بسم الله الرحمن الرحيم

Sudan University of Science & Technology

College of Graduate Studies

Association of Interleukin-10 (1082G/A) (rs1800896) Polymorphism in Acute Myeloid Leukemia Patients in Sudanese Population.

العلاقة بين المحول للإنترليوكين 10 في مرضى سرطان الدم الأبيض النخاعي الحاد بالسودان.

A dissertation submitted in partial fulfillment for the requirements of the degree of M.Sc. in medical laboratory science.

(Hematology)

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الآية

بسم الله الرحمن الرحيم

قال تعالى :

"..... وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ"

صدق الله العظيم

سورة هود الآية (88)

Dedication

To the soul of my father (**Mohamed Sharif Alamin**)

To the candles of my life my mother (**Sittna Abdullah**)

To my brothers and my lovely sister (**Fatima)**

To my colleagues' and friends

I dedicate this work

Acknowledgment

My grateful thanks for **Allah.** Then, I deeply grateful indebted to my supervisor **D. Ibrahim Khider Ibrahim** for his supervision, advice and critical review of the manuscript. Also I thanks **Nada Dawood, Israa Abuobaida** to help me in AML sample and those were become volunteer to give me control blood sample, principally includes **all staff members of Sudan University research laboratory and hematology department**, my grateful appreciation should be extended to **UZ. Sohair Ramadan, my brother Hamza and his wife Azza** with them I have had the ability to complete this work. Finally my deeply grate to all who have given me their time and energy to supply me by the facts and opinions.

Abstract

This is prospective case control study conducted in Radio isotope center Khartoum Sudan to study a single nucleotide polymorphism (SNP) in IL-10 -1082G/A (rs1800896) in Sudanese acute myeloid leukemia patients in Khartoum state in the period from January to May 2017.

Thirty samples were collected from newly diagnosed patients of acute myeloid leukemia and 30 samples were collected from normal individuals as control group, all samples were collected in Ethylene di-amine tetra acetic acid (EDTA) container, then Deoxyribonucleic Acid (DNA) was extracted by using salting out method. And polymorphism analysis for IL-10(-1082G/A) (rs1800896) was determined by using allele specific-PCR. The results were analyzed by statistical package for social sciences (SPSS) computer program.

The results showed that (36.7%) of cases have homogenous IL-10 (1082G/A) genotype, (43.3%) have heterogeneous (IL-10 -1082G/A) genotype, (20.0%) have normal IL-10(-1082G/A) genotype. The association between IL-10 (-1082G/A) polymorphism and AML is statistically significant, heterogeneous genotype of IL-10(-1082G/A) polymorphism is risk factor of AML.

The study concluded that there is no association between IL-10(-1082G/A) polymorphism and AML sub-type, gender, age group, mean of hematological parameter (TWBs, Blast%, Hb and platelet count).

مستخلص البحث

هذه دراسة مقارنة حالة ضابطة تحليلية أجريت في ولاية الخرطوم في الفترة ما بين يناير الي مايو 2017 وذلك لفحص علاقة الجين المحول للإنترليوكين10 في مرضي السرطان الابيض النخاعي الحاد عند المرضى المشخصين حديثا بالسودان.

أختير عدد ثلاثون مريضا شخصوا بسرطان الدم الابيض النخاعي الحاد الذين حضرو الي مستشفي الخرطوم للأورام ،وثلاثون متطوعين أصحاء تم اختيارهم كعينة ضابطة . جمعت عينة الدم الوريدي من كل متبرع في حاوية مضاد تجلط يحتوي علي ايثايلين ثنائي الامين رباعي حامض الاستيك. استخرج الحمض النووي منزوع الاكسجين للتحليل الجزئي عن طريق الترسيب بواسطة الملح. تغيير شكل الجين المحول للانترليوكين حدد بإستخدام أليل البلمرة المتسلسلة . حللت النتايج بإستخدام الحزم الإحصائية للعلوم الإجتماعية لمحوسب.

أظهرت النتائج ان 36.7% من المرضي يمتلكون الجين المتجانس و43.3% يمتلكون الجين الغير متجانس و20.0% يمتلكون الجين الطبيعي . وكانت هذة النسب ذات دلالة احصائية وبينت أن الجين الغير متجانس يمثل عامل خطورة للاصابة بمرض سرطان الدم الابيض النخاعي الحاد. لا توجد دلالة إحصائية لوجود علاقة بين الجين المحول للانترليوكين10 وكل من عناصر الجنس،المجموعات العمرية ،أنواع السرطان ، (حساب كريات الدم البيضاء،نسبة خلايا العلة، هيموجلوبين الكرية الوسطي ،نسبة الصفائح الدموية) .

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ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
ARMS	Amplification-refractory mutation system
ASPCR	Allele specific polymerase chain reaction
ATRA	All-trans retinoic acid
CGH	Comparative genomic hybridization
DCs	Dendrites cell
EDTA	Ethylene di-amine tetra acetic acid
DNA	Deoxyribonucleic Acid
FAB	French American-British
FISH	Fluorescence in situ hybridization
HLA	Human leukocyte antigen
IARC	International agency for research on cancer
ILs	Interleukins
ITD	Internal Tandem Duplications
MDS	Myelodysplastic syndrome
MPD	Myeloproliferative disorder
MPO	Myeloperoxidase
NK	Natural killer
NOS	Not otherwise specified
RCLB	Red cells lysis buffer
RT-PCR	Reverse transcriptase-polymerase chain reaction
SBB	Sudan black B

SCT	Stem cell transplantation
SNPs	Single nucleotide polymorphism
SPSS	Statistical package for social sciences
TCR	T-cell receptor
TTP	Tris tetra prolin
UTR	Un translated region
WCLB	White cells lysis buffer
WHO	World health organization

Chapter One

Introduction & Literature review

1.1 Introduction:

Acute leukemia includes acute lymphoblastic leukemia and acute myeloid leukemia (AML), AML is the most common type of acute leukemia affecting adults. Interleukins (ILs) are a diverse set of small cell signaling protein molecules, or cytokines, that function to alter the immune system in humans, ILs are predominantly produced by antigen-presenting cells, monocytes, macrophages, and endothelial cells, which are involved in the regulation of immune cell responses against infections, as well as governing the inflammation, differentiation, proliferation, and secretion of antibodies for tumor development. Single nucleotide polymorphisms (SNPs) of ILs may alter their function, thus changing cytokine function and dysregulating their expression (Fei *et al*., 2015) .

Interleukin-10 (IL-10) is a multifunctional cytokine with both immunosuppressive and anti-angiogenic functions and may have both tumor-promoting and -inhibiting properties (Howell and Rose-Zerilli, 2006).

The gene encoding IL-10 is located on chromosome 1 (1q31-1q32) and displays a few polymorphisms in the promoter region of the transcriptional start site, including IL-10 -1082G/A (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872). Only one study has indicated that polymorphisms in IL-10 were associated with AML(Fei *et al*., 2015) .

1.2 Literature review:

1.2.1 Leukemia:

The leukemia's are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood (Hoffbrand and Moss, 2011).

1.2.2 classification of leukemia:

The main classification is into four types: acute and chronic leukemia's, which are further subdivided into lymphoid or myeloid (Hoffbrand and Moss, 2011).

1.2.3 Molecular basis of leukemia:

Genetic predisposition to cancer operates through inherited genetic variants that are either oncogenic themselves or which accelerate the rate of acquisition of somatic oncogenic mutations (Hoffbrand *et al*., 2016).

1.2.4 Types of somatic mutations:

Cancer is a product of somatic mutations, which can be large - scale (e.g. chromosomal translocations, inversions and numerical aberrations) or small - scale (e.g. point mutations, micro deletions and epigenetic changes) (Hoffbrand *et al.*, 2016).

1.2.4.1 Chromosomal translocations:

Chromosomal translocations are probably the most extensively studied genetic abnormalities in hematological malignancies. Balanced translocations involve a reciprocal exchange of genetic material between two chromosomes and may result in aberrant function of genes adjacent to the breakpoint. Two common mechanisms have been described first; a fusion gene may be generated encoding a fusion protein with oncogenic properties. This mechanism is seen in many of the translocations associated with myeloid malignancies

and some associated with acute lymphoblastic leukemia (ALL). The second category of translocations results in a structurally intact gene being placed next to regulatory elements from a gene on the partner chromosome. This scenario is frequently observed in lymphoid malignancies in which a malfunction in the normal process of antigen receptor rearrangement results in translocations involving immunoglobulin or TCR loci (Hoffbrand *et al.*, 2016).

1.2.4.2 Large deletions and aneuploidy:

Chromosome deletions and disorders of chromosome number (aneuploidy) are frequently seen in hematological malignancies. It is thought that quantitative chromosomal changes of this sort contribute to tumorigenesis by altering the expression levels of key oncogenic or tumor - suppressor genes. However, it is likely that in many cases such chromosomal changes may not be directly pathogenic in themselves, but simply a consequence of genomic instability(Hoffbrand *et al*., 2016).

Hyperdiploidy is the most frequent cytogenetic abnormality in childhood ALL and can involve any chromosome; thus it may not have a specific pathogenetic function in this disease. Trisomy 8 is the most common numerical abnormality of clonal myeloid disorders and can be seen in AML, myelodysplasia and myeloproliferative disorders(Hoffbrand *et al.*, 2016).

1.2.4.3 Submicroscopic mutations:

Much of our initial understanding of the molecular pathogenesis of hematological malignancies came from studies of cytogenetically visible chromosomal abnormalities. However, it has since become clear that submicroscopic mutations also play a similarly critical role in many

malignancies. Small - scale mutations such as small deletions can disrupt two or more genes; however, most mutations in this group affect single genes by disrupting their coding sequence, which in turn enhances, attenuates or entirely alters the function of the coded protein. Many of these mutations have been described, but it is widely agreed that many examples are yet to be identified. Activating mutations commonly involve oncogenes encoding tyrosine kinases and members of the RAS pathway and are usually missense mutations or tandem duplications. For example, around 90% of cases of systemic mastocytosis, particularly those with bone marrow involvement, have an activating point mutation in the KIT (c -KIT) gene (Hoffbrand *et al*., 2016).

1.2.5 Acute myeloid leukemia:

Acute myeloid leukemia (AML) is a heterogeneous group of leukemia's that result from clonal transformation of hematopoietic precursors through the acquisition of chromosomal rearrangements and multiple gene mutations (Rubnitz *et al*., 2010).

1.2.5.1 Incidence and epidemiology:

Incidences rate of leukemia word wide is 2.5% and mortality rate is 3.2%. for African the Incidence rate 2.8% and mortality rate is 3.6%. While, in Sudan the leukemic rate is 5.4% and mortality rate is 6.5% with increase the incidence among male which is 7.5% and mortality is 8.4% than in female is 3.5% and mortality is 4.5% according to International Agency for Research on Cancer (IARC) database (Ferlay *et al*., 2015).

AML can occur in patients of any age, but in general, both the overall incidence and the proportion of total acute leukemia's that are myeloid increase with age. Thus, acute lymphoblastic leukemia (ALL) predominates in children, with only one

case of AML diagnosed for every 5 cases of ALL(Foucar *et al.*, 2008). For childhood AML, peak incidence occurs in the first year of life, then decreases until age 4, and thereafter remains relatively constant until adulthood. The incidence of AML then increases through adulthood, during which period 70% - 80% of acute leukemia's are AML, with a marked spike in incidence in the elderly. Much of this increased incidence is attributable to AML with myelodysplasia-related changes, which becomes more common with age, while the incidence of de novo AML remains approximately constant across all adult age groups (Foucar *et al.*, 2008) .

1.2.5.2 Pathophysiology:

AML is a malignant clonal disorder of immature cells in the haemopoietic hierarchical system. Leukemic transformation is assumed to occur in many cases at, or near, the level of the haemopoietic stem cell before it has embarked on any lineage commitment. Some cases may originate at a slightly later stage in cells that are committed to lineage differentiation. These cell have abnormal functions characterized by failure to progress through the expected differentiation programme and/or to die by the process of apoptosis. Associated with this may be retention of the stem cell characteristic of self - renewal. This leads to the accumulation of a clone of cells, which dominates bone marrow activity and leads to marrow failure. The potential for arrest of hemopoiesis at different time points partially explains why there can be such variation in the leukemic or 'blast' population characterizing the individual case.

Adenopathy or organomegaly can occur but are not usual features (Hoffbrand *et al*., 2016).

1.2.5.3 Risk factors of AML:

The development of AML has been associated with several risk factors, as summarized in Table. These include age, antecedent hematologic disease, and genetic disorders; as well as exposures to viruses as well as radiation, chemical, or other occupational hazards and previous chemotherapy leukemogenesis is a multistep process that requires the susceptibility of a hematopoietic progenitor cell to inductive agents at multiple stages. The different subtypes of AML may have distinct causal mechanisms, suggesting a functional link between a particular molecular abnormality or mutation and the causal agent of most cases of AML arise de novo without objectviable leukemogenic exposure (Deschler and Lübbert, 2006).

Table(1.1): Risk factors associated with AML (Deschler and Lübbert, 2006).

Risk factors	
Genetic disorders	Down syndrome Klinefelter syndrome Patau syndrome Ataxia telangiectasia Shwachman syndrome Kostman syndrome Neurofibromatosis Fanconi anemia

	Li-Fraumeni syndrome
Physical and chemical exposures	Benzene Drugs such as pipobroman Pesticides Cigarette smoking Embalming fluids Herbicides
Chemotherapy	Alkylating agents Topoisomerase-II inhibitors Anthracyclines Taxanes
Radiation exposure	Nontherapeutic,therapeutic radiation

1.2.5.4 Classification of AML:

The World Health Organization (WHO) classification includes different biologically distinct groups based on immunophenotype, clinical features, and cytogenetic and molecular abnormalities in addition to morphology. In contrast to used French American-British (FAB) scheme , the WHO classification places limited reliance on cytochemistry (FAUCI, 2010). The most significant difference between the WHO classification and FAB classification that shown in table(1.2) is the WHO recommendation that blast percentage for diagnose of AML at least 20% blasts in the blood or bone marrow. The FAB scheme required blast percentage in the blood or bone marrow to be at least 30% (FAUCI, 2010).

Table (1.2): Classification of acute myeloid (AML) according to the French-American-British (FAB) groups(Hoffbrand and Moss, 2011).

M0	Undifferentiated
M1	without maturation
M2	with granulocytic maturation
M3	acute promyelocytic
M4	granulocytic and monocytic maturation
M5	monoblastic (M5a) or moi10cytic (M5b)
M6	Erthroleukaemia
M7	Megakaryoblastic

1.2.5.5 WHO classification:

There are two categories of WHO classification of AML which are:

1.2.5.5.1 Biologic Subtypes of AML:

There are essentially two ways to categorize specific biologic subtypes of AML:

❖ Molecular Genetic Biologic Subtypes:

- AML with t (8; 21) (q22; q22); RUNX1-RUNX1T1.
- AML inv. (16) (p13.1; q22) t (16; 16) (p13.1; q22) CBFB MYH11.
- AML t (15;17) (q22;q21); PML-RARA
- AML t (9;11) (p22;q23); MLLT3-MLL.
- AML t (6;9) (p23;q34); DEK-NUP214.
- AML inv (3) (q21; q26.2) or t(3;3) (q21;q26.2) RPN1-EVI1.
- AML t (1;22) (p13;q13); RBM15-MKL1.
- AML with mutated NPM1.
- AML with mutated CEBPA.

❖ Clinical Biologic Subtypes of AML:

- AML with Characteristic Genetic Abnormalities.
- AML with Mltilineage Dysplasia.
- AML and MDS Therapy Related: Secondary to Alklating agent or Topoisomerase π inhibitor therapy (Foucar *et al*., 2008).

1.2.5.5.2 AML, not otherwise categorize:

Despite attempts to utilize a biologic-based classification of AML to the greatest extent possible, the 2008 WHO classification has included 11 subtypes of AML, not otherwise specified (NOS) A lineage-based system is used to sub classify those cases of AML that lack any specific AML-defining biologic characteristic:

- AML with minimal differentiation.
- AML without maturation.
- AML with maturation.
- AMML.
- AMML t (8; 16) (p11; p13): MYST3 (8p11) and CREBBP.
- Acute monocytic leukemia.
- Acute erythroid leukemia.
- Acute mega karyoblastic leukemia.
- Acute basophilic leukemia.
- Acute panmyelosis with myelofibrosis (Foucar *et al*., 2008).

1.2.5.6 WHO diagnostic criteria of AML:

The presence of ≥20% myeloblasts in the bone marrow or peripheral blood, if there are associated characteristic cytogenetic abnormalities such as the t(15;17)(q22;q12), t(8;21)(q22;q22), inv16(p13;q22), or t(16;16)(p13;q22), then the diagnosis may be made even if there are <20% marrow blasts(Foucar *et al*., 2008).

1.2.5.7 Prognostic factors of AML:

The heterogeneity of disease has also become apparent with respect to differences in relapse risk. On multivariate analysis a number of factors have emerged that can predict the risk of relapse irrespective of treatment schedules (Hoffbrand *et al*., 2016).

1.2.5.8 Cytogenetic factors:

Pretreatment conventional cytogenetic studies identify an acquired clonal abnormality in approximately 50% - 60% of patients with de novo AML, of which 10% - 20% are complex (≥3 chromosomal aberrations). In approximately 40% - 50% of cases, no karyotypic abnormality is detected using typical banding techniques, yielding an AML with normal karyotype molecular submicroscopic) characterization of the karyotypically normal AMLs is an area of active investigation Based on several large studies, a cytogenetic risk stratification system has been proposed for AML that categorizes specific karyotypic abnormalities as favorable, intermediate, or unfavorable The majority of large studies agree that the AML patients with t(15;17), inv(16)/t(16;16), or t(8;21) have a favorable prognosis compared with those that have a complex karyotype and monosomy 7. Of note, while additional chromosomal changes with t (8; 21), inv(16)/t(16;16), or t(15;17) may be seen, these have not been generally shown to affect prognosis. FLT3 mutations may occur in t(8;21) and inv(16), and although not well established, may suggest an adverse prognosis. Amonosomal karyotype in AML may portend a particularly unfavorable prognosis in patients ≥60 years with de novo AML, the significance of some of cytogenetic risk groups may differ from younger patients. A very complex karyotype, defined as ≥5 chromosomal abnormalities, is associated with an unfavorable risk in the older age group,

are non-complex karyotypes showing a rare aberration such as trisomy 4, abnormalities of 3q, t(6;9)(p23;q34), and double minutes (Foucar *et al.*, 2008).

1.2.5.9 Molecular genetics factor:

In addition to the gene mutations (NPM1 and CEBPA) that currently define provisional biologic subtypes of AML, there is an ever-expanding catalogue of additional genetic alterations that occur in significant numbers in AML of various subtypes. These include mutations (e.g., TET2, MLL, KRAS, NRAS, WT1) and alterations in gene expression levels (e.g., BAALC, ERG, MN1, EVI1, MN1, PRAME, MLL, WT1, RHAMM) KIT mutations are particularly associated with a relatively adverse prognosis in the core binding factor AMLs [t(8;21) and inv(16)/t(16;16)] . In general, these alterations contribute to leukemogenesis and carry prognostic significance, but, in contrast to NPM1 and CEBPA mutations, they do not presently define distinct biologic or clinical entities (Foucar et al., 2008).

Activating FLT3 mutations occur in 2 forms, only 1 of which is at present incontrovertibly significant for AML prognosis. So-called internal tandem duplications (ITD) affecting the juxta membrane portion of the protein correlate with poor prognosis, an association that has been particularly documented in karyotypically normal cases (Foucar *et al.*, 2008).

1.2.5.10 Flow cytometry:

Aside from the fact that certain immune phenotypic profiles tend to “track” with specific AML categories, certain aberrant immune phenotypic findings have prognostic significance. These distinctive immune phenotypic features are also useful in the assessment for minimal residual disease The prognostic value of immune phenotyping in AML is controversial .Many of the studies have been small, single-institution

studies, with few results being confirmed in large clinical trials. The different results likely stem from a variety of technical, analytic, and population based factors. In particular, how the blast population is gated and the cut-off point (or percentage) for selection of antigen positivity is problematic (Foucar *et al.*, 2008).

1.2.5.11 other prognostic factors:

Additional prognostic factors delineated in various AML outcome studies in adults include age, WBC, de novo vs. secondary AML, performance status, and rapidity in the clearance of blasts from either blood or bone marrow. Although overall survival in adults with AML has improved over time, the survival time for advanced elderly patients (≥80 years) with AML has not improved " survival times for children with AML have also been noted, although factors predictive of inferior survival include age >16 years, non-white ethnicity, absence of a related donor, WBC ≥100 × 109/L, and adverse karyotype (Foucar *et al.*, 2008).

1.2.5.12 Clinical manifestation:

Acute presentation usual; often critically ill due to effects of BM failure. Symptoms of anemia: weakness, lethargy, breathlessness, lightheadedness, and palpitations. Infection: particularly chest, mouth, perianal, skin (*Staphylococcus*, *Pseudomonas*, HSV, *Candida*). Fever, malaise, sweats. Hemorrhage (especially APL due to DIC): purpura, menorrhagia and epistaxis, bleeding gums, rectal, retina. Gum hypertrophy and skin infiltration (monocytic leukemia's (M4, M5). Signs of leukostasis, e.g. hypoxia, retinal hemorrhage, confusion, or diff use pulmonary shadowing. Hepatomegaly occurs in 20%, splenomegaly in 24%; the latter should raise the question of transformed

CML; lymphadenopathy is infrequent (17%). CNS involvement at presentation is rare in adults with AML(Provan *et al.*, 2009) .

1.2.5.13 Laboratory investigation:

Many laboratory techniques had been improved to apply for investigation and diagnosis of AML. These techniques give accurate specific results which help in management of the disease.

1.2.5.13.1 Counting system:

There are two type of counting system are known that using for automated blood counters.

1.2.5.13.2 Impedance counting:

Impedance counting, first described by Wallace Coulter in 1956,depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors; this difference forms the basis of the counting systems used in Beckman Coulter, Sysmex, Abbott, Horiba Medical and a number of other instruments (Bain *et al.*, 2016).

For a cell count, blood is highly diluted in a buffered electrolyte solution. The flow rate of this diluted sample is controlled by a mercury siphon or by displacement of a tightly fitting piston. This results in a measured volume of the sample passing through an aperture tube of specific dimensions. By means of a constant source of electricity, a direct current is maintained between two electrodes, one in the sample beaker or the chamber surrounding the aperture tube and another inside the aperture tube. As a blood cell is carried through the aperture, it displaces some of the conducting fluid and increases the electrical resistance (Bain *et al.*, 2016).

1.2.5.13.3 Light scattering:

Red cells and other blood cells may be counted by means of electro-optical detectors. A diluted cell suspension flows through an aperture so that the cells pass, in single file, in front of a light source; light is scattered by the cells passing through the light beam. The scattered light is detected by a photomultiplier or photodiode, which converts it into electrical impulses that are accumulated and counted. The amount of light scattered is proportional to the surface area and therefore the volume of the cell so that the height of the electrical pulses can be used to estimate the cell volume (Bain *et al*., 2016).

1.2.5.13.4 Blood film:

Blood films can be prepared from fresh blood with no anticoagulant added or from Ethylene Di-amine Tetra-Acetic acid (EDTA)-anticoagulated blood. Heparinized blood should not generally be used because its staining characteristics differ from those of EDTA-anticoagulated blood. Good films can be made in the following manner, using clean slides, if necessary wiped free from dust immediately before use. Slides should measure 75 25 mm and be approximately 1 mm thick; ideally, they should be frosted at one end to facilitate labeling the film should be about 3 cm in length. It is important that the film of blood finishes at least 1 cm before the end of the slide. Film should be fixed as soon as possible after they have dried. Methyl alcohol (methanol) is fixation of choice. Romanowsky stain are used universally for routine staining of the blood film and satisfactory result can be obtained(Bain *et al*., 2016) .

1.2.5.13.5 Bone marrow aspiration and trephine biopsy:

Bone marrow aspiration and trephine biopsy are simple and safe procedures that can be repeated many times and can be performed on outpatients. The morphological assessment of aspirated or core biopsy

specimens of bone marrow is based on two principles. First, that bone marrow has an organized structure such that in normal health, bone marrow cells display distinct numerical and spatial relationships to each other. Second, that individual bone marrow cells have distinctive cytological appearances that reflect the lineage and stage of maturation. The specimens obtained by bone marrow aspiration or by bone marrow trephine biopsy are very different samples and contribute differently to diagnosis (Bain *et al.*, 2016).

1.2.5.13.6 Cytochemistry:

Leukocyte cytochemistry encompasses the techniques used to identify diagnostically useful enzymes or other substances in the cytoplasm of haemopoietic cells. These techniques are particularly useful for the characterization of immature cells in AML and the identification of maturation abnormalities in the myelodysplastic syndromes (MDS) and myeloproliferative disorder (MPD)(Bain et al., 2016) .The most important leukocyte cytochemistry stains are:

1.2.5.13.6.1 Myeloperoxidase:

Myeloperoxidase (MPO) is located in the primary and secondary granules of neutrophils and their precursors, in eosinophil granules and in the azurophilic granules of monocytes. The MPO in eosinophil granules is cyanide resistant, whereas that in neutrophils and monocytes is cyanide sensitive. MPO splits H2O2 and in the presence of a chromogenic electron donor forms an insoluble reaction product (Bain *et al.*, 2016) .

1.2.5.13.6.2 Sudan black B:

Sudan Black B (SBB) is a lipophilic dye that binds irreversibly to an undefined granule component in granulocytes, eosinophils and some monocytes. It cannot be extracted from the stained granules by organic dye

solvents and gives comparable information to that of MPO staining (Bain *et al*., 2016) .

1.2.5.13.6.3 Neutrophils alkaline phosphatase:

Alkaline phosphatase activity is found predominantly in mature neutrophils, with some activity in metamyelocyte Early methods of demonstrating alkaline phosphatase relied on the use of glycerophosphate or other phosphomonoesters as the substrate at alkaline pH, with a final black reaction product of lead sulphide (Bain *et al*., 2016) .

1.2.5.13.6.4 Acid phosphatase reaction:

Cytochemically demonstrable acid phosphatase is ubiquitous in haemopoietic cells. The staining intensity of different cell types is somewhat variable according to the method used. The reaction product is red with a mixture of granular and diffuse positivity Granulocytes are strongly positive. Monocytes, eosinophils and platelets show variable positivity (Bain *et al*., 2016) .

1.2.5.13.7 Immunophenotyping:

There are several techniques for identifying antigens expressed by leucocytes:

1. Flow cytometry to test suspensions of viable cells or fixed cells.
2. Immunocytochemistry to examine cells on cytospinmade slides or directly on blood or bone marrow films.
3. Immunohistochemistry to study cells in frozen or paraffin-embedded sections from bone marrow biopsy specimens or other haemopoietic tissues (Bain *et al*., 2016).

1.2.5.13.8 Molecular genetic analysis:

Molecular genetic analysis includes Southern blotting (now little used in routine diagnosis), the polymerase chain reaction (PCR) to study genomic DNA, and reverse transcriptase polymerase chain reaction (RT - PCR) to study RNA after its reverse transcription (Hoffbrand *et al*., 2016).

1.2.5.13.9 Cytogenetic analysis:

Classical cytogenetic analysis is carried out on cells that have entered mitosis and have been arrested in metaphase so that individual chromosomes can be recognized by their size and their banding pattern following staining (e.g. Giemsa staining [G-banding] or staining with a fluorescent dye). Alternating dark and light bands are numbered from the centromere toward the telomere to facilitate description of any abnormalities detected. The results of cytogenetic analysis may be displayed visually (a karyogram) or written according to standard conventions (a karyotype) (Bain *et al*., 2016).

1.2.5.13.10 Florescence insite hyprbridization:

Fluorescence in situ hybridization (FISH) bridges classical cytogenetic analysis and molecular diagnostic techniques. Chromosomes can be stained and visualized but the technique is also dependent on the recognition of specific DNA sequences by means of a fluorescent probe that can anneal to a specific DNA sequence. FISH can be carried out on metaphase preparations or on cells in interphase (Bain *et al.*, 2016).

1.2.5.13.11 Whole-genome scanning:

There are a number of molecular techniques available for whole - genome scanning. These techniques include comparative genomic hybridization (CGH) and microarray analysis (Hoffbrand *et al.*, 2016).

1.2.5.14 Laboratory finding:

Hematological investigations may reveal a normochromic, normocytic anemia with thrombocytopenia in most cases. The total white cell count may be decreased, normal or increased to 200 x 109/L or more. Blood film examination typically shows a variable numbers of blast cells. The bone marrow is hypercellular with >20% leukemic blasts. The blast cells are characterized by morphology, immunological tests and cytogenetic analysis Biochemical tests may reveal a raised serum uric acid, serum lactate dehydrogenase or, less commonly, hypercalcaemia. Liver and renal hmction tests are performed as a baseline before treatment begins (Hoffbrand and Moss, 2011) .

1.2.6 Interleukin-10:

Interleukin-10 (IL-10) is a Type II cytokine and the 'founding' member of a family of cytokines that include IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. Human B cells are also a potentially important source of IL-10; as are some granulocytes, including eosinophils and mast cells .Non-immune cell sources of IL-10 include keratinocytes, epithelial cells, and even tumor cells**.** It is difficult to determine which cells are the most important producers of IL-10. The human IL-10 gene spans about 4.7 kb on chromosome 1q21–32 and contains five exons that are separated by four introns. The murine IL-10 gene is organized in a similar fashion with 5.1 kb spanning chromosome 1E4. There are several genes adjacent to IL-10 gene within a segment of approximately 200 kb that forms the IL-10 gene cluster (Mosser and Zhang, 2008).

1.2.6.1 The IL-10 receptor:

IL-10 signals through a two-receptor complex consisting of two copies each of IL-10 receptor 1 (IL-10R1) and IL-10R2 IL-10R1 binds IL-10 with a relatively high affinity (50–200pM), and the recruitment of IL-10R2 to

the receptor complex makes only a marginal contribution to ligand binding. Most hematopoietic cells constitutively express low levels of IL-10R1, and receptor expression can often be dramatically up regulated by various stimuli. Non-hematopoietic cells, such as fibroblasts and epithelial cells, can also respond to stimuli by up regulating IL-10R1. The IL-10R2 is expressed on most cells, and therefore a large number of diverse cells have the ability to bind to and consume IL-10. The binding of IL-10 to the receptor complex activates the Janus tyrosine kinases, JAK1 and Tyk2, associated with IL 10R1 and IL-10R2, respectively, to phosphorylate the cytoplasmic tails of the receptors. This results in the recruitment of STAT3 to the IL-10R1 (Mosser and Zhang, 2008).

1.2.6.2 IL-10 Bioactivity:

The main biological function of IL-10 appears to be exerted on DCs and macrophages. IL-10 is a potent inhibitor of antigen presentation. It inhibits major histocompatibility complex class II expression as well as the up regulation of co stimulatory molecules CD80 and CD86. IL-10 inhibits the differentiation of DCs from monocyte precursors, and it also inhibits DC maturation. It is important to also note that not all IL-10 bioactivity results in a suppression of immune responses. IL-10 can co stimulates B-cell activation, prolong B-cell survival, and contribute to class switching in B cells. It can also costimulate natural killer (NK) cell proliferation and cytokine production (Mosser and Zhang, 2008).

1.2.6 .3 IL-10 Transcriptional regulations:

IL-10 is made by many different immune cells, and there are aspects of IL-10 gene regulation that are conserved among all these cells, while other

mechanisms that appear to be cell specific. The promoters for IL-10 in all the cells producing IL-10 are essentially the same and therefore the transcription factors that initiate transcription are conserved. Epigenetic modifications of IL-10 gene locus, the influence of epigenetic on IL-10 gene expression has been studied in a variety of different cells, and in all of these cells, epigenetic mechanisms appear to play significant roles in IL-10 gene regulation. However, in non-dividing cells, such as macrophages and DCs, these mechanisms confer transient regulation of gene expression, whereas in committed T cells, epigenetic mechanisms may stabilize gene expression and maintain it over several generations of daughter cells (Mosser and Zhang, 2008).

1.2.6.4 Post-transcriptional regulation of IL-10:

Similar to the majority of cytokine genes, IL-10 mRNA contains a long segment of 30-untranslated region (UTR) (702bp) for the murine IL-10 and 1033bp for the human one) that plays a role in mRNA instability. IL-10 mRNA becomes more stable after the 30-UTR is deleted, extending the half-life from 1 h to 4 12 h. IL-10 mRNA contains class II AU-rich elements (AREs) in its 30-UTR, which are classified based on the number and spacing of the canonical AUUUA pentamer. Class II AREs have a cluster of 4–7 overlapping pentamers within the U-rich context, typically an extended UUAUUUAUU nonamer. IL-10 30-UTR contains six AUUUA pentamers, four of which are surrounded by U residues to form octameric motifs that are very similar to the nonamer. Tristetraprolin (TTP), an RNA-binding protein, is known to bind nonamers and trigger the rapid degradation of mRNA (Mosser and Zhang, 2008).

1.2.6.5 Polymorphisms in the IL-10 promoter:

There have been several studies showing strong correlations between disease prevalence and polymorphisms in the IL-10 gene. Five SNPs tagging the promoter haplotypes of the IL-10 gene have been widely studied. They are 3575, 2763, 1082, 819, and 592, which are defined relative to their positions in the IL-10 gene sequence upstream of the transcription initiation site.IL-10 and the potential for therapeutics. Numerous studies using mice that are genetically deficient in IL-10 have illustrated the importance of this cytokine in limiting autoimmune pathologies (Mosser and Zhang, 2008).

1.2.7 IL -10 polymorphism and AML:

Interleukin-10 (IL-10) is a multifunctional cytokine with both immunosuppressive and anti-angiogenic functions. In consequence, IL-10 can have both tumor-promoting and tumor-inhibiting properties. Raised levels of serum and peritumoral IL-10 production have been reported in many malignancies, which have been interpreted in support of a role for IL-10 in tumor escape from the immune response (Howell, 2013).

Multivariate regression analyses conducted by (Fei et al., 2015) showed that subjects carrying the rs1800871 CC genotype and C allele had a significantly increased risk of AML, and Those carrying the rs1800872 G allele had a slightly increased risk of AML. Moreover, genotyping results demonstrated that subjects carrying both the rs1800871 C allele and rs1800872 G allele had a moderately increased risk of AML, indicating that The two genotypes had a synergistic effect on AML risk.

1.3 Rationale:

Single nucleotide polymorphisms (SNPs) of ILs may alter their function, thus changing cytokine function and dysregulating their expression. The gene encoding IL-10 is located on chromosome 1 (1q31-1q32. There is no study in Sudan has indicated that polymorphisms in IL-10(1082G/A)(rs1800871) was associated with AML. Therefore, I investigated the association between polymorphisms in IL-10 -1082G/A (rs1800896) and the risk of AML in a Sudanese population.

1.4 Objectives:

1.4.1General objective:

To study polymorphisms in interleukin-10 (IL-10) -1082G/A (rs1800896) in acute myeloid leukemia (AML) in a Sudanese population.

1.4.2 Specific objective:

- To detect SNP in interleukin-10 (IL-10) -1082G/A (rs1800896) in acute myeloid leukemic patients.
- To compare the SNP in (IL-10)-1082G/A (rs1800896) between acute myeloid leukemic case and control.
- To compare the SNP in (IL-10)-1082G/A (rs1800896) between acute myeloid leukemic age group.
- To compare the SNP in (IL-10)-1082G/A (rs1800896) between acute myeloid leukemic gender.
- To correlate the SNP in (IL-10)-1082G/A (rs1800896) with acute myeloid leukemic hematological data and subtype.

Chapter Two

Materials and Methods

2.1 Study design:

A case control study conducted in Khartoum state in 2017 to detect a SNP in interleukin-10 (IL-10) -1082G/A (rs1800896) in Sudanese acute myeloid leukemia patients.

2.2 Study population:

Thirty AML patients referring to Radio isotope center Khartoum Sudan were recruited to participate in this study as well as 30 apparently healthy volunteers were enrolled as control group.

2.3 Inclusion criteria:

All newly diagnosed AML (Acute Myeloid Leukemia) patients.

2.4 Exclusion criteria:

Patients of Acute Myeloid Leukemia under treatment.

2.5 Study duration:

This study was conducted in the period from January to May 2017.

2.6 Ethical consideration:

The consent of the selected individuals to the study was taken (verbally) after being informed with all detailed objectives of the study and its health benefit in future.

2.7 Data collection:

Data was collected from hospital record.

2.8 Sampling:

2.8.1 Sample technique:

Non-probability sampling method was used (only who coming to hospital) were involved in sample.

2.8.2 Sample collection:

The biological sample is blood specimen was collected in EDTA containers.

2.9 Study Variable:

- Age.
- Gender.
- Hematological parameter (Hb, WBCs, PLTs, Blast).
- AML-subtype.
- Genotypes and allele frequencies of SNP of IL-10(-1082 G/A).

A venous blood (1.5ml) sample was collected in plastic vacationer containing anticoagulant Ethylenediamine Tetra Acetic Acid (EDTA) for molecular analysis.

2.10 Data presentation:

The data were presented in tables and figures.

2.11 Data analysis:

The data were analyzed using the SPSS computer program version 16. Chi square done to determine the association and risk factor.

2.12Methodology:

2.12.1 DNA extraction by salting out method:

2.12.1.1 Principle steps:

RBCS were Hemolyzed by alkaline solution (Red Cells lysis buffer) ,then the membranes were digested by solution containing detergent and

proteases (White Cells Lysis buffer), then protein was precipitated out by saturated NaCL and centrifugation , finally DNA was precipitated by absolute ethanol ,washed by 70% ethanol and eluted in 50 µL of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 .

2.12.1.2 Procedure:

300 µl of blood sample was Placed in 1.5 ependorf tube, 1000 µl RCLB was added to the tube, Mixed well, centrifuged at 2500 rpm for 10 minutes, Supernatant was discarded and the pellet (WBCs) washed again with 1000µl of RCLB (repeated until clear pellet was obtained). WCLB, 10µlprotinase K and 10 µl SDS were added to the clear white pellets. The mixture was incubated for 2 Hours at 56^0C.100 µl of 6 M NaCL was added to precipitate the protein and mixed well by vortex. 200µl of ice cold chloroform were added to tube and centrifuged at 12000 rpm for 6 minutes.The aqueous phase was transferred carefully to clean ependorf tube, and to which double volume of cold absolute ethanol was added to precipitate the DNA. The tube was centrifuged at 12000 rpm for 5minutes.The supernatant was poured off without disturbing the precipitate and then washed with 600µl 70% ethanol. The tube content was centrifuged at 7000 rpm for 5 minutes, the ethanol was discarded and the tube was left to air dry.The pellets were re suspended in 50µl of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 and leaved to dissolve overnight.

2.12.3 Determination of DNA quality and purity:

Part of the DNA solution was mixed with loading dye 5 in 1 and DNA quality and purity was determined using gel electrophoresis.

DNA was transferred into 1 ml eppindrof tube.

2.12.4 DNA storage:

DNA was transferred into 1ml eppindroff tube and preserved at -20°C until PCR is performed**.**

2.12.5 Polymerase chain reaction (PCR):

2.12.5.1 ARMS- PCR Principle and protocol:

The amplification-refractory mutation system (ARMS), also known as allele-specific polymerase chain reaction (ASPCR) or PCR amplification of specific alleles,. is a simple, rapid, and reliable method for detecting any mutation involving single base changes or small deletions. ARMS is based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample and will not amplify the non target allele. Following an ARMS reaction the presence or absence of a PCR product is diagnostic for the presence or absence of the target allele (Little, 2001).

Since the ARMS PCR is mostly done to identify a mutation or a polymorphism it is also important that it should be able to identify whether the change in DNA is heterozygous or homozygous. A heterozygote or homozygote is differentiated by using ARMS primers for the smutant/polymorphic and the normal (wild type) alleles. The reactions for the mutant and the normal alleles are usually carried out in separate tubes (Newton, 1989). .

2.12.5.2 Detection of IL-10-1082G/A (rs1800896) genotype:

All patients with AML and control were screened for the presence of AA, GA, AA genotype using allele specific PCR.

Table 2.1: The primers sequence used were as follow:

Polymorphism and Allele location	**Sequence**	**Product size (bp)**
IL-10 common (reverse)	5'-CAGCCCTTCCATTTTACTTTC-3'	550 bp
IL-10 G (forward)	5'-TACTAAGGCTTCTTTGGGAG-3'	
IL-10 A (forward)	5'-CTACTAAGGCTTCTTTGGGAA-3'	
Internal control primer (forward)	5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3'	823 bp
Internal control primer (reverse)	5'-AACCAGCGGGAAGAGGTCAAGGG-3'	

PCR mixture of 20 µl was prepared using premix master mix tubes (Maxime^{TM}PCR premix Kit{i-Taq}) for each sample, genomic DNA was amplified in two different PCRs for each polymorphism; each reaction employed a generic antisense primer and one of the two allele-specific sense primers. To assess the success of PCR amplification in both reactions, one internal control of 823 bp was amplified using a pair of primers designed from the nucleotide sequence of the vitamin-D receptor (Bsm1).

Table 2.2: master mix tube preparation:

Reagents	**Volume**
Double D.W	15µl
IL common (reverse primer)	0.5µl
IL-A or IL-G (forward primer)	0.5µl
Internal control forward primer	0.5µl
Internal control reverse primer	0.5µl
Template DNA	3µl
Total reaction volume	20µl

Table 2.3: Optimized cycling protocol for PCR analysis as follow:

Profile	**Temperature**	**Time duration**	**Number of Cycles**
Initial Denaturation	94°C	5 minutes	1
Denaturation	94°C	30 seconds	35
Annealing	58°C	30seconds	
Extension	72°C	40 seconds	
Final Extension	72°C	5 minutes	

2.11.5.3 Demonstration of PCR product:

Five µl of the PCR product (ready to load) was electrophoresed on 1.5% agarose gel, and was stained with ethedium bromide, 1X TBE buffer was used as a running buffer. The Voltage applied to the gel was 100 volt with time duration of 30S minutes. 50 pb DNA ladder was used as molecular

weight marker with each patch of samples .Finally, PCR product was demonstrated by gel system**.**

Chapter Three

Results

3.1 Demographic data:

Study included 60 sample 30 of them were cases and 30 of them were control. The results showed that 14/30 (46.7%) of cases were males and 16/30 (53.3%) were females and 16/30(53.3%) of controls were males and 14/30 (46.7%) were females.

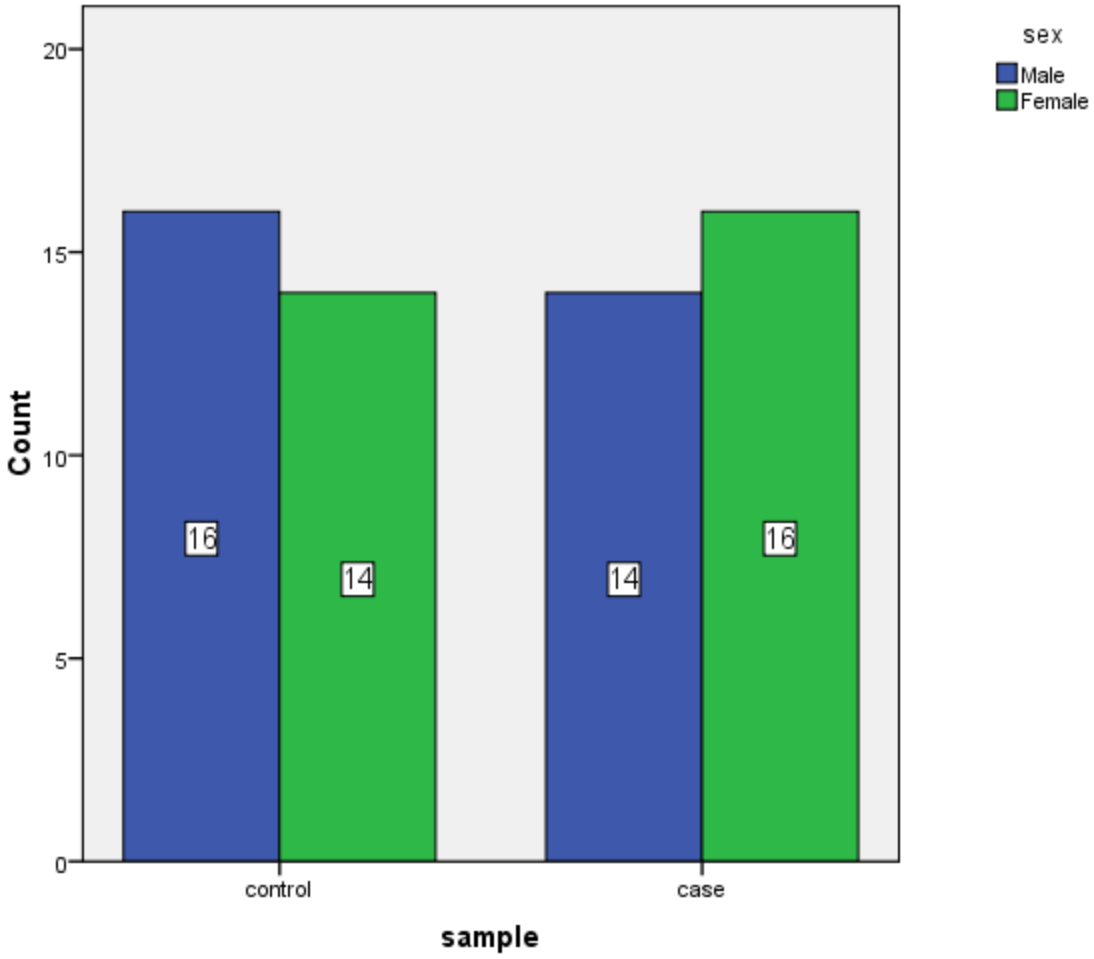

Figure (3.1): Gender distribution among the samples

In figure (3.2) the results showed that 9/30 (30%) of cases were children and 21/30 (70%) were adults and 8/30(26.7%) of controls were children and 22/30 (73.3%) were adults.

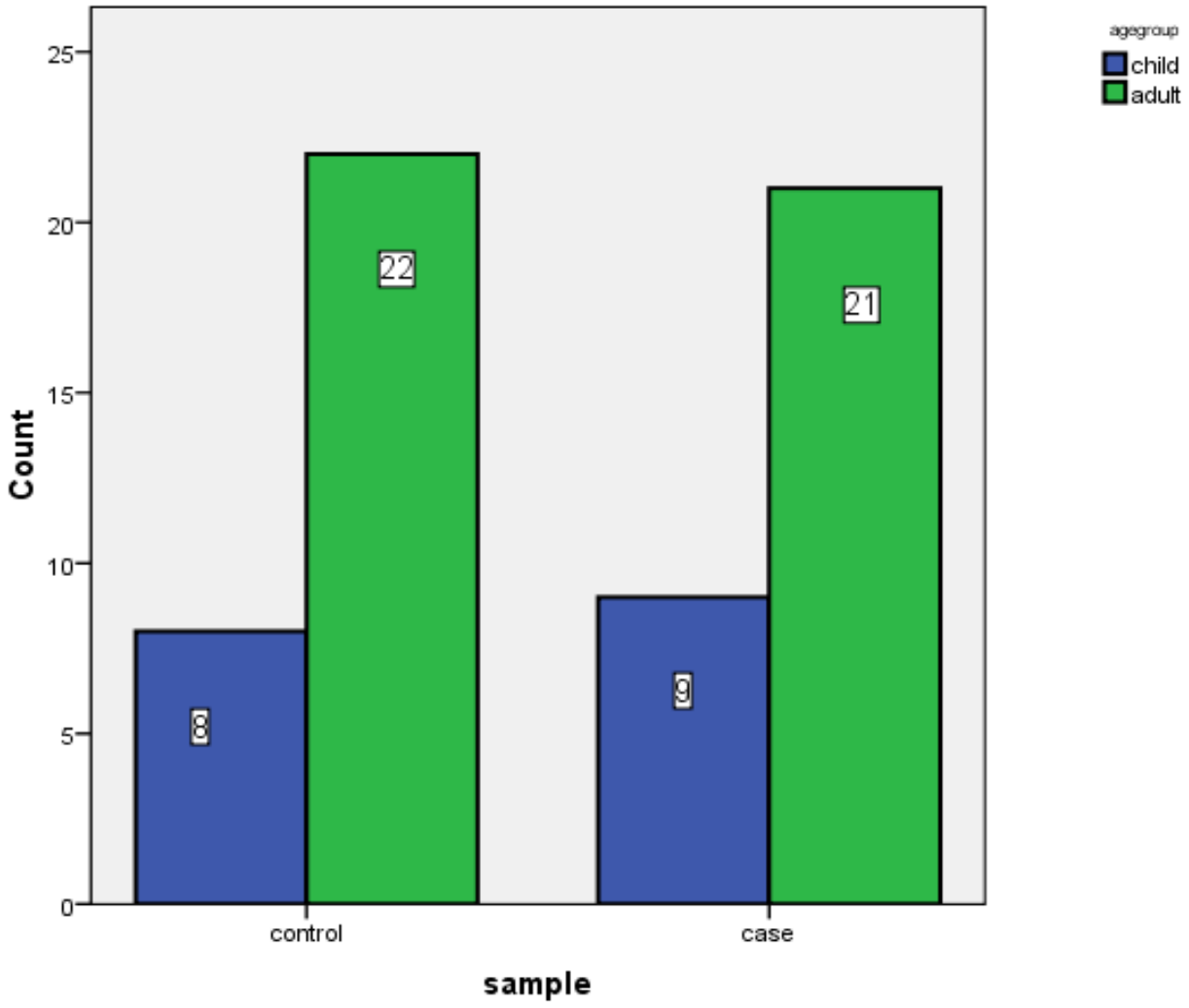

Figure (3.2): Age groups distribution among the samples

3.2 Distribution of Genotypes and Allele Frequencies of IL-10 (A-1082 G) (rs1800896) Polymorphism in Acute myeloid leukemic Patients and Controls:

Table 3.1 shows that there was statistically significant correlation between Co-dominant genotypes of IL-10 (-1082 G/A) Polymorphism and AML patients(p.value=0.005).

Table 3.1 Correlation between Co-dominant genotypes of IL-10 (A-1082 G) Polymorphism in Acute myeloid leukemic Patients and controls

Sample	Co-dominant genotype			Total	p.value
	AA	GA	GG		
Case	6 20%	13 43.4%	11 36.6%	30 100%	0.005
Control	16 53.3%	3 10%	11 36.7%	30 100%	

Table3.2 showed that IL-10-(1082G/A) dominant genotype (GA+GG) was increased risk of AML and there was statistically significant correlation between dominant genotypes (GA+GG) and AML patients (odd ratio=4.6, p.value=0.007).

Table3.2 Correlation between dominant genotypes in IL-10 (-1082 G/A) Polymorphism in Acute myeloid leukemic Patients and control

Sample	dominant genotype		Total	p.value
	AA	GA+GG		
Case	6(20 %)	24(80%)	30(100%)	0.007
Control	16(53.4%)	14(46.6%)	30(100%)	

Table 3.3 showed that there was no statistically significant correlation between Allele frequency of IL-10 (-1082 G/A) (rs1800896) Polymorphism in Acute myeloid leukemic Patients and controls (odd ratio=1.9, p.value=0.196)

Table 3.3 Correlation between Allele frequency of IL-10 (-1082 G/A) Polymorphism in Acute myeloid leukemic Patients and controls

Sample	Allele Frequency		Total	p.value
	A	G		
Case	13 43.4%	17 56.6%	30 100%	0.196
Control	18 60%	12 40%	30 100%	

3.3 Correlation between Genotypes and Allele Frequencies of IL-10(-1082 G/A) (rs1800896) Polymorphism and AML Age groups:

Table 3.4 showed that there was no statistically significant correlation between IL-10 (-1082 G/A) (rs1800896) Polymorphism and AML patient age group (p.value=0.763, 0.842, 0.936)

Table 3.4 Correlation between Genotypes and Allele Frequencies of IL-10 (-1082 G/A) Polymorphism and Age groups

IL-10(-1082 G/A) Polymorphism		Age groups		p.value
		Child	Adult	
Co-dominant genotype	AA	2 (6.7%)	4 (13.3%)	0.763
	GA	3 (10%)	10 (33.3%)	
	GG	4 (13.3%)	7 (23.3%)	
Dominant genotype	GA+GG	7 (23.3%)	17 (56.7%)	0.842
Allel frequency	A	4 (13.3%)	9 (30%)	0.936
	G	9 (30%)	12 (70%)	

3.4 Correlation between Genotypes and Allele Frequencies of IL-10 (1082 G/A) (rs1800896) Polymorphism and AML Gender:

Table 3.5 showed that there was no statistically significant correlation between IL-10 (-1082 G/A) Polymorphism and AML patient gender (p.value= 0.722, 0.855, 0.431).

Table 3.5 Correlation between Genotypes and Allele Frequencies of IL-10 (-1082 G/A) Polymorphism and Gender

il10(-1082 G/A) Polymorphism		Gender		p.value
		Male	Female	
Co-dominant genotypes	AA	3 (10%)	3 (10%)	0.722
	GA	5 (16.7%)	8 (26.7%)	
	GG	6 (20%)	5 (16.7%)	
Dominant genotype	GA+GG	11 (36.7%)	13 (43.3%)	0.855
Allele frequencies	A	5 (16.7%)	8 (26.7%)	0.431
	G	9 (30%)	8 (26.7%)	

3.5 Correlation between Genotypes and Allele Frequencies of IL-10(-1082 G/A) (rs1800896) Polymorphism and AML sub-types:

Table 3.6 showed that there was no statistically significant correlation between IL-10 (-1082 G/A) Polymorphism and AML sub-types (p.value=0.711, 0.752, 0.396)

Table 3.6 Correlation between Genotypes and Allele Frequencies of IL-10 (-1082 G/A) Polymorphism and AML sub-types

IL10(-1082 G/A) Polymorphism		AML sub-type							p.value
		M0	M1	M2	M3	M4	M5	M7	
Co-dominant genotype	AA	1(3.3%)	1(3.3%)	1(3.3%)	1(3.3%)	1(3.3%)	1(3.3%)	0(0%)	0.711
	GA	1(3.3%)	2(6.7%)	2(6.7%)	6(20%)	2(6.7%)	0(0%)	0(0%)	
	GG	1(3.3%)	0(0%)	0(0%)	5(16.7%)	3(10%)	1(3.3%)	1(3.3%)	
Dominant genotype	GA + GG	2(6.7%)	2(6.7%)	2(6.7%)	11(36.7%)	5(16.7%)	1(3.3%)	1(3.3%)	0.752
Allele frequency	A	1(3.3%)	2(6.7%)	3(10%)	4(13.3%)	2(6.7%)	1(3.3%)	0(0%)	0.396
	G	2(6.7%)	1(3.3%)	0(0%)	8(26.7%)	4(13.3%)	1(3.3%)	1(3.3%)	

3.6 Comparisons of hematological finding in Genotypes and Allele Frequencies of IL-10 (-1082 G/A) (rs1800896) Polymorphism:

Table 3.7 showed that mean of Hb was no statistically significant (p. value=0.552, 0.276) highest in patients with AA genotype(mean±SD:9.7±2.9) compared to other genotypes and allele frequencies, mean of TWBCs count was no statistically significant(p. value=0.276, 0.697) highest in patients with A allele(mean±SD:55.3 ±64.1) compared to genotypes and other allele frequency, mean of PLTs was no statistically significant(p. value=0.875, 0.610) highest in patients with GA genotype (mean ±SD: 102.4±179.4) compared to other genotypes and allele frequencies, mean of Blast percentage was no statistically significant(p. value=0.380, 0.774) highest in patients with GG genotype(mean±SD:63.9 ±22.8) compared to other genotypes and allele frequencies.

Table 3.7Comparisons of hematological finding in Genotypes and Allele Frequencies of IL-10 (-1082 G/A) Polymorphism

Hematological parameters	il10(-1082G/A) Polymorphism genotype and allele frequency		Mean	Std.Deviation	p.value
Hb	Co-dominant	AA	9.7	±2.9	0.552
		GA	8.3	±2.8	
		GG	8.4	±2.1	
	Dominant	GA+GG	8.4	±2.5	0.276
	Allele	A	8.8	±2.9	0.276
		G	8.5	±2.4	
TWBCs	Co-dominant	AA	45.9	±37.1	0.896
		GA	38.1	±65.3	
		GG	31.5	±65.3	
	Dominant	GA+GG	35.5	±63.9	0.697
	Allele	A	55.1	±64.1	0.697
		G	23.4	±52.9	
PLTs	Co-dominant	AA	66.8	±44.9	0.875
		GA	102.4	±179.4	
		GG	95.9	±118.1	
	Dominant	GA+GG	99.4	±151.2	0.610
	Allele	A	99.3	±169.9	0.610
		G	88.0	±110.1	
Blast	Co-dominant	AA	60.2	±20.4	0.380
		GA	51.6	±21.2	
		GG	63.9	±22.8	
	Dominant	GA+GG	57.3	±22.4	0.774
	Allele	A	60.7	±16.9	0.774
		G	55.6	±25.0	

Genotype	Expected	Observed
AA	5.21	6
AG	14.58	13
GG	10.21	11
p allele freq = 0.42; q allele freq = 0.58		
$\chi^2 = 0.35$; $P = 0.55$		

The allelic frequencies of the A and G alleles as obtained from actual counts were computed to be 0.42 and 0.58, respectively. Chi-square analysis revealed that there was no significant difference ($P = 0.55$) between the observed and Hardy–Weinberg expectations.

Chapter Four

Discussion, Conclusions & Recommendations

4.1Discussion

This case control study was conducted to detect SNP in interleukin-10 (IL-10) -1082G/A (rs1800896) among firstly diagnosed patients with AML and to examine the association between SNP in interleukin-10 (IL-1082G/A (rs1800896) and risk of AML.

In this study the result showed that dominant genotype (GA+GG) of IL-10 -1082G/A (rs1800896) polymorphism was risk factor for AML(odd ratio=4.6, p.value=0.007) and this finding disagree with study of Fei et al., 2015 which investigated the association between polymorphisms in interleukin-10 (IL-10) -1082G/A (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) and the risk of acute myeloid leukemia (AML) in a Chinese population and they found no statistically association between 1082G/A (rs1800896) polymorphism and risk of AML.

Also our findings disagree with study which done by Martin W. Howell which study relationship between Interleukin-10 Gene Polymorphisms and Cancer which found no evidence role of IL-10 -1082 SNPs in determining susceptibility to or disease progression in AML.

In the co-dominant genotype percent of GA was most increase than GG and AA genotypes in AML patient (43.3%, 36.3%, 20.0%) respectively with statistically significant association with AML (p.value=0.005).

In the allele frequency (G, A) percent of G allele was slightly increase than A allele in AML patient (56.7%, 43.3%) respectively with no statistically significant association with AML (odd ratio=1.9, p.value=0.196) and this finding disagree with Nursal et al., 2016 which study The Associations of

IL-6, IFN-c, TNF-a, IL-10, and TGF-b1 Functional Variants with Acute Myeloid Leukemia in Turkish Patients and they found in IL-10) -1082G/A (rs1800896) polymorphism there was statistically significant increased in A allele rather than G allele in AML patient (72.6%, 27.4%) respectively (p.value=0.024).

There were no statistically significant association between (co-dominant (AA,GG,GA), dominant (AA,GA+GG) genotypes and allele frequencies (A,G) of IL-10 (-1082 G/A) Polymorphism and gender (p.value= 0.722, 0.855, 0.431)) respectively.

There were no statistically significant association between (co-dominant (AA, GG, GA), dominant (AA,GA+GG) genotypes and allele frequencies (A,G) of IL-10 (-1082 G/A) Polymorphism and age group (p.value= 0.763, 0.842, 0.936) respectively.

There were no statistically significant association between (co-dominant (AA, GG, GA), dominant (AA,GA+GG) genotypes and allele frequencies (A,G) of IL-10 (-1082 G/A) Polymorphism and AML sub-group (M0-M7) (p.value= 0.711, 0.752, 0.396) respectively.

The mean of Blast percentage in (co-dominant (AA, GG, GA), dominant (AA, GA+GG) genotypes and allele frequencies (A, G) among cases was no statistically significant highest in patients with GG genotype (p. value=0.380, 0.774, 0.774).

The mean of Hb in (co-dominant (AA, GG, GA), dominant (AA, GA+GG) genotypes and allele frequencies (A, G) among cases was no statistically significant highest in patients with AA genotype (p. value=0.552, 0.276, 0.276).

The mean of TWBs count in (co-dominant (AA, GG, GA), dominant (AA, GA+GG) genotypes and allele frequencies (A, G) among cases was no

statistically significant highest in patients with A allele (p. value=0896, 0.697, 0.697).

The mean of platelets count in (co-dominant (AA, GG, GA), dominant (AA, GA+GG) genotypes and allele frequencies (A, G) among cases was no statistically significant highest in patients with GA genotype (p. value=0.875, 0.610, 0.610).

4.2 Conclusions:

- Dominant (GA+GG) genotype of IL-10 -1082G/A (rs1800896) polymorphism is a risk factor for AML.
- G allele is insignificantly higher than A allele in AML patient.
- IL-10 -1082G/A (rs1800896) polymorphism is not correlated with patient´s age group, gender and AML sub-type.
- In IL-10 -1082G/A (rs1800896) polymorphism (GG, GA) genotypes associated with statistically insignificant higher (blast percent, platelet) and statistically insignificant lower (Hb, TWBCs).

4.3 Recommendations:

- Another study should be conducted to investigate the role of variants of IL-10 -819T/C (rs1800871), and -592A/C (rs1800872) in the pathogenesis of AML.
- Further large-scale studies with large sample sizes from different ethnic populations are required to confirm my results

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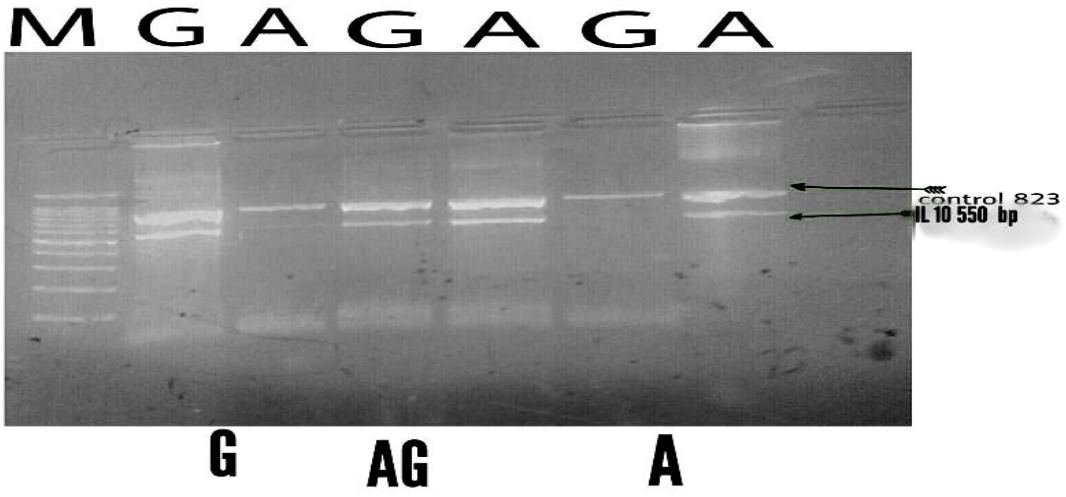
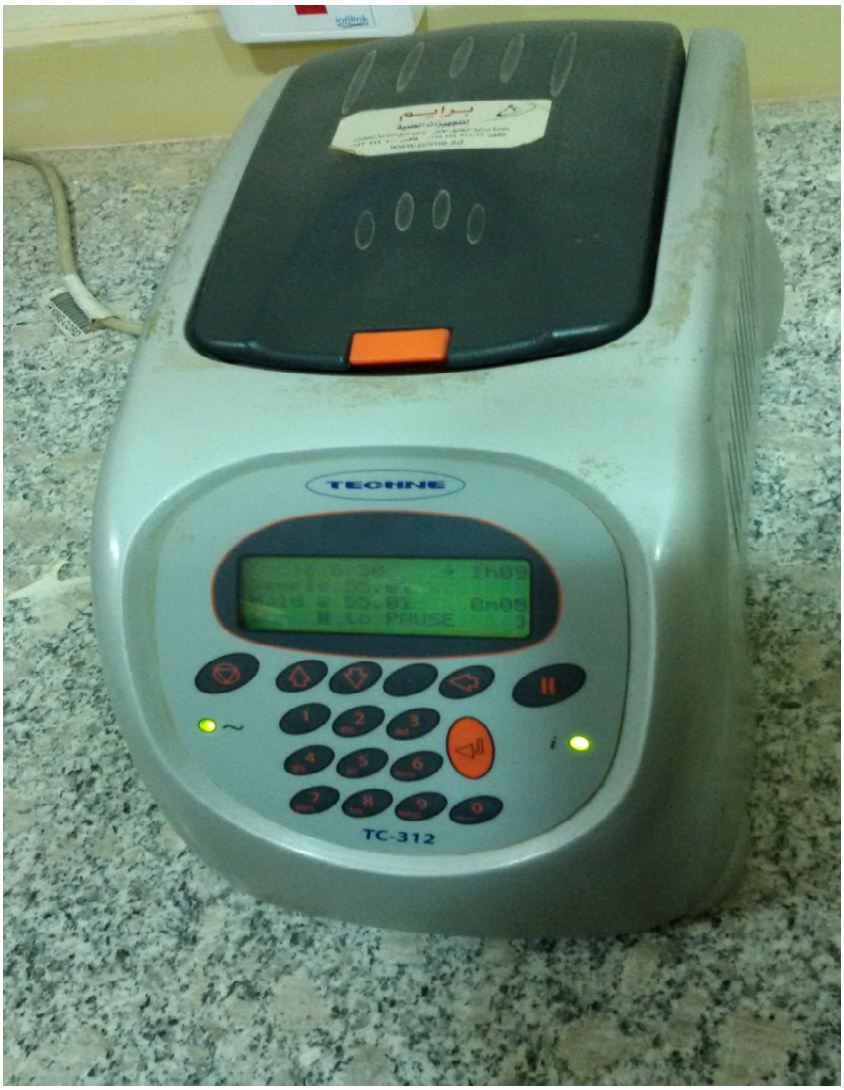
Appendix1:

A representative agarose (1.5%) gel electrophoresis of ARM-PCR products DNA marker:



Appendix2: Images

PCR machine:



Gel electrophoresis: