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Allelic Analysis of HLA-A,B and IL-12A (rs583911) Polymorphism in Pediatric Patients with Acute Lymphoblastic Leukemia

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Dedications

To…

My dear father , mother and all my family..

To…

My love "Zaydoon" and my daughter "Reemas"..

For your support and encouragement, I hope this thesis serves to repay your contributions in some small part….

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Summary

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid progenitor cells and the most common type of malignant neoplasms in children, this disease is a multifactorial disease that results from interaction between genetic and environmental factors.

This study was conducted on 60 pediatric patients with ALL (40 males and 20 females) their age ranges between 2-14 years, attended to Central Child Teaching Hospital in Baghdad for the period from February2018 to June 2018. Other 60 apparently healthy subjects (42 males and 18 females) were included as a control group. Blood samples were collected from both groups, genomic DNA was extracted from blood leukocytes for further molecular study to reveal any association between two HLA loci and IL-12A (rs583911) gene polymorphism and predisposition to ALL. Allele specific -polymerase chain reaction (AS-PCR) technique was used for IL-12A (rs583911) gene polymorphism and Sequence Specific Primers-polymerase chain reaction (SSP) –PCR technique was used for HLA-A and B allelic analysis.

The current study showed that the peak percentage of the disease happened at (4-5) years of age. The results also revealed that 67% of patients were males. The data showed that 56.7% of children with ALL had positive family history of malignant disorder.

This study detected that the most prevalence IL12-A rs583911 genotype was AA so it was considered as the wild type (reference) whereas the least frequent IL12-A rs583911 genotype was GG, therefore it regarded as the variant (mutant) genotype. Genotype GG was significantly more frequent in study group than in control group 56.7 % versus 23.3% (P = 0.001). There was highly significant difference in rate of allele G between study and control groups (P=0.002). It acts as risk factor for ALL with an Odds ratio of (2.94);(95 %)

confidence interval of (1.71 -5.05) and the etiologic fraction of allele G as a risk factor for ALL was (0.40). While , variant allele A was more frequent in control than in study group, 51.7% versus 26.7% respectively and may be considered as a preventive fraction (0.40).

The results suggests that the frequency distribution and contribution to acute lymphoblastic leukemia of HLA-A alleles *1, *23, *24, *26 and *30 have no significant association with the disease. While alleles *2, *3,*11 and *33 were considered as risk factors for ALL. Whereas the alleles *26,*32 and *68 plays significant role in protection against ALL.

In other hand the frequency distribution and contribution to acute lymphoblastic leukemia of HLA-B alleles *13, *27, *42 and *58 which have no significant association with acute lymphoblastic leukemia. While the alleles *35, *44, *50, *51 and *52 were found to be risk factors for ALL, and alleles *7, *15, *18, *38, *40 and *53 have significant role in protection against ALL.

Also the data showed there was no significant association between family history of malignancy and IL12-A rs583911 genotypes ($P = 0.273$) and any of HLA-A,B genotypes ($P > 0.05$).

In conclusion the study revealed that the variant allele G of IL12-A (rs583911) polymorphism considered as etiological factor for acute lymphoblastic leukemia, also Human Leukocyte Antigen-A alleles (*01, *03, *11 and *33) and alleles (*35, *44, *50, *51 and *52) of HLA-B have significant association as risk factors for ALL.

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List of Abbreviations

Abbreviation	*Meaning*
ALL	**Acute Lymphoblastic Leukemia**
AMLs	**Acute Myeloid Leukemias**
APCs	**Antigen-Presenting Cells**
Bp	**Base pair**
B cell	**Bursa cell**
Cm	**Centimeter**
CNS	**Central Nervous System**
CSF	**Cerebrospinal Fluid**
CNTF	**Ciliary Neutropic Factor**
CD	**Cluster of Differentiation**
CI	**Confidence Interval**
dATP	**Deoxy Adenosine Tri Phosphate**
dCTP	**Deoxy Cytosine Tri Phosphate**
dGTP	**Deoxy Guanine Tri Phosphate**
dNTP	**Deoxy Nucleotide Tri Phosphate**
dTTP	**Deoxy Thymine Tri Phosphate**
DNA	**Deoxyribonucleic Acid**
EtBr	**Ethidium Bromide Solution**
EDTA	**Ethylene Diamine Tetra Acetic Acid**
EF	**Etiologic Fraction**
FAB	**French American British**
GSB	**Gel Sample Buffer**
GD column	**Genomic DNA column**
GM-CSF	**Granulocyte-Macrophage Colony-Stimulating Factor**

HS	**Highly Significant**
HCl	**Hydrochloride**
IFN-g	**Interferon gamma**
KDa	**Kilo Dalton**
LD	**Linkage Disequilibrium**
LBL	**Lymphoblastic Lymphoma**
MHC	**Major Histocompatibility Complex**
Mb	**Mega bases**
mRNA	**messenger Ribonucleic Acid**
µg/ml	**Microgram Per Milliliter**
µl	**Microliter**
Ml	**Milliliter**
mM	**MilliMolar**
MRD	**Minimal Residual Disease**
MLL	**Mixed Lineage Leukemia**
MPO	**Myeloperoxidase**
ng	**Nanogram**
nm	**Nanometer**
NMDP	**National Marrow Donor Program**
NHL	**Non-Hodgkins lymphoma**
NS	**Not Significant**
OR	**Odd Ratio**
Ph-like ALL	**Philadilphia- like Acute Lymphoblastic Leukemia**
Pmol	**Pico-mole**
PCR	**Polymerase Chain Reaction**
PF	**Preventive Fraction**

Rpm	**Round per minute**
SSP-PCR	**Sequence Specific Primers-Polymerase Chain Reaction**
SNPs	**Single Nucleotide Polymorphisms**
SD	**Standard Deviation**
SE	**Standard Error**
SPSS	**Statistical Package For Social Science**
Th	**T helper**
Tdt	**Terminal deoxynucleotidyl transferase**
T cell	**Thymus cell**
TBE	**Tris/Borate/EDTA**
TNF-α	**Tumor Necrosis Factor Alpha(TNFA)**
UV	**Ultra –Violet**
U/µl	**Unit per microliter**
UK	**United Kingdom**
USA	**United States America**
V	**Volt**
WHO	**World Health Organization**

1. Introduction and Literature Review:

1.1. Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid progenitor cells and the most common type of malignant neoplasms in children (Tomizawa & Kiyokawa, 2017).

Its incidence peaks between the ages of 2 and 5 years; rates are lower during later childhood, adolescence and young adulthood .It is the most common leukemia in children representing 23% of cancer diagnosis among children younger than 15 years (Pui *et al.*, 2008) .

The researchers find that the cause of this disease is related to different environmental and genetic factors: Environmental factors, such as ionizing radiation, electromagnetic field, certain diets (e.g., bioflavonoids), seem to have little association in most of the ALL cases (Kendall *et al.,* 2011). However, two infection-based hypotheses, Greaves' "delayed infection" hypothesis and Kinlen's "population-mixing" hypothesis that childhood ALL arise as a consequence of an abnormal immune response in susceptible individuals to common infections, are well supported by epidemiological data (Kinlen, 1995; Greaves, 2006),while the genetic factors play an important role in the etiology of ALL and this arise from in utero chromosomal abnormalities that can lead to clonal expansion of pre-leukemic precursor cells (Tulstrup *et al.*, 2017). Although it has been long recognized that majority of ALL patients have no apparent clinical inherited factors, recent genome-wide association studies have identified several germline single nucleotide polymorphisms (SNPs) in several genes that are associated with significantly higher risk of developing ALL (Perez-Andreu *et al.*, 2013).

Interleukin-12 (IL-12) is a pro-inflammatory cytokine that is mainly secreted by antigen-presenting cells.IL-12 targets T-helper (Th) cells and natural

killer cells, and stimulates the synthesis and secretion of interferon gamma (IFN-γ),which is a well established anti tumor factor. Additionally, lower serum IL-12 levels have been observed in patients with various types of cancer , suggesting that IL-12 functions as a potent tumor suppressive factor. Biologically active IL-12 consists of two functional subunits, p35 and p40, which are encoded by the IL-12A and IL-12B genes, respectively (Croxford *et al*., 2014; Fang *et al.,*2015).Since the tumor-suppressive effect of IL-12 is well documented, functional polymorphisms of the IL-12A and IL-12B genes are thought to be good genetic candidates for cancer susceptibility. Extensive studies have explored the potential associations of IL-12A and IL-12B genetic variants with cancer risk (Wu & Brewer, 2012 ; Wang *et al.*, 2013).A meta-analysis of many case-control studies show that risk of many types of cancer is associated with interleukin 12 gene polymorphism (Shi *et al.*, 2017).

Human leukocyte antigen (HLA) genes encode cell surface glycoproteins associated with antigen presentation that selectively interact with short peptide fragments derived from non-self and self-proteins. The HLA class I molecules (A, B, and C) present intracellular antigens to CD8 T cells, while class II molecules (DR, DQ, and DP) present extracellular antigens toCD4+ T cells, which activate macrophages and B cells. HLA has a major role in regulating host responses to infections. It has been hypothesized that the HLA alleles may have an important role in predisposal to ALL (Hosking *et al.*, 2011).The HLA genes are the most polymorphic genes in the human genome **.**An association between ALL and HLA alleles has been shown ; however, the data are not conclusive so far (Ozdilli *et al.*, 2010; Uçar *et al.*, 2011).

Aim of Study:

The aim of this study is to investigate the associations of two HLA loci with pediatric acute lymphoblastic leukemia patients and IL 12A (rs583911) polymorphism in a selected sample of Iraqi population and to see if these parameter act as a predisposing factors in the occurrence of the disease. To achieve this goal, the following steps were used:

- Studying sample subjects, personal and disease characteristics like; age, sex, disease onset, family history of any cancer.
- Identifying an association between single nucleotide polymorphism (SNP) of the selected Th1-cell cytokine focused on IL 12A (rs583911) polymorphism with acute lymphoblastic leukemia.
- Investigate the allelic frequency of human leukocyte antigen (HLA) class 1 (A&B) alleles in patients with acute lymphoblastic leukemia and control group.

1.2. Literatures Review

1.2.1. Overview of Acute Lymphoblastic Leukemia

Acute leukemias are characterized by the proliferation and accumulation of malignant, transformed, immature, hematopoietic cells, so-called blasts, in the blood and the bone marrow. All other lymphatic organs (e.g. lymph nodes, spleen) and non-lymphatic organs (e.g. liver, CNS, skin, bone, etc.) can also be affected. The leukemic blasts displace the normal hematopoietic bone marrow and cause cytopenias in all three cell lineages anemia, thrombocytopenia, granulocytopenia (Gökbuget, 2011).Both T-cell and B-cell precursors can give rise to ALL; B-cell ALL represents about 88% of all cases (Terwilliger & Abdul-Hay, 2017).

The overall incidence rate of ALL amounts to 1.1/100,000 per year. The peak incidence lies in childhood at an age of less than 5 years (5.3/100.00). Thereafter the incidence rate declines continually. In patients over 50 years it rises a second time and reaches a peak at the age of over 80 years (2.3/100.000). There is a slight predominance of males 1.4:1.0 (Campana, 2010).

1.2.2. Epidemiology

Most of ALL cases happen in children, with an incidence of 3 to 4/100,000 in patients 0 to 14 years of age and 1/100,000 in patients which are older than 15 years. In children, ALLs represent more than75% of all acute leukemias (which in turn represent 34% of all cancers in this group of age), with a peak incidence at 2 to 5 years of age (Pui *et al.*,2008 ; Hunger & Mullighan, 2015).

Generally, the incidence of the disease is higher in boys than in girls (four times for T-cell ALL), except that girls have a higher (1.5 times) incidence of leukemia in the first year of life (Linet *et al.*, 2003).The percentage of incidence is much lower in adults, in whom acute myeloid leukemia (AMLs) and chronic lymphocytic leukemia are more common. There is a slight male predominance

in all age groups and a significant excess incidence among white children (Pui *et al.*, 2008; Campana & Pui, 2011).

Age-adjusted incidence rates for ALL is different several-fold, internationally, with the highest rates occurring in Spain, among Hispanics in Los Angeles, and in Caucasians in Quebec and Ontario, Canada, and in New Zealand (Lim *et al.*, 2014).The most low rates are found in developing countries, among US blacks, Israeli Jews, and Chinese and Asian Indians, whose rates may be many times lower than those in more affluent developed countries (Ward *et al.* , 2014).

Acute Lymphoblastic Leukemia presents primarily as *de novo* disease, with only rare cases occurring as secondary neoplasms (Shivakumar *et al.,* 2008). Studies in the pediatric population have identi ed genetic syndromes that predispose to a minority of cases of ALL, such as Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia and Nijmegen breakdown syndrome (Bielorai *et al.*, 2013; Buitenkamp *et al.*, 2014).

Other predisposing factors include exposure to ionizing radiation, pesticides, certain solvents or viruses such as Epstein-Barr Virus and also congenital Immunode ciency disease (Sehgal *et al.*, 2010; Margolin *et al.*, 2010; Urayama *et al.*, 2011).

1.2.3.Survival and Mortality of ALL

Less than half of all adult leukemias survive 5 years after diagnosis, but nearly two-thirds of all adult ALL cases survive at least 5 years after diagnosis (Katz *et al.* , 2015).

Since 1975, the survival rates for pediatric, adolescent, and young adult patients with ALL have increased, but as of 2006, still fewer than half of AYAs survived 5 years from diagnosis. From 2000 to2011 the 5-year relative survival rate of ALL declined most strikingly between the ages of 16 and 21 years; this was true both for males and females. Additionally, the average annual percent

change (APC) in death rate declined from 1998 to 2011 in patients of all ages diagnosed with ALL, except for young adults aged 25–45 years (McNeer *et al.*, 2017).

Childhood ALL survival is one of the most dramatic success stories in the history of chemotherapy, showing a remarkable improvement due to innovative treatments (Lewis *et al.*, 2014).

1.2.4. Etiology and risk factors of ALL

Among children, only ionizing radiation and certain genetic disorders are known risk factors (Margolin *et al.*, 2010). Many other risk factors have been suggested but remain under investigation, such as exposure to pesticides, automobile exhaust, certain chemicals such as benzene, non ionizing radiation (e.g., magnetic fields), parental exposures (e.g., cigarette smoking, alcohol consumption and use of some pharmaceuticals), and even parental consumption of certain dietary constituents. There is growing evidence that this cancer may arise from in utero chromosomal abnormalities that can lead to clonal expansion of pre-leukemic precursor cells. The risk factors for ALL in children are multiple, most notably common germline polymorphisms and rare genetic syndromes that directly influence hematopoiesis and cell cycling, as well as possibly infection-related aberrant DNA editing (Tulstrup *et al.*, 2017).

1.2.4.1. Biological Factors

1.2.4.1.1. Genetics of Childhood ALL

Leukemia, like other forms of cancer, is ultimately a disease of the DNA. Although single-gene mutations (e.g., BRCA1 and BRCA2) are known to predispose to solid tumors (e.g., carcinomas of breast and ovary), no such single-gene mutations have been linked to childhood ALL, which tends instead to be associated with chromosomal anomalies. Genetic factors ranging from predisposing highly penetrant mutations to low penetrant, genetic

polymorphisms have been shown to significantly influence the interindividual variation in cancer incidence (Iacobucci & Mulligha, 2017).

1.2.4.1.2. Cytogenetic Abnormalities

Cytogenetic abnormalities frequently found in ALL cases include germ-line karyotypic abnormalities, somatic karotypic abnormalities, translocations, and deletions. The germ-line abnormalities associated with childhood leukemia include Down syndrome (trisomy21) (Buitenkamp *et al.*, 2014),Bloom syndrome, Fanconi anemia, Klinefelter syndrome, and ataxia-telangiectasia (Bielorai *et al.*, 2013). The somatic abnormalities associated with childhood leukemia include aneuploidy (changes in chromosome number) chromosomal rearrangements that deregulate gene expression or result in expression of chimeric fusion proteins, deletions and gains of DNA, and DNA sequence mutations (Harrison, 2009). Hypodiploidy (<44 chromosomes) occurs in 2 to 3% of children with B-cell ALL and is a strong negative prognostic factor (Nachman *et al.*, 2007).Low hypodiploidy (30 to 39 chromosomes), which is associated with the presence of TP53 mutations that are frequently inherited, is a manifestation of the Li–Fraumeni syndrome (Holmfeldt *et al.* , 2013).

Chromosomal translocations and intra-chromosomal rearrangements are early, possibly initiating events in leukemogenesis. Several can be detected in neonatal blood samples years before there are clinical manifestations of leukemia**.** These translocations and rearrangements are usually present in all leukemic cells, are retained at relapse (Ma *et al.*, 2015).Translocations frequently found in ALL cases include the TEL-AML1 translocation (found in about20–25% of B-lineage childhood ALLs)**.**In short, the chromosomes that are known to be involved in karyotypic abnormalities found in childhood ALL are 1, 4, 6–9, 11, 12, 14, 19, 21, and 22 (Tulstrup *et al.*, 2017).

Neither X nor Y is known to be involved with childhood ALL. Translocations are especially common in childhood ALL. Triggers for molecular

anomalies may be inherited during pregnancy, and may develop during infancy or early childhood (Buffler *et al.*, 2005; Mullighan *et al.*, 2008).

1.2.4.1.3. Infectious Etiology

The most widely accepted current theory of causation of childhood ALL is based on an infectious etiology associated with decreased immune function. Three variations on this theme of the "infection" that have been put forward are:

(1) Exposure to a specific infectious agent post natally, proposed by Kinlen (Kinlen, 1988).

(2) Exposure to a specific infectious agent prenatally or around the time of birth, proposed by Smith *et al.*, (1998).

(3) A delay in the initial exposure to infectious agents in general beyond the first year of life, proposed by Greaves & Alexander, (1993).

1.2.4.2. Physical Factors

1.2.4.2.1. Ionizing Radiation

The importance of ionizing radiation as an etiologic agent for leukemia and other lymphohematopoeitic cancers has been known since the early 1900s from studies of radiologists (Berrington *et al.*, 2001). However, the most compelling evidence for this association has come from studies of survivors of the atomic bomb blasts in Hiroshima and Nagasaki (Preston *et al.*, 1994)**.**These types of exposures, leukemias (other than chronic lymphocytic) were noted as early as 3 years after exposure, with peak incidence occurring 5–10 years after exposure, and additional cases were diagnosed even 30 years after exposure (Kipen & Wartenberg, 2005).

Also there is evidence for leukemia risk associated with occupational exposure to ionizing radiation among radiation-monitored workers (Leuraud *et al.*, 2015).

1.2.4.2.2. Nonionizing Radiation

Concern also has been raised over the apparent elevated leukemia incidence among children and workers exposed to electric and magnetic fields (EMF) (IARC, 2005).The risk was documented in a Case-control study of children who had lived in homes with high magnetic fields (Kabuto *et al.*, 2006).Some studies did not show this association (Kheifets *et al.*, 2017). The interpretation of these studies remains controversial. Because no viable mechanism has been postulated for non ionizing radiation to cause leukemia (Huss *et al.*, 2018).

1.2.4.3. Chemical Factors

1.2.4.3.1. Solvents

The most important solvents that known to cause leukemia is benzene, although exposure to benzene has been shown to cause leukemia, most studies have reported excesses of AML rather than ALL (Loomis *et al.,* 2017).

1.2.4.3.2. Pesticides

Numerous studies have examined the association between lymphohematopoeitic cancers, pesticides and farming, and they report limited evidence to suggest an association with ALL. Recent studies, however, have suggested an association for all types of leukemia, and specifically for exposure during pregnancy, indoor exposure, prenatal exposure to insecticides and whatever the age at diagnosis (VanMaele-Fabry *et al.*, 2018).

1.2.4.3.3. Outdoor Air Pollution

Some researchers have suggested that outdoor air pollution may be a risk factor for leukemia. A Canadian study found an association between exposure to ambient air pollution during pregnancy, especially in the first trimester and an increased risk of astrocytoma and ALL (Lavigne *et al.*, 2017).Various

hypotheses exist, most notably that the risk is attributable to benzene, a component of automobile exhaust (Wartenberg *et al.* ; 2008).

1.2.4.3.4. Tobacco Smoke

The association of tobacco smoke and lymphohematopoietic cancers has been assessed in several studies . However, several studies, including more than a dozen case control studies and two large cohort studies, have looked at the association between passive smoke exposure and childhood leukemia, and have reported inconsistent results (Buffler *et al.*, 2005; Metayer *et al.*, 2013). A study reported statistically significant association between Preconception paternal smoking and ALL (Orsi *et al.*, 2015).

1.2.4.3.5. Diet

There have been only a limited number of studies investigating the role of diet in the occurrence of leukemia among adults. In one study, Abiri *et al.*, (2016) reported maternal diet composed largely of vegetables, fruits, and protein sources before and during pregnancy can reduce the risk of ALL in offspring.

1.2.4.3.6. Maternal Pharmaceutical Use

There are limited data on the possible association between maternal use of pharmaceuticals and the risk of ALL. Milne *et al.*, (2010) studied in a meta analysis study, the association between maternal folate and other vitamin supplements before and during pregnancy and the risk of ALL in children, this study suggest that vitamin supplements in general during pregnancy may protect against childhood ALL but, this effect is unlikely to be large due specifically to folate .Another study in Egypt found that the risk of ALL was increased with the mother's use of medications for ovulation induction (Ezzat *et al.*, 2016)

1.2.5. Clinical Presentation

1.2.5.1. Clinical Features

The clinical onset of ALL is most often acute, although a small percentage of cases may evolve insidiously over several months (Pui, 2013). The presenting symptoms and signs correlate with the leukemic cell burden and the degree of marrow replacement, leading to cytopenias. The most common symptoms include fever (caused by leukemia or a secondary infection secondary to neutropenia), fatigue and lethargy (as a result of anemia), bone and joint pain, and a bleeding diathesis (related to thrombocytopenia). Patients with precursor T-cell ALL/LBL often present with a mediastinal mass with or without associated pleural effusions, which may lead to respiratory distress and other signs of superior vena cava syndrome. Common extramedullary sites of involvement include lymph nodes, liver, spleen, and meninges, whereas less commonly, ALL may infiltrate orbital tissues, testes, tonsils, and adenoids (Shivakumar *et al.*, 2008). Less commonly, bone pain is caused by recurring episodes of bone marrow necrosis. Approximately 2% of children present with marrow findings consistent with aplastic anemia, followed by overt leukemia within weeks to months; the majority of these children are girls <10 years old. Weight loss rarely is severe and is seen only in patients with long-standing symptoms (Baer & Greer, 2009).

Central nervous system involvement by leukemia, usually asymptomatic, occurs in ~2 to 3% of patients at diagnosis. Symptoms, when present, include headaches, vomiting, or cranial nerve palsies. Physical findings include various combinations of pallor, petechiae or purpura, mucous membrane bleeding, fever, lymphadenopathy, splenomegaly, hepatomegaly, tenderness over the sternum and other bones, and retinal hemorrhages or infiltrates. Organomegaly is rarely symptomatic but may cause abdominal pain or loss of appetite. Skin involvement is rare; when it occurs, it is associated with a pre-B-cell phenotype (Hoffbrand & Moss, 2011).

1.2.5.2. Laboratory Features

The most common laboratory abnormalities in ALL include anemia, thrombocytopenia, neutropenia, and leucopenia or leukocytosis, with hyperleukocytosis (>100X109/L) present in approximately 15% of the pediatric patients .Biochemical tests may reveal raised serum uric acid and lactate dehydrogenase levels, correlating with the tumor burden and degree of tumor lysis. Liver and renal function tests are performed as a baseline before treatment begins. Radiology may reveal lytic bone lesions or a mediastinal mass caused by enlargement of the thymus and/or mediastinal lymph nodes characteristic of T-ALL (Baer & Greer, 2009 ; Hoffbrand & Moss, 2011).

1.2.6. Classification of Acute Lymphoblastic Leukemia

The rst attempt at classifying ALL was the French American British (FAB) morphological criteria that divided ALL into 3 subtypes (L1, L2 and L3) based on cell size, cytoplasm, nucleoli, vacuolation and basophilia (Bennett *et al.*, 1976). In 1997, the World Health Organization proposed a composite classi cation in attempt to account for morphology and cytogenetic pro le of the leukemic blasts and identi ed three types of ALL: B lymphoblastic, T lymphoblastic and Burkitt-cell Leukemia (Harris *et al.*, 1999). Later revised in 2008, Burkitt-cell Leukemia was eliminated as it is no longer seen as a separate entity from Burkitt Lymphoma, and B-lymphoblastic leukemia was divided into two subtypes: B-ALL with recurrent genetic abnormalities and B-ALL not otherwise speci ed. B-ALL with recurrent genetic abnormalities is further delineated based on the speci c chromosomal rearrangement present (Table 1.1) (Vardiman *et al.*, 2009).

In 2016, two new provisional entities were added to the list of recurrent genetic abnormalities and the hypodiploid was rede ned as either low hypodiploid or hypodiploid with TP53 mutations. In adults, B-cell ALL

accounts for ~ 75% of cases while T-cell ALL comprises the remaining cases (Arber *et al.*, 2016).

Table (1.1) WHO classi cation of acute lymphoblastic leukemia[a]

B-cell lymphoblastic leukemia/lymphoma, not otherwise speci ed.
B-cell lymphoblastic leukemia/lymphoma, with recurrent genetic abnormalities.
* B-cell lymphoblastic leukemia/lymphoma with hypodiploidy.
* B-cell lymphoblastic leukemia/lymphoma with hyperdiploidy.
*B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2)[BCR-ABL1].
*B-cell lymphoblastic leukemia/lymphoma with t(v;11q23)[MLL rearranged].
*B-cell lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22)[ETV6 RUNX1].
*B-cell lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3)[TCF3-PBX1].
*B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32)[IL3-IGH].
*B-cell lymphoblastic leukemia/lymphoma with intrachromosomal ampli cation of chromosome 21 (iAMP21)[b].
*B-cell lymphoblastic leukemia/lymphoma with translocations involving tyrosine kinases or cytokine receptors (BCR-ABL1–like ALL)[b].
T-cell lymphoblastic leukemia/lymphomas
*Early T-cell precursor lymphoblastic leukemia[b].
Abbreviations: ALL, acute lymphoblastic leukemia; WHO, World health Organization.[a]On the basis of The 2016 revision to the World Health Organization. Classi cation of myeloid neoplasms and acute leukemia. [b]Provisional entity.

The FAB and WHO classifications of acute lymphoblastic leukemias (Mughal *et al.*, 2009)

(a) FAB classification of ALL

L1 Small homogeneous, high nuclear: cytoplasmic ratio, smallnucleoli

L2 Larger, pleomorphic, low nuclear: cytoplasmic ratio, prominent nucleoli

L3 Larger, vacuolated basophilic cytoplasm, large vesicular nucleus, large nucleoli; resemble Burkitt's lymphoma cells

(b) WHO classification of ALL

-Precursor B-lymphoblastic leukemia/lymphoblastic lymphoma* (precursor B-cell acute lymphoblastic leukemia)(Equivalent to B-cell ALL, FAB L1 and L2).

-Precursor T-lymphoblastic leukemia/lymphoblastic lymphoma*(precursor T-cell acute lymphoblastic leukemia).

*The FAB ALL L3 category has been put into the Burkitt lymphoma/leukemia group by the WHO (Mughal *et al.,* 2009).

1.2.7. Pathogenesis of acute lymphoblastic leukemia

ALL is characterized by an uncontrolled proliferation of early lymphoblastic progenitor cells in the bone marrow, whose maturation is blocked at a distinct level of differentiation (Katz *et al.*, 2015).

The leukemic blasts of a given patient in general display individually specific genetic markers. These so-called clonal markers indicate that the origin of ALL is to be found in one transformed lymphatic stem cell. The transformation might occur on various levels of lymphoid cell maturation as shown in (figure 1.1) (Bhojwani *et al.*, 2015). As a result the leukemia cells of ALL subgroups display a variety of phenotypic features, for example, a set of cell surface markers which are correlated with the stage of maturation and also with the disease's clinical manifestation. More than 60% of all adult ALL have cytogenetic aberrations which are also often characteristic of certain phenotypic and clinical manifestations and, in part, are also of prognostic

relevance. In addition, they give indications to genes connected to the pathogenesis of the disease (Lo Nigro, 2013) . Genes affected by aberrations, and/or their gene products, are involved in signal transduction, regulation of transcription, cell-cycle control, and/or the regulation of apoptosis. The alteration of single genes has impact on the transcription of downstream genes and subsequent regulatory mechanisms. It must be assumed that several genetic aberrations are necessary for the malignant transformation of lymphoid progenitor cells. They result in differentiation disorders increase of proliferative functions, and/or a loss of mechanisms which lead to apoptosis. These alterations ultimately lead to a survival advantage of the malignant clone and result in a differentiation block on a certain level of maturation, in analogy to regular lymphoid progenitor cells. The most important example of the pathogenetic and prognostic significance of a single aberration is the translocation t(9;22) (Philadelphia chromosome), which is associated with the formation of the BCR-ABL fusion gene. In this case a protein with aberrant tyrosine kinase activity is expressed, which is responsible for the development of Ph/BCR-ABL-positive ALL (Brüggemann *et al.*, 2010).

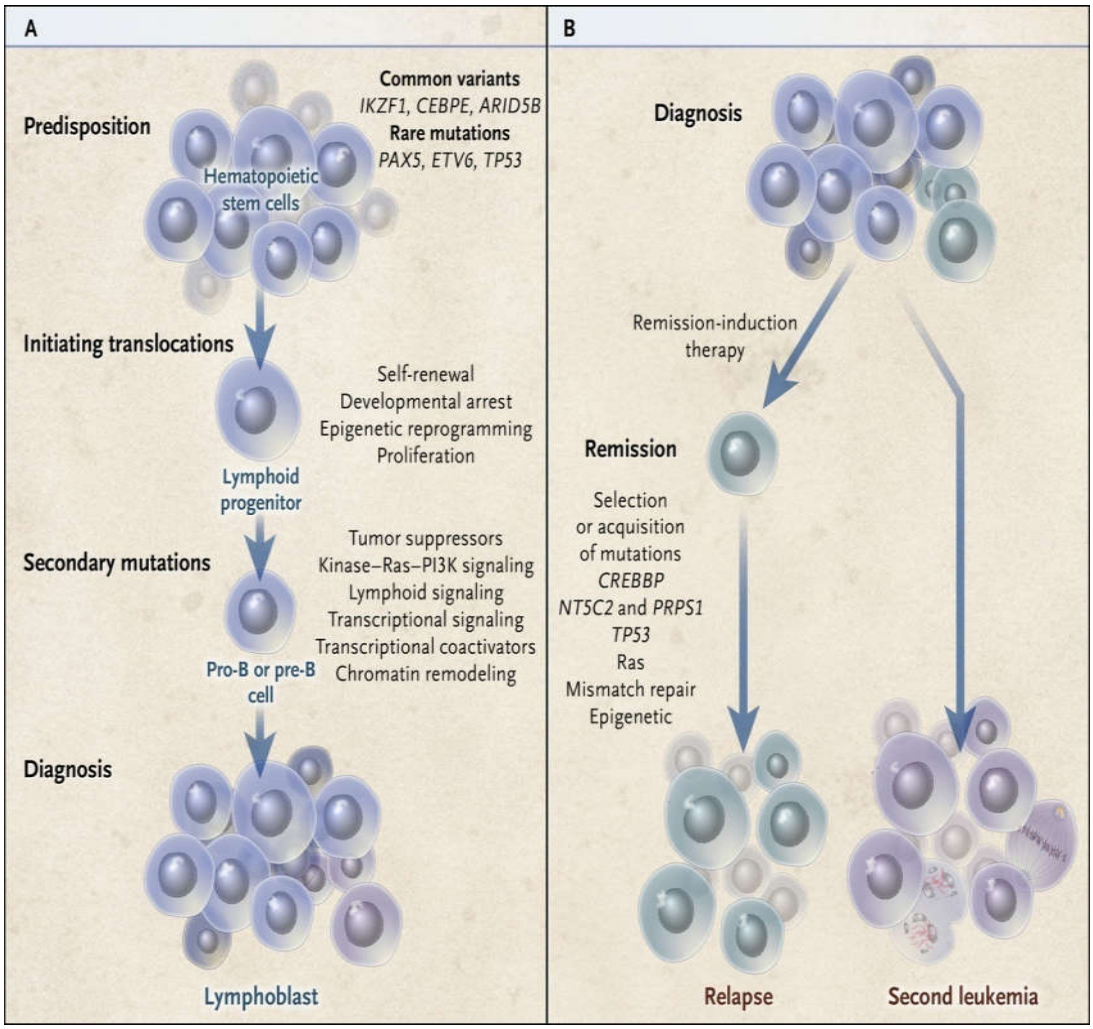

Figure (1.1) Proposed Sequential Acquisition of Genetic Alterations Contributing to the Pathogenesis and Relapse of ALL (Hunger & Mullighan, 2015).

1.2.7.1.Leukemia and immunity

Although little is known about the etiology of leukemia, this has a multifactorial behavior with risk factors that may contribute to its development such as ionizing radiation, chemotherapy and chromosomal abnormalities(Han *et al*., 2010). By other hand, there are three hypotheses as we mentioned previously, one called delayed infection, the second population mixing and the third hygiene hypothesis (Strachan, 1989; Kinlen, 1995; Greaves, 2006),the first two suggest that the immune system deficiency in an early stage of development can cause an abnormal immune response to infections which may arise in the

development of human beings. Both hypotheses are similar to third called hygiene hypothesis, which explains an increase in allergies in Western populations (Chang *et al.*, 2010).Although most studies support to infections and immune system factors in the etiology of ALL, little is known about the role of genes in this etiology.

The relation of immune system in the ALL is a complex process that involves the interaction of many cells that including leukocytes, epithelial barriers, complement proteins, colexinas, pentraxins, cytokines (TNF, IL-1, chemokines, IL-2, IFN type I,IFN etc.), Th1, Th2, Treg and Th17 cells, CD28, FCGR2, GATA3, STAT4, STAT6 and many other (Chang *et al.*, 2010). Variations in the genes of these cells can affect their development and function in the immune response and therefore it may increase susceptibility to developing ALL (Han *et al.*, 2010; Chang *et al.,* 2010). Moreover it was found that the CD47molecule protects the macrophage leukemic clones to bind to a molecule on the surface of these cells. The interaction between macrophages and leukemic cells inhibits macrophage specific action which allows the cancer cell to proliferate. So that although the macrophage plays an important role in the destruction of cancer cells, leukemic cells with greater metabolic potential, and the potential escapes annihilating the macrophage (Tesniere *et al.,* 2008; Jaiswal *et al.,*2009). In the innate immune system, macrophages and other immune cells involved in immune surveillance protect the body permanently cell rate unexpectedly mutates. In contrast, the adaptive immune system through T and B cells upon activation attempt to destroy leukemic cells; however these also evade cellular immunity (Kalender *et al.,* 2012).

1.2.7.1.1.The role of IL12 and HLA in the pathogenesis of ALL

Interleukin-12 (IL-12) is a heterodimeric protein, first recovered from EBV transformed B cell lines. It is a multifunctional cytokine (figure 1.2) , the properties of which bridge innate and adaptive immunity, acting as a key

regulator of cell-mediated immune responses through the induction of T helper 1differentiation. By promoting IFN-g production, proliferation, and cytolytic activity of natural killer and T cells, IL-12 induces cellular immunity. In addition, IL-12 induces an antiangiogenic program mediated by IFN-g inducible genes and by lymphocyte-endothelial cell cross-talk. The immunomodulating and antiangiogenic functions of IL-12 have provided the rationale for exploiting this cytokine as an anti cancer agent. In contrast with the significant antitumor and anti metastatic activity of IL-12, documented in several preclinical studies, clinical trials with IL-12, used as a single agent, or as a vaccine adjuvant, have shown limited efficacy in most instances. More effective application of this cytokine, and of newly identified IL-12 family members (IL-23 and IL-27),should be evaluated as therapeutic agents with considerable potential in cancer patients (Del Vecchio *et al.*, 2007).

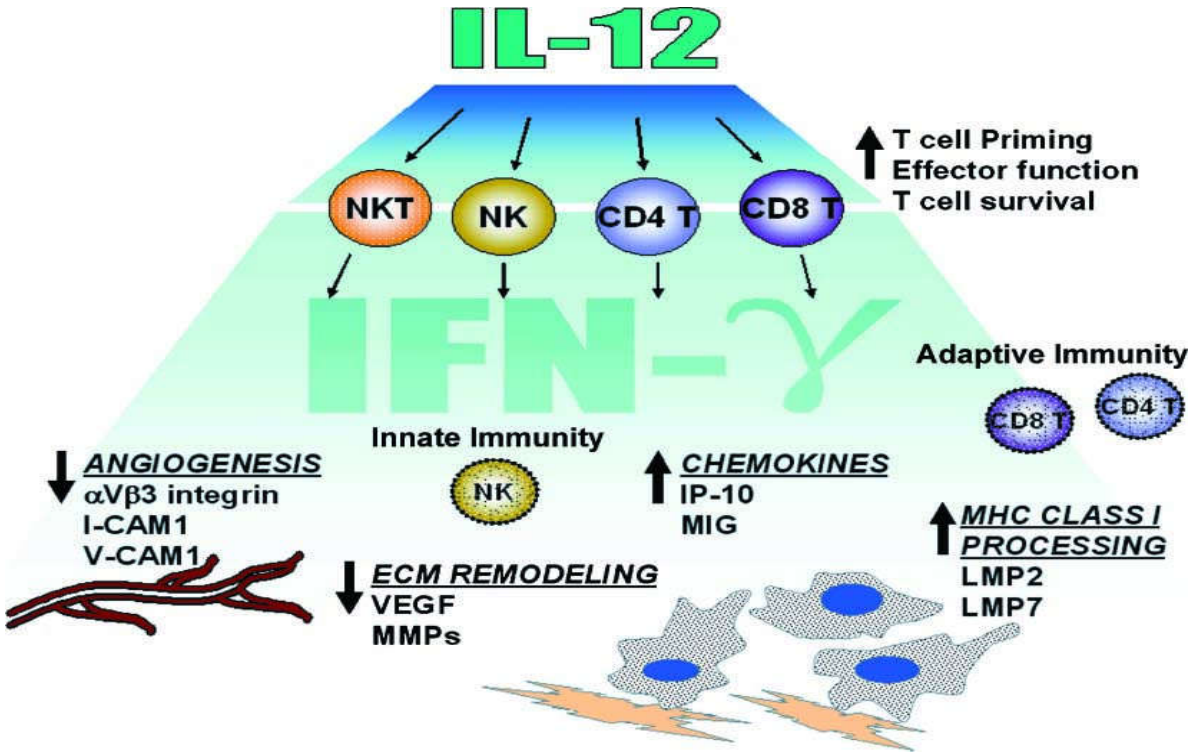

Figure (1.2) A model of mechanisms involved in the antitumor effects of IL-12 (Del Vecchio *et al.*, 2007).

Also HLA (Human Leukocyte Antigen) found to have an association as an immunologic factor in the pathogenesis of childhood ALL, studies investigating the role of polymorphic alleles of the human leukocyte antigen (HLA) class II genes have reported evidence of an association with childhood ALL. The HLA class II genes encode highly polymorphic cell surface glycoproteins that play an important role in adaptive immune response to infections. The earliest study revealed that childhood ALL cases were reportedly more likely to have HLA-DPB1 alleles coding specific polymorphic amino acids than normal infants or cases with solid tumors (Malcolm Taylor *et al.*, 2002).

This suggests that susceptibility to childhood ALL may involve the presentation of specific antigenic peptides derived from infectious agents. As a result, activation of helper T cells occurs, which mediates proliferative stress on pre-leukemic cells (Buffler *et al.*,2005).

1.2.7.2. Genetic role in pathogenesis of leukemia

Much of what we know about the great influence of certain mutant genes, in the origin of leukemia, is derived from mouse transgenesis studies in molecular virology, with gene transfection and the generation of leukemia in vivo. These studies are based on bacterial recombinant DNA methods. This knowledge has increased our understanding of leukemogenesis and prognosis, and additionally has served as foundation for the development of targeted therapy. However, the comprehension of how genetic alterations that collaborate to induce leukemic transformation is not clear yet (Pui *et al*, 2011).

Most mutations in leukemia are acquired, and occur *de novo* in the lymphoid progenitor cells, less frequently (1% to 5% of leukemias) the mutated genes are inherited (vgr, p53, DNA ligase),or a numeric chromosomal abnormality is involved, for example constitutive trisomy 21. Acute leukemias are the most studied malignant disorders from a genetic standpoint, the results of whole-genome studies, e.g.: gene expression analysis of high-resolution,

genome-wide alterations in DNA copy number variation , loss of heterozygosity, epigenetic changes, and complete genome sequencing have favored the recognition of new genetic alterations, then virtually all patients with ALL can be classified according to the specific genetic abnormality, as shown in figure (1.3), which is evident in children with ALL the high frequency of genetic abnormalities (Mullighan *et al*, 2007; Pui *et al*, 2011).

The altered genes in leukemia can result in loss or gain of function, and this is achieved through various mechanisms, for example, abnormal recombination (chromosomal translocation, inversion, or insertion), loss of genetic material (deletion), gain of genetic material (duplication), or mutation. Also can be present additional copies of certain chromosomes, as in the case of hyperdiploidy. With these chromosomal alterations, the activation of oncogenes is favored. Oncogenes can be activated by: chromosomal rearrangements, gene mutation and gene amplification (Croce, 2008).

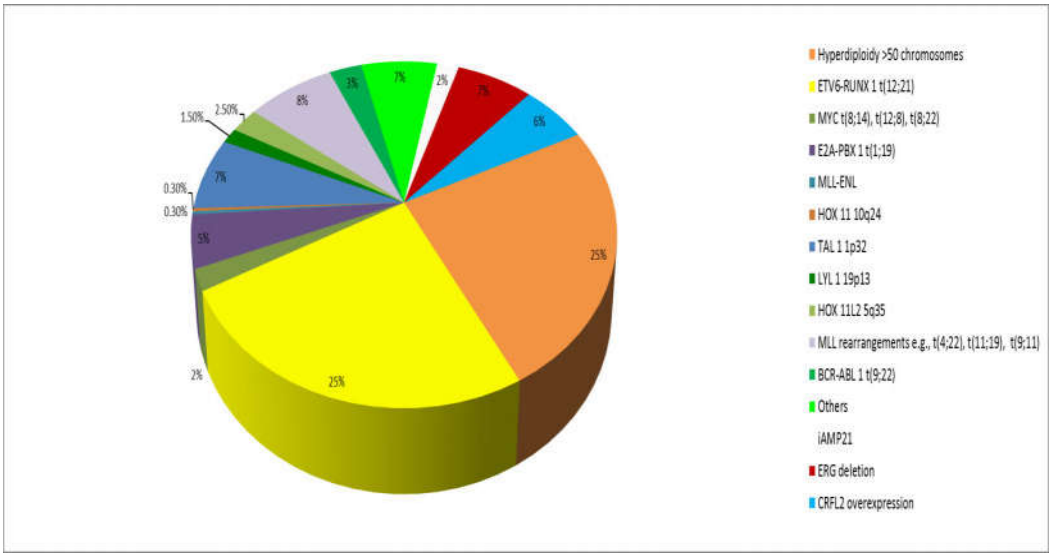

Figure (1.3) Frequency of genetic abnormalities in children with Acute Lymphoblastic Leukemia. (modified of Pui *et al*, 2011).

1.2.7.2.1.Genetic role of IL12 polymorphism in pathogenesis of leukemia

As previously explained the interleukin 12 is heterodimeric molecule, and the genes encoding the two-subunit chains of IL-12, p35 and p40, were cloned (Yan *et al.*, 2018).

The heterodimeric IL-12 is often referred to as IL-12 p70. The two genes encoding IL-12p40 and p35 are located on separate chromosomes (chromosomes 11 and 6,respectively, in humans and mice, respectively) as shown in figure (1.4) and are not evolutionary duplicates. The p35 cDNA encodes a 209 amino acid polypeptide corresponding to a mature protein of 27.5 kDa. It contains seven cysteine residues and 3 potential N-glycosylation sites. The p40 cDNA sequence encodes a 328 amino acid polypeptide with a 22 amino acid signal peptide which generates a mature protein of 34.7 kDa. It contains ten cysteine residues and four potential N-glycosylation sites, and one consensus heparin-binding site (Liu *et al*, 2005).

The p35 gene has some homology to IL-6 and granulocyte colony-stimulating factor (G-CSF).Whereas the p40chain is homologous to the extracellular portion of the α chain of ciliary neurotropic factor(CNTF) receptor as well as to those of the IL-6 and G-CSF receptors. The p40 chain carries the hallmarks of the hematopoietin receptor family: one tryptophan and four cysteine residues in conserved positions and a WSEWAS sequence, similar to the consensus motif WSXWS in the hematopoietic receptor family. Most of the members of this transmembrane receptor family can be released from the producer cells in soluble forms containing the extracellular portion truncated after the WSXWS motif by either alternative splicing of the mRNA transcripts or by proteolytic digestion of the receptor (Taga & Kishimoto, 1992).

Cytokine polymorphisms and cancer is small but growing rapidly. A number of studies reported associations between TNFA SNPs and particular cancers, including chronic lymphocytic leukemia, non-Hodgkin's lymphoma (NHL), and breast cancer. These associations are, however, refuted by others .A

well-documented association exists between IL-1B and IL-1RN polymorphisms and the development of gastric adenocarcinoma subsequent to *Helicobacter pylori* infection (Howell & Rose-Zerilli, 2007).

However, IL12 polymorphisms are of particular interest in relation to cancer because of the pleiotropic activities of IL-12,dozens of molecular epidemiologic studies have explored the influences of IL-12 polymorphisms on susceptibility of various cancers, including hepatocellular carcinoma, colorectal cancer and gastric cancer (Zheng *et al.*, 2017).

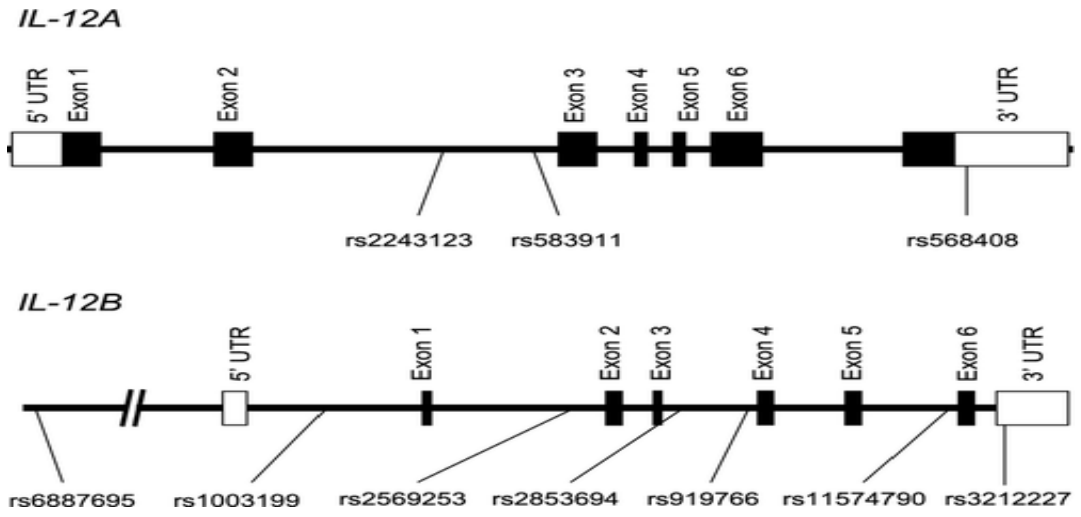

Figure (1.4) IL-12 gene and the location of SNPs (Öhman *et al.,* 2015).

1.2.7.2.2 Genetic role of HLA in pathogenesis of leukemia

Findings from genetic susceptibility studies targeting immune-related genetic loci have fuelled an ongoing suspicion of a role for immunologic response involving infections. Foremost among the candidate loci are those residing within the major histocompatibility complex (MHC), a genomic region well-known for its high density of genes, complex linkage disequilibrium (LD), and highly polymorphic nature. The human MHC region spans approximately 4 Mega bases (Mb) on the short arm of chromosome 6 (6p21.3) and contains about 250 gene loci with a disproportionately large number encoding genes that

play a role in immune function and regulation (The MHC Sequencing Consortium, 1999).

This region first received attention following the discovery of cell surface molecules called the human leukocyte antigens (HLA) involved in tissue graft and organ transplant rejection. HLA is now well-understood to have a primary functional role in the protection against pathogens through selective binding and presentation, in allele selective fashion, of processed peptides to T lymphocytes critical to both cellular and humoral immune responses. Scrutiny of the HLA system in leukemia susceptibility in humans began in the 1960's following landmark studies by Lilly and colleagues who demonstrated that leukemogenesis by the Gross leukemia virus in mice is strongly influenced by H-2 (the MHC system in mice) type. The earliest studies in humans showed a link with class I loci such as HLA-A2 antigen serotype (Urayama *et al.*, 2013) , after these studies many other studies are done to find an association between HLA class I and childhood ALL (Ozdilli *et al.*, 2010) and these studies revealed a spectrum of HLA associations conferring predisposition to or protection from refractory disease (Klitz *et al.*, 2012).

1.2.8. Pathologic Diagnosis

1.2.8.1. Morphology

The classic morphologic features of ALL have been well described in the literature and are best summarized by the categories outlined by the first classification of ALL, the French-American-British (FAB) system, which was based primarily on the microscopic appearance of the leukemic cells, as seen on Wright-Giemsa–stained smears (Lilleyman *et al.*, 1986). Small subset of precursor B-cell neoplasms, often associated with hypodiploidy, may also present with FAB L3 features. When seen in hematoxylin and eosin-stained histologic sections of bone marrow biopsies and tissue infiltrates, the neoplastic lymphoblasts of ALL/LBL may show features that correspond to the FAB

categories. This classification cannot accurately distinguish between ALL and non - lymphoid acute leukemia, and has no prognostic or therapeutic relevance with contemporary therapy (Campana & Pui, 2011).

1.2.8.2. Cytochemistry

Cytochemical staining has been used with decreasing frequency in the diagnosis of ALL due to the availability of immunophenotyping. The leukemic cells of ALL are uniformly negative for myeloperoxidase (MPO), Sudan Black-B, chloroacetate esterase, and nonspecific esterases, while they are positive for periodic acid Schiff in about 70% of cases (Campana & Pui, 2011).

1.2.8.3. Immunophenotype

Immuno-phenotypic studies are an essential component of the diagnostic workup of ALL/LBL. As opposed to the morphologic features, the lineage of ALL established in this manner subdivides this disease into two broad, clinically and biologically meaningful categories: precursor B-cell ALL (B-ALL) and precursor T-cell ALL (T-ALL) (Swerdlow *et al.*, 2008).

B-ALL is characterized by the expression of a variety of B-cell–specific antigens, which often include PAX-5 (B-cell–specific activator protein), CD19, CD20, CD22 (surface and cytoplasmic), CD24, and CD79a (cytoplasmic).T-ALL is characterized by expression of T-lineage–associated antigens (CD2, CD3, CD4, CD5, CD7, CD8) as well as CD1a, CD10, CD34, CD99, HLA-DR, and terminal deoxynucleotidyl transferase (Tdt). The pattern of antigen expression may be used to subclassify T-ALLs according to the stages of normal thymocyte development that they resemble (Onciu, 2009).

1.2.8.4. Genetics

Approximately 75% of childhood ALL cases harbor a recurring chromosomal alteration detectable by karyotyping or molecular techniques

(Figure 1.5). In B-progenitor ALL, these include hyperdiploidy with greater than 50 chromosomes, hypodiploidy with less than 44 chromosomes, and chromosomal rearrangements including t (12; 21) ETV6-RUNX1 (TEL-AML1), t (1; 19) TCF3-PBX1 (E2A-PBX1), t (9; 22) BCR-ABL1, and rearrangement of MLL at 11q23 to a diverse range of fusion partners. T-lineage ALL is characterized by activating mutations of NOTCH1 and rearrangements of transcription factors TLX1 (HOX11), TLX3 (HOX11L2),LYL1,TAL1, and MLL. High hyperdiploidy, ETV6-RUNX1 (both associated with favorable outcome), and TCF3-PBX1 are less common in adult ALL. Although these rearrangements are important initiating events in leukemogenesis and are widely used in diagnosis and risk stratification algorithms, they are insufficient to fully explain leukemogenesis. Rearrangements such as ETV6-RUNX1 are present years before the development of leukemia, and many do not alone result in the development of leukemia in experimental models. It is now known that the majority of ALL cases are characterized by distinct constellations of structural and submicroscopic genetic alterations and sequence mutations (Mullighan, 2012).

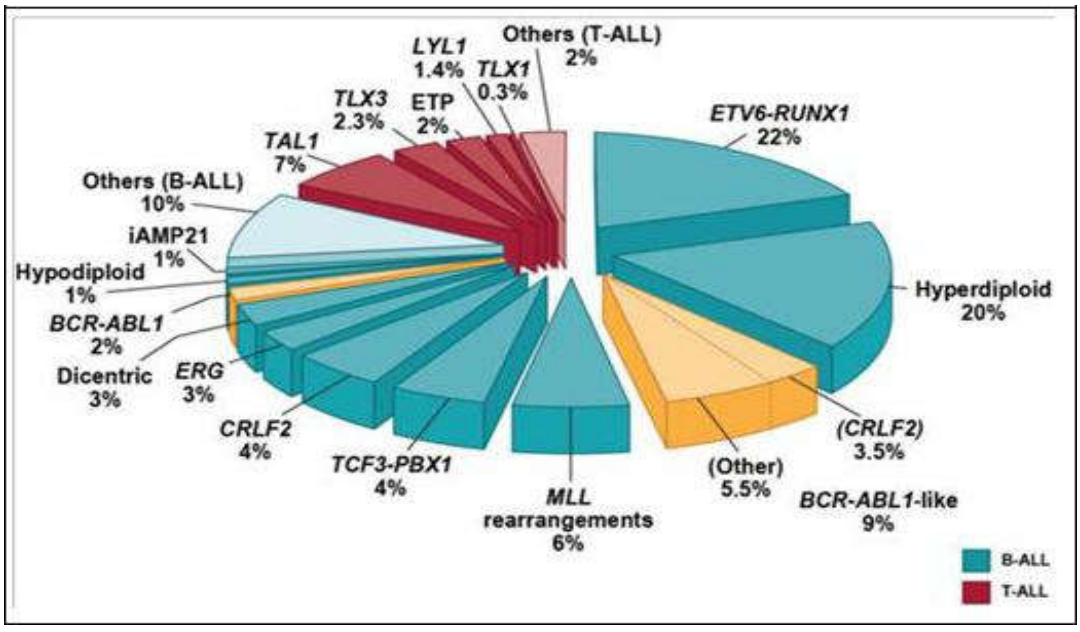

Figure (1.5) Frequency of cytogenetic subtypes of pediatric Acute Lymphoblastic Leukemia (Mullighan, 2012)

1.2.9. Prognosis

The prognosis of ALL has improved dramatically over the past several decades as a result of adapting therapy to the level of risk for relapse, improvements in supportive care, and optimization of the existing chemotherapy drugs. Major prognostic factors include the clinical features that are present at diagnosis, biologic and genetic features of leukemia cells, and early response to treatment (Hunger & Mullighan , 2015) .

Prognosis is very variable. ALL patients are stratified into low, high and standard risk and treated according to algorithms that integrate:

1. **Presenting features:**

   Age Prognosis is best in children between the ages of 1 and 10 years. It is adverse in adults and in infants less than a year of age**. Leukocyte counts** A high white cell count is associated with a worse prognosis. **Presence or absence of central nervous system [CNS], or testicular involvement:** Leukaemic blast cells are identified morphologically at diagnosis in the cerebrospinal fluid (CSF) of approximately one -third of children with ALL, most of whom have no neurological symptoms. Although CNS leukemia is defined by the presence of at least five leucocytes /mL of CSF and the detection of leukaemic blast cells, or by the presence of cranial nerve palsy, the presence of any leukaemic cells in CSF (even from iatrogenic introduction due to a traumatic lumbar puncture) predicts an increased risk of ALL relapse (Baruchel, 2011).

2. **Biologic and genetic features**

   Several genetic alterations are associated with the outcome in children with ALL. High hyperdiploidy associated with a favorable outcome. Hypodiploidy with Less than 44 chromosomes (Baer & Greer, 2009). MLL rearrangement, Ph-like ALL and early T- cell precursor ALL

are associated with high-risk clinical features or a poor outcome (Roberts *et al.*, 2014).

3. **Early therapy response**

Induction failure or persistent minimal residual disease (MRD).The risk of treatment failure and death is 3 to 5 times as high among children with levels of minimal residual disease that are 0.01% or higher at the end of induction therapy and at later time points than among those with levels that are lower than 0.01% (Schrappe *et al.*; 2011).Intensification of therapy for patients with higher levels of minimal residual disease improves their outcome (Pui *et al*., 2015).

2. Materials and Methods

2.1. Materials

2.1.1. Equipment and Instruments

The equipment and instruments used in this study are listed in table (2.1).

Table (2.1): Equipments and Instruments with their Remarks

Equipment & instrument	Company
Cabinet Hood	Telstar/U.S.A
Digital camera	Samsung/ china
Disposable syringe 10 ml /5 ml	Sterile EO. / China
EDTA tube (anticoagulant tube)	AFNA-Dispo/Jordan
Eppendorf centrifuge	Eppendorf Ag/Germany
Eppendorf tube	Biobasic/ Canada
Exispin centrifuge	Bioneer/ Korea
Gel electrophoresis	Bioneer/ Korea
High Speed Cold Centrifuge	Eppendorf /Germany
Incubation tray	Innoginetics/Bilgium
Incubator	Mammert/Germany
Micropipettes 5-50, 0.5-10, 100-1000µl	CYAN/ Belgium
Nanodrop	THERMO/ USA
Oven	Mammert/Germany
Refrigerator	Concord /Lebanon

Sensitive Balance	Sartorius/Germany
Sterile test tube	Superestar/ India
Thermo electric	Jouhn/U.S.A
Thermocycle	GeneAmp/U.S.A
Thermocycler PCR	BioRad /USA
UV Transilluminator	ATTA/ Korea
Vortex	CYAN/ Belgium
Water Bath	Mammert/Germany

2.1.2. Chemical and Biological Materials

The chemical and biological materials used in this study are listed in table (2.2).

Table (2.2): Chemical and Biological Materials with their Remarks

Chemical	Company and Origin
Agarose	Promega (USA)
DNA ladder (100bp)	Biolabs/ UK
Ethanol (96%)	Himedia (India)
Ethidium bromide	BIO BASIC INC/ USA
Free nuclease water	Bioneer (Korea)

TBE buffer	BIO BASIC INC/ USA

2.1.3. The DNA extraction and polymerase chain reaction kits

In table (2.3) chemical materials that used in AS-PCR and SSP-PCR work of this study with their companies and countries of origin are listed.

Table (2.3): DNA extraction and PCR kits with their Remarks

gSYAN Blood DNA Extraction Kit GST buffer, GSB buffer, W1 buffer, Wash buffer, Elution buffer, GD column, Collection tube 2ml, Proteinase K 11mg	Geneaid	Taiwan
Maxime PCR PreMix Taq DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), Tris-HCl pH 9.0, KCl, $MgCl_2$, Stabilizer and Tracking dye	iNtRON	Korea
QIA amp DNA extraction Kit Binding buffer (GC) ,Proteinase K 25 mg W1 buffer,Wash buffer, Elution buffer Binding column, Collection 2ml tube	QIAGEN	USA
HISTO TYPE SSP Kit HISTO TYPE plates/strips for the HLA typing(allele specific primers, internal control primers and nucleotides),PCR strips (á 8)(contamination control with internal control primers and amplificate specific primers), 10x PCR-buffer,Strip-caps or PCR foil,	BAG	Germany

Happy Taq (Taq DNA polymerase).		

2.1.4. Primers

Allele-specific PCR primer for detection of IL12-A rs583911 (A/G) were designed by (Hirschfield *et al.*, 2009). These primers were provided from (Macrogen company, Korea) as following table (2.4):

Table (2.4):The Multiplex PCR Primers with their Sequence and amplicon Size

Primers	Sequence (5'-3')		Amplicon
IL12-Agene	Wild type R	CGTTGGATGAGCTTGTCTTAAGGGTTTGC	100bp wild type allele (A)
	Mutant type R	ACGTTGGATGCAAGTATAACTTCTAAAGGG	100bp Mutant type allele (G)
rs583911Allele Common Forward Primer	TTTGCATGTTTGTTATATCCATCA		

2.1.5. The Human Leukocytes Antigen (HLA) A&B Typing

2.1.5.1. HISTO TYPE SSP HLA Class 1-A,B kit

All the Reagents and materials that used in the amplification of purified DNA and electrophoresis of amplification products for HLA Class 1 –A,B allele typing by using HISTO TYPE SSP Kit are listed in tables (2.5),(2.6).

Table (2.5):HISTO TYPE SSP HLA-A,B Reagents and materials

Component	Description
HISTO TYPE plates/strips for the	pre-dropped and dried reaction

HLA typing	mixtures consist of allele specific primers, internal control primers and nucleotides.
PCR strips	(á 8) contamination control with internal control primers and amplificate specific primers.
10x PCR-buffer	
Strip-caps or PCR foil	
Happy Taq	Containing Taq DNA polymerase (5 U/µl).
piston pipettes	(0.5-250 µl)
sterile tips with integrated filter	

Table (2.6) Devices and materials used in gel electrophoresis

Component	Description
DNA agarose	2.0%
0.5 x TBE buffer	Containing 45 mM of Tris base, 45 mM of boric acid, 0.5 mM of EDTA
Ethidium bromide (EtBr)	0.5 µg/ml of EtBr in H2O or TBE buffer
submarine electrophoresis unit	

power supply	200 - 300 V
DNA-length standard	10 µl for size comparison

2.2. Methods

2.2.1. Patients and Controls

2.2.1.1. Study Design

The study is a case –control study was conducted during the period from first of February2018 to the first of June 2018, 60 pediatric patient who diagnosed as acute lymphoblastic leukemia by hematology and pediatric consultant in the oncology unit in the Central Child Hospital. For each patient a questionnaire form was done regarding age, sex, family history of the disease, and then the retrieval of the required amount of blood sample. Also a60 apparently healthy control individuals, were included in this study. Verbal informed consent obtained from all participant.

2.2.1.2. Inclusion and Exclusion Criteria

2.2.1.2.1. Inclusion Criteria of Patients

1. Patients were below 13 years old.
2. Patients were newly diagnosed as ALL and confirmed diagnosis by cytochemical stains.

2.2.1.2.2. Exclusion Criteria of Patients

All patients who have exposured to chemotherapy were excluded. So cases were *de novo* cases, not received steroid or chemotherapy before sample collection.

2.2.1.2.3. Inclusion and Exclusion Criteria of Control Group

A random sample of healthy unrelated subjects with no family history of acute lymphoblastic leukemia were considered as a control group.

2.2.2. Sample Collection

Four ml of blood obtained from forearm vein, the 4 ml is divided in to 2 ml sample in each EDTA tubes (figure 2.1) which was immediately deeply frozen, one for HLA class1 (A&B) typing and the other for IL 12A SNP analysis.

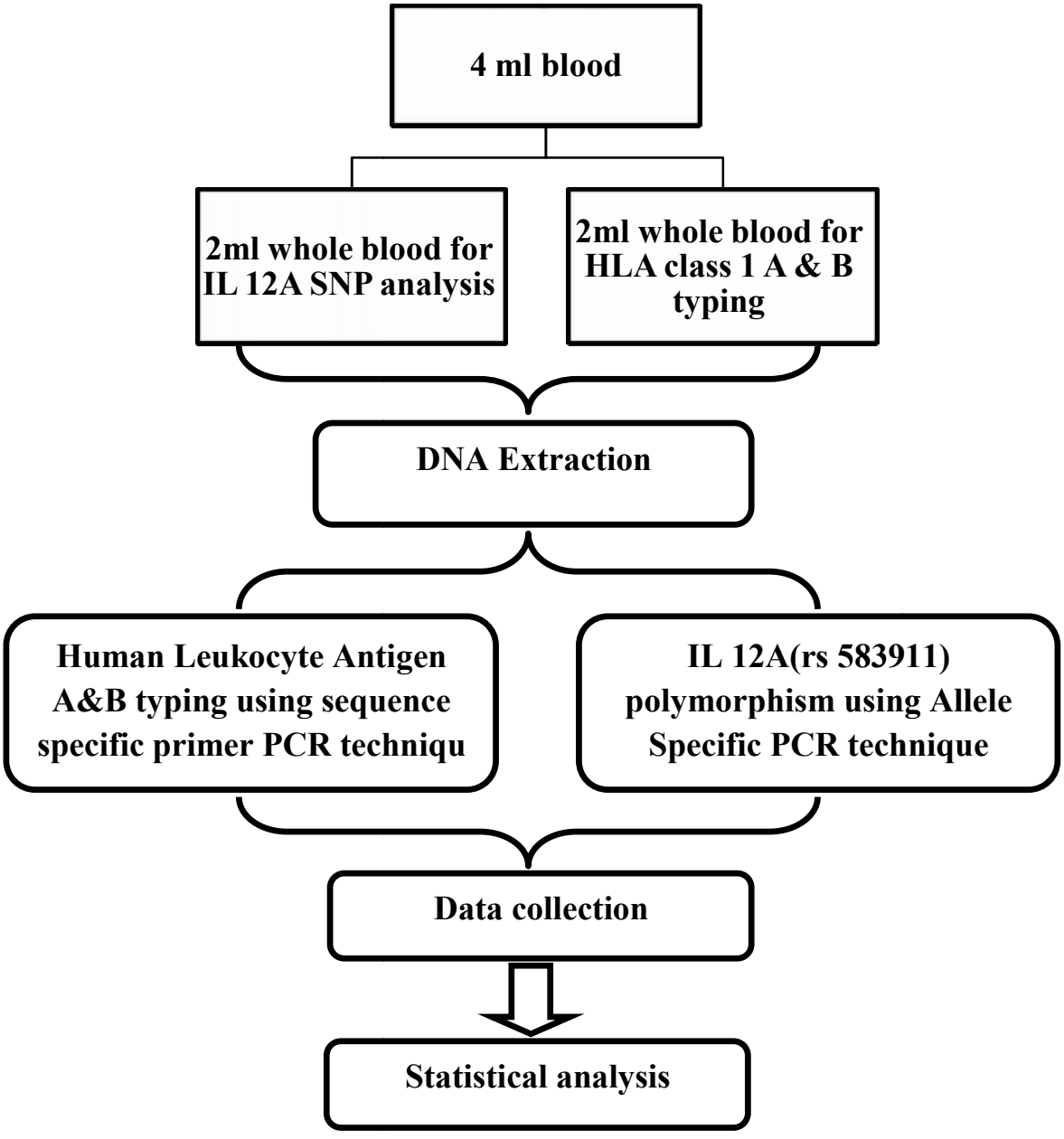

Figure (2.1): The Study Design

2.2.3. Allele-specific PCR (AS-PCR)

The AS-PCR technique was performed for detection IL12-A rs583911 (A/G) gene polymorphism from 60 patients and 60 healthy control blood samples . This method was carried out according to method described by (Hirschfield *et al.*, 2009) as following steps:

2.2.3.1. Blood genomic DNA Extraction

Blood genomic DNA from blood samples were extracted by using gSYAN DNA kit extraction kit (Frozen Blood protocol) Geneaid. USA, and done according to company instructions as following steps:

1. A 200µl of frozen blood was transferred to sterile 1.5ml micro centrifuge tube, and then added 20µl of proteinase K and mixed by vortex. And incubated at 60℃for 15 minutes.

2. After that, 200µl of GSB cell lysis buffer was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70℃for 15 minutes, and inverted every 3 minutes through incubation periods.

3. Then A 200µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously.

4. Filter column of DNA was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tube containing the flow through were discarded and placed the column in a new 2 ml collection tube.

5. Then 400µl W1 buffer were added to the DNA filter column, then centrifuge at 10000 rpm for 30 seconds. The flow through was discarded and placed the column back in the 2 ml collection tube.

6. After that a 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow through was discarded and placed the column back in the 2 ml collection tube.

7. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix.

8. The dried DNA filter column was transferred to a clean 1.5 ml micro centrifuge tube and 50 µl of preheated elution buffer were added to the center of the column matrix.

9. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA.

2.2.3.2.Assessment of DNA purity and concentration

The extracted genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).

2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.

3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of blood genomic DNA was added to measurement.

2.2.3.3.Allele Specific- PCR master mix preparation

Allele Specific-PCR master mix was done two reactions for each sample (Wild type allele master mix reaction) and (Mutant type allele master mix reaction) using Maxime PCR PreMix and done according to company instructions as following table (2.7), (2.8):

Table (2.7): AS-PCR Master mix reaction for wild type allele

PCR master mix	**Volume**
Genomic DNA (5-50ng)	5µL
Common Forward Primer (10pmol)	1µL
Wild type Reverse Primer primers (10pmol)	1µL
PCR water	13 µL
Total	20µL

Table (2.8): AS-PCR Master mix reaction for mutant type allele

PCR master mix	**Volume**
Genomic DNA (5-50ng)	5µL
Common Forward Primer (10pmol)	1µL
Mutant type Reverse Primer primers (10pmol)	1µL
PCR water	13 µL
Total	20µL

After that, these PCR master mix reaction components that mentioned above, placed in standard PCR tubes containing the PCR PreMix as lyophilized materials containing all other components needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, stabilizer, and tracking dye). Then the tube placed in Exispin vortex centrifuge for 3 minutes. Then transferred in PCR thermocycler.

2.2.3.4.Allele Specific - PCR thermocycler reaction conditions

Allele Specific -PCR Thermocycler conditions was done by touchdown protocol using (Optimase Protocol Writer™) as following table (2.9):

Table (2.9) : Thermocycling conditions

PCR cycle	Repeat	Temp.	Time
Initial denaturation	1	95 ºC	5min
Denaturation	15	95 ºC	30sec.
Annealing		59.7ºC decrease 0.5ºC per cycle	30sec
Extension		72 ºC	10sec
Denaturation	20	95 ºC	30sec.
Annealing		52.7ºC	30sec
Extension		72 ºC	10sec
Final extension	1	72 ºC	5min
Hold	1	4 ºC	Forever

2.2.3.5.Gel electrophoresis

The PCR products were analyzed by loading in 2% agarose as following steps:

1- The 2% agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µL of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5ul of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80AM for 1hour.

5- The PCR products were visualized by using ultraviolet transilluminator.

2.2.4. Human Leukocytes Antigen Class I(A&B) Typing

The HISTO TYPE SSP kit, for *in vitro* use, is intended for the nucleic acid amplification of the human leukocytes antigen (HLA) A&B locus. The reaction is performed by using the Sequence Specific Primers (SSP) –PCR. This method is based on the fact that primer extension and hence successful PCR relies on an exact match at the 3'-end of both primers. Therefore, only if the primers entirely match the target sequence is amplification obtained which is subsequently visualized by agarose gel electrophoresis.

The test is essentially performed by the following steps:-

2.2.4.1. Genomic DNA Extraction

Genomic DNA was extracted from frozen blood of the study population, by using QIA amp DNA kit (QIAGEN, USA) and done according to the company instructions.

2.2.4.2. Polymerase Chain Reaction Mix Preparation

The extracted DNA was amplified for the HLA –A,B genes by using HISTO TYPE SSP kit, the reagents used are summarized in table (2.10).

Table (2.10): PCR Master-Mix Reaction

Component	Quantity
Aqua dest.	8 µl
10x PCR buffer	1 µl
DNA-solution (25-50 ng/µl)	1 µl
Happy-Taq (Taq polymerase) (5U/µl)	0.08 µl
Total reaction volume	10 µl

All pre-aliquoted and dried reaction mixtures already contain allele and control-specific primers and nucleotides. These are supplied dried down in the reaction vial. PCR amplification done according to the company instructions as following:

1. After removing the required number of PCR plates / strips and the 10x PCR buffer from the Kit. The Master-Mix is pipetted which consist of 10x

PCR buffer, DNA solution, Taq-Polymerase and Aqua dest. and mixed well.

2. The Master-Mix was placed in the vortex, after vortexing10 µl of this mixture was added to the pre-dropped and dried reaction mixtures in the reaction vial. After each pipetting step, changing the tip, and tightly close the tubes with the respective caps or foil.

3. After the second step the reaction tubes placed firmly into the thermal cycler and tighten the lid well, and Starting the PCR program as in table (2.11) .

Table (2.11): Thermocycling Condition for HLA Class I Gene detection

Program-step	Temp.	Time	No. of cycles
First Denaturation	96°C	5 min.	1 cycle
Denaturation	96°C	20 sec.	5 cycle
Annealing+Extension	68°C	1 min.	
Denaturation	96°C	20 sec.	10 cycle
Annealing	64°C	50 sec.	
Extension	72°C	45 sec.	
Denaturation	96°C	20 sec.	15 cycle
Annealing	61°C	50 sec.	
Extension	72°C	45 sec.	
Final extension	72°C	5 min.	1 cycle

5. In the end of amplification process, the sample was used immediately in gel electrophoresis , and the same protocol was repeated for amplification of HLA-B locus using HISTO TYPE B KIT, except the use of HLA-B multiplex primer solution instead.

2.2.4.3. Gel electrophoresis

Separation of the amplification products is done by electrophoresis via a (horizontal) agarose gel. As electrophoresis buffer, 0.5 x TBE (45 mM of tris, 45 mM of boric acid, 0.5 mM of EDTA). The gel concentration was 2.0% of agarose, and the gel allowed to polymerize at least 30 minutes before sample loading. After finishing the amplification, the samples taken out of the thermal cycler and loaded carefully in each slot of the gel. In addition, 10 µl of the DNA length standard was applied for size comparison. Electrophoretic separation was done at 10 - 12 V/cm (with 20 cm distance between the electrodes approx. 200 - 240 V), for 20 - 40 minutes. After the run had been completed, the complete gel was stained by ethidium-bromide (EtBr) solution (0.5 µg/ml of EtBr in H2O or TBE buffer) for (30 – 40) minutes.

Documentation and interpretation :- For documentation, visualizing of the PCR amplification was done by using an UV transilluminator (220 - 310 nm).The results were analyzed by HISTO MATCH software.

2.2.5. Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. **Qualitative (categorical) variables** were expressed as number and percentage, whereas, **quantitative (numeric) variables** were first evaluated for normality distribution using **Kolmogorov-Smirnov** test, and then accordingly normally distributed numeric variables were expressed as **mean** (an index of central tendency) and **standard deviation** (an index of dispersion), while those numeric variables that are not normally distributed were expressed as **median** (an index of central tendency) and **inter-quartile range** (an index of dispersion).

The following statistical tests were used:

1. **Chi-square test** was use to evaluate association between any two categorical variables provided that less than 20 % of cells have expected count of less than 5. However, **Fischer exact test** was used instead when chi-square test was not valid (in case that more than 20 % of cells have expected count of less than 5).
2. **Independent samples t-test** was used to evaluate the difference in mean of numeric variables between any two groups provided that these variables were normally distributed; otherwise **Mann Whitney U test** would be used instead if those variables were not normally distributed.
3. **Risk was estimated using odds ratio statistic with 95% confidence interval together with assessment of etiologic as well as preventive fractions.**

The level of significance was considered at *P*-value of equal or less than 0.05. The level of high significance was considered at *P*-value of equal or less than 0.01.

3. Results and Discussion

3.1. Distribution of patients and control subjects according to age and gender

The present study included 60 children with acute lymphoblastic leukemia with a mean age of 6.13 ±3.00 years and an age range of 2-14 years; the median age and inter-quartile range were 5.5 (4.00) years, as shown in table (3.1). The control group was composed of 60 apparently healthy children with a mean age of 5.64 ±3.20 years and an age range of 2-12 years; the median age and inter-quartile range were 4.50 (5.00). Despite some difference in mean age between control and study group, there was not statistical significance (P = 0.164), as shown in table (3.1). The lack of significance difference in age between the two groups is essential in such case control study to ensure statistical matching.

The distribution of children with ALL according to one year interval is shown in figure(3.1). It appeared that the peak incidence of the disease happened at 4-5 years of age.

Table (3.1): Mean age of acute lymphoblastic leukemia patients and control subjects

Age (years)	Control group n = 60	Study group n = 60	P*
Mean ±SD	5.64 ±3.20	6.13 ±3.00	0.164 NS
Range	2-12	2-14	
Median (IQR)	4.50 (5.00)	5.5 (4.00)	

SD: standard deviation; n: number of cases; *: Mann Whitney U test; NS: not significant at $P \leq 0.05$

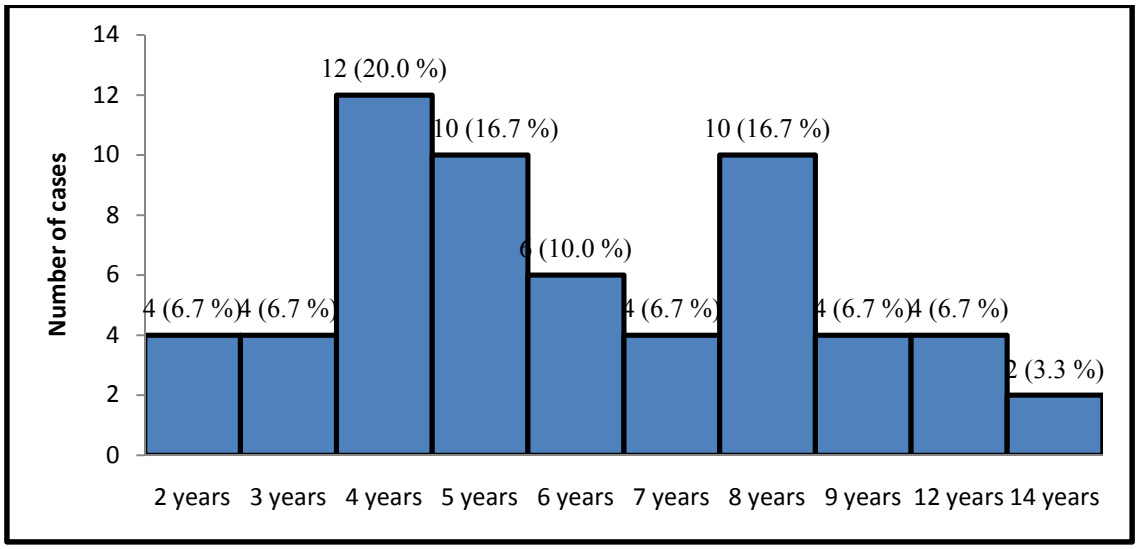

Figure (3.1): The distribution of children with Acute Lymphoblastic Leukemia according to age

The distribution of children with ALL according to one year interval is shown in figure (3.1). It appeared that the peak incidence of the disease happened at 4-5 years of age.

This median age and peach age of incidence is in accordance with Snodgrass *et al*.,(2018) who did a cohort study in Calgary, Alberta, Canada, and found that ALL incidence varies considerably by age, with peak incidence rates for children aged 0–4with 7.55 and 3.32 cases per 100,000 person-years for males and females, respectively. And over 50% of cases occurring in children 10 years old or under. The results also agreed with the results of Yasmeen and Ashraf, (2009) which showed that the median age of Acute Lymphoblastic Leukemia was 5 years. This present study also agreed with several other reports throughout the globe (Inaba *et al*., 2013 ; Hossain *et al*., 2014 ; Kumar *et al*., 2015; Wang *et al*., 2016).

Study group included 40 boys and 20 girls; in terms of statistics the difference was highly significance with male predilection ($P = 0.010$), as shown in figure 3.2. Control group included 42 boys and 18 girls, accounting for 70 % and 30 %, respectively. Indeed, there was no significant difference in male to

female ratio between control and study groups (P = 0.695), as shown in table (3.2).

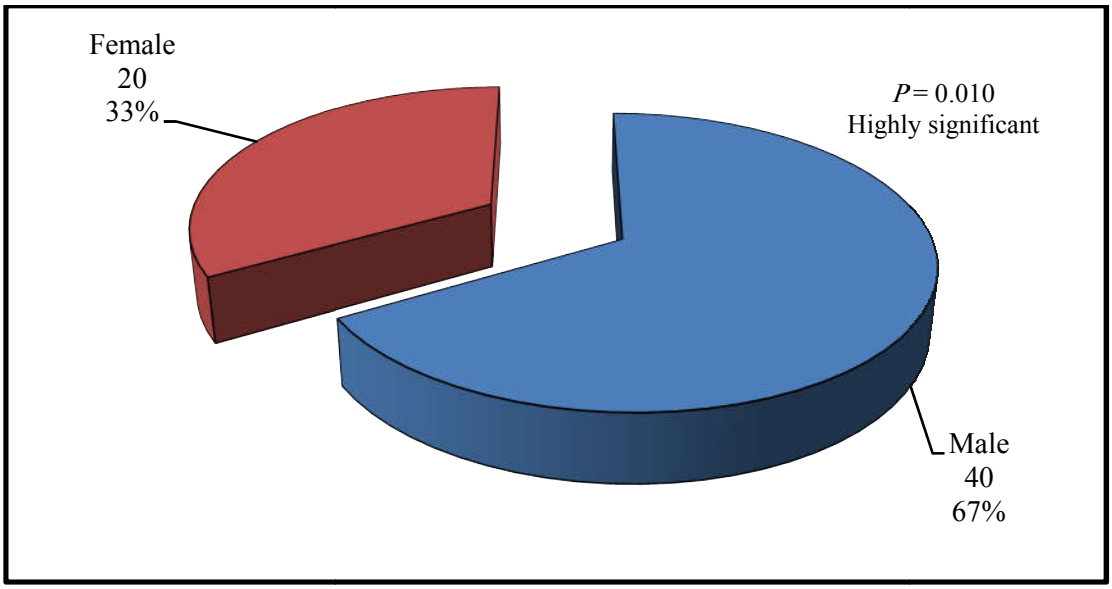

Figure (3.2): The frequency distribution of male and female children with acute lymphoblastic leukemia

Table (3.2): Distribution of Acute Lymphoblastic Leukemia patients and control subjects according to gender

Gender	Control group n = 60	Study group n = 60	P*
Male, n (%)	42 (70.0 %)	40 (66.7 %)	0.695 NS
Female, n (%)	18 (30.0 %)	20 (33.3 %)	

n: number of cases; *: Chi-square test; NS: not significant at $P \leq 0.05$

There was slight difference in age between boys and girls in control as well as study groups, as described in table (3.3), even though, the difference was statistically insignificant (P> 0.05).

Table (3.3):Comparison of median age between male and female children in control and study groups

Groups	Male	Female	*P**
Control Median age (IQR) Years	4.00 (4.50)	3.90 (6.00)	0.067 NS
Study Median age (IQR) Years	4.75 (6.50)	4.38 (4.00)	0.449 NS

IQR: inter-quartile range; *: Mann Whitney U test; NS: not significant at $P \leq 0.05$

The significant predilection to boys by acute lymphoblastic leukemia in our study is in agreement with most reports (Esparza and Sakamoto, 2005; Foà, 2011; Abbasi *et al*., 2013;Singh *et al*., 2016).

3.2. Family history of malignancy

In the questionnaire, we included survey about family history of any malignant tumor experienced by close relatives of children included in the current study and results were outlined in table (3.4). Actually, all children, belonging to control group, had negative family history, whereas 56.7 % of children with ALL had positive family history of malignant disorder, therefore the difference in rate of positive family history between control and study groups was highly significant ($P< 0.001$).

Table (3.4):Family history of malignancy in control and children with Human Leukocyte Antigen

Family history	Control group *n* = 60	Study group *n* = 60	*P**
Positive, *n* (%)	0 (0.0 %)	34 (56.7 %)	< 0.001 HS
Negative, *n* (%)	60 (100.0 %)	26 (43.3 %)	

n: number of cases; *: Chi-square test; HS: highly significant at $P \leq 0.01$

Despite that most studies deny significant association between family history of cancer and predisposition to acute lymphoblastic leukemia in children (Zierhut *et al*., 2011); several reports supported our findings that ALL children have strong family history of overall malignancy (Infante-Rivard and Guiguet, 2004; Couto *et al*., 2005; Ripert *et al*., 2007).

3.3. Detection ofIL12-A rs583911(A/G) gene polymorphism:

Distribution of IL12-A rs583911 (A/G) gene polymorphism was detected by Allele-specific PCR primer technique from patients and healthy control blood samples, at this locus there were three genotypes: Lane (AA) wild type homozygote genotypes at 100bp band, while (GG) mutant type homozygote genotypes at 55bp band and (A/G) heterozygote genotypes at 100 and 55 bp bands as shown in figure (3.3). This result consistent with Hirschfield *et al.* (2009) .

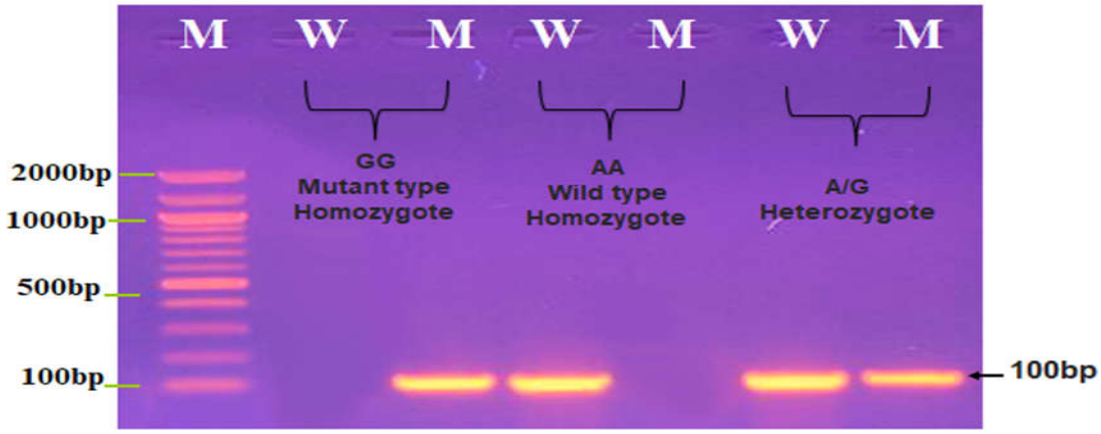

Figure (3.3): Agarose gel electrophoresis image that showed the AS-PCR product analysis of IL12-A rs583911 (A/G) at 2% agarose. Where M: marker (2000-100bp). Lane (GG) mutant type homozygote genotypes was showed amplification only with mutant reverse primer. Lane (AA) wild type homozygote genotypes was showed amplification only with wild reverse primer. Whereas, Lane (A/G) heterozygote genotypes was showed amplification with wild and mutant reverse primer. Positive alleles amplification at 100bp product size.100 volt and 80AM for 1hour.

3.3.1. Frequency distribution of IL12-A rs583911 genotypes AA, AG and GG in control and study groups

In order to be sure about the random selection of control subjects enrolled in the present study, Hardy Weinberg equilibrium was carried out and showed no significant difference between observed and expected genotype frequencies (P=0.444), as shown in table (3.5). The genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups. The most prevalence IL12-A rs583911 genotype was AA so it will be considered the wild type (reference) whereas the least frequent IL12-A rs583911 genotype was GG, therefore it will be regarded as the variant (mutant) genotype. Considering control group, the frequency distribution of IL12-A rs583911 genotype AA,AG and GG were 16, 30 and 14, respectively and this observed distribution did not differ significantly from the expected distribution according to Hardy Weinberg equation (P = 0.444), as shown in table (3.5).

Table (3.5): Hardy Weinberg equilibrium of IL12-A rs583911 genotype in control group

Genotypes	Observed	Expected	χ^2	*P*
AA (wild)	16	25.4	0.587	0.444 NS
AG	30	27.3		
GG (mutant)	14	7.4		

NS: not significant at $P \leq 0.05$

Considering study group, the frequency distribution of IL12-A rs583911 genotype AA, AG and GG were 6, 20 and 34, respectively and this observed distribution differed significantly from the expected distribution according to Hardy Weinberg equation , as shown in table (3.6). Indeed, genotype GG was significantly more frequent in study group than in control groups, 56.7 % versus 23.3% (P = 0.001), as shown in table (3.6).

Table (3.6): Comparison of IL12-A rs583911 genotype between control and study groups

Genotype	Control group $n = 60$	Study group $n = 60$	χ^2	*P**
AA	16 (26.7 %)	6 (10.0 %)	14.879	0.001 HS
AG	30 (50.0 %)	20 (33.3 %)		
GG	14 (23.3 %)	34 (56.7 %)		

n: number of cases; *: Chi-square test; HS: Highly significant at $P \leq 0.01$

There was highly significant difference in rate of allele G between study and control groups (*P*=0.002). It acts as risk factor for ALL with an Odds ratio of 2.94;95 % confidence interval of 1.71 -5.05 and the etiologic fraction of allele G as a risk factor for ALL was 0.40, as shown in table (3.7). Nevertheless, variant allele A was more frequent in control than in study group, 51.7% versus 26.7% respectively and may be considered as a preventive fraction (0.40).

Table (3.7): Comparison of IL12-A rs583911 alleles between control and study groups

Allele	Control $n = 120$	Study $n = 120$	*P*	OR	95% CI	EF	PF
A	62 (51.7 %)	32(26.7 %)	0.002 HS	0.34	0.20 - 0.58	---	0.40
G	58 (48.3 %)	88 (73.3 %)		2.94	1.71 -5.05	0.40	---

n: number of alleles; OR: Odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; *: Chi-square test; HS: Highly significant at $P \leq 0.01$

In one study, of the 208 SNPs, only rs583911 of *IL12A*, which encodes a critical modulator of T-cell development, remained significant after accounting for multiple testing (odds ratio for each copy of the variant G allele = 1.52, 95% confidence interval: 1.25–1.85, $p = 2.9 \times 10^{5}$) (Chang *et al*., 2010). This result supports our finding that allelic variation exists in the gene encoding IL-12 A in children with ALL; and agree that the variant allele G may considered as a risk factor for ALL in children.

Previous studies have shown that newborns have Th2-skewed immune profiles (Ribeiro-do-Couto *et al.*, 2001). Furthermore, during the normal course

of immune development, a shift from Th2-dominant to Th1-dominant immune profiles occurs with increasing age (Prescott *et al*., 1998). It is thought that the major driving force for this immune shift is the production of IL12 by innate immune cells (e.g. dendritic cells) after exposure to microbial challenges (Romagnani, 2004). The IL12 protein is a heterodimer that consists of two subunits, IL12A (p35) and IL12B (p40) (Trinchieri, 2003). In the current study, we observed significant variation with rs583911 of *IL12A*. Functional impact of the *IL12A* SNPs has yet to be characterized. A study by Pistiner *et al*. (2008) showed that rs2243123 of *IL12A*, a SNP in intron 2 that is 739 base pairs away from rs583911, is associated with immune sensitization to cockroach antigen; this lends further support that the region around rs583911 may be important in either the function or the expression of IL12A and may be a promising candidate region to perform fine mapping and functional studies to determine causal variants.

The hypotheses of childhood leukemia propose that childhood leukemia may result from abnormal responses to microbial challenges due to lack of immune priming during early childhood (Greaves, 2006). Consistent with this hypothesis are the findings showing a reduced risk of childhood ALL associated with higher birth order (Dockerty *et al*., 2001) and early daycare attendance (Ma *et al*., 2005).

3.4. Frequency distribution of HLA-A and HLA-B alleles in control and study groups

3.4.1. Frequency distribution of HLA-A alleles in control and study groups

The frequency distribution of HLA-A alleles in control and study groups is shown in table (3.8). The frequency distribution was as following:

1. Allele *1 was less frequent in study group than in control group, 24 versus 36, and the difference was not significant ($P = 0.074$).

2. Allele *2 was more frequent in study group than in control group, 28 versus 12, and the difference was highly significant ($P = 0.006$) . By this way, HLA-A *2 allele is a risk factor for ALL with an odds ratio of 2.74.
3. Allele *3 was more frequent in study group than in control group, 28 versus 12, and the difference was highly significant ($P = 0.006$). By this way, HLA-A *3 allele is a risk factor for ALL with an odds ratio of 2.74.
4. Allele *11 was limited to study group since it was not found in control group, 8 versus 0, and the difference was highly significant ($P = 0.007$). By this way, HLA-A *11 allele is a risk factor for ALL with an approximated odds ratio of 18.21.
5. Allele *23 was limited to study group since it was not found in control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
6. Allele *24 was less frequent in study group than in control group, 8 versus 12, and the difference was not significant ($P = 0.350$).
7. Allele *26 was limited to control group since it was not found in study group, 24 versus 0, and the difference was highly significant ($P < 0.001$) . By this way, HLA-A *26 allele is protective against ALL with an approximated odds ratio of 0.02 reducing the risk by 0.98.
8. Allele *30 was limited to study group since it was not found in control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
9. Allele *32 was limited to control group since it was not found in study group, 12 versus 0, and the difference was highly significant ($P < 0.001$) . By this way, HLA-A *32 allele is protective against ALL with an approximated odds ratio of 0.04 reducing the risk by 0.96.
10. Allele *33 was limited to study group since it was not found in control group, 8 versus 0, and the difference was highly significant ($P = 0.007$).By this way, HLA-A *33 allele is risk factor for ALL with an approximated odds ratio of 18.21.

11. Allele *50 was limited to study group since it was not found in control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
12. Allele *51 was limited to study group since it was not found in control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
13. Allele *68 was limited to control group since it was not found in study group, 12 versus 0, and the difference was highly significant ($P < 0.001$). By this way, HLA-A *68 allele is protective against ALL with an approximated odds ratio of 0.04 reducing the risk by 0.96 .

Table (3.8): The frequency distribution of HLA-A alleles in control and study groups

HLA-A Alleles	Study *n* = 120	Control *n* = 120	*P*	OR	95% CI
*1	24	36	0.074* NS	0.58	0.32 - 1.06
*2	28	12	0.006* HS	2.74	1.32 - 5.69
*3	28	12	0.006* HS	2.74	1.32 - 5.69
*11	8	0	0.007† HS	18.21	---
*23	4	0	0.122† NS	9.31	---
*24	8	12	0.350* NS	0.66	---
*26	0	24	<0.001* HS	0.02	---
*30	4	0	0.122† NS	9.31	---
*32	0	12	<0.001* HS	0.04	---
*33	8	0	0.007† HS	18.21	---
*50	4	0	0.122† NS	9.31	---
*51	4	0	0.122† NS	9.31	---

*68	0	12	<0.001* HS	0.04	---

3.4.2. Frequency distribution of HLA-B alleles in control and study groups

The frequency distribution of HLA-B alleles in control and study groups is shown in table (3.9). The frequency distribution was as following:

1. Allele *7 was less frequent in study group than in control group, 4 versus 24, and the difference was highly significant ($P < 0.001$). By this way, HLA-B *7 allele is protective against ALL with an odds ratio of 0.14, reducing the risk to 0.86.
2. Allele *13 was more frequent in study group than in control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
3. Allele *15 was less frequent in study group than in control group, 4 versus 12, and the difference was significant ($P = 0.038$). By this way, HLA-B *15 allele is protective against ALL with an odds ratio of 0.31, reducing the risk to 0.69.
4. Allele *18 was limited to control group since it was not found in study group, 12 versus 0, and the difference was highly significant ($P < 0.001$). By this way, HLA-B *18 allele is protective against ALL with an approximated odds ratio of 0.04, reducing the risk to 0.96.
5. Allele *27 was limited to study group since it was not found in the control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
6. Allele *35 was more frequent in study group than in control group, 28 versus 12, and the difference was highly significant ($P = 0.006$). By this way, HLA-B *35 allele is a risk factor for ALL with an odds ratio of 2.74.
7. Allele *38 was less frequent in study group than in control group, 4 versus 24, and the difference was highly significant ($P < 0.001$). By this way, HLA-B *38 allele is protective against ALL with an odds ratio of 0.14, reducing the risk to 0.86

8. Allele *40 was less frequent in study group than in control group, 4 versus 12, and the difference was highly significant (P = 0.038). By this way, HLA-B *40 allele is protective against ALL with an odds ratio of 0.31, reducing the risk to 0.69.
9. Allele *41 was less frequent in study group than in control group, 4 versus 12, and the difference was highly significant (P = 0.038). By this way, HLA-B *40 allele is protective against ALL with an odds ratio of 0.31, reducing the risk to 0.69.
10. Allele *42 was limited to study group since it was not found in the control group, 4 versus 0, and the difference was not significant ($P = 0.122$).
11. Allele *44 was limited to study group since it was not found in the control group, 12 versus 0, and the difference was highly significant ($P < 0.001$). By this way, HLA-B *44 allele is a risk factor for ALL with an approximate odds ratio of 27.76.
12. Allele *50 was limited to study group since it was not found in the control group, 8 versus 0, and the difference was highly significant ($P = 0.007$). By this way, HLA-B *50 allele is a risk factor for ALL with an approximate odds ratio of 18.21.
13. Allele *51 was limited to study group since it was not found in the control group, 24 versus 0, and the difference was highly significant ($P < 0.001$). By this way, HLA-B *51 allele is a risk factor for ALL with an approximate odds ratio of 74.25.
14. Allele *52 was limited to study group since it was not found in the control group, 8 versus 0, and the difference was highly significant ($P = 0.007$). By this way, HLA-B *50 allele is a risk factor for ALL with an approximate odds ratio of 18.21.
15. Allele *53 was less frequent in study group than in control group, 4 versus 12, and the difference was highly significant ($P = 0.038$). By this

way, HLA-B *53 allele is protective against ALL with an odds ratio of 0.31, reducing the risk to 0.69.

16. Allele *58 was limited to study group since it was not found in the control group, 4 versus 0, and the difference was not significant (P = 0.122).

Table (3.9): The frequency distribution of HLA-B alleles in control and study groups

HLA-B Alleles	Study n = 120	Control n = 120	P	OR	95 % CI
*07	4	24	<0.001 HS	0.14	0.05 - 0.41
*13	4	0	0.122† NS	9.31	---
*15	4	12	0.038* S	0.31	0.10 - 0.99
*18	0	12	<0.001* HS	0.04	---
*27	4	0	0.122† NS	9.31	---
*35	28	12	0.006* HS	2.74	1.32 - 5.69
*38	4	24	<0.001 HS	0.14	0.05 - 0.41
*40	4	12	0.038* S	0.31	0.10 - 0.99
*41	4	12	0.038* S	0.31	0.10 - 0.99
*42	4	0	0.122† NS	9.31	---
*44	12	0	<0.001* HS	27.76	---
*50	8	0	0.007† HS	18.21	---
*51	24	0	<0.001* HS	74.25	---
*52	8	0	0.007† HS	18.21	---
*53	4	12	0.038* S	0.31	0.10 - 0.99

*58	4	0	0.122† NS	9.31	---

In summary, HLA-A alleles *1, *23, *24, and *30 have no significant association with acute lymphoblastic leukemia. This study agrees with (Patıroğlu and Akar, 2016) that HLA-A *1, *24, *26 and *30 alleles play no role in pathogenesis of ALL; however, this study disagree with (Patıroğlu and Akar, 2016) about the role of HLA-A *23 allele in ALL. We found no significant association, whereas,(Patıroğlu and Akar, 2016) considered it as protective since it happened more frequently in control group than in ALL group. We agree with (Klitz *et al*., 2012) that HLA-A *26 has no significant association with ALL; however they found that HLA-A *1 allele has a protective role against ALL and that HLA-A *23, 24 and *30 alleles were risk factors in striking contradiction to our findings.

Back to summary of our results, we found that HLA-A alleles *2, *3,*11 and *33 were risk factors for ALL. We agree with (Patıroğlu and Akar, 2016) that HLA-A *3 allele is a risk factor; however, (Patıroğlu and Akar, 2016) found, in contrary to our results, that neither HLA-A *2 nor HLA-A *11 had significant association with ALL. We agree with (Klitz *et al*., 2012) that HLA-A *11 is a risk factor for ALL; however they found that HLA-A *2 allele has no significant association with ALL and that HLA-A *3, plays a protective role in ALL in clear contradiction to our observation. In other hand we disagree with (Klitz *et al*., 2012) who found HLA-A *33 as protective factor.

Again, our results suggested that HLA-A alleles *32, *26 and *68 plays significant role in protection against ALL. This is in contradiction to the findings of (Patıroğlu and Akar, 2016) who described no significant association between ALL and *32, *33 and *68 alleles; *50 and *51 were not evaluated by (Patıroğlu and Akar, 2016). however, we disagree with them regarding alleles

*26 and *68 since they described no significant contribution by *26 allele and a significant risk by *68.

Regarding HLA-B alleles, we can summarize our findings in that alleles *13, *27, *42 and *58 have no significant association with acute lymphoblastic leukemia. This study agrees with (Patıroğlu and Akar, 2016) that HLA-B *27 and *58 alleles play no role in pathogenesis of ALL; however, this study disagree with (Patıroğlu and Akar, 2016) about the role of HLA-B *13 allele in ALL. We found no significant association, whereas,(Patıroğlu and Akar, 2016) considered allele *13 as protective since it happened more frequently in control group than in ALL group and that allele *42 was not mentioned in their study. We agree with (Klitz *et al*., 2012) that HLA-B *27 and *58 alleles have no significant association with ALL; however they found that HLA-B *13 allele is a risk factor for ALL and that HLA-B *42 was mentioned in their study. Moreover, we totally agree with (Fernández-Torres *et al*., 2013) that HLA-B *13, *27, *42 and *58 have no significant association with ALL.

Back to summary of our results, we found that HLA-B alleles *35, *44, *50 ,*51 and *52 were risk factors for ALL. Therefore, we disagree with (Patıroğlu and Akar, 2016) that these alleles, *35, *44, *50, *51 and *52, have no role in association with ALL. We agree with (Klitz *et al*., 2012) that HLA-B *51 is a risk factor for ALL; however they found that both HLA-B *35 and *50 alleles have no significant association with ALL and that HLA-B *44, plays a protective role in ALL in clear contradiction to our observation. Moreover, we totally disagree with (Fernández-Torres *et al*., 2013) who found no significant association between ALL and HLA-B *35, *44, *50 and *51 alleles.

Again, our results suggested that HLA-B alleles *7, *15, *18, *38, *40 ,*41 and *53 plays significant role in protection against ALL. We agree with (Patıroğlu and Akar, 2016) that HLA-B *40 allele has a protective role against ALL; however, we disagree with them that HLA-B *7, *15, and *38 alleles have

no significant association with ALL. Patıroğlu and Akar (2016) did not mention HLA-B 53 in their study. In addition, we agree with (Klitz *et al*., 2012) that HLA-B *7 and *38 alleles are protective against ALL; however, we disagree with them regarding alleles *15, *40 and *53 since they considered these alleles as risk factors for ALL. In addition we disagree with (Fernández-Torres *et al*., 2013) who found no significant association between ALL and HLA-B *7, *15, *38 and *53 alleles; they also found that HLA-B *40 was a risk factor in clear contradiction to our result.

A number of studies described the association between ALL and HLA-A and HLA-B; however, these studies were based on serotypes rather than allelic level classifications. The HLA-A2 antigen association found in the previous generation of studies of ALL (all age groups) was not observed in subsequent studies conducted specifically in children. In one of the earliest studies of HLA and childhood ALL risk, Davey *et al.* in 1981 observed no difference in distribution of HLA-A and HLA-B antigens between patients and controls. Among four additional studies that investigated HLA-A antigens in childhood ALL (von Fliedner *et al*., 1983; Muller *et al*., 1988; Cameron *et al*., 1990; Ozdilli *et al*., 2010), only one reported an association with this locus, but with the HLA-A23 serotype (Ozdilli *et al*., 2010). An association with the *HLA-B* locus was found in three of these studies (von Fliedner *et al*., 1983; Cameron *et al*., 1990; Ozdilli *et al*., 2010), one of which was a family-based study comprising 55 families with a child affected with ALL (von Fliedner *et al*., 1983). A comparison made between observed vs. expected numbers of shared antigens among parents with ALL offspring showed increased compatibility for HLA-B antigens, suggesting a role for this locus as a recessive determinant. Using the case-control design, one study reported higher HLA-B40 and lower HLA-B5 antigen frequencies in Trinidadian cases compared to controls

(Cameron *et al*., 1990), and another study conducted in Turkey reported a reduced risk associated with the HLA-B7 antigen (Ozdilli *et al*., 2010).

The lack of consistent results for HLA class I loci from these mostly small studies (all less than 100 patients) utilizing low-resolution HLA typing complicates interpretation. Addressing these shortcomings, in the largest study to date evaluating HLA and childhood ALL risk, Klitz *et al.* (2012) utilized HLA allelic data from the National Marrow Donor Program (NMDP) in an analysis of 2,438 medically refractory childhood ALL patients being considered for or undergoing hematopoietic stem cell transplantation and 41,750 controls that were matched on age (2–15 years), sex, and geographical location in the United States. This comprehensive analysis of the *HLA-A* and *HLA-B* loci revealed a spectrum of HLA associations conferring predisposition to or protection from refractory disease. Four significantly (p-value <0.01) protective *HLA-A* alleles (*A*33, A*01, A*03*, and *A*26*) and six predisposing-alleles (*A*24, A*31, A*23, A*30, A*68*, and *A*74*) were found, with 50% of alleles as a whole either predisposing or protective in this high-risk ALL population. *HLA-B* allelic associations revealed six protective alleles (*B*14, B*38, B*08, B*07, B*57*, and *B*44*) and eight predisposing (*B*15, B*18, B*40, B*51, B*53, B*39, B*48*, and a binned category "rare" with <50 copies). As with *HLA-A*, they showed a continuum of odds ratios ranging from 0.57 to 3.20, with more predisposing than protective alleles, and 24.0% of alleles being neutral with respect to disease. These analyses show a spectrum of HLA associations and indicate that studies with larger sample sizes might clarify the predisposing, neutral, or protective status of alleles whose confidence intervals are close to, or include, an odds ratio of 1.0.

3.5. The association between family history of malignancy and the HLA and IL-12 A genotypes and alleles

There was no significant association between family history of malignancy and IL12-A rs583911 genotypes (P= 0.273), as shown in table

(3.10). In addition, there was no significant association between family history of malignancy and any of HLA-A genotypes ($P > 0.05$), as shown in table (3.11). Moreover, there was no significant association between family history of malignancy and any of HLA-B genotypes ($P > 0.05$), as shown in table (3.12).

Table (3.10): Association between family history of malignancy and IL12-A rs583911 genotypes

Genotype	Total *n* = 60	Family history		*P**
		Positive *n* = 34	Negative *n*= 26	
AA	16	9 (56.3 %)	7 (43.8 %)	0.273 NS
AG	20	14 (70.0 %)	6 (30.0 %)	
GG	24	11 (45.8 %)	13 (54.2 %)	

n: number of cases; *: Chi-square test; NS: not significant at $P \leq 0.05$

Table (3.11): Association between family history of malignancy and HLA-A genotypes

HLA-A	Total *n* = 60	Family history		*P*
		Positive *n* = 34	Negative *n* = 26	
*01	4	2 (50.0 %)	2 (50.0 %)	1.000
*01-*02	8	3 (3.8 %)	5 (4.2 %)	0.428
*01-*11	4	2 (50.0 %)	2 (50.0 %)	1.000
*01-*30	4	4 (100.0 %)	0 (0.0 %)	0.126
*01-*68	0	0 (0.0 %)	0 (0.0 %)	---
*02-*03	8	3 (3.8 %)	5 (4.2 %)	0.428
*02-*23	4	3 (75.0 %)	1 (25.0 %)	0.807
*02-*24	4	2 (50.0 %)	2 (50.0 %)	1.000
*02-*32	0	0 (0.0 %)	0 (0.0 %)	---
*03	8	5 (4.2 %)	3 (3.8 %)	0.428
*03-*24	4	3 (75.0 %)	1 (25.0 %)	0.807
*03-*26	0	0 (0.0 %)	0 (0.0 %)	---

*11-*33	4	3 (75.0 %)	1 (25.0 %)	0.807
*24-*26	0	0 (0.0 %)	0 (0.0 %)	---
*50-*51	4	2 (50.0 %)	2 (50.0 %)	1.000
*02-*33	4	2 (50.0 %)	2 (50.0 %)	1.000

Table (3.12): Association between family history of malignancy and HLA-B genotypes

HLA-B	Total *n* = 60	Family history		*P*
		Positive *n* = 34	Negative *n* = 26	
*07-*40	0	0 (0.0 %)	0 (0.0 %)	---
*07-*51	4	3 (75.0 %)	1 (25.0 %)	0.807
*07-*53	0	0 (0.0 %)	0 (0.0 %)	---
*13-*27	4	0 (0.0 %)	4 (100.0 %)	0.126
*15-*35	4	2 (50.0 %)	2 (50.0 %)	1.000
*18-*41	0	0 (0.0 %)	0 (0.0 %)	---
*35	4	2 (50.0 %)	2 (50.0 %)	1.000
*35-*40	4	1 (25.0 %)	3 (75.0 %)	0.807
*35-*41	4	3 (75.0 %)	1 (25.0 %)	0.807
*35-*52	4	2 (50.0 %)	2 (50.0 %)	1.000
*38	0	0 (0.0 %)	0 (0.0 %)	---
*38-*51	4	2 (50.0 %)	2 (50.0 %)	1.000
*42-*51	4	4 (100.0 %)	0 (0.0 %)	0.126
*44	4	2 (50.0 %)	2 (50.0 %)	1.000
*44-*51	4	3 (75.0 %)	1 (25.0 %)	0.807
*50-*51	4	2 (50.0 %)	2 (50.0 %)	1.000
*50-*58	4	2 (50.0 %)	2 (50.0 %)	1.000
*51-*35	4	3 (75.0 %)	1 (25.0 %)	0.807
*52-*53	4	3 (75.0 %)	1 (25.0 %)	0.807

Conclusions:

1. Childhood acute lymphoblastic leukemia increases in children with age range (4-5) years in Iraq.

2. Males appear to be more affected than females by acute lymphoblastic leukemia and often associated with family history of any tumor malignancy in Iraq.

3. There was no association between family history of malignancy and IL12-A rs583911 genotypes and any of HLA-A,B genotypes.

4. Variant allele G of IL12-A (rs583911) polymorphism was more frequent in ALL patients and it acts as risk factor for ALL.

5. Human Leukocyte Antigen-A Alleles (*02, *03, *11 and *33) were contribute to ALL pathogenesis ,while Alleles (*26,*32, and *68) were protective against ALL.

6. Alleles (*35, *44, *50, *51 and *52) of HLA-B were risk factors for ALL. And alleles *7, *15, *18,*38, *40 and *53 plays significant role in protection against ALL.

Recommendations:

1. A multicentric study based on large sample size is required to better understanding the role of IL12-A(rs583911) polymorphism and HLA-A,B alleles in development of ALL.

2. With the evidence that genetic factors play a role in the pathogenesis of ALL as confirmed by family and twin studies, the evaluation of other genetic polymorphisms involving other cytokines and immune response mediators properly addresses the need for a more rigorous and objective studies.

3. Study the association between other SNPs of IL12 gene and ALL.

4. Evaluating the effect of IL12-A(rs583911) polymorphism and HLA-A,B alleles according to subtypes of ALL, evaluate each one separately (T cell-ALL and B-cell ALL).

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Patients' Questionnaire Form

Name :

Age :

Sex:

Address:

Family history of cancer :

Other notes:

الخلاصة

سرطان الدم الليمفاوي الحاد (ALL) هو اضطراب خبيث من الخلايا السلف اللمفاوية ونوع الأورام الخبيثة الأكثر شيوعًا لدى الأطفال ، وهذا المرض هو مرض متعدد العوامل ينتج عن التفاعل بين العوامل الجينية والبيئية.

أجريت هذه الدراسة على ٦٠ مريضا ممن يعانون من متلازمة سرطان الدم الليمفاوي الحاد (٤٠ ذكور و ٢٠ إناث) تراوحت أعمارهم بين (٢-١٤) سنة ومن الراقدين في مستشفى الطفل المركزي التعليمي في بغداد للفترة من الأول من شهر شباط لسنة ٢٠١٨ حتى بداية شهر يونيو لسنة ٢٠١٨. وتمت المقارنة مع (٦٠)شخص من الأصحاء (٤٢ ذكور و ١٨ إناث) كمجموعة سيطرة. تم جمع عينات الدم من كلتا المجموعتين وتم عزل الحمض النووي من كريات الدم البيض لغرض الدراسة الجينية والكشف عن وجود أي ارتباط بين تعدد أشكال الاليل الجيني لانترلوكين ١٢(rs583911) ومواقع مستضد خلايا البيضاء البشريه(أ،ب)والاستعداد لسرطان الدم الليمفاوي الحاد.وقد تم استخدام تقنية (AS-PCR) لتعدد اشكال الاليل الجيني لأنترلوكين ١٢(rs583911) وتقنية (SSP-PCR) لتحليل الاليلي لمواقع مستضد خلايا البيضاء البشرية (أ،ب).

أظهرت الدراسة الحالية أن ذروة الاصابة بالمرض تكون بين الفئات العمرية (٥-٦) سنة، كشفت النتائج أيضا أن (٦٧٪) من المرضى كانوا من الذكور.وأظهرت البيانات ان (٥٦.٧٪)من المرضى الذين يعانون من السرطان الدم اللمفاوي الحاد كان لهم تاريخ عائلي وراثي لأمراض خبيثة.

كما بينت هذه الدراسة أن النوع الوراثي AA كان الاكثر انتشارا لذلك كان يعتبر نوع البري (المرجع) في حين أن النمط الوراثي الأقل تكرارا IL12-A rs583911 كان GG، لذلك تم اعتباره النوع الوراثي البديل(المتنحية). النوع الوراثي GG كان الاكثر تكرارا في مجموعة الدراسة والذي كان بنسبة (٥٦.٧%) مما هو عليه في المجموعة السيطره حيث كانت النسبة لديهم (٢٣.٣%). كان هناك اختلاف كبير للغاية في معدل أليل G بين مجموعات الدراسة والسيطرة (P = 0.002).حيث ان الاليل G يعمل كعامل مخاطرة لـلاصابه بالمرض مع نسبة الأرجحية (٢.٩٤) ؛ فاصل الثقة (٩٥٪) من (١.٧١ -٥.٠٥) والكسر المسبب للأليل G كعامل خطر لـلاصابه بهذا المرض (0.40) . كما إن الأليل البديل Aكان الأكثر تكرارا في مجموعة السيطره مما هو عليه في مجموعة الدراسه ، ٥١.٧% مقابل ٢٦.٧% ويعمل بمثابة عامل الحمايه ضد الأصابه بسرطان الدم اللمفاوي الحاد.

تشير النتائج إلى أن توزيع التردد و المساهمة في سرطان الدم الليمفاوي الحاد لأليلات المستضدات الخلايا البيضاء البشريه-أ (*١،*٢٣،*٢٤،*٢٦ والاليل *٣٠)ليست لهم علاقه بالمرض ،. بينما كان

الأليلات *٢،*٣ والاليل*١١ اعتبروا كعامل يزيد من نسبة الاصابة بالمرض.في حين أن الأليلات (*٣٢،*٣٣،*٥٠،*٥١ والاليل *٦٨) وجدت انها تعمل على الحماية ضد الاصابه بمرض سرطان الدم اللمفاوي الحاد .

من ناحية أخرى ، كان توزيع التردد و المساهمة في سرطان الليمفاوية الحادة لأليلات الخاصة بمستضدات الخلايا البيضاء البشرية-ب(* ١٣،*٢٧،*٤٢ والاليل *٥٨) والتي وجدت ليس لها علاقه بالمرض. كما لوحظ إن الاليلات (*٣٥،*٤٤،*٥٠ والاليل *٥١) تمثل عامل الخطوره للاصابه بمرض سرطان الليمفاويه الحاد، و يعتبر الأليلات (*٧،*١٥،*٣٨،*٤٠،*٥٣) كحماية ضد هذا المرض.

كما أظهرت البيانات عدم وجود ارتباط كبير بين تاريخ العائلة بالاصابة بالورم الخبيث و الأنماط الجينية لأنترلوكين ١٢-A rs583911 وأي من الأنواع الوراثية لمستضد الخلايا البيضاء البشرية أ،ب .

استنتاج هذه الدراسه ان الاليل G في تعدد الاشكال لانترلوكين ١٢-A rs583911 اعتبر كعامل مسبب للاصابه بالمرض، كذلك وجدت ان الاليلات الخاصه بمستضدات الخلايا البيضاء البشريه –أ(*٢،*٣ و*١١) والاليلات (*٣٥،*٤٤،*٥٠ و*٥١) الخاصه بمستضدات الخلايا البيضاء البشريه –ب لها علاقه كبيره للاصابه بمرض السرطان الليمفاوي الحاد في الاطفال.

وزارة التعليم العالي والبحث العلمي
جامعة القادسية
كلية الطب

التحليل الأليلي لمستضدات خلايا البيضاء البشرية (أ،ب) وتعدد الأشكال لأنترلوكين ١٢-A(rs583911) في الأطفال المصابين بسرطان الدم الليمفاوي الحاد

رسالة مقدمة إلى
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كجزء من متطلبات نيل درجة الماجستير
في علم الأحياء المجهرية الطبية

من قبل
تبارك سلام عبد الرؤوف
بكالوريوس علوم في علوم الحياة / جامعة المثنى-٢٠١٠

بإشراف
أ. د. منال محمد كاظم

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