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Molecular and Biochemical Study of Toxoplasmosis among Beta Thalassemia Major Patients in Babylon Province

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By

Wurood Alwan Kadhim Juaifer

(B.Sc. Biology, University of Babylon, 2011**)**

Supervised by

Prof. Dr.

Ahmed Khudhair Obayes Al-Hamairy

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ﴾

صدق الله العليّ العظيم

سورة الزمر الاية (٩)

Supervisor Certification

I certify that this thesis entitle **(Molecular and Biochemical Study of Toxoplasmosis among Beta Thalassemia Major Patients in Babylon Province)** by (**Wurood Alwan Kadhim Juaifer**) was prepared under my supervision at the department of Biology, College of Science for Women, University of Babylon as a partial requirement for the degree of Master in Biology.

Signature

Dr. Ahmed Khudhair Al-Hamairy

Scientific order: Prof.

Date: / / 2022

Department Head of Biology Recommendation

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature:

Name: Hadi Meziel Kudhair

Scientific order: Prof. Dr.

Address: Head of Biology Department College of Science for Women/ University of Babylon

Date: / / 2022

Dedication

This thesis is dedicated to my

Family and friends……..

Researcher

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Researcher

Summary

The current study aimed to evaluate toxoplasmosis in thalassemia patients based on epidemiological, hematological and immunological criteria by IgG and IgM, and to determine the interleukin-10 gene polymorphism and its relationship to toxoplasmosis in thalassemia patient.

66 samples were collected for patients with beta thalassemia major, and 30 samples as a control group in healthy males and females during the month of October to December at the Maternity and Children Hospital, in Babylon، and 96 samples were examined in the Cobas e411 device for both IgG and IgM for *Toxoplasma*, at Al-Amin Center in Najaf, and 20 positive samples were obtained for IgG test in patients with beta thalassemia major and one sample was positive for IgM, and uric acid test was conducted for all patients and healthy ones. Twelve samples were taken from males and females containing a high percentage of uric acid, six of them were positive for toxoplasmosis and six samples were negative for the disease. High-performance liquid chromatography (HPLC) was performed at the Ministry of Science and Technology in Baghdad, using the standard allantoin to monitor the relationship between uric acid and allantoin, as the relationship is inverse between them, in case of infection and the presence of the parasite of interest in the blood.

The study samples were divided into three age groups. The results showed that the highest percentage of females was 58.33% in the age groups 10-15 years, and the percentage was 50% in males in the 16-20 years of age in thalassemia patients without toxoplasmosis, while the percentage reached the rate is 25% in males in the age groups 21-25 years in patients with thalassemia with toxoplasmosis.

According to the presence of cats, the highest incidence of males was 12.5% in thalassemia patients with toxoplasmosis, and the absence of cats 90.91% in thalassemia patients without toxoplasmosis, while the incidence of females was higher with the presence of cats 16.67% in thalassemia patients with toxoplasmosis, and the absence of cats 95.83 % in thalassemia patients without toxoplasmosis.

It was found that the effect of blood transfusion factor is higher percentage in females, the rate of blood transfusion once a month is 58.33% in thalassemia patients with and without toxoplasmosis, blood transfusion twice a month in males 62.5% in thalassemia patients with *toxoplasma*, and blood transfusion four times a month in males, 4.55% in thalassemia patients without toxoplasmosis.

For biochemical parameters, the concentration of uric acid and allantoin correlated as markers for oxidative stress in beta-thalassemia patients with or without toxoplasmosis, to reveal the highest concentration (5.10) mg/dL of uric acid in males compared to females (4.66) mg/dL in beta-thalassemia patients groups with toxoplasmosis, the highest concentration was seen in males (5.36) mg/dL compared to females (3.76) mg/dL in beta-thalassemia patients without toxoplasmosis. Allantoin concentration in females (59.52±1.6) ppm was higher compared to males (56.39±1.9) ppm in beta thalassemia patients with *toxoplasma*, while the allantoin concentration in beta thalassemia patients without *toxoplasma* was higher in males (39.78 ± 1.3) ppm compared to females (37.53 ± 1.2) ppm.

Genotyping results of wild and mutated alleles of a single nucleotide polymorphism of the interleukin-10 gene and assessment of their relationship to toxoplasmosis and thalassemia. It was found that the presence of two single nucleotide polymorphisms (SNPs) in the promotor region of the respective gene are rs1800871 and rs1800872. The homozygous CC mutant genotypes showed a greater distribution in toxoplasmosis and thalassemia patients compared to the control group, and the odds ratio (OR) values support that the C allele in CC is associated with the disease of interest and is considered a risk allele. The odds ratio is higher in the CC genotype = 2.3 (0.6-8.5) with p-value= 0.2.

The final result showed that the analysis of IL-10 polymorphisms has a clear effect on thalassemia patients infected with toxoplasmosis. In this study, the interleukin-10 polymorphism showed high efficacy in the occurrence of toxoplasmosis and thalassemia, the frequency of interleukin-10 rs1800871 T>C and the interleukin-10 rs1800872 A>C alleles were not similar between patients and the control group.

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List of Abbreviations

Term	Meaning
ANOVA	Analysis of Variance
bp	base pair
β-thalassemia	Beta-thalassemia
βTM	Beta -Thalassemia Minor
°C	Centigrade
CBC	Complete Blood Count
CI	Confidence Interval
COI	Cutoff index
CSIF	Cytokine Synthesis Inhibitory Factor
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
fl	Femtoliter
g/dl	gram/deciliter
Hb	Hemoglobin
HbA_2	Hemoglobin A_2
HCL	Hydrochloride acid
HPLC	High Performance Liquid Chromatography

HSCT	Hematopoietic Stem Cell Transplantation
IFA	Immunofluorescence Assay
IgG	Immunoglobin G
IgM	Immunoglobin M
IL-10	Interleukin-10
INF	Interferon
IU	International Unite
LAT	Latex Agglutination Test
µl	Microliter
MCH	Mean Corpuscular Hemoglobin
MHC II	Major Histocompatibility Complex class II
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
mg/dl	milligram/ deciliter
ml	Milliter
NCBI	National Center for Biotechnology Information
ng/µl	ninogram/microliter
NK	Natural killer
NO	Nitric Oxide

ODS	Octadycyl-Silica
OD	Optical Density
OR	Odd Ratio
OS	Oxidative Stress
P	Probability
PCR	Polymerase Chain Reaction
PCV	Packed Cells Volume
pg	Pictogram
PLTs	Platelets
PPM	Part Per Million
R	Correlation coefficient
RBCs	Red Blood Corpuscles
RDW	Red cell Distribution Width
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
rpm	revolution-per minute
rs	reference strain
RT	Retention Time
SE	Standard Error
SNPs	Single Nucleotide Polymorphisms

SPSS	Statistical Package for the Social Sciences
T. gondii	*Toxoplasma gondii*
TNF	Tumour Necrosis Factor
USA	United States of America
UV	Ultra-Violate
WBCs	White Blood Cells

List of Appendixes

Chapter One

Introduction

CHPTER ONE

1- Introduction

1-1: General Introduction

Toxoplasma gondii, the parasite protozoan that causes toxoplasmosis, is an obligate intracellular parasitic protozoan that infects a wide range of warm-blooded vertebrates and causes disease in people and domestic animals (Dubey, 2016). Immunoassay studies estimate that up to 33% of the world's population has been exposed to *T. gondii* and may be permanently infected, despite the fact that infection rates vary greatly between countries (Pappas *et al*., 2009). *T. gondii* is transmitted mostly through the eating of undercooked meat with tissue lesions, as well as through the consumption of tainted vegetables/water containing oocysts (Bouchard *et al*., 2019).

All animals are susceptible to the disease, and fowls are a major source of zoonotic infection (Polley and Thompson, 2009). Globally, zoonotic parasite infections continue to be a major public health, veterinary, and socioeconomic concern (Ben-Harari and Connolly, 2019).

Toxoplasmosis, by instance, affects approximately a third of the world's population (Flegr *et al*., 2014). More worrisome, Montazeri *et al*. (2020) indicated that 35% to 76% of wild and domestic felids are infected with *T. gondii*, implying that *T. gondii* infection in humans and animals is on the rise globally, and that more research is needed to combat these parasite infections. Management, prevention, and therapy with safe and efficient pharmaceutical inhibitors are all necessary measures, even though there are some medications capable of treating *T.gondii* infections in humans and other animals, they have drawbacks such as toxicity, high cost, and more importantly, most drugs are unsuccessful in treating the latent stage (bradyzoite) that persists in the brain (Shiojiri *et al*., 2019; Angel *et al*., 2020; Secrieru *et al*., 2020).

Patients with beta thalassemia major who receive blood transfusions on a regular basis are at risk of contracting transplant-transmitted diseases like toxoplasmosis, which is a common and serious parasitic disease with a high prevalence that can be transmitted through blood donation from healthy asymptomatic contributors (Mousa and Nahab, 2020).

Thalassemia is an autosomal recessive condition that causes hemolytic anemia caused by a lack of normal hemoglobin production (Desouky *et al*., 2009). The problem of transfusion-transmitted infection, which is directly proportional to the prevalence of infection in the blood donor, may contribute to the increase in morbidity and death among thalassemia patients, including toxoplasmosis (Simon *et al*., 2009).

The interleukin-10 (IL-10) is cytokine produced by immune cells such as macrophages, T lymphocytes, mast cells, granulocytes, dendritic cells, NK cells and keratinocytes. This cytokine possesses immunosuppressive, it leads to tumor proliferation and metastasis through immunosuppression by means of TNF, IL-1 and IL-12. IL-10 is also helpful in enhancing the expression of IL-6 that leads to cells proliferation, at the same time; it also prevents the access of antigen presenting cells to tumor antigens (Sheikhpour *et al*., 2018).

1-2: The Aim of the Present Study

The present study aims to evaluate the relationship of IgG and IgM in toxoplasmosis among β-thalassemia patients, based on age and gender and detecting the role of polymorphism of promoter region of gene IL-10 based of genotyping mutant alleles in two SNPs in thalassemia with *T.gondii* and *T.gondii* alone, compared with control group based on the following objectives:

1- Detecting the positive toxoplasmosis in β-thalassemia patients by using immunological cobas e411 device for IgG and IgM.

2- Determining the relationship between positive toxoplasmosis and some epidemiological criteria (age groups, gender, residence area, and genetic factor, number of blood transfusion, and presence or absence of cats).

3- Making a biochemical study by determining some antioxidants such as uric acid by special kits and allantoin concentrations in patients and control group by HPLC technique for allantoin only.

4- Calculating the genotyping, allele frequency based evaluated the Odd Ratio (OR) on polymorphism of IL-10 in patient's thalassemia that infected with toxoplasmosis and comparing them with control group.

Chapter Two

Literatures Review

CHAPTER TWO

2- Literatures Review

2-1: Historical View

Nicolle and Manceaux in Tunisia and Splendore in Brazil were the first to characterize *T. gondii* in 1908 (Weiss and Dubey, 2009). The protozoan was discovered first in a rabbit by Splendore, and it was discovered later in a North African rodent by Nicolle and Manceaux. The nickname "gundi" comes from the scientific name for the rodent (*Ctenodactylus gundi*). Nicolle and Manceaux distinguished the protozoan from *Leishmania* in 1909. *T. gondii* was called after the arcate form of its infectious phase (Greek root 'toxon'=bow) by Nicolle and Manceaux (Ferguson, 2009).

The first instance of congenital toxoplasmosis was reported in 1923, although it was not linked to *T. gondii*. The postmortem findings of an 11-month-old boy who had gone to the hospital with hydrocephalus were published in detail by scholar Jank in 1923 (De Souza and Attias, 2015).

The boy developed chorioretinitis, which is a classic sign of toxoplasmosis (inflammation of the choroid and retina of the eye). Scholar Jank could not recognize the "sporocytes" as *T. gondii*, and it was not until 1937 that the first full scientific investigation of *T. gondii* was performed applying techniques previously developed for analyzing viruses. Sabin and Olitsky studied *T. gondii* in laboratories monkeys and mice in 1937 (Abbas *et al*., 2020). The degree of sickness in adulthood, which varies between ocular toxoplasmosis to myocarditis, encephalitis, or hydrocephalus and can be deadly, is dictated by the virulence of the strain and the sensitivity of the host species (Maenz *et al*., 2014).

2-2: Classification

According to Hill and Dubey (2014), *T. gondii* is classified as follows:

Kingdom: - Protista

Phylum: - Apicomplexa

Class: - Sporozoasida

Sub class: - Coccidiasina

Order: - Eucoccidiida

Sub order: - Eimeriidae

Family: - Toxoplasmatidae

Genus: - *Toxoplasma*

Species: - *gondii*

2-3: *Toxoplasma gondii* Phases

2-3-1: Tachyzoites

The crescent-shaped stage of the tachyzoite is also known as the trophozoite, rapid proliferative shape, feeding shape, and endozoite (Wang *et al*., 2015). It can infect practically every cell in the body, including the nucleus, which is found in the center of the organism. Endodyogeny is a specialized process that separates it (Arefkhah *et al*., 2019).

Despite the availability of Golgi apparatus, ribosomes, and mitochondria, tachyzoite contains the apical complex (conoid) at the pointy end of the parasites and the back end of the round, and needs habitats within the cells to maintain life (Paniker and Ghosh, 2013).

2-3-2: Bradyzoite or Tissue Cysts

The term "bradyzoite" refers to a slow-reproducing organism; cystozoites are another name for bradyzoites (Kadhim *et al*., 2016). To minimize confusion with oocysts, it is proposed that cysts can be referred to as tissue cysts. *T. gondii* can live in tissues for months in "cysts," but there has long been a misunderstanding about the difference amongst "pseudocysts" (a type of tachyzoite) and tissue cysts. Cysts are organisms with a subterminal nucleus and periodic acid Schiff-positive granules encircled by an argyrophilic cyst wall, according to cytology (Lemgruber *et al*., 2009). Because carnivorous hosts can become afflicted by ingesting infected meat, the organisms are resistant to digestion by gastric juices (pepsin HCl), whilst the tachyzoites are destroyed instantly, tissue cysts are seen to be essential in the life cycle of *T. gondii*, and excretion is added to the biological characterization of the cyst (Dukaczewska *et al*., 2015).

2-3-3: Oocyst

After consuming tissue cysts or bradyzoites, cats excrete oocysts with a brief prepatent period (3-10 days), however after eating tachyzoites or oocysts, the prepatent period is greater (18 days), regardless of the quantity of organisms (Dubey, 2006). The intake of transitional stages among tachyzoite and bradyzoite is thought to cause prepatent durations of 11-17 days (Dubey, 2005).

When cats ingest an infected animal with tissue cysts and subsequently discharge these created oocyst (immature) into the environment, where the oocyst is identified and developed in the gut of the ultimate host (cats), oocysts include two sporocysts, each of which contains four produce sporozoites about eight sporozoties, spherical shape, with a size of 10-12 µm, and comprising sporocysts in the final host intestines through the generation of gametes process known as (sexual reproduction) (Feng *et al*., 2013).

Scanning and transmission electron microscopy are utilized to collect a large amount of pictures of the different developmental phases of *T. gondii*, i.e., tachyzoites, bradyzoites, and oocyst as show in Figure (2-1) below (Attias *et al*., 2020).

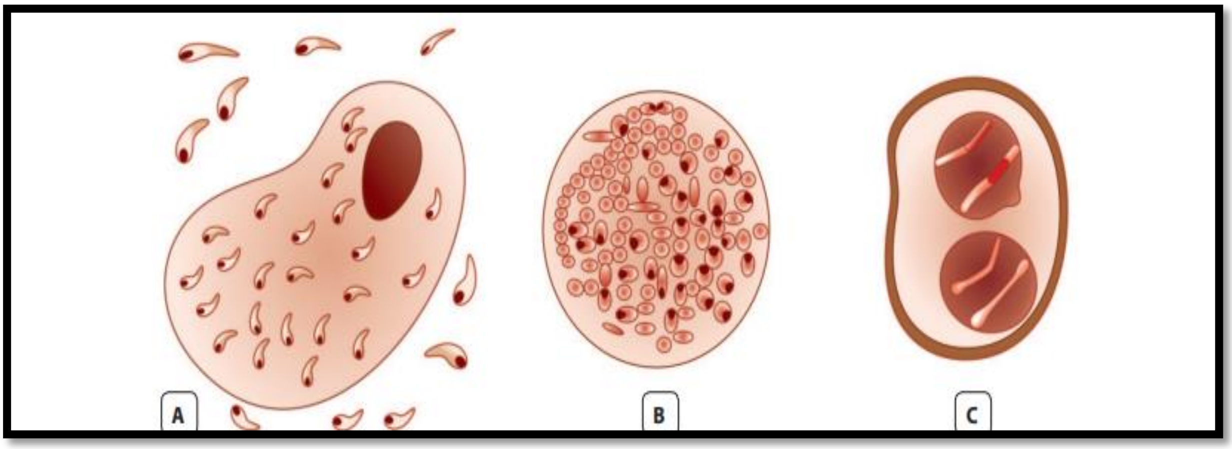


Figure (2-1): ***T. gondii*** **(A) Tachyzoites (B) Bradyzoites (C) Oocyst** (Ghosh, 2013).

2-4: Life Cycle

T. gondii has a complex life cycle that includes an asexual and sexual cycle; the asexual cycle takes place in a wide spectrum of intermediate hosts, but the sexual cycle occurs exclusively in feline hosts (cats), who shed infectious oocysts in their feces (Gissot, 2022).

The tachyzoite, a highly versatile, asexual stage of *T. gondii*, is capable of adhering, invading, altering, and multiplying rapidly within a variety of nucleated cells, allowing it to infect a wide range of warm-blooded intermediate hosts (Dubey, 2014). Intermediate hosts produce strong immune responses, causing tachyzoites to transform into bradyzoites, which are long-lived, asexual, and contagious parasites that live in cysts within cerebral and muscle cells (Miller *et al*., 2009).

T. gondii is only found in felids (cats) that serve as final host and develops through a conventional coccidian life cycle (Ferguson and Dubremetz, 2014). Predation of an infected intermediate host by a definitive host results in bradyzoite attack of enterocytes, going to initiate several rounds of quick asexual reproduction, generating merozoites, accompanied by transformation into male (micro-) and female (macro-) gametes in the epithelia of the small intestine, in addition to spreading systemically as in intermediate hosts (Yongzhen *et al*., 2018).

Fertilization of macrogametes by microgametes produces zygotes, the only one diploid stage of the life cycle. Resistant bi-layered walls grow around the zygotes, resulting in oocysts. The latter is initially unsporulated and non-infectious, but with enough temperature, moisture, and oxygen. It will perform meiosis for sporulate, generating two sets of four haploid sporozoites, which are housed inside a second set of walled structures known as sporocysts (Gazzinelli *et al*., 2014).

Although these sporulated oocysts are infectious, it should be emphasized that there is some debate over whether fertilization is required for the production of sporulated oocysts (Walker *et al*., 2015).

Furthermore, cats producing oocysts in farming communities infect cattle and cause microscopic, infectious cysts in tissues. When all of these data are considered, it is clear that human exposure to *T. gondii* infectious stages is common whether via oocysts or tissue cysts. It is still debatable whether route of infection is more essential, but it is known that every human infection is caused by cats releasing oocysts into the surroundings, either directly or indirectly see Figure (2-2) (Hussein *et al.*, 2017).

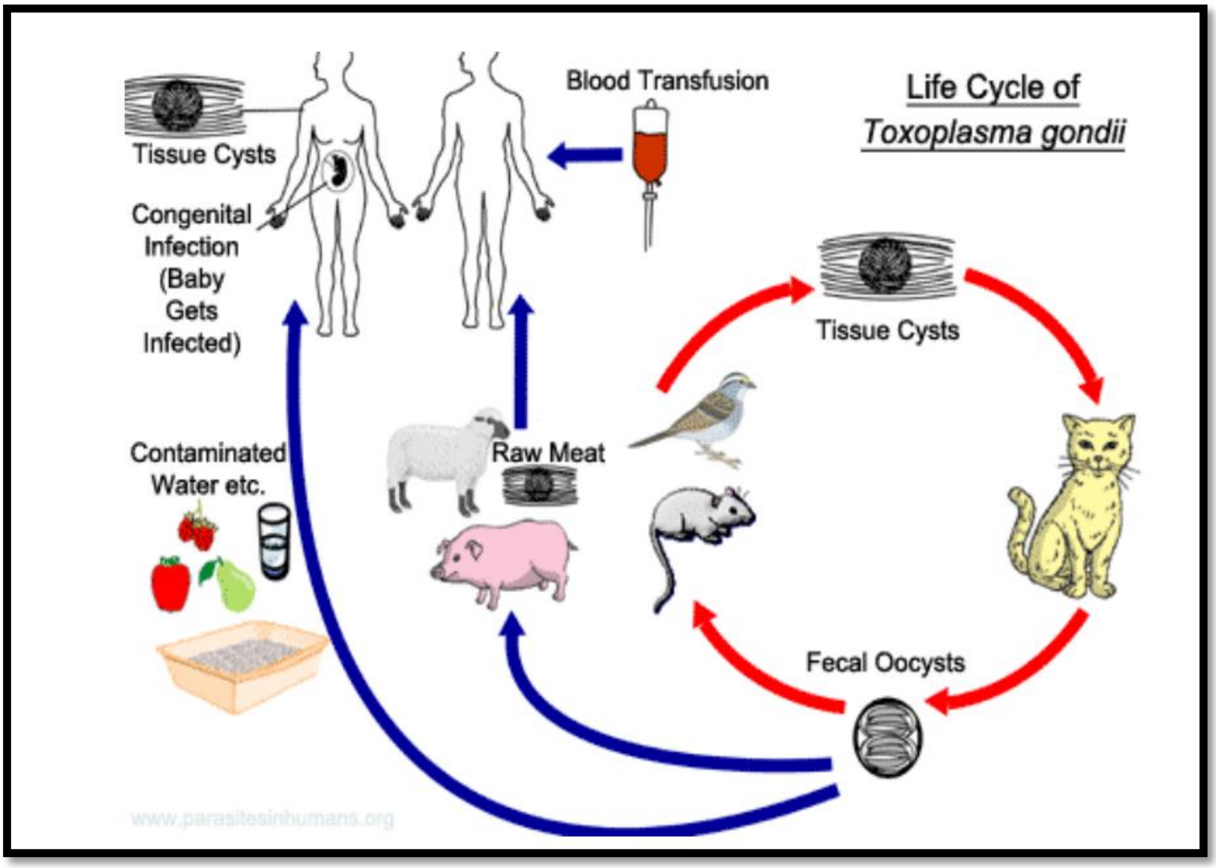


Figure (2-2): ***Toxoplasma gondii*** **Life cycle**

https://www.cdc.gov/dpdx/toxoplasmosis/index.html

2-5: Modes of transmission

Toxoplasmosis is spread in a variety of ways, as shown below:

1. During consumption raw or undercooked meat containing bradyzoites or formed tissue cysts, including such sheep, cows, and chickens (Mahami *et al.*, 2017). Working in the dairy business, as well as meat packing employees and those who sell meat, creates a risk for contracting this parasite (Amissah-Reynolds, 2020).

2. Fresh fruits and vegetables that have not been properly cleaned and are infected with infective phase oocysts are consumed (Shapiro *et al*., 2019).
3. Transmission from an infected woman to her fetus via the placenta (Omidian *et al*., 2021).
4. Through oocysts in polluted water and soil (Pinto-Ferreira *et al*., 2019). When someone eats an oocyst, he will get infected. This oocyst is resistant to difficult environmental living conditions and can survive in contaminated soils for further than one year (Gotteland *et al*., 2014).
5. Animal milk and bird eggs could be a cause of toxoplasmosis infection to humans (Stelzer *et al*., 2019).
6. During transfusions of blood and organ transplants (Manouchehri *et al*., 2019).

2-6: Epidemiology

Toxoplasmosis is a worldwide infection that affects 500 million people, with an estimated affected (12-90 %), and increase with age, low education, overcrowding, health, social, economic, traditions and ethnic issues, intake of undercooked meat, exposure to cat excrement, and immune impaired individuals more susceptible to infection were all factors (Gaetano *et al*., 2010). It can boost all of these elements, as well as climate circumstances, to create antibodies against parasites. As a result, oocysts can thrive in warm, wet soil from heated, dry places (Jones *et al*., 2006; Petersen, 2007).

2-6-1: Toxoplasmosis in the Worldwide

According to recent seroprovenance data, *T. gondii* prevalence in the Korean population ranges from 0.97% to 12.9%, with latitudes fluctuating in the south of Korea from 1960 to 1999, In Korea, it affects residents, children, and patients in hospitals at a lower rate, spanning from 1.1% to 7.7%, as compared to American and European locations (Tenter *et al*., 2000).

In the United States Jones *et al*. (2001) reveal that majority of the published statistics on seroprevalence are among women of reproductive age and/or those who are pregnant. According to information from 88 countries, the parasite seroprevalence rises with age in the majority of human populations, and it varies by sexual identity.

Toxoplasmosis is a major public health issue in Brazil, with a serological frequency of 50-54% in adulthood (Bahia-Oliveira *et al.,* 2003). Toxoplasmosis prevalence was researched in several countries of the world, with the highest rate found among pregnant women in France, spanning from 87% in 1984 (Hegab and Al-Mutawa, 2003).

On the other hand, the high prevalence rate of the indian population, Malaysia, and Nepal has been recorded. Toxoplasmosis IgG seroprevalence was 45 % overall. Just seven women (3.3%) had IgM antibodies, but only two of these had low IgG avidity, indicating a recent infection of less than four months (Singh and Pandit, 2004). The infection had indicated *T. gondii* consider the expansion in regions competent Iran and seropositivity 5.7 through 78%, according to reports (Ali *et al*., 2007).

Around 88% of the population in France is infected, owing to widespread ingestion of enormous amounts of raw and minimally cooked meat (Larosa *et al*., 2008), however, from 2000 to 2009, it exhibited a modest increase in toxoplasmosis seroprevalence (Shin *et al*., 2009).

In 2008, the Iranian Ministry of Health reported that the rates of toxoplasmosis were 40.7% in Isfahan, 44.2% in Lorestan, and 34.2% in Bandar-e-Abbas (Shin *et al*., 2009). In an another study, pregnant women utilizing IFA in Chaharmahal and Bakhtyari, Iran, had a seroprevalence of *T. gondii* antibodies of 27.6% (Ebrahimzadeh *et al*., 2013). In Pakistan, Sadiqui *et al.* (2018) state that among 500 pregnant women, the total seroprevalence of toxoplasmosis was 24.8% and the percentage infection for acute infection (IgG) was 8%.

2-6-2: Toxoplasmosis in Arab World

Many studies have been conducted at the national level of Arab nations to assess the seroprevalence rates of parasite infection. Elnahas *et al*. (2003) declare that anti-*Toxoplasma* antibodies were detected in 34.1% of Sudanese people in a research. Another study is carried out by Al – Qurashi (2004) which states that a high *T. gondii* rate of 52.1% was recorded among donors in the Asir region of Saudi Arabia, and Nimri *et al*. (2004) shows in a study conducted on 148 women in Jordan who have had a previous pregnancy is normal and ranged percentages recorded for my body antibody IgG and IgM between 45% and 27% for chronic and acute infection, respectively.

Also Jumaian (2005) shows that toxoplasmosis is shown to be prevalent in 31.6% of pregnant women in one study, and 53.1% in another one in Kuwait by (Iqbal and Khalid, 2007). Just a few researches have been carried out in Saudi Arabia to determine the prevalence of *T. gondii*, including investigations in Riyadh, the eastern area, and Jeddah (Tonkal, 2008). In Qatar, Abu-Madi *et al.* (2010) explain that the rate of toxoplasmosis infection amongst 823 women is 35.1% for IgG and 5.2% for IgM, respectively.

2-6-3: Toxoplasmosis in Iraq

In Mosul, Al-Obeidi (2004) makes a study on a total of 406 patients with serum; three distinct laboratory procedures have been used to diagnose a positive serum. A latex test revealed active infection in 79% of cases, followed by a dye test in 46 % of cases, and an ELISA test in 37% of cases.

Razzak *et al*. (2005) in the Kirkuk province, states that the infection rates are found to be 61.2% among pregnant women and 33.3% among nonmarried women. Also a study in the province of Dohuk by Razzak *et al*. (2005) Using the ELISA technique, it is discovered that the infection rate among women with a single fetus or many losses is 0.97%. Yacoub *et al*. (2006) in Basrah, the infection rate amongst aborted women is reported to be 59.1%.

Al Dalawi (2007) explains that by utilizing mini VIDAS technique, researcher discovers that the proportion of illness toxoplasmosis of IgG is 29.2% in Baghdad. Kareem (2008) concentrates on using the ELISA technology, it is discovered that the rate of infection in Baghdad is 31.04 % amongst previously and suddenly aborted women, apparently healthy pregnant women, and apparently healthy non married women. Khalil (2008) in Baghdad, on the other hand, explains that by using ELISA method, it is discovered that the prevalence of acute toxoplasmosis disease among women had been reduced by antibody immunoglobulin (21.5%).

Al-Rawi (2009) indicates that 4.16% of aborted women with IgM antibodies have toxoplasmosis, while 25.83% has IgG antibodies. In Baghdad, by using the ELISA technology, it is 13.33% for both (IgM and

IgG). Al-Shikhly (2010) explains that by using the latex agglutination test (LAT), ELISA IgM and IgG procedures, the rate of toxoplasmosis among healthy females in Baghdad is 51.52%, 41%, and 16.2%, respectively. In Iraq, the parasite is expanded to 36.6% in Basrah, 58% in Kirkuk, and 29.2% in Tikrit in 2011 (Al-Dory, 2011; Al-Mousawi and Shani, 2011; Kadir *et al*., 2011).

In Kut Al-Mayahi (2011) states that by using the ELISA IgM and IgG techniques, researchers discover that the incidence of toxoplasmosis in women is 66.35% and 44.71%, respectively. In Babylon province, Al-Mosawi (2012) declares that by using the ELISA technology, it is discovered that the rate of toxoplasmosis amongst pregnant women infected with diabetes for IgG, IgM, and both (IgG, IgM) is 69.1%, 17.4%, and 48.8%, respectively. In Babylon province, Al-Ghargholi (2014) says that by using the ELISA IgM and IgG techniques, researchers discover that the rate of *T. gondii* infection in diabetes mellitus patients is 51.4% and 11.4%, respectively.

Mahmood (2014) adds that by using the ELISA technique, it is discovered that the rate of toxoplasmosis among aborted women in Najaf province was 49.12% for each (IgG, IgM). From 1992 to 2012, he discovered *Toxoplasma* antibodies among persons in Kirkuk province, reporting an overall toxoplasmosis rate of 31.35%, with a high incidence rate of 63.80% in 2005, which is compared to 17.59% in 2012 (Salman, 2014). Al-Jumaili (2015) states that by employing the ELISA technique, the infection of toxoplasmosis on marriage candidates and blood donors is found to be (24%, 24.4%, 2%, 2.22%), (14.9%, 14.1%, 1.5%, and 4%), respectively, in Babylon province.

In Baghdad province Al-Rawazq (2017) explains that the seroprevalence of *T. gondii* IgG antibodies resulted 63.4% of 110 pregnant women being examined. In Tikrit province Tawfiq *et al*. (2019) state that IgG-*Toxoplasma* is found to be positive in 35.5% of pregnant women.

2-7: Diagnosis

2-7-1: Serological and immunological diagnosis

The presence of trophozoites and cysts in tissue and bodily fluids, as well as serological techniques, are used to diagnose acute and chronic toxoplasmosis (Gov *et al*., 2013). The simultaneous presence of IgM and IgG antibodies can be used to diagnose acute and chronic *T. gondii* infections, respectively (Hamza *et al*., 2020).

A positive IgM antibody titer suggests an early primary infection; the serum IgM titer can be determined using an IgM ELISA kit, which is equally specific and sensitive; a negative IgM titer and a positive IgG titer suggest a remote infection (Tait and Hunter, 2009; Hunter and Sibley, 2012).

Although infection acquired after birth is usually asymptomatic, lymphadenopathy is the most common symptom of acute acquired toxoplasmosis, with the cervical lymph nodes being the most commonly impacted. Fever, headache, myalgia, and splenomegaly are common symptoms, the disease resembles mild influenza and is self-limiting, though lymphadenopathy may remain. A typhus-like exanthema with pneumonitis, myocarditis, and meningoencephalitis may occur in certain cases, which can be deadly (Dubey, 2021).

Both humoral (antibody-mediated) and cellular responses are involved in the host's respone against *Toxoplasma* infection, a specific IgG antibody can lyse extracellular trophozoites, T cells and natural killer cells that have been activated appear to be more essential in limiting the infection and preventing clinical illness (Lambert, 2009). Cobas e411 analysis (Electro-chemiluminescence immunoassay ECLIA) for IgG and IgM antibodies can be used to diagnose *T. gondii* immunologically. The seropositivity of *Toxoplasma* IgG antibodies by Electro-chemiluminescence immunoassay is 52.6%, while the seropositivity of *Toxoplasma* IgM antibodies is 5.1%. Although the electro-chemiluminescence immunoassay remains a highly specific and sensitive approach for diagnosing toxoplasmosis, the high cost of both the instrument and the test reagents may limit its use in rural areas. As a result, the *Toxoplasma*-Latex agglutination test may be useful in disease screening due to its ease of use (My *et al*., 2017).

2-7-2: Molecular diagnosis

The polymerase chain reaction (PCR) is a molecular way of detecting *T. gondii* DNA in clinical and epidemiological contexts. Nevertheless, the sensitivity and accuracy of PCR are affected by the type of material used; for example, given the random distribution of tissue cysts, so, it is difficult to determine which organ is best for PCR (Contini *et al*., 2005). Because of the recently developed Polymerase Chain Reaction (PCR) alternative, molecular approaches are now used to diagnose congenital toxoplasmosis, a clinically significant condition, despite the fact that the performance of these strategies is liable to change due to the huge number of target genes (Sterkers *et al*., 2010).

2-8: Toxoplasmosis in the Immunocompromised Patients

Many *T. gondii* infections in immunocompetent persons are asymptomatic, but they can have devastating consequences in immunocompromised patients, including life-threatening opportunistic infections (Halonen and Weiss, 2013).

Even though most cases of toxoplasmosis are asymptomatic, infection with *T. gondii* can have catastrophic implications in immunocompromised people and in cases of congenital infection, ocular infection, or posterior retinochoroiditis, is the most common clinical symptom of congenital and acquired toxoplasmosis in the latter instance (Olariu *et al*., 2011; Dubey *et al*., 2012).

The intensity and prevalence of the condition varies widely, and it is thought to be influenced by the host's immune system's state (Garweg and Candolfi, 2009). In immunocompromised adults, severe toxoplasmosis develops as a result of acute infection or reactivation from dormant tissue cysts (Ajzenberg *et al*., 2009). There is an additional evidence of a link between *toxoplasma* infection and immunocompromised patients (Ibrahim *et al*., 2015). The immune system in immunocompetent people regulates parasite proliferation and prevents them from spreading. Furthermore, cytokines have been demonstrated to play a significant role in the pathogenesis of toxoplasmosis, favoring the transition of tachyzoites into bradyzoites and, subsequently, the production of tissue cysts (Costa-Silva *et al*., 2012; Sullivan and Jeffers, 2012).

T. gondii antibodies are found in considerable numbers in healthy blood donor participants in several researches (Eser and Yay, 2006; Elhence *et al*., 2010).

Children with thalassemia who require regular, frequent, and numerous transfusions are given multiple units of blood from different donors on a regular basis for survival (Moghimi *et al*., 2015). Nevertheless, a *T. gondii* screening program prior to blood transfusion has not yet been addressed. Also, children with blood illnesses such as thalassemia have a compromised immune system, rendering them more susceptible to opportunistic *Toxoplasma* infection (Al-Ofairi *et al*., 2011).

2-9: Thalassemia

Thalassemia is a term used to describe a group of genetic blood diseases that are characterized by anemia due to enhanced red platelet obliteration. Hemoglobin, the oxygen-carrying component of red platelets, is made up of tetrad distinct protein chains, 2 α globin and 2 β globin (Passarello *et al*., 2012).

In many countries, thalassemia is considered a serious public health issue; and the prevalence rate is particularly high in the Middle East, Southeast Asia, Burma, and India (Simon *et al*., 2009).

According to research studies on patients with Mediterranean anemia in Babylon province, the majority of the patients are beta medial anemia, with the average being alpha type. As a result, only patients with beta mediated anemia are included in the study (Omar *et al*., 2005).

Statistics show that according to the Iraqi Health ministry, the number of people with Mediterranean anemia has risen to 15,000 (Babylon province 395 patients, 283 of whom have been classified with Mediterranean great Beta anemia) (Al-Saray, 2012). Patients with thalassemia in AL-Muthana province rely on transfusions when their blood cells are low and there is no other option (Rummel and Ellsworth, 2016).

El-Beshlawy and Youssry (2009) state that, in Egypt, thalassemia illness is shown to be the most frequent cause of chronic hemolytic anemia (85.1%), with a carrier incidence of 5.3-9% and 1,000 every 1.5 million live births each year. Loss of appetite, jaundice, an enlarged spleen or liver, and various bone abnormalities are common in thalassemia patients (Joly *et al.*, 2014). Because blood transfusions are the most common treatment for thalassemia, excessing iron builds up in the bloodstream (Habibian *et al.*, 2014). Excess iron in the body can lead to a variety of cardiac disorders, including irregular heartbeats, heart attacks, and even death from heart failure (Harteveld and Higgs, 2010).

2-10: Types of Thalassemia

2-10-1: Beta-Thalassemia Carrier State

Microcytosis (reduced red blood cell volume) is a hallmark hematological trait of beta-thalassemia carriers who are clinically asymptomatic, increased HbA2 level (the minor component of adult Hb, which is made up of two alpha and two delta chains), hypochromia (reduced Hb content in red blood cells), in vitro synthesis of radioactive-labeled globin chains produced a slightly imbalanced alpha/beta, gamma globin chain synthesis ratio is (1.5–2.4) (Thein and Menzel, 2009).

β-thalassemia, on the other side, are haemoglobin synthesis disorders caused by mutations in regulatory globin genes on chromosome 11 short arm, resulting in reduced synthesis of normal globin proteins as shown in Figure (2-3) (Kamil *et al.*, 2014).

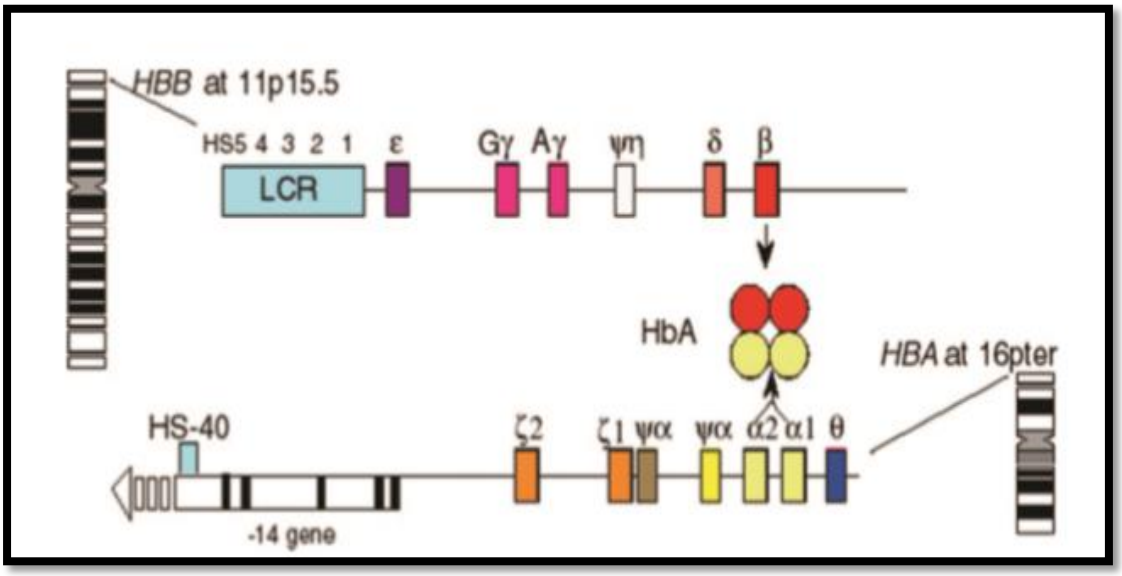


Figure (2-3): The location and organization of alpha and beta globin gene clusters on the chromosome (Farashi and Harteveld, 2018).

2-10-2: Beta-Thalassemia major

Patients with thalassemia major suffer from severe microcytic and hypochromic anemia, which is associated by an decreased number of red blood cells as well as low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) (De Sanctis *et al*., 2013).

Borgna-Pignatti *et al*. (2010) show that since β-thalassemia is a group of frequent genetic illnesses that cause the production of few or no-globin chains, researchers are working on new approaches to repair the ensuing /β globin chain imbalance. In an effort to go beyond palliative care for this illness and associated therapeutic consequences (for example, life-long red blood cell transfusions, iron reduction, splenectomy), which are costly to healthcare systems (Hattab, 2013).

Three options are being considered: pharmacological agents administered into patients throughout their lives to reactivate the fetal globin gene, allogeneic hematopoietic stem cell transplantation (HSCT), and gene therapy (Rachmilewitz and Giardina, 2011). HSCT is currently the only treatment that has been proved to offer a permanent cure for β-thalassemia. Nevertheless, because this operation is dangerous and only a tiny percentage of patients have histocompatible donors, novel pharmacological substances are being investigated. However, none have yet made it into routine clinical practice for the treatment of β-thalassemia major, and gene therapy is still in its early stages (Singh *et al*., 2013).

Study of Mousa and Nahab, (2020) states that patients with β-thalassemia major who get blood transfusions on a regular basis are at risk of contracting transplant-transmitted illnesses such as toxoplasmosis. This is a common and dangerous parasitic disease with a high frequency that can be spread via blood transfusions from healthy asymptomatic donors (However, there is no data on *T. gondii* testing prior to blood donation).

2-10-3: Thalassemia Intermedia

Thalassemia intermedia or non-transfusion-dependent thalassemia is a term used to label patients who do not require lifelong regular transfusions for survival, although they may require occasional or even frequent transfusions in certain clinical settings and usually for defned periods of time (surgery, pregnancy, infection) (Weatherall, 2012).

Patients with thalassemia intermedia have a mild anemia and a hematological picture that is strikingly diverse, ranging in severity from β-thalassemia minor to thalassemia major (Menzel *et al*., 2007).

Ineffective erythropoiesis, chronic anemia, and iron overload contribute to the clinical complications of thalassemia intermedia through stepwise pathophysiological mechanisms. These complications, including splenomegaly, extramedullary erythropoiesis, iron accumulation, leg ulcers, thrombophilia, and bone abnormalities can be managed via fetal hemoglobin induction, occasional transfusions, chelation, and in some cases, stem cell transplantation (Asadov *et al*., 2018).

2-10-4: Beta-thalassemia Minor

β-thalassemia minor (βTM) is a term used to describe heterozygotes who have inherited a single gene that causes them to produce less beta globin. βTM patients are typically asymptomatic and have relatively mild anemia. When a blood count is taken for another purpose, this problem is frequently identified. As a prevalent and often symptom-free condition, Countries in the Mediterranean region, such as Turkey, have the highest prevalence of β-thalassemia minor (Benz, 2012).

Carriers of thalassemia minor are normally asymptomatic, however a slight anemia can occur. When both parents are carriers, there is a 25% chance of having children with homozygous thalassemia during each pregnancy (Lechauve *et al*., 2019).

Thalassemia minor causes varying degrees of illness, based on the rate at which chains are produced. Asymptomatic moderate anemia (hemoglobin 1–2 g/dL lower than normal people of the same age and gender) is the most common sign (Verma *et al*., 2011).

2-11: Oxidative Stress

An imbalance between the processes that contribute to the creation of free radicals (pro-oxidant) and the systems that seek to eliminate them (antioxidant) is known as oxidative stress (OS). This imbalance can be caused by the first mechanisms being activated, the second mechanisms being inhibited, or both processes being active at the same time, for all of these instances of free radical buildup that are characterized by a high capacity to destroy tissue cellular components (Dikalov and Dikalova, 2019).

Oxidative stress (OS) is a primary cause of spontaneous and threatened abortion, and spontaneous abortion may be linked to (OS) in the maternal circulation (Bagavathiammal *et al*., 2017).

Free radicals from oxygen easily interact with DNA, proteins, and lipids in the cell envelope, causing conformational changes in cellular structures and resulting in disruption and failure. Many diseases, including cancer, arthritis, aging, and diabetes, are induced by oxidative stress (Stulce *et al*., 2019).

2-12: Free Radicals

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals (Jomova and Valko, 2011).

Free radicals are formed in the mitochondria and white blood cells, as well as by interfering with detoxification mechanisms as a result of toxic material or radiation exposure. This free radicals they target and attack cellular components, producing substantial damage that can results in cell death. Due to their high activity, because damage to proteins generates an imbalance of enzymatic processes, it works to destroy membrane phospholipids and impair membrane functioning, and also free radicals' propensity to alter the structure of DNA, which has an impact on protein synthesis (Valko *et al*., 2006). Throughout metabolic processes, cells produce modest levels of free radicals and active species, which are regulated by an antioxidant defense mechanism (Franco *et al*., 2019).

In the defense of the host, neutrophilic cells and macrophages play a significant role. These cells have the ability to produce vast quantities of extremely poisonous chemicals, like reactive oxygen species (ROS) including peroxide (O_2) radicals, hydrogen peroxide (H_2O_2) radicals, and hydroxyl radicals (•OH). Bacteria, parasites, and cancer cells trigger macrophages in large quantities of reactive nitrogen species (RNS), including nitric oxide (NO) (Terra *et al*., 2019). ROS and RNS, on the other hand, can degrade a wide range of biomolecules, including DNA, carbohydrates, and proteins. Because lipid peroxidation leads to the creation of various degradation products, including malondialdehyde (MDA). ROS and RNS can target the polyunsaturated fatty acids of membrane lipids, producing lipid peroxidation and disruption of cytoskeletal function (Rahman *et al*., 2012).

2-13: Uric acid (Structure and clinical significant)

Uric acid has the formula $C_5H_4N_4O_3$ and is a heterocyclic chemical comprising carbon, nitrogen, oxygen, and hydrogen as shown in Figure (2-4). It produces urates and acid urates, such as ammonium acid urate, which are ions and salts, the metabolic breakdown of purine nucleotides produces uric acid, which is a typical component of urine. High uric acid levels in the blood can cause gout and are linked to other medical issues such diabetes and the creation of ammonium acid urate kidney stones (Rada *et al*., 2019).

Uric acid as an antioxidant against ROS.Where the accumulation of uric acid considerably increases the free radical-scavenging activity and resistance against oxidative stress (Tasaki *et al*., 2017).

Function of uric acid are alleviating oxidative damage and providing protection to cells during injury and disease (Settle and Klandorf, 2014).

The normal range for uric acid in human blood plasma is typically 3.4–7.2 mg per 100 mL (200–430 µmol/l) for males and 2.4–6.1 mg per 100 mL (140–360 µmol/l) for females. Hyperuricemia and hypouricemia are respectively the terms for uric acid concentrations in blood plasma that are above and below the range of normal, similarly, hyperuricosuria and hypouricosuria are terms for uric acid concentrations in urine that are above and below normal, respectively. Uric acid concentrations in saliva may be linked to blood uric acid levels (Zhao and Huang, 2015).

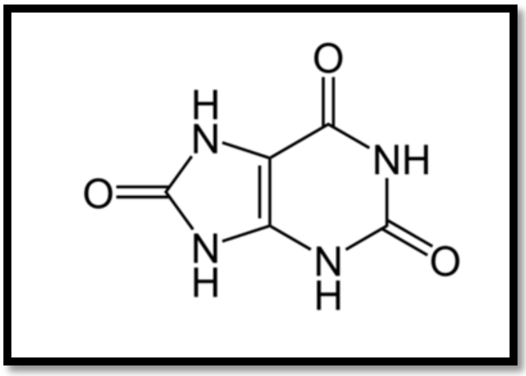


Figure (2-4): The chemical structure of uric acid (Grases *et al*., 2014).

2-14: Allantoin Compound

Allantoin ($C_4H_6N_4O_3$) is a chemical compound that called 5-uridohydantoin. It is also known as glyoxylidoride, and it is a kind of uridohydantoin in most living creatures such as animals, plants, and microorganisms. Allantoin is a significant modulator of metabolic pathways (Audra *et al*., 2017). It is created from uric acid, which is formed when nitrogenous bases are dissolved by the enzyme uricase or urate oxidase as show in Figure (2-5) (Ramazzina *et al*., 2006).

Michele Francesco Boniva (1761-1834), an Italian physician, and Louis Nicolas Vauquelin, a French chemist, discovered allantoin for the first time in 1800, who mistakenly assumed that it was present in the amniotic fluid. It was discovered in the fluid allantoic in 1821 by French scientist Jean-Louis Lassaigne, Friedrich Waller and Justus Liebig. The two German scientists manufactured it from uric acid and renamed it allantoin, which is found in perennial plant extracts and also most mammals' urine (Rekha and Sinha, 2021).

Chemically synthesized allantoin blocks are chemically equivalent to natural allantoin in terms of safety, non-toxicity, compatibility with cosmetic raw ingredients, and adherence to American personal care products council standards. Allantoin is mentioned in over 10,000 patents (Zhong *et al*., 2016).

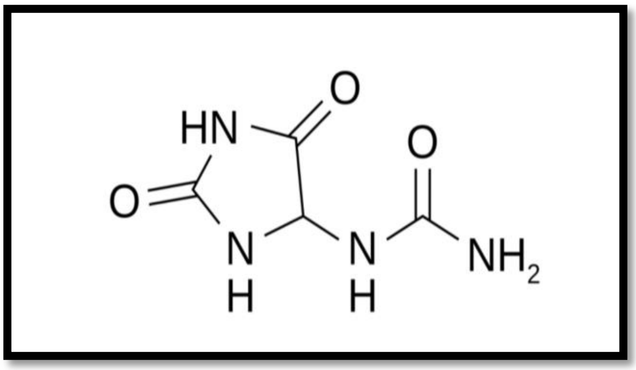


Figure (2-5): Chemical structure of the allantoin (Menezes *et al*., 2020).

Allantoin can be found in a variety of cosmetic products and creams, as well as toothpaste, mouthwash, and other oral health products, as well as shampoos, lipsticks, acne-fighting products, sunscreens, purifying moisturizers, and other pharmaceuticals (Savić *et al*., 2015).

2-14-1: Allantoin as a biomarker of Oxidative Stress

Allantoin is generated in humans by nonenzymatic oxidation of urate; given uric acid is the end result of purine metabolism in humans. It may be useful in measuring oxidative stress, allantoin is a suitable biomarker for detecting oxidative stress in chronic disease and aging since it is produced only by non-enzymatic processes that involve reactive oxygen species. In primates, including humans, urea is the last result of purine metabolism. Urate is also a good oxidant scavenger and is regarded to be a significant antioxidant in human bodily fluids (Kozlik *et al*., 2020).

Allantoin, the major oxidation product of urate, has been suggested as a candidate biomarker of oxidative stress because it is not produced metabolically (Kand'ár and Záková, 2008).

2-15: Interleukin-10

Interleukins (ILs) are a group of tiny cell signaling protein molecules, also known as cytokines that modify the immune response in humans (Tsai *et al*., 2013). Single nucleotide polymorphisms (SNPs) in ILs may change their function, increasing vulnerability to various diseases and, as a result, altering cytokine function and their expression (Zhou *et al*., 2008). Interleukin 10 (IL-10) is an anti-inflammatory cytokine also referred to as human cytokine synthesis inhibitory factor (CSIF) (Kang *et al*., 2009). IL-10 main biological activities are to reduce or regulate the inflammatory reactions generated by dendritic cells and macrophages, as well as to reduce T cell adaptive responses; its cytokine inhibits antigen presentation by decreasing dendritic cell production of the major histocompatibility complex class II (MHC II) and accessory co-stimulatory molecules. The overall impact is to prevent these cells from maturing (Stanic *et al*., 2015).

Many studies have found that immune-regulatory cytokines play an important role in hematology malignancies. Cytokines are a varied collection of low molecular weight regulating proteins or peptides that are mostly soluble, cytokines have a role in cell-to-cell contact, organize inflammatory and immunological responses, and function on their own. They are secreted in response to a variety of stimuli, the molecular structure or function of cytokines is used to classify them, in to IL-1 family, IL-17 family, tumor necrosis factor (TNF) family, and four alpha-helix family, which includes IL-2, interferon (INF), and IL-10, is the four structural families of cytokines. Cytokines are either part of antibody-mediated (humoral) immunity or cellular-mediated immunity, depending on their function (Al-Khatib *et al*., 2020).

IL-10 possesses a variety of inhibitory and stimulatory properties (Wilke *et al*., 2011). The gene that codes for IL-10 is found on chromosome (1) at the junction of (1q31-1q32) and contains a few polymorphisms in the promoter region of the transcriptional start point, including IL-10, -1082G/A (rs1800896), and -819T/C (rs1800871), and -592A/C (rs1800872) (Yao *et al*., 2013).

IL-10 It can also activate B cells and encourage the growth of natural killer cells (NK cells). When IL-10 is generated and secreted, it interacts with the IL-10 receptor, which is made up of two subunits: IL-10 receptor 1 and IL-10 receptor 2, IL-10 has emerged as a major immune regulator during infection with viruses, bacteria, fungi, protozoa, and helminths, which cause much of the immunopathology associated with diseases such as *T. gondii* (Eidenschenk *et al*., 2014).

Chapter Three

Materials and Methods

Chapter Three

3- Materials and Methods

3-1: The Equipment's and Tools

Table (3-1): The Equipment's and Tools used in the Current Study.

No.	Instruments	Company	Origin
1	Autoclave	Hirayama	Japan
2	Buchner funnel	LAB.	Germany
3	CBC device	Mindray	China
4	Cobas c111	Roche	Germany
5	Cobas e411	Roche	Germany
6	Digital camera	Sony	Japan
7	EDTA tube	Argelik	Jordan
8	Electrophoresis unit	Labnet	Taiwan
9	Eppendorf tubes	Afco	Jordan
10	Filter paper	Broche	Turkey
11	Flask	Marienfeld	Germany
12	FTIR-8400S(Fourier Transform Infra- red Spectrophotometer	Shimadzu	Japan
13	Gel tubes	Afco	Jordan
14	HPLC	Sykamn	Germany

15	Incubator	Memmert	Germany
16	Macro centrifuge	Hitachi	Germany
17	Mechanical stirrer	LabTech	Korea
18	Melting point apparatus (SMP30)	Stuart	English
19	Micro centrifuge	Hitachi	Germany
20	Micropipette	Eppendorf	Germany
21	Microwave	Argos	Germany
22	Nanodrop2000	Thermo	USA
23	Rack	Afco	Jordan
24	Refrigerator	Hitachi	Japan
25	Sample cup	Roche	Germany
26	Sensitive balance	Precisa	Switzerland
27	Syringes	Medo	U.A.E
28	Thermo cycle (PCR)	Labnate	USA
29	Tips	Sterile	UK
30	U.V Light	Quantum	France
31	Vortex	Labinco	Japan
32	Water bath	Memmert	Germany

3-2: Biological and Chemical Materials

Table (3-2): Biological and Chemical Materials used in the Current Study.

Parameters	Company	Origin
Agarose	Biobasic	Canada
Distilled water	Iraq	Iraq
DNA Ladder (DNA marker 100bp)	Promega	USA
Ethanol (96~100%)	Flukachemika	India
Ethidium bromide	Biobasic	Canada
FABG buffer	Favorgen	Taiwan
Glacial acetic acid	TCI	China
Green master mix	Promega	USA
Humen *Toxoplasma* IgG kit	Hitachi	Germany
Humen *Toxoplasma* IgM kit	Hitachi	Germany
Manganese dioxide	CDH	China
Norite	CDH	China
Nuclease free water	Promega	USA
Potassium permanganate	CDH	China

Primers	Promega	USA
Proteinase K	Biobasic	Canada
Sodium hydroxide	CDH	China
Tris Borate-EDTA–Buffer solution (TBE)10x	Biobasic	Canada
Tris-EDTA Buffer solution (TE) or Elution buffer	Favorgen	Taiwan
Uric acid kit	Hitachi	Germany
Uric acid 100 gm	BDH	China
Wash 1 buffer	Favorgen	Taiwan
Wash 2 buffer	Favorgen	Taiwan

3-3: Experimental Design of the Study

The overall procedures of the study summarized in Figure (3-1).

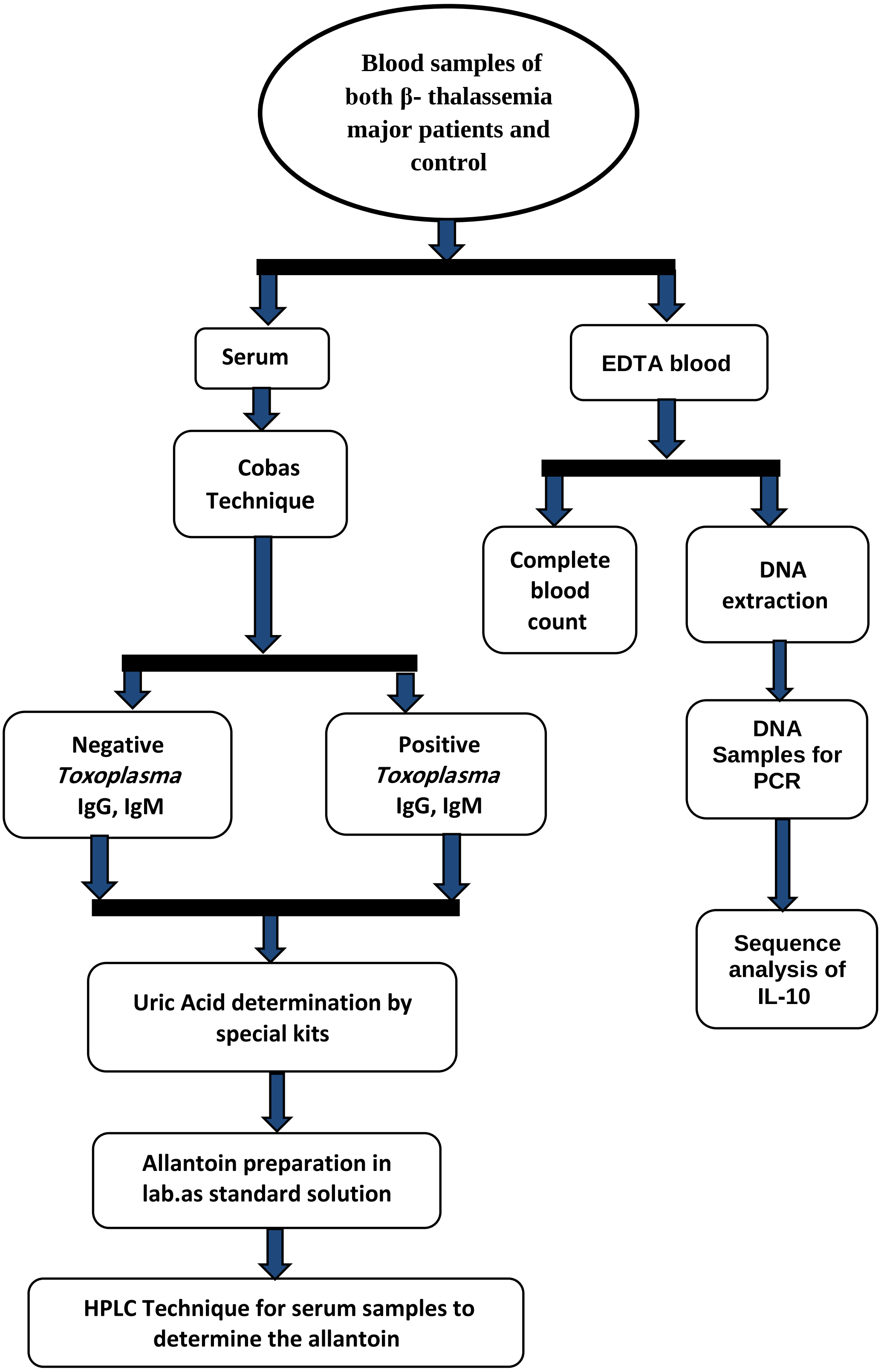


Figure (3-1): Experiment design of the study.

3-4: Methods

3-4-1: Samples

A total of 96 blood samples (sixty-six samples from β-thalassemia major patients diagnosed by a specialist physician and 30 samples of healthy peoples as control group) were chosen from the Maternity and Children's Hospital in the Al-Hilla city, Babylon province. Their age groups ranged from 10 to 25 years during the period from October till December 2021.Before receiving a transfusions quantity of healthy people (control) to compare them. Blood specimens were saved under -20°C in frozen stat until uses, and the samples were divided into two part as the following :-

1- Filling an EDTA tube with two mL of blood, for the purpose of a blood test for hematological assay and molecular study for IL-10.

2- To get serum, place three mL of blood in a gel tube and allowed to clot for 20 minutes at room temperature before being centrifuged at 3000 rpm for five minutes to extract serum for serological and biochemical tests (Jones *et al*., 2014).

3-4-2: Blood Samples Collection

Before obtaining a blood sample, all men and women must provide some information as questionnaire sheet. Appendix (1) (2).

1. Five (5) milliliters of blood were drawn from each patient by a sterile syringe was used to take blood from the antecubital vein
2. Took five milliliter of blood for β-thalassemia patients, including men and women (Lima-Oliveira *et al*., 2015).

3-4-3: Sterilization Methods

Sterilization of the tools by autoclave at 121°C for and pressure 1.5 bar for 15 min.

3-5: Hematological Assay

The blood test includes the following tests red blood corpuscles count (RBCs), hemoglobin concentration (Hb), packed cells volume(PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells count (WBCs) and platelets count (PLT) were examined using CBC device (Mindray company). After depositing two mL of blood in an EDTA tube in the device's chosen location and then giving the start instruction, as the device analyzes the results automatically , and when it appears the results given a directive (print) for the device (Yamsri *et al*., 2015).

3-6: Complete Blood Counts (CBC)

The hematological diagnosis shows anemia due to microcytic anemia. Mediterranean anemia is characterized by a decrease in the MCV normal level (80-96) fl/cells, hemoglobin Hb normal level (12-18) gm/dl, and MCH normal level (27-33) pg/cells (Galanello *et al*., 2001).

3-7: Immunological assay

3-7-1: Cobas e 411 analyzer to detection the IgG and IgM

This assay was performed by using two kits (Al-Amin Center in Najaf), one for detection of *Toxoplasma* IgG antibodies, and the other for detection of *Toxoplasma* IgM specific antibodies against *Toxoplasma* antigen in the patient's serum.

3-7-2: Detection of Anti-*T. gondii* Antibody IgG and IgM by Cobas e 411 Technique

1- The detector *Toxoplasma* IgG, *Toxoplasma* IgM and diluted is selected inside the device.

2- Put one ml of the serum in the sample cup and then put it in a designated place in the device.

3- The device takes 20 microliters of sample volume.

4- The device is programmed and left for 20 minutes for each sample.

5- The readings are recorded on the device screen and read with a unit *Toxoplasma* IgG (Iu/ml) and *Toxoplasma* IgM (CoI). Photo (3-1).

3-7-3: Quality control

Based on the measurements of calibration-1 and calibration-2, the cut-off was calculated automatically. Readings: IgG: >1 (non-reactive), > 1.0 - > 30 (Intermediate), > 30 (Reactive). Readings: IgM: > 0.8 (non-reactive), > 0.8 - >1.0 (Intermediate), >1 (Reactive). Values obtained were fall within the established parameters (Elshiekh, 2015).

Photo (3-1): Cobas e411

3-8: Biochemical assay

3-8-1: Uric Acid Measurement

After completing the examination of samples (IgG, IgM), the uric acid was measured by Cobas c111 device (Al-Amin Center in Najaf) by taking (10 µl) of the patient's serum or the control group, where the device analyzes the sample for a period of (5 minutes) after programming the device and reading the result automatically (Bowling and Katayev, 2010). Photo (3-2).

Photo (3-2): Cobas c111.

3-8-2: Preparation of Allantoin from Uric acid by oxidation as (standard material) Procedure depends on (Czauderna and Kowalczyk, 1997; Caussé *et al*., 2007)

1- 100 grams of uric acid and 4.5 l. of hot water (70-85°C) are placed in a 12 l. round-bottomed flask equipped with a mechanical stirrer. The stirrer is started, and a solution of 80 gm of commercial sodium hydroxide in 120 ml of water is added. Stirring is continued until the uric acid is in solution.

2- After which the solution is cooled by means of a stream of water directed against the flask. When the temperature has fallen to 25-30°C.

3- 50 gm of potassium permanganate is added all at once to the vigorously stirred solution. Stirring is continued for 15-20 min.

4- The mixture is filtered at once through a 19-cm büchner funnel. The first fraction of the filtrate contains a small amount of manganese dioxide. This fraction must be collected separately and returned to the funnel.

5- As soon as the filtrate becomes clear it is collected in a 12 l. round bottomed flask which contains (137 gm) of glacial acetic acid. The filtrate is tested with litmus to be sure that it is acid, and evaporated to a volume of 1.5-2 l. on a steam bath under reduced pressure (20-30 mm).

6- The solution thus obtained is allowed to stand in a cool place overnight, and the allantoin which crystallizes is filtered on a büchner funnel.

7- The allantoin is dissolved in 800-900 ml. of boiling water, treated with five gm. of norite, and filtered rapidly through a filter paper in a steam funnel. The filtrate is allowed to stand in a cool place overnight, and the white crystals of allantoin are separated by filtration.

8- To conform that this white crystals its allantoin perform the following steps. The yield of product melting at 230-231°C is 60-71 gm (64-75%) of the theoretical amount. If the filtrate from the purification liquors is concentrated to 100 ml, there is obtained an additional 3-5 gm of allantoin. And then tested some gram of allantoin in melting point apparatus (SMP30) to ensure that this material its allantoin that melting at 230-231°C.

9- by FTIR-8400S (Fourier Transform Infrared Spectrophotometer) put the sample and then by this device and from peaks revealed known that this samples return to allantoin, U.V. visible of uric acid its equal 294.46 nm and λmax=380 nm whereas the U.V. visible its 175-800 nm and λmax=299.01 nm.

3-8-3: Determination of Allantoin level concentration in serum patients and control groups by High Performance Liquid Chromatography technique (HPLC)

3-8-3-1: Sample preparation

A 100 µm serum sample was combined with 400 µm solvent C (KH_2PO_4/H_3PO_4-buffer with 50 mmol/1 phosphate, pH 4.60) and filtered through a 0.22 µm membrane filter. An aliquot (50 µm) of the filtrate was directly injected into the HPLC injector, and the quantification was done using the peak areas determined for the wavelength (Kirchherr and Kühn-Velten, 2006; Mosch *et al*., 2010; Westberg *et al*., 2014) Photo (3-3).

Photo (3-3): HPLC technique

3-8-3-2: Preparation of Standard Solutions

By using the dilution law $C_1 V_1 = C_2 V_2$, 2 mg of each standard was placed in a volumetric flask (25 ml) and the volume was supplemented with methanol (HPLC 99.9%) till the point where the stock solution concentration (80 ppm) was reached. The quantities that were introduced into the HPLC were prepared (Ministry of Science and Technology/Department of Environment and Water) (Li *et al*., 2010).

3-8-3-3: Diagnostics of separated compounds: Injection process

After turning on the device and allowing it to settle, the solvent that dissolved the substance is injected, followed by the standard substance with a known concentration, followed by the solvent being injected once more to ensure that no residue of the standard material remains, and finally the samples are injected one by one.

After analyzing the standard material, it will be shown as a peak written at the top of the curve. The peak is the retention time (Rt), this is the time it takes for the material to exit the detector after it has been injected, and which is the substance's description function under verified operating conditions.

The sample is injected after the standard substance has been injected multiple times to set its retention time. It is worth noting that a solvent is injected after each injection of the standard sample to ensure that no prior sample residue remains. The very first sample is then injected, and the standard substance's retention time (Rt) is compared to the retention time separated materials. The standard item will be whichever has the same retention time as the standard material. This is a descriptive diagnosis is what it's termed (Cao *et al*., 2015).

3-8-3-4: HPLC Condition

HPLC model SYKAMN (High Performance Liquid Chromatography) (Germany) it was used to analyses adds detection of vincristine and vinblastine. The mobile phase was an isocratic acetonitrile-0.1 M phosphate buffer containing 0.5% glacial acetic acid (30: 70) at a flow rate of 1.2 mL/min, the column was C18-ODS (25 cm * 4.6 mm), and the detector was UV-360 nm (Salah and Abdul-RedhaIsmaiel, 2022).

20/01/2022 12:52 م Calibration F:\allantoin.CAL Page 2 of 2

allantoin - Signal 1 - 3.46 min.

Compound Type : Ordnr
Left Window : 0.2 min
Right Window : 0.2 min
Response Base : Area
Curve Fit Type : Linear
Origin : Curve passes through Origin
Weighting Method : None
Equation : Y = 21.16*X
Correlation Factor : 0.9999722
Residuum : 1.8 [mV.s]
Linearisation X : None
Linearisation Y : None

	Response	Amount	Resp. Factor	Rec No.	Used
1	540.0000	15.0000	0.0476	1	☒
2	1088.0000	30.0000	0.0474	1	☒
3	1632.0000	45.0000	0.0470	1	☒
4	2170.0000	60.0000	0.0473	1	☒
5	0.0000	0.0000	0.0000	1	☒
6	0.0000	0.0000	0.0000	1	☒
7	0.0000	0.0000	0.0000	1	☒
8	0.0000	0.0000	0.0000	0	☒
9	0.0000	0.0000	0.0000	0	☒
10	0.0000	0.0000	0.0000	0	☒
11	0.0000	0.0000	0.0000	0	☒
12	0.0000	0.0000	0.0000	0	☒
13	0.0000	0.0000	0.0000	0	☒
14	0.0000	0.0000	0.0000	0	☒
15	0.0000	0.0000	0.0000	0	☒
16	0.0000	0.0000	0.0000	0	☒
17	0.0000	0.0000	0.0000	0	☒
18	0.0000	0.0000	0.0000	0	☒
19	0.0000	0.0000	0.0000	0	☒
20	0.0000	0.0000	0.0000	0	☒
BL	0.0000	Blank	0.0000	0	☒

20/01/2022 12:52 م Calibration F:\allantoin.CAL Page 1 of 2

Clarity - Chromatography SW
DataApex
www.dataapex.com

Calibration : F:\allantoin By : Administrator
Description :
Created : 20/01/2022 12:40:00 م Modified : 20/01/2022 12:51:59 م

Calculation : ESTD Mode : Calibrate
Calibrate : Automatic Recalibration Type : Replace
Change Response : Enable Weight : 0.25
Update Reten. Time : Enable Search Criteria : 0.00%
Deviation Limit : Not Used Correlation Limit : Not Used
Default Injected Volume : Not Used

Calibration Summary Table (ESTD - F:\allantoin - Signal 1)

Used	Compound Name	Reten. Time	Left Window	Right Window	Peak Type	Peak Color	LOD	LOQ	RB	Resp. Factor
☒	allantoin	3.46	0.200 min	0.200 min	Ordnr		0.1	0.000	A	0.0000

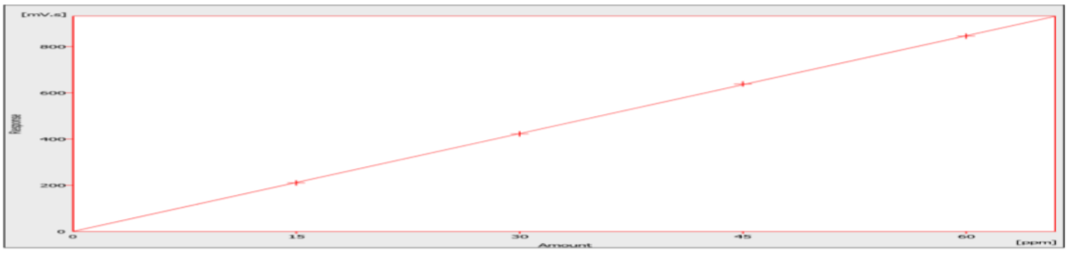
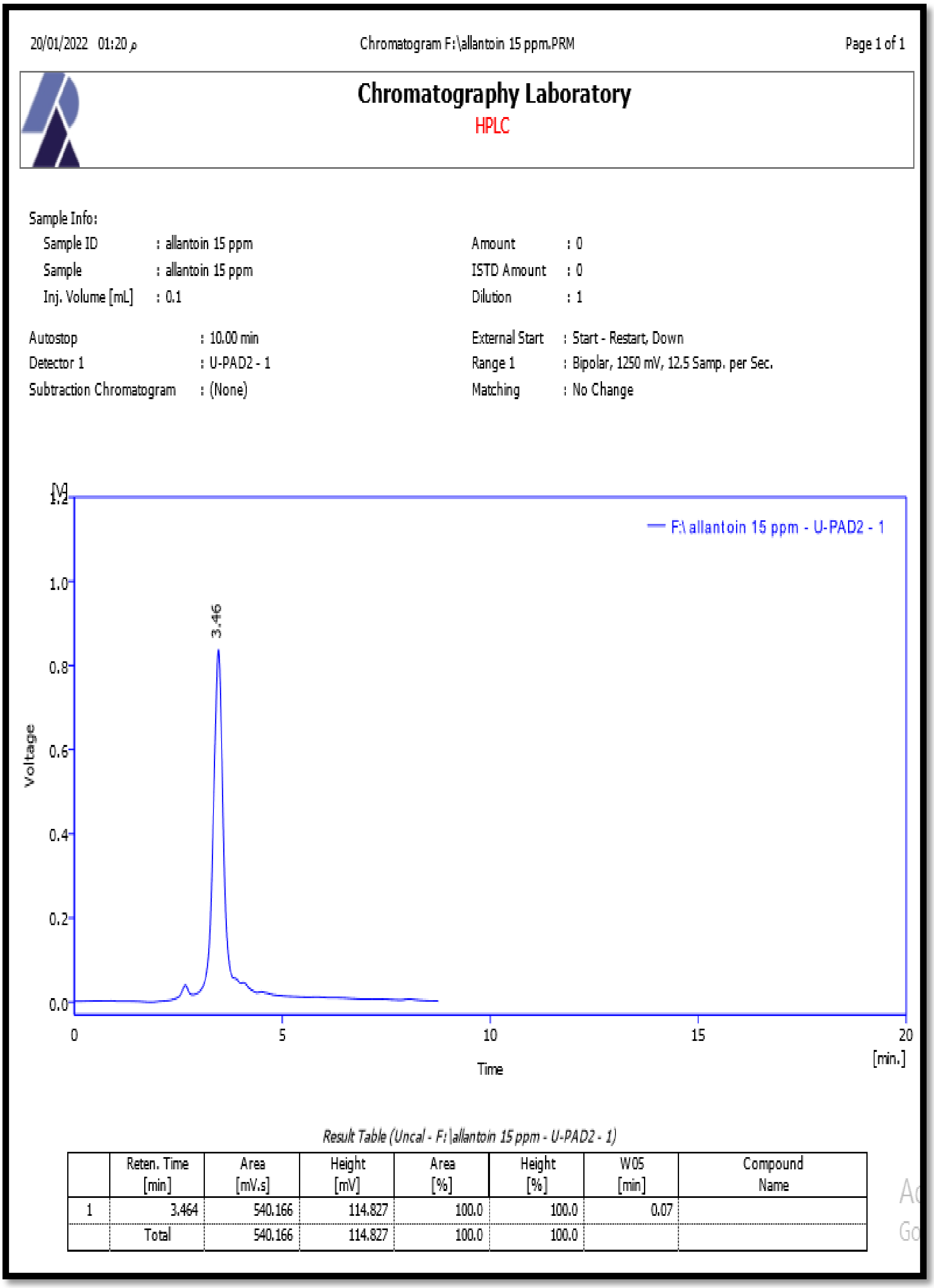


Figure (3-2): Standard curve for determination of allantoin concentration


20/01/2022 01:20 م Chromatogram F:\allantoin 15 ppm.PRM Page 1 of 1

Chromatography Laboratory
HPLC

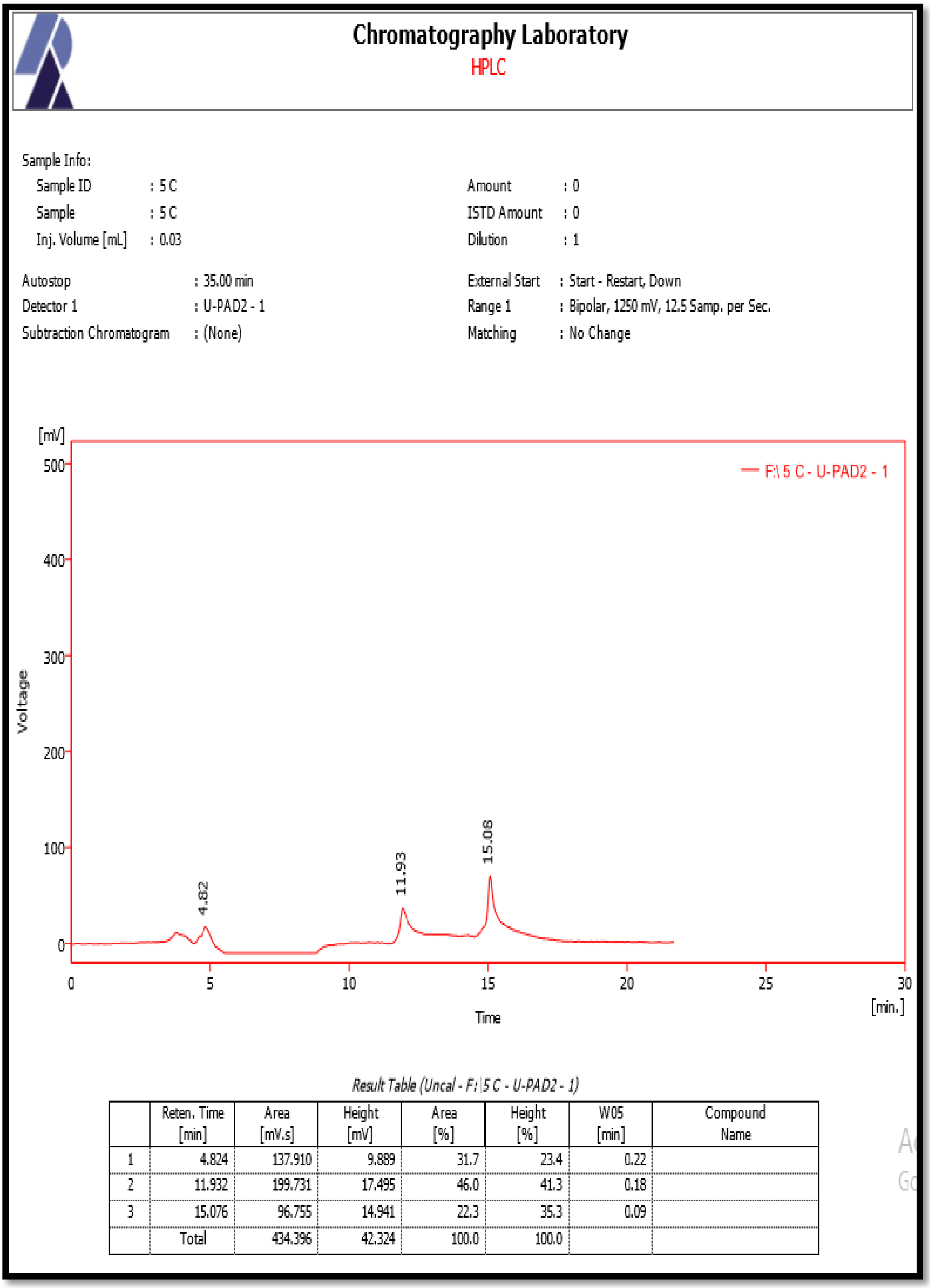
Sample Info:
Sample ID : allantoin 15 ppm
Sample : allantoin 15 ppm
Inj. Volume [mL] : 0.1
Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 10.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change

Result Table (Uncal - F:\allantoin 15 ppm - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.464	540.166	114.827	100.0	100.0	0.07	
	Total	540.166	114.827	100.0	100.0		

Figure (3-3): Typical chromatogram of prepared allantoin (standard material) that used in HPLC technique



Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: 5 C	Amount	: 0
Sample	: 5 C	ISTD Amount	: 0
Inj. Volume [mL]	: 0.03	Dilution	: 1
Autostop	: 35.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change

Result Table (Uncal - F:\5 C - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.824	137.910	9.889	31.7	23.4	0.22	
2	11.932	199.731	17.495	46.0	41.3	0.18	
3	15.076	96.755	14.941	22.3	35.3	0.09	
	Total	434.396	42.324	100.0	100.0		

Figure (3-4): Typical chromatogram of allantoin in control (healthy) serum

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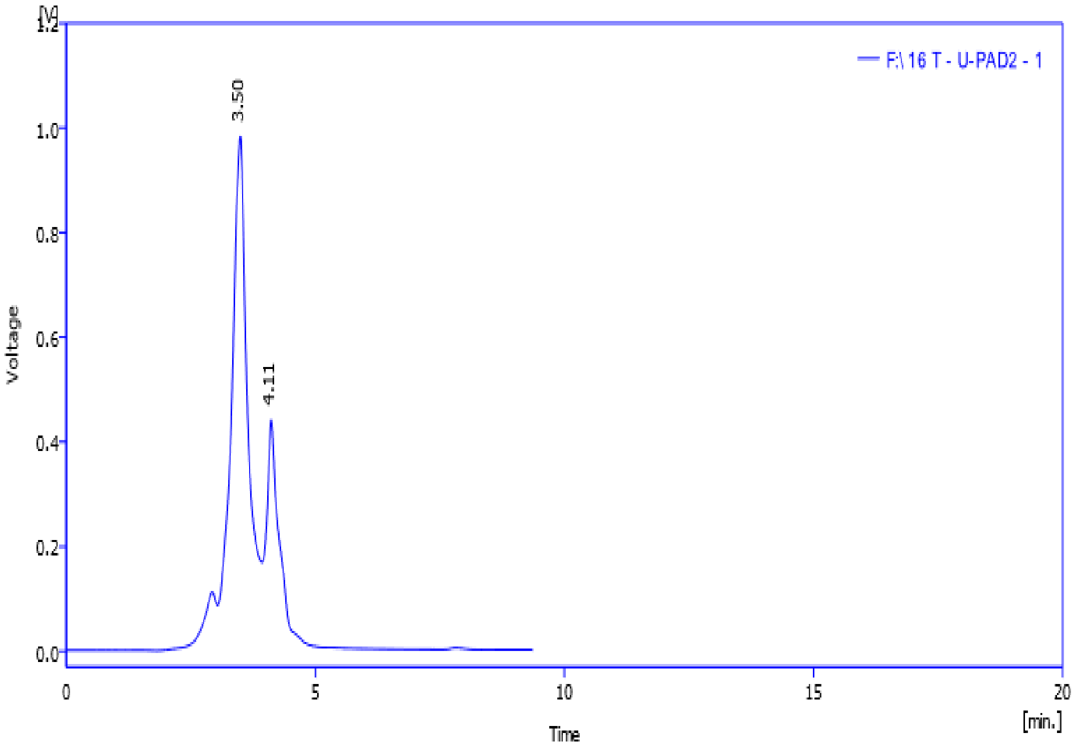
Chromatography Laboratory

HPLC

Sample Info:

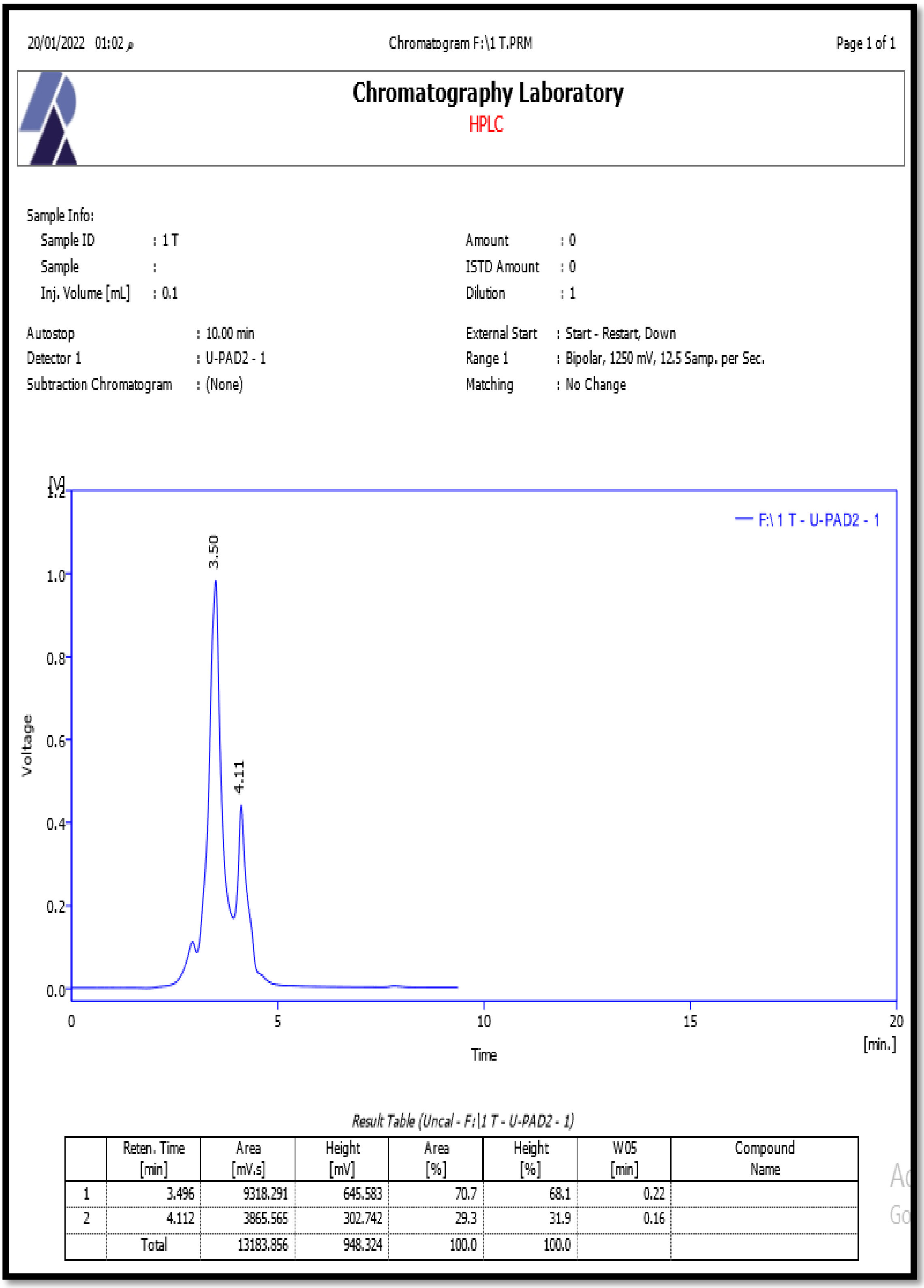
Sample ID	: 16 T	Amount	: 0
Sample	: 16 T	ISTD Amount	: 0
Inj. Volume [mL]	: 0.1	Dilution	: 1
Autostop	: 10.00 min	External Start	: Start - Restart, Down
Detector 1	: U-PAD2 - 1	Range 1	: Bipolar, 1250 mV, 12.5 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - F:\16 T - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.496	12148.327	768.313	75.1	71.3	0.25	
2	4.112	4019.794	309.314	24.9	28.7	0.16	
	Total	16168.121	1077.627	100.0	100.0		

Figure (3-5): Typical chromatogram of allantoin in male patient serum.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.496	9318.291	645.583	70.7	68.1	0.22	
2	4.112	3865.565	302.742	29.3	31.9	0.16	
	Total	13183.856	948.324	100.0	100.0		

Figure (3-6): Typical chromatogram of allantoin in female patient serum

The following procedure was used to determine the concentration of the substance in the serum samples:

Material Concentration= ((Standard substance concentration × Sample area \ Standard material area)) × (Dilution factor \ Sample volume)

3-9: Molecular assay

3-9-1: The extraction of DNA from blood

1. Placed frozen human blood in an anticoagulant-treat collection tube and allowed it to defrost at room temperature before gently mixing it.
2. For each sample, 200 µl of blood was transferred to a micro centrifuge tube .
3. For each sample, 30 µl of proteinase K was added and gently mixed .
4. Incubated the samples for 15 minutes at 60°C temperature .
5. A 200 µl FABG buffer was added and vortex combined .
6. For 15 minutes, the samples were incubated in a water bath at 70°C. During incubation, invert the sample every 3-5 minutes .
7. The sample was supplemented with 200µl ethanol (96~100%). Mix vigorously for 10 seconds.
8. Pick up spin column tubes and place them in their respective collection tubes.
9. For each sample, transfer all lysate into a spin column tube of samples.
10. For one minute, spin the micro centrifuge at 14000 rpm. Replace the collection tubes after removing the filtrate lysate.
11. Wash FABG column tube with 400µl wash1 buffer (ethanol added) by centrifuge for 30 seconds at speed 14000 rpm.
12. Wash FABG column tube with 600µl wash 2 buffer (ethanol added) by centrifuge for 30 seconds at speed 14000 rpm.

13. To dry the column tube, it is centrifuged for an additional 3 minutes at 14000 rpm.
14. For each FABG Column tube, a new 1.5ml microcentrifuge tube was used.
15. For the membrane core of the FABG column, 100 µl of preheated elution buffer or TE was added. Incubate the FAGB column at 37°C for 10 minutes, or until the buffer has been absorbed by the membrane.
16. Centrifuge it for 60 seconds at full speed 14000 rpm to elute the pure DNA.
17. The DNA fragment was kept at 20°C.

3-9-2: Estimation the DNA concentration and purity

The purity of samples was determined by observing the ratio of optical density (OD) 260/280 nm to detect protein contamination. The DNA concentration of samples was estimated using the Nano drop by placing 1 µl of extracted DNA in the machine to detect concentration in ng/µl . For purifying DNA, the standard 260/280 ration was between 1.7 and 1.9 (Sambrook and Russell, 2001).

3-9-3: Primers

The primer pair was used in the current study are listed in Table (3-3).

Table (3-3): Primers used in the current study.

Primer	Sequence (5' → 3')	Size	Reference
IL-10	IL-10 F: ATCCAAGACAACACTACTAA IL-10 R: TAAATATCCTCAAAGTTCC	588bp	Yoo *et al.*, 2009

3-9-4: Dissolving of Primers

Nuclease free water was used to dissolve primer pair utilized in this study (Promega). The primer stock tube would be prepared first, and then the working solution would be created from the primer stock tube.

Nuclease free water was added according to the primer manufacturer's instructions to achieve a primer stock solution concentration of 100 picomol/microliter. The working solution prepared from stock by dilution 10µl primer stock solution with 90 microliter of nuclease free water to get 10 picomol/microliter (work solution).

3-9-5: Prepared the Reaction Mixture

Amplification of DNA was carried out in a final volume of 25 µl reaction mixture for detection of IL-10 polymorphism as mentioned in Table (3-4) as mentioned adding (1µl) from each diluted primer.

Table (3-4): Contents of the Reaction Mixture (Promega) of PCR for IL-10 gene polymorphism.

No.	Contents of reaction mixture	Volume (µl)
1.	Master mix	12.5
2.	Forward primer	1
3.	revers primer	1
4.	DNA	1.5
5.	Nuclease free Water	9
Total volume		25

3-9-6: Thermal Cycling Conditions

The PCR reaction is shown in Table (3-5).

Table (3-5): Thermal cycling conditions for Specific Primers for IL-10 gene under interest.

Step Type	Temperature °C	Time	Cycling
Initial Denaturation	93	3 min.	1
Denaturation	93	30 Sec.	32
Annealing	47	30 Sec.	
Extension	72	30 Sec.	
Final Extension	72	3 min.	1
Hold	4	∞	

3-10: Validity of the IL-10 genotype

The genotypes of IL-10 SNP (NCBI SNP CLUSTER ID: rs1800871, rs1800872) were analyzed by polymerase chain reaction (PCR) and sequencing investigation according to the conventions depicted before based on Govatati *et al*. (2012) method. PCR were performed in a complete volume of 25 µl containing 50 ng genomic DNA, 1µl of primers, 12.5 µl master mix in Table (3-4). The PCR result of 588 bp was electrophoresis by 1.5% agarose gel staining with ethidium bromide and afterward sequenced in Macrogen Lab. (Korea).

3-11: Detection of Amplified Products by Agarose Gel Electrophoresis

The amplified products of PCR were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Agarose gel was prepared by dissolving 1.5 g of agarose powder in 100 ml of 1X TBE buffer in flask then placed in microwave for 2 minutes, and waiting for cool to 50^{o}C and adding about 0.5µl of ethidium bromide stain concentration (0.5 mg/ml) (Sambrook and Russell, 2001).

First the 4µl of DNA ladder (100-10kbp) was put. The electric voltage 75 volt at 45 minute (Sambrook and Russell, 2001). Gel documentation system was used captures the pictures of the gel and observation of DNA bands under UV light.

3-12: Interpretation of sequencing data

The sequencing results of the PCR products of different samples were edited, aligned, and analyzed with the respective sequences in the reference database using bioedit for multiple alignment sequence based on Editor software version 7.1 (DNASTAR, Madison, WI, USA). Multiple alignment chromatograms were performant by geneious primer software, the observed variations in each sequenced sample were numbered in PCR amplicons as well as in their corresponding position within the referring genome.

3-13: Checking the validity of SNPs

The observed SNPs were submitted to the dbSNP database to check their originality. Each particular SNP was re-positioned according to its place in the reference genome subsequently, the determination of the presence of previous SNP was performed by viewing its corresponding data base SNP position. Then, all data base SNP positions for all observed SNPs were documented respectively.

3-14: Statistical Analysis

Data was analyzed using SPSS (version 23, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard error), and differences were compared by two-way ANOVA. As well as it was carried out using student's-t test for comparing between two groups. In addition, post hoc-tests such as least significant difference (LSD) were used when the P value is less than 0.05. It was followed by chi-square for some cases. The value of $P \leq 0.05$ was considered to be statistically significant. The relation ship between studied parameters were determined by Pearson's correlation coefficient (r).

Chi square test was used to test the deviation from Hardy-Weinberg Equilibrium of SNPs by comparing the observed and expected frequencies. The association of different genotypes was estimated by calculation of odds ratios (OR) with 95% confidence intervals (CI).

Chapter Four

Results and Discussion

Chapter Four

4- Results and Discussion

4-1: Epidemiological Criteria

According to residence area Table (4-1) shows the highest rate in males for (β-thalassemia with *Toxoplasma*) fall in rural area 75% p-value = 0.068, whereas the highest rate in females fall in urban area 66.67%, as well as, the lowest infection rate reveals in males with urban area 25%, compared with females that fall in rural area 33.33%. From the above there is no significant difference, whereas in (β-thalassemia without *Toxoplasma*) reveal the highest rates were in rural area 63.64%, 54.17% for males and females respectively, while the lowest rates in urban area 36.36%, 45.83% in males and females respectively p-value = 0.515, and there are no significant difference.

Different toxoplasmosis readings according to where people live may be caused by poor health education in some rural areas, inadequate treatment, and close contact with cats and other animals since some areas breed animals in their backyard parks. Due to the more crowded living conditions in metropolitan areas, the existence of poultry and the consumption of sheep meat may be a probable explanation for the increased infection exposure (Mortensen *et al.*, 1999). The reason may be due to the size of the sample that was selectively selected for patients so that it does not give a true picture of the extent of the disease's prevalence, whether in the rural area or the urban, and due to the low immunity in thalassemia patients, the toxoplasmosis parasite spreads rapidly in these patients regardless of the look if they come from the rural area or the urban, and this compatible with Hamza (2006) and Jassam (2010), and disharmonious with study of Al-Jubori (2005), Al-Gharrgholi (2014) and

Al-Jumaili (2015) revealed that the explanatory variable chosen among the schizophrenia group's positivity rate of anti-*Toxoplasma* IgG antibodies was non-significant when compared to urban residence.

Table (4-1): Epidemiological parameter percentage rate infection of prevalence of toxoplasmosis with two groups (β-thalassemia major patients with *T. gondii* and without *T. gondii*).

Groups / Characteristics	β-thalassemia patients with *T. gondii*		β-thalassemia patients without *T. gondii*	
	Male (male=8)	**Female (n=12)**	**Male (n=22)**	**Female (n=24)**
		No. (%)		
Resident area				
Urban	2(25%)	8(66.67%)	8(36.36%)	11(45.83%)
Rural	6(75%)	4(33.33%)	14(63.64%)	13(54.17%)
Total	8	12	22	24
p-value	**0.068**		**0.515**	
Age (years)				
10-15	4(50%)	6(50%)	9(40.91%)	14(58.33%)
16-20	2(25%)	4(33.33%)	11(50%)	7(29.17%)
21-25	2(25%)	2(16.67%)	2(9.09%)	3(12.5%)
Total	8	12	22	24
p-value	**0.479**		**0.033***	
Cats presence				
Yes	1(12.5%)	2(16.67%)	2(9.09%)	1(4.17%)
No	7(87.5%)	10(83.33%)	20(90.91%)	23(95.83%)
Total	8	12	22	24
p-value	**0.021***		**0.023***	
Genetics				
Father	7(87.5%)	9(75%)	18(81.82%)	14(58.33%)
Mother	1(12.5%)	2(16.67%)	2(9.09%)	3(12.5%)
Father and mother	0(0%)	1(8.33%)	2(9.09%)	7(29.17%)
Total	8	12	22	24
p-value	**0.816**		**0.031***	
Blood transport				
One every month	3(37.5%)	7(58.33%)	10(45.45%)	14(58.33%
Two every month	5(62.5%)	5(41.67%)	11(50%)	10(41.67%)
Four every month	0	0(0%)	1(4.55%)	0(0%)
Total area	8	12	22	24
p-value	**0.041***		**0.311**	

According to age groups, it has been shown that the highest rate in males 50% in 10-15 years in patients with *T. gondii*, 50% in 16-20 years in patients without *T. gondii*, and 25% in both age groups 16-20, 21-25 years in patients with *T. gondii*, whereas the highest rate in females 58.33% in 10-15 years in patients without *T. gondii*, and 33.33% in 16-20 years, and 16.67% in 21-25 years in patients with *T. gondii*. From the above information, there is significant difference between the three groups of age in the patients without *T. gondii*. Table (4-1). The present study agreed with pervious study Al-Dulaimy (2015) was most frequent age group for single women was 15-19 years, and represent 40.0%, and with Abdullah (2001) and Al-Jumaili (2015) who concluded that the main age of seropositive of toxoplasmosis cases were between 11-20 years.

These differences between the previous results and the current results may be due to longer risk factors associated with exposure to the parasite, as the older individuals are exposed during the period in their lives long-infection more than younger ages, so their chance to be more infections.

The highest percentage of acute infections were found in the age groups 17-21, 22-26, and chronic infections were found in the age groups 37-41, 42 years. It may be due to the increase in infection at young ages more than others, because these people suffer from genetic diseases such as thalassemia that exhaust their immune system by breaking red blood cells, and thus the lack of nutrients and oxygen necessary to build the body and the immune system, and basically that these ages are in the process of building the immune system.

This differences may be due to variable of size specimen were taken was males and females from β-thalassemia that infected or not with toxoplasmosis, the fluctuation of infection according to different epidemiological criteria so that once the infection increased in males and once in females in both groups (thalassemia group without toxoplasmosis and thalassemia group with toxoplasmosis) may be due to the size and selectivity of the sample when collecting samples in the current study, or may be due to of probably both males or females the same susceptibility with toxoplasmosis, according to mode of live or residence area or not to follow hygiene habits. Previous studies such as study of Al-Jubori (2005), Al-Gharrgholi (2014) and Al-Jumaili (2015) were targeting the effect of toxoplasmosis on females than in males fact that infection toxoplasmosis have no symptoms no significant risk to healthy males and most risk in women during pregnancy and childbirth.

Accordingly, cats presence show the highest rate in males 12.5% in patients with *T. gondii*, and no cats presence 90.91% in patients without *T. gondii*, whereas in females the highest rate in cats presence 16.67% and no cats presence 95.83% in patients without *T. gondii*, from the above there is significant difference between cats presence and no cats presence in both the patients with and without *T. gondii*, Table (4-1) and agreement with Ghasemian *et al*. (2007) was 6.1% not keeping animals (cats) and 3.3% keeping animals (cats), and in conflict with Al-Maamuri (2014), who discovered that those who own cats are more likely to own them 88.43% versus 59.7%. Breeding various animals (chicken, sheep, and rabbit) in the study revealed elevated number of people who were seropositive for *T. gondii* was 91.67% compared to people who did not 54.41%, various studies have also reported a statistical correlation between toxoplasmosis prevalence and close contact with cats, as Al-Shikhly (2012) observed no statistically significant differences among university students who had animal contact, which suggested a high risk of infection among women who had no contact with animals especially with cats.

According to the family history (father, mother, father and mother), it has been show that the highest rate is in males (first group), in father 87.5%, and in mother 12.5% in patients with *T. gondii*, and the highest rate in father and mother 9.09% in patients without *T. gondii*, whereas the highest rate in females (second group), in father 75%, and in mother 16.67% in patient with *T. gondii*, and the highest rate in father and mother 29.17% in patients without *T. gondii*. From the above information there is significant difference between the genetic factors in the patients without *T. gondii*. Table (4-1). Perhaps the genetic factor, whether from the father or the mother, is less influential if it is isolated from one of them, as it comes to their children from one of the parents, especially thalassemia patients, but its impact is strong if it comes from both, which negatively affects the immune system and thus increases the chance of contracting toxoplasmosis.

But the current study showed that the first group has thalassemia with toxoplasmosis, and that the genetic factor that comes from the father is more susceptible to toxoplasmosis, perhaps because the factor that comes from the father has a stronger effect on the genetic characteristics and the emergence of the disease with thalassemia, and perhaps the reason is due to the size and selectivity of the sample When collecting samples in the current study.

According to the blood transport it has been shown the highest rate is in males, in blood transfusion every once a month 45.45% in patients without *T. gondii*, in blood transfusion twice every month 62.5% in patients with *T. gondii*, and in males the percentage rate of blood transfusion four times a month 4.55% in patients without *T. gondii*, whereas in females the percentage rate of blood transfusion every once a month 58.33% equal percentage in patients with and without *T. gondii*, and the percentage rate of blood transfusion twice every month 41.67% equal percentage in patients with and without *T. gondii*, and no presence rate to blood transfusion four times a month 0% in patients with and without *T. gondii*, from the above there is significant difference between the blood transport in the patients with *T.gondii*. Table (4-1).

The increased incidence of toxoplasmosis for thalassemia patients who replace their blood twice a month or four times a month is due to the fact that these people, according to the recommendation of the specialist doctor, cannot continue to live if their blood is replaced once a month, which indicates the collapse of their immune system and thus great exposure to toxoplasmosis, or that they are exposed to toxoplasmosis from the same donors, as these donors are mainly infected with toxoplasmosis, and health authorities do not take into account the examination of donors for toxoplasmosis, which explains the increased rates of infection in the current study.

4-2: Hematological Criteria

4-2-1: Complete Blood Count

β-thalassemia patients with *T. gondii* in males, there are decrease level in RBCs($\times10^6$/mm^3), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.25±0.4), (7.8±1.1), (0.45±0.01), (77.7±6.5), (25.2±2.4), (32.4±1.1) respectively, whereas increase level of the following WBCs($\times10^3$/mm^3), PLT($\times10^3$/mm^3), RDW(%), show (7.79±0.6), (366.0±114.5), (15.7±5.4) respectively compared to the control group.

β-thalassemia patients with *T. gondii* in females, there are decrease level in RBCs($\times10^6$/mm^3), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.22±0.5), (8.2±1.1), (0.39±0.01), (78.6±2.9), (25.6±1.4), (32.7±1.8) respectively, whereas increase level of the following WBCs($\times10^3$/mm^3), PLT($\times10^3$/mm^3), RDW(%), show (10.33±1.0), (371.68±21.2), (13.13±0.5) respectively compared to the control group.

β-thalassemia patients without *T. gondii* in males, there are decrease level in, RBCs($\times10^6$/mm^3), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.16±0.3), (7.9±0.7), (24.8±2.3), (79.46±3.2), (24.9±1.3), (31.4±1.6)) respectively, whereas increase level of the following WBCs($\times10^3$/mm^3), PLT($\times10^3$/mm^3), RDW(%), show (21.73±2.6), (459.8±31.1), (14.1±3.3) respectively compared to the control.

β-thalassemia patients without *T. gondii* in females, there are decrease level in RBCs($\times10^6$/mm^3), Hb(g/dl), PCV(%), MCV(fl), MCH(pg), MCHC(g/dl), show (3.24±0.2), (8.1±0.7), (25.2±3.1), (80.0±3.3), (25.0±1.5), (31.3±1.6) respectively, whereas increase level of the following WBCs($\times10^3$/mm^3), PLT($\times10^3$/mm^3), RDW(%), show

(15.36±1.3), (495.5±26.7), (13.5±2.6) respectively compared to the control group. Table (4-2).

Table (4-2): Hematological parameter related with prevalence of toxoplasmosis with two groups (β-thalassemia major patients with *T. gondii* and without *T. gondii*).

Groups / Characteristics	β-thalassemia patients with *T. gondii*		β-thalassemia patients without *T. gondii*		Control		LSD (0.05) (group*Gender)
	Male	Female	male	Female	Male	Female	
	Mean±S.E						
WBCs($\times10^3$/mm^3)	7.79±0.6	10.33±1.0	21.73±2.6	15.36±1.3	7.25±1.8	8.01±3.1	5.351
RBCs($\times10^6$/mm^3)	3.25±0.4	3.22±0.5	3.16±0.3	3.24±0.2	5.69±0.2	4.92±0.1	0.157
PLT($\times10^3$/mm^3)	366.0±114.5	371.68±21.2	459.8±31.1	495.5±26.7	318±22.4	331.2±12.0	79.121
Hb (g/dl)	7.8±1.1	8.2±1.1	7.9±0.7	8.1±0.7	15.03±0.5	13.04±0.2	0.367
PCV (%)	0.45±0.01	0.39±0.01	24.8±2.3	25.2±3.1	25.1±2.9	25.6±2.3	0.822
MCV(fl)	77.7±6.5	78.6±2.9	79.46±3.2	80.0±3.3	79.7±4.1	81.08±2.1	2.342
MCH(pg)	25.2±2.4	25.6±1.4	24.9±1.3	25.0±1.5	26.76±1.2	26.92±0.7	0.888
MCHC(g/dl)	32.4±1.1	32.7±1.8	31.4±1.6	31.3±1.6	33.60±0.3	33.02±0.3	0.562
RDW(%)	15.7±5.4	13.13±0.5	14.1±3.3	13.5±2.6	12.93±0.7	12.4±1.2	1.076

The study's findings revealed in Table (4-2) that individuals with beta-mediated severe anemia had a considerable reduction in both the quantity of red blood corpuscles and hemoglobin Hb. This is explained by a reduction in the production of beta-globin chains in the molecules of patients. Hemoglobin Hb are described as an increase in the dysregulated globin protein in the cell membranes, which makes it vulnerable to destruction by phagocytic cells in the bone marrow that have the capacity to distinguish and destroy abnormal cells, and this results in a significant number of red blood corpuscles are destroyed during the process of their formation, since the volume of the packed blood cells PCV depends on the number and size of erythrocytes and is also reduced them (Alemu *et al.*, 2006)**,** as well as are consistent with many other studies Pavlova *et al.*(2007); Ali (2008); Al-Saray (2012); Al-Haddad (2012); Al-Mousawi (2014) and Elias (2016).

The results also demonstrated a significant decrease in the level of average weight MCH globular hemoglobin due to a defect in the production of globin protein, which results in a reduction in the average red blood cell volume MCV in patients. This is due to the small size of the microcytic erythrocyte due to a deficiency in the production of the beta chain globin caused by a mutation in the beta globin gene. These results agree with those of other research Al-Saray (2012), Al-Haddad (2012), Elias (2016), Taher *et al.* (2008), and Pavlova *et al.* (2007). The normal level of MCHC is When compared to the healthy, it was normal and remained unchanged, and the outcomes supported the findings studies by Hussain *et al.* (2005), Pavlova *et al.* (2007), Al-Haddad (2012), and Elias (2016), who saw a drop in (Hb, PCV, MCV, MCH) levels and discovered that the MCHC criteria was within normal ranges in patients with minor Mediterranean anemia (Mohammed *et al.*, 2020).

Aslan *et al.* (2002), Al-Haddad (2012), and Elias (2016) found that the presence of small-sized globules with an uncompensated and unequal deficiency for beta-globin chains was indicated by an increase in the value of RDW (Mankad *et al.*, 2013). Regarding the rapid rise in blood platelets (Wandersee *et al.*, 2001). The spleen creates aberrant cells that are concentrated there and have a limited lifespan and swift degradation.

This highlights the vital function the spleen plays in eliminating platelets, these findings concur with those from Eldor and Rachmilewitz (2002), Al-Haddad (2012), and Elias (2016). These results are consistent with those of Pavlova *et al.* (2007), Elhams (2010), Al-Haddad (2012), Al-Saray (2012), Al-Mousawi (2014), and Elias(2016). As for the significant increase in the number of WBCs in patients, its cause is due to the presence of a large number of immature red blood corpuscles (RBCs), which contain nuclei that may be mistakenly counted as WBCs.

4-2-2: Blood Groups

According blood groups (A^+, A^-, B^+, AB^+, O^+, O^-) it has been shown that the highest rate in males ,in A^+ 12.5%, in B^+ 25%, in O^- 50% in thalassemia patients with *T. gondii*, whereas the highest rate in A^- 4.55%, in AB^+ 36.36%, in O^+ 4.55% in thalassemia patients without *T. gondii*, and in females the highest rate in B^+ 33.33%, in AB^+ 16.67%, in O^+ 4.17% in thalassemia patients without *T. gondii*, whereas in A^- 16.67%, in O^- 41.67% in thalassemia patients with *T. gondii*, and equal percentage in both A^+ 16.67% in thalassemia patients with and without *T. gondii*, from the above there is no significant difference between blood groups. Table (4-3).

Table (4-3): Hematological parameter of blood groups percentage rate related with prevalence of toxoplasmosis with two groups (β- thalassemia major patients with *T. gondii* and without *T. gondii*).

Groups / Characteristics	β-thalassemia patients with *T. gondii*		β-thalassemia patients without *T.gondii*	
	Male	**Female**	**male**	**Female**
			No.(%)	
Blood groups				
A⁺	1(12.5%)	2(16.67%)	2(9.09%)	4(16.67%)
B⁺	2(25%)	3(25%)	4(18.18%)	8(33.33%)
A⁻	0(0%)	2(16.67%)	1(4.55%)	0(0%)
AB⁺	1(12.5%)	0(0%)	8(36.36%)	4(16.67%)
O⁻	4(50%)	5(41.67%)	6(27.27%)	7(29.17%)
O⁺	0(0%)	0(0%)	1(4.55%)	1(4.17%)
Total	8	12	22	24
p-value	0.564		0.641	

However, these findings not concur with those of Al-Saadii (2013), who found that blood group O had the lowest percentage of *toxoplasma* infection 15.7% and blood group AB had the highest percentage 41.32%, with toxoplasmosis occurring in 30% of cases and blood group O and A having the lowest percentage 20%, concentrate on blood groups AB and B and agreement with Chiang *et al*. (2012) that blood group AB had the highest percentage 13% and blood group B had the lowest percentage 8.5%.

Toxoplasma was more prevalent high percentage by ELISA IgG among blood group AB 41.32% and lowest percentage was among blood group O 15.70% and by ELISA IgM prevalent high among A and O 30%

and lowest percentage was among blood group AB and B was 20%, according to a study by Mahmood *et al.* (2013), and disagreement with Al-Shikhly *et al.* (2013) regarding the blood group B+ high percentage by ELISA IgG 30.6% and blood group AB low percentage AB 6.3%.

In a study by Meymand *et al.* (2015), they found that blood group O had the highest prevalence of IgG 36% and the blood group AB had the lowest 10%. In a study by Obaid (2014), samples from married and unmarried women revealed that blood group A was most prevalent 66.6%, followed by blood group AB 60% and blood group O had the lowest percentage 0%.

There could be a number of reasons why these results and those from other studies disagree. It's likely that the molecular diversity of the strains in the patients from Iraq or the study's usage of solely male patients led to results that may be different from those found in other populations. Or, it's probable that AB antigens have a significant impact on *T. gondii* ability to cling to the gastrointestinal mucosa, as evidenced by the high prevalence of *T. gondii* infection in the Iraqi population. The reason may be due to the size of the sample selected for thalassemia patients, as well as the selectivity of these patients. They may have duplicate and similar blood groups, which does not give a realistic picture of the relationship of toxoplasmosis infection with blood groups.

4-3: Immunological Criteria

4-3-1: Percentage rate of IgG, IgM

The study included an investigation of the antibodies generated against infection with the parasite *Toxoplasma gondii*. The results showed that the rate of chronic infection (IgG antibody prevalence) was 30.30%

among β -thalassemia patients compared to the control group (0%) by using Cobas e411 technique. The statistical analysis by chi-square test showed that there were significant ($P<0.05$) differences between the two groups under study. While the rates of acute infection (prevalence of IgM antibody) and mixed (prevalence of antibody IgM and IgG) were 1.51% for each, with one infection among β -thalassemia patients, and no case was recorded in the control group and no significant differences ($P>0.05$) appeared Table (4-4).

Table (4-4): Percentage of IgG, IgM and mixed (IgG and IgM) to *T. gondii* by using Cobas e411 technique in β-thalassemia patients and control.

Groups	N	IgG +ve	%	IgM +ve	%	IgG and IgM +ve	%
β-thalassemia	66	20	30.30	1	1.51	1	1.51
Control	30	0	0	0	0	0	0
Total	96	20	20.83	1	1.04	1	1.04
P- value		0.0007		0.497		0.497	

4-3-2: Immunological parameters concentration of IgG and IgM

In the Table (4-5) results mentioned below it reveals that there are three groups in both from IgG and IgM , for IgG the highest concentration of IgG(Iu/ml) in males is (164.63±25.0) that falls in reactive groups in thalassemia patients with *T. gondii*, whereas the lowest concentration of IgG(Iu/ml) in females is (98.276±19.4) that falls in reactive groups in thalassemia patients with *T. gondii*, and shows the highest concentration of IgG(Iu/ml) in males is (0.79±0.17), whereas the lowest concentration of IgG(Iu/ml) in females is (0.62±0.15) in thalassemia patients without *T. gondii*.

For IgM, the highest concentration of IgM (COI) in males is (0.458±0.03), whereas the lowest concentration of IgM (COI) in females is (0.300±0.04) in thalassemia patients with *T. gondii*, and shows that the highest concentration of IgM (COI) in males is (0.296±0.01), whereas the lowest concentration of IgM (COI) in females is (0.291±0.02) in thalassemia patients without *T. gondii*. Table (4-5).

In Cobas e411 device, the IgM (COI) concentration shows that there is one sample give positive result with reactive group (1.35 COI), whereas all samples of IgM show non-reactive.

The low level of IgM antibody may be caused by the immune response in some patients, and they may be able to limit the disease from its onset to elimination. Additionally, the low level of IgM antibody may be faster than the lower level of IgG antibody and may be present for many years in individuals with ineffective immune systems after infection. Due to the antibody IgG ability to replace big molecules in the absence of antibody producers and the fact that its half-life is longer than that of the antibody IgM, IgG antibody has the ability to persist in the body for a very long period after infection, in addition to the differences between the antibodies IgM and IgG in terms of the number of units and weights of the molecular as well as to the types of chains peptide and splits (Wilson *et al.*, 1997).

The reason for the high IgG antibody is because most thalassemia patients are predisposed to any chronic infections, and as we know that the fast-reproducing phase (Tachyzoites) does not remain for a long time in the blood, but rather goes to the tissues of all organs and turns into a phase of slow reproduction (Bradyzoites). This explains the low concentration of IgM antibody.

Table (4-5): Concentration of IgG and IgM in two groups (β-thalassemia patients with *T. gondii* and without *T. gondii*) and control groups.

Groups / Characteristics	β-thalassemia patients with *T. gondii*		β-thalassemia patients without *T. gondii*		Control		LSD (0.05) (group*Gender)
	Male	Female	male	Female	male	Female	
	Mean±S.E						
IgG (Iu/ml)	164.63±25.0	98.276±19.4	0.79±0.17	0.62±0.15	0.128±0.02	0.128±0.02	5.612
IgM(COI)	0.458±0.03	0.300±0.04	0.296±0.01	0.291±0.02	0.31±0.01	0.30±0.01	0.040

4-4: Biochemical Criteria

Table (4-6) and Figures (4-1, 4-2, 4-3) shows the concentration of uric acid and allantoin as oxidative stress related with β-thalassemia patients infected with *T. gondii* and β-thalassemia patients non infected with *T. gondii*, reveals the highest concentration (5.10±0.3) mg/dl of uric acid in males compared with females (4.66±0.2) mg/dl in the groups of β-thalassemia patients infected with *T. gondii*, whereas the highest concentration shows in males (5.36±0.6) mg/dl compared with females (3.76±0.3) mg/dl in β-thalassemia patients without *T. gondii*.

Proximal tubular injury may produce hyperuricemia in these patients in addition to accelerated erythrocyte turnover because the filtered uric acid can be reabsorbed from the proximal tubules (Al-Haddad, 2012; Rasool *et al*., 2016).

As uric acid has little interaction with it and produces the compound allantoin from this oxidation, whose concentrations were high for the group of thalassemia patients, the increase in uric acid levels in the current study for the patient group compared to the control group may be caused by the presence of free radicals (ROS). Uric acid is the by product of purine metabolism.

Table (4-6): Biochemical parameter (uric acid and allantoin concentration) related with prevalence of toxoplasmosis with two groups (β-thalassemia major patients with *T.gondii* and without *T. gondii*).

Groups / Characteristics	β-thalassemia patients with *T. gondii*		β-thalassemia patients without *T. gondii*		Control		LSD (0.05) (group*Gender
	male	Female	male	Female	male	Female	
	Mean±S.E						
Uric acid (mg/dl)	5.10±0.3	4.66±0.2	5.36±0.6	3.76±0.3	4.33±0.3	3.47±0.2	0.571
Allantoin (ppm)	56.39±1.9	59.52±1.6	39.78±1.3	37.53±1.2	3.08±0.4	1.97±0.3	0.685

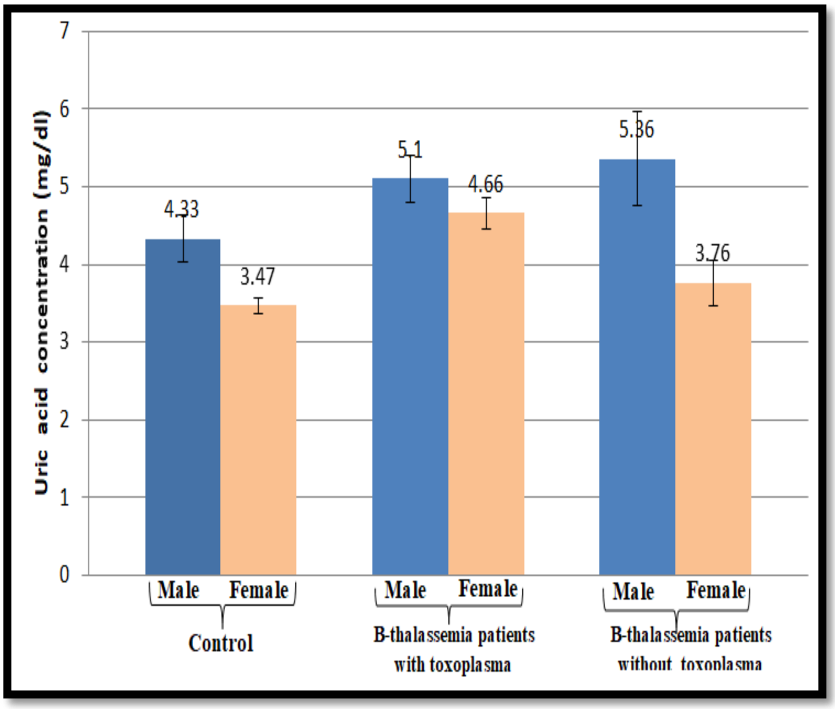


Figure (4-1): Uric acid concentration in two group's thalassemia patients

The allantoin concentration reveals highest concentration compared with uric acid, that shows highest concentration in females (59.52±1.6) ppm compared with males (56.39±1.9) ppm in β-thalassemia patients with *T. gondii*, where as the concentration of allantoin in β-thalassemia patients without *T. gondii* reveals the highest concentration in males (39.78±1.3) ppm compared with females (37.53±1.2) ppm, Figure (4-2).

As an antioxidant, uric acid interacts with reactive oxygen metabolites to produce allantoin, which can be measured as a sign of oxidative damage (Ismael, 2001). Another justification for doing oxidative stress fingerprint testing is: For instance, the hydroxyl radical on DNA causes a pattern of chemical changes to all four purine and pyrimidine bases that appear characteristic of H when the ROS react with the biological molecule. Other oxygen-derived molecules such as $^{\bullet -}O_2$ and H_2O_2 either completely avoid attacking DNA bases or simply change guanine (Al-Sayegh, 2021).

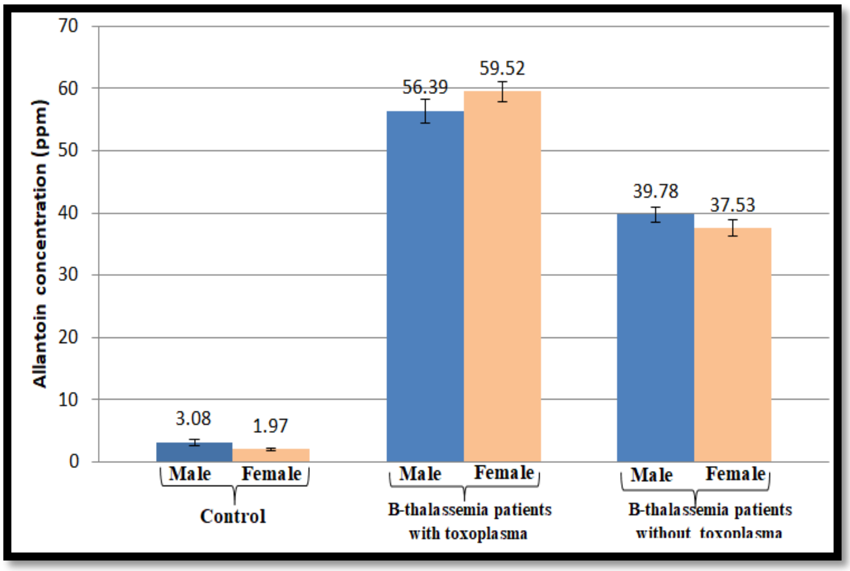
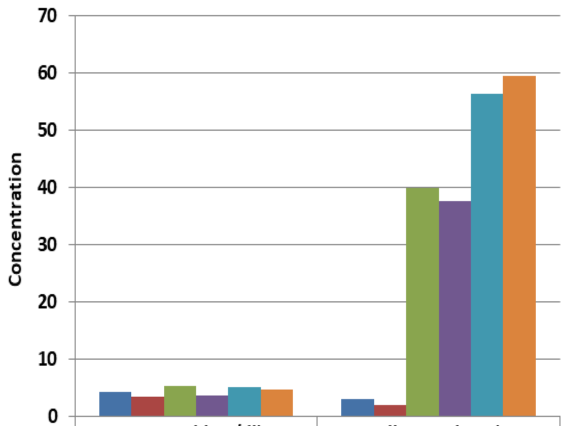


Figure (4-2): Allantoin concentration in two group's thalassemia patients



	Uric acid (mg/dl)	Allantoin (ppm)
Control(male)	4.33	3.08
control(female)	3.47	1.97
patients without toxoplasma (male)	5.36	39.78
patients without toxoplasma (female)	3.76	37.53
patients with toxoplasma (male)	5.1	56.39
patients with toxoplasma (female)	4.66	59.52

Figure (4-3): Relationship between uric acid and allantoin in groups (males and females) in thalassemia patients and control.

Uric acid is produced in the human body by the oxidation of purines, but there are no enzymes available to further oxidize it. When compared to equivalent levels in controls, the levels of uric acid and allantoin were considerably lower and higher in patients who were male and female, respectively. This discrepancy is seen in patient serum, where the uric acid ratio has a non-significant negative association (r = -0.496), as seen in Table (4-7), Figure (4-4).

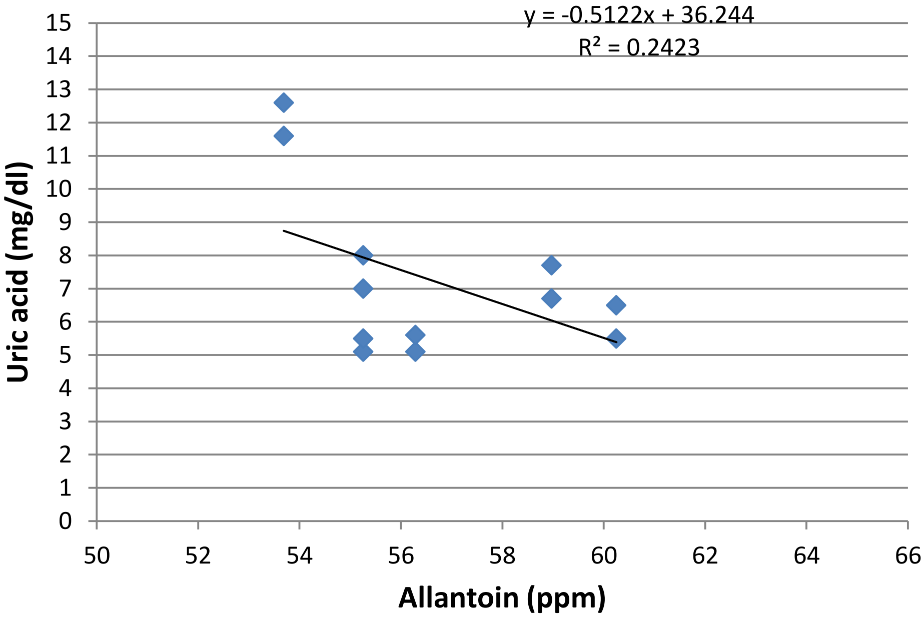


Figure (4-4): Correlation between allantoin and uric acid

This finding is extremely significant because it showed that both male and female thalassemia patients have severely depleted scavenger systems due to the production of RNS and ROS. It also showed that male thalassemia patients are more likely than female thalassemia patients to experience oxidative stress due to ROS. The literature on this subject is totally lacking because the assessment of the uric acid-allantoin pathway is being done for the first time in thalassemia patients. Uric acid is a well-known scavenger that is water soluble and has been shown to have significant antioxidant activity (Ismael, 2001; Mohammed and Abd-Elrasoul, 2020).

It is crucial to mention the details of the uric acid metabolic process here:

1. Under physiological circumstances, xanthine dehydrogenase is activated to produce uric acid. The xanthine oxidase enzyme is activated by ROS as a result of pathological conditions and some physiological circumstances Therefore, it is most likely that the activation of the xanthine oxidase enzyme in this study is related to thalassemia and lead to rise of the uric acid level.

2- Uricase, which is not present in physiological settings, is the enzyme responsible for uric acid breakdown in humans. There is no evidence of this enzyme in bodily fluids, nor is there any evidence of its uric acid byproduct, particularly allantoin.

The findings of this study demonstrated that the uric acid-allantoin pathway works to alleviate oxidative stress in thalassemia patients as seen by the decrease in uric acid and rise in allantoin levels. Further investigation showed that thalassemia patients have a 1/4 fold reduction in the activity of the uricase-like enzyme compared to controls (Zekavat *et al.*, 2018).

4-5: Correlation and association between hematological, biochemical and immunological parameters in patient group

The results of the current study revealed all relationships its non-significant correlation except the following correlation its positive correlation and negative correlation which were significant correlation as shown in Table (4-7) and Figures (4,5,6,7,8,9,10,11 ,12,13,14,15,16,17 ,18,19, 20 and 21).

4-5-1: Positive correlation

1- allantoin and (PCV)
2- (IgG) and (IgM)
3- (WBC) and (PLT)
4- (WBC) and (MCV)
5- (WBC) and (RDW)
6- (RBC) and (Hb)
7- (RBC) and (RDW)
8- (PLT) and (MCV)
9- (PLT) and (RDW)
10- (MCV) and (MCH)
11- (MCH) and (MCHC)

4-5-2: Negative correlation

1-(WBC) and (MCHC)

2-(RBC) and (MCV)

3-(RBC) and (MCH)

4-(PLT) and (MCHC)

5- (MCV) and (RDW)

6- (MCH) and (RDW)

The positive and negative between the correlations above-mentioned criteria indicate a deterioration in the numbers, shapes and sizes of red blood corpuscles as a result of thalassemia disease , as well as the concentration of hemoglobin and the increase in the number of white blood cells and platelets, in addition to infection with toxoplasmosis, an increase in the concentration of IgG and decrease IgM antibodies and an increase in oxidative stress as a result of thalassemia and an increase in leukocytes as immune responses gave additional concentrations of free radicals, such as free oxygen $\bar{\dot{O}}_2$ and hydrogen peroxide H_2O_2 , thus giving positive and negative interactions.

In addition to some large molecules like albumin and small ones like uric acid, whose concentration decreased and increased respectively as a result of oxidation and turned into allantoin, this suggests the presence of free radicals generated in thalassemia patients, which are counteracted by some enzymes whose concentration is reduced, evidence of their response to these radicals.

There were other positive and negative relationships, but they did not achieve significance.

Table (4-7): Association between hematological, biochemical and immunological aspects in thalassemia patients infected with toxoplasmosis

		IgG	IgM	uric	WBC	RBC	PLT	Hb	PCV	MCV	MCH	MCHC	RDW
Allantoin	**r**	-0.329	-0.268	-0.496	0.425	0.253	0.515	0.256	0.941**	0.087	-0.191	-0.362	0.618
	Sig.	0.524	0.608	0.317	0.401	0.628	0.295	0.624	0.005	0.870	0.717	0.480	0.191
IgG	**r**	1	.613**	.067	-.135	-.140	-.073	-.109	-.100	.103	.045	-.048	-.127
	Sig.		.000	.594	.281	.263	.559	.382	.423	.412	.718	.700	.310
IgM	**r**		1	.173	-.101	.100	-.096	.079	-.080	-.031	-.058	-.043	-.085
	Sig.			.165	.418	.422	.444	.530	.525	.802	.641	.730	.496
uric	**r**			1	.162	-.198	.097	-.135	-.166	.058	.190	.173	.225
	Sig.				.194	.110	.438	.278	.184	.643	.126	.164	.069
WBC	**r**				1	.089	.659**	.132	.118	.398**	.033	-.334**	.339**
	Sig.					.480	.000	.290	.345	.001	.795	.006	.005
RBC	**r**					1	.201	.761**	.189	-.337**	-.409**	-.179	.262*
	Sig.						.105	.000	.128	.006	.001	.152	.034
PLT	**r**						1	.204	.066	.265*	-.011	-.274*	.266*
	Sig.							.100	.596	.031	.928	.026	.031
Hb	**r**							1	.138	.080	.164	.116	.054
	Sig.								.271	.522	.187	.355	.665
PCV	**r**								1	.056	-.151	-.239	.224
	Sig.									.654	.227	.053	.070
MCV	**r**									1	.588**	-.239	-.263*
	Sig.										.000	.053	.033
MCH	**r**										1	.644**	-.265*
	Sig.											.000	.031
MCHC	**r**											1	-.079
	Sig.												.528

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

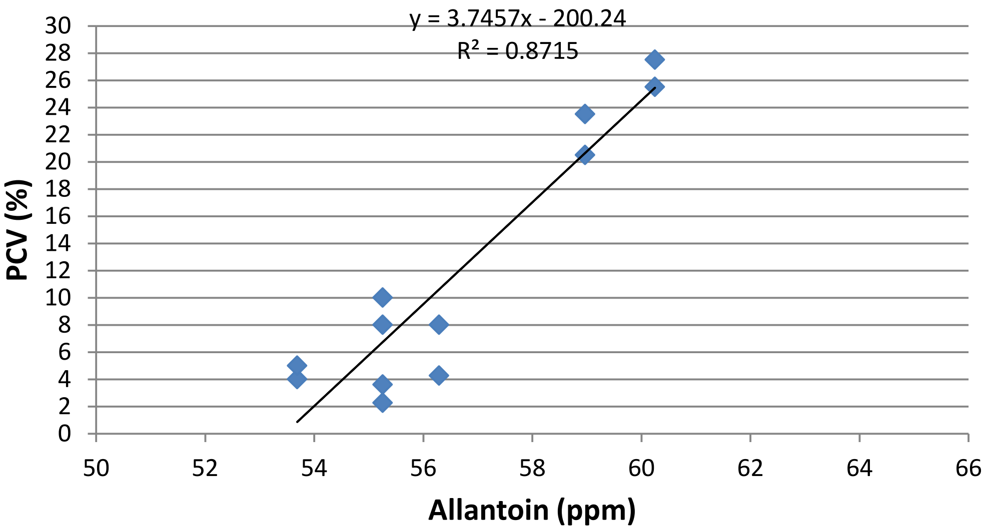


Figure (4-5): Correlation between allanation and PCV

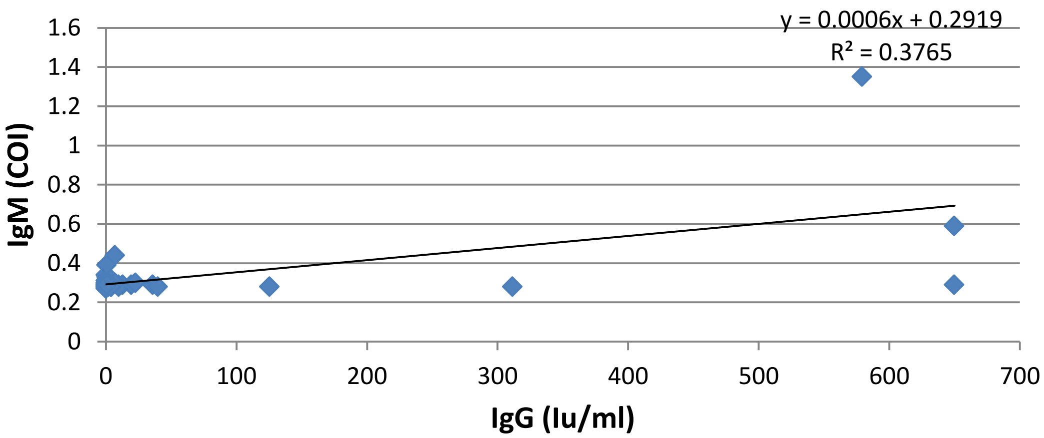


Figure (4-6): Correlation between IgG and IgM

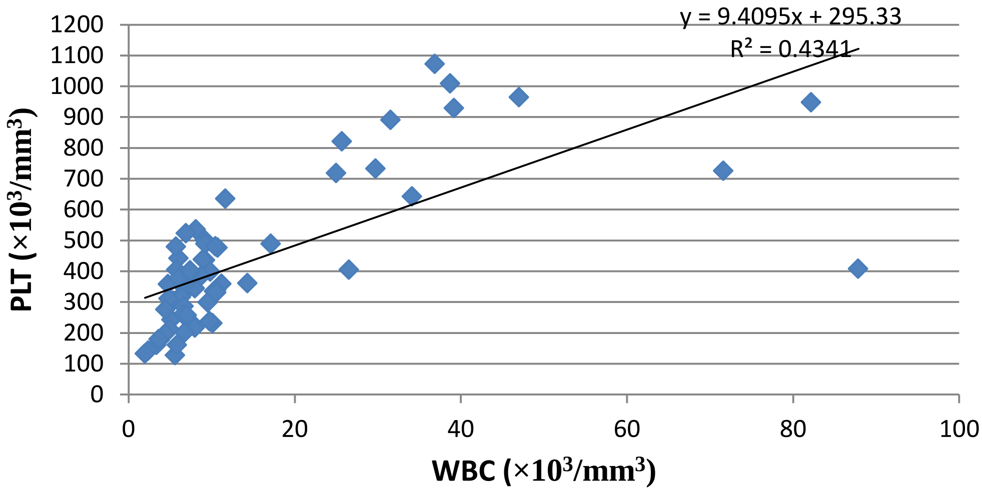


Figure (4-7): Correlation between WBC and PLT

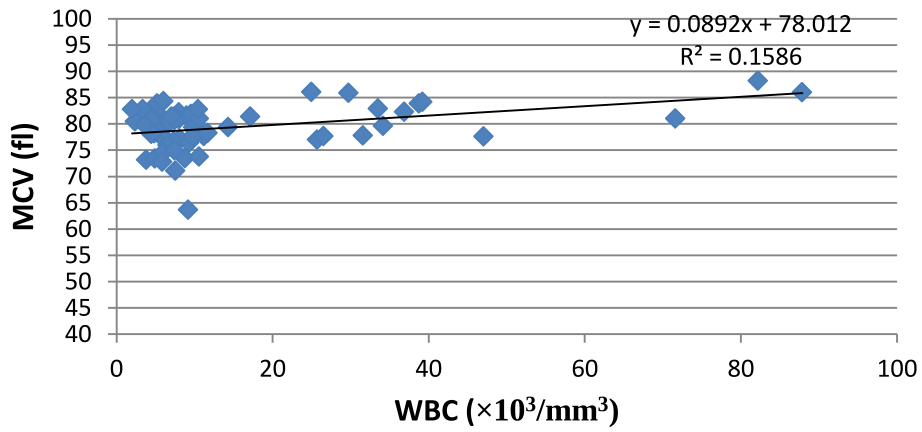


Figure (4-8): Correlation between WBC and MCV

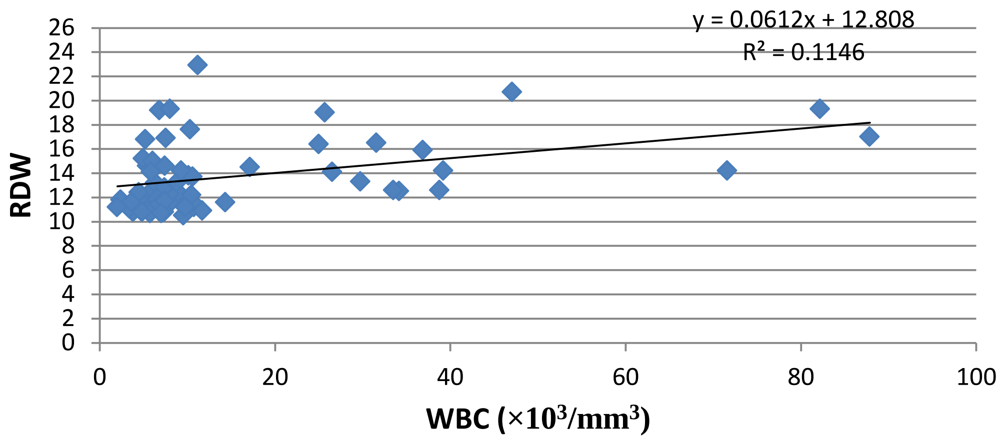


Figure (4-9): Correlation between WBC and RDW

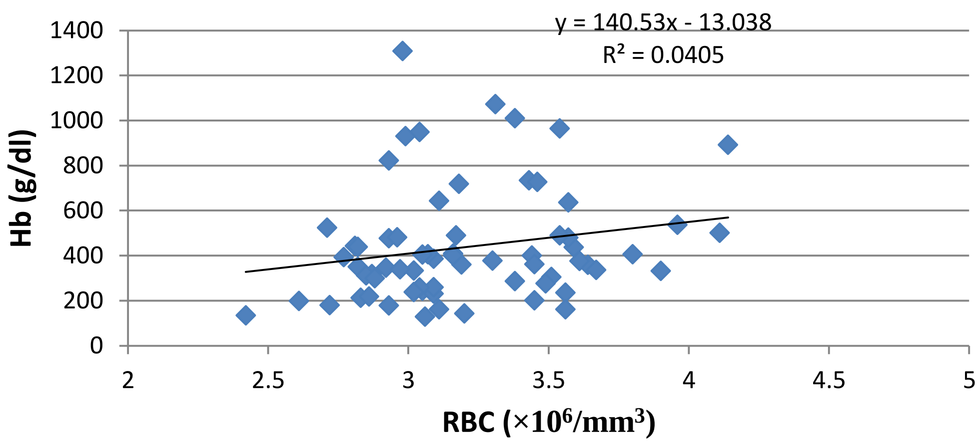


Figure (4-10): Correlation between RBC and Hb

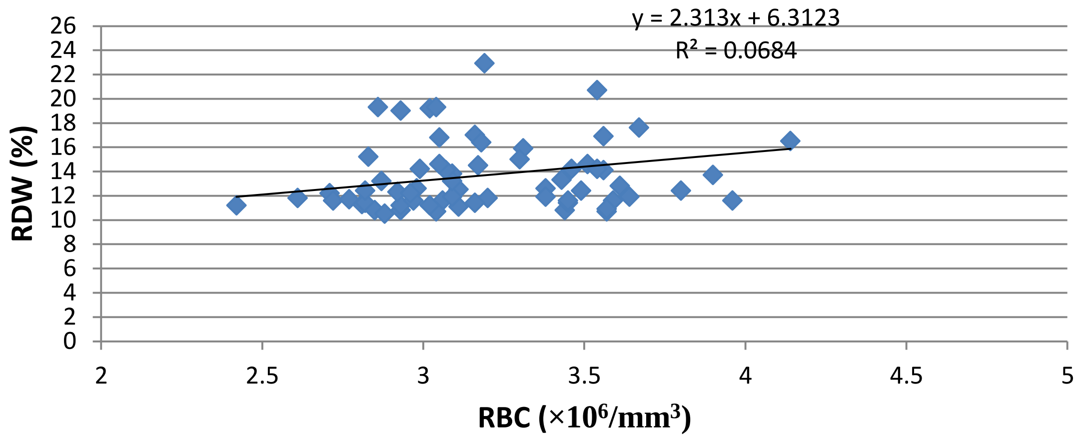


Figure (4-11): Correlation between RBC and RDW

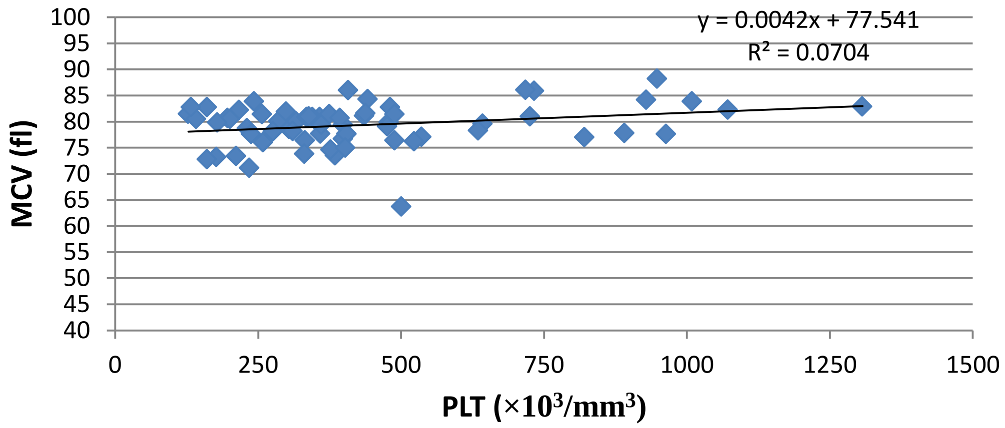


Figure (4-12): Correlation between PLT and MCV

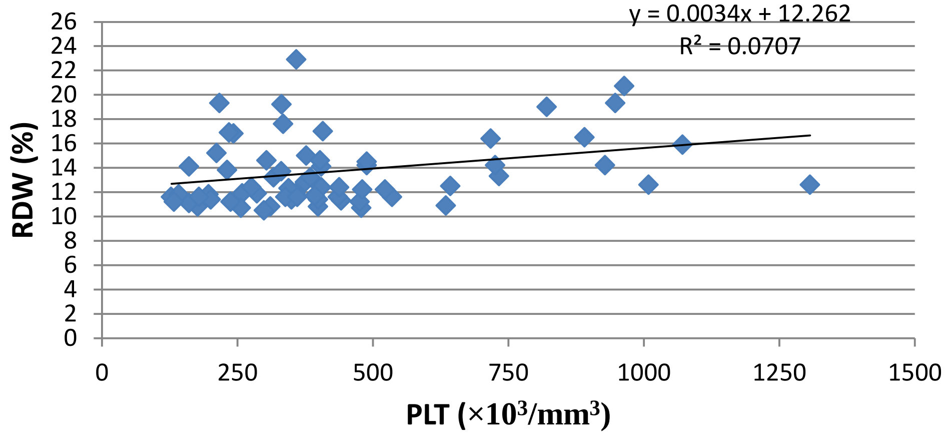


Figure (4-13): Correlation between PLT and RDW

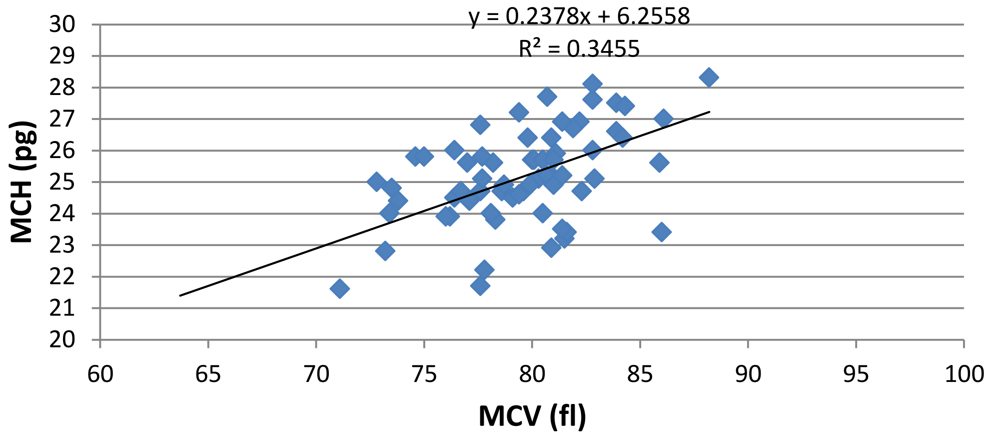


Figure (4-14): Correlation between MCV and MCH

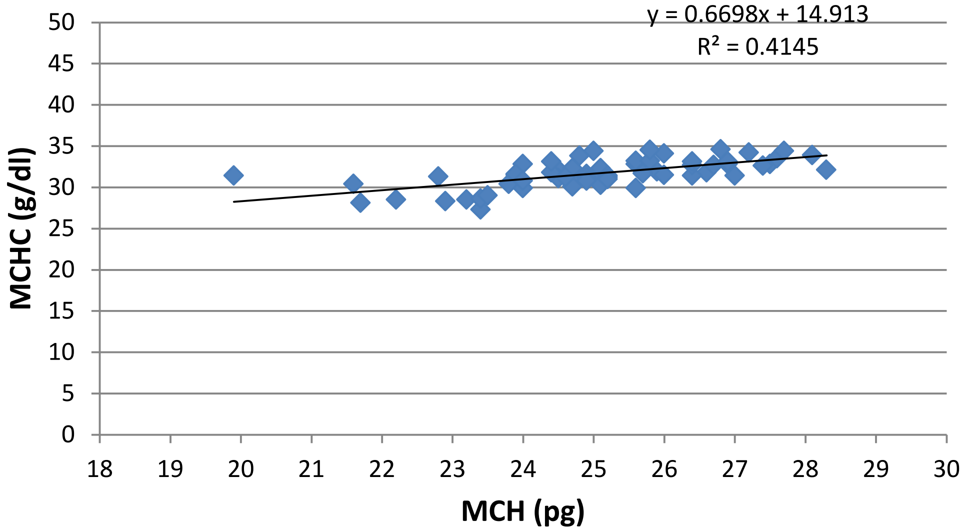


Figure (4-15): Correlation between MCH and MCHC

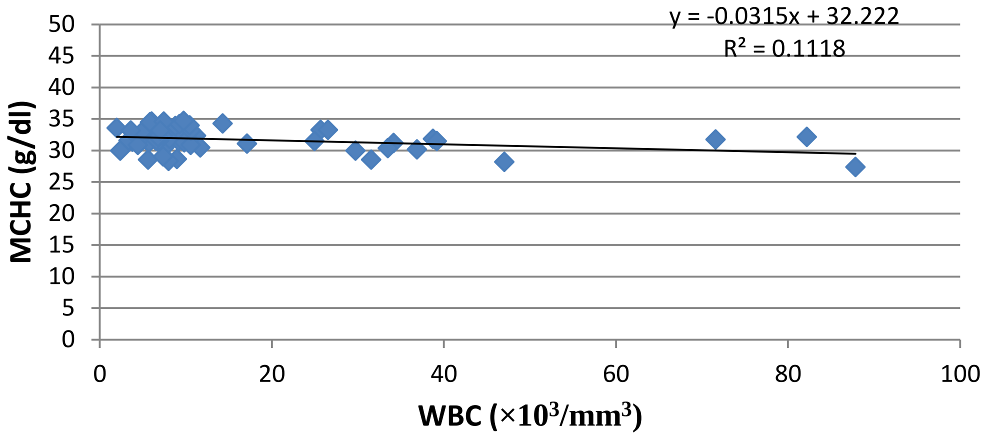


Figure (4-16): Correlation between WBC and MCHC

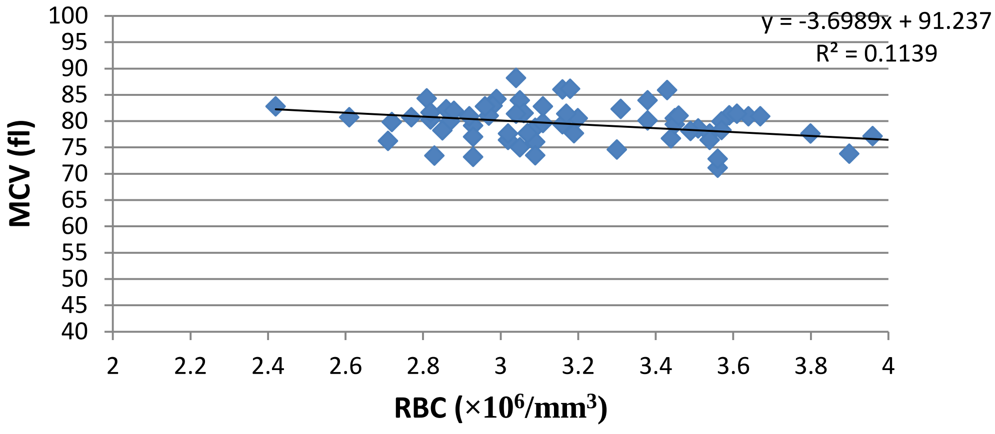


Figure (4-17): Correlation between RBC and MCV

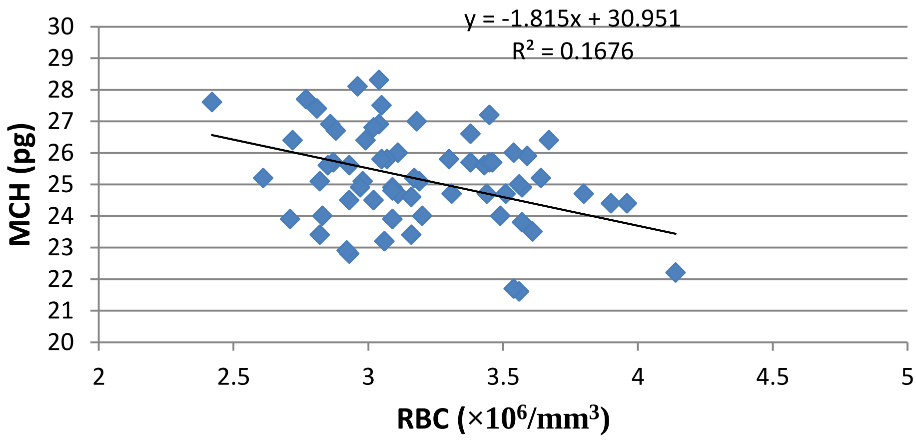


Figure (4-18): Correlation between RBC and MCH

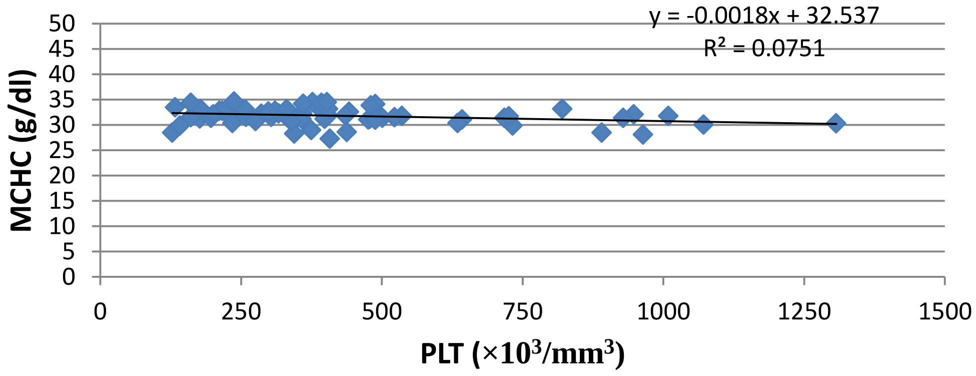


Figure (4-19): Correlation between PLT and MCHC

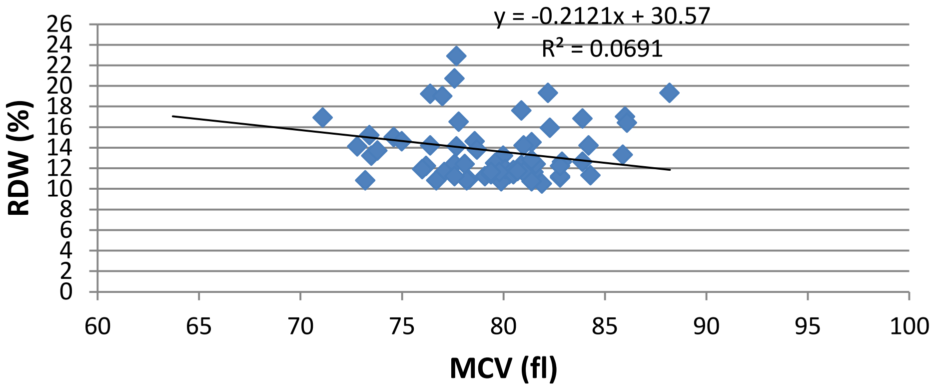


Figure (4-20): Correlation between MCV and RDW

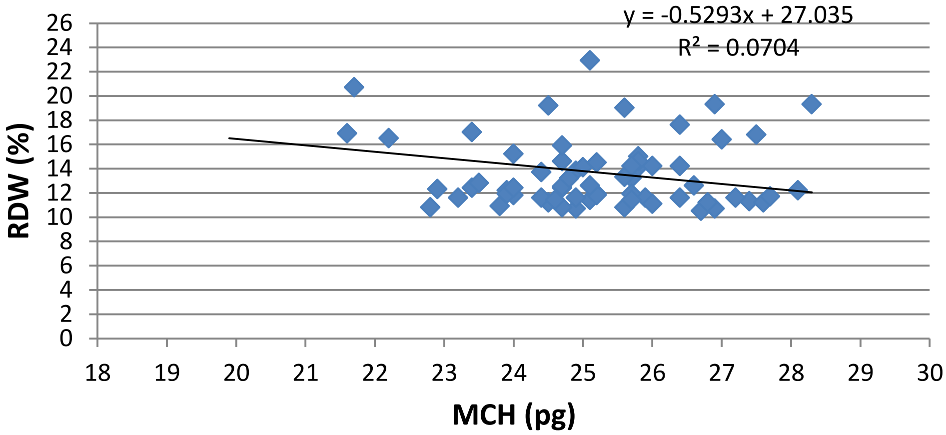


Figure (4-21): Correlation between MCH and RDW

4-6: Polymorphism of IL-10

4-6-1: locations of IL-10 and flanking region of primers

The IL-10 gene locates on short part of chromosome 1 at site 1q32.1 between 206767602-206772494 with 5 exons, also knowing names: CSIF, TGIF, GVHDS, IL-10, IL-10A. IL-10 has length 4892 bp Figure (4-22).

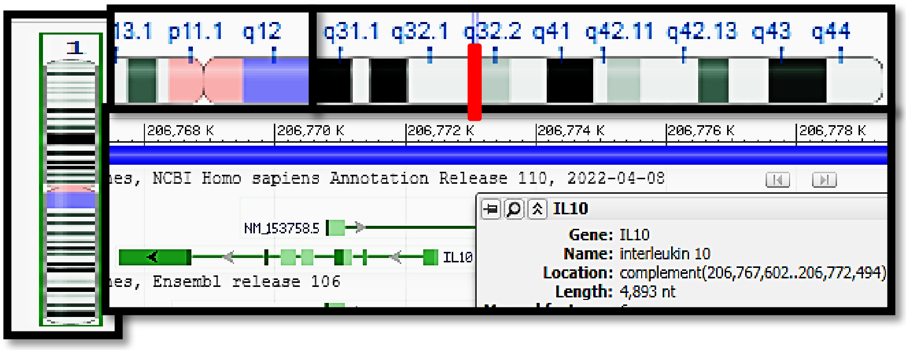


Figure (4-22): Chromosome (1) parts, the locations of IL-10 on Chromosome (1), sites it on short arm between q32.1-q32.2, length of gene, exons and introns.

4-6-2: The targeted amplicon on promoter region of IL-10

The targeted SNPs: rs1800871 T>C and rs1800872 A>C on promoter region of IL-10 were located in the spanning sequence between 206772998-206773585, the amplicon with length 588bp Figure (4-23).

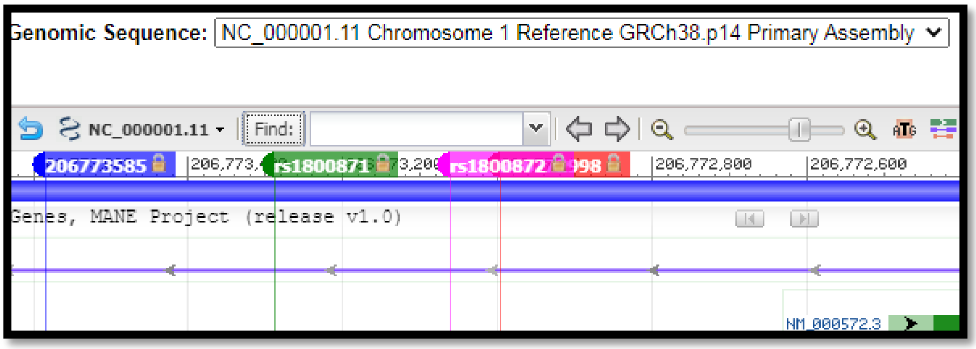


Figure (4-23): The targeted amplicon (588bp) spanning from 206772998-206773585 on promoter region of IL-10 included two SNPs: rs1800871 T>C and rs1800872 A>C.

4-7: Quality DNA extracted from patients group

The result of electrophoresis of DNA is shown the profile gel-electrophoresis 23 blood samples Figure (4-24) shows huge bright bands of DNA.

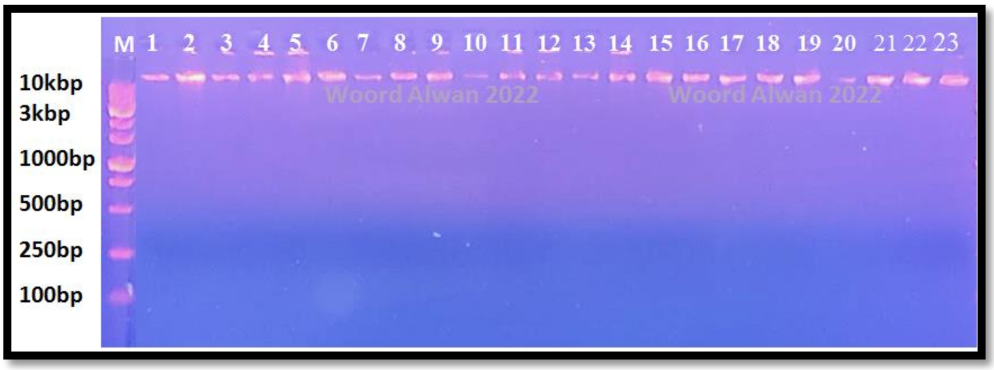


Figure (4-24): Gel-electrophoresis illustration quality of DNA extracted from patients group, 1-23 patient samples, M=molecular marker first step100bp.

4-8: Amplification part sequence of IL-10 gene included SNPs rs1800871 T>C, rs1800872 A>C

4-8-1: Amplification of targeted region of thalassemia with *T. gondii*

The primer pair flanking targeted region of promoter of IL-10 gene was successfully amplified. The PCR products of target of IL-10 shown 588bp for 20 PCR product of IL-10 of thalassemia with *T. gondii* 1-20, Figure (4-25).

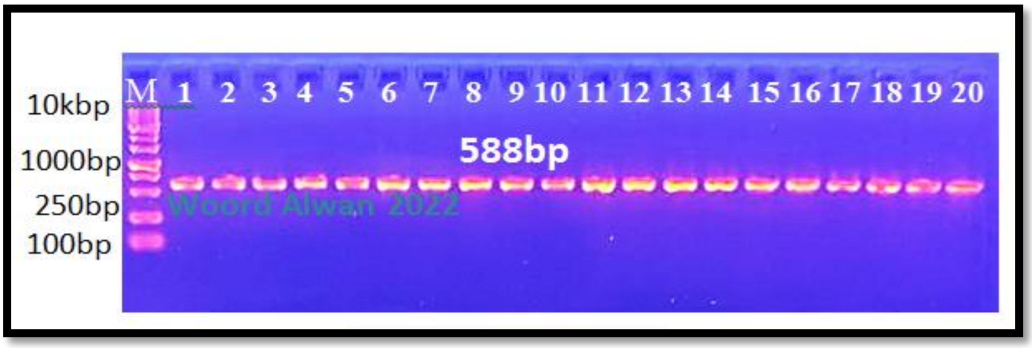


Figure (4-25): Profile of PCR products of target of IL-10 shown 588bp for 20 PCR product of IL-10 of thalassemia with *T. gondii*. 1-20 patient samples, M=molecular marker first step100bp.

4-8-2: Amplification of targeted region of thalassemia patients

The primer pair flanking targeted region of promoter of IL-10 gene was successfully amplified. The PCR products of target of IL-10 shown 588bp for 20 PCR product of IL-10 of thalassemia Figure (4-26).

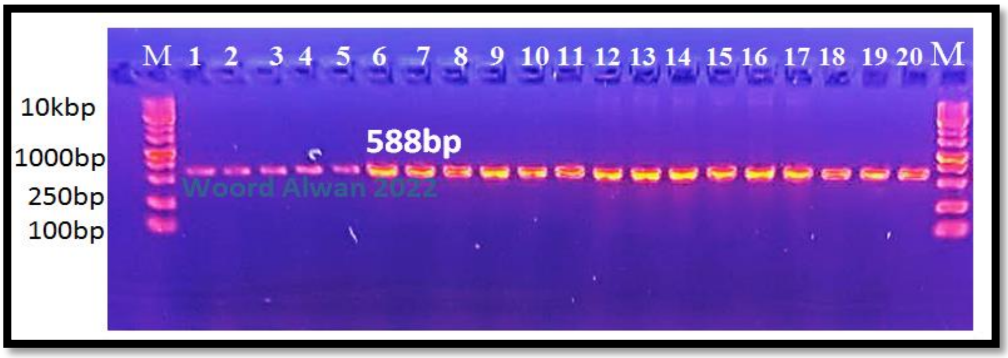


Figure (4-26): Profile of PCR products of target of IL-10 shown 588bp for 20 PCR product of IL-10 of thalassemia. 1-20 patients samples, M=molecular marker first step100bp.

4-8-3: Amplification of targeted region of healthy group

The primer pair flanking targeted region of promoter of IL-10 gene was successfully amplified. The PCR products of target of IL-10 show 588bp for 20 PCR product of IL-10 of healthy group Figure (4-27).

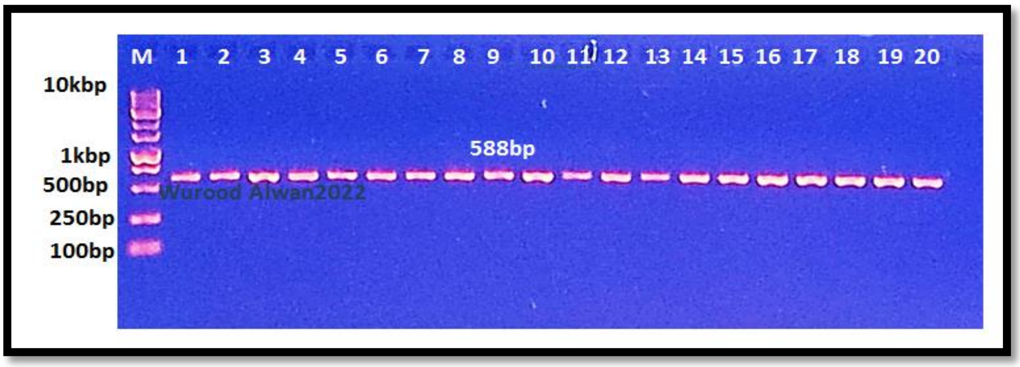


Figure (4-27): Profile of PCR products of target of IL-10 shown 588bp for 20 PCR product of IL-10 of healthy group. 1-20 samples, M=molecular marker first step100bp.

4-9: Sequence results

In order to perform sequence of PCR products, about 18-20µl of PCR product were send to macrogen laboratory (South Korea). After receiving the sequence, the results were subjected for bioinformatics analysis by geneious prime software.

4-9-1: The multiple alignments of chromatograms *T. gondii* with thalassemia group

The multiple alignment results of patients thalassemia with *T. gondii* of targeted region 206772998-206773585 was shown two ways SNPs observed: rs1800871 T>C, rs1800872 A>C. Both SNPs: rs1800871 T>C, rs1800872 A>C showed high frequent to be correlated risk allele disease. A combination grouping were performed to illustrate both SNPs: rs1800871 T>C, rs1800872 A>C Figure (4-28).

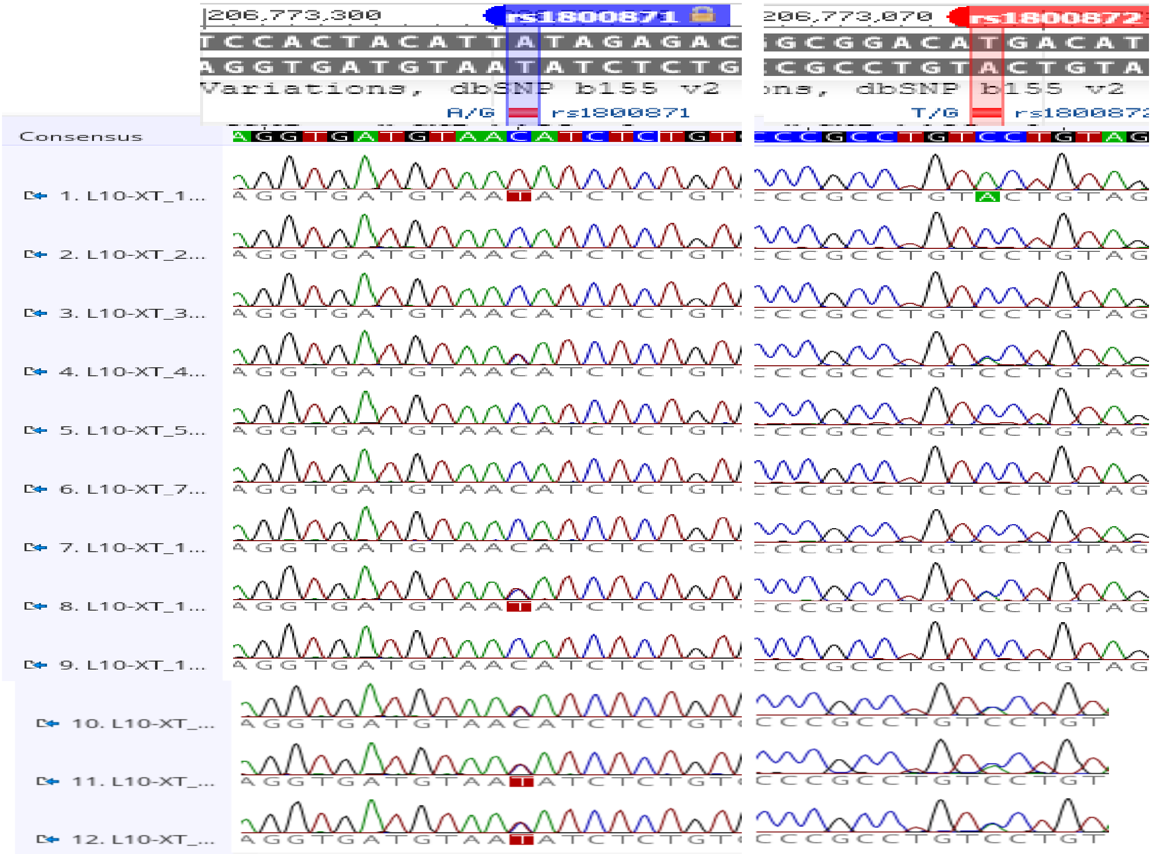


Figure (4-28): The multiple alignments of chromatograms of targeted region of IL-10 shown two SNPs: rs1800871 T>C, rs1800872 A>C, 1-12 *T. gondii* with thalassemia group. Alignment performed by geneious prime software. Red and green color referred to wild allele based on the option of software.

The results of multiple alignments of chromatograms were shown in three genotypes, TT, TC and CC. The allele C was considered mutant allele based on valid SNP: rs1800871 and pointed on chromosome 1 at site 20677311 which mutant allele A>G on upstream DNA while mutant the opposite allele C>T on downstream DNA Figure (4-28).

The genotype TC and CC showed more distribution in patients undergo both *T. gondii* and thalassemia compared with control group, the values of odd ratio (OR) support that C allele in genotype CC was correlated with disease under interest and considered as risk allele. The odd ratio was higher in genotype CC, OR=2.3(0.6-8.5) with P-value=0.2, and the allele frequency was higher with allele C= 23(57.5%) in patient group with high value of OR=1.6(0.6-4.0) P-value=0.2, while C allele frequency was low 18(45%) in control group Table (4-8).

Table (4-8): Genotypes distribution and allele frequency of wild type allele and mutant allele of SNP: rs1800871 C>T, OR values and p values for *T. gondii* with thalassemia patients.

rs1800871 T>C	**Thalassemia patients with *T.gondii* N=20**		**Control N=20**	**OR(95%CI)**	**P-value**
genotypes	TT	7(35%)	8(40%)	Reference group	
	TC	3(15%)	6(30%)	0.41(0.08-1.9)	0.2
	CC	10(50%)	6(30%)	2.3(0.6-8.5)	0.2
Allele Frequency	T	17(42. 5%)	22(55%)	0.6(0.2-1.4)	0.2
	C	23(57. 5%)	18(45%)	1.6(0. 6-4.0)	0.2

Also, the results of multiple alignments of chromatograms for *T. gondii* and thalassemia patients showed three present genotypes, AA, AC and CC. The allele C was considered mutant allele based on valid SNP: rs1800872 pointed on chromosome 1 at site 206773078 which mutant allele T>G on upstream DNA while mutant the opposite allele A>C on downstream DNA Figure (4-28).

Table (4-9) shows the genotypes AC and CC, and shows more distribution in patients undergoes both *T. gondii* and thalassemia compared with control group. The values of Odd Ratio (OR) support that C allele in both AC and CC is correlated with disease under interest. The OR was higher in genotypes AC, OR=1.9(0.5-7.07), P-value=0.3 and for CC with OR=2.3(0.6-8.5) with low significance P-value=0.2, the allele frequency was higher with allele C= 47(72.5%) in patient group with high value of OR=3.2(1.2-8.1), p-value=0.01, while the allele frequency low 18(45%) in control group.

Table (4-9): Genotypes distribution and allele frequency of wild type allele and mutant allele of SNP: rs1800872 A>C, OR values and p-values for *T. gondii* with thalassemia patients.

rs1800872 A>C	**thalassemia patients with *T.gondii* N=20**		**Control N=20**	**OR(95%CI)**	**P-value**
genotypes	AA	1(5%)	8(40%)	Reference group	
	AC	9(45%)	6(30%)	1.9(0.5-7.07)	0.3
	CC	10(50%)	6(30%)	2.3 (0.6-8.5)	0.2
Allele Frequency	A	10(27.5%)	22(55%)	0.3 (0.1-0.7)	0.01
	C	29(72.5%)	18(45%)	3.2(1.2-8.1)	0.01

4-9-2: The multiple alignments of chromatograms thalassemia group

The results of multiple alignments of patients with thalassemia are shown in Figure (4-29), and the two SNPs were observed: rs1800871 T>C, rs1800872 A>C. Both SNPs rs1800871 T>C, rs1800872 A>C shown high frequent to be correlated risk allele disease. A combination grouping was performed to illustrate both SNPs: rs1800871 T>C, rs1800872 A>C.

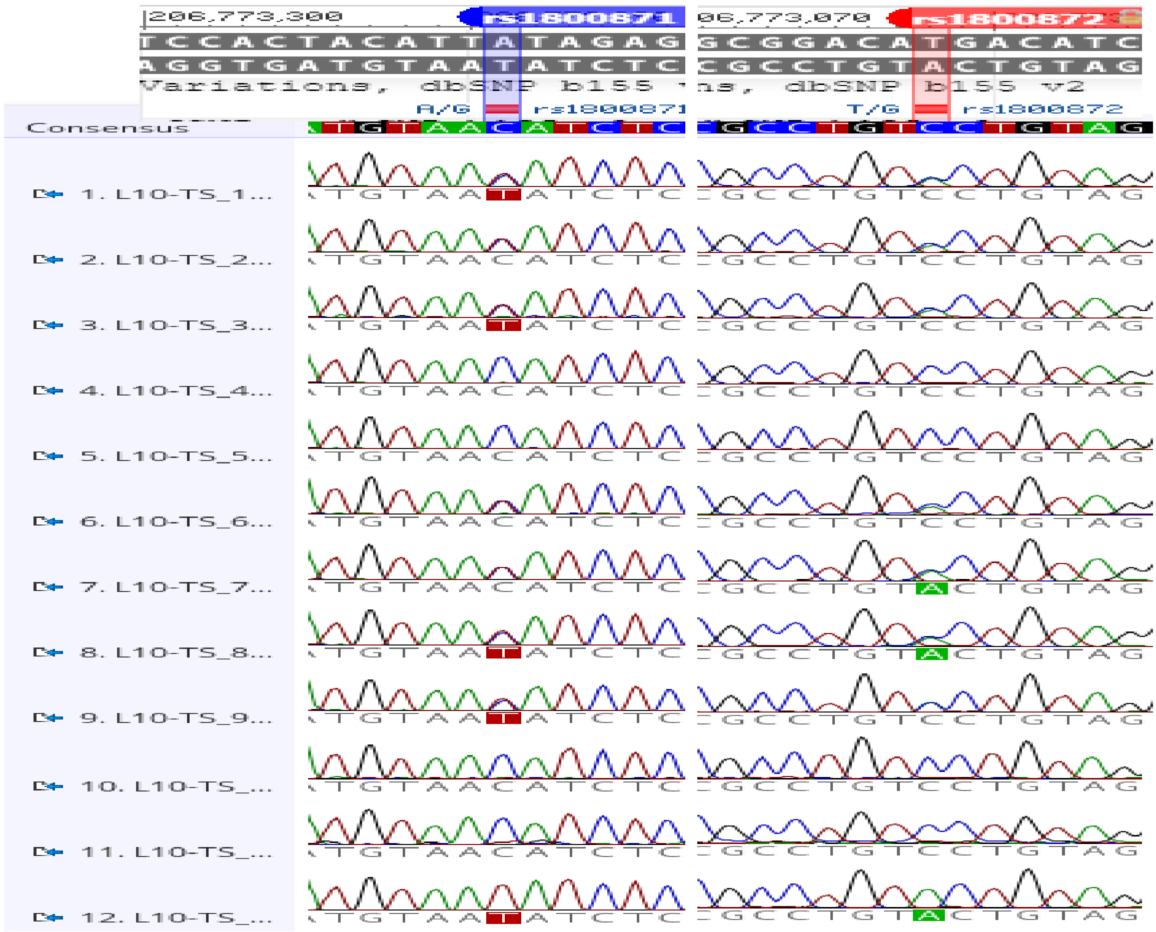


Figure (4-29): The multiple alignments of chromatograms of targeted region of IL-10 shown two SNPs: rs1800871 T>C, rs1800872 A>C, 1-12 thalassemia groups. Alignment performed by geneious prime software. Red and green color referred to wild allele based on the option of software.

4-9-3: The multiple alignments of chromatograms in healthy group

The results of multiple alignment for patients with healthy group are shown in Figure (4-30) of targeted region 206772998-206773585 that shows two SNPs observed: rs1800871 T>C, rs1800872 A>C. Both SNPs: rs1800871 T>C, rs1800872 A>C show low frequent in healthy group.

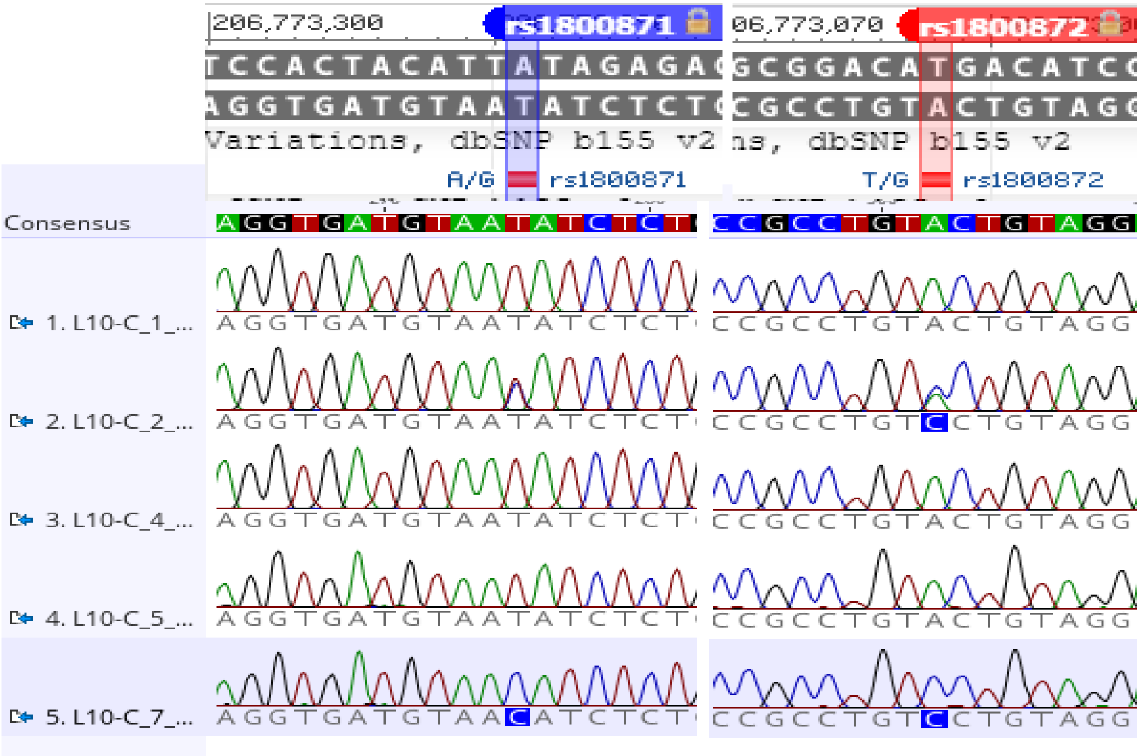


Figure (4-30): The multiple alignments of chromatograms of targeted region of IL-10 shown two SNPs: rs1800871 T>C, rs1800872 A>C, 1-5 control groups, alignment performed by geneious prime software. Blue color referred to mutant allele based on the option of software.

The results of multiple alignments of chromatograms show three present genotypes, TT, TC and CC. The allele C is considered mutant allele that is based on valid SNP rs1800871 pointed on chromosome 1 at site

20677311 which mutant allele A>G on upstream DNA while mutant the opposite allele C>T on downstream DNA.

The genotype TC and CC show more distribution in patients undergoes both *T. gondii* and thalassemia compared with control group, the values of odd ratio (OR) were supports that C allele in genotype CC is correlated with disease under interest. The OR is higher in genotype CC, OR=1.25(0.3-4.7) with high significance P-value= 0.8, and the allele C frequency is higher =19(47.5%) in patient group with high value of OR=1.1(0.4-2.6), P-value=0.8, while the allele frequency was lower 18(45%) in control group as shown in Table (4-10).

Table (4-10): Genotypes distribution and allele frequency of wild type allele and mutant allele of SNP: rs1800871 C>T, OR values and p-values for thalassemia patients.

Rs1800871 T>C	**thalassemia patients N=20**		**Control N=20**	**OR(95%CI)**	**P-value**
genotypes	TT	8(40%)	8(40%)	Reference group	
	TC	5(25%)	6(30%)	0.7 (0.19-3.1)	0.7
	CC	7(35%)	6(30%)	1.25(0.3-4.7)	0.8
Allele Frequency	T	21(52.5%)	22(55%)	0.9(0.37-2.2)	0.8
	C	19(47.5%)	18(45%)	1.1(0.4-2.6)	0.8

The allele distribution and allele frequency in thalassemia patients show more variations, the results of multiple alignments of chromatograms show three present genotypes, AA, AC and CC. The allele C is considered mutant allele based on valid SNP: rs1800872.

The homozygous genotype CC were shown more correlated with thalassemia patients, the risk allele in homozygous genotypes were shown high 1.2 (0.3-4.7). The allele frequency of mutant allele C shown higher 26(55%) with OR= 1.76(0.7-4.1) in patients less than wild allele A 18(45%) with OR= 0.57(0.2-1.3). Table (4-11).

Table (4-11): Genotypes distribution and allele frequency of wild type allele and mutant allele of SNP: rs1800872 A>C, OR values and p values for thalassemia patients.

Rs1800872 A>C	thalassemia patients N=20		Control N=20	OR(95%CI)	P-value
genotypes	AA	5(25%)	8(40%)	Reference group	
	AC	8(40%)	6(30%)	1.5 (0.4-5.7)	0.5
	CC	7(35%)	6(30%)	1.2 (0.3-4.7)	0.5
Allele Frequency	A	18(45%)	22(55%)	0.57 (0.2-1.3)	0.1
	C	26(55%)	18(45%)	1.76 (0.7-4.1)	0.1

As seen in Figures (4-28), (4-30), the IL-10 SNPs polymorphism the presence of TT genotype of rs1800871 and AA genotype in rs180072 of IL-10 allele is fundamentally low in control group than patients groups.

This study was taken up two important diseases; first one is thalassemia and the second is toxoplasmosis and correlated with polymorphism of IL-10.

Toxoplasmosis, caused by *T. gondii*, is a common foodborne opportunistic pathogen that can cause serious illness in immunocompromised patients. In strains from around the world, the pathology and immunological responses associated with the resulting illness have not been thoroughly documented (Lieberman and Hunter, 2002).

In this study, the high frequency of allele risk allele C in homozygous genotype CC in SNP rs1800871 based on the odd ratio was higher in genotype CC, OR=2.3(0.6-8.5) with p-value=0.2, and the allele frequency is higher with allele C=23(57.5%) in patient group with high value of OR=1.6(0.6-4.0) p-value=0.2, while the allele frequency is low 18(45%) in control group as shown in Table (4-8). The same observation in case (AC and AA) genotypes in SNP of rs1800872 based on present results, the OR is higher in genotypes AC, OR=1.9(0.5-7.07), p-value= 0.3 and for CC the OR= 2.3(0.6-8.5) with high significance p-value=0.2, the allele frequency is higher with allele C=29(72.5%) in patient group with high value of OR=3.2(1.2-8.1), P-value=0.01, while the allele frequency is low 18(45%) in control group as shown in Table (4-9). These causes deviation the role of IL-10, this deviation was correlated with the function of IL-10.

In general, IL-10 levels are enhanced during the intense and continuous disease, while immunosuppressed patients' IL-10 levels decreased. This anti-inflammatory cytokine can stimulate T helper 1 (Th1) reactions, IL-10 is thought to be a suppressor of Th1 and Th2 insusceptible responses (O'Garra and Vieira, 2007). Thus, in both immunocompetent and immunosuppressed mice, the role of IL-10 cytokines released by macrophages, monocytes, B cells, and CD4+ and CD8+ T cells during both the intensive and continuous phases of contamination is investigated is to downregulate or limit the consequences of an exaggerated provocative reaction and important histocompatibility complex and costimulatory atom articulation by acting broadly on embellishment cells and versatile cell reactions (Mose *et al*., 2019). This cytokine has also been identified as a component triggered by *T. gondii* infection, as it prevents tissue safe obliteration through immunomodulation (Kim *et al*., 2007; Mose *et al*., 2019). Unfortunately, no more studies are performed on the relation between IL-10 and thalassemia, the main study in Iraq, the study of Surhan *et al*. (2018) in Najaf.

Thalassemia is the most common congenital hemolytic anemia caused by mutations that disrupt the synthesis of globin chains, resulting in a partial or complete absence of globin chains. The aim of this study is the detection of IL-10-rs1800896 A\G polymorphism and IL-10 serum level with it correlation to pathogenesis. A case-control study was conducted on 60 individuals with β-thalassemia major who were diagnosed at the AL-Zahra hospital in AL-Najaf, Iraq (Surhan *et al*., 2018).

Insofar as cytokine production is genetically controlled, and IL-10 promoter polymorphisms have been linked to a variety of disorders (Daher *et al*., 2003; Kamali-Sarvestani *et al*., 2005), here IL-10 gene polymorphisms has been investigated in toxoplasmosis and thalassemia and control group. These results demonstrate a role for SNPs cases under interest (Moore *et al*., 2001).

The present results are consistent with results of Madeshiya *et al*. (2017), when he refer to the role of Interleukin-10 (IL-10) which an important anti-inflammatory cytokine, the genetic polymorphisms include three polymorphisms (rs1800896), A>C (rs1800872) and T>C (rs1800871).

Based on the role of IL-10, which is the standard suppressor cytokine of the responses resistant structures, it has been proven that their levels are linked to bacterial infection in individuals (Rizzuti *et al*., 2015).

The reification obtained in persons is sponsored by exploratory verification, which has established a causal link between IL-10 levels and human disorders (Murdock *et al*., 2014).

Final finding shows that the IL-10 polymorphisms analysis has clear impact on any human diseases. In this study, the polymorphism of IL-10 shows high effect on occurrence of toxoplasmosis and thalassemia, and the results illustrated in Figures (4-28), (4-29), (4-30), and Tables (4-8), (4-9), (4-10), (4-11) were show the role and polymorphism and distribution of IL-10 rs1800871 T>C and rs1800872 A>C genotypes. The frequency of IL-10 rs1800871 T>C and C alleles, together with IL-10 rs1800872 C alleles are similar between patients and controls. In contrast, the frequency of the IL-10 rs1800872 (mutant) C allele (OR of AC=1.5(0.4-5.7), CC=1.2(0.3-4.7), P- value= 0.5 is higher among patients 26(55%) see Table (4-11). The frequencies of IL-10 -592C/A, -819C/T and -1082A/G genotypes are comparable between patients and controls (Zammiti *et al*., 2006).

The other light point in this study, it finds that allele risk of C allele in both heterozygous and homozygous SNP rs1800872 more than risk of allele C in SNP rs1800871 only with homozygous allele CC= 2.3 with thalassemia and *T. gondii* and same with thalassemia 0.7 and 1.25 for TC and CC respectively. See Tables (4-8), (4-10). While, OR of risk allele C in heterozygous AC and homozygous CC both disease cases thalassemia and *T.gondii* with OR 1.9, 2.3 and thalassemia alone OR 1.5, 1.2 for AC and CC respectively as shown in Tables (4-9), (4-11). This means SNP: rs1800872 A>C more risk than rs1800871 T>C on patients under interest.

Conclusions

And

Recommendations

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Conclusions

1- Thalassemia patients have been high exposure to toxoplasmosis because depression of immune system, and age group 10-20 years have high incidence of toxoplasmosis.

2- Patients (males and females) bear thalassemia were shows different percentage, and have the same chance of infection with toxoplasmosis.

3- There are some criteria such as cat existence in home of infected persons or genetic characters and blood transfusion have not effected on rates of infection with patients with toxoplasmosis.

4- Beta thalassemia patients with *Toxoplasma*, there are decreasing level in RBCs, Hb, PCV, MCV, MCH, MCHC, whereas increasing level of the following WBCs, PLT, RDW.

5- According blood groups (A^+, A^-, B^+, AB^+, O^+, O^-) show the highest rate in males has been shown, in A^+, in B^+, in O^- in thalassemia patients with *Toxoplasma*.

6- The study reveal the decrease the uric acid and increase the allantoin concentration.

7- The IgG predominant in all samples in the present study that indicates infection of all patients.

8- The IL-10 polymorphisms analysis had clear impact on human diseases, and show high effect on occurrence of toxoplasmosis and thalassemia, polymorphism and distribution of IL-10 rs1800871 T>C and rs1800872 A>C.

9- The frequency of IL-10 rs 1800871 and alleles C, together with IL-10 rs1800872 and alleles C. Risk allele C more frequent in patients than in control group.

10- The allele frequency of the IL-10 rs 1800872 mutant allele C (OR of AC=1.9 (0.5-7.07); CC=2.3 (0.6-8.5), were higher among patients 22(55%).

Recommendations

1- Designing specific primers for detecting present of *Toxoplasma gondii* in suspected patients.

2- Expanding studies on relationship the parasites with thalassemia due to poor studies on this disease in Iraq.

3- Expanding studies that aim to immunogenic explanation of many interleukins like IL-6, IL-8, IL-12 and IL-17.

4- Studying another biochemical tests such as (albumin, ascorbic acid, zinc, copper, selenium, etc.) and antioxidant enzyme like catalase and SOD (superoxide dismutase).

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Appendixes

Appendix (1): Sample collection form

Sample collection form	
Name	
Gender	
Age	
Region (urban – rural)	
Sample collection date	
Genetic factor (Does the mother or father have thalassemia)	
Blood group	
Number of blood transfusions (monthly or twice a month)	
Are cats in the house or not	
Eating meat (raw - undercooked - eating canned meat)	
Are there other accompanying diseases (blood pressure - diabetes - arthritis ... etc)	

Paper that reveal the agreement of patient before taken the samples as ethics.

Ethics

You are invited (e) to participate in the clinical scientific research that will be conducted at -------------------- Please take (j) enough time to read the following information carefully before deciding (j) To participate or not. You can request clarifications or additional information about anything in this form or about this study from your doctor.

In the event that you agree and accept to participate in this study, your name, address and phone number will remain.

Agree ☐ **Not agree** ☐

I have read this acceptance form and know what it is about. All my questions were answered. I authorize the conduct of this study and agree to participate in it.

Subscriber name:

Subscriber signature:

Appendix (2): The ethical paper given to thalassemia patients to take agree or not to take blood samples.

جمهورية العراق

وزارة الصحة والبيئة
دائرة صحة محافظة بابــــل
المديـــر العـــام
مركـــز التدريب والتنمية البشرية
وحدة أدارة البحوث

Ministry Of Health
Babylon Health Directorate
Email:-
Babel_Healthmoh@yahoo.com
Tel:282628 or 282621

العدد : ١٠٤٥
التاريخ: ٩ / ١١ /٢٠٢١

إلى / مستشفى بابل التعليمي للنسائية والاطفال
م/ تسهيل مهمة

السلام عليكم ...

أشــارة إلــى كتــاب جامعــة بابــل/ كليــة الدراســات العليــا / شــؤون الطلبــة / العلــوم الطبيعية ذي العدد ٤٠٢٠ في ٢٠٢١/١١/٨

نرفــق لكــم ربطــا اســتمارات الموافقــة المبدئيــة لمشــروع البحــث العائــد لطالبــة الدراســات العليا الماجستير (ورود علوان كاظم) قسم علوم الحياة.

للتفضــل بــالاطلاع وتســهيل مهمــة المومــا أليــه مــن خــلال توقيــع وخــتم اســتمارات اجــراء البحــث المرفقــة فــي مؤسســاتكم وحســب الضــوابط والإمكانــات لاستحصــال الموافقــة المبدئيــة ليتســنى لنــا أجــراء الــلازم علــى أن لا تتحمــل مؤسســاتكم أيــة تبعــات ماديــة وقانونيــة .... مع الاحترام

المرفقات :
استمارة عدد /٢

الدكتور
محمد عبد الله عجرش
مدير مركز التدريب والتنمية البشرية
/ / ٢٠٢١

نسخة منه إلى:

- مركز التدريب والتنمية البشرية / وحدة أدارة البحوث مع الأوليات ...

سوزان ١١/٩

دائرة صحة محافظة بابل / مركز التدريب والتنمية البشرية // ايميل المركز babiltrainning@gmail.com

Appendix (3): A letter facilitating a task addressed to Babylon Hospital for Maternity and Children (Center for Genetic Blood Diseases).

Ministry of Higher Education and Scientific Research

University of Babylon

College of Science for Women

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية العلوم للبنات

UNIVERSITY OF BABYLON

Ref. No.:

Date: / /

العدد : ٣٢٥

التاريخ: ١٢/١/٢٠٢٢

إلى/ وزارة العلوم والتكنلوجيا/دائرة البيئة والمياه

م/ تسهيل مهمة

تحية طيبة:

نرجو تفضلكم بتسهيل مهمة طالبة الدراسات العليا/الماجستير/علوم الحياة (ورود علوان كاظم) المقبولية في كلية علوم البنات بجامعتنا للعام الدراسي(٢٠٢٠-٢٠٢١) وذلك لإكمال متطلبات البحث تفضلكم بالاطلاع واجراء ما يقتضي من قبلكم

..مع الاحترام..

أ.د. عبير فوزي مراد الربيعي

معاون العميد للشؤون العلمية

١٢/١/٢٠٢٢

نسخة منه إلى//

- الدراسات العليا (ملفة الطالبة).

- الصادرة.

STARS

E-mail: grlsci@uobabylon.edu.iq

Appendix (4): A letter facilitating a task to the Ministry of Science and Technology / Department of Environment and Water for the purpose of examining serum samples of Thalassemia patients using the HPLC technique.

الخلاصــــــة

هدفت الدراسة الحالية إلى تقييم داء المقوسات في مرضى الثلاسيميا بناءً على المعايير الوبائية والدموية والمناعية بواسطة IgG وIgM، وتحديد تعدد أشكال جين انترلوكين- 10 وعلاقته بداء المقوسات في مرضى الثلاسيميا.

تم جمع 66 عينة لمرضى بيتا ثلاسيميا الكبرى، و30 عينة كمجموعة سيطرة في ذكور وإناث أصحاء خلال شهر تشرين الاول والى شهر كانون الاول في مستشفى الولادة والاطفال، في بابل، وتم فحص 96 عينة في جهاز Cobas e411 لكل من IgG و IgM خاصه بالتوكسوبلازما، في مركز الامين في النجف، وتم الحصول على 20 عينة موجبة لاختبار IgG في مرضى بيتا ثلاسيميا الكبرى وعينة واحدة منها موجبة لـ IgM، كما تم إجراء اختبار حمض اليوريك لجميع المرضى والأصحاء. اخذت 12 عينة من ذكور واناث تحتوي على نسبة عالية من حمض اليوريك، 6 منها كانت موجبة لداء المقوسات و 6 عينات سالبة للمرض. تم إجراء كروماتوكرافيا سائلة عالية الأداء (HPLC) في وزارة العلوم والتكنولوجيا في بغداد، باستخدام المادة القياسية النتوين لرصد العلاقة بين حمض اليوريك والنتوين، اذ تكون العلاقة عكسية بينهما، في حالة الإصابة ووجود الطفيلي المثير للاهتمام في الدم.

قسمت عينات الدراسة إلى ثلاث فئات عمرية، فقد أظهرت النتائج أن أعلى نسبة للاناث هي58.33٪ في الفئات العمرية 10-15 سنة، وكانت النسبة 50٪ عند الذكور في الفئات العمرية 16-20 سنة في مرضى الثلاسيميا غير المصابين بداء المقوسات، بينما بلغت النسبة 25٪ عند الذكور في الفئات العمرية 21-25 سنة في مرضى الثلاسيميا مع داء المقوسات.

وفقا لوجود القطط أظهرت أعلى نسبة إصابة عند الذكور 12.5٪ في مرضى الثلاسيميا بداء المقوسات، وغياب القطط 90.91٪ في مرضى الثلاسيميا غير المصابين بداء المقوسات، بينما تكون نسبة الإصابة عند الإناث أعلى مع وجود القطط 16.67٪ في مرضى الثلاسيميا بداء المقوسات، وغياب القطط 95.83٪ في مرضى الثلاسيميا غير المصابين بداء المقوسات.

وجد ان تأثير عامل نقل الدم أعلى نسبة في الاناث، تتساوى نسبة نقل الدم مرة في الشهر 58.33٪ في مرضى الثلاسيميا مع وبدون التوكسوبلازما، ونقل الدم مرتين في الشهر في الذكور 62.5٪ في مرضى الثلاسيميا مع التوكسوبلازما، ونقل الدم أربع مرات في الشهر في الذكور 4.55٪ في مرضى الثلاسيميا المصابين بدون التوكسوبلازما.

بالنسبة للمعايير الكيموحيوية، يرتبط تركيز حمض اليوريك والنتوين كمعلمات لجهد الاكسدة لمرضى بيتا ثلاسيميا المصابين او غير المصابين بداء المقوسات، للكشف عن أعلى تركيز (5.10) ملغم/ ديسيلتر من حمض اليوريك عند الذكور مقارنة بالإناث (4.66) ملغم/ ديسيلتر في مجموعات مرضى بيتا ثلاسيميا المصابين بداء المقوسات، بينما يظهر أعلى تركيز عند الذكور (5.36) ملغم/ ديسيلتر مقارنة بالإناث (3.76) ملغم/ ديسيلتر في مرضى بيتا ثلاسيميا غير المصابين بداء المقوسات. تركيز النتوين في الاناث (59.52±1.6) جزء بالمليون كان اعلى مقارنة مع الذكور(56.39±1.9) جزء بالمليون في مرضى بيتا ثلاسيميا مع توكسوبلازما بينما تركيز النتوين في مرضى بيتا ثلاسيميا بدون توكسوبلازما كان اعلى في الذكور (39.78±1.3) جزء بالمليون مقارنة بالاناث(37.53±1.2) جزء بالمليون.

نتائج التنميط الجيني للأليلات البرية والمتحورة لتعدد أشكال النوكليوتيدات المفردة لجين إنترلوكين-10 وتقييم علاقتها بداء المقوسات والثلاسيميا. وجد أن وجود اثنين من تعدد اشكال النيوكليوتيدات المفردة في المنطقة المحركة للجين المعني هما rs1800871 و rs1800872. اظهرت الأنماط الجينية الطافرة المتجانسة CC توزيعًا أكبر في مرضى التوكسوبلازما والثلاسيميا مقارنة بمجموعة السيطرة، وتدعم قيم نسبة الأرجحية (OR) أن الأليل C في CC مرتبط بالمرض محل الاهتمام ويعتبر أليل خطر. نسبة الأرجحية أعلى في النمط الجيني CC = 2.3 (0.6- 8.5) مع قيمة p=0.2 .

اظهرت النتيجة النهائية أن تحليل تعدد الأشكال للإنترلوكين-10 له تأثير واضح على مرضى الثلاسيميا المصابين بداء المقوسات. في هذه الدراسة، أظهر تعدد الأشكال للإنترلوكين-10 فعالية عالية في حدوث داء المقوسات والثلاسيميا، لا يتشابه تكرار إنترلوكين-10 rs1800871 T>C مع أليلات الإنترلوكين-10 rs1800872 A>C بين المرضى ومجموعة السيطرة.

وزارة التعليم العالي والبحث العلمي

جامعة بابل – كلية العلوم للبنات

قسم علوم الحياة

دراسة جزيئية وكيموحيوية لداء المقوسات في مرضى بيتا ثلاسيميا الكبرى في محافظة بابل

رسالة مقدمة الى

مجلس كلية العلوم للبنات، جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير علوم

في علوم الحياة

من قبل

ورود علوان كاظم جعيفر

(بكالوريوس علوم، علوم الحياة، جامعة بابل،2011)

بإشراف

أ.د. احمد خضير عبيس الحميري

محرم ١٤٤٤ هـ

آب ٢٠٢٢ م