipokine expression (Gui *et al*., 2004).

Nishizawa *et al*. (2002) showed the effect of sex hormones on the production of adiponectin in human subjects the testosterone reduces the production of adiponectin in plasma, which may relate to the high risks of insulin resistance and atherosclerosis in men.

Adiponectin concentrations were found to be 6.26-3.94 mg/ml in males and 8.84-4.71mg/ml in females, with the latter having a substantially greater concentration. Males adiponectin levels associated favorably with age and testosterone while adversely correlating with body mass index and free testosterone. Males had a greater body mass index than females, despite the fact that there was no statistical difference in age between the sexes Isobe *et al*. (2005).

Research in mice suggested that androgens, specifically testosterone and estrogen, might inhibit adiponectin production, and that a decline in sex hormones with aging might result in a gender difference in the process of adiponectin elevation. The regulation by estrogen was weak compared to that by testosterone. In males, testosterone was shown to have inverse associations with adiponectin, and adiponectin levels were found to decline in tandem with the onset and development of puberty (Böttner *et al*., 2004).

Also the elevated concentration of resistin and adiponectin in infected females and males for the same reason are shown in the discussion Table (4-8).

5-2-4: The effect of *E. histolytica* on physiological and Immunological parameters according to the age groups.

The result in Table (4-10) showed that the difference in the concentration of adiponectin, resistin, IL8,and IL22 was not significant according to age group.

The result of study Obata *et al*. (2013) showed that serum adiponectin levels in healthy subjects are positively correlated with age in both men and women with type 2 diabetes. Previous studies have reported that serum adiponectin levels are increased in elderly individuals such as (Adamczak *et al*., 2005 ; Arai *et al*., 2006).

While the studies of Nishizawa *et al*. (2002), and Ryan *et al*. (2003) and Adamczak *et al*. (2005) showed that plasma adiponectin concentration in females did not change significantly with age group.

While the study by Treat *et al*. (2019) found that mean resistin is negatively associated with age group implying that younger women must have higher resistin levels than older in diabetes and breast cancer patient .

5-2-5:The Correlation among physiological and Immunological parameters in the infected patients with *E.histolytica*.

Tables (4–11) reveal a significant negative connection between IL-18 and resistin at the $p \leq 0.05$ level. Pathological inflammatory situations may be associated with increased expression of human resistin, suggesting a connection between the two parameters. Numerous cell and tissue types are impacted by resistin's autocrine, paracrine, and endocrine actions. In patients with type 2 diabetes, rheumatoid arthritis, chronic kidney disease, sepsis,

and coronary atherosclerosis, the level of resistin in the blood plasma is positively correlated with common inflammatory and fibrinolytic biomarkers such as C-reactive protein (CRP), tumor necrosis factor alpha (TNF-a), and interleukin 6 (IL-6), and with disease severity in the case of sepsis and pancreatitis (Tripathi *et al*., 2020).

The anti-inflammatory and immunosuppressive effects of human resistin extend to different immune cell types. As a result of human resistin, the pro-inflammatory pathway in dendritic cells is dampened (Son *et al*., 2008). By modulating the IRF-1-mediated TREG pathway, human resistin may also dampen the immunological response of T cells (Son *et al*., 2010).

5-3:Molecular study

5-3-1: Study the Associated factors of Genotypes of IL-18,and Resistin (single-nucleotide polymorphisms) in *E.histolytica* Patients Compared to the control group

Four IL-18 genetic polymorphisms, SNP1(rs1866694757), SNP4 (rs196518), SNP6 (rs1946519), and SNP7 (rs1215648807) showed a high risk of *E. histolytica* infection, as shown in Table (4-12) of the current study, suggesting that these polymorphisms play causative roles in the development and progression of *E. histolytica* infection.

SNP 2 (rs 940255648), SNP 3 (rs 1037707423), SNP 5 (rs 1213044637), SNP 8 (rs 1866697972), SNP 9 (rs 1866698066), and SNP 10 (rs 1866698286) were found to be protective factors against *E. histolytica* infection progression

Results of the genotype of resistin shown in Table (4-13) indicated that the mutation in the polymorphism of SNP1(rs1862513), SNP2 (rs567367264), and SNP3 (rs536392382) demonstrated a risk factor for intestinal parasites in the patient group than in the control group. While the genotype of resistin polymorphism of SNP 4 (rs2032442393) was shown to be a protective factor.

5-3-2:Association between Biochemical markers and gene polymorphisms.

5-3-2-1:Association between IL-18 concentration and polymorphisms of IL-18 Gene.

The result in Table (4-14) showed although IL-18 gens polymorphisms were never shown any statistically significantly differences with any of gene polymorphisms SNPs. The results showed increased in level of IL-18 in SNP1/**(**rs1866694757)(heterozygous/CA);SNP2/(rs940255648)(heterozygous/GC);SNP3/(rs1037707423)(heterozygous/CT);SNP4/(rs1946518)(heterozygous,homozygous/TG,GG);SNP5/(rs1213044637)(heterozygous/GA);SNP6/(rs1946519)(heterozygous,homozygous/AC,CC);SNP7/(rs1215648807)(SNP8/(rs1866697972)/AC);SNP9/(rs1866698066)(heterozygous/GT); SNP10/(rs1866698286) (homozygous /TT).

The current study's findings agree with many other studies that discovered relationships between IL-18 levels and SNPs. The previous study such as the study of Wang *et al*.(2017) in Qilu Hospital of Shandong University determined that IL-18 (rs1946518) polymorphisms have been shown to have a role in the susceptibility and severity of acute myeloid

leukemia (AML) by showing that the bone marrow blasts of individuals having IL-18 polymorphisms are more likely to develop into leukemia (rs1946518). It was shown that patients with the GG or GT genotypes fared better than those with the TT genotype. Individuals carrying the IL-18 (rs1946518) GT or TT genotype had greater plasma IL-18 levels compared to those carrying the GG genotype. Additionally, AML-specific survival was significantly worse in those with the GT genotype of IL-18 (rs1946518).

rs1946519 IL-18 single-nucleotide polymorphisms were also found to be associated with recurrent spontaneous miscarriage (RSM) in additive, dominant, and recessive models (Al-Khateeb *et al*., 2011). Lower serum IL-18 levels were seen between patients and controls and were more pronounced in rs1946519 heterozygous and homozygous genotypes.

Bone marrow blasts of patients carrying the IL-18 (rs1946518) polymorphism were found to be more aggressive than those of patients without the polymorphism. This suggested that IL-18 polymorphisms contribute to the susceptibility and severity of acute ulcerative colitis UC (rs1946518). Patients with genotypes of GG or GT fared better than those with the TT genotype. Patients with the IL-18 (rs1946518) GT or TT genotype had higher plasma IL-18 levels compared to those with the GG genotype. More so, having the AML-specific poorer survival associated with the GT genotype of IL-18 (rs1946518) (Wang *et al*.2014).

Maroufi *et al*. (2019) found no significant differences in the genotype frequencies of the rs1946518 C/A polymorphism between breast cancer (BC) patients and healthy controls under the codominant (p=0.333; OR =0.95; 95% CI =0.88-1.51), dominant (p=0.677; OR =1.18; 95% CI =0.69-

1.31), recessive (p=0.522; OR =0.99.IL-18 rs1946518 and rs1946519 polymorphisms were found to be significantly associated with a predisposition to coronary artery disease (CAD) in overall combined East Asians (Zheng *et al*., 2020).

Also refering Kadi *et al*.(2021) to the change of homozygote wild type to homozygote mutant for(rs1946519) significantly affected the serum IL-18 level, with a median sIL-18 level higher in infants with acute kidney infection. Interleukin-18 (IL-18) is a member of the IL-1 cytokine superfamily and functions as a pro-inflammatory cytokine by activating innate immunity and inflammatory pathways as well as polarizing T cells into Th1 or Th2. (Shaheen *et al*., 2014). IL-18 is mainly produced by macrophages, dendritic cells, keratinocytes, osteoblasts, and intestinal epithelial cells (Naik & Wala, 2013). Interleukin IL-18 was first found as a factor that promotes interferon (IFN)- production by anti-CD3-stimulated T helper 1 (Th1) cells, especially in conjunction with IL-12. Interleukin-12 (IL-12) is a cytokine that promotes the maturation of Th1 cells. Although IL-18 cannot directly stimulate the formation of Th1 cells, it may activate preexisting Th1 cells to secrete IFN- when combined with IL-12. So, IL-18 is considered a proinflammatory cytokine that promotes type 1 reactions. (Smith, 2011; Nakanishi, 2018).

To add insult to infection, interleukin-18 (IL-18) suppresses the production of the anti-inflammatory cytokine interleukin-10 (IL-10) and enhances the proinflammatory cytokine tumor necrosis factor alpha (TNF-) and T cell differentiation toward the proinflammatory Th1 phenotype, respectively (Veenbergen *et al*., 2010). Epidemiological evidence suggests that interleukin-18 (IL-18) may have a role in the etiology of chronic

inflammatory illnesses associated with T helper 1 (Th1) and T helper 2 (Th2) responses(Pastorelli *et al*., 2010).

Six exons and five introns make up the human IL-18 gene, which have been located on chromosome 11q22.2-q22.3(Giedraitis *et al*., 2001). The IL-18 gene, being about 19.5 kb in length, is home to a number of SNPs. The Th1/Th2 cytokine balance is disrupted by single nucleotide polymorphisms (SNPs) in the IL-18 gene that affect IL-18 production and function (Gao *et al*., 2015).

The cytokine interleukin IL-18, along with IL-1, contributes to host defense against infections by boosting the antimicrobial properties of phagocytes and kicking off Th1 and Th17 adaptive immune responses, all of which may be related to the effects (SNPs) on progression in *E. histolytica* infections (van de Veerdonk *et al*., 2011). Multiple prior epidemiological studies have shown that IL-18 has a role in the etiology of autoimmune and inflammatory illnesses because of its ability to regulate both innate and acquired immune responses. It is important to highlight that IL-18 may promote T cell development to the pro-inflammatory Th phenotype by stimulating the production of TNF- and IL-1 (Gao *et al*., 2015).

5-3-2-2:Association between Resistin concentration and polymorphisms of Resistin Gene.

The result in Table (4-15) showed resistin gens polymorphisms were statistically significantly different SNP1/(rs1862513)(CG/GG)with wild type(CC) and polymorphisms SNP2/(rs567367264)(GC)with wild type(GG) and SNP4/ (rs2032442393)(CT) with wild type(CC). The results showed increased in resistin level, while the SNP3/(rs536392382)(CT) although not

shown significant differences with wild type (CC) the level of resistin was high too. Also it has been reported to relate with resistin concentration in some studies such as Menzaghi *et al.*(2015); Asano *et al*.(2010).

The result of present study agrees with Hashemi *et al*. (2018) Stratification analysis by cancer type revealed that the rs1862513 variant significantly increased the risk of colorectal and breast cancer.

Type 2 diabetes (T2DM) susceptibility in the general Japanese population was shown to be linked with the G/G genotype of a single nucleotide polymorphism (SNP) (rs1862513) in the promoter region of human RETN by Onuma *et al*.(2013). The plasma resistin levels were greatest in those who had the G/G genotype of rs1862513, followed by those who carried the C/G and C/C genotypes. A higher risk of developing type 2 diabetes is associated with the G/G genotype of rs1862513 in RETN, likely because this variant enhances promoter activity of the gene. While the result disagree with Chen *et al*. (2010) who showed that RETN promoter polymorphism rs34861192 was associated with elevated circulating resistin levels, but rs1862513 was not.

Allele and genotype frequencies of rs1862513 were not significantly different between rheumatoid arthritis (RA) patients and healthy controls ($P \leq 0.05$) in a Chinese population study conducted by Li *et al*. (2018). No significant connection was found ($P \leq 0.05$) when we looked at the impact of genotypes in dominant and recessive models. Polymorphisms in the resistin gene may influence the production and activity of inflammatory cytokines, which may play a role in the onset of autoimmune disorders. However, the present study has a number of caveats that might potentially affect the reliability of the findings. These include the diversity of patients population, the severity of their condition, how long it has been treated, and their

ethnicity. Therefore, further researches are required to replicate the findings, ideally with bigger samples across a variety of populations.

Conclusions:

1- The findings indicated that the overall infection rate with intestinal parasites among people hospitalized and attending hospital about 13 %, the present study along whole year, this proportion is thus consider to be low.

2- Infection with *E. histolytica* is more prevalent than with other intestinal parasites, making this parasite the most prevalent in this location for protozoa while the lowest *T. hominis* and *H .nana* consider the highest rates helminth in this study while *E. vermicularis* was the lowest.

3-The infection of intestinal parasites was impacted by the majority of epidemiological factors, including gender, season, and age group of infected children.

4. The results indicated that the infection of *E. histolytica* is effect in most of the blood, immunological and physiological parameter (adiponectin, resistin, IL-18 ,IL-22).

5- The IL-18 and resistin polymorphism can be considered as one of the genetic factor responsible for the progression of *E. histolytica* infection.

Recommendations

1-Finding the relationship between infection with intestinal parasites and other physiological and immunological parameters such as IL-33.

2-Conducting a molecular study to study the effect of IL-22 and adiponectin gene polymorphisms on the progression of intestinal parasite infection.

3-Studying of other diarrheal causes such as *Blastocystic hominis* and *Cryptosporidium parvum*.

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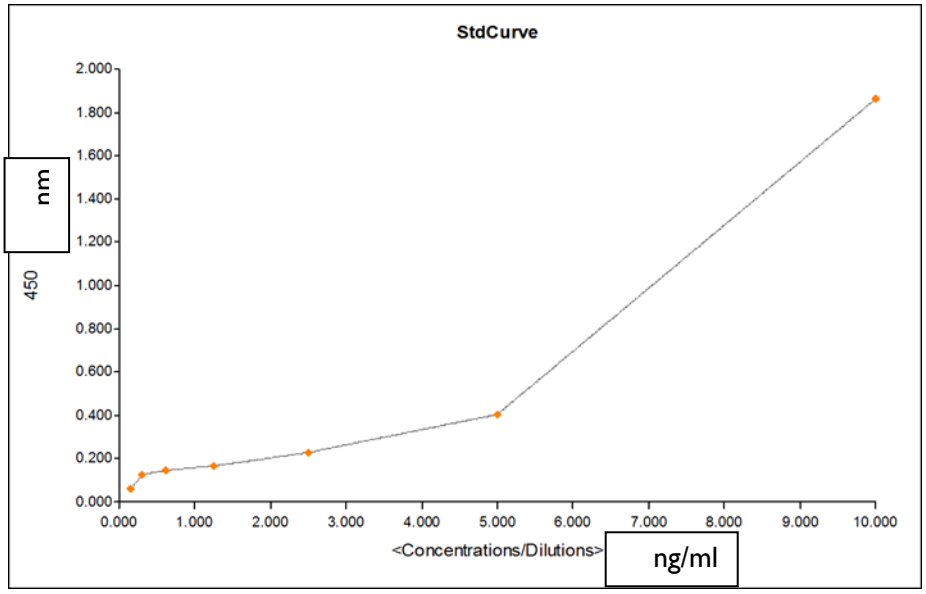
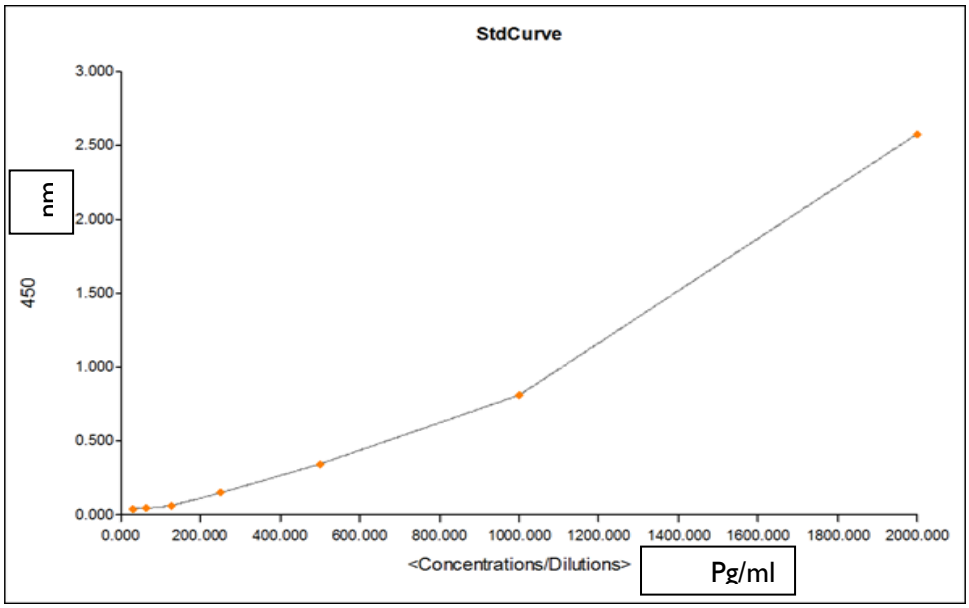
Appendix

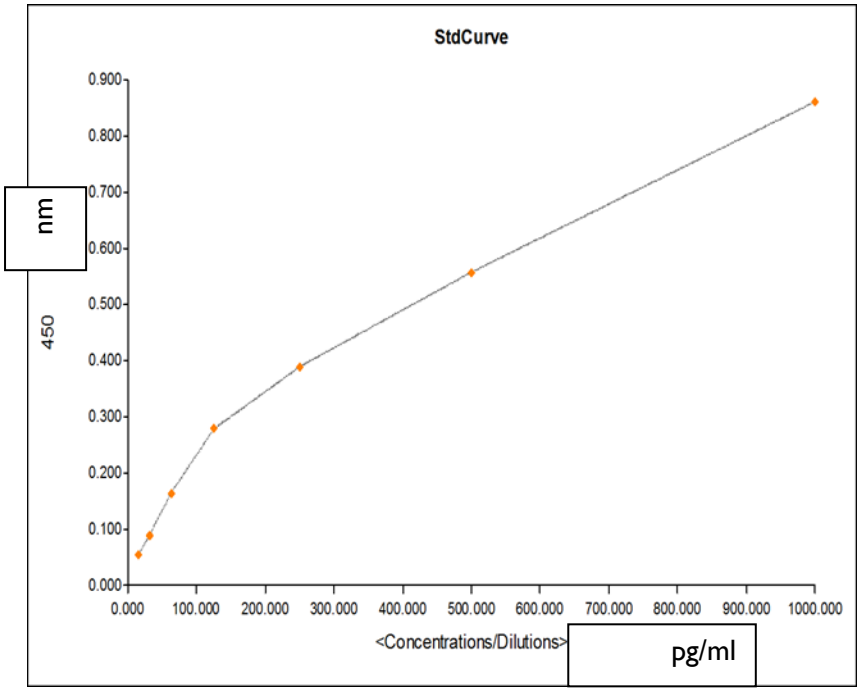
Appendix



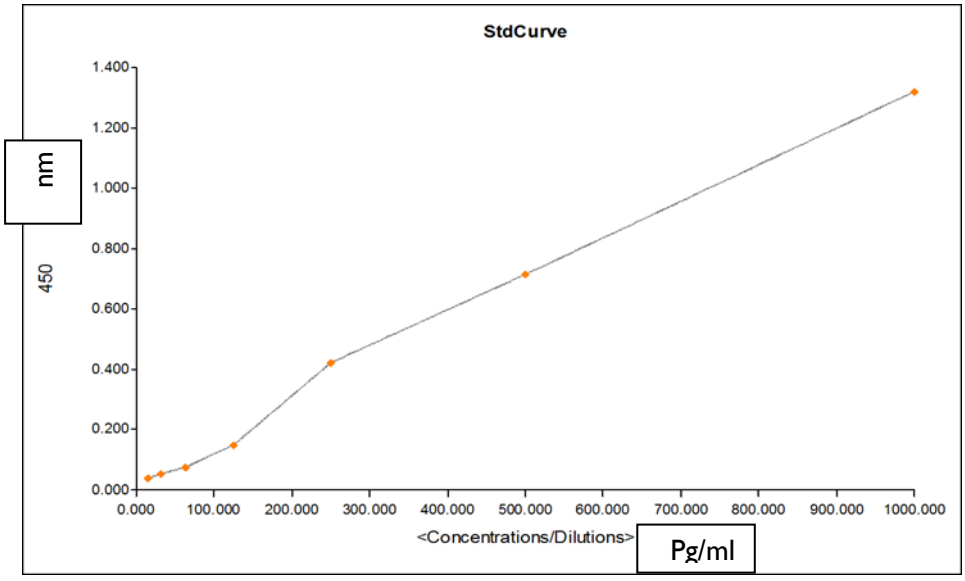
Appendix (1): Stander curve of Adiponectin.



Appendix: (2) Stander curve of resistin.

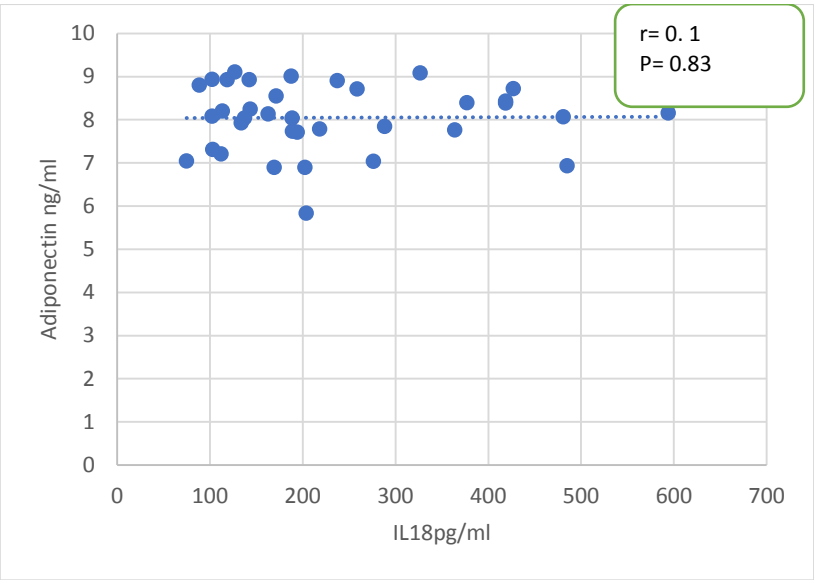


Appendix (3): stander curve of IL-18

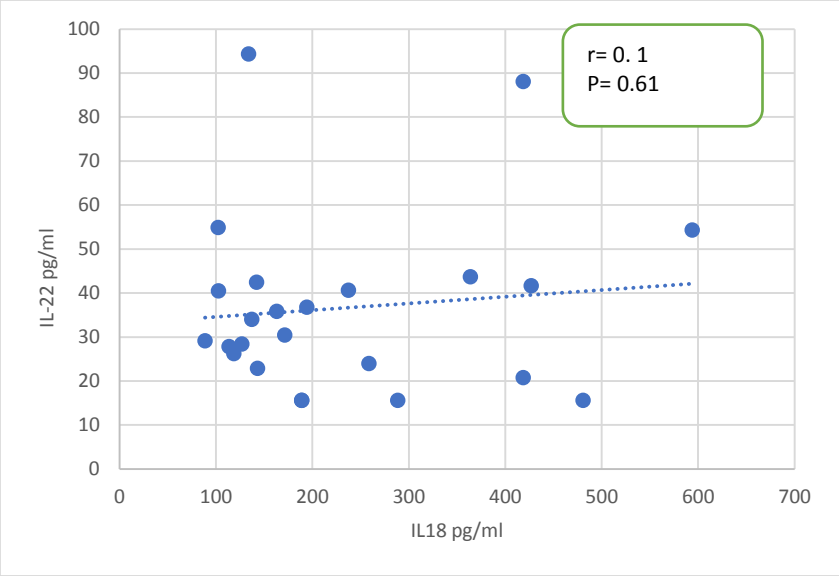


Appendix(4): stander curve of IL-22

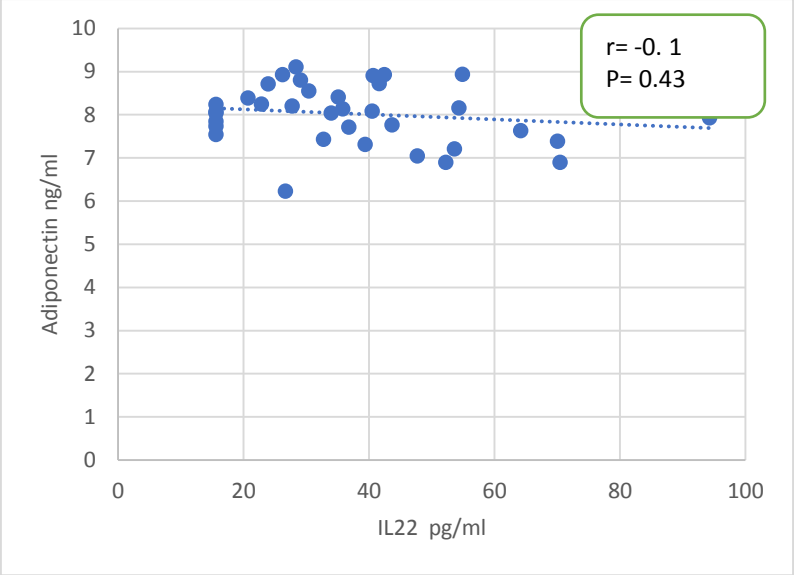
Appendix



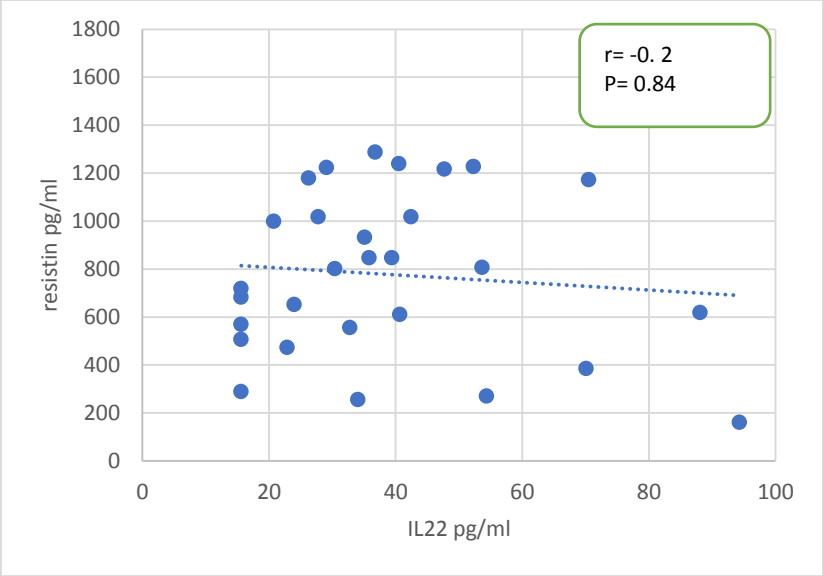
Appendix(5): Correlation between of IL-18 and Adiponectin concentration in infected patients



Appendix(6): Correlation between of IL-18 and IL-22concentration in infected patients

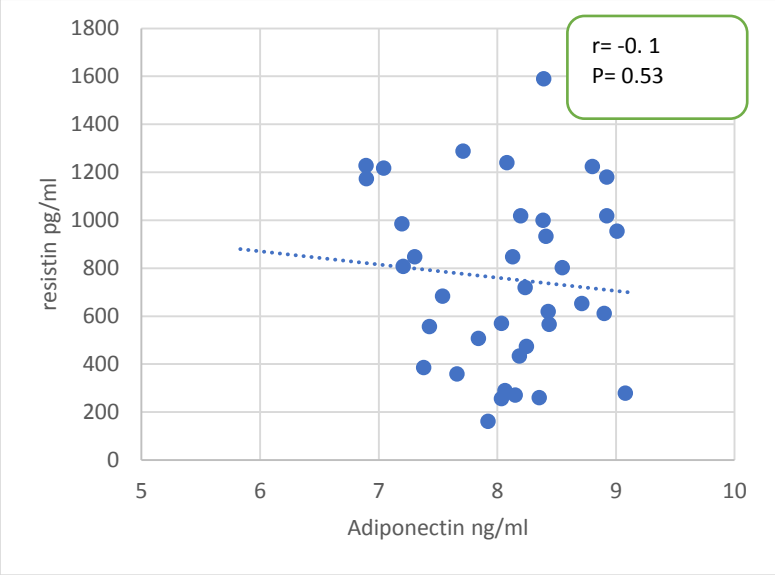


Appendix(7): Correlation between of Adiponectin and IL-22concentration in infected patients



Appendix(8): Correlation between of resistin and IL-22concentration in infected patients

Appendix



Appendix(9): Correlation between of resistin and Adiponectin concentration in infected patients

الخلاصة:

اجريت هذه الدراسة للفترة من شباط 2021 حتى كانون الثاني 2022 لتحديد بعض المقاييس الدموية والمناعة والفسيولوجية وعلاقتها بالعدوى الطفيلية المعوية لدى الأطفال في المراجعين والراقدين في مستشفى الاطفال في محافظة كربلاء المقدسة. بالإضافة إلى ذلك ، تم فحص تعدد أشكال النوكليوتيدات المفردة فيما يتعلق بالعلامات المناعية والفسيولوجية. الأطفال الذين تتراوح أعمارهم بين (1 - 15) سنة ، وباستخدام تقنية المسحة المباشرة والاختبار السريع ، تم فحص إجمالي 3748 عينة براز.

أظهرت النتائج ان النسبة الكلية للإصابة بالطفيليات المعوية للأطفال المراجعين والراقدين في مستشفى الاطفال والمشمولين بالدراسة حوالي13% وكانت نسبة اصابة الذكور اعلى من نسبة اصابة الاناث حيث بلغت نسبة اصابة الذكور 14.1% والاناث 11.7% على التوالي. تم تسجيل خمسة انواع من الطفيليات المعوية وكانت نسبها كالاتي الاميبا الحالة للنسج *Entamoeba histolytica* 10.54% والجياردیا لامبيليا *Giardia lamblia* 2.46% وطفيلي الابواغ الخبيئة *Cryptosporidium parvum* 0.4% والمتحرشفة القزمة *Hymenolepis nana* 0.24% والدودة الدبوسية *Enterobius vermicularis* 0.13% والمشعرة البشرية *Trichomonas hominis* 0.03%

كما بينت النتائج ان نسبة الاصابة بالطفيليات المعوية المفردة اكثر شيوعا من الاصابات المزدوجة. وسجلت أكبر نسبة من الإصابات بالطفيليات المعوية في ايلول 2022 وبنسبة 30.1٪ ، بينما سجلت أقل نسبة في شباط 2021 بواقع 3.5٪. بالإضافة إلى ذلك ، فإن الفئة العمرية 5-10 سنوات كان لها أعلى معدل انتشار لعدوى الطفيليات المعوية ، بنسبة 17.2٪ مقارنة بالفئات العمرية الاخرى.

ولإجراء الفحص الدموي والمناعي والفسيولوجي ، تم اختيار 48 من الاطفال المصابين بالاميبا الحالة للنسج و42 من الاطفال الاصحاء ، أظهرت نتائج التحليل الأحصائي للمعايير الدموية ارتفاعًا كبيرًا ($p\leq 0.05$) في خلايا الدم البيضاء ، العدلات ، والخلايا الليمفاوية ، وانخفاض معنوي ($p\leq 0.05$) في الهيموكلوبين Hb ، مقارنة مع الاطفال الاصحاء (مجموعة السيطرة).

كما بينت النتائج بالنسبة لمجموعة الاطفال المصابين بالاميبا الحالة للنسج، ارتفاع في كل من تراكيز الانترلوكين 18 , resistin وadiponectin كما اظهرت النتائج زيادة معنوية في تركيز كل من الاديبونكتين وresistin في كلا الجنسين الذكور والاناث،كما اظهرت النتائج عدم وجود فروق معنوية بين الفئات العمرية قيد الدراسة.

أظهرت النتائج توزيع النمط الجيني لتعدد أشكال النيوكليوتيدات المفردة IL-18 في المرضى المصابين بالطفيليات المعوية على عكس مجموعة السيطرة. يشير هذا إلى أن الطفرة في تعدد الأشكال لـ (rs1866694757) SNP 1 و (rs 1946518) SNP 4 و (rs1946519) SNP 6 و (SNP 7 rs1215648807) أثبتت أنها عامل خطر في مجموعة الاطفال المصابين بالطفيليات المعوية مقارنةً بمجموعة السيطرة ،وبمجال ثقة 95٪ (OR = 1.333، 1.800، 1.200 و 1.750 ؛ (0.321- 5.538) ، (0.264-12.296) ، (0.396-7.732) و (0.396-7.732) على التوالي. بينما اظهرت النتائج ان تعدد الاشكال (rs 940255648) SNP 2 و (rs 1037707423) SNP 3 و (rs1213044637) SNP 5 و (1866697972) SNP 8 و (1866698066) SNP 9 و (rs1866698286) SNP 10 عاملًا وقائيًا (OR = 0.364، 0.308، 0.955، 0.500، 0.393، 0.073 ؛ بمجال الثقة 95٪ = (0.084- 1.583) ، (0.052- 1.829) ، (0.235- 3.878) ، (0.101-2.477) ، (0.081-1.909) و (0.008- 0.689) على التوالي ومن المثير للاهتمام ، أن النمط الجيني لـ (rs1866698286) SNP 10 AA (النوع البري) / AT (متغاير الزايكوت) هو الوحيد الذي أظهر فرقًا مهمًا إحصائيًا لمجموعة المرضى.

أظهر تعدد الأشكال لـجين resistin (rs1862513) SNP1 و (rs567367264) SNP2 و (rs536392382) SNP3 عامل خطر للاطفال المصابين بالطفيليات المعوية مقارنة بمجموعة السيطرة (OR = 2.068 و 1.360 و 1.360) 95٪ فاصل الثقة = (0.477) - 8.973) و (0.344- 5.379) و (0.344-5.379) ,على التوالي بينما في النمط الوراثي لتعدد الأشكال لجين resistin (rs2032442393) SNP 4 أظهر عامل وقائي (النسبة الفردية = 0.952 ؛ 95٪ = CI 0.056 - 16.279)) لم تكن هناك فروق ذات دلالة إحصائية عبر النمط الجيني لتعدد الاشكال لجين resistin.

على الرغم من أن تعدد الأشكال في جين IL-18 لم يظهر أبدًا أي فروق ذات دلالة إحصائية مع أي من الأشكال الجينية متعددة الأشكال ($p \leq 0.05$) الا ان هناك زيادة في مستوى IL-18 في (SNP1 / CA و SNP2 / GC و SNP3 / CT و SNP5 / GA و SNP7 / AC و SNP8 / SNP9 / GT و GT) مقارنة مع النوع البري على التوالي.

كما اظهرت النتائج ان الشكل الجيني (SNP6 AC / CC ،SNP4 / TG / GG) قد اظهر فروق معنوية مقارنة بين النوع البري وغير المتجانس على التوالي، و (SNP10 TT / AC) مقارنة بين النوع البري وغير المتجانس على التوالي .

كان تركيز resistin مختلفًا بشكل كبير إحصائيًا بين تعدد الأشكال الجيني للجين ، SNP 1 و SNP5. هناك زيادات كبيرة من CC (rs1862513) SNP 1 إلى CG ، ومن SNP 1 (rs1862513) CG إلى GG ، وكان هناك فرق ذو دلالة إحصائية بين مستوى resistin و SNP4 (rs2032442393). كشف اختبار LSD اللاحق عن وجود اختلاف في تركيز resistin في SNP 4 (rs2032442393) من CC (النوع البري) إلى CT (متغاير الزيجوت المتحور) ،عند ($p = .019$) ، بينما لا يوجد فرق ذو دلالة إحصائية مع بقية جين SNPs resistin ($p \leq 0.05$).

وفقًا لنتائج هذه الدراسة ان للإصابة بالطفيليات المعوية ، ولا سيما الاميبا الحالة للنسج ، تأثير كبير على غالبية العوامل الدموية والمناعة والفسيولوجية لدى الأطفال المصابين.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل ـ كلية العلوم

قسم علوم الحياة

وبائية بعض الطفيليات المعوية ودراسة جزيئية لبعض المعايير المناعية والفسلجية للأشخاص المصابين بـ *Entamoeba histolytica*

أطروحة

مقدمة الى مجلس كلية العلوم / جامعة بابل

وهي جزءا من متطلبات نيل درجة الدكتوراه فلسفة

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