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Immunophenotypic and IL-17 Genetic Evaluation in Iraqi Patients with Chronic Myeloid Leukemia

A Thesis

Submitted to the council of Science College / Al-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology.

By

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Dedication

To the one who enlivens my life with her affection and patience..

My Mother

To the one who taught me that knowledge is a light..

My Father

To the one who filled my life with love and happiness..

My Wife

To the apple of my eye, the cutest human being ever..

My Daughter

To those who supported and helped me in my tough moments..

My Brothers and Friends

To every candle that burns itself to enlighten the way to others..

My great teachers

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// Summary

This study was designed to shed light on the association between the genetic variations in IL-17A and IL-17F genes, and their levels in chronic myeloid leukemia (CML).

Sixty two patients (38 males, and 24 females), with an age range 21 – 73 years old, were diagnosed with chronic myeloid leukemia, and 28 healthy individuals (18 males and 10 females), with an age range 24 – 72 years old, were enrolled in this study. Blood samples were collected from Oncology Teaching Hospital and Baghdad Teaching Hospital – Baghdad Medical City, and National Center of Blood diseases – Al-Mustansiriya University.

Cytokine gene polymorphism analysis by sequencing revealed 13 registered variants for IL-17A exon 1; the rs199815459 SNP in IL-17A exon 1, which linked specifically to the CML with a genotype TG while healthy subject showed a GG genotype. The rs749745973 detected with GA genotype with (100%) frequency in CML and healthy unlike the registered genotype, which was GC. Also, results showed some unregistered genetic variations detected in IL-17A exon1, first one was in the promoter, the second and third variations were recorded in the coding sequence, while the last one was an intronic variation. These variations were detected in CML and healthy. IL-17F exon 3 sequencing results showed a registered rs2397084, which was found in AG genotype in (9.1%) frequency in CML and AA genotypes which was detected in CML with frequency (90.9%) and (100%) in healthy.

Also, serum level of IL-17A and IL-17F were estimated by ELISA. Results exhibited IL-17A level in newly diagnosed recorded a significant elevation in comparing with healthy individuals, while the level was significantly lower in non-complete treatment patients compared with healthy individuals ($P \leq 0.05$). Serum IL-17F levels in newly diagnosed showing a significant increase while the under treatment patients showed a significant lower level compared with pretreated.

Also, immunophenotypic features of CD4+ and CD8+ were calculated by flow cytometer in this study. Results revealed CD4 and CD8 count in newly diagnosed and under treatment CML patients' blood was also different from the healthy, giving a significant increase count in newly diagnosed patients and a significant decrease count in non-complete treatment patients.

List of Contents

Item.	Subject	Page
	List of Contents	*iii*
	List of Tables	*vi*
	List of Figures	*vii*
	List of Abbreviations	*viii*
Chapter One: Introduction and Literature Review		
1.1.	Introduction	**1**
1.2.	Cancer	**3**
1.3.	Leukemia	**3**
1.3.1.	Definition	**3**
1.3.2.	History of Leukemia	**4**
1.3.3.	Classification of Leukemia	**4**
1.3.4.	Leukemia Causes	**6**
1.3.5.	Leukemia Diagnosis	**8**
1.4.	Chronic Myeloid Leukemia (CML)	**9**
1.4.1.	Discovery of Chronic Myeloid Leukemia	**9**
1.4.2.	Chronic Myeloid Leukemia Patients	**10**
1.4.3.	Physiology	**11**
1.4.4.	Diagnosis of Chronic Myeloid Leukemia	**12**
1.4.5.	Chronic Myeloid Leukemia Phases	**15**
1.4.6.	Therapy	**16**
1.4.7.	Imatinib	**17**
1.5.	Cytokines and Interleukins	**18**
1.5.1.	IL-17 Family	**19**
1.5.1.A.	IL-17 Family Review	**19**
1.5.1.B.	Biological Function of IL-17 Family	**21**
1.5.2.	IL-17A and IL-17F properties	**21**
1.6.	Cluster of Differentiation Markers	**23**
Chapter Two: Materials and Methods		
2.1.	Subjects	**25**
2.2.	Materials	**25**

2.2.1.	Equipments	**25**
2.2.2.	Chemicals and Solutions	**27**
2.2.3.	Primers	**27**
2.2.4.	Kits Reagents	**28**
2.2.4.A.	DNA isolation Kit	**28**
2.2.4.B.	IL-17A estimation Kit	**28**
2.2.4.C.	IL-17F Estimation Kit	**29**
2.3.	Methods	**30**
2.3.1.	Molecular Steps	**30**
2.3.1.A.	Selection of a functional sequence in IL-17A andIL-17F genes	**30**
2.3.1.B.	Primers Designation	**30**
2.3.1.C.	Blood Sample Collection	**31**
2.3.1.D.	DNA isolation	**31**
2.3.1.E.	Agarose Gel Electrophoresis	**33**
2.3.1.F.	Polymerase Chain Reaction (PCR)	**34**
2.3.1.G.	Sequencing	**36**
2.3.1.H.	Detection of IL-17A and IL-17F variants	**36**
2.3.2.	Immunological tests	**36**
2.3.2.A.	Estimation of serum IL-17A	**36**
2.3.2.B.	Estimation of serum IL-17F	**39**
2.3.2.C.	Analysis of T-Lymphocyte Subset By Flow Cytometer	**42**
2.3.3.	Statistical analysis	**43**
Chapter Three: Results and Discussion		
3.1.	Distribution of Subjects	**44**
3.1.1.	Distribution by Gender	**44**
3.1.2.	Distribution by Age	**44**
3.2.	Molecular Analysis of IL-17A and IL-17F	**45**
3.2.1.	DNA isolation	**45**
3.2.2.	Thermal Gradient PCR	**46**
3.2.3.	PCR products	**46**
3.2.3.A.	IL-17A Gene Exon-1 PCR	**46**
3.2.3.B.	IL-17F Gene Exon-3 PCR	**47**
3.2.4.	IL-17A Exon 1 Sequencing Results	**48**
3.2.4.A.	Registered Variants	**48**
3.2.4.B.	Unregistered Variants	**51**

3.2.5.	IL-17F Exon 3 Sequencing Results	**53**
3.3.	IL-17A and IL-17F Estimation	**55**
3.3.1.	Serum Level of IL-17A	**55**
3.3.2.	Serum Level of IL-17F	**55**
3.4.	CD4+ and CD8+ Lymphocytes Counting by Flow Cytometer	**56**
3.4.1.	Blood picture of control and CML samples	**56**
3.4.2.	CD4 and CD8 Count	**57**
3.4.3.	CD4/CD8 Ratio	**58**
Chapter Four: Conclusions and Recommendations		
4.1.	Conclusions.	**61**
4.2.	Recommendations	**62**
	References	**63**

List of Tables

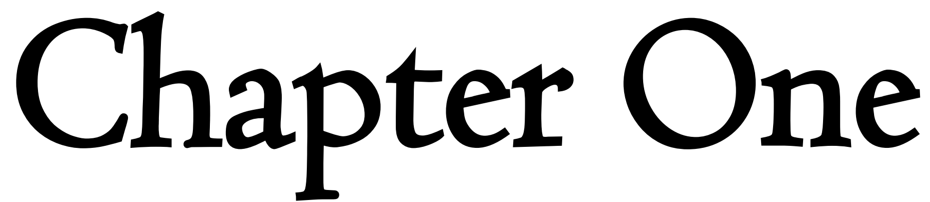
No.	Title	Page
1-1	Amino acid sequence homology between human IL-17 family	**22**
2-1	The equipments used in this study	**25**
2-2	Chemicals used in this study	**27**
2-3	Sequences of the two primers used to amplify IL-17A and IL-17F genes	**27**
2-4	Registered SNPs in IL-17A exon 1 and IL-17F exon 3	**30**
2-5	PCR components for the amplification of coding sequences in IL-17A and IL-17F gene	**35**
2-6	PCR amplification program for both IL-17A primers and IL-17F primers	**35**
3-1	The detected registered variations and genotype frequency within the targeted sequence in IL-17A gene exon 1 in CML and healthy	**50**
3-2	The detected unregistered variants and genotype frequency within the targeted sequence in IL-17A gene exon 1 in CML and healthy	**52**
3-3	The detected SNPs that are with frequency exactly as in ensembl Data Base	**53**
3-4	The detected registered variants and genotype frequency in CML and healthy within the targeted sequence in IL-17F gene exon 3	**54**
3-5	Serum level of IL-17A and IL-17F in CML and healthy	**55**
3-6	Total and differential leucocyte count (mean ± SE) in CML and healthy	**57**
3-7	CD4+ and CD8+ count (mean ± SE) in CML patients and healthy	**58**

List of Figures

No.	Title	Page
1-1	Age-specific incidence rates of chronic myeloid leukemia in the period 2007-2011	11
1-2	The Ph chromosome in CML and associated molecular abnormalities	12
1-3	Mechanism of action for Tyrosine kinase inhibitor	17
2-1	Standard curve IL-17A	38
2-2	Standard curve of IL-17F	42
3-1	Distribution of CML patients according to Gender	44
3-2	Distribution of CML patients according Age	45
3-3	Genomic DNA bands visualized under UV after staining with ethidium bromide on 1% agarose gel at 100 volt for 15 minutes	46
3-4	PCR product electrophoresed on 2% agarose gel 70 volt/hr, showing clear 251 bp bands of IL-17A exon-1 for 10 samples. Marker used in this gel is (100 bp)	47
3-5	PCR product electrophoresed on 2% agarose gel 70 volt/hr, showing clear 355 bp bands of IL-17A exon-1 of 6 samples. Marker used in this gel is (100 bp)	47
3-6	Sequencing result of IL-17A gene exon 1 in CML and healthy.	48
3-7	Sequencing result showing unregistered variations in IL-17A gene exon 1 in CML and healthy.	53
3-8	Sequencing result showing variants in IL-17F in CML and healthy.	55
3-9	Flow cytometer results of CD4+ and CD8+ in newly diagnosed CML subjects.	59
3-10	Flow cytometer results of CD4+ and CD8+ in non-complete treatment CML subjects.	59
3-11	Flow cytometer results of CD4+ and CD8+ in healthy subjects.	60

List of Abbreviations

Abbreviation	Name
Bp	Base Pair
CD	Cluster of Differentiation
CML	Chronic Myeloid Leukemia
ELISA	Enzyme-linked Immunosorbent Assay
G-CSF	Granulocyte Colony-Stimulating Factor
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HRP	Horse Radish Peroxidase
IL	Interleukin
NK cell	Natural Killer Cell
PCR	Polymerase Chain Reaction
SNP	Single-Nucleotide Polymorphisms
TK	Tyrosine Kinase
TNF	Tumor Necrosis Factor



Introduction and Literature Review

1. Introduction and Literature Review.

1.1. Introduction.

Leukemia is a type of cancer, which is defined as an accumulation of hematopoietic cells in lymph or blood stream (Kindt *et al.*, 2007). Leukemia is ranked in Iraq as the third among other ten types of cancer types in 2011 (Ministry of Health, Iraqi Cancer Board, 2011). Leukemia was occurred when there is a lymphoid lineage malfunction, which the immune cells T-cells (such as CD4+ T cells, CD8+ T cells), B-cells, dendritic cells, and natural killer cells. Leukemia can be classified into acute and chronic leukemia, and myeloid or lymphoid (Hoffbrand *et al*., 2006).

Chronic myeloid form of leukemia is one important type of leukemia, in which is resulted from acquired chromosomal abnormality, by reciprocal translocation of a small part of the long arm of chromosome 9, to the long arm of chromosome 22 (Mughal and Goldman, 2004).

Cytokines are low molecular weight soluble proteins have a fundamental role in communication within the immune system and involved in a wide array of biological activities specially that regulate growth, differentiation and activation of immune cells (Chokkalingam *et al*., 2013).

IL-17 is proinflammatory cytokine and produced by Th17 cells and other cells: CD8+ T cells, γδT cells, invariant NKT cells, mast cells, and granulocytes. IL-17 has pleiotropic functions and multiple targets, and has pro- and anti-tumor actions. Functions of IL-17 to cancer include angiogenesis, granulopoiesis, osteoclast induction, and induction of cytokines such as IL-6, TGF-β, granulocyte colony-stimulating colony-stimulating factor (GM-CSF) as well as matrix metalloproteinase and intercellular adhesion molecule-1 in a variety of cell types, including bone marrow stromal cells (Iwakura, *et.al*. 2011).

Gene expression of cytokines and cytokine receptors is tightly regulated and aberrant expression has been implicated in susceptibility to a range of infectious diseases and some cytokine Single-Nucleotide Polymorphisms (SNPs)

have been demonstrated to be important in altering expression or function of the cytokine gene. Genetic alterations in cytokine genes may lead to high or low production of certain cytokines that may influence native antitumor immune responses or tumor progression by acting on pathways of tumor angiogenesis (Kaur *et al.,* 2012).

Aims of study

The present study was designed to shed light on the following aims:

1- Investigation of genetic variants in IL-17A and IL-17F genes in chronic myeloid leukemia.
2- Estimation IL-17A and IL-17F serum levels in CML patients to detect variation that is related to genes polymorphisms.
3- Immunophenotypic evaluation of CD4 and CD8 markers.

1.2. Cancer

Cancer is a disorder or a disease that is known with abnormal cells that divide continuously and in uncontrolled way. Cancer cells can be spread into other organs or places in body, by invasion, which means direct growth into adjacent tissues, or it can be implant into further places rather than adjacent tissues, through a mechanism called Metastasis, in which cancer cells are transported through blood in blood vessels, or by the lymphatic system (Blachford, 2002).

Cancer continues to be a worldwide killer, despite the enormous amount of research and rapid developments seen during the past decade. According to statistics, cancer accounts for about 23% of the total deaths in the USA and is the second most common cause of death after heart disease (Jemal *et al.,* 2007).

There are two types of cancer according to the nature of malignant cell, solid tumor and soft cancer. Solid tumors usually consist of a mass of malignant and nonmalignant cells with a large number of blood veins around them such as stomach cancer, while soft cancer is defined with malignant single cells like Leukemia, which is the highest mortality rates among other types of cancers (Schrck, 2007).

1.3. Leukemia.

1.3.1.Definition.

It's a malignant disorder of hemopoetic cells that inhibits the normal production of blood cells (Debra, 2005), and characterized by an abnormal proliferation (production by multiplication) of leukocytes. It is part of a broad group of diseases called hematological neoplasms (Goldman, 1998). Leukemia general primary signs can be attributed to other diseases such as: low grade fever, chills, night sweats and other flu-like symptoms, weakness, swollen or bleeding gums, neurological symptoms (headaches), enlarged liver and spleen, frequent infection, bone pain, joint pain, dizziness, nausea, swollen tonsils, diarrhea ,paleness and weight loss (Kurzrock *et al.,* 2003). Excessive number of cells can

also interfere with the level of other cells, causing a harmful imbalance in blood cells count, such as: low count of platelets and red blood cells (Connor, 1984; Goldman, 1998 and Vincernt *et al.*, 2004).

1.3.2. History of Leukemia.

Leukemia was first observed in 1845, by the pathologist Rudolf Virchow (Virchow, 1845). He noticed a large number of abnormal white bold cells in a blood sample of a patient. Virchow termed this condition *Leukämie* in Dutch, a term composed of two Greek words, *Leukos*, which means white, and *aima*, meaning blood (Patlak, 2010).

Around ten years after Virchow’s findings, a pathologist named Franz Ernst Christian Neumann found that one diseased leukemia patient’s bone marrow have different color, dirty green-yellow, rather than a normal bone marrow color, that is red. This finding led Neumann to conclude that the abnormal blood of leukemia patients was because of a defect in the bone marrow (Eyal and Attar 2010).

1.3.3. Classification of Leukemia.

Leukemia is subdivided clinically and pathologically into many large groups. One type of dividing is to make two types of leukemia, Acute and Chronic.

- Acute Leukemia is characterized by a rapid increase in the immature blood cells amount, in which lead to a defect in the production of new healthy blood cells in bone marrow. Moreover, progression and accumulation of malignant cells; immature blood cells, will make them later to spill over into blood stream, and then to be spread in the body. Forms of acute leukemia are commonly found in children (Jameson 2005).

- Chronic Leukemia is characterized by the excessive buildup of relatively mature, but still abnormal white blood cells. Typically taking months or years to progress (Sawyers *et al.*, 2002, Gorer *et al.,* 2007). The cells are produced at a much higher rate than normal, resulting in many abnormal

white blood cells (Rai *et al.,* 1975). Chronic leukemia mostly occurs in older people, but can theoretically occur in any age group (Binet *et al.,* 1981).

Other form of division split the leukemia into either Lymphoblastic or Lymphocytic leukemia, in which cancerous mutations occur in a type of bone marrow cell responsible of originating lymphocytes, specially B cell, or myeloid or myelogenous leukemia, which the mutation occur in the marrow cells that are responsible for red blood cells formations, some types of white blood cells, and platelets (Jameson *et al.,* 2005).

In General, there are four major types of leukemia:

a. Acute Lymphocytic Leukemia (ALL):

This type of leukemia is the most familiar one in children, but it also can be diagnosed in older adults, those ages 65% and older. Rates of survival are different according to the age, starting from 85% of surviving chance to children, to 50% in adults (Bennett *et al.,* 1989, Harrison 2007). This form of leukemia can be occurring in blast cells of bone marrow (B cells), thymus (T-cells), and lymph nodes. It begins with immature lymphocytic blood cells, progresses very fast, and accumulates in bone marrow, leading to stop it from making new blood cells properly (Harris *et al.,* 1994).

b. Chronic Lymphocytic Leukemia (CLL):

Chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. It is the most common form of leukemia found in adults in Western countries (Elter *et al.,* 2006). This form of leukemia appears mostly in adults those over the age of 55 years, and in a less rate of younger adults, but it is almost never appeared in children (Matutes *et al.,* 1994). Most of the patients are men, a ratio that reach two of three patients. The five-year survival rate is 75% (Rai *et al.,* 1975).

c. Acute Myelogenous Leukemia (AML):

It occurs more commonly in adults than in children, and more commonly in men than women. AML is treated with chemotherapy. The five-year survival rate is 40% (Colvin and Elfenbein, 2003). An activation of Ras-oncogene due to a mutation in its coding sequence is one reason of this kind of leukemia (Bos *et al.,* 1985).

d. Chronic Myeloid Leukemia (CML):

Chronic myeloid leukemia (CML), a clonal myeloproliferative disorder, has its origin in the neoplastic transformation of primitive hematopoietic stem cells (Fialkow et al., 1997). CML results from an acquired (not present at birth) or a genetic injury to the DNA of a single bone marrow cell. The mutated cell multiplies into many cells (CML cells). The result of the uncontrolled growth of CML cells in the marrow is an increase in the number of CML cells in the blood (Hirji *et al.,* 2013).

1.3.4. Leukemia Causes:

There is no single certain cause of leukemia (Kurzrock *et al.,* 2003). In General, leukemia is like other types of Cancer, result from a mutations. Some mutations can trigger leukemia by either activating some oncogenes, or in an opposed way, deactivating tumor suppressor genes, such as P^{53}. This action will lead to a major disrupting of the regulation of many cellular operations, like cell death, cell differentiation, or cell division. These mutations may occur spontaneously or a result of exposure to a carcinogenic factor, such as chemicals and radiation (WHO 2002).

There are some causes of leukemia, like ionized radiation, some types of viruses, and chemicals (Stass *et al*., 2000, Robinette *et al*., 2001, Wiernik 2001).

- **Ionized Radiation:** For a dangerously long period, however, the correlation between radiation exposure and leukemia incidence and mortality was merely anecdotal. Significant evidence only began emerging in Life Span Studies following cohorts of Japanese atomic bomb survivors and patients receiving high doses of therapeutic radiation for cervical cancers, tinea capitis, and ankylosing spondylitis (Hall, *et al*., 2012; Preston *et al*., 1994). After Chernobyl disaster, many researches started to study patients of leukemia of the disaster, trying to get full information about age dependence, doses, and latencies (Noshchenko, *et al*., 2010; Ivanov, *et al*., 1997). These studies led to the identification of salient features common to all ionizing radiation-induced leukemia. Results came with most of adult patients observed with AML and CML, younger children, with 5 to 9 years old, are more likely to develop ALL, while older ones are more susceptible to AML (Preston, *et al*., 1994; Little, *et al*., 2009). Interestingly, the incidence of CLL does not seem to be influenced by radiation (Hall, *et al*., 2006).
- **Viruses:** Many studies on mice and some mammals regarding viruses causing leukemia demonstrated that many retroviruses can cause leukemia. One of these viruses is human T-lymphotropic virus, or HTLV-1, which is known to cause adult T-cell leukemia (Leonard 1998**).** Human immunodeficiency virus (HIV) played a role in causing leukemia in 3% of all reported AIDS cases and in HIV-positive hemophiliacs (Beral *et al.,* 1991, Ragni *et al.,* 1993). In cell, oncogenes are found but in an inactive status, but some viruses, such as *Rous sarcoma* viruses can trigger these oncogenes to be activated, and cause leukemia (Barbacid, 1987).
- **Chemicals:** Chronic exposure to some chemicals in the environment, have reported to be a factor that can cause leukemia (Hagstedt *et al*., 1986). Workers those who work in an environment with benzene, petroleum derivatives, solvents, herbicides, and pesticides, have a risk of 20% of

having leukemia (Saracci *et al*., 1991). Agricultural chemicals are also linked with leukemia. For instance, the tobacco smoke active compounds, such as benzene, polonium-210, and polycyclic aromatic hydrocarbons, can induce acute myeloid leukemia in the body, in a rate of one of four patients (Ardito *et al.*, 1980, Severson 1987, and Sylvia 1998).

- **Genetics:** Some genetic studies on a number of leukemia patients have suggested that a few genes to be mutated are only needed to develop leukemia, or can maintain the growth of malignant cell (Mittleman and Heim, 2007). In some cases, some families may tend to have the same kind of leukemia as other members of the same family, while in other families they tend to have a different type of leukemia (Elaine *et al*., 2001).
- **Chromosomal Abnormalities:** It is believed that chromosomal abnormalities play a role in causing leukemia; for example, people suffering of Down syndrome have a tendency of having acute leukemia, especially AML (Roninette *et al*., 2001). In fact, children with Down syndrome have a leukemia risk increased 15 times (Miller, 1967 and Rowley, 1981).
- **Other causes:** A few cases of maternal-fetal transmission have been reported (Koren and Lishner, 2010). A study in France has revealed that mothers, who use some fertility drugs that induce ovulation, give children that are twice as likely to develop leukemia in their childhoods that other children (Rudant *et al*., 2012).

1.3.5. Leukemia Diagnosis.

The basis of leukemia diagnosis is repeated complete blood counts, or a bone marrow examination. These two tests are done if the symptoms were observed. However, in some rare case, the blood tests may not give an accurate results of leukemia, or do not show if the patient have leukemia. This happen in case of a very early stage of leukemia, or leukemia entered a remission stage.

Other tests like lymph node biopsy are also can be used in order to diagnose and examine some certain types of leukemia in specific situations (Abbott 2005)

Following diagnosis, other more specific tests are used, such as blood chemistry tests, to determine the degree of damage of liver and kidney, beside the effects of chemotherapy on the patient like reticulocytes count, examination of the peripheral blood smear, iron studies including Fe (Ferrous) and ferritin, erythropoietin level and bone marrow biopsy with cytogenetic. If a visible damage occurs due to leukemia, doctors may use X-ray, MRI, to view the effects of leukemia in bones and brain respectively. Other body organs, like liver, kidneys, and spleen, can be view by the use of ultrasound. Lymph nodes in chest can be checked rarely by the use of CT scans (Eyal and Attar 2010).

1.4. Chronic Myeloid Leukemia (CML).

Generally, it is a clonal disease that results from an acquired genetic change in a pluripotential haemopoietic stem cell (Goldman*,* 1998). It occurs mainly in adults, but though, there was a small number of affected children to this kind of leukemia. A mutant stem cell will proliferate and generates some differentiated cells that will gradually take the normal haemopoietic stem cell place, leading to an expending in the total myeloid mass (Nowell and Hungerford 1960, Buchdunger *et al.,* 2001).

1.4.1. Discovery of Chronic Myeloid Leukemia.

The first reasonably convincing description of leukemia was reported by Velpeau in France in 1827 (Velpeau, 1827), although it is likely that forms of leukemia had been recognized as early as 1811 (Piller 1993). This was followed by the observation of Barth and Donne (Donne 1842) and of Craigie (Craigie 1845). Nevertheless, the definition of leukemia as a distinct entity is attributed to the virtually simultaneous autopsy reports in 1845 by John Hughes Bennett of a 28-

year-old slater from Edinburgh and by Rudolph Virchow in Berlin of a 50-year-old cook (Bennett 1845, Virchow 1846).

Both patients had been unwell for 1 and a half to 2 years, and their condition had progressively worsened with increasing weakness, bleeding, and other problems. In both cases, the remarkable features at autopsy were the large size of spleen and the consistency of blood, in particular the white cell content. Bennett's case may have been CML, while Virchow's was CLL.

A dispute about the priority of the discovery arose, but was eventually settled cordially, when Virchow acknowledged Bennett's priority and even wrote a letter to support his promotion (Degos 2001).

1.4.2. Chronic Myeloid Leukemia Patients.

The frequency of CML increases with age, as shown in figure (1-1) on US patients in the period 2007 to 2011, from about less than 1.2 in 100,000 people until about 40 years, to about 2.4 in 100,000 people at 55 years, to about 9.6 in 100,000 people at 80 years and older. In coming years, the incidence of CML may increase, as a sizable portion of the US population is made up of people born between 1946 and 1964. These individuals have reached, or are approaching, the age range associated with increased CML incidence (Howlader *et al.,* 2013).

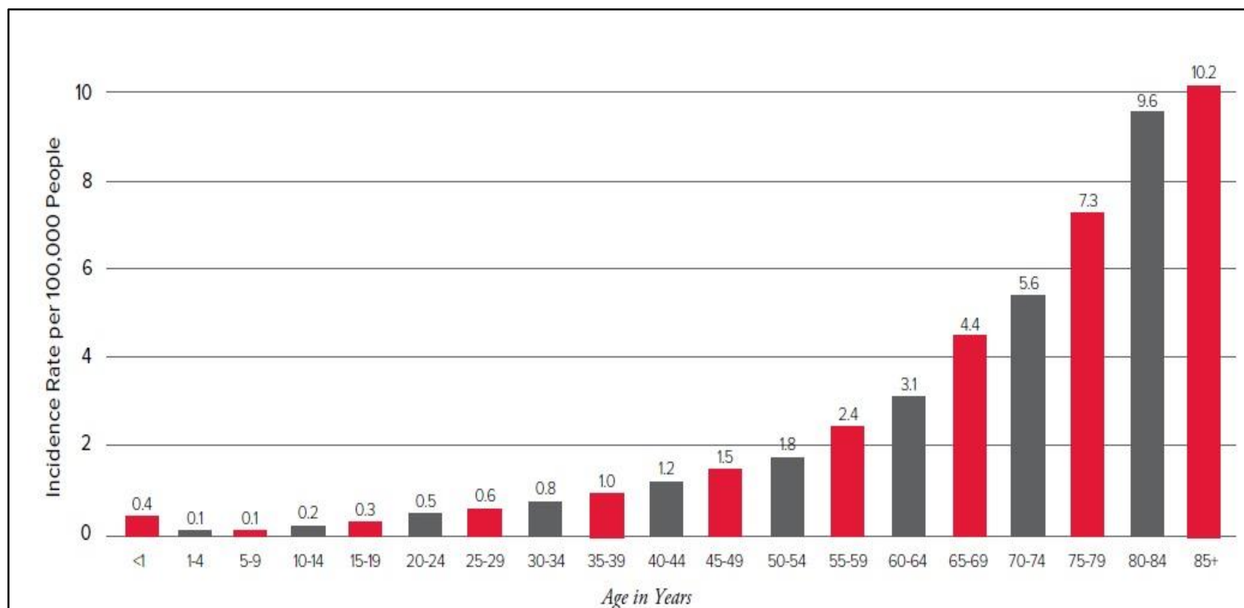


Figure (1-1): Age-specific incidence rates of chronic myeloid leukemia in the period 2007-2011 (Howlader *et al.,* 2013).

1.4.3. Physiology.

In 1960, two doctors from the University Of Pennsylvania School Of Medicine in Philadelphia have discovered the 22nd chromosome abnormality in people with CML was shorter than it was in healthy people, they are Peter Nowell of the University of Pennsylvania and David Hungerford of Fox Chase Cancer Center (Nowell 2007). This shortened 22nd chromosome was later named the "Philadelphia chromosome" or "Ph chromosome" due to the place of the discovery.

The Ph chromosome initially described a shorter long arm of chromosome 22. It is the result of breaks on chromosomes 9 and 22, with a reciprocal translocation of the distal genetic material, t(9;22)(q34;q11) (Nowell and Hungerford 1960, Rowley 1973). This translocation transposes the c-ab1 proto-oncogene from its normal location, on chromosome 9, to a new position on chromosome 22, in proximity to the breakpoint cluster region (bcr) as shown in figure (1-2). A new hybrid BCR-ABL oncogene is formed. It produces an abnormal 8.5-kb RNA that encodes for a 2 IO-Kd (p210) fusion protein. The latter,

presumably through its increased tyrosine kinase activity, changes normal hematopoietic cells into CML cells (Cannistra 1990).

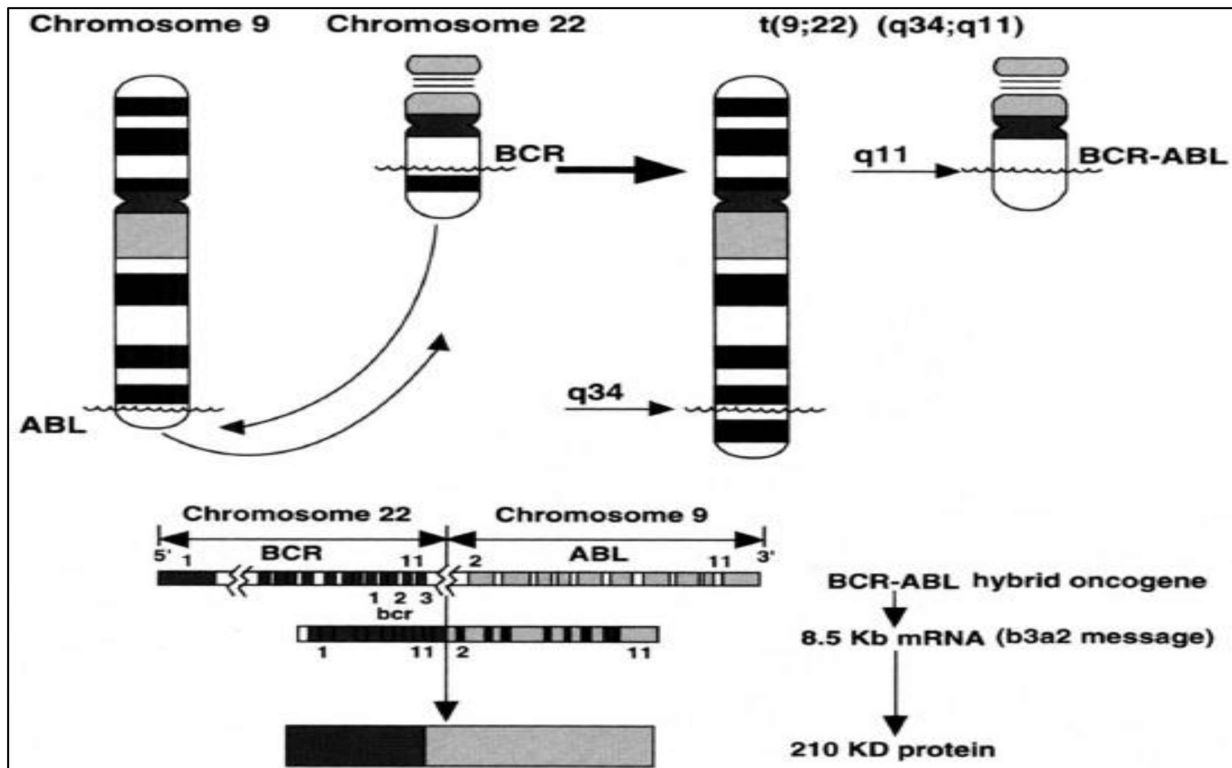


Figure (1-2): The Ph chromosome in CML and associated molecular abnormalities.

1.4.4. Diagnosis of Chronic Myeloid Leukemia.

To diagnose CML, doctors use a variety of tests to analyze the blood and marrow cells (Shah and Arceci, 2014).

A- Complete Blood Count (CBC). This test is used to measure the number and types of cells in the blood. People with CML often have

- Decreased hemoglobin concentration
- Increased white blood cell count, often to very high levels
- Possible increase or decrease in the number of platelets depending on the severity of the person's CML.

Blood cells are stained (dyed) and examined with a light microscope.

These samples show a:

- Specific pattern of white blood cells
- Small proportion of immature cells (leukemic blast cells and promyelocytes)
- Larger proportion of maturing and fully matured white blood cells (myelocytes and neutrophils).

These blast cells, promyelocytes and myelocytes are normally not present in the blood of healthy individuals.

B- Bone Marrow Aspiration and Biopsy. These tests are used to examine marrow cells to find abnormalities and are generally done at the same time. The sample is usually taken from the patient's hip bone after medicine has been given to numb the skin. For a bone marrow aspiration, a special needle is inserted through the hip bone and into the marrow to remove a liquid sample of cells. For a bone marrow biopsy, a special needle is used to remove a core sample of bone that contains marrow. Both samples are examined under a microscope to look for chromosomal and other cell changes.

C- Cytogenetic Analysis. This test measures the number and structure of the chromosomes. Samples from the bone marrow are examined to confirm the blood test findings and to see if there are chromosomal changes or abnormalities, such as the Philadelphia (Ph) chromosome. The presence of the Ph chromosome (the shortened chromosome 22) in the marrow cells, along with a high white blood cell count and other characteristic blood and marrow test findings, confirms the diagnosis of CML.

D- FISH (Fluorescence In Situ Hybridization). FISH is a more sensitive method for detecting CML than the standard cytogenetic tests that identify the Ph chromosome. FISH is a quantitative test that can identify the presence

of the *BCR-ABL* gene. Genes are made up of DNA segments. FISH uses color probes that bind to DNA to locate the *BCR* and *ABL* genes in chromosomes. Both *BCR* and *ABL* genes are labeled with chemicals each of which releases a different color of light. The color shows up on the chromosome that contains the gene—normally chromosome 9 for *ABL* and chromosome 22 for *BCR*—so FISH can detect the piece of chromosome 9 that has moved to chromosome 22 in CML cells. The *BCR-ABL* fusion gene is shown by the overlapping colors of the two probes. Since this test can detect *BCR-ABL* in cells found in the blood, it can be used to determine if there is a significant decrease in the number of circulating CML cells as a result of treatment.

E- Polymerase Chain Reaction (PCR). The *BCR-ABL* gene is also detectable by molecular analysis. A quantitative PCR test is the most sensitive molecular testing method available. This test can be performed with either blood or bone marrow cells. The PCR test essentially increases or "amplifies" small amounts of specific pieces of either RNA or DNA to make them easier to detect and measure. So, the *BCR-ABL* gene abnormality can be detected by PCR even when present in a very low number of cells. About one abnormal cell in one million cells can be detected by PCR testing.

Quantitative PCR is used to determine the relative number of cells with the abnormal *BCR-ABL* gene in the blood. This has become the most used and relevant type of PCR test because it can measure small amounts of disease, and the test is performed on blood samples, so there is no need for a bone marrow biopsy procedure.

Blood cell counts, bone marrow examinations, FISH and PCR may also be used to track a person's response to therapy once treatment has begun. Throughout treatment, the number of red blood cells, white blood cells, platelets and CML cells is also measured on a regular basis.

1.4.5. Chronic Myeloid Leukemia Phases.

CML is usually a triphasic disease, with chronic, accelerated, and blast phase (White *et al.,* 2006). The precise definition of these phases is still a subject of debate, but regardless of the definition or treatment used, patients whose CML is chronic phase do better than patients with CML in blast or accelerated phase (Kantarjian *et al.,* 2003, White *et al.,* 2007, Wang *et al.,* 2008).

A. Chronic Phase.

This phase is the initial phase of CML, lasts for 4 to 5 years, and symptoms are mild (Bonthron 1998, Pasternak 2006). The bone marrow in this phase is typically hypercellular with marked myeloid predominance (Talpaz 2002). High risk of this phase includes amount of platelets to be more than 1000×10^{9} /L before the start of therapy, while in low risk state, peripheral or bone marrow blasts are less than 10%, and peripheral or bone marrow basophils are less than 20% (Cortes *et al.,* 2006).

B. Accelerated Phase.

The definition for the accelerated phase is not uniform (Kantarjian *et al*., 1988). Specific criteria associated with a survival shorter than 18 months by multivariate analysis have been proposed, including the presence of 15% or more blasts in peripheral blood, 30% or more blasts and promyelocytes in the blood, 20% or more basophils in the blood, or a platelet count less than 100,000/µL (Kantarjian *et al.,* 1988). 75% to 80% of patients go to accelerated phase before entering blast crisis phase (Kantarjian *et al.,* 1993).

C. Blast crisis phase:

The blastic phase resembles an acute leukemia. Its diagnosis requires the presence of at least 30% of blasts in the bone marrow or peripheral blood (Kantarjian *et al.,* 1987). In some patients, the blastic phase is characterized by extramedullary deposits of leukemia called myeloblastomas (Jacknow *et*

al., 1985)**.** These usually appear in the CNS, lymph nodes, or bones and occasionally occur in the absence of blood or bone marrow evidence of blastic transformation. Most of these cases, however, will have hematologic manifestations within a few months. Patients in the blastic phase usually die within 3 to 6 months. Approximately 50% of patients have a myeloid blastic phase, 25% lymphoid, and 25% undifferentiated (Terjanian *et al.,* 1987).

1.4.6. Therapy.

A. Interferon-α

In the early 1980s, IFN-alfa was introduced into the therapy for CML following observations of in vitro inhibition of myeloid colony formation when normal or CML progenitors were cultured in its presence (Verma *et al.,* 1979). The first reports in humans used partially pure IFN-alfa to treat 51 patients in chronic phase. More important, however, was the fact that cytogenetic responses (ie, suppression of the Ph-positive clone) were observed in 39% of patients (Talpaz *et al.,* 1987).

B. Antimetabolites therapy.

Busulfan and Hydroxyurea were used as a therapy for CML for a long time. Busulfan therapy was always related to lung, marrow, and heart fibrosis. The dose of busulfan is usually 0.1 mg/kg/d until WBC counts decrease by 50%, and then the dose is reduced to 50%)**.** Hydroxyurea is a less toxicity, but with shorter control of hematologic manifestations, in which need more to follow-up (Rushing *et al.,* 1982). It is given at a dose of 40 mg/kg/d and then it is reduced to 50% when WBC count drops to be below 20,000/µL, and then it is adjusted individually to keep WBC count at 5,000/µL to 10,000/µL. A study to compare between the two drugs showed that hydroxyurea has a median duration of chronic phase of 47 months, and a longer overall survival of 58 months, while busulfan has 37 months, and 45 months respectively (Hehlmann *et al.,* 1993).

C. Tyrosine Kinase Inhibitors (TKI).

Current standard therapy for chronic phase of Chronic myeloid leukemia (CML) is the chronic oral administration of tyrosine kinase inhibitor (TKI) drug (Baccarani *et al.,* 2013)**.** They are drugs that target the abnormal BCR-ABL protein "tyrosine kinase", that is located on or near the surface of cells, and inhibit (block) this protein from sending the signals that cause the growth of abnormal cells as shown in figure (1-3) (Abruzzese *et al.,* 2014).

Tyrosine kinase inhibitors are effective in the targeted treatment of various malignancies. Imatinib was the first to be introduced into clinical oncology, and it was followed by drugs such as gefitinib, erlotinib, sorafenib, sunitinib, dasatinib , and some others (Hartmann *et al.,* 2009).

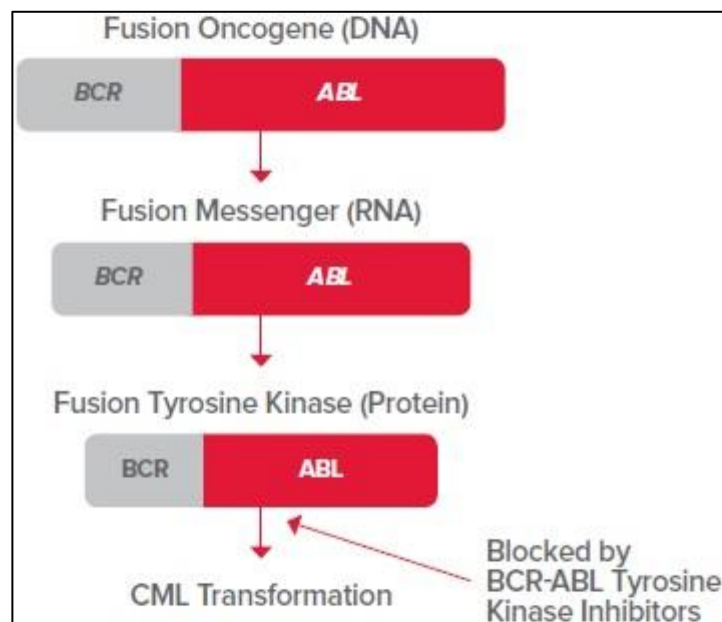


Figure (1-3): Mechanism of action for Tyrosine kinase inhibitor (Abruzzese *et al.,* 2014).

1.4.7. Imatinib.

In 2001, Imatinib mesylate (known as Gleevec or Glivec) was approved by the U.S. Food and Drug Administration (FDA) for treating CML patients (Druker *et al.,* 2001)**.** The rate of complete cytogenetic response among patients receiving

imatinib was 87% after 5 years of treatment (Druker *et al.,* 2006). Since the advent of Imatinib, CML became the first type of cancer that a standard medical treatment may give a normal life to the patient (Gambacorti-Passerini *et al.,* 2011). Despite of its ability to stop BCR-ABL kinase activity and increases rates of survival of CML patients, imatinib still doesn’t lead to a cure of CML, because BCR-ABL expression leukemia cells can still be noted in complete cytogenetic remission patients. One explanation of this status is that these surviving leukemia cells were not dependent on BCR-ABL kinase activity (Holyoake *et al.,* 1999, Graham *et al.,* 2002).

This kind of CML patients are expected most likely to take imatinib for the rest of their lives (Rousselot *et al.,* 2007**).** This fact made it apparent that imatinib treatment alone for such patients could not cure CML (Graham *et al.,* 2002).

1.5. Cytokines and Interleukins.

Cytokines are low molecular weight protein mediators involved in cell growth and differentiation, inflammation, immunity, and repair (Nicod 1993). They are soluble proteins or glycoproteins that are produced from some kinds of leukocytes to serve as chemical communicators from one cell to another (Julius 2004). They differ from endocrine hormones, which act throughout the body, by their locally limited acting, near by the cells that produce them (Narins 2003).

Cytokines are produced by immune cells like B lymphocytes, activated T lymphocyte, macrophage, mast cells, and natural killer cell, as well as fibroblast, endothelial cells, and various stromal cells (Mantovani and Dejana, 1989**).** Cytokines include chemokines, interferons, interleukins, lymphokines, tumour necrosis factor (Ma *et al.,* 2015).

Interleukins (ILs) are immunemodulatory proteins that induce many cellular and tissue responses. ILs elicit a response to the target cell by high-binding-affinity receptors on the cell surface**.** ILs can act as an autocrine or a paracrine, rather than enocrine, which is common with hormones. Unlike chemokines and interferons

(IFNs), in which chemokines direct immune cells to the site of inflammation, and IFNs initiate cellular responses to viral infection, ILs main functions is to modulate growth, differentiation, and activation of cells in the immune response (Commins *et al.,* 2010**).** ILs can induce two opposite actions, for instance, they can exert both inflammatory and anti-inflammatory. Some of them work as chemoattractants for T helper cells, just like chemokines. Others are involved in actions familiar to IFNs, helping in initiating the cellular response to viral pathogens (Brocker *et al.,* 2010).

1.5.1. IL-17 Family.

A. IL-17 Family Review.

IL-17 (or IL-17A) is a proinflammatory cytokine that is produced by activated T cells (Yao *et al.,* 1995, Fossiez *et al.,* 1996). It is secreted as a disulfide-linked homodimeric glycoprotein with a dimer molecular weight of 30-35 kDa (Fossiez *et al.,* 1996).

IL-17A was first identified from a rodent as a cDNA transcript isolated from an activated T-cell hybridoma, and named CTLA8 (Rouvier *et al.,* 1993**).** It was noted that this factor can act as an activator and promoter of other cytokines and chemokines, such as IL-6, IL-8, and granulocyte-colonystimulating factor (G-CSF) from a variety of endothelial, epithelial, and fibroblastic cell types. This cytokine was unique comparing to other ILs, in which no similarity to other known ILs. Moreover, a receptor that binds IL-17A was isolated and it was found that it is not relate to any other cytokines receptors, and required for the signaling of IL-17A, it was termed IL-17 receptor (IL-17R**).** Thus, the IL-17 system appeared to be a distinct and potent signaling system involved in the control of the immune response.

The large-scale sequencing of expressed sequence tags and genomes of several vertebrate species has led to the identification of additional genes that bear clear homology to IL-17 and thus define an emerging cytokine family (Li *et al.,*

2000, Lee *et al.,* 2001**).** There are six members, including IL-17A, of the IL-17 family dispersed throughout the human genome. They are IL-17B, IL-17C, IL-17D, IL-17F, and IL-25(IL-17E) (Brocker *et al.,* 2010).

First, IL-17A and IL-17F were reported to be both predominantly expressed in activated T cells. Linking IL-17A-producing CD4+ T cells and IL-17F-producing CD4+ T cells to IL-23 effector function led to the concept that Th17 cells belong to a distinct CD4+ T cell subset (McGeachy and Cua, 2008). There are many cytokines that control the process of differentiation of naïve CD4+ T cell to Th17 cell. These cytokines including TGF-β, IL-6, and IL-21, which activate Stat3- and IRF4-dependent expression of retinoic acid receptor-related orphan receptor-gt (RORgt) (Hirahara *et al.,* 2010; Korn *et al.,* 2009; Zhou and Littman, 2009). Other transcription factors that play a role in regulation of Th17 differentiation in corporation with RORgt are RORa, basic leucine zipper transcription factor (Batf), Runx1, and IkBz (Iwakura *et al.,* 2011).

Both IL-1 and IL-23 are also critical for Th17 cell differentiation, growth, survival, and effector functions. In humans, IL-1 β, IL-21, IL-23, and TGF- β are required for the development of Th17 cells expressing IL-17A, IL-17F, IL-22, and RORgt, although the requirement for TGF- β in human Th17 cell development still remains elusive (Korn *et al*., 2009). The mechanisms that regulate IL-17A and IL-17F production also differ; IL-17F is expressed earlier than IL-17A during Th17 cell development (Lee *et al.,* 2009). A deficiency of RORa will lead to a remarkable reduction in IL-17A production (Yang *et al*., 2008).

T cell receptor (TCR) is also have a role in IL-17A production as a mediator, in which it was found that IL-17A expression was more sensitive to the strength of TCR signaling (Gomez-Rodriguez *et al.,* 2009).

In addition to Th17 cells, IL-17A and IL-17F are both can be produced from a wide variety of T cells. For instance, CD8 T cells (Tc17) can also produce these cytokines under conditions that are similar to those required for Th17 in order to produce IL-17A and IL-17F. However, these conditions are much different from

those needed to produce IFN-g producing CD8+ T cells (Tc1). Others cells producing these cytokines are distinct population of gdT cells (gd-17) and Natural Killer T cells (NKT-17) (Cua and Tato, 2010).

B. Biological Function of IL-17 Family.

IL-17 biological actions are proinflammatory in character. The family members increase chemokines local production of, such as IL-8 (Fossiez *et al.,* 1998; Spriggs, 1997), monocyte chemoattractant protein-1 (MCP-1) (Woltman *et al*., 2000, Van Kooten *et al.,* 1998), leading of promoting monocytes and neutrophils recruitments (Jovanovic *et al.,* 2001; Fridman and Tartour, 1998). Furthermore, they stimulate the production of the hematopoietic cytokines G-CSF and granulocyte macrophages (GM)-CSF which promote the expansion of these myeloid lineages (Linden *et al*., 2000; Cai *et al.,* 1998). Also, some other actions such as the stimulation of IL-6 and PGE2 production, enhance the local inflammatory environment (Fossiez *et al.,* 1996; Yamamura *et al.,* 2001). Moreover, IL-17 also drives T-cell responses, notably through the induction of the costimulatory molecule intercellular adhesion molecule (ICAM) (Albanesi *et al.,* 1999; Teunissen *et al.,* 1998).

IL-17B is expressed in several peripheral tissues, as well as immune tissues (Li *et al.,* 2000). IL-17C expression is highly regulated in inflammatory conditions. IL-17D appears to be highly expressed in skeletal muscles and nervous system. IL-17E is expressed in low amounts in peripheral tissues (Lee *et al.,* 2001). IL-17F is also expressed in low amounts, and is clearly detectable in activated population of T cells (Hymowitz *et al.,* 2001, Starnes *et al.,* 2001).

1.5.2. IL-17A and IL-17F properties.

IL-17A and IL-17F are both located in adjacent places in chromosome 6p12 (Hymowitz *et al.,* 2001). They both share a high similarity in their amino acid identity, with 44% of similarity, while the other IL-17 family members share 15-

27% of amino acid similarity (Aggarwal and Gurney, 2002), as shown in table (1-1), suggesting that both IL-17A and IL-17F form a distinct subgroup within the IL-17 family.

Table (1-1): Amino acid sequence homology between human IL-17 family (Aggarwal and Gurney, 2002).

	IL-17A	IL-17B	IL-17C	IL-17D	IL-17E
IL-17B	25.9%				
IL-17C	26.4%	24.9%			
IL-17D	20.3%	26.1%	22.3%		
IL-17E	25.0%	18.3%	26.3%	21.2%	
IL-17F	45.7%	25.0%	25.5%	20.6%	19.8%

IL-17A and IL-17F can both be secreted as disulfide-linked homodimers or heterodimers. Thus, these two molecules are likely to have similar biological activities (Iwakura *et al.,* 2008; Reynolds *et al.,* 2010). IL-17A and IL-17F are both involved in the inflammation development and host defense against infection by inducing the expression of genes encoding proinflammatory cytokines such as tumor necrosis factor (TNF), IL-1, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage colony-stimulating factor (GM-CSF), chemokines (CXCL1, CXCL5, IL-8, CCL2, and CCL7), antimicrobial peptides (defensins and S100 proteins), and matrix metalloproteinases (MMP1, MMP3, and MMP13) from fibroblasts, endothelial cells, and epithelial cells (Iwakura *et al.,* 2011).

IL-17F has also been shown to induce transforming growth factor-beta (TGF-β) expression in human umbilical vein endothelial cells and decrease their ability to undergo capillary tube formation, suggesting a potential ability to inhibit angiogenesis (Starnes *et al.,* 2001). IL-17A is able to stimulate nuclear factor κB (NFκB), a transcription factor that contributes to the signal transduction of several important proinflammatory molecules including TNF, IL-1, and Toll-related

receptors (Cao *et al.,* 1999, Schuster and Nelson, 2000). IL-17A was reported to have an action in regulation of the activities of p44 and p42 extracellular regulated kinase (ERK) 1, ERK2, c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinases (MAPKs) (Shalom-Barak *et al.,* 1998, Awane *et al.,* 1999).

1.6. Cluster of Differentiation Markers (CDs).

CD-Markers are used for the determination the identity of cell types based on the protein express on their cell surface. Using these markers, cells can be separated and stored based on their cell surface protein by application of flow cytometry . Depending on cell type, on marker alone or a combination of marker con be used to isolate a particular cell (Chia *et al*, 2010).

The CD-markers are glycoprotein characterized in two population of lymphocytes (T and B –lymphocyte) and Natural killer (NK) cells. Most T helper cells express CD4,whereas most T- cytotoxic cells express CD8, NK cells express CD16 and CD5 and B cells express CD19,CD21,CD32 and CD35 (Ferreira *et al.*,2010). The CD4 cells involved via the regulation of the immune response and the T-CD8 cells have suppressive and cytotoxic activity. T- cell function involves the respective recognition of CD4 and CD8 by class II and class I MHC molecules, respectively which represent their natural ligands. The flow cytometry as well as alternative evaluation methods such as immunoperoxidase staining and immunofluorescent of lymphocytes enumeration are based on the immunological detection of the markers of the cell surface with labeled specific monoclonal antibodies (Saleh, 2012).

The flow cytometry gives access to the percentage of CD4+ and CD8+ cells. The number of TCD4 and TCD8 decrease from birth and stabilize in the healthy adult. A mean value of a normal CD4 and CD8 count ranges from 500-1,000 cells/mm3. When the CD4 or CD8 count is 350 cells/mm3 or less, it’s time to consider treatment and when the count is fewer than 200 cells/mm3 is one of the

qualifications for a diagnosis of AIDS. The count can vary from day to day. It can also vary depending on the time of day and on the infections or illnesses.

However, CD4 and CD8 percentage is a more accurate measurement of the immune function, so the percentage that is greater than 29% usually means that the immune system is functioning normally, which mean that the CD4 count is roughly > 500 cell/mm3). The percentage of (14-28%) typically means the count is in the range of 200-500 cells/mm3. When the count is below 200 cells/mm^3, the percentage is likely to be below 14% (Riemann *et al.,* 2000).

Chapter Two

Subjects, Materials and Methods

2. Materials and Methods.

2.1. Subjects.

Chronic Myeloid Leukemia Blood samples were collected from 62 male and female CML patients, age range of 21 – 73 years old. The samples were collected during the period January 2015 to October 2015, from three different places: Oncology Teaching Hospital-Baghdad Medical City, Baghdad Teaching Hospital– Baghdad Medical City, and The National Center for Blood Diseases – Al-Mustansiriya University.

Twenty out of 62 CML patients were newly diagnosed CML cases, 12 males and 8 females, with an age range of 25 - 37 years old (according to physicians consultant)

The rest 42 were non-completed treatment CML patients. They were with an age range of 21 - 73 years old, with 26 males and 16 females. These Patients were in Chronic Phase of CML, and in the 5rd month of treatment with Gleevec 400mg (Imatinib Mesylate) provided by Novartis Pharmaceuticals Corporation, Hanover, Germany.

Twenty Eight blood samples were collected from healthy persons, taken from volunteers from University of Baghdad. Their age range was 24 - 72 years old, and they were 18 males and 10 females.

2.2. Materials.

2.2.1. Equipments.

The equipments used and their sources are given in table (2-1)

Table (2-1): The equipments used in this study.

Name of Equipment	Company	Origin
Balance	MettlerAE240	Swiss
Blood collection plain Tubes	AFMH	England

Blood collection plastic can tubes	AFMH	England
Blood collection pyrex test tubes	AFMH	England
Centrifuge	Hettich	Germany
EDTA containing tubes	AFMH	England
Eppendorff bench Centrifuge	Hermle	Germany
Eppendorff tube	Eppendorf	Germany
Flow cytometry	Apogee	England
Gel electrophoresis system	Major Science	Japan
Gel tubes	Ardh Al-Rafidain Labs.	Iraq
Micro ELISA system (reader)	Thermo	Germany
Micropipette(Automatic)	Eppendorf 0.5-10, 5-20, 20-200, 1000 μL	Germany
Thermal Cycler (Veriti)	AB Applied Biosystems	Singapore
Plastic disposable Syringes	Meheco	China
Refrigerator (4°C) and freezer (-18°C)	Beko	Turkey
Sensitive balance	Sartorius	Germany
Tips	JRL	Lebanon
U.V transilluminater	LKB	Sweden
Volumetric cylinders	Volac	England
Volumetric Flasks	Volac	England
Vortex	Clay Adams	Germany
Water bath	Memmert	Germany

2.2.2. Chemicals and Solutions.

The chemicals used and their sources are given in table (2-2):

Table (2-2): Chemicals used in this study.

Name of chemicals	Company	Origin
Absolute ethanol	Fluka	Germany
Absolute Isopropanol	Sigma	USA
Agarose	Promega	USA
DNA ladder marker (100 bp)	Promega	USA
Ethidium Bromide	BioNeer	Korea
Free nuclease distilled water	BioNeer	Korea
Loading dye	Promega	USA
PCR master mix	Promega	USA
Primers	Alpha DNA	Canada
TBE buffer (10x) (Tris-Borate EDTA)	Promega	USA

2.2.3. Primers.

Specific primers, created by Alpha DNA Company, were used to amplify to certain fragments IL-17A gene exon 1, and IL-17F gene exon 3, and are shown in table (2-3).

Table (2-3): Sequences of the two primers used to amplify IL-17A and IL-17F genes.

Gene	Exon	Primer	Sequence	Product Size
IL-17A	Exon 1	Forward	**5'-GGTGTCACCCCTGAACCCAC-3'**	251
		Reverse	**5'-CGTTTCATGCCCACGGTCCA-3'**	
IL-17F	Exon 3	Forward	**5'-GGCTCTCATTCGCTGTCTTC-3'**	355
		Reverse	**5'-GGGTAAGGAGTGGCATTTCTA-3**	

2.2.4. Kits Reagents.

A. DNA isolation Kit.

DNA was isolated from samples by using ***AccuPrep***® Genomic DNA Extraction Kit, BiONEER Company, Korea. The kit contents are:

- Proteinase K, lyophilized 25 mg X 2 vial.
- Tissue Lysis buffer (TL) 25 ml.
- Binding buffer (GC) 25 ml
- Washing buffer 1 (W1) 40 ml
- Washing buffer 2 (W2) 20 ml
- Elution buffer (EL) 30 ml: 10 mM Tris-Cl (pH 8.5).
- 1.5 and 2 ml tubes, and Binding column tubes.

B. IL-17A estimation Kit.

abcam® IL-17A (Interleuking-17A) Human ELISA Kit, USA, was used to measure the amount of IL-17A in the serum of the patients. The kits contents are:

- 10X IL-17A Capture Antibody 2 x 300 µL
- 10X IL-17A Detector Antibody 2 x 300 µL
- IL-17A Human Lyophilized Recombinant Protein 2 Vials
- Antibody Diluent 5BI 2 x 3 mL
- 10X Wash Buffer PT 20 mL
- 5X Cell Extraction Buffer PTR* 10 mL
- 50X Cell Extraction Enhancer Solution* 1 mL +2-8ºC
- TMB Substrate 12 mL +2-8ºC
- Stop Solution 12 mL +2-8ºC
- Sample Diluent NS 12 mL +2-8ºC
- Sample Diluent 50BS 2 x 12 mL +2-8ºC
- SimpleStep Pre-Coated 96 Well Microplate
- Plate Seal 1 +2-8ºC

C. IL-17F Estimation Kit.

abcam® IL-17F (Interleuking-17F) Human ELISA Kit, USA, was used to indicate IL-17F in the patients serums. The kits contents are:

- IL-17F Microplate (12 x 8 wells) 96 wells -20°C
- 20X Wash Buffer Concentrate 25 mL -20°C
- Recombinant Human IL-17 Standard 2 vials -20°C
- Assay Diluent C 30 mL -20°C
- 5X Assay Diluent B 15 mL -20°C
- Biotinylated anti-Human IL-17F 2 vials -20°C
- 200X HRP-Streptavidin Concentrate 200 µL -20°C
- TMB One-Step Substrate Reagent 12 mL -20°C
- Stop Solution 8 mL -20°C

2.3. Methods.

2.3.1. Molecular Steps.

A. Selection of a functional sequence in IL-17A andIL-17F genes.

The present study focused on specific sequences of each IL-17A and IL-17F genes. Many researchers studied targeted sequences in the two genes and linked them to some diseases such as AML, Rheumatoid Arthritis. Zhu *et al* (2015) studied a SNP located upstream and close to exon 1 in IL-17A gene and focused on some SNPs in the exon 3 of IL-17F. FASTA sequences from NCBI database of both genes were gained and exon 1 and exon 3 were studied (by ensembl database) with concentration. It was found that both exons have several SNPs sites within their sequences. Table (2-4) showed some of the SNPs found in each IL-17A exon 1, and IL-17F exon 3 according to ensemble Data Base.

Table (2-4): Registered SNPs in IL-17A exon 1 and IL-17F exon 3.

IL-17A exon 1 SNPs	IL-17F exon 3 SNPs
rs199815459, rs749745973, rs554117879, rs554295086, rs774834222, rs369837714, rs752836946, rs771480019, rs759094625, rs375071669, rs532385992, rs376028065, rs781660057, rs775271736, rs7600224158, rs763918114, rs568834044, rs761536762.	rs768255263, rs746645424, rs775184787, rs142962486, rs376780230, rs756602791, rs748486078, rs781668456, rs755441459, rs751817035, rs766577007, rs200163061, rs758436603, rs146083682, rs148940532, rs137981743, rs767452983, rs536968854, rs550486674, rs533677359, rs755388522, rs2397084, rs780259967, rs758566976, rs201184682, rs141798304, rs147873628, rs368500268.

B. Primers Designation.

This step was done according to Primer Blast feature of NCBI database.

Exon 1 for IL-17A was selected and intronic sequences upstream and downstream exon 1 was included in the targeted sequence. The first half of exon 3 was selected for IL-17F and targeted sequence included only an upstream intronic sequence.

C. Blood Sample Collection.

Five ml of Blood samples were collected from patients, using 5 ml syringe, and then were divided to 1 ml put in EDTA tube (for DNA isolation / PCR / Sequencing), 2 ml in gel tube (for IL-17A and IL-17F levels estimation) , and 2 ml in EDTA tube (for CD4 and CD8 markers estimation). The blood was poured to in the tubes slowly and on the wall of the tube, to avoid hemolysis.

The plain-tubes-samples were then centrifuged at 5000 rpm for 2 minutes, in order to separate the serum (to be used in ELISA tests). Then, the separated serum was kept in 1.5 ml eppindorff tube. Serum-containing eppindorff tubes and blood-containing EDTA tubes were stored for at 4 °C till the using time (after 1 day)

For CD4/CD8 measurements, 2 ml of blood were taken in EDTA tubes. The samples were stored in 4 °C.

D. DNA isolation.

After blood collection, blood-containing EDTA tubes are transported to the laboratory. DNA was extracted by using ***AccuPrep***® Genomic DNA Extraction Kit techniques, BiONEER Company.

1- The Kit Reagents Preparations.

- Proteinase K was dissolved in 1.25 ml of nuclease-free water.
- Binding buffer (GC) was mixed thoroughly by shaking before using it.
- Thirty ml of absolute ethanol was added to concentrated Washing buffer 1 (W1).
- Eighty ml of absolute ethanol was added to concentrated Washing buffer 2 (W2).

- Elution buffer (EL) was preheated to 60°C before the extraction process.

2- Procedure (according to BiONEER Company).

- Twenty µl of Proteinase K was added to a clean 1.5 ml tube.
- Two hundreds µl of whole blood dded was added to the tube containing proteinase K. If the sample volume was less than 200 µl, make the total volume 200 µl by adding PBS.
- Two hundreds µl of Binding buffer (GC) was added to the sample and mixed immediately by vortex mixer. (Samples must be completely resuspend to achieve maximum lysis efficiency).
- The mixture was incubated at 60℃ for 10 min. Water bath can be used in this step. Then 100 µl of Isopropanol was added and mixed well by pipetting.
  After this step, briefly spin down to get the drops clinging under the lid. (Don't vortex as this might reduce DNA yield).
- The lysate was transferred carefully into the upper reservoir of the Binding column tube (fit in a 2 ml tube) without wetting the rim. Then the tube was closed and centrifuged at 8,000 rpm for 1 min. (It must be closed each Binding column tube to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (>10,000 rpm) until the binding column tube is empty).
- The tube was opened and the Binding column tube was transferred to a new 2 ml tube for filtration.
- Five hundred µl of Washing buffer 1 (W1) was added without wetting the rim. Then the tube was closed and centrifuged at 8,000 rpm for 1 min.
- The solution was poured into from the 2 ml tube into a disposal bottle.
- Five hundred µl of Washing buffer 2 (W2) was added without wetting the rim. Then the tube was closed and centrifuged at 8,000 rpm for 1 min. Then,

centrifuged once more at 12,000 rpm for 1 min to completely remove ethanol, and checked that there was no droplet clinging to the bottom of Binding column tube. Residual W2 in the Binding column tube may cause problems in later applications.

- The Binding column tube was transferred to a new 1.5 ml tube for elution and 200 µl of Elution buffer was added (EL, or nuclease-free water) onto Binding column tube, and left for at least 1 min at room temperature (15~25℃) until EL is completely absorbed into the glass fiber of Binding column tube and centrifuge at 8,000 rpm for 1 min.

  To increase DNA yield, you should wait for 5 min after adding Elution buffer (EL). The volume of EL added can be adjusted from 50 µl to 100 µl. A smaller volume will result in a more concentrated solution, but total yield may be reduced). About 180 µl ~ 200 µl of eluent can be obtained when using 200 µl of Elution buffer (or nuclease-free water). For an improved yield, the sample was eluted twice and used after concentration process.
- About 6 µg of DNA in 200 µl of eluent (30 ng/µl) with an *A*260/*A*280 ratio of 1.6 ~ 1.9 can be typically obtained from 200 µl of whole blood (~ 1 X 10^6 leukocytes/ml).

E. Agarose Gel Electrophoresis.

After genomic DNA isolation or PCR amplification, agarose gel electrophoresis was used to detect the presence and the integrity of the extracted DNA, and the presence of PCR product (Sambrook *et al.,* 1989).

1- Preparation of 1X TBE Buffer.

The 1X TBE buffer was prepared from 10X TBE buffer (stock solution) by adding 100 ml of this stock solution to 900 ml of distilled water.

2- Agarose Gel Preparation.

- Fifty ml of 1X TBE buffer was taken in a beaker.

- Agarose powder was added to 50ml buffer, in an appropriate concentration: A quantity of 0.5 gm to prepare 1% agarose for DNA detection and 1.0 gm to prepare 2% agarose for PCR product detection.
- The solution was heated to boiling by using microwave until all gel particles were dissolved.
- The solution was left to cool down at 50-60 °C.
- Ethidium bromide (10mg/ml), 1 µl, was added to the agarose solution, and then the agarose was stirred in order to be mixed and avoid making bubbles.

3- Casting of the Horizontal Agarose Gel.

After sealing both edges of the gel tray with a cellophane tapes and fixing the comb in 1 cm away from one edge, the agarose solution was poured into the gel tray. The agarose was allowed to solidify at room temperature at 30 minutes. The fixed comb was carefully removed and the gel tray was placed in the gel tank. The tank was filled with 1X TBE buffer until the buffer reached 3-5 mm over the surface of the gel.

4- DNA Loading and Electrophoresis.

Seven µl of DNA was mixed with 3µl of loading dye. Samples were loaded carefully into the individual wells of the gel, and then electrical power was 5 volt/cm^2 for 30 minutes afterwards the DNA moved from cathode (-) to anode (+) poles due to the negative charge of the DNA. The Ethidium Bromide stained-bands in the gel were visualized using UV transiluminator at 350 nm and photographed.

F. Polymerase Chain Reaction (PCR).

PCR was carried out for both primers groups in a total volume of 25 µl. The reaction components, which described in table (2-5), were mixed together in PCR

eppendroff tubes and then placed in PCR device. Two PCR devices are recommended.

Table (2-5): PCR components for the amplification of coding sequences in IL-17A and IL-17F gene.

Component	Quantity for normal DNA bands (µl)	Quantity for weak DNA bands (µl)
PCR Master Mix (promega)*	12.5	12.5
Forward Primer	0.7	0.7
Reverse Primer	0.7	0.7
D.W.	8.1	4.6
DMSO	-	0.5
DNA	3.0	6.0
Total	**25.0**	**25.0**

**Taq*DNA polymerase , dNTPs, MgCl2 and reaction buffer (pH 8.5)

After that, PCR amplification started according to the program of each primers group separately, as described in table (2-6).

Table (2-6): PCR amplification program for both IL-17A primers and IL-17F primers.

Step	Temperature °C		Time in minutes	Number of cycles
	IL-17A primers	IL-17F primers		
Initial denaturation	**95.0**	**95.0**	**5:00**	**1**
Denaturation	**95.0**	**95.0**	**0:30**	**35**
Annealing	**60.0**	**57.0**	**1:00**	
Extension	**72.0**	**72.0**	**0:30**	
Final extension	**72.0**	**72.0**	**9:00**	**1**

Then, PCR product of each was detected as 251 bp and 355 bp for IL-17A primers PCR product and IL-17F primers PCR product respectively on two separated 2% agarose gels for electrophoresis process for almost two hours. Later, the gels were visualized by UV transiluminator due to ethidium bromide staining. A 100 bp DNA ladder is applied in both agarose gels as marker.

G. Sequencing.

The PCR products of 30 samples (22 Non-complete treatment CML and 8 healthy samples) were sent for macrogen company, Korea, for sequencing purposes to detect gene polymorphisms and mutation of both IL-17A *Exon1* and IL-17F *Exon 3*. 20µl of each PCR product, and forward primer of each of the genes, were sent too.

H. Detection of IL-17A and IL-17F variants.

The Codon-Codon Aligner software program was used to analyze the DNA sequences comes from macrogen company, Korea. After that, NCBI BLAST was used to detect any DNA alterations in sequences of the genes in current study.

2.3.2. Immunological tests.

A. Estimation of serum IL-17A.

IL-17A was estimated by ELISA technique, by using abcam® IL-17A (Interleuking-17A) Human ELISA Kit, USA.

1- Principle of the Assay.

Abcam's IL-17A (Interleukin-17A) *in vitro* SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for an accurate quantitative measurement of the IL-17A protein in serum samples, plasma samples, and human cell culture supernatant. The SimpleStep ELISA™ uses an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This whole complex (capture

antibody/analyte/detector antibody) is immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, standards or samples are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. Then, TMB substrate is added and during incubation is catalyzed by HRP, giving a blue coloration. Stop solution addition is then stops this reaction, making a color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm.

2- Reagent Preparation.

All reagents were equilibrated to room temperature (22 – 25 °C) prior to use

i. 1X Wash Buffer PT

1X Wash Buffer PT was prepared by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT and 5 mL 10X Wash Buffer PT was combined with 45 mL deionized water. Then mixed thoroughly and gently.

ii. Antibody Cocktail

Antibody Cocktail was prepare by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody was combined with 2.4 mL Antibody Diluent 5BI. Then mixed thoroughly and gently.

iii. Serum Preparation.

Neat serum can be assayed without dilution, or it can be diluted with Sample Diluent 50BS.

3- Assay Procedure.

It was recommended to assay all standards, controls and samples in duplicate.

i. Fifty µL of all sample or standards (1000, 500, 250, 125, 62.5, 31.25, and 15.625 pg/mL) were added to appropriate wells.

ii. Fifty µL of the Antibody Cocktail was added to each well.

iii. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker set to 400 rpm.

iv. Each well was washed with 3 x 350 μL 1X Wash Buffer PT. The washing was by aspirating or decanting from wells and then dispensing 350 μL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash, the plate was inverted and blotted against clean paper towels to remove excess liquid.

v. One hundred μL of TMB Substrate was added to each well and then incubated for 10 minutes in the dark on a plate shaker set to 400 rpm.

vi. One hundred of Stop Solution was added to each well. Then, the plate was shaken on a plate shaker for 1 minute to mix.

vii. The optical density was read at 450 nm.

4- Calculation of Results

Average zero standard was subtracted from all readings. The duplicate readings of the positive control dilutions were also averaged and plotted against their concentrations. Then, the best smooth curve through these points was drown to construct a standard curve (Figure 2-1).

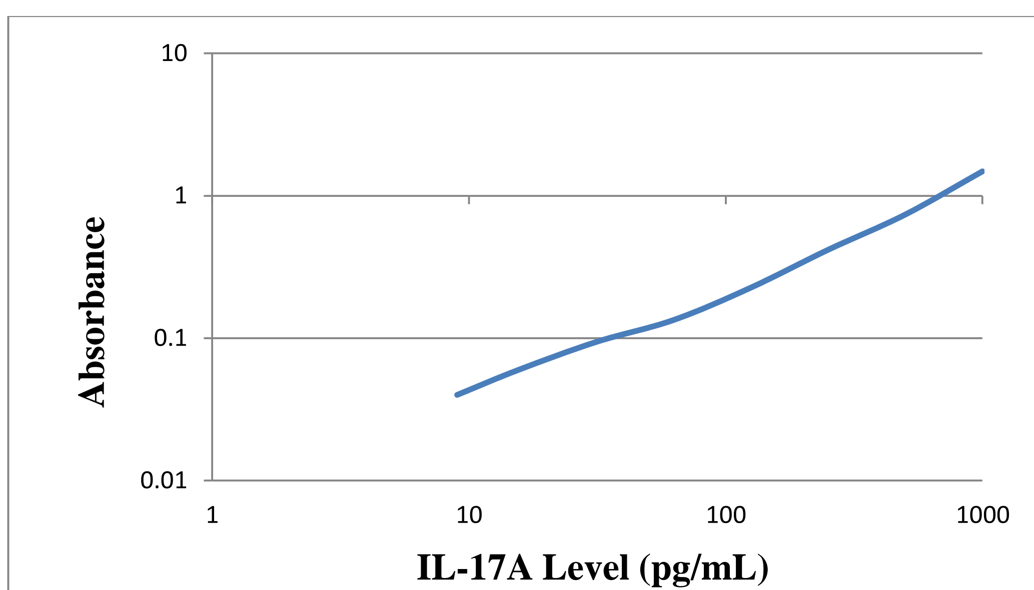


Figure (2-1): Standard curve IL-17A

Protein concentrations for unknown samples from the standard curve were plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, and then multiplying the concentration was found by the appropriate dilution factor.

B. Estimation of serum IL-17F.

IL-17F was estimated by ELISA technique, by using abcam® IL-17F (Interleuking-17F) Human ELISA Kit, USA.

1- Principle of the Assay

Abcam's IL-17F (Interleukin 17F) Human ELISA (Enzyme- Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for a quantitative estimation of Human IL-17F in plasma, serum, and cell culture supernatants.

This assay employs a 96-well plate coated with an antibody that is specific for Human IL-17F. Samples and standards are pipetted into the wells. IL-17F present in a sample will be bound to the wells by the immobilized antibody. The wells are then washed and biotinylated anti-Human IL-17F antibody is added. After washing of the away unbound biotinylated antibody, HRP-conjugated Streptavidin is pipetted into the wells. Then, wells are washed again. TMB substrate solution is then added to the wells and color appears in proportion to the amount of IL-17F bound. Later, the color is changed from blue to yellow once stop solution is added, and the intensity of the color is measured at 450 nm.

2- Reagent Preparation.

i. 1X Assay Diluent B

Five X Assay Diluent B was diluted 5-fold with deionized or distilled water before use.

ii. 1X Wash Solution.

Twenty X Concentrate Wash was equilibrated to room temperature and mixed gently until dissolved in case it contained visible crystals. Twenty mL of 20X Wash Buffer Concentrate was diluted into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

iii. 1X Biotinylated IL-17F Detection Antibody.

The Biotinylated anti-Human IL-17F vial was briefly spun before use. One hundred μL of 1X Assay Diluent B was added into the vial to prepare a concentrate detection antibody. Then the solution was pipetted up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The concentrate detection antibody was diluted 80-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

iv. 1X HRP-Streptavidin Solution.

Two hundreds X concentrate HRP-Streptavidin vial was briefly spun and pipetted up and down to mix gently before use. Concentrate HRP-Streptavidin was diluted 200-fold with 1X Assay Diluent B prior to use in the Assay Procedure and then mixed. It should not store for next day use.

v. Serum Preparations.

Assay Diluent C was used for serum dilution to a ratio 1:2.

3- Assay Procedure.

i. One hundred μL of each standard (10000, 3333, 1111, 370.4, 123.5, 41.15, and 13.71 pg/mL) and sample into was added to appropriate wells. Then, wells were sealed and incubated for 2.5 hours in room temperature or overnight at 4°C with gentle shaking.

ii. The solution was thrown away. Then, wells were washed 4 times with 1X Wash Solution. The washing was done through filling each of the wells with 1X Wash Solution (300 μL), using an auto washer or a multi-channel

Pipette. It was ensured that a complete removing of the liquid at each step is important for good performance. After the last wash, aspirating or decanting was done to ensure any remaining Wash Buffer was perfectly removed. The plate was then inverted and blotted against clean paper towels.

iii. One hundred µL of 1X Biotinylated IL-17F Detection Antibody was added to each well. Then, wells were incubated for an hour at room temperature with gentle shaking.

iv. The solution was discarded and washing is repeated as in step “b” again.

v. One hundred µL of 1X HRP-Streptavidin solution was added to each well. Then, wells were incubated with gentle shaking for 45 minutes at room temperature.

vi. The solution was discarded and washing is repeated as in step “b” again.

vii. One hundred µL of TMB One-Step Substrate Reagent was added to each well. Then, wells were incubated in the dark with gentle shaking for 30 minutes at room temperature.

viii. Fifty µL of Stop Solution was added to each well. The optical density was read at 450 nm.

4- Calculation of Results.

The mean absorbance was calculated for each set of duplicate standards, controls and samples, and the average zero standard optical density was subtracted. Then, the standard curve on log-log graph paper was blotted, with standard concentration on the x-axis and absorbance on the y-axis. Finally, the best-fit straight line was drawn through the standard points. Figure (2-2) shows the standard curve.

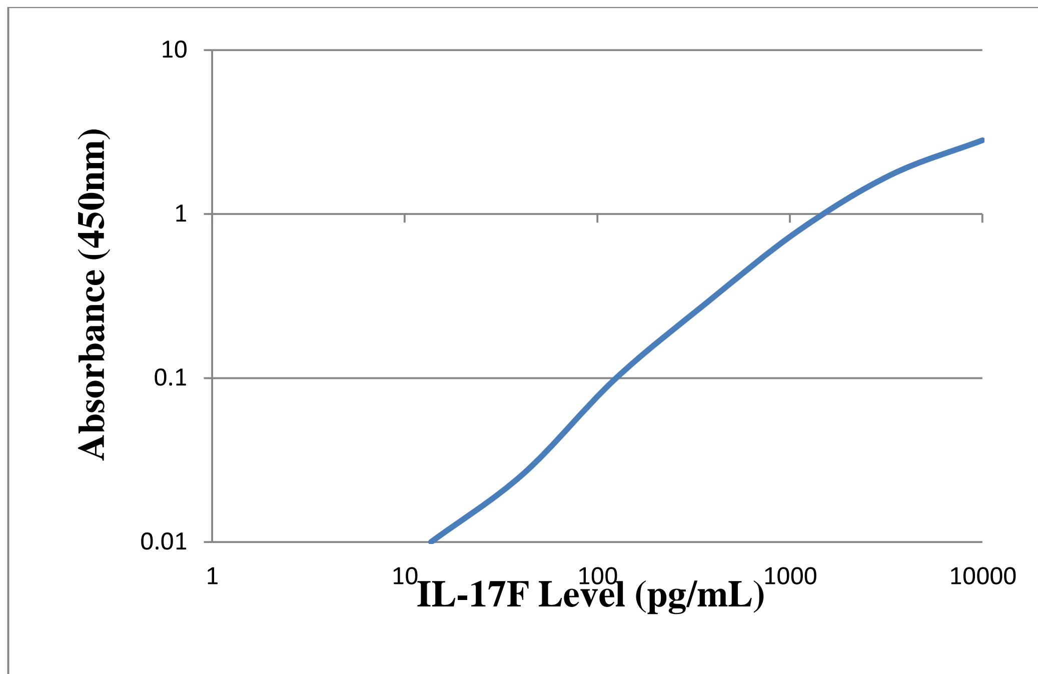


Figure (2-2): Standard curve of IL-17F

C. Analysis of T-Lymphocyte Subset by Flow Cytometer.

1- Description.

Cylyse stands for an erythrocyte lysing reagent kit for wash and no wash procedure with a complete preservation of the surface proteins and practically no loss of cells. Cylyse is perfect for absolute cell counting and for assays, ensuring a minimum loss of leukocytes. Because of the features of the lysing reagent buffer, there is no need for centrifugation to remove the residual debris. Fixative reagent stabilizes and fixes the leukocytes. The fixed samples will be suitable to be stored for up to 24 hours at 2-8°C before analysis.

2- Assay Procedure.

i. Antibody labeling: an aliquot of 100 µl of whole blood or isolate leukocytes was mixed with conjugated antibodies (e.g. 10 µl or as recommended by the antibody supplier) in a test tube. Then mixed thoroughly and incubated for 15 minutes in the dark at room temperature.
ii. Leukocyte fixation: an aliquot of 100 µl of fixative reagent A was added, then mixed thoroughly and incubated for 10 minutes in the dark.

iii. Erythrocyte lysis: an aliquot of 2.5 ml of reagent B was added, then shook gently and incubated for 20 minutes in the dark.

The samples were analyzed using flow cytometer. Fixed samples were stored at 2- 8 °C, protected from light, up to 24 hours until analysis.

3- Storage and Calculations.

Samples were stored at room temperature. Temperatures below 17°C could lead to form a white precipitation in lysing reagent B. The precipitate could be re-dissolved at 35°C (for e.g. in a water bath). Total and differential counting was done to get WBC count of each subject.

For measuring CD4 count, the following formula was used:

Lymphocyte Count = (Percentage of Lymphocyte / 100) × WBC Count.

CD4 or CD8 count = (Percentage of CD4 / 100) × Lymphocyte Count.

2.3.3. Statistical analysis.

Data were analyzed statistically using SPSS program version 23. Results were expressed using simple statistical parameters such as mean and standard error. Differences between means were assessed by ANOVA, followed by Dunkan and Tukey's tests.

Chapter Three
Results and Discussion

3. Results and Discussion.

3.1. Distribution of Subjects.

3.1.1. Distribution by Gender.

Distribution of CML patients according to gender showed a significant difference between males and females patients. Thirty Eight (61%) were males and 24 (39%) were females out of 62. The overall ratio of males/females was 1.6:1. Goldman, 1998 reported that the ratio males/females should be 2:1. The reason of this difference here might be attributed to the small numbers of samples collected, as in figure (3–1).

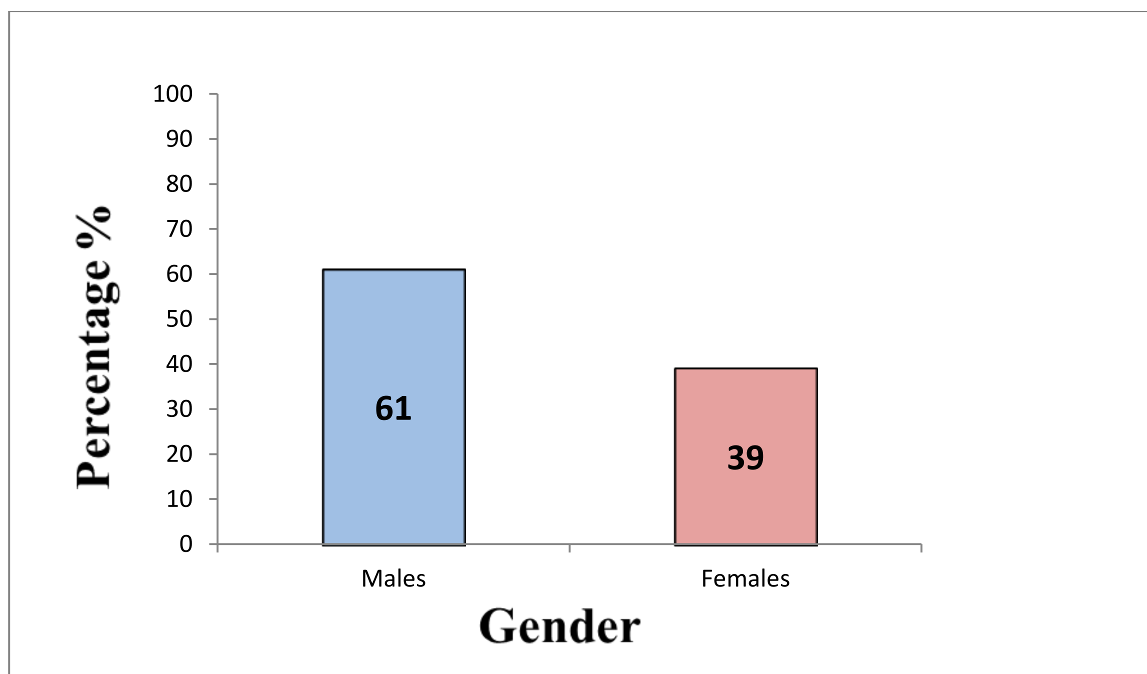


Figure (3-1): Distribution of CML patients according to Gender.

3.1.2. Distribution by Age.

The results of studied groups have shown that the minimum age of onset in our study was 21 years old, while the maximum age registered was 73 years, making age groups ranging from 21-73 years old, with a median age of 47 years old. As shown in figure (3-2), it is obvious that the distribution of the patients mostly occurred between 31-40 years old and 41-50 years old.

According to Goldman (1998) studies, due to the loss of genetic material in each cell division, CML affects old ages mostly, beside the increasing chance of

exposure or accumulation of carcinogenic materials, such as benzene and other petro-products (Harvey, 1991), accompanied with increasing age, according to (Mundhada, 2006). Older ages patients, especially over sixty, were few and this attributed mostly to death of patient before reaching old age.

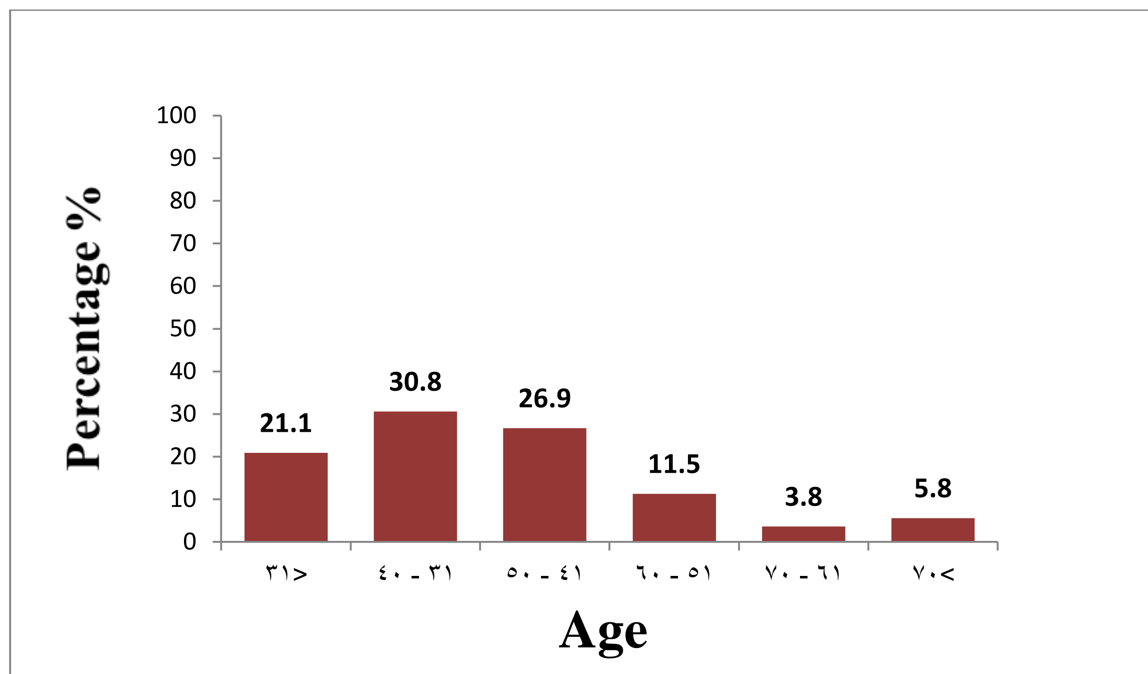


Figure (3-2): Distribution of CML patients according Age.

3.2. Molecular Analysis of IL-17A and IL-17F.

3.2.1. DNA isolation.

DNA isolation was done by *AccuPrep*® Genomic DNA Extraction Kit techniques to prepare a pure DNA for PCR from the frozen blood samples. This procedure involves lyses of red blood cells (RBCs) and removing dissolving undesirable contaminants including proteins and RNA. The purity was 1.6 (figure 3-3).

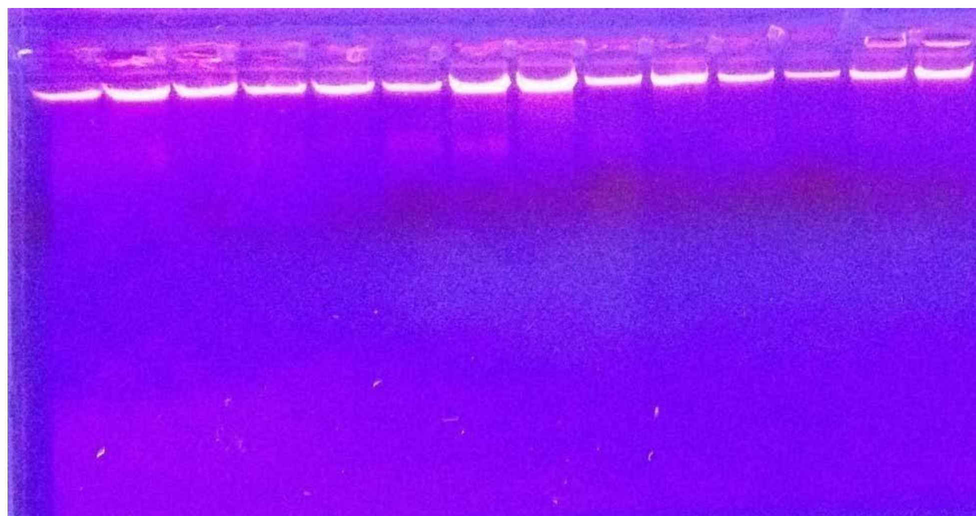


Figure (3–3): Genomic DNA bands visualized under UV after staining with ethidium bromide on 1% agarose gel at 100 volt for 15 minutes.

3.2.2. Thermal Gradient PCR.

Running a thermal gradient PCR for IL-17A and IL-17F primers allows identifying a single annealing temperature that will provide efficient, specific amplification of the targeted sequences in each gene.

Therefore, the procedure was done for both primers at the same time, by using of gradient-featured PCR system for 2 hours and a half, and depending on melting temperature of each of the primers. The thermal gradient was 56°C, 57°C, 58°C, 59°C, 60°C, and 61°C. Then, gel electrophoresis procedure was done for all to check the clear bands and to find out the best temperature for each primer.

It was found that the optimum melting temperature for IL-17A exon 1 primer was 60°C and 57°C for IL-17F exon-3 primer.

3.2.3. PCR products.

A. IL-17A Gene Exon-1 PCR.

PCR technique was used in the present study in order to amplify a targeted sequence of Exon-1 of IL-17A gene. The PCR results showed clear bands related

to the targeted sequence. PCR amplified regions revealed a molecular size of 251 bp for the primer used for this exon, as shown in Figure (3-4).

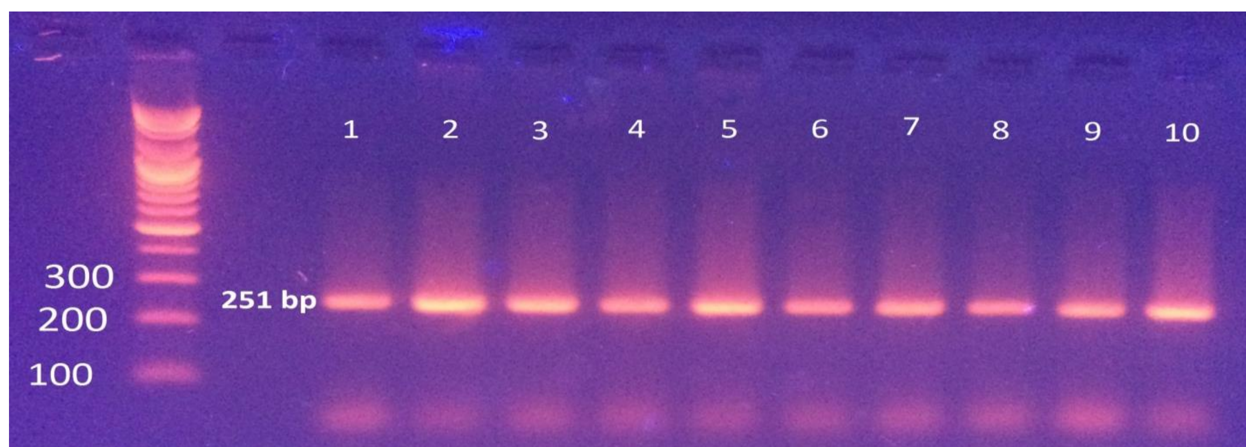


Figure (3–4): PCR product electrophoresed on 2% agarose gel 70 volt/hr, showing clear 251 bp bands of IL-17A exon-1 for 10 samples. Marker used in this gel is (100 bp).

B. IL-17F Gene Exon-3 PCR.

PCR technique was used in the present study in order to amplify a targeted sequence of Exon-1 of IL-17A gene. The PCR results showed clear bands related to the targeted sequence. PCR amplified regions revealed a molecular size of 355 bp for the primer used for this exon, as shown in figure (3-5).

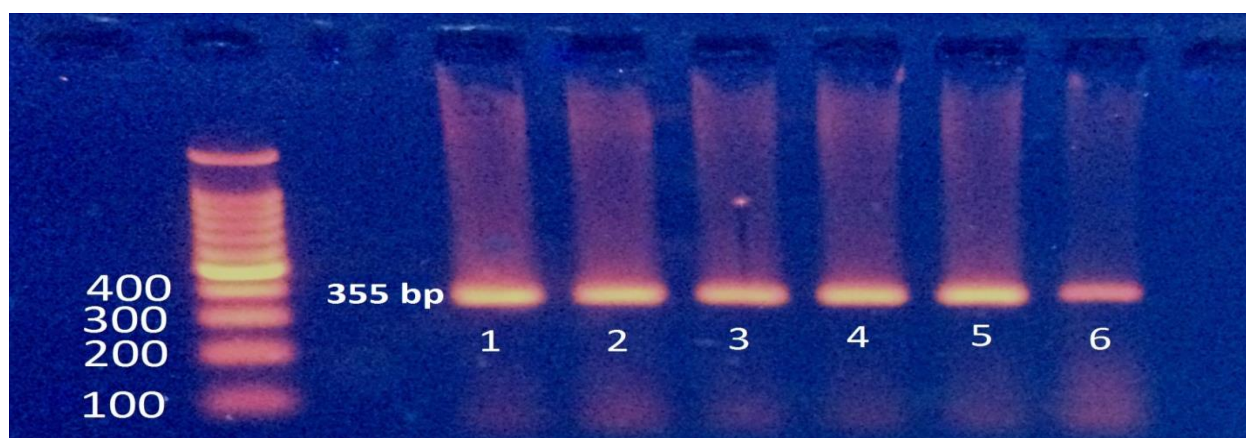


Figure (3–5): PCR product electrophoresed on 2% agarose gel 70 volt/hr, showing clear 355 bp bands of IL-17A exon-1 of 6 samples. Marker used in this gel is (100 bp).

3.2.4. IL-17A Exon 1 Sequencing Results.

The present study detected some specific variant for CML. Some of them are already registered according to ensembl Data base, others are not.

A. Registered Variants.

The sequencing results showed 13 variants were detected in the exon 1 of IL-17A gene in CML patients and 12 variants were detected in healthy, leaving one single variants be specific to CML, termed rs199815459 (T > G) as shown in table (3-1) and responsible for changing amino acid Proline codon CCT to another Proline codon CCG. It was located inside exon 1, at the site 5054 of the gene IL-17A (and at the site 52186440 of chromosome 6) as shown in figure (3–6). This SNP has three genotypes, TG genotype which was found in 22 CML with (100%) frequency, GG genotype which is found in 8 healthy subjects with (100%) frequency and TT genotype which wasn't detected in any subjects.

Table (3–1): The detected registered variations and genotype frequency within the targeted sequence in IL-17A gene exon 1 in CML and healthy subjects.

Site	Codon	Variants ID	Genotype	Source	Subject			
					CML No.	%	Con. No.	%
4987	Intron 1	rs376028065	TT		22	100	8	100
			CC		0	0	0	0
			TC		0	0	0	0
5014	Promoter	rs532385992	CC		22	100	8	100
			AA		0	0	0	0
			CA		0	0	0	0
5016	Promoter	rs375071669	TT		0	0	0	0
			CC		0	0	0	0
			TC	T > C	22	100	8	100
5018	Promoter	rs773802580	CC		22	100	8	100
			TT		0	0	0	0
			CT		0	0	0	0
5022	Promoter	rs759094625	CC		22	100	8	100
			TT		0	0	0	0
			CT		0	0	0	0

5025	Promoter	rs771480019	AA		22	100	8	100
			GG		0	0	0	0
			AG		0	0	0	0
5054	Codon 3	rs199815459	TT		0	0	0	0
			GG		0	0	8	100
			TG	T > G	22	100	0	0
5067	Intron 2	rs752836946	TT		22	100	8	100
			CC		0	0	0	0
			TC		0	0	0	0
5100	Intron 2	rs369837714	CC		0	0	0	0
			TT		0	0	0	0
			CT	C > T	22	100	8	100
5117	Intron 2	rs749745973	AA		0	0	0	0
			GG		0	0	0	0
			GA	G > A	22	100	8	100
5119	Intron 2	rs774834222	TT		0	0	0	0
			AA		0	0	0	0
			TA	T > A	22	100	8	100
5128	Intron 2	rs554295086	TT		0	0	0	0
			GG		0	0	0	0
			TG	T > G	22	100	8	100
5146	Intron 2	rs554117879	CC		0	0	0	0
			GG		0	0	0	0
			CG	C > G	22	100	8	100

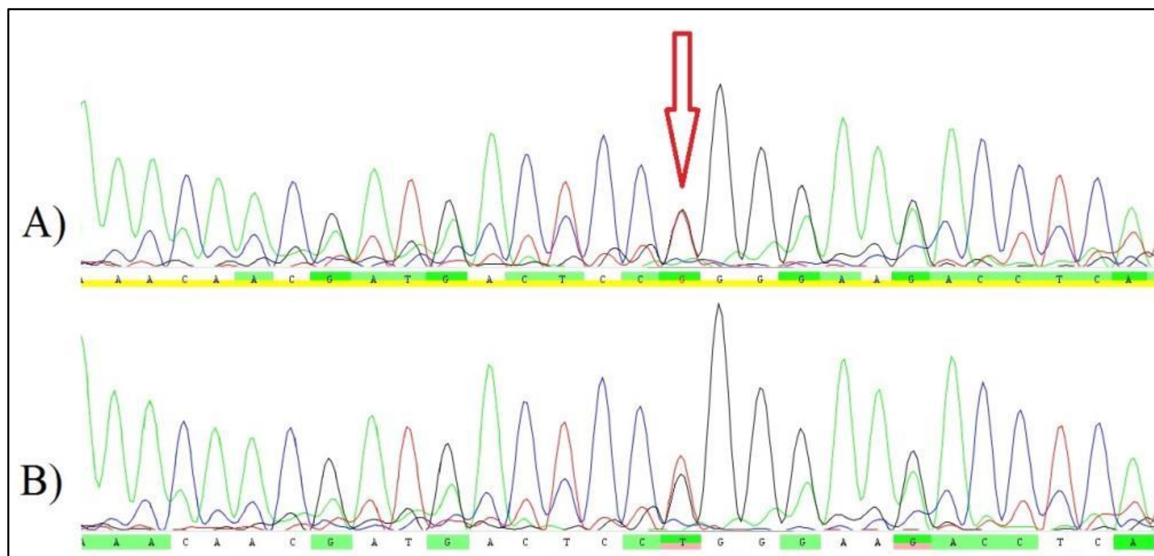


Figure (3-6): Sequencing result of IL-17A gene exon 1 in CML and healthy subjects. A) Showing variant of rs199815459 SNP with a “T > G” change in CML. B) Representing a normal sequence of a healthy individual sequencing result.

Results showed also four important variants that were globally registered, but with genotypes differs from the genotypes in the present study. They are rs774834222, rs554295086, rs554117879, and rs749745973. The rs774834222 SNP (located in 5119 bp site of the gene, and at 52186505 bp site on chromosome 6) was registered with a variation of T > C and a TC genotype, while the present study showed a T > A variation for this position, with a genotype TA was present in CML and healthy with (100%) frequency. The other genotypes TT and AA were not recorded in CML and healthy. The rs554295086 SNP (at 5128 bp site of the gene, and at 52186514 bp site on chromosome 6) was registered as T > C variation and a TC genotype, unlike the present study which a T > G variation was detected, with a genotype of TG with (100%) frequency in CML and healthy. The genotype TT and GG were not recorded in CML and healthy. The rs554117879 SNP (at 5146 bp site of the gene, and at 52186532 bp site on chromosome 6) was detected with a C > G variation and a CG genotype. But, the present study detected a variation of C > T, with a genotype of CG with (100%) frequency in CML and healthy. The other genotypes CC and GG were not recorded in CML and healthy. The rs749745973 SNP (at 5117 bp site of the gene, and at 52186503 bp site on chromosome 6) was registered with a variation of G > C and genotype of GC, while the present study recorded a different variation, with a G > A, with a genotype of GA was recorded with (100%) frequency in CML and healthy, while, GG and AA genotypes were not recorded in CML and healthy.

There were some other variations detected. These variations were detected with the same allelic or genotypic information that are registered globally. The present study classified them into two groups: Intronic SNPs, and Promoter-within SNPs.

Intronic SNPs were three. The rs376028065, an intronic SNP located upstream exon 1, was found with a TT genotype with (100%) frequency in CML and healthy, while other genotypes CC and TC were not found in any case. rs752836946, an intronic SNP found downstream exon 1, was detected in a TT

genotype with (100%) frequency for CML and healthy, while the other genotypes, CC and TC were not recorded in any CML and healthy. The rs369837714, an intronic SNP located downstream exon 1, was found as a C > T variation and CT genotype with (100%) frequency for in CML and healthy, while the other genotypes, CC and TT were not found in CML patients and healthy.

Promoter-within SNPs were five. The rs532385992, located within the promoter, was found at a genotype of CC with (100%) frequency in CML patients and healthy, while the genotypes AA and CA were not found in any subjects. The rs375071669, a SNP within the promoter, was found in a T > C variation and TC genotype with (100%) frequency in all CML and healthy. The other TT and CC genotypes of this SNP were not found in any CML patients and healthy subject. The rs773802580, a SNP within the promoter, was found in a CC genotype with (100%) frequency in all CML and healthy, while the other genotypes, TT and CT, were not found in any CML patients and healthy subjects. The rs759094625, a SNP located within the promoter, was detected in a CC genotype (100%) frequency in CML and healthy. Its other TT and CT genotypes were not found in CML patients and healthy subjects. The rs771480019, a SNP within the promoter was found in an AA genotype with (100%) frequency in CML and healthy, while the other genotypes, GG and AG, were not found in any CML and healthy subjects.

B. Unregistered Variants.

Results showed four 4 unregistered variants in exon 1 of IL-17A gene. Three of them were located inside exon 1, while the other one was outside the exon, as shown in Table (3–2).

The first one was located in 5037bp site of the gene (and in 52186423 bp site of chromosome 6). It was located at the promoter site in GA genotype and responsible for changing the amino acid Glutamic acid to be Lysine. However, this genotype was detected in both CML and healthy subjects with (100%) frequency

Table (3–2): The detected unregistered variants and genotype frequency within the targeted sequence in IL-17A gene exon 1 in CML and healthy subjects.

Origin	Codon	Variants ID	Genotype	Source	Subject			
					CML No.	%	Control No.	%
5037	Promoter	Novel	AA	/	0	0	0	0
			GG	/	0	0	0	0
			GA	G > A	22	100	8	100
5060	Codon 5	Novel	GG	/	0	0	0	0
			AA	/	0	0	0	0
			GA	G > A	22	100	8	100
5071	Codon 9	Novel	TT	/	0	0	0	0
			GG	/	0	0	0	0
			TG	T > G	22	100	8	100
5135	Intron 2	Novel	TT	/	0	0	0	0
			GG	/	0	0	0	0
			TG	T > G	22	100	8	100

in CML and healthy, while the other genotypes, AA and GG, were not found in any CML patients and healthy subjects. The second one was located at codon 5 site, at 5060 bp site of the gene (in 52186446 bp site of the whole chromosome 6). This variation was found in a GA genotype, and responsible for giving the amino acid Lysine as the original sequence gives. This genotype was found with (100%) frequency in CML and healthy, while the other GG and AA genotypes were not found in any CML and healthy subjects. The third one was found within codon 9, at 5071 bp site of the gene (at 52186457 of chromosome 6). This variation was found in a T > G variation and TG genotype, giving Glycine amino acid instead of Valine. It was also found in both CML patients and healthy subjects with (100%) frequency, while the other TT and GG genotypes were not found in any CML and healthy subjects. The last variant was an intronic variant, located downstream exon 1, at 5135 bp site of the gene (on 52186521 bp site of chromosome 6). This variation was found in a T > G variation and TG genotype, giving the amino acid Arginine as the original sequence gives. This variation was found in both CML

patients and healthy subjects with (100%) frequency in CML and healthy, while the other TT and GG genotypes were not found in CML and healthy subjects. Figure (3-7) shows an unregistered variant.

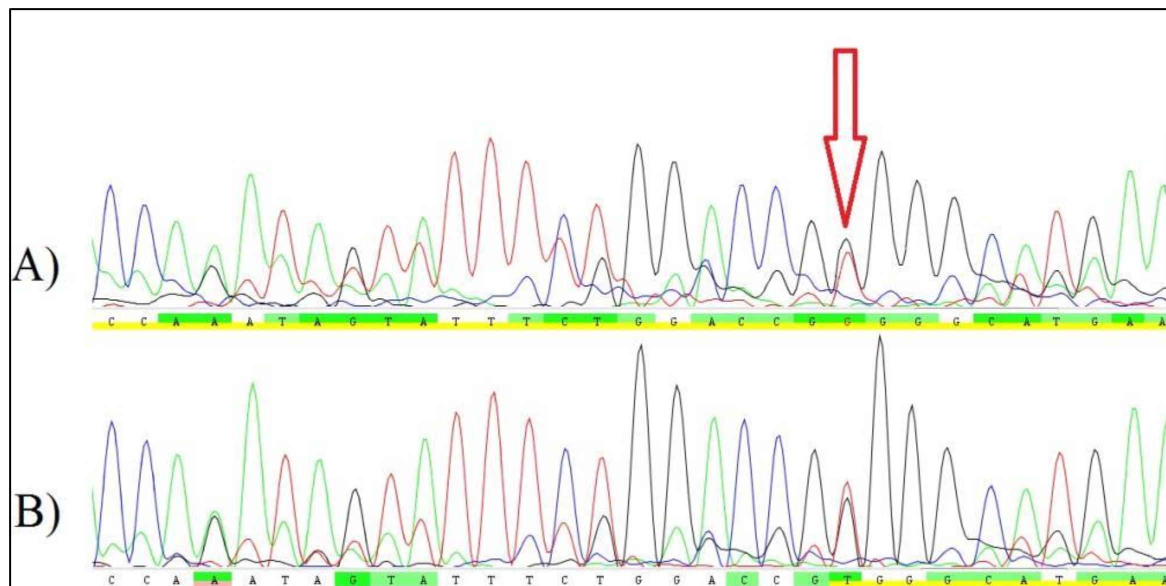


Figure (3-7): Sequencing result showing unregistered variations in IL-17A gene exon 1 in CML and healthy subjects. A) Representing CML sequencing results showing a "T > G" change in. B) Representing a normal sequence of a healthy.

3.2.5. IL-17F Exon 3 Sequencing Results.

For IL-17F Exon 3, there were more than 70 variations loci considered as SNPs. These SNPs are shown in Table (3-3).

However, the analysis of sequencing results of IL-17F exon 3 showed one remarkable variant for IL-17F exon 3 among all known SNPs, in comparing with the dominant alleles, which was rs2397084, a registered SNP, found normally in a TT genotype. In the present study, this genotype was founded in 20 CML with frequency (90.9%) and in all healthy subjects with (100%) frequency. TC genotype was not recorded in all subjects. Interestingly, TC genotype was found in 2 of CML patients with frequency of (9.1%). This genotype was a TC SNP. This variation causes a change in amino acid codon, gave Glycine instead of Glutamic acid, as shown in able (3–4) and figure (3-8). The other CC genotype was not detected in any of the samples.

Table (3–3): The detected SNPs that are with genotype exactly as in ensembl Data Base.

Gene / Exon	Detected SNPs
IL-17F / Exon 3	rs772871633, rs762490751, rs766130634, rs571639953, rs759430564, rs767197513, rs763780, rs149025136, rs753929271, rs757261387, rs144576902, rs746053425, rs11465553, rs780312510, rs769299818, rs777210315, rs762731838, rs148267766, rs541568544, rs774103459, rs759080928, rs767158385, rs11465552, rs368500268, rs760408480, rs764026426, rs147873628, rs141798304, rs765197624, rs201184682, rs758566976, rs780259967, rs2397084, rs755388522, rs570911210, rs533677359, rs550486674, rs536968854, rs767452983, rs137981743, rs775060397, rs760533244, rs763865065, rs776587391, rs148940532, rs146083682, rs750479457, rs758436603, rs200163061, rs766577007, rs751817035, rs755441459, rs781668456, rs748486078, rs756602791, rs376780230, rs142962486, rs775184787, rs746645424, rs768255263.

The other SNPs detected in this exon were in the same frequency in most web sites, such as ensembl Data Base.

Table (3–4): The detected registered variants and genotype frequency in CML and healthy subjects within the targeted sequence in IL-17F gene exon 3.

Site	Codon	Variant ID	Genotype	Source	Subject			
					CML No.	%	Control No.	%
8644	41	rs2397084	TT	T > T	20	90.9	8	100
			CC	/	0	0	0	0
			TC	T > C	2	9.1	0	0

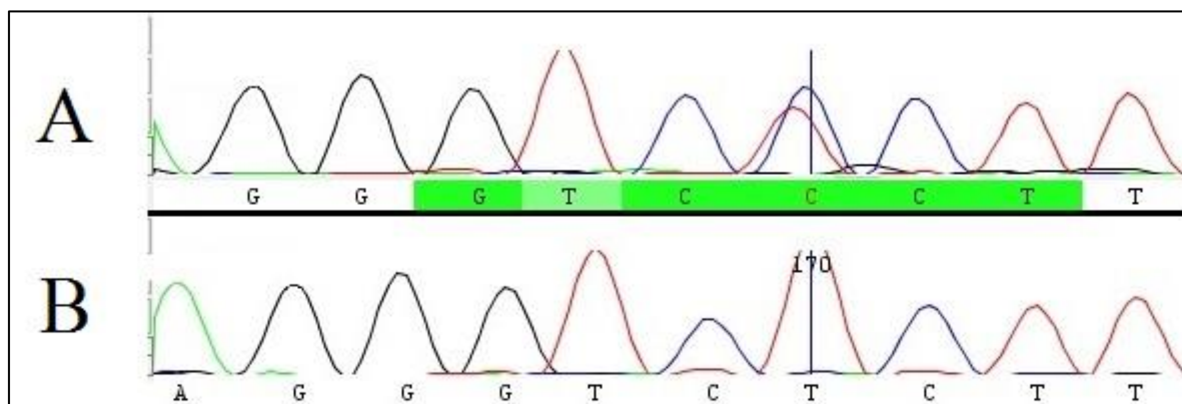


Figure (3-8): Sequencing result showing rs2397084 in IL-17F in CML and healthy subjects. A) Representing CML sequencing results showing a “T > C” change in. B) Representing a normal sequence of healthy subjects.

3.3. IL-17A and IL-17F Estimation.

3.3.1. Serum Level of IL-17A.

Results showed the mean serum level of IL-17A in newly diagnosed was (118.6 ± 30.18^b) pg/ml higher than healthy individuals (29.0 ± 16.67^a) pg/ml. Non-complete treatment cases showed level of IL-17A with (31 ± 18.4^a) pg/ml which was normalized in comparison with newly diagnosed group ($p \leq 0.05$), as shown in table (3-5).

3.3.2. Serum Level of IL-17F.

As shown in table (3-5), results revealed that IL-17F level was significantly higher in newly diagnosed CML patients (101.9 ± 21.58^b) pg/ml when compared with healthy with mean level (26.3 ± 6.64^a) pg/ml. After treatment there was a significant decrease in serum levels (28.4 ± 8.01^a) pg/ml with no significant differences between treated and healthy as shown in table (3-5).

A significant increase in IL-17 concentration may be accompanied by an increase of CD4 (Th17) cell number in CML patients and normalized level was recorded in non-complete treatment patients may be due to decrease in CD4 (th17) in patients undergoing chemotherapy. Recently, Abousamra *et al*. (2013) suggested that the number of circulating Th17 cells significantly increased in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients in

Table (3-5): Serum level of IL-17A and IL-17F in CML and healthy subjects.

Parameter	Healthy Subjects	Newly Diagnosed CML Subjects	Non-complete Treatment CML Subjects
IL-17A	29.0 ± 16.67[a]	118.6 ± 30.18[b]	31 ± 18.41[a]
IL-17F	26.3 ± 6.64[a]	10.1.9 ± 21.58[b]	28.4 ± 8.01[a]

***Difference letters represent significant difference between means (Duncan test).**

***Values were expresses as mean ± SE.**

comparing with healthy controls. In these patients, Th17 cell number decreased obviously in patients with complete remission after treatment.

Wu *et al*. (2009) revealed that the number of peripheral Th17 cells increased significantly in AML patients comparing with healthy controls. They also found that IL-17 concentration might have an increase, and Th17 cell number decreased in patient with complete response.

It's known that inflammations are believed in promoting tumor development and progression through affecting of cytokines, chemokines, and growth factors directly or indirectly, in tumor cells or their environment. In healthy hematopoietic cells, the cell surface receptors activation by chemokines, cytokines, and growth factors regulates the activity of signal transduction pathway (STP). Abnormalities in signaling by STP are casual events in leukemia and are thought to participate to leukemogenesis (Van, 2007).

3.4. CD4+ and CD8+ Lymphocytes Counting by Flow Cytometer.

3.4.1. Blood picture of healthy and CML samples.

A complete blood picture of the three groups was applied to get full information about white blood cells count. Results showed that all parameters were

significantly elevated in newly diagnosed CML subjects. Treatment managed to normalize these values, as shown in table (3-6).

Table (3-6): Total and differential leucocyte count in CML and healthy subjects.

Parameter	Healthy (Mean ± SE) cell/1ml.blood	Newly diagnosed CML cell/1ml.blood	Treated CML cell/1ml.blood
WBC Count (10^9/L)	8 ± 0.3438[a]	13.06 ± 0.6916[b]	7.32 ± 0.8734[a]
Neutrophils %	66.22 ± 1.3398[a]	81.5 ± 1.4395[b]	65.3 ± 2.1190[a]
Eosinophils %	1.4 ± 0.1633[a]	2.3 ± 0.2134[b]	0.8 ± 0.2906[a]
Basophils %	0.65 ± 0.15[a]	3.9 ± 0.2769[b]	0.3[b] ± 0.1528[a]
Lymphocytes %	32.6 ± 1.3182[a]	35.8 ± 0.5734[b]	31.7 ± 2.2462[a]

***Difference letters represent significant difference between means (Duncan test).**

***Values were expresses as mean ± SE.**

3.4.2. CD4 and CD8 Count.

As shown in table (3-7), newly diagnosed CML showed a significant increase in CD4+ count (1.624 ×10^9/L ± 0.05[b]) in comparing to healthy count (1.27 ×10^9/L ± 0.04[a]), while treated CML showed significantly lower mean count (0.451 ×10^9/L ± 0.11[c]) than the mean of pretreated.

CD8 count showed a slight significant increase in CD8+ (0.819 ×10^9/L ± 0.05[a]) in newly diagnosed CML subjects comparing to healthy subjects (0.698 ×10^9/L ± 0.04[ab]), while non-complete treatment CML subjects showed a slight significant decrease (0.504 ×10^9/L ± 0.12[b]) compared with the mean of healthy subjects.

Figure (3-9) is showing some results of Flow Cytometer of CD4+ and CD8+ counts in some patients.

3.4.3. CD4/CD8 Ratio.

Results showed, as in table (3-7), a significant increase in CD4/CD8 ratio (2.017 ± 0.12^{b}) in newly diagnosed CML subjects, compared to healthy subjects (1.845 ± 0.07^{a}), while non-complete treatment CML showed significantly decreased ratio (0.94 ± 0.08^{c}).

Table (3-7): CD4+ and CD8+ count (mean ± SE) in CML patients and healthy.

CD marker	Healthy Subjects	Newly diagnosed CML Subjects	Non-complete Treatment Subjects
CD4+ (10^9/L)	1.27 ± 0.04^{a}	1.624 ± 0.05^{b}	0.451 ± 0.11^{c}
CD8+ (10^9/L)	0.698 ± 0.04^{ab}	0.819 ± 0.05^{a}	0.504 ± 0.12^{b}
CD4/CD8	1.85 ± 0.07^{a}	2.18 ± 0.12^{b}	0.94 ± 0.08^{c}

***Difference letters represent significant difference between means (Duncan test).**

***Values were expresses as mean ± SE.**

The present study showed that there is a significant increase in CD4+ count and a slight increase with no significant in CD8+ counts in newly diagnosed CML patients, in comparing to healthy. An elevation may represent a secondary immune response of the body in order to control leukemia cells due to the defect on the body immune homeostatic mechanism by tumor cells (Mos and Gillespie, 2002). Other clinical studies suggested a presence of activated leukemia-specific CD4+

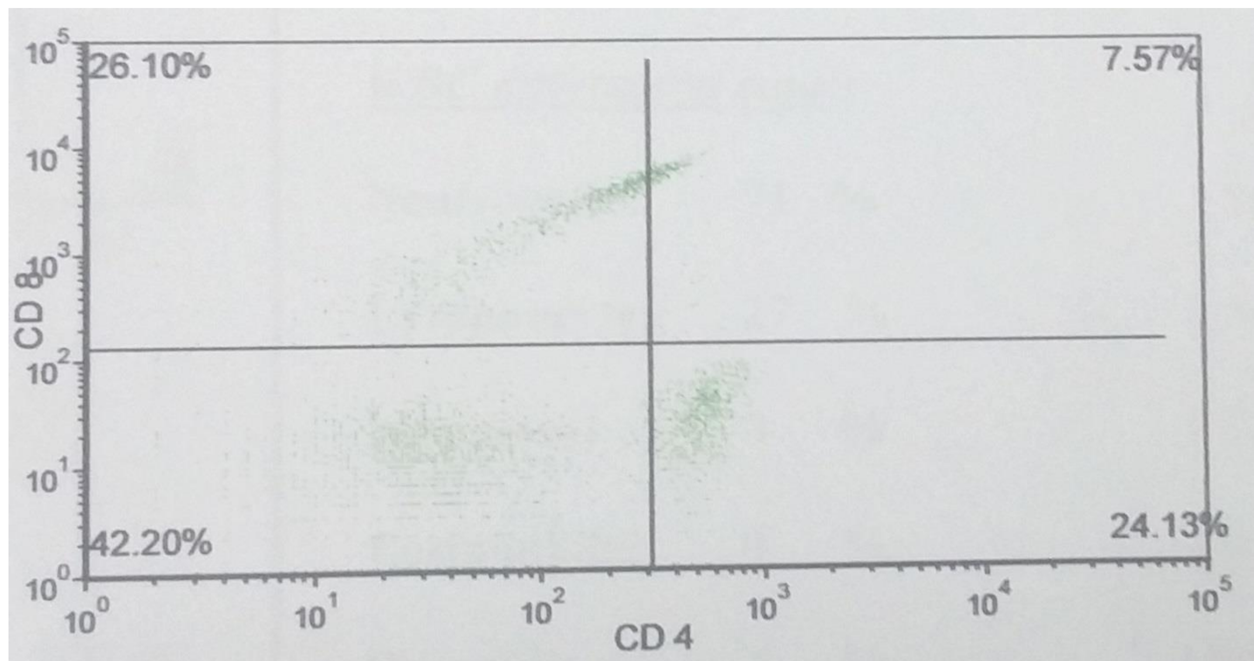


Figure (3-9): Flow cytometer results of CD4+ and CD8+ in newly diagnosed CML subjects.

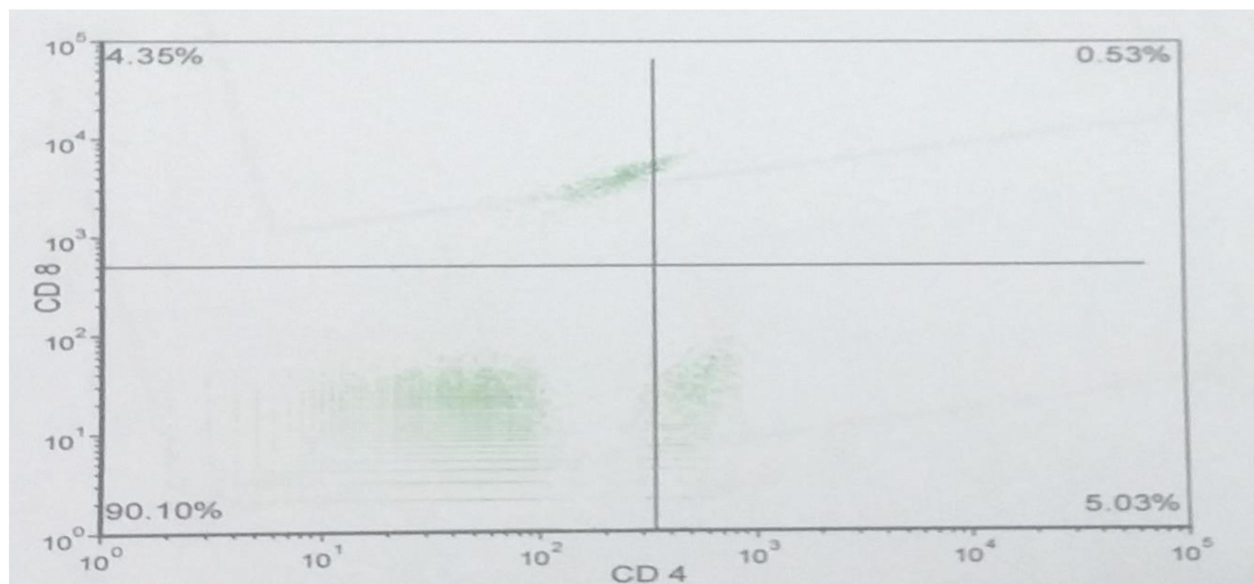


Figure (3-10): Flow cytometer results of CD4+ and CD8+ in non-complete treatment CML subjects.

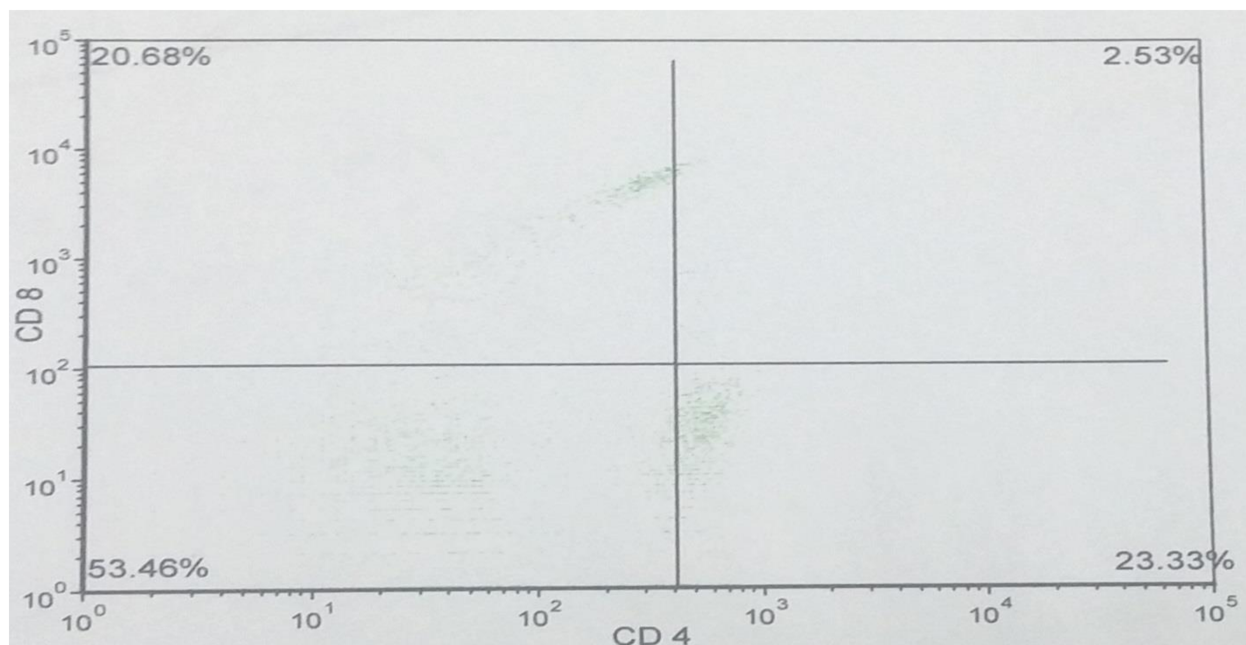


Figure (3-11): Flow cytometer results of CD4+ and CD8+ in healthy subjects.

and CD8+ T cells (Schürch *et al.,* 2013, Kreutzman *et al.*, 2014, Burkholder *et al.*, 2014).

Treated CML patients showed a significant decreased level for CD4+ counts. The decrease is referred to the effect of TKI, such as Imatinib. This finding supports Boissel *et al* (2006) and agrees with Seggewiss *et al* (2005) who revealed that imatinib decreases ZAP70 (ZAP70 is essential for TCR signal transduction), reducing tyrosine phosphorylation, and a cause of reduction of CD4+ and CD8+ activity.

Cwynarski *et al* (2004) also mentioned that imatinib inhibits the activation and proliferation of normal T lymphocytes, including CD4+ and CD8+ T lymphocytes, beside CD3 and CD28 T lymphocytes. Gao *et al* (2005) proved that T-cells activation in blood of imatinib-treated or under treatment patients, results in a remarkable decrease in CD4+ T-cells percentage in comparing with controls.

The CD4+/CD8+ T-lymphocytes ratio for newly diagnosed patients showed elevation in comparing to healthy. TKI-treating CML patients showed a major decrease in CD4+/CD8+ T-lymphocytes, leading to a significant difference with healthy CD4+/CD8+ ratio. This decrease is axiomatic due to the effect of TKI as

mentioned before in the present study. This imbalance in the ratio is considered evidence on the immunosuppressant effect (Miao *et al.,* 2004 and Ali, 2007).

Conclusions

And Recommendations

4. Conclusions and Recommendations.

4.1. Conclusions.

- Registered and unregistered variants in IL-17A gene exon 1 and IL-17F gene exon 3 may have a role in the etiology of CML by alteration IL-17 level.
- Higher CD4+ cell counts have a role elevating level of IL-17.
- TKI drug (Imatinib) plays a role in normalizing the count of CD4+ and CD8+ counts in non-complete treatment patients.

4.2. Recommendations

- Further studies on the variants detected in both IL-17A and IL-17F studied exons in the present study.
- More investigation of the role of higher IL-17 levels in CML.
- Studies focusing on the possibility of IL-17 have probable therapeutic actions in CML.

References

References

-A-

Abbott, B. (2005). Advances in the diagnosis and treatment of Chronic Lymphocytic Leukemia. *Hematological Oncology, 23*:34 – 40.

Abousamra N. K.; Salah El-Din, M. and Helal, R (2013). Prognostic value of Th17 cells in acute leukemia. Med Oncol.30:732.

Abruzzese, E.; Trawinska, M. M.; Perrotti, A. P. and De Fabritiis, P. (2014). Tyrosine kinase inhibitors and pregnancy. *Mediterranean Journal of Hematology and Infectious Diseases* 6(1):e2014028.

Aggarwal, S. and Gurney, A. L. (2002). IL-17: prototype member of an emerging cytokine family. J Leukoc Biol 71: 1–8

Al-Askari, M.A. (2002). Cytogenetic and biochemical study on lymphoma patients. MSc. Thesis . College of Science. Baghdad University.

Albanesi, C.; Cavani, A., and Girolomoni, G. (1999). IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-gamma and TNF-alpha. J. Immunol. 162, 494–502.

Ali, E. A. (2007). Studying the Association between HLA Alleles and Acute Leukemia in a Sample of Iraqi Arab Patient. PhD. Thesis, College of Science, AL-Mustansirya University, Iraq.

Angelica, **A.** (2011). Analysis of current and potential treatments for chronic lymphocytic leukemia Senior .Thesis submitted in partial fulfillment of the requirements for graduation in the honors program liberty university.5-35.

Ardito, G.; Lomberti, L.; Ansaldi, E. and Ponzetto (1980). Sister chromatid exchange in cigarette-smoker. *Mutant Research.*vol (78):209-212.

Awane, M.; Andres, P. G.; Li, D. J. and Reinecker, H. C. (1999) NF-kappa B-inducing kinase is a common mediator of IL-17-, TNF-alpha-, and ILbeta-induced chemokine promoter activation in intestinal epithelial cells. J. Immunol. 162, 5337–5344.

-B-

Baccarani, M.; Deininger, M. W.; Rosti, G.; Hochhaus, A.; Soverini, S.; Apperley, J. F.; Cervantes, F.; Clark, R. E.; Cortes, J. E.; Guilhot, F.; Hjorth-Hansen, H.; Hughes, T. P.; Kantarjian, H. M.; Kim, D. W.; Larson, R. A.; Lipton, J. H.; Mahon, F. X.; Martinelli, G.; Mayer, J.; Müller, M. C.; Niederwieser, D.; Pane, F.; Radich, J. P.; Rousselot, P.; Saglio, G.; Saußele, S.; Schiffer, C.; Silver, R.; Simonsson, B.; Steegmann, J. L.; Goldman, J. M. and Hehlmann, R. (2013). European LeukemiaNet recommendations for the management of chronic myeloid leukemia: Blood 122:872–84.

Barbacid, M. J. (1987). *ras* genes. *Annual Review of Biochemistry*.vol (56): 779-827.

Bennett, J. H. (1845). Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood. Edinb Med Surg J. 64:413-423.

Bennett, J. M.; Catovsky, D. and Daniel, M. T. (1989). Proposals for the classification of chronic (mature) B and T lymphoid leukemias. *Journal of clinical pathology*,vol (42),567-584.

Beral, V.; Peterman, T.; Berkelman, R. G. and Jaffe, H. (1991). AIDS –associated non-Hodykin's lymphoma. *Lancet*; vol(37)805-809.

Binet, J.; Auquier ,A. and Dighiero, G. (1981). A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*; 48:198-200.

Blachford, S. L. (2002). The Gale encyclopedia of genetic disorders. Gale group, Thomson leaming.U.S.A. 1.

Boissel, N.; Rousselot, P.; Raffoux, E.; Cayuela, J. M.; Soulier, J.; Mooney, N.; Charron, D.; Dombret, H.; Toubert, A. and Rea, D. (2006). Imatinib mesylate minimally affects bcr-abl+ and normal monocyte-derived dendritic cells but strongly inhibits T cell expansion despite reciprocal dendritic cell-T cell activation. J. Leukoc. Biol;79:747–756.

Bonthron, D. (1998). Cytogenetics. Textbook. Plenum Medical Book Com. :pp: 133-156.

Bos, J.; Takosz, D. and Marshall, J. (1985). Amino acid substitutions at codon 13 of the N-ras oncogene in human myeloid leukemia. *Nature*; vol(52)315:725.

Brocker, C.; Thompson, D.; Matsumoto, A.; Nebert, D. W. and Vasiliou V. (2010). Evolutionary divergence and functions of the human interleukin (IL) gene family. Hum Genomics 5(1): 30-55.

Buchdunger, E.; Matter, A. and Druker, B.J. (2001). Bcr-Abl inhibition as a modality of CML therapeutics. *Biochimestry and Biophysics* .vol(15):11–18.

Burkholder, B.; Huang, R. Y.; Burgess, R.; Luo, S.; Jones, V. S.; Zhang, W.; Lv, Z. Q.; Gao, C. Y.; Wang, B. L.; Zhang, Y. M. and Huang, R. P. (2014). Tumor-induced perturbations of cytokines and immune cell networks. Biochemical at Biophysica Acta 1845:182-201.

-C-

Cai, X. Y.; Gommoll Jr., C. P.; Justice, L.; Narula, S. K. and Fine, J. S. (1998). Regulation of granulocyte colony-stimulating factor gene expression by interleukin-17. Immunol. Lett. 62, 51–58.

Cannistra, S. (1990). Chronic myelogenous leukemia as a model for genetic basis for cancer. Hematol Oncol Clin North Am 4:337.

Cao, Z.; Tanaka, M.; Regnier, C.; Rothe, M.; Yamit-hezi, A.; Woronicz, J. D.; Fuentes, M. E.; Durnin, M. H.; Dalrymple, S. A. and Goeddel, D. V. (1999). NF-kappa B activation by tumor necrosis factor and interleukin-1. Cold Spring Harbor Symp. Quant. Biol. 64, 473–483.

Chokkaalingam , V.; Tel., J.; Wimmers, F.; Liu.,X.; Semenov, S.; Thiele, J.; Fidgor, C. and W., Huck (2013). Probing Cellular Heterogeneity in Cytokine Secreting Immune Cells using Droplet- based Microfluids . Lab Chip., 24:4740-4744.

Colvin, G. and Elfenbein, G. (2003). "The latest treatment advances for acute myelogenous leukemia". *Med Health* 86: 243–246.

Commins, S. P.; Borish, L. and Steinke, J. W. (2010). Immunologic messenger molecules: Cytokines. interferons, and chemokines. J Allergy Clin Immunol 125:S53–S72.

Connor, A. S. (1984).Cytogenetic of Cancer. Druk . New Yourk.

Cortes, J. E.; Talpaz, M.; O'Brien, S.; Faderl, S.; Garcia-Manero, G.; Ferrajoli, A.; Verstovsek, S.; Rios, M. B.; Shan, J. and Kantarjian, H. M. (2006). Staging of chronic myeloid leukemia in imatinib era: an evaluation of the World Health Organization proposal. Cancer 106: 1306-1315.

Craigie, D. (1845). Case of disease of spleen in which death took place consequent on the presence of purulent matter in the blood. Edinb Med Surg J. 64:400-412.

Cua, D. J., and Tato, C. M. (2010). Innate IL-17-producing cells: The sentinels of the immune system. Nat. Rev. Immunol. 10, 479–489.

Cwynarski, K.; Laylor, R.; Macchiarulo, E.; Goldman, J.; Lombardi, G.; Melo, J. V.; and Dazzi, F. (2004). Imatinib inhibits the activation and proliferation of normal T lymphocytes in vitro. Leukemia vol(8):1332-9.

-D-

Debra, R. N. (2005). Cancer in Depth the leukemia and lymphoma society. *Leukemia*.vol(6):190-193.

Degos, L (2001). "John Hughes Bennett, Rudolph Virchow... and Alfred Donné: the first description of leukemia". *The Hematology Journal* 2 (1): 1.

Derderian, P. M.; Kantarjian, H. M.; Talpaz, M.; O'Brien, S.; Cork, A.; Estey, E.; Pierce, S. and Keating, M. (1993). Chronic myelogenous leukemia in the lymphoid blastic phase: Characteristics, treatment response, and prognosis. Am J Med 94(1):69-74.

Di Giovine, F. S. and Duff, G. W. (1990). Interleukin 1: the first interleukin. *Immunol. Today* **11**(1): 13–20.

Donne, A. (1842). The origin of blood cells, their formation, their end. CR Acad Sci. 4:366.

Dowlati, Y.; Hermann, N.; Swardfager, W.; Liu, H.; Sham, L.; Reim, E. K. and Lanctôt, K. L. (2010). A meta-analysis of cytokines in major depression. J. Biol. Psychiatry, 67: 446-457.

Druker, B. J.; Guilhot, F.; O'Brien, S. G.; Gathmann, I.; Kantarjian, H.; Gattermann, N.; Deininger, M. W.; Silver, R. T.; Goldman, J. M.; Stone, R. M.; Cervantes, F.; Hochhaus, A.; Powell, B. L.; Gabrilove, J. L.; Rousselot, P.; Reiffers, J.; Cornelissen, J. J.; Hughes, T.; Agis, H.; Fischer, T.; Verhoef, G.; Shepherd, J.; Saglio, G.; Gratwohl, A.; Nielsen, J. L.; Radich, J. P.; Simonsson, B.; Taylor, K.; Baccarani, M.; So, C.; Letvak, L.; Larson, R. A. and IRIS Investigators (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Eng J Med 355:2408–2417.

Druker, B. J.; Sawyers, C. L.; Kantarjian, H.; Resta, D. J.; Reese, S. F.; Ford, J. M.; Capdeville, R. and Talpaz, M. (2001). Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Eng J Med 344:1038–1042.

-E-

Elaine, J.; Nancy, H.; Harald, S. and Vardiman, W. (2001). World health organization, international agency for research on cancer. Pathology and genetics of tumors of haematopoietic and lymphoid tissues. 832:2411-2416.

Elter, T.; Hallek, M. and Engert, A. (2007). Fludarabine in chronic lymphocytic leukaemia. *Expert Opin Pharmacother*. 7(12):1641-51.

Eyal, C. and Attar, D. (2010). Massachusetts general hospital cancer center leukemia review: Types, Diagnostics, Treatments.Cancer statastics .*CA. J .Clin* .50:7-33).

-F-

Fialkow, P. J.; Jacobson, R. J.; and Papayannopolou, T. (1997). Chronic myelocytic leukemia clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte macrophage. Am J Med 1997; 63:125-130.

Fossiez, F.; Banchereau, J.; Murray, R.; Van Kooten, C.; Garrone, P. and Lebecque, S. (1998). Interleukin-17. Int. Rev. Immunol. 16, 541–551.

Fossiez, F.; Djossou, O.; Chomarat, P.; Flores-Romo, L.; Ait-Yahia, S.; Maat, C.; Pin, J. J.; Garrone, P.; Garcia, E.; Saeland, S.; Blanchard, D.; Gaillard, C.; Das Mahapatra B.; Rouvier, E.; Golstein, P.; Banchereau, J. and Lebecque, S. (1996). T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J. Exp. Med. 183, 2593–2603.

Fridman, W. H. and Tartour, E. (1998). Macrophage- and lymphocyte-produced Th1 and Th2 cytokines in the tumour microenvironment. Res. Immunol. 149, 651–653.

-G-

Gambacorti-Passerini, C.; Antolini, L.; Mahon, F. X.; Guilhot, F.; Deininger, M.; Fava, C.; Nagler, A.; Della Casa, C. M.; Morra, E.; Abruzzese, E.; D'Emilio, A.; Stagno, F.; le Coutre, P.; Hurtado-Monroy, R.; Santini, V.; Martino, B.; Pane, F.; Piccin, A.; Giraldo, P.; Assouline, S.; Durosinmi, M. A.; Leeksma, O.; Pogliani, E. M.; Puttini, M.; Jang, E.; Reiffers, J.; Valsecchi, M. G. and Kim, D. W. (2011). Multicenter Independent Assessment of Outcomes in Chronic Myeloid Leukemia Patients Treated with Imatinib. JNCI Journal of the National Cancer Institute 103 (7): 553–561.

Gao, H.; Lee, B. N.; Talpaz, M.; Donato, N. J.; Cortes, J. E.; Kantarjian, H. M. and Reuben, J. M. (2005). Imatinib mesylate suppresses cytokine synthesis by activated CD4 T cells of patients with chronic myelogenous leukemia. Leukemia 19: 1905–1911.

Goldman, J. M. (1998). Leukemia and related disorders, third edition. Black well.pp:235-267.

Gomez-Rodriguez, J.; Sahu, N.; Handon, R.; Davidson, T. S.; Anderson, S. M.; Kirby, M. R.; August, A. and Schwartzberg, P. L. (2009). Differential expression of interleukin-17A and -17F is coupled to T cell receptor signaling via inducible T cell kinase. Immunity 31, 587–597.

Gorer, P. A.; Lyman, S. and Snell, G. D. (2007). Studies on the genetic and antigenic basis of tumour transplantation. Linkage between a histocompatibility gene and "fused" in mice. Proceedings of the Royal Society of London - Series B*: Biological Sciences* vol (135) 499-505.

Graham, S. M.; Jorgensen, H. G.; Allan, E.; Pearson, C.; Alcorn, M. J.; Richmond, L. and Holyoake, T. L. (2002). Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 99:319–325.

-H-

Hagstedt, C.; Aringer, L. and Gustavsson, A. (1986). Epidemiologic support for ethylene oxide as a cancer-causing agent. *Journal of the American Medicine Association*; 255, 1575-1578.

Hall, E. J.; and Giaccia, A. J. (2012). Radiobiology for the Radiologist. 7th edition. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Harris, N. L.; Jaffe, E. S. and Stein, H. (1994). A Revised European American classification of lymphoid neoplasms proposal from the international lymphoma study Group. *Blood*, vol(84), 1361-1392.

Harrison, T. (2007). Harrison's Principles of Internal Medicine, 16th Edition, Chapter 97. Malignancies of Lymphoid Cells. Clinical Features, Treatment, and Prognosis of Specific Lymphoid Malignancies. Black-well scientific publication.

Hartmann, J. T.; Haap, M.; Kopp, H. G. and Lipp, H. P. (2009). Tyrosine kinase inhibitors - a review on pharmacology, metabolism and side effects. Curr Drug Metab 10(5):470-81.

Hehlmann, R.; Heimpel, H.; Hasford, J.; Kolb, H. J.; Pralle, H.; Hossfeld, D. K.; Queisser, W.; Löffler, H.; Heinze, B. and Georgii, A. (1993). Randomized comparison of busulfan and hydroxyurea in chronic myelogenous leukemia: Prolongation of survival by hydroxyurea. Blood 83:398–407.

Hirahara, K.; Ghoreschi, K.; Laurence, A.; Yang, X. P.; Kanno, Y. and O’Shea, J. J. (2010). Signal transduction pathways and transcriptional regulation in Th17 cell differentiation. Cytokine Growth Factor Rev. 21, 425–434.

Hirji, I.; Gupta, S.; Goren, A.; Chirovsky, D. R.; Moadel, A. B.; Olavarria, E.; Victor, T. W. and Davis C. C. (2013). Chronic myeloid leukemia (CML): association of treatment satisfaction, negative medication experience and treatment restrictions with health outcomes, from the patient's perspective. Health Qual Life Outcomes 11:167. doi: 10.1186/1477-7525-11-167

Hoffbrand, A. V.; Moss, P. A. H., and Pettit, J. E. (2006). Essential Haemotology, ed 5, Oxford, Blackwell Publishing.

Holyoake, T.; Jiang, X.; Eaves, C. and Eaves, A. (1999). Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood 94:2056–2064.

Howlader, N.; Noone, A. N.; Krapcho, M., Garshell, J.; Neyman, N.; Altekruse, S. F.; Kosary, C. L.; Yu, M.; Ruhl, J.; Tatalovich, Z.; Cho, H.; Mariotto, A.; Lewis, D. R.; Chen, H. S.; Feuer, E. J.; Cronin, K. A. (eds). SEER Cancer Statistics Review, 1975-2011, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2011/, based on November 2013 SEER data submission, posted to the SEER web site, April 2014. Accessed September 10, 2014.

Hymowitz, S. G.; Filvaroff, E.; Yin, J. P.; Lee, J.; Cai, L.; Risser, P.; Maruoka, M.; Mao, W.; Foster, J.; Kelley, R. F.; Pan, G.; Gurney, A. L.; De Vos, A. M. and Starovasnik, M. A. (2001). IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. EMBO J., 20, 1–10.

-I-

Ivanov, V. K.; Tsyb, A. F.; Gorsky, A. I.; Maksyutov, M. A.; Rastopchin, E. M.; Konogorov, A. P.; Korelo, A. M.; Biryukov, A. P. and Matyash, V. A. (1997). Leukaemia and thyroid cancer in emergency workers of the Chernobyl accident: estimation of radiation risks (1986–1995). Radiat Environ Biophys 36(1):9–16.

Iwakura, Y.; Ishigame, H.; Saijo, S. and Nakae, S. (2011). Functional specialization of interleukin-17 family members. Immunity 34(2):149–62.

Iwakura, Y.; Nakae, S.; Saijo, S. and Ishigame, H. (2008). The roles of IL-17A in inflammatory immune responses and host defense against pathogens. Immunol. Rev. 226, 57–79.

-J-

Jacknow, G.; Frizzera, G.; Gajl–Peczalska, K.; Banks, P. M.; Arthur, D. C.; McGlave, P.B. and Hurd, D. D. (1985). Extramedullary presentation of the blast crisis of chronic myelogenous leukemia. Br J Haematol 61:225–236.

Jameson, C.; Dennis, L. ;Kasper, H. ; Tinsley Randolph , B.; Eugene, F.; Anthony, S.; Hauser, L.; and Longo, L. (2005). Harrison's principles of internal medicine. New York: McGraw-Hill Medical Publishing Division. *ISBN*07:140235-140237.

Jemal, A.; Siegel, R.; Ward, E.; Murray, T.; Xu, J. and Thun, M. J. (2007). Cancer statistics.CA Cancer J. Clin.57:43–66 (2007).

Jovanovic, D. V.; Di Battista, J. A.; Martel-Pelletier, J.; Reboul, P.; He, Y.; Jolicoeur, F. C. and Pelletier, J. P. (2001). Modulation of TIMP-1 synthesis by antiinflammatory cytokines and prostaglandin E2 in IL-17 stimulated human monocytes/macrophages. J. Rheumatol. 28, 712–718.

Julius, M. Cruse **(2004)**. Atlas of Immunology. 2nd Edition; by CRC Press LLC.

-K-

Kantarjian, H. M.; Deisseroth, A.; Kurzrock, R.; Estrov, Z. and Talpaz, M. (1993). Chronic myelogenous leukemia: A concise update. Blood 82:691-703.

Kantarjian, H. M.; Dixon, D.; Keating, M. J.; Talpaz, M., Walters, R. S., McCredie, K. B. and Freireich E. J. (1988). Characteristics of accelerated disease in chronic myelogenous leukemia. Cancer 61, 1441-1446.

Kantarjian, H. M.; Keating, M. J.; Talpaz, M.; Walters, R. S.; Smith, T. L.; Cork, A.; McCredie, K. B. and Freireich, E. J. (1987). Chronic myelogenous leukemia in blast crisis. Analysis of 242 patients. Am J Med 83:445–454.

Kantarjian, H. M.; Smith, T. L.; O'Brien, S.; Beran, M.; Pierce, S. and Talpaz, M. (1995). Prolonged survival following achievement of a cytogenetic response with alpha interferon therapy in chronic myelogenous leukemia. Ann Intern Med 122(4):254-61.

Kantarjian, H. M.; Talpaz, M.; O'Brien, S.; Giles, F.; Garcia-Manero, G.; Faderl, S.; Thomas, D.; Shan, J.; Rios, M. B. and Cortes, J. (2003). Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. Blood 101:473-475.

Kaur, G. and Mehra, N. (2012). Cytokine gene polymorphism: methods of detection and biological significance. Methods Mol Biol. ;882 (10):549-568.

Kindt, T.; Goldsby, R. and Osborne, B. (2007). Immunology. New York: W.H. Freeman and Company.65-114.

Koren, G. and Lishner, M. (2010). "Pregnancy and commonly used drugs in hematology practice". *Hematology Am Soc Hematol Educ Program* 2010: 160–5.

Korn, T.; Bettelli, E.; Oukka, M. and Kuchroo, V. K. (2009). IL-17 and Th17 Cells. Annu. Rev. Immunol. 27, 485–517.

Kreutzman, A.; Ilander, M.; Porkka, K.; Vakkila, J. and Mustjoki, S. (2014). Dasatinib promotes Th1- type responses in granzyme B expressing T-cells. Oncoimmunology 3: e28925:1-9.

Kurzrock, R.; Kantarjian, H. M.; Druker, B. J. and Talpaz, M. (2003). Phildelphia Chromosome-positve leukemias: from basic mechanisms to molecular therapeutics. *Internal Medicine*. Vol(138):819-30

-L-

Lee, J.; Ho, W-H.; Maruoka, M.; Corpuz, R. T.; Baldwin, D. T.; Foster, J.; Goddard, A. D.; Yansura, D. G.; Vandlen, R. L.; Wood, R. I. and Gurney, A. L. (2001). IL17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL17Rh1. J. Biol. Chem. 276, 1660–1664.

Lee, Y. K.; Turner, H.; Maynard, C. L.; Oliver, J. R.; Chen, D.; Elson, C. O. and Weaver, C. T. (2009). Late developmental plasticity in the T helper 17 lineage. Immunity 30, 92–107.

Leonard, B. (1998). Leukemia: A Research Report. *DIANE* Publishing. *ISBN*. 788-7189.

Li, H.; Chen, J.; Huang, A.; Stinson, J.; Heldens, S.; Foster, J.; Dowd, P.; Gurney, A. L. and Wood, W. I. (2000). Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. Proc. Natl. Acad. Sci. USA 97, 773–778.

Liles, W. C. and Van Voorhis, W. C. (1995). Review: Nomenclature and biologic significance of cytokines involved in inflammation and the host immune response. J Infect Dis 172:1573–1580.

Linden, A.; Hoshino, H., and Laan, M. (2000). Airway neutrophils and interleukin-17. Eur. Respir. J. 15, 973–977.

Little, M. P.; Wakeford, R.; Tawn, E. J.; Bouffler, S. D. and de Gonzalez, B. A. (2009). Risks associated with low doses and low dose rates of ionizing radiation: why linearity may be (almost) the best we can do. Radiology 251(1):6–12.

-M-

Ma, H.; Yang, Y. and Young, M. (2015). Cytokines**.** *Rep Opinion* 7(2):86-89.

Mantovani, A. and Dejana, E. (1989). Cytokines as communication signals between leukocytes and endothelial cells. Immunol Today 10:370-5.

Matutes, E.; Owes–Arkoma, K. and Morilla, R. (1994). The Immunological profile of B-cell disorders and proposal of as coring system for the diagnosis of CLL. *Leukemia*; 8, 1640-1645.

McGeachy, M. J. and Cua, D. J. (2008). Th17 cell differentiation: The long and winding road. Immunity 28, 445–453.

Miao, X. Y.; Jia, L. and Wang, Y. C. (2004). Expression of T cell markrs in hematological diseases and Solid tumor. *Zhongguo-Shi-yan–Xue-Ye-Xue-Za-Zhi.*, vol(12): 79-82.

Miller R.W. (1967) Person of exceptionally high risk of leukemia. *Cancer Reseach*; vol(27): 2420-2423.

Ministry of Health, Iraqi Cancer Board. (2011). Results of Iraqi Cancer Registry.

Mittleman, F. and Heim, S. (2007).Quantitative acute leukemia cytogenetics Genes. Chromosomes and Cancer, 5, 57-66.

Mos, P. A. and Gillespie, G. (1997). Clonal populations of T cells in patients with B-cells malignancies. Leuk. Lymphoma 27 (3-4): 231-8.

Mughal, T. I. and Goldman, J. M. (2006). Chronic myeloid leukemia: Why does it evolve from chronic phase to blast transformation?. Frontiers in Bioscience. (1):198-208.

Mundhada, B. (2006). Association of HLA Class I and Class II genes with *bcr-abl* transcripts in leukemia patients with t(9;22)(q34;q11). *Biology* vol(9): 39-46.

-N-

Narins, Brigham **(2003)**. World of Microbiology and Immunology; by Gale Inc., a division of Thomson Learning Inc.

Nicod, L. P. (1993). Cytokines: 1. Overview. Thorax 48:660-667.

Noshchenko, A. G.; Bondar, O. Y. and Drozdova, V. D. (2010). Radiation-induced leukemia among children aged 0–5 years at the time of the Chernobyl accident. Int J Cancer 127(2):412–426.

Nowell, P. C. and Hungerford, D.A. (1960). A minute chromosome in human chronic granulocytic leukaemia. *Science*;vol (3):132:1497.

Nowell, P. **C**. (2007). Discovery of the Philadelphia chromosome: a personal perspective". Journal of Clinical Investigation 117 (8): 2033–5.

-P-

Pasternak, G. (2006). Chronic myelogenous leukemia: molecular and cellular aspects *.Journal of Cancer Research. ;*vol(124):643-660.

Patlak, M. (2010). “Targeting Leukemia: From Bench to Beside”. Break throughs in Bioscience. The Federation of American Societies for Experimental Biology.

Piller, G. (1993). The history of leukemia: a personal perspective. *Blood Cells*. 19:521-529.

Preston, D. L.; Kusumi, S.; Tomonaga, M.; Izumi, S.; Ron, E.; Kuramoto, A.; Kamada, N.; Dohy, H.; Matsui, T.; Nonaka, H.; Thompson, D. E.; Soda, M. and Mabuchi, K. (1994). Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma. Radiat Res 1994, 137(2 Suppl):S68–S97.

-R-

Ragni, M. V.; Belle S. H. and Jaffe R. A. (1993). Acquired immunodeficiency syndrome–associated non-Hodykin's lymphomas and other malignancies in patients with Hemophilia. *Blood;* vol(81):1889 -1897.

Rai, K.; Sawitsky, A. and Cronkite, E. (1975). Clinical staging of chronic lymphocytic leukemia. *Blood*,46:219

Reynolds, J. M.; Angkasekwinai, P. and Dong, C. (2010). IL-17 family member cytokines: Regulation and function in innate immunity. Cytokine Growth Factor Rev. 21, 413–423.

Robinette, M.; Susan, C. and Van de ,W. (2001). Quick look series in veterinary medicine,*Hematology*, Teton NewMedia. 101- 105.

Rousselot, P.; Huguet, F.; Rea, D.; Legros, L.; Cayuela, J. M.; Maarek, O.; Blanchet, O.; Marit, G.; Gluckman, E.; Reiffers, J.; Gardembas, M. and Mahon, F. X. (2007). Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood 109:58–60.

Rouvier, E.; Luciani, M. F.; Mattei, M. G.; Denizot, F. and Golstein, P. (1993). CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. J. Immunol. 150: 5445–5456.

Rowley, J. D. (1981). A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining, *Nature* . vol (24):290-307.

Rowley, J. D. (1973). A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 243:290.

Rudant, J.; Alicia, A. ;Laurent, O.; Thomas, A.; Guy, L.; André, B.; Yves, B.; Brigitte, N.; Geneviève, P.; Gérard, M.; Nicolas, S.; Pascal, C.; Stéphane, D.; Xavier, R.; Denis, H. and Jacqueline, C. (2012). "Fertility treatments, congenital malformations, fetal loss, and childhood acute leukemia: The ESCALE study (SFCE)". *Curr . Opin. Biotechnol*. 11:586-592.

Rushing, D.; Goldman, A.; Gibbs, G.; Howe, R. and Kennedy, B. J. (1982). Hydroxyurea versus busulfan in the treatment of chronic myelogenous leukemia. Am J Clin Oncol 5:307–313.

-S-

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989). Molecular cloning: A laboratory manual. *2nd edition, Cold spring harbor laboratory press, NewYork.*

Saracci, R.; Kogevinas, M. and Bertazzi P-A. (1991) Cancer mortality in workers exposed to chlorophenoty herbicides and chlorophenols. *Lancet*; vol(33): 1027-1032.

Sawyers, C.L.; Hochhaus, A.; and Feldman, E.; Goldman, J. M.; Miller, C. B.; Ottmann, O. G.; Schiffer, C. A.; Talpaz, M.; Guilhot, F.; Deininger, M. W.; Fischer, T.; O'Brien, S. G.; Stone, R. M.; Gambacorti-Passerini, C. B.; Russell, N. H.; Reiffers, J. J.; Shea, T. C.; Chapuis, B.; Coutre, S.; Tura, S.; Morra, E.; Larson, R. A.; Saven, A.; Peschel, C.; Gratwohl, A.; Mandelli, F.; Ben-Am, M.; Gathmann, I.; Capdeville, R.; Paquette, R. L. and Druker, B. J. (2002). Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* .May vol(10): 3530-9.

Schrck, E. (2007). Spectral karyotyping and multi-colorfluorescence in situ hybridization reveal new tumor-specific chromosomal abnormalities. Hematology.vol(37), 334-47.

Schürch, C. M; Riether, C. and Ochsenbein, A. F. (2013). Dendritic cell-based immunotherapy for myeloid leukemias. Front Immunol; 4: 496.

Schuster, J. M. and Nelson, P. S. (2000). Toll receptors: an expanding role in our understanding of human disease. J. Leukoc. Biol. 67, 767–773.

Seggewiss, R.; Loré, K; Greiner, E.; Magnusson, M. K.; Price, D. A.; Douek, D. C.; Dunbar, C. E. and Wiestner, A. (2005). Imatinib inhibits T-cell receptor-mediated T-cell proliferation and activation in a dose-dependent manner. Blood 105: 2473–2479.

Severson, R. K. (1987). Cigarette smoking and leukemia. *Cancer;* vol **(**18**)** 141-144.

Shah, N. P. and Arceci, R. J. (2014). Chronic Myeloid Leukemia Booklet. Leukemia & Lymphoma Society.

Shalom-Barak, T.; Quach, J. and Lotz, M. (1998). Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-kappaB. J. Biol. Chem. 273, 27467–27473.

Spriggs, M. K. (1997). Interleukin-17 and its receptor. J. Clin. Immunol. 17, 366–369.

Starnes, T.; Robertson, M. J.; Sledge, G.; Kelich, S.; Nakshatri, H.; Broxmeyer, H. E. and Hromas, R. (2001). IL-17F, a novel cytokine selectively expressed in activated T-cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. J. Immunol. 167, 4137– 4140.

Stass, A.; Schumacher, R. and Rock, R. (2000). Handbook of hematologic pathology. New York, N.Y: Marcel Dekker. 193–194.

Sylvia, S. M. (1998). Regulation of gene activity–causes and preventation of cancer. Biology.16, 264-265.

-T-

Talpaz, M.; Kantarjian, H. M.; McCredie, K. B.; Keating, M. J.; Trujillo, J.; and Gutterman, J. (1987). Clinical investigation of human alfa interferon in chronic myelogenous leukemia. Blood 69:1280–1288.

Talpaz, M.; Silver, R. T.; Druker, B. J.; Goldman, J. M.; Gambacorti-Passerini, C.; Guilhot, F.; Schiffer, C. A.; Fischer, T.; Deininger, M. W.; Lennard, A. L.; Hochhaus, A.; Ottmann, O. G.; Gratwohl, A.; Baccarani, M.; Stone, R.; Tura, S.; Mahon, F. X.; Fernandes-Reese, S.; Gathmann, I.; Capdeville, R.; Kantarjian, H. M. and Sawyers, C. L. (2002). Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 99:1928-1937.

Terjanian, T.; Kantarjian, H.; Keating, M.; Talpaz, M.; McCredie, K. and Freireich, E. J. (1987). Clinical and prognostic features of patients with Philadelphia chromosome-positive chronic myelogenous leukemia and extramedullary disease. Cancer 59(2):297–300.

Teunissen, M. B., Koomen, C. W., de Waal Malefyt, R., Wierenga, E. A., and Bos, J. D. (1998). Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J. Investig. Dermatol. 111, 645–649.

-V-

Van Etten, R. A. (2007). *Aberrant cytokine signaling in leukemia. Oncogene 26(47):6738-6749.*

Van Kooten, C.; Boonstra, J. G.; Paape, M. E.; Fossiez, F.; Banchereau, J.; Lebecque, S.; Bruijn, J. A.; De Fijter, J. W.; van Es, L. A. and Daha, M. R. (1998). Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. J. Am. Soc. Nephrol. 9, 1526–1534.

Velpeau, A. (1827). Sur la resorption du pus et sur lálteration du sang dans les maladies cliniques de persection nenemant. Premier observation. Rev Med 2:216-218.

Verma, D. S.; Spitzer, G.; Gutterman, J. U.; Zander, A. R.; McCredie, K. B. and Dicke, K. A. (1979). Human leukocyte interferon preparation blocks granulopoietic differentiation. Blood 54:1423–1427.

Vincernt, T.; Samuel, H. and Steaven, A. (2004). Cancer principle and practice of Oncology.5th edition-Lippincott-Raven,Philadelaphia U.S.A..

Virchow, R. (1846). Weisses Blut and Milztumoren. Med Z. 15:157.

-W-

Wang, L.; Giannoudis, A.; Lane, S.; Williamson, P.; Pirmohamed, M. and Clark, R. E. (2008). Expression of the uptake drug transporter hOCT1 is an important clinical determinant of the response to imatinib in chronic myeloid leukemia. *Clin Pharmacol Ther* 83: 258–264.

White, D. L.; Saunders, V. A.; Dang, P.; Engler, J.; Venables, A.; Zrim, S.; Zannettino, A.; Lynch, K.; Manley, P. W. and Hughes, T. (2007). Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: Higher doses of imatinib may overcome the negative impact of low OCT-1 activity. Blood 110:4064–4072.

White, D. L.; Saunders, V. A.; Dang, P.; Engler, J.; Zannettino, A. C.; Cambareri, A. C.; Quinn, S. R.; Manley, P. W. and Hughes, T. P. (2006). OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): Reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. Blood 108:697-704.

WHO (World Health Organization) (2002). Non-ionizing radiation.Part 1: static and extremely low-frequency (ELF) electric and magnetic fields (IARC Monographs on the Evaluation of the Carcinogenic Risks) . Geneva: World Health Organisation, 332:333-338.

Wiernik, H. (2001). Adult leukemias. New York: B. C. Decker. pp. 3–15.

Woltman, A. M.; de Haij, S.; Boonstra, J. G.; Gobin, S. J.; Daha, M. R. and van Kooten, C. (2000). Interleukin-17 and CD40-ligand synergistically enhance cytokine and chemokine production by renal epithelial cells. J. Am. Soc. Nephrol. 11, 2044–2055.

Wu, C.; Wang, S.; Wang, F.; Chen, Q.; Peng, S.; Zhang, Y.; Qian, J.; Jin, J. and Xu, H. (2009). Increased frequencies of T helper type 17 cells in the peripheral blood of patients with acute myeloid leukaemia. Clin Exp Immunol. 158:199–204.

-Y-

Yamamura, Y.; Gupta, R.; Morita, Y.; He, X.; Pai, R.; Endres, J.; Freiberg, A.; Chung, K. and Fox, D. A. (2001). Effector function of resting T cells: activation of synovial fibroblasts. J. Immunol. 166, 2270–2275.

Yang, X. O.; Pappu, B. P.; Nurieva, R.; Akimzhanov, A.; Kang, H. S.; Chung, Y.; Ma, L.; Shah, B.; Panopoulos, A. D.; Schluns, K. S., Watowich, S. S.; Tian, Q.; Jetten, A. M. and Dong, C. (2008). T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity 28, 29–39.

Yao, Z.; Fanslow, W. C.; Seldin, M. F.; Rousseau, A. M.; Painter, S. L.; Comeau, M. R.; Cohen, J. I. and Spriggs, M. K. (1995). Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 3, 811–821.

-Z-

Zhou, L. and Littman, D.R. (2009). Transcriptional regulatory networks in Th17 cell differentiation. Curr. Opin. Immunol. 21, 146–152.

Zhu, B.; Zhang, J.; Wang, X.; Chen, J. and Li, C. (2015). Correlation between acute myeloid leukemia and IL-17A, IL-17F, and IL-23R gene polymorphism. International Journal of Clinical and Experimental Pathology 8(5):5739-5743.

الملخص

صممت هذه الدراسة لتسليط الضوء على العلاقة بين التغايرات الوراثية في جين IL-17A و IL-17F، وتقدير مستوياتهم في مرض سرطان الدم النخاعيني المزمن.

شملت هذه الدراسة اثنان وستون شخصا مصابين بمرض سرطان ابيضاض الدم النخاعيني المزمن، بالاضافة لثمان وعشرون شخصا أصحاء. جمعت عينات الدم من مستشفى الاورام التعليمي ومستشفى بغداد التعليمي في مدينة الطب – بغداد، و المركز الوطني لأمراض الدم – الجامعة المستنصرية.

أظهر تحليل التعدد الوراثي للجين بواسطة تقنية تتابع تسلسل الدنا ظهور 13 تغاير مسجل في المنطقة المشفرة 1 لجين IL-17A: سجل التعدد الأليلي rs199815459 وجود النمط الوراثي T/G في المرضى و النمط الوراثي G/G لدى الأصحاء. بينما التعدد الأليلي rs749745973 سجل ظهور أنماط وراثيية مختلفة عن الأنماط الوراثية المسجلة وكان النمط الوراثي G/A بتردد 100% في المرضى والأصحاء، والذي يختلف عن النمط الوراثي المسجل عالميا وهو G/C.

أظهرت النتائج وجود بعض التعددات الأليلية الغير مسجلة. فكانت الأولى في منطقة الحفاز، والثانية والثالثة في المنطقة المشفرة، بينما كانت الرابعة في المنطقة الغير مشفرة.

أظهرت نتائج تتابع تسلسل الدنا لجين IL-17F المنطقة المشفرة 3، وجود تعدد أليلي مسجل rs2397084 والذي سجل بالنمط الوراثي A/G بتردد 9.1% في المرضى والنمط الوراثي A/A بتردد 90.1% في المرضى وبتردد 100% في الأصحاء.

أما بالنسبة للحركيات الخلوية (IL-17A و IL-17F)، فقد أظهرت النتائج اختلافا معنويا واضحا في تركيزها في مصل الدم لدى المرضى المشخصين حديثا والمرضى تحت العلاج.

أظهرت نتائج الأنماط المناعية المظهرية ل +CD4 و +CD8 بعد قياسها بجهاز تعداد تدفق الخلايا وجود زيادة معنوية في المرضى المشخصين حديثا، وانخفاضا معنويا في المرضى تحت العلاج.

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تقييم النمط المظهري المناعي والوراثي للانترلوكين 17 في المرضى العراقيين المصابين بابيضاض الدم النخاعيني المزمن

الأطروحة

مقدمة الى مجلس كلية العلوم/جامعة النهرين، كجزء من متطلبات نيل درجة الماجستير في العلوم/التقانة الاحيائية

من قبل

هشام عبد الحسين الفيصل

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