Republic of Iraq
Ministry of Higher Education and
Scientific Research
AL-Nahrain University
College of Science

Molecular Genetic Study of Leukemia Patients

A thesis
Submitted to the College of Science, AL-Nahrain University in partial Fulfillment of the Requirements for the Degree Master of Science in Biotechnology

By
Noha Mohammed Saleh Shebeeb

August /2008 **Sha`ban /1429**

Supervisors Certificate

We certify that this thesis was prepared under our supervision at the College of Science, Al-Nahrain University in partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology.

Signature:
Supervisor: Dr. Mohammed A. Ibrahim
Scientific degree: Professor
Date:

Signature:
Supervisor: Dr. Essmaiel Archoukieh
Scientific degree: Professor
Date:

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

Signature:
Name: Dr. Kadhim M. Ibrahim
Scientific degree: Professor
Title: Head of Biotechnology Department
Date:

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the Degree of Master of Science in Biotechnology.

Signature:
Name: Dr. Hayfa H. Hassansi
Scientific Degree: Assistant professor
Date:

(Chairman)

Signature:
Name: Dr. Abdul Kareem Al-Kazaz
Scientific Degree: Assistant professor
Date:

(Member)

Signature:
Name: Hameed M. Jasim
Scientific Degree: Assistant professor
Date:

(Member)

Signature:
Name: Dr. Mohammed A. Ibrahim
Scientific Degree: Professor
Date:

(Member)

I here by certify upon the decision of the examining committee

Signature:
Name: Dr. Laith Abdul Al-Aziz. Al-Ani
Scientific Degree: Assistant Professor

Title: the dean of college of science
Date:

The present work comprised molecular genetic analysis of four types of leukemia, Acute Lymphoid leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphoid Leukemia (CLL) and Chronic Myeloid Leukemia (CML).

Samples of blood were obtained from 65 Syrian patients affected with one of the four types of leukemia, admitted to Al-Bairuni University Hospital and Al-Assad University Hospital.

The Wizard Promega kit was found suitable for DNA isolation from leukemia patients and normal individuals. By this method suitable quantities of DNA approximately (100 – 600 µg) were obtained from 300 µl of whole blood. The purity of isolated DNA ranged from (1.7 - 1.9). However only 45 patients of the total number of leukemia cases gave good yield of DNA. The rest of cases were distributed between ten cases showed clotted blood, and ten cases showed low W.B.C count with low DNA yield.

RAPD-PCR analysis was carried out with 22 different arbitrary primers of decamer oligonucleotides. These primers were evaluated for their usefulness in detecting DNA polymorphism among leukemia patients. The study

involved optimization for RAPD reaction for DNA template, primer concentration and run program. The obtained results were as following :

A- Thirteen primers showed no amplified products in all normal individuals and leukemia patients.

B- Nine primers amplified genomic DNA in the normal individuals and leukemia patients with different efficiencies. The generated DNA fragments (when using these primers) were scored by their presence or absence and the differences in their molecular weight across the normal individuals and leukemia patients. The total number of amplified bands generally ranged between (1 - 15) and the molecular weights varied between (95 – 3162) bp according to the primer used. The minimum number of amplified bands was one band which was observed when using primers OPB-18 and OPZ-02; on the other hand the highest number of bands were (15) bands which were observed when using primer OPJ-05. The lowest molecular weight of amplified band was 95 bp which was obtained with primers OPB-15 and OPJ-05, and the highest molecular weight of amplified band was 3162 bp which was obtained with primer OPA-09.

C- In addition it was possible to observe decrease or increase in the relative intensities of the bands amplified from the genomic DNA of leukemia patients as compared with normal DNA.

D- Among the leukemia patients tested, 37.8% of patients showed the highest DNA polymorphism because they were detected by nine

primers used in this study. 48.8% of patients were detected by eight primers, 8.9% of patients were detected by seven primers and 4.5% of patients were detected by six primers.

E- The ability to detect genetic alteration by each primer was variable, the highest value of efficiency was shown by primer OPB-17 (0.059), whereas primer OPE-05 showed the lowest value (0.016).

F- The study had shown that genetic instabilities were frequent in genomic DNA of leukemia patients and could be detected by using suitable RAPD primers, e.g. primer OPA-09 could be used as a marker for detection of ALL and CLL patients. Primers OPB-17 and OPW-17 gave a specific band in all AML patients which was absent in normal individuals and other leukemia patients. On other hand, primer OPW-17 gave another specific band in AML and CLL patients, while, it was absent in normal individuals and other leukemia patients. Whereas, primers OPB-15 and OPW-17 showed another specific band which was present in normal individuals and in other leukemia patients, but was lost in AML patients. The result of primer OPZ-02 showed a specific band was present in normal individuals, and in all leukemia patients, however in CML patients was lost .

G- Primer OPJ-05 could amplify specific band in normal male individuals but it could not amplify this band in normal female individuals.

RAPD-PCR analysis was applied to investigate polymorphism in DNA methylation of CML leukemia patients and normal individuals. The results of digested genomic DNA with *Hpa*II and *Msp*I showed clear differences in the methylation of the genomic DNA of the CML patients and normal individuals, suggesting variation in the proportion and pattern of methylated and unmethylated CGs. The results revealed differences in banding patterns which include: banding shifts , missing bands and / or banding intensity changes

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Meaning
A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
ABL	v-abl Abelson murine leukemia viral oncogene homolog
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APC	Adenomatous polyposis coli
bp	Base pair
BRCA	Breast cancer
BSA	Bovine serum albumin
c-abl	Cellular Abelson proto-oncogen
CLL	Chronic lymphatic leukemia
CML	Chronic myeloid leukemia
c-Oncogen	Cellular oncogen
CpG	Cytosine proceed Guanine
Del	Deletion
DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleoside triphosphate
EDTA	Ethylene diaminetetraacetic acid

EGFR	Epidermal growth factor receptor

Abbreviations	Meaning
FAB	French-American-British
*Hpa*II	Haemophius parainfluenzae
Inv	Invertion
*Msp*I	Moraxella species
MYC	v-myc avian myelocytomatosis viral oncogene homologue
OD	Optical density
PCR	Polymerase Chain Reaction
Ph	Philadelphia chromosome
RAPD	Random Amplified Polymorphism DNA
RAS	Retrovirus Associated Sequences
RNase	Ribonuclease
RE Buffer	Restriction enzyme buffer
Src	Rous sarcoma virus oncogene
SDS	Sodium dodecyl sulfate
UV	Ultra violet light
WBC	White blood cell count

Introduction

Cancer is a disorder of cell growth that lead to invasion and distraction of healthy tissue by abnormal cells. Although cancer has affected human since early time, it was a rare disease until the twentieth century. Cancer now ranks second only to heart disease as a major cause of death in the world (Sinclair *et al.*, 2000; Jemal *et al*., 2007).

One of the common cancers in human is leukemia. In this regard leukemia is considered as neoplastic proliferation of leukocyte precursor in the bone marrow. Leukemia are grouped by the type of white blood cell affected (either lymphoid cells or myeloid cells) and by the rate of cell growth (either acute or chronic). Acute leukemia involves an overgrowth of very immature blood cells and get worse quickly, whereas chronic leukemia involves an overgrowth of mature blood cells and get worse slowly (Hoffbrand *et al.*, 2002). There are four types of leukemia:

1- Acute lymphoblastic leukemia (ALL): accounts for about 3 - 4 new cases / 100,000 per year. It is the most common type of leukemia in young children (Pui *et al*., 2004; Cardinez *et al*., 2006).

2- Acute myeloid leukemia (AML): accounts for about 7.4 new cases/100,000 per year. It occurs in both adults and children (Lowenberg *et al.*, 1999; Cardinez *et al*., 2006).

3- Chronic lymphoid leukemia (CLL): accounts for about 7.5 new cases per 100,000 each year. Most often, people diagnosed with the disease are over age 55. It almost never affects children (Kipps, 2000; Cardinez *et al*., 2006).

4- Chronic myeloid leukemia (CML): accounts for about 3 new cases per 100,000 each year. It affects mainly adults (Goldman and Melo, 2003; Cardinez *et al*., 2006).

Recent studies indicated that the initiation and progression of cancer is due to accumulation of genetic alteration and to epigenetic changes (Hahn and Weinberg, 2003).

The genetic alterations include mutations in three groups of genes. The first group, called proto-oncogenes which produce protein products that normally enhance cell division or inhibit normal cell death. The mutated forms of these genes are called oncogenes (Croce, 2008). The second group, called tumor suppressors which make proteins that normally prevent cell division or cause cell death (Payne and Kemp, 2005). The third group contains DNA repair genes, which help to prevent formation of mutations that lead to cancer (Sieber *et al*., 2003).

Epigenetic changes which include DNA methylation that modify the DNA without altering the native nucleotide sequence (Jones and Baylin, 2002). The role of DNA methylation in oncogenesis has been explained by one or more mechanisms. Firstly, tumor suppressor genes and DNA repair genes can be inactivated by hypermethylation of CpG islands in promoter regions (Herman and Baylin, 2003). Secondly, activation of oncogenes due to hypomethylation (Nephew and Huang, 2003; Ducasse and Brown, 2006).

The identification of genes and pathways involved in tumor initiation and progression will not only enhance our understanding of the biology of this process, it will also provide new targets for early diagnosis and facilitate treatments' design. Genomic approaches have proven to be effective in detecting chromosomal alterations and identifying disrupted genes in cancer (Garms *et al.*, 2004).

The technique of polymerase chain reaction (PCR) (Saiki *et al*., 1985) has been extensively utilized for the detection and characterization of mutations associated with tumor development (Boultwood and Fidler, 2002). RAPD-PCR is one of the techniques that has benefited from the advent of the PCR (Williams *et al*., 1990). The RAPD method can simply and rapidly detect genetic alterations in the entire genome without knowledge of specific DNA sequence information (Papadopoulos *et al.,* 2002). Recently, the RAPD method was used as a mean for identifying the genetic alterations in human tumors and revealed that genetic alterations occurred frequently in different kind of tumors (Wang *et al*., 2001; Wang *et al*., 2002), for example, lung cancer (Ong *et al.,* 1998), squamous cell carcinoma of the head and neck (Maeda *et al.,* 1999), brain tumor (Dil-Afroze *et al.,* 1998) , ovarian cancer (Sood and Buller, 1996), breast cancer (Papadopoulos *et al.,* 2002; Singh and Roy, 2001), hepatocellular carcinoma (Xian *et al.,* 2005; Zhang *et al.,* 2004) or human aberrant crypt foci and colon cancer (Luo and Pretlow, 2003). RAPD-PCR analysis has been also applied in other aspects of cancer research (Navarro & Jorcano 1999); for example, detection of DNA damage and mutation (Atienzar *et al*., 2002); genotoxicity and carcinogensis studies (Atienzar & Jha 2006), and identifying novel DNA amplifications (Garnis *et al*., 2004) in lymphoma (Scarpa *et al*., 2001) and in leukemia (Odero *et al*., 2001).

There are various methods which have been developed to detect DNA methylation in genomic DNA. These methods can be divided into two groups: firstly, using methylation-sensitive restriction endonucleases, and secondly, using bisulfite modifications of cytosine (Clark *et al.*, 1994). The former is based on the principle that methylation-sensitive restriction endonucleases cannot cut methylated DNA, in this respect; methylation-based polymerase chain reaction (PCR) assays are included in this group (Singer-Sam *et al*., 1990). Methylation-sensitive arbitrarily primed PCR (MS-AP-PCR) is a simple and rapid method that can be used to screen for methylation changes in normal and tumor DNA. The method is based on the digestion of genomic DNA with methylation sensitive enzyme (such as *Hpa*II), and then arbitrarily amplified using random primers. Similarly, the same DNA is digested with isoshizomer *Msp*I, which digests both methylated and unmethylated DNA, and then DNA amplified by the same above mentioned primers. After separation on agarose gel, a band present in *Hpa*II digested DNA but missing from the *Msp*I digested DNA indicates the DNA fragment containing methylated *Hpa*II sites (Liang *et al*., 2002; Gonzalgo *et al*., 1997).

The aim of study

The purpose of the work is to study the molecular genetic polymorphism of leukemia patients and to investigate the possible use of RAPD-PCR analysis in detection genetic and epigenetic variation in leukemia patients.

1.1 Cancer

Cancer has afflicted humans throughout recorded history. The oldest description of cancer was discovered in Egypt and dates back to approximately 1600 B.C. The origin of the word cancer is credited to the earliest medical scientists such as Hippocrates, Galen and Paracelsus who described cancer as a crab like uncontrolled growth, hence the name (Thomas, 2006). Cancer is the second leading cause of death in the world, a total of 1,444,920 new cancer cases and 559,650 deaths for cancers are projected to occur in the United States in 2007 (Jemal *et al*., 2007).

Cancer develops through a multi step process in which normal , healthy cells in the body go through stages that eventually change them to abnormal cells that multiply out of control. In most cases, cancer takes many years to develop. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled (Lodish *et al.*, 2003). These altered cells divide and grow in the presence of signals that normally inhibit cell growth; therefore, they no longer require special signals to induce cell growth and division. As these cells grow they develop new characteristics, including changes in cell structure, and production of new enzymes. Such changes allow the cancer cells to spread and invade other tissues (Yeang, 2001).

The abnormalities in cancer cells usually result from mutations in protein-encoding genes that regulate cell division (tumor suppressor genes and proto-oncogenes) (Hahn and Weinberg, 2003), thus over time more genes become mutated. This is often because the genes that make the proteins that normally

repair DNA damage are themselves not functioning normally because they are also mutated. Consequently, mutations begin to increase in the cell, causing further abnormalities in that cell and the daughter cells. This enhanced growth describes most cancer cells, which have gained functions repressed in the normal, healthy cells (Hanahan and Weinberg, 2000).

Recent studies have also highlighted the importance of epigenetic alterations of certain genes that result in the inactivation of their functions in some human cancers, Like transcriptional silencing of tumor suppressor genes, DNA repair genes, and metastasis inhibitor genes. These aberrations lead to the abnormal behavior common to all neoplastic cells (Jones and Baylin, 2002; Nephew and Huang, 2003; Ducasse and Brown, 2006).

1.2 Leukemia

Leukemia is a cancer of blood. The term itself was first used over a century ago by a German physician called Rudolf Virchow in 1859 , who observed that some patients seemed to be affected by a condition leading to 'weisses blut' (German for 'white blood', later translated into Greek as 'Leukemia'). The term 'white blood' was used because at that time it was thought that the high number of white blood cells in the blood actually made the blood appear to be white. Although this was later shown to be untrue, the term 'leukemia' continued to be used to describe this disease (Goldman *et al*., 1999).

Leukemia is a neoplastic proliferation of leukocyte precursor in the bone marrow, usually recognized in blood smear by the presence of many abnormal white blood cells. The neoplastic cells will take nutrition from normal hematopoietic tissue and grow more rapidly than normal bone marrow cells.

As a result the production of normal bone marrow elements will be decreased and eventually will be displaced by the cancerous cell line. This is an important consequence such as decrease in the normal red blood cells (R.B.Cs) production which leads to anemia. Decrease in the normal white blood cells (W.B.Cs) production will make the patient more susceptible to infections and bleeding due to not enough platelets available to produce homeostatic plugs (Hoffbrand *et al.*, 2002).

The neoplastic cell escape into blood (like normal leukocyte) where they may be very numerous will pave the way for their infiltration into various other tissues producing general organ enlargement or less commonly tumor masses, most of the clinical effects of leukemia are due to tissue infiltration or to interference with normal marrow function by the proliferation of neoplastic leukocyte (Mackie *et al*., 1999).

1.3 Classification of Leukemia

Leukemia is classified into two major types, acute and chronic leukemia, each of the two major types is further subdivided into lymphoid and myeloid.

Acute leukemia are usually aggressive diseases in which the malignant transformation causes accumulation of early bone marrow haemopoietic progenitors, called blast cells. The dominant clinical feature of these diseases is usually bone marrow failure caused by accumulation of blast cells, although tissue infiltration also occurs. If acute leukemias are untreated, then these malignancies are usually rapidly fatal, but, they are also easier to cure than chronic leukemias. The chronic leukemia are distinguished from acute

leukemia by their slower progression and the neoplastic cells are more mature (Hoffbrand *et al.*, 2002).

1.3.1 Acute Lymphoid Leukemia (ALL)

Acute lymphoblastic leukemia is caused by accumulation of lymphoblasts and is the common malignancy in childhood. There are three subtypes of ALL (L1, L2 and L3) according to French-American-Britain (FAB) classification on the basis of morphology of cells (Table 1 - 1). It accounts for 80% of all childhood leukemia and its incidence is highest at the age of 3 - 7 years, falling off by age of 10 years. This type of leukemia is most usual in children and has an equal sex incidence. 70-80% of children can be expected to be cured, whereas in adults this ratio is decreased to less than 5% over the age of 65 years (Hoffbrand *et al.,* 2002). The incidence of ALL is 3 - 4 new cases / 100,000 children per year and one death case per 100,000 (Cardinez *et al*., 2006). The disease is characterized by abnormalities of the lymphoid cell precursor leading to an excessive accumulation of lymphoblasts in the marrow and other organs, particular spleen and liver (Pui *et al*., 2004). Hepatomegaly and splenomegaly are due to leukemia infiltration and are present in approximately one-half to three-fourth of patients with ALL. The common manifestations of ALL are anemia, neutropenia, thrombocytopenia and pain of the bones (Xinias *et al*., 2005). It is worth to mention that Philadelphia chromosome is found in 95% of CML patients, it is also found in 5 percent of children and in 15 to 30 percent of adults with acute lymphoid leukemia. The other more frequent chromosomal abnormality are t (1 ; 14), t (8 ; 14), t (11 ; 14) and t (7 ; 9) (Specchia *et al*., 1995).

Table (1 – 1): The French-American-Britain (FAB) classification of ALL (Hoffbrand *et al.,* 2002).

FAB Type	Morphology
L1	Blast cells small, uniform high nuclear to cytoplasmic ratio.
L2	Blast cells larger, heterogeneous, lower nuclear to cytoplasmic ratio.
L3	Vaculated blasts, basophilic cytoplasm.

1.3.2 Acute Myeloid Leukemia (AML)

Acute myeloid leukemia occurs in all age groups, however it is the most common form of acute leukemia in adults and its occurrence increased with age. AML forms only a minor fraction (10-15%) of the leukemias in childhood. It had been reported that 50% of children and young adults may expect a long-term cure. The incidence of acute myeloid leukemia in the United State is 7.4 cases per 100,000 inhabitants per year and the death cases is 5.8 per 100,000 (Cardinez *et al*., 2006). The clinical signs and symptoms of AML are diverse and nonspecific, but they are usually directly attributable to the leukemic infiltration of the bone marrow, with resultant cytopenia (anemia, granulocytopenia, or thrombocytopenia), with or without leukocytosis). Typically, AML patients showed signs and symptoms of fatigue, hemorrhage, or infections and fever due to decreases in red cells, platelets, or white cells, respectively. Leukemic infiltration of various tissues, including the liver (hepatomegaly), spleen (splenomegaly) and lymph nodes (lumphoadenopathy) (Lowenberg *et al.*, 1999). The most commonly used

method of classification is that developed by the French–American–British (FAB) group which divides AML into eight distinct subtypes (M0, M1, M2, M,3, M4, M5, M6 and M7) that differ with respect to the particular myeloid lineage involved and the degree of leukemic-cell differentiation (Hoffbrand *et al.,* 2002). The more frequent chromosomal abnormality shown in AML patients were t (8 ; 21), t (6 ; 9), inv (16), del (11q) and t (9 ; 11) (Harrison, 2000; McCormack *et al.,* 2008).

Table (1 – 2): The French-American-Britain (FAB) classification of AML (Hoffbrand *et al.,* 2002)

FAB Type	Morphology
M0	Acute myeloid leukemia with minimal evidence of myeloid differentiated.
M1	Acute myeloid leukemia without maturation.
M2	Acute myeloid leukemia with granulocytic maturation.
M3	Acute promyelocytic leukemia.
M4	Acute myeloid leukemia with granulocytic and monocytic maturation.
M5	Acute monocytic leukemia.
M6	Acute erythro leukemia.
M7	Acute megakaryoblastic leukemia.

1.3.3 Chronic Lymphoid Leukemia (CLL)

Chronic lymphoid leukemia accounts 25% of all leukemia in adults with a peak incidence in age group between 60 and 80 years, the male to female ratio is 2:1. The median survival of patient with CLL is about nine years. The CLL can be classified to B-cell (B-cell chronic lymphocytic leukemia, B-cell prolymphocytic leukemia, hairy cell leukemia and plasma cell leukemia) and T-cell (large granular leukemia and T-cell prolymphocytic leukemia) (Hoffbrand *et al.,* 2002). The incidence of CLL in U.S. is 7.5 cases per 100,000, and the death cases is 3.2 case per 100,000 (Cardinez *et al*., 2006). The aetiology is unknown but there are geographical variations in incidence. It is the most common type of leukemia in the West but rare in the far East (Keating, 1999). There is no higher incidence with previous radiotherapy or chemotherapy. The tumor cell appears to be a relatively mature B cell with weak surface expression of immunoglobulin M (IgM) or IgD (Kipps, 2000). The cells accumulate in the blood, bone marrow, liver, spleen and lymph nodes as a result of prolonged lifespan with impairment of normal apoptosis. Symmetrical enlargement of superficial lymph nodes is the most frequent clinical sign. Bacterial or fungal infections are common because of immune deficiency and neutropenia (caused by marrow infiltration). Lymphocytosis with features of anemia may be present e.g pallor. Patients with thrombocytopenia may show bruising (Rozaman and Montserrat, 1995). The common chromosome abnormalities are deletion of 13q14, trisomy 12, deletions at 11q23 and structural abnormality of 17p involving the p53 gene (Dohner *et al*., 2000).

1.3.4 Chronic Myeloid Leukemia (CML)

Chronic myeloid leukemia comprises 15% of leukemias and may occur at any age. This disease occurs in either sex, the ratio of male to female is 1.4:1, however it occurs most frequently between the ages of 40 and 60 years. Moreover, it may occur in children and neonates, and in the very old individuals and the median survival is 5-6 years. There are many variants of CML such as chronic myelomonocytic leukemia, juvenile CML, ph-negative CML and eosinophilic leukemia (Hoffbrand *et al.*, 2002). The incidence of CML in U.S. is 3 cases per 100,000, and the death cases is 1 case per 100,000 (Cardinez *et al*., 2006). In most cases of CML there are no known predisposing factors which might cause the disease but the incidence was increased in survivors of the atom bomb explosions in Japan at the end of second world war (Sawyers, 1999). This disease is characterized by an overproduction of cells of the granulocytic series leading to splenomegaly and very high white blood cells counts, moreover, basophilia, thrombocytosis, renal impairment and anemia are common (Faderl *et al.,* 1999; Goldman and Melo, 2003). The diagnosis of CML is rarely difficult and is assisted by the characterstic presence of the Philadelphia (Ph) chromosome, which result from reciprocal translocation between chromosomes 9 and 22. As a result of this translocation part of the Abelson proto-oncogene ABL is moved to the breakpoint cluster region BCR gene on chromosome 22 and part of chromosome 22 moves to chromosome 9. The abnormal chromosome 22 is the Ph chromosome. In the Ph chromosome the BCR gene are fused to ABL gene (Martein *et al.,* 1999). The resulting chimeric BCR-ABL genes codes for a fusion protein which have leukemogenic activity. This chromosomal abnormality is found in up to 95 percent of CML patients. Another 5 percent have complex or variant translocations involving additional chromosomes that

have the same end result. The Ph chromosome is found in cells from the myeloid, erythroid, megakaryocytic, and B lymphoid lineages (Kurzrock *et al.,* 1988; Melo, 1996).

1.4 The genetic basis of cancer

1.4.1 Oncogene

Is a gene that can cause cancer. It causes the transformation of normal cells into cancerous tumor cells, specially a viral gene that can transform a host cell into tumor cell. It is worth to mention that oncogene is altered or mutated from its original form, the proto-oncogene. In this respect, proto-oncogene promote the differentiation and proliferation of normal cells. A variety of proto-oncogenes are involved in different crucial steps of cell growth, and a change in the proto-oncogenes sequence or in the amount of protein it produces can interfere with its normal role in cellular regulation, which might result in uncontrolled cell growth, or neoplastic transformation, thus ultimately resulting in the formation of cancerous tumor (shovlin *et al.*, 1999). The first oncogene was discovered in 1970 and was termed *src* (pronounced SARK). This gene was in fact first discovered as an oncogene in a chicken retrovirus. In 1976 Bishop and Varmus demonstrated that oncogenes were defective proto-oncogenes, found in many organisms including humans (Bishop, 1987).

Oncogenes have various cellular functions and include growth factors, growth factor receptors, genes that function in intracellular signal transduction, transcription factors, and positive regulators of the cell cycle (McManus and Alessi, 2004).

In human proto-oncogene can be transformed into oncogenes by three ways (Robertson, 1983; Pedraza-Fariña, 2006; Croce, 2008):

- Point mutation

Oncogene is activated by a mutation, the structure of the encoded protein is changed in a way that enhances its transforming activity. Many types of mutation occur in oncogenes such as deletion, base substitution or insertion. Example are the *RAS* oncogenes (*KRAS, HRAS,* and *NRAS*). When the *RAS* genes are mutated which encode a protein that remains in the active state. These activated proteins induce continuous cell growth. Mutation of oncogenes in the RAS family has been associated with exposure to environmental carcinogens. Mutations of *KRAS* are common in carcinomas of the lung, colon, and pancreas, whereas mutations of *NRAS* occur principally in acute myelogenous leukemia and the myelodysplastic syndrome (Weber and McClure, 1987). Activating point mutations of the *BRAF* gene occur in 59% of melanomas, 18% of colorectal cancers, 14% of hepatocellular carcinomas, and 11% of gliomas. (Davies *et al*., 2002; Croce, 2008).

- Chromosomal translocations

The transfer of a gene from its normal position to one on another chromosome is called translocation, and chromosome translocations are common cytogenetic abnormalities in cancer cells. In hematopoietic cancers and solid tumors, the translocations and inversions increase or deregulate transcription of the oncogene. In chronic myeloid leukaemia there is precise translocation of genes, including another oncogene, *abl*, from chromosome 9 to

chromosome 22; this is the molecular basis of the Philadelphia chromosome. In Burkitt's lymphoma there is a translocation between chromosome 8 and the immunoglobulin genes on chromosomes 2, 14, or 22. In both of these examples the break occurs at the chromosomal site of the proto-oncogene; in Burkitt's lymphoma it is the c-myc gene that is brought close to the immunoglobulin genes. This rearrangement is probably associated with developing the specific B cell malignant phenotype. In prostate cancer, gene fusion occurs between a gene that carries a promoter that is very active in the target cells, and another that carries the oncogenic activity (e.g., *ERG1*) (Hall, 1984; Croce, 2008).

Amplification

Amplification means the repeating of the DNA sequences of the gene in question, sometimes by as many as 50-100 times; this may lead to overexpression of the gene product. The c-myc oncogene is associated with acute leukaemia and was the first oncogene to show amplification in malignant change compared with normal cells from the same patient (Weber and McClure, 1987). *MYC* is also amplified in small-cell lung cancer, breast cancer, esophageal cancer, cervical cancer, ovarian cancer, and head and neck cancer, whereas amplification of *NMYC* correlates with an advanced tumor stage. There are different oncogene families are often amplified: cyclin D1 (or CCND1), EGFR, and RAS. *CCND1* amplification occurs in breast, esophageal, hepatocellular, and head and neck cancer. *EGFR* (*ERBB1*) is amplified in glioblastoma and head and neck cancer. Amplification of *ERBB2* (also called *HER2/neu*) in breast cancer correlates with a poor prognosis (Croce, 2008; Weinberg, 1989).

1.4.2 Tumor suppressor genes

Is a gene that reduces the probability that human cell will turn into tumor cell, a mutation or deletion of such a gene will increase the probability the formation of a tumor. Tumor suppressor genes, or more precisely, the protein for which they code, have a repressive effect on the regulation of the cell cycle or promote apoptosis, and sometimes do both (Levine, 1993; Weinberg, 1991). Tumor suppressor genes play a critical role in regulating when cells are allowed to divide and increase in number. When DNA damage is detected in a cell, some tumor suppressor genes can stop the cell from multiplying until the damage is repaired. Also, specific tumor suppressor genes can stimulate cells with damaged DNA to commit "cell suicide". When tumor suppressor genes don't function correctly, the cells with DNA damage continue to divide and can accumulate further DNA damage that can eventually lead to the formation of a cancer cell (Fearon and Vogelstein, 2000; Payne and Kemp, 2005).

Disruption of the function of a tumor-suppressor gene, as defined by Knudson, requires a complete loss of function of both copies of the involved gene. When caused solely by genetic changes, such losses are the consequence of germ-line mutations (in familial cancers) or somatic mutations (in non-inherited tumors) within the coding region of one copy of the gene. This is the first hit of the Knudson two-hit hypothesis. The second hit generally involves somatic loss of the chromosomal region containing the other copy of the gene. The loss of both alleles of a tumor suppressor gene thus sets the scene for malignant transformation of the cell (Fig. 1 - 1). Abnormal promoter hypermethylation can have the same effect as a coding-

region mutation in one copy of the gene (the first hit); often, loss of the other copy serves as the second hit (Fig. 1 - 1). In familial cancer, epigenetic change is not a mechanism of the first hit, but it can cause the second hit (Knudson, 2001).

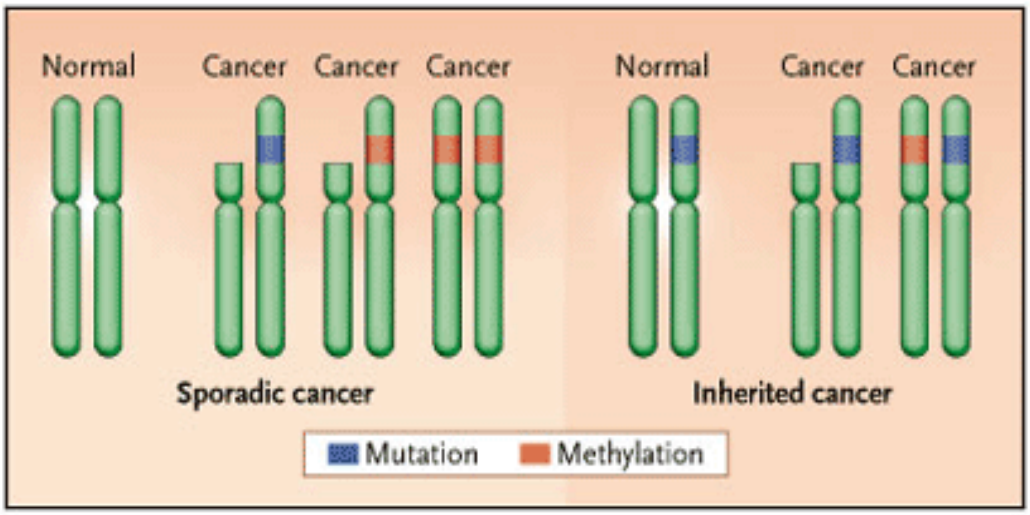


Figure (1 – 1): Genetic and epigenetic changes that inactivate tumor-suppressor genes according to the Knudson two-hit hypothesis (Knudson, 2001).

The first tumor-suppressor genes discovered was the Rb gene in hereditary retinoblastoma, a serious cancer of the retina that occurs in early childhood. In addition to retinoblastomas, mutations in the Rb gene have been detected in osteosarcomas, bladder carcinomas, small-cell lung carcinomas, prostate carcinomas, breast carcinomas, some types of leukemias, and cervical carcinomas (Krug *et al*., 2002).

Another tumor suppressor gene is p53, a gene found to be mutated in a large proportion of human cancers such as, in lung, colon, esophageal, ovarian, pancreatic, skin, stomach, head and neck, bladder, sarcoma, prostate, hepatocellular, brain, breast, renal, thyroid, hematological malignancies, melanoma and cervical cancers. Germ line mutation of p53 has been linked to

an inherited predisposition to cancer Li-Fraumeni syndrome of cancer including breast carcinoma, sarcoma, brain tumors, osteosarcoma and leukemia. increased amounts of cellular p53 protein after DNA damage have been associated with cell-cycle arrest and programmed cell death (apoptosis) and mutations or losses of p53 have been result in development of cancer (Velculescui and Eldeiry, 1996; William and Foulkes, 2007). Figure (1 – 2) illustrates the roles of wild type and mutant alleles of tumor suppressor gene P53 in DNA repair, apoptosis and carcinogenesis.

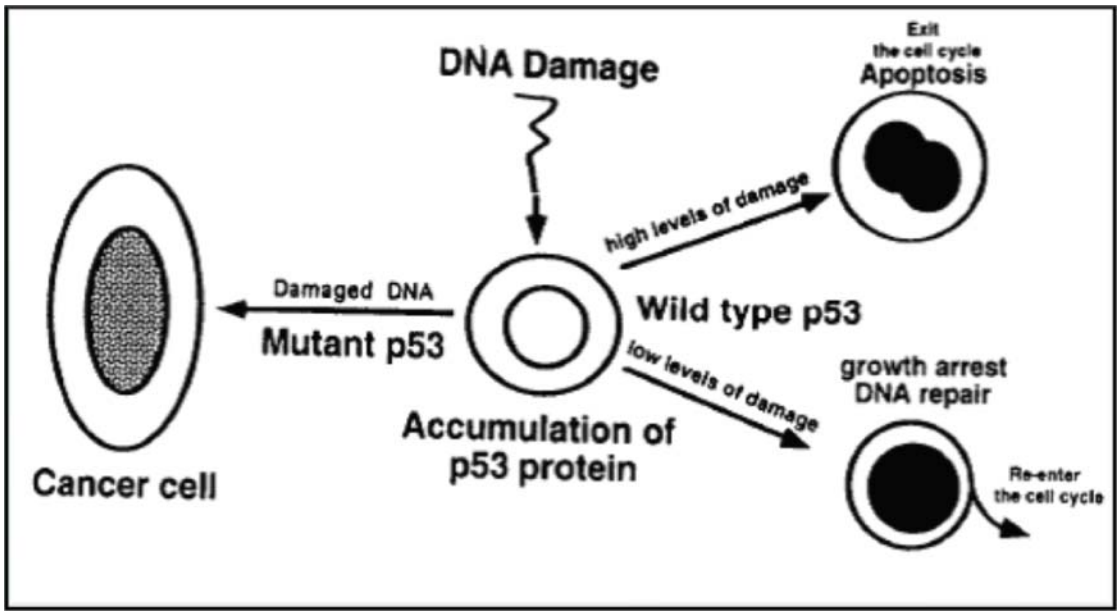


Figure (1 – 2): The functions of wild type and mutant of tumor suppressor gene P53 in DNA repair, apoptosis and carcinogenesis (William and Foulkes, 2007).

The other cancers associated with defects in tumor suppressor genes include familial adenomatous polyposis of the colon (FPC), which results from mutations to both copies of the *APC* gene; hereditary breast cancer, resulting from mutations to both copies of *BRCA2*; and hereditary breast and ovarian cancer, resulting from mutations to both copies of *BRCA1* (Fearon and

Vogelstein, 2000; Sherr, 2004). There are other types of tumor suppressor genes some of them are listed in table (1 – 3).

Table (1 – 3): Some genes associated with cancer.

Genes	Function	Example	Type of cancer gene	Referances
APC	regulates transcription of target genes	Familial Adenomatous Polyposis	tumor suppressor	Fearon and Vogelstein, 2000
BCL2	involved in apoptosis; stimulates angiogenesis	Leukemia; Lymphoma	oncogene	Croce, 2008
BLM	DNA repair	Bloom Syndrome	DNA repair	Lodish *et al*., 2003
BRCA1	may be involved in cell cycle control	Breast, Ovarian, Prostatic, & Colonic Neoplasms	tumor suppressor	Sherr, 2004
BRCA2	DNA repair	Breast & Pancreatic Neoplasms; Leukemia	tumor suppressor	Sherr, 2004
HER2	tyrosine kinase; growth factor receptor	Breast, Ovarian Neoplasms	oncogene	Croce, 2008
MYC	involved in protein-protein interactions with various cellular factors	Burkitt's Lymphoma	oncogene	Croce, 2008
p16	cyclin-dependent kinase inhibitor	Leukemia; Melanoma; Multiple Myeloma; Pancreatic Neoplasms	tumor suppressor	William and Foulkes, 2007
p21	cyclin-dependent kinase inhibitor	-	tumor suppressor	William and Foulkes, 2007
p53	apoptosis; transcription factor	Colorectal Neoplasms; Li-Fraumeni Syndrome	tumor suppressor	William and Foulkes, 2007
RAS	GTP-binding protein; important in signal transduction cascade	Pancreatic, Colorectal, Bladder Breast, Kidney, & Lung Neoplasms; Leukemia; Melanoma	Oncogene	Croce, 2008
RB	regulation of cell cycle	Retinoblastoma	tumor suppressor	Krug *et al*., 2000

SIS	growth factor	Dermatofibrosarcoma; Meningioma Skin Neoplasms	oncogene	Croce, 2008
XP	DNA repair	Xeroderma pigmentosum	DNA repair	Lodish *et al*., 2003

1.4.3 DNA repair genes:

A third type of gene associated with cancer is the group involved in DNA repair and maintenance of chromosome structure. In this respect, it is known that environmental factors, such as ionizing radiation, UV light, and chemicals, can damage DNA. Errors in DNA replication can also lead to mutations. Certain gene products can repair damage occurring to chromosomes, thereby minimizing mutations in the cell. When a DNA repair gene is mutated its product is no longer made, preventing DNA repair and allowing further mutations to accumulate in the cell. These mutations can increase the frequency of cancerous changes in a cell (Loeb, 1991; Loeb, 2001).

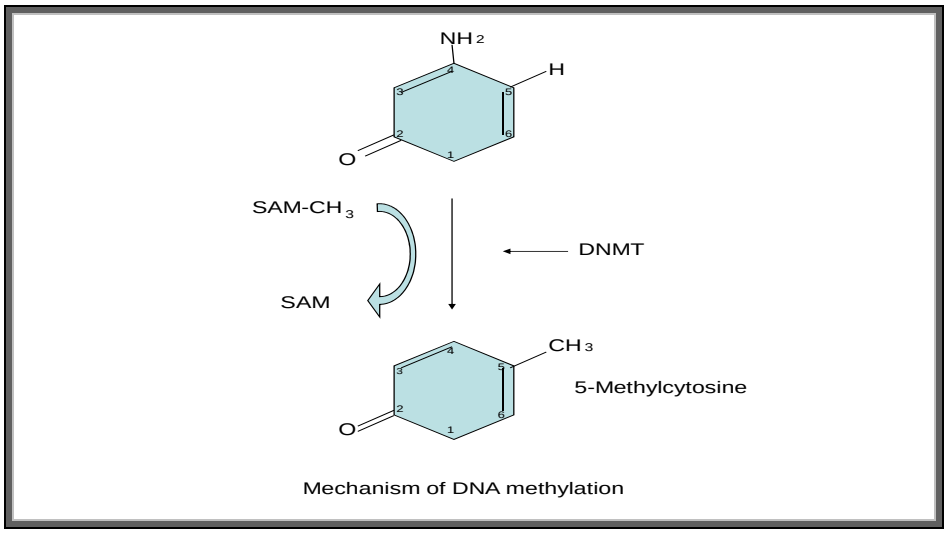
The mutations in DNA repair genes can drive tumorigenesis which is shown by the existence of human cancer syndromes with germ line mutations in genes involved in maintaining genomic integrity. These include xeroderma pigmentosum which result from defect in a DNA repair gene called *XP* results in individuals who are very sensitive to UV light and have a thousand-fold increase in the incidence of all types of skin cancer. There are seven *XP* genes, whose products remove DNA damage caused by UV light and other carcinogens (Sieber *et al.*, 2003).

Another example of a disease that is associated with loss of DNA repair is Bloom syndrome, an inherited disorder that leads to increased risk of cancer, lung disease, and diabetes. The mutated gene in Bloom syndrome, *BLM*, is required for maintaining the stable structure of chromosomes. Individuals

with Bloom syndrome have a high frequency of chromosome breaks and interchanges, which can result in the activation of oncogenes. In addition, somatic changes in some DNA-repair genes have been shown in a small number of cancer types. Most of these mutations are recessive (requiring two 'hits') at the cellular level (Fridman, 1996; Sieber *et al.*, 2003).

1.5 Epigenetic Changes

Epigenetic change is a heritable change in the pattern of gene expression that is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene (Bird, 2002). Epigenetic changes include DNA methylation and histone modifications (acetylation and methylation), which influence chromatin structure or modify the DNA without altering the native nucleotide sequence. DNA methylation involves the addition of the methyl group to the number 5 carbon of a cytosine in a CG dinucleotide (CpG) by a DNA methyltransferase enzymes. Figure (1 - 3) illustrate the mechanism of methylation of cytosine to 5- methylcytosin (Esteller, 2008; Ducasse and Brown, 2006).



Mechanism of DNA methylation

Figure (1 – 3): DNA methylation, the DNA methyltransferases (DNMTs), catalyzes the methylation of the 5 position of the cytosine ring, using *S*-adenosyl-methionine as the donor molecule for the methyl group (CH_3) (Esteller, 2008).

Methylation patterns are established during normal embryonic development, and participate in X chromosome inactivation and genomic imprinting. Tissue-specific methylation also occurs and methylation has been shown to increase in some tissues with aging (Moss and Wallrath, 2007).

Hypermethylation of promoter CpG islands is correlated with transcriptional inactivation of the associated gene, and this inactivation is equivalent to a loss-of-function genetic alteration such as a deletion or mutation. If the gene has tumor suppressor properties or is involved in DNA repair genes, differentiation, or apoptosis, aberrant methylation can play a central role in tumorigenesis (Herman and Baylin, 2003). However, hypomethylation of a normally methylated gene promoter can lead to activation of that gene, such as oncogenes and anti-appoptotic genes (Jones and Baylin, 2002; Nephew and Huang, 2003).

There is a critical difference between mutations and epigenetic gene silencing: the former are irreversible and the latter are potentially reversible. The potentially reversible epigenetic changes in neoplasia present new opportunities for the clinical management of cancer. The reaction of DNA methylation can be blocked by the drug 5-azacytidine . When this compound is integrated into DNA, replacing the natural base cytidine, it acts as a direct and irreversible inhibitor of the DNMTs, since it contains a nitrogen in place

of carbon at the 5 position of the cytidine ring. (Novak, 2004; Lund and Lohuizen, 2004). Figure (1 - 4) shows the inhibitory effect of 5-Azacytidine on methylation of cytosine.

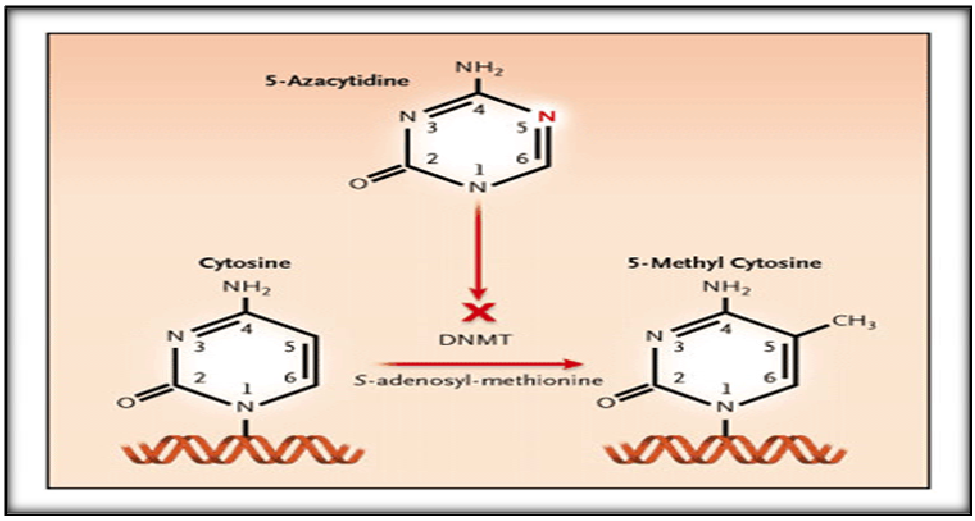


Figure (1 – 4): Pathway for the methylation of cytosine in the mammalian genome and effects of inhibiting methylation with 5-Azacytidine (Herman and Baylin, 2003).

Various methods to detect DNA methylation have been developed. These can be divided into two groups: those using methylation-sensitive restriction endonucleases, and those using bisulfite modification of cytosine. The former is based on the principle that methylation-sensitive restriction endonucleases cannot cut methylated DNA. methylation-based polymerase chain reaction (PCR) assays (Singer-Sam *et al*., 1990) are included in this group. The latter is based on the principle that unmethylated cytosines are modified by bisulfate and changed to uracil, whereas methylated cytosines escape from bisulfate modification and remain as cytosine (Clark *et al*., 1994). Methylation-sensitive arbitrarily primed PCR (MS-AP-PCR) is a simple and rapid method that can be used to screen for methylation changes in normal

and tumor DNA, which is based on the digestion of genomic DNA with methylation sensitive enzyme (such as *Hpa*II), and then arbitrarily amplified using random primers with low annealing temperatures. Similarly, the same DNA is digested with isoshizomer *Msp*I, which digest both methylated and unmethylated DNA, and then PCR amplified by same primers. After separation on agarose, a band present in *Hpa*II digested DNA but missing from the *Msp*I digested DNA indicates the DNA fragment containing methylated *Hpa*II sites (Liang *et al*., 2002; Gonzalgo *et al*., 1997).

1.6 Isolation of mammalian genomic DNA

Isolation of genomic DNA is an essential step in many molecular biology experiments. However, there are many different methods and technologies for the isolation of genomic DNA from different organisms; for example bacteria (Kieser, 1995), fungi (Cenis, 1992), plant (Murray and Thompson, 1991) and mammalian cells (Laird *et al*., 1991). All the procedures for DNA isolation depend on two important characters of the nucleic acids: first, they don't dissolve in organic solvents and the second precipitated DNA in the presence of alcohol and high salt concentration. These characters permit the DNA isolation from other cell components (Parish, 1972).

In general, all DNA isolation methods have common steps, which involve chemical and mechanical disruption methods, followed by one of several basic enzymatic and chemical methods to remove contaminating proteins, RNA, and other macromolecules. The enzymatic methods depend on using of proteiolytic enzymes (Gross-Bellard *et al.,* 1973; Enrietto *et al*., 1983). whereas the chemical methods almost depend on using of detergents such SDS which result in membranes solubilization and proteins denaturation (Blin

and Stafford, 1976) and then the DNA is separated from other cell components by centrifugation (Parish, 1972).

There are several methods for DNA purification from cell lysate. The standard method is used for deproteinizing DNA is to extract with phenol / chloroform / Isoamyl alcohol. The using of phenol which efficiently denature proteins and probably dissolved denaturated protein is first described by Kirby in 1957; it is worth to mention that, chloroform is also a useful protein denaturant. The phenol/chloroform mixture reduces the amount of aqueous solution retained in the organic phase(compared to a pure phenol phase), maximizing the yield (Penman, 1966; Palmiter, 1974). In this respect, isoamyl alcohol, prevents foaming of the mixture upon vortexing and aids in the separation of the organic and aqueous phases (Marmur, 1961). Since denatured proteins form a layer at the interface between the aqueous and organic phases, thus, proteins could be isolated from the bulk of the DNA in the aqueous layer. The standard method to recover DNA from aqueous solutions is to precipitate with ethanol (McCarty, 1985); isopropanol may also be used to precipitate DNA. However, isopropanol is less volatile than ethanol and is therefore more difficult to remove; moreover, some salts are less soluble in isopropanol (as compared to ethanol) and will precipitate with DNA (Moore *et al.,* 2002). There are many methods which use phenol in DNA extraction from different animal tissues, in 1973 Gross-Berallard *et al*., described a method for DNA extraction from cell culture by using phenol which depends on culture treated with proteiolytic enzymes like proteinase K in the presence of SDS and EDTA. In 1976 Blin and Stafford found that the efficient extraction with phenol was increased when liquid nitrogen was used, which led to fast and complete destruction of cells. The extraction of nucleic acids with phenol and chloroform is the standard method to remove proteins

and lipids from cellular digests. However, there are many drawbacks to the use of these organic solvents, for example their caustic and toxic qualities. Phenol can cause severe burns while chloroform is hepatotoxic and carcinogenic (Tilzer *et al.,* 1989; Thomas *et al.,* 1989). Therefore there are several alternative methods which are developed, these methods depend on preparation of cell lysate with different material for DNA extraction and purification without using of organic solvent such as, salting-out method (salting-out of cellular proteins by dehydration and precipitation with a saturated NaCl solution) (Miller *et al.,* 1988; Jeanipierre, 1987) or by using of sodium perchlorate and chloroform instead of phenol in extraction of DNA from whole blood (Johns, 1989); or by dialyzing nucleic acid containing solutions polyethylene glycol (PEG) (this method includes removal of detergent and proteolytic digestion products by dialyzing nucleic acid containing solutions against 20% (w/v) polyethylene glycol (PEG)) (Longmire, 1987); or by using of enzymatic procedure for the isolation of genomic DNA (which involves the incubation of nuclei with only proteinase K) (Grimberg *et al*., 1989); or purification of genomic DNA by filtration of cells lysate through an aluminum oxide membranes surface (AOM) (Elgort, 2004).

Blood is considered a common source of genomic DNA for molecular studies and various approaches were used for isolation of DNA from blood. In this respect, DNA can be isolated from a variety of blood's sources including anti-coagulated whole blood (Singer *et al*., 1988; Lahiri and Nurnberger, 1991; Grimberg *et al*., 1989; Parzer and Mannhalter, 1991; Elgort, 2004; Johns and Paulus-Thomas, 1989), buffy coat cells (Sambrook and Russell, 2001; Miller *et al.*, 1988; Tilzer *et al*., 1989) and clotted whole blood (Kanai *et al.,* 1994).

There are many parameters affecting the yield of DNA from human blood; for example, the yield of genomic DNA will vary depending on the quantity of white blood cells present (Alanne *et al.*, 2004). Other factors which might influence the yield is the effect of storage conditions and period of storage. Therefore blood samples collected in the field for isolating DNA for molecular analysis, need special care in storage conditions and handling. It has been reported that the collected blood in suitable containers with anti-coagulatant could be stored at 4°C for short term, from a few days to a few weeks. However, long-term storage for months usually involves blood being frozen at -20°C, in this case DNA yield will be reduced with length of time's storage (Richardson *et al*., 2006). DNA from blood samples that had undergone more than four freeze-thaw cycles were found to be partially degraded (Lahiri, 1993).

Various kinds of anticoagulants are commonly used for blood sampling, such as acid citrate dextrose (ACD), EDTA, and heparin. In this respect, some studies have indicated that the type of anticoagulant that is used when preparing genomic DNA from whole blood effects both the DNA yield and quality. ACD was found superior to EDTA in preserving high-molecular weight DNA (Gustafson *et al*., 1987). Another study pointed out that the EDTA is a better anticoagulant than heparin or citrate for delayed blood processing for plasma DNA analysis (Lam, 2004).

1.7 Polymerase Chain Reaction (PCR)

Since the discovery of the double helix structure of DNA, no single event has had the same impact on the field of molecular biology as the discovery by Kary Mullis in the early 1980_s of the polymerase chain reaction (PCR) (Mullis *et al.*, 1986). This elegant technology with its apparent simple theory

has revolutionized almost every aspect of classical molecular biology (Lisby, 1997). The polymerase chain reaction (PCR) is defined as an *in vitro* technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of known DNA sequence enzymaticaly by a DNA polymerase using oligonucleotid primers which are complementary to the ends of a defined sequence of DNA template (Newton and Graham, 1997).

A basic PCR set up requires several components and reagents (Saiki *et al*., 1990). These components include:

- DNA template: that contains the DNA fragment (target) to be amplified.
- Deoxynucleotide triphosphate ($dNTP_S$): the building blocks from which the DNA polymerases synthesize a new DNA strand.
- Primers: which are complementary to the DNA regions at the 5' and 3' ends of the DNA region
- PCR buffer: which provides a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- DNA polymerase: such as *Taq polymerase* or another DNA polymerase with a temperature optimum at around 70°C.

The original protocol for PCR used the klenwo frgment of *E.coli* DNA polymeras to extend the annealed primers. This enzyme was inactivated high temperature required to denature DNA consequently, fresh enzyme had to be added during every cycle (Saiki *et al*., 1988). Later, this original PCR process was greatly improved by the use of

DNA polymerase taken from *Thermophilic bacteria* grown in geysers at a temperature of over 110°C. The DNA polymerase taken from these organisms is stable at high temperatures and, when used in PCR, does not break down when the mixture was heated to separate the DNA strands. Since there was no longer a need to add new DNA polymerase for each cycle, the process of copying a given DNA strand could be simplified and automated. Recently the recombinant *Taq* DNA polymerase is produced from a genetically engineered *E. coil* where the desired gene is introduced in to this bacterium after recovering it from *Thermus aquaticus* (Lawyer *et al*., 1989).

There are three major steps in a PCR, which are repeated for 30 or 40 cycles. Each cycle of PCR includes steps for template denaturation, primer annealing and primer extension (Figure 1 - 5). The initial step denatures the target DNA by heating it to 94°C or higher for 15 seconds to 2 minutes. In the denaturation process, the two strands of DNA separate from one another, producing the necessary single-stranded DNA template for replication by the thermostable DNA polymerase. In the next step of a cycle, the temperature is reduced to approximately 40–60°C. At this temperature, the oligonucleotide primers can form stable associations (anneal) with the denatured target DNA and serve as primers for the DNA polymerase. This step lasts approximately 15–60 seconds. Finally, the synthesis of new DNA begins as the reaction temperature is raised to the optimum for the DNA polymerase. For most thermostable DNA polymerases, this temperature is in the range of 70–74°C. The extension step lasts approximately 1–2 minutes (Newton and Graham, 1997).

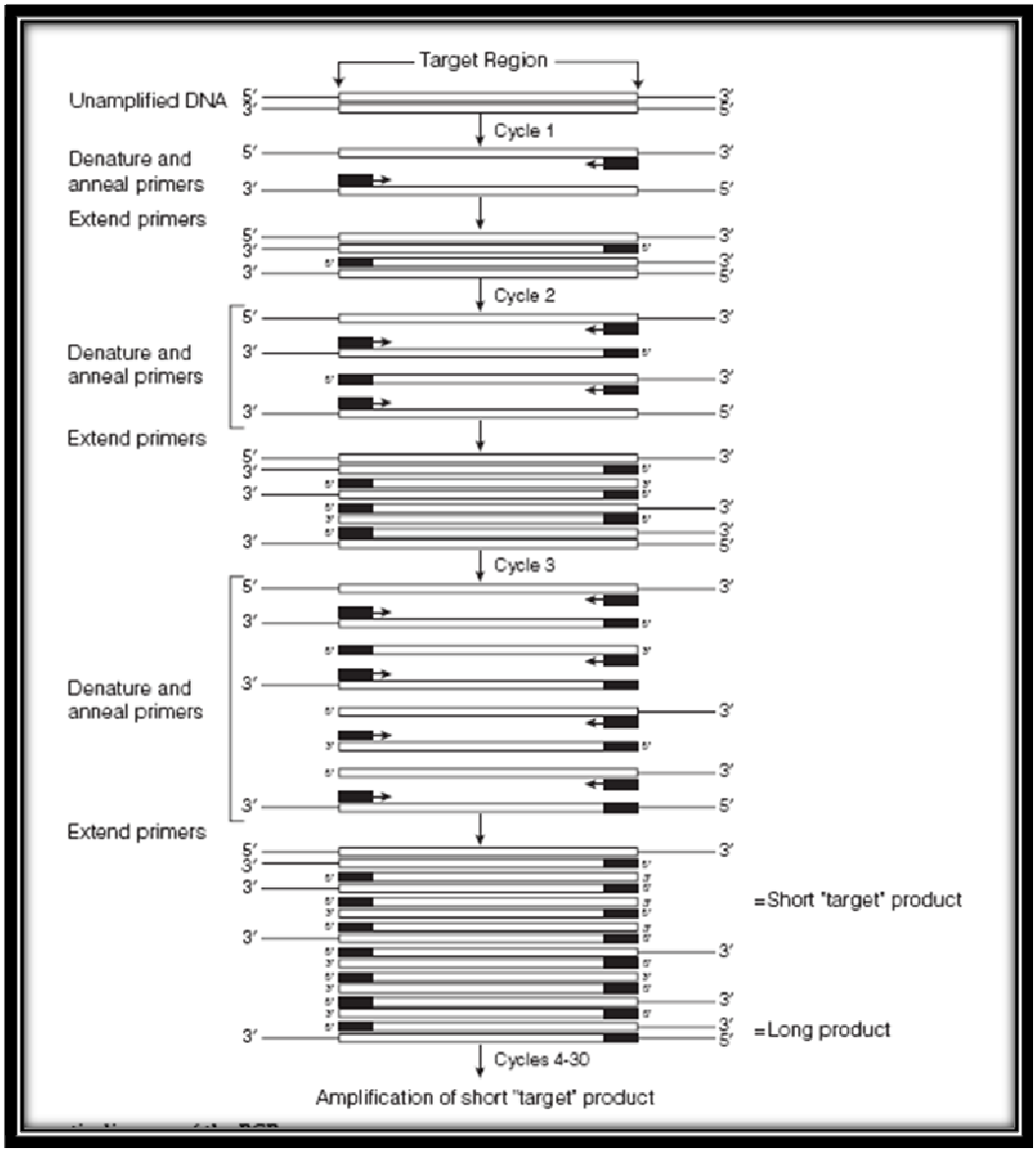


Figure (1 – 5): Schematic diagram of the PCR process. (1) Denaturing at 94-96°C. (2) Annealing at ~65°C. (3) Elongation at 72°C (Newton and Graham, 1997).

PCR techniques have a wide range of applications such as:

1- PCR allows early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest developed in cancer research and is already being, used routinely (Zhu *et al*., 2005; Liu *et al*., 2007; Osumi *et al*., 2002; Diss and Pan, 1997; Lee *et al*., 1987).

PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity which is at least 10,000 fold higher than other methods (Lee *et al*., 2002; Qin *et al*., 2007; Nasedkina *et al*., 2003; Li *et al*., 2003; Albinger-Hegyi *et al*., 2002).

2- PCR allows also the detection of residual disease after treatment of leukemia to identify patients at risk for relapse (Kolomietz *et al*., 2003; Cave *et al*., 1998).

3- PCR used in mutations screening of cancer-related genes such as activated oncogenes (Bos *et al*., 1987; Gunnarsson *et al*., 2003; Bernard and Wittwer, 2002) and tumor suppressor genes such as p53 to aid understanding of tumor biology and the role of specific genes in malignant transformation (Lukas *et al*., 2000).

4- PCR used to reveal the genetic disorders such as cystic fibrosis (Ballabio *et al*., 1990); sickle cell anemia (Saiki *et al.* 1985; Costa *et al*., 2003) and thalassaemia (Settin *et al*., 2006; Vrettou *et al*., 2003).

5- PCR used to identify many pathogenic microorganisms of medical importance for example *Helicobacter pylori* (He *et al*., 2002); *Mycobacterium tuberculosis* (Desjardin *et al*., 1998; Folgueira *et al*., 1996; Mehrotra *et al*., 2002); *Bordetella pertussis* (Kösters *et al*, 2002); *Streptococcus pneumonia* (Friedland *et al.*, 1994); human brucellosis (Queipo-Ortuño *et al*., 1997); *Legionella pneumophila* (Reischl *et al*., 2002); *Entamoeba histoliytica* (Mirelman *et al*., 1991); HIV (Ou *et al*., 1988); Human cytomegalovirus (Boom *et al*., 1999); Hepatitis B virus (Brechtbuehl *et al*., 2001) and Human papillomavirus (Wang-Johanning *et al*., 2002).

Variationts on the basic PCR technique

- Hot-start PCR: This is a technique that reduces non-specific amplification during the initial set up stages of the PCR. The technique may be performed manually by heating the reaction components to the melting temperature (e.g. 95˚C) before adding the polymerase (Chou *et al,.* 1992). Specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature, either by the binding of an antibody or by the presence of covalently bound inhibitors that only dissociate after a high-temperature activation step (Sharkey *et al*., 1994).

- Intersequence-specific (ISSR) PCR: A PCR method for DNA fingerprinting that amplifies regions between some simple sequence repeats to produce a unique fingerprint of amplified fragment lengths (Zietkiewicz *et al.,* 1994).

- Methylation-specific PCR (MSP): The MSP method was developed by Stephen Baylin and Jim Herman at the Johns Hopkins School of Medicine (Herman *et al.*, 1996) and is used to detect methylation of CpG islands in genomic DNA. DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is recognized by PCR primers as thymine. Two PCRs are then carried out on the modified DNA, using primer sets identical except at any CpG

islands within the primer sequences. At these points, one primer set recognizes DNA with cytosines to amplify methylated DNA, and one set recognizes DNA with uracil or thymine to amplify unmethylated DNA.

- RT-PCR(Reverse Transcription PCR): Is a method used to amplify, isolate or identify a known sequence from a cellular or tissue RNA. The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites and, if the genomic DNA sequence of a gene is known, to map the location of exons and introns in the gene (Myers and Gelfand, 1991). Rapid amplification of cDNA ends (RACE) is a variation of RT-PCR that amplifies unknown cDNA sequences corresponding to the RNA (Troutt *et al*., 1992).

- Quantitative PCR (Q-PCR): Is a modification of the PCR used to rapidly measure the quantity of DNA, cDNA or RNA present in a sample. PCR theoretically amplifies DNA exponentially, doubling the number of molecules present with each amplification cycle. The number of amplification cycles and the amount of PCR end-product should allow one to calculate the initial quantity of genetic material. The most sensitive quantification methods are done by the real-time polymerase chain reaction, where the amount of DNA is measured

after each cycle of PCR by use of fluorescent markers (Ding and Cantor, 2004).

- Touchdown PCR: A variant of PCR that aims to reduce nonspecific background by gradually lowering the annealing temperature as PCR cycling progresses. The annealing temperature at the initial cycles is usually a few degrees (3-5˚C) above the T_m of the primers used, while at the later cycles, it is a few degrees (3-5˚C) below the primer T_m. The higher temperatures give greater specificity for primer binding, and the lower temperatures permit more efficient amplification from the specific products formed during the initial cycles (Don *et al*., 1991).

- *In situ* PCR : First described in 1990, combines the sensitivity of PCR or RT-PCR amplification with the cellular or histological localization associated with in situ hybridization techniques (Haase *et al*., 1990). These features make in situ PCR a powerful tool for detecting proviral DNA, gene rearrangements and single gene copy. The technique is amenable to analysis of fixed cells or tissue cross-sections. Detection of amplified products can be accomplished indirectly by subsequent hybridization using either radiolabeled, fluorescently labeled or biotin-labeled nucleic acid probes. PCR products can also be detected directly by the incorporation of a labeled nucleotide, although this method is subject to higher background levels (Haase *et al*., 1990; Bagasra *et al*., 1993).

- RAPD PCR (Random Amplified Polymorphic DNA) : Is a DNA fingerprint technique based on polymerase chain reaction (PCR) amplification of random fragments of genomic DNA with single short primer of arbitrarily nucleotides sequence usually 10-bases (Williams *et al.* 1990). In this process a single species of primer binds to the genomic DNA at two different sites on opposite strands of the template DNA. In order for PCR to occur, annealing of primers must be in a particular orientation (such that they point each other) fig. (1 – 6), and the primers must anneal within a reasonable distance of one another (Tingey *et al.*, 1994). Primers are usually able to amplify fragments from 3 – 10 genomic sites simultaneously (Williams *et al.,* 1990). Generated RAPD fragments were commonly separated using agarose gel electrophoresis and then visualized by staining with ethidium bromide and observed under UV light (Lowe *et al.*, 1996).

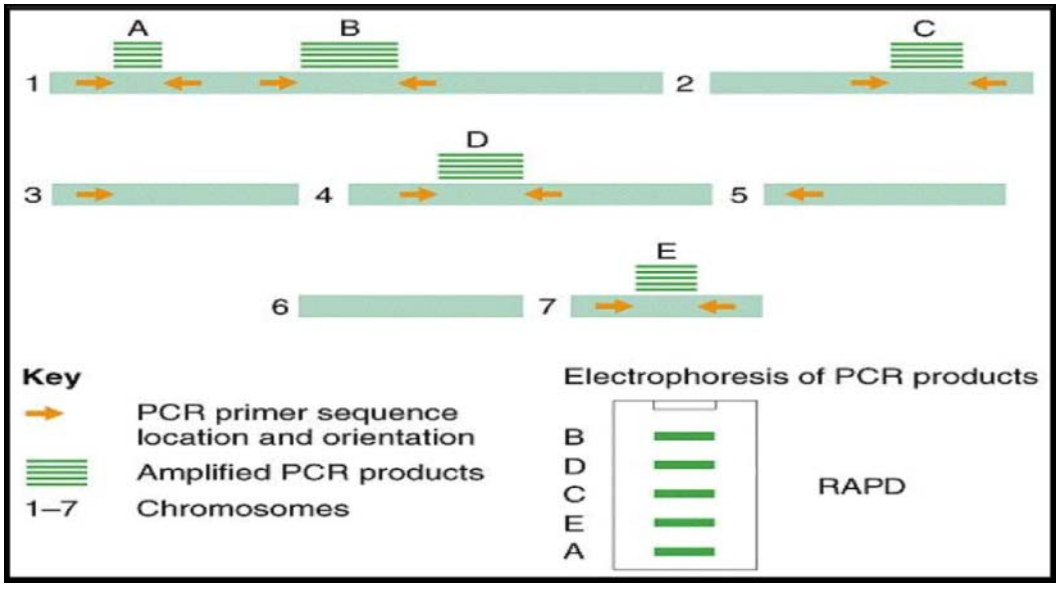


Figure (1 – 6): Schematic diagram of random amplified polymorphic DNA (Tingey *et al.*, 1994).

The product of the reaction depends on the sequence and the length of the oligonucleotide, as well as the reaction condition (Williams *et al.*, 1993), in which amplification with RAPD primers is extremely sensitive to single-base change in the primer-target site, i.e. a single base change in the primer sequence or in the genome may prevent amplification and cause complete change in the pattern of amplified DNA segments (Williams *et al.*, 1990; Williams *et al.*, 1993). The number of generated fragments is depending on the size of the genome and the length of the primer. The amplified products usually consist of 1 – 10 discrete bands of length between 200 – 2000 bp (Rafalski and Tingey, 1993; Jones *et al.*, 1997).

Hence, RAPD primers can detect polymorphisms that are usually noted by the presence or absence of an amplification product from a single locus (Tingey *et al.*, 1994). Polymorphism between individuals can be arising through:

1. Nucleotide change that prevent amplification by introducing by mismatch at one primer-site.
2. Deletion of a priming-site.
3. Insertions that render priming sites too distance to support amplification.
4. Insertions or deletions that change the size of the amplified product (Williams *et al.,* 1990).

DNA amplification reaction is repeated on a set of DNA samples with several different primers, under condition that results in several amplified bands from each primer (Williams *et al.*, 1993).

RAPD analysis offers the following advantages (Rafalski, 1997):

1. Non radio- active detection.

2. No prior DNA sequence information for genome is required.
3. Very small amounts of genomic DNA are sufficient.
4. It works with universal primer at any genome.
5. Experimental simplicity.
6. No need for expensive equipment beyond a thermo cycler and transilluminater.

Because of the advantages offered by RAPD technique, it has been found many applications in a wide variety of fields. RAPD fingerprints was used for identification of *Streptococci* strain (Truong *et al.,* 2000), RAPD-PCR analysis, also was used for genetic comparison of *Mycobacterium abscessus* strains (Zhang *et al.*, 1997). RAPD analysis was used to differentiate between two *Lactobacillus* species (Van-Reenen and Disks, 1996). RAPD technique was used in the genetic analysis of many fungi species, e.g. 19 isolates of *Aspergillus fumigatus* (London *et al*., 1993). Simplicity of this technique facilitated its use to reveal the genetic structure of different planet species (Klein-Lankhorst *et al*., 1991; Chaparro *et al*., 1992).

The RAPD method can simply and rapidly detect genetic alterations in the entire genome without knowledge of specific DNA sequence information (Papadopoulos *et al.,* 2002). Recently, the RAPD method was used as a mean for identifying the genetic alterations in human tumors and revealed that genetic alterations occurred frequently in different kinds of tumors (Wang *et al*., 2001; Wang *et al*., 2002); lung cancer (Ong *et al.*, 1998); squamous cell carcinoma of the head and neck (Maeda *et al.,* 1999); brain tumor (Dil-Afroze *et al.*, 1998); ovarian cancer (Sood and Buller, 1996); breast cancer and uveal melanoma (Papadopoulos *et al.,* 2002; Singh and Roy, 2001); hepatocellular carcinoma (Xian *et al.,* 2005; Zhang *et al.*, 2004) or human aberrant crypt foci and colon cancer (Luo and Pretlow, 2003); lymphoma (Scarpa *et al.*, 2001), in

leukemia (Odero *et al.*, 2001). It has been also applied in cancer research (Navarro and Jorcano, 1999) ; Detection of DNA damage and mutation (Atienzar *et al*., 2002); genotoxicity and carcinogensis studies (Atienzar and Jha, 2006), and found application in identification of novel DNA amplifications (Arribas *et al*., 1997; Garnis *et al.,* 2004).

Variation in the RAPD technique have been developed which share the same principle as RAPD analysis but detecting methods use different longer primers. These methods are called arbitrarily primed polymerase chain reaction (AP-PCR) and DNA amplification fingerprinting (DAF). AP-PCR methods used primers about 15 nucleotides long and acrylamide gels for product separation, and detection was achieved by using autoradiography. On the other hand primers shorter than 10 nucleotides were used in DAF method and products were also separated on acrylamide gels but were detected by using silver staining methods (Welsh and McClelland, 1990; Caetano-Annoles *et al*., 1991).

2.1 Materials

2.1.1 Apparatuses

Various apparatuses were used in this work are shown in table (2 - 1).

Table (2 - 1): The apparatuses used in this work

Apparatus	Company / Country
Analytical balance	Mettler Toledo / Switzerland
Autoclave	Lab tech. / Korea
Distiller	Mettler Toledo / Switzerland
Electrophoresis equipment	Biorad / U.S.A
Heater – magnetic stirrer	Neolab / India
Laminar flow hood	Micro flow / Germany
Microfuge	Boeco / Germany
Oven	Jrad / India
PCR (thermo cycler)	Appolo / U.S.A
pH- meter	Mettler Toledo / Switzerland
Shaker	Boeco / Germany
UV-Spectrophotometer	Genow / Switzerland
UV – transilluminator	Vilber lourmat / France
Vortex mixer	Spinixc / India
Water bath	Nickel electro ltd / India

2.1.2 Chemicals

The chemicals which were used in this work are shown in table (2 - 2).

Table (2 - 2): The chemicals used in this work

Chemicals	Company / Country
Agarose	USB / Spain
Boric acid	USB / Japan
Ethanol	BDH / England
Ethidium bromide	USB / U.S.A
Ethylene diaminetetra acetic acid (EDTA)	Scharlau / Spain
Glacial acetic acid	BDH / England
Hydrochloric acid	BDH / England
Isopropanol	SCP / England
Sodium hydroxide	BDH / England
Potassium acetate	Prolabo / E.C.C
Sodium dodecyl sulfate (SDS)	USB / U.S.A
Tris-base	SCP / England

2.1.3 Enzymes

The enzymes used in this study are shown in table (2 - 3).

Table (2 - 3): The enzymes which were used in this work.

Enzymes	Company/ Country
*Msp*I	Promega / U.S.A
*Hpa*II	Promega / U.S.A
Proteinase K	Applichem / Germany
RNase	Promega / U.S.A

2.1.4 Stock solutions.

The following solutions were prepared according to Sambrook and Russell (2001).

2.1.4.1 Tris-HCl (1 M, pH 8.0)

It was prepared by dissolving 60.55 g of Tris base in 400 ml of distilled water. The pH was adjusted to 8.0 by adding concentrated HCl (about 21 ml). The volume was completed to 500 ml with distilled water, dispensed into aliquots and sterilized by autoclaving.

2.1.4.2 Tris-HCl (1 M, pH 7.6)

It was prepared by dissolving 60.55 g of Tris base in 400 ml of distilled water. The pH was adjusted to 7.6 by adding concentrated HCl (about 30 ml). The volume was completed to 500 ml with distilled water, dispensed into aliquots and sterilized by autoclaving.

2.1.4.3 EDTA (0.5 M, pH 8.0)

It was prepared by dissolving 93.05 g of EDTA-2H_2O in 400 ml of distilled water with vigorous stirring on a magnetic stirrer. The pH was adjusted to 8.0 with NaOH (~20 g of NaOH pellets). The volume was completed to 500 ml with distilled water, dispensed into aliquots and sterilized by autoclaving.

2.1.5 Buffers and solutions for isolation of genomic DNA from blood (Sambrook and Russell, 2001).

The following buffers and solutions were used for isolation of genomic DNA from blood cells:

2.1.5.1 Red blood cell lysis buffer {Tris-HCl (20 mM)}

Tris-HCl (1 M, pH 7.6)	10 ml
Distilled water	490 ml

Store the buffer at room temperature.

2.1.5.2 Cell lysis buffer {Tris-HCl (10 mM), EDTA (1 mM) and SDS (0.1%(w/v))}

Tris-HCl (1 M, pH 8.0)	5 ml
EDTA (0.5 M, pH 8.0)	1 ml
SDS	0.5 g
Distilled water	494 ml

The buffer was stored at room temperature.

2.1.5.3 TE Buffer (pH 7.6) {Tris-HCl (10 mM), EDTA (1 mM)}

Tri-HCL (1M, pH 7.6)	5 ml
EDTA (0.5 M, pH 8.0)	1 ml
Distilled water	494 ml

The buffer was stored at room temperature.

2.1.5.4 Potassium acetate Solution

Potassium acetate (5 M)	60 ml
Glacial acetic acid	11.5 ml
Distilled water	28.5 ml

The buffer was stored at room temperature.

2.1.5.5 Proteinase K stock solution (20 mg/ml)

Proteinase K	20 mg
Distilled water	1 ml

The stock solution was divided into small aliquots and stored at -20^{o}C.

2.1.5.6 RNase stock solution (4 mg/ml)

RNase	4 mg
Distilled water	1 ml

The stock solution was divided into small aliquots and stored at -20^{o}C.

2.1.5.7 Isopropanol

Absolute isopropanol was stored at 4°C.

2.1.5.8 70% Ethanol

It was prepared by mixing 70 ml of absolute ethanol with 30 ml distilled water and stored at 4°C.

2.1.6 Genomic DNA isolation kit

The genomic DNA isolation kit was provided by Promega company / U.S.A. The components of the kit are the following:-

1. Cell lysis solution.
2. Nuclei lysis solution.
3. Protein precipitation solution.
4. DNA rehydration solution.
5. RNase A which is dissolved in DNA rehydration solution at a concentration of 4 mg/ml.

2.1.7 Solutions and buffers used in agarose gel electrophoresis (Sambrook and Russell, 2001).

2.1.7.1 5X TBE buffer (Tris / borate / EDTA) electrophoresis buffer

Tris – base	54 g
Boric acid	27.5 g
EDTA (0.5 M, pH 8.0)	20 ml

Distilled water	980 ml

2.1.7.2 Ethidium Bromide (10 mg/ml)

One gram of ethidium bromide was added to 100 ml of distilled water and stirred on a magnetic stirrer for several hours to ensure that the dye was dissolved. The container was warped with aluminum foil or kept in a dark bottle and stored at room temperature.

2.1.7.3 6X Loading buffer

It was provided by Fermentas Company/ Lithuania. Which was composed of Tris-HCl (10 mM, pH 7.6) , bromophenol blue (0.03%), xylene cyanol FF (0.03%), glycerol (60%) and EDTA (60 mM) .

2.1.8 RAPD-PCR amplification

The following chemicals were used for RAPD-PCR amplification.

2.1.8.1 2X PCR Master Mix

It was provided by Fermentas Company /Lithuania, with following composition:-

- *Taq* DNA Polymerase (0.05 units / µl).
- $MgCl_2$ (4 mM).

• dNTPs (dATP, dCTP, dGTP, dTTP), (0.4 mM of each).

2.1.8.2 Primers

Primers were provided by Alfa DNA Company / Canada in a lyophilized form which were diluted with sterile distilled water to a final concentration of 10 pmol/ml. The primers and their sequences are listed in table (2– 4).

Table (2 – 4): Primers sequences which were used for PCR amplification

Primer	Sequences
OPA- 01	5¯ CAGGCCCTTC 3¯
OPA- 09	5¯ GGGTAACGCC 3¯
OPA- 11	5¯ CAATCGCCGT 3¯
OPA- 12	5¯ TCGGCGATAG 3¯
OPA- 13	5 ¯ CAGCACCCAC 3¯
OPA-14	5¯ TCTGTGCTGG 3 ¯
OPB -11	5¯ GTAGACCCGT 3¯
OPB -12	5¯ CCTTGACGCA 3 ¯
OPB -15	5¯ GGAGGGTGTT 3 ¯
OPB -17	5¯ AGGGAACGAG 3 ¯
OPB -18	5 ¯ AGGTGACCGT 3 ¯
OPC-01	5¯ TTCGAGCCAG 3¯
OPE-05	5¯ TCAGGGAGGT 3¯
OPF-18	5¯ TTCCCGGGTT 3¯
OPI -18	5¯ TGCCCAGCCT 3¯
OPJ -01	5¯ CCCGGCATAA 3¯
OPJ -05	5¯ CTCCATGGGG 3¯
OPK -08	5¯ GAACACTGGG 3¯
OPW -17	5¯ GTCCTGGGTT 3¯
OPY-10	5¯ CAAACGTGGG 3¯
OPZ-02	5¯ CCTACGGGGA 3¯
OPZ-19	5¯ GTGCGAGCAA 3¯

2.1.9 DNA methylation analysis

2.1.9.1 Restriction Enzymes

Two restriction enzymes were used in this study (*Hpa*II and *Msp*I) which were provided with their buffers from Promega Company / U.S.A.

2.1.9.1.1 *Msp*I Enzyme:

It was supplied in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mg/ml BSA and 50% glycerol with the concentration of 10 U/µl. The *Msp*I buffer composed of 60 mM Tris-HCl (pH 7.5), 60 mM NaCl, 60 mM $MgCl_2$ and 10 mM DTT, with the concentration of 10X.

2.1.9.1.2 *Hpa*II Enzyme:

It was supplied in 10 mM Tris-HCl (pH7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol with the concentration of 8 U/µl. The *Hpa*II buffer composed of 60 mM Tris-HCl (pH7.5), 500 mM NaCl, 60 mM $MgCl_2$ and 10 mM DTT, with the concentration of 10 X.

2.1.9.2 Acetylated Bovine Serum Albumin (BSA):

It was provided from Promega Company / U.S.A with concentration of 0.1 mg/ml.

2.1.10 λ DNA ladder

λ DNA Ladder was used for DNA molecular size estimation. It was provided by Fermentas Company / Lithuania, with concentration of 0.5 µg/µl. Which was supplied in storage buffer composed of 10 mM Tris-HCl (pH 7.6), 1 mM

EDTA. The DNA ladder contained 10 discrete fragments (in base pair) as follow: 1031, 900, 800, 700, 600, 500, 400, 300, 200, and 100.

2.2 Methods

2.2.1 Sterilization methods

2.2.1.1 Autoclaving

Buffers and solutions were sterilized by pressure vessel (autoclave) at 121^{o}C and 15 bar for 15 minutes.

2.2.1.2 Dry heat

A laboratory oven was used for glass wares sterilization. The temperature of the oven was fixed at 200^{o}C for two hours.

2.2.2 Blood samples collection

Blood samples were collected from 65 patients with acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphoid leukemia (CLL). The patients were attending Al-Bairuni University Hospital and Al-Assad University Hospital from different regions of Syria. The period of blood sample collection was from 20/3/2007 to 30/9/2007. Five-milliliters of blood were obtained from each patients and controls (normal individuals), placed in tubes containing anti-coagulant (K_3EDTA) and kept at -20^{o}C till further use for genomic DNA extraction.

2.2.3 Genomic DNA isolation

2.2.3.1 Isolation of genomic DNA from whole blood (Sambrook and Russell, 2001).

1- 300 µl aliquot of whole blood was transferred to each of two microfuge tubes, then 900 µl of red blood cell lysis buffer (2.1.5.1) was added to each tube. The capped tubes were inverted to mix the contents of tube and incubated at room temperature for 10 minutes, with occasional inversion of the tubes.

2- The tubes were centrifuged at 13000 rpm for 20 seconds at room temperature in a microfuge, then most of the supernatant was discarded from each tube but left behind about 20 µl of each supernatant above the pellet.

3- The pellets of white cells were resuspended in the small amount of supernatant left in each tube, the resuspended cell pellets of two tubes were combined in a single tube.

4- The resuspended white blood cell pellets were then transferred to a microfuge tube containing 600 µl of ice-cold cell lysis buffer (2.1.5.2). The suspension was homogenized quickly with 30-50 strokes.

5- Three microliters of proteinase K solution (2.1.5.5) was added to the lysate, and incubated for three hours at 55^{o}C.

6- The tubes was allowed to cool at room temperature and then three microliters of 4 mg/ml RNase (2.1.5.6) was added, and incubated for 30 minutes at 37^{o}C.

7- The samples were allowed to cool at room temperature, then 200 µl of potassium acetate solution (2.1.5.4) was added and the contents were mixed by vortex vigorously for 20 seconds.

8- The precipitated protein /SDS complex was pelleted by centrifugation at 13000 rpm for 3 minutes at 4^{o}C in a microfuge.

9-The supernatant was transferred to a fresh microfuge tube containing 600 µl of isopropanol (2.1.5.7). The solution was mixed well and then the precipitated DNA was recovered by centrifuging the tube at 13000 rpm for one minutes at room temperature in a microfuge.

10- The supernatant was removed by aspiration and 600 µl of 70% ethanol (2.1.5.8) was added to the DNA pellet. The tube was inverted several times and then was run at 13000 rpm for one minute at room temperature in a microfuge.

11- The supernatant was removed by aspiration and the DNA pellet was allowed to dry in air for 15 minutes.

12- The pellet of DNA in microfuge tube was redissolved in 100 µl of TE buffer (2.1.5.3) and stored at -20^{o}C until used.

2.2.3.2 Isolation of genomic DNA by Promega kit

The protocol for genomic DNA isolation by promega kit was as following:-

1- 900 µl of cell lysis solution was added to a sterile 1.5 ml microfuge tube.

2- The tube of blood was gently rocked until thoroughly mixed, then 300µl of blood was transferred to the tube containing the cell lysis solution. The tube was inverted 5 - 6 times to mix.

3- The mixture was incubated for 10 minutes at room temperature (inverted 2-3 time during the incubation) to lyse the red blood cells. After that the mixture was centrifuged at 13,000 rpm for 20 seconds at room temperature.

4- As much as possible of supernatant was discarded with caution to avoid disturbing the visible white pellet. Usually approximately 10-20 µl of residual liquid will remain in the tube.

5- The contents of the tube were mixed vigorously by vortex mixer until the white blood cells were resuspended (10-15 seconds).

6- 300 µl of nuclei lysis solution was added to the tube containing the resuspended cells. The solution was pipetted 5 - 6 times to lyse the white blood cells.

7- 1.5 µl of RNase solution was added to the nuclear lysate and the sample was mixed by inverting the tube 2-5 times. The mixture was incubated at 37°C for 15 minutes, and then chilled on ice for 5 minutes.

8- Protein precipitation solution 100 µl was added to the nuclear lysate and vortexed vigorously for 10-20 seconds, then centrifuged at 13,000 rpm for three minutes at room temperature.

9- The supernatant was transferred to a clean tube containing 300 µl of isopropanol (2.1.5.7).

10- The solution was mixed gently by inversion until the white thread-like strands of DNA form a visible mass, then the tube was centrifuged at 13000 rpm for one minute at room temperature. Then the DNA will be visible as a small white pellet.

11- The supernatant was removed, and 300 µl of 70% ethanol was added to the DNA pellett. The tube was inverted gently several times to wash the DNA pellet completely from the microfuge tube, and then centrifuged at 13000 rpm.

12- The ethanol was aspirated using a drawn Pasteur pipette. The tube was inverted on clean absorbent paper and air-dries the pellet for 10-15 minutes.

13- 100 µl of DNA rehydration solution was added to the tube and rehydration of the DNA was carried out by incubating at 65°C for 1 hour. Periodical mixing of the solution was done by gently tapping the tube.

2.2.4 DNA Purity and concentration

Measurement of DNA purity and concentration were carried out by using jenaw spectrophotometer which designed for such measurement. Moreover, estimation of DNA concentration and purity were determined according to the procedure reported by Sambrook and Russell (2001).

2.2.4.1 Estimation of DNA concentration

10 µl of prepared DNA sample was added to 990 µl of TE buffer (pH 7.6) and mixed thoroughly, then the optical density (O.D) was measured in a UV spectrophotometer at wavelength of 260 nm. The DNA concentration in the solution was calculated according to the following formula:

DNA concentration (µg/µl) = ($O.D_{260}$ X 100 X 50 µg/ml) / 1000

Theoretically, O.D value of one is corresponding to approximately (50 µg/ml) for double strand DNA.

2.2.4.2 Estimation of DNA purity

The ratio between the readings at 260 nm and 280 nm provides an estimate of the purity of nucleic acid, and was measured as following:-

$$\text{DNA Purity} = O.D_{260} / O.D_{280}$$

Pure preparations of DNA samples have ($O.D_{260}$ / $O.D_{280}$) values of 1.7 and 2.0 (Manchester, 1995).

2.2.5 Estimation of molecular size of DNA bands

The λ DNA ladder which was used in this study contained 10 discrete fragments (in base pair): 1031, 900, 800, 700, 600, 500, 400, 300, 200, and 100 from nearest distance to the well to the far one from the well respectively. This Ladder was used as a molecular size indicator in the experiments of this study. The molecular sizes of DNA bands were estimated according to the standard curve as seen in fig.(2 – 1) represents the relationship between band molecular weight and distant of migration. The molecular weights (in units of base pairs (bp) for DNA) is plotted on the Y-axis and the distance the molecule migrated (in mm) is plotted on the X-axis (Sambrook and Russell, 2001).

Distance (mm)	Size (bp)
47	1031
52	900
56	800
60	700
66	600
70	500
64	**?**

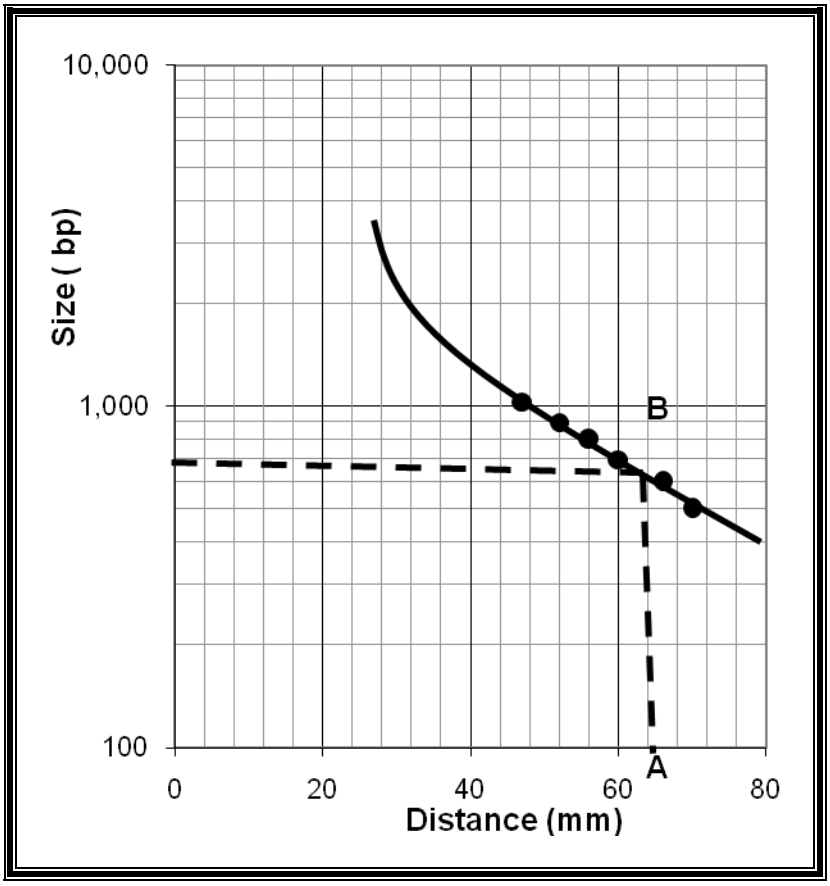


Figure (2 - 1): Standard curve for estimation of molecular sizes of DNA bands (Sambrook and Russell, 2001).

2.2.6 Agarose gel electrophoresis

Agarose gel (1.0-1.5%) containing ethiduim bromide with final concentration 0.5 µg/ml were prepared in 1X TBE buffer (2.1.7.1). Samples of DNA were mixed with 1/5 volume of loading buffer and added to the well on the gel. Generally, the gel buffer added up to the level of horizontal gel surface and gels were run for 2 - 3 hours at 3-5 v/cm. DNA bands were visualized by UV illumination at 366 nm wavelength on UV illuminator. Gels were destained in distilled water for 30-60 minutes to get rid of excess of stain before photographed (Sambrook and Russell, 2001).

2.2.7 RAPD PCR amplification

The reaction mixture of RAPD PCR was prepared according to the addition order shown in table (2 – 5).

The reaction mixture samples were mixed gently by vortex and centrifuged at 13000 rpm for few seconds to collect all drops to the bottom of the tubes. The tubes were then placed in the Appollo thermal cycler (with heating lid) to carry out amplification. The amplification was run according to the program shown in table (2 – 6). 20 µl of the amplified DNA was drawn into another tube and analyzed by agarose electrophoresis (2.2.8) .

Table (2 - 5): Reagents and their addition order for preparation of reaction mixture used in RAPD PCR amplification.

Addition order	Component	Volume	Concentration
1 -	Water, nuclease free	8.0 µl	-
2 -	PCR Master Mix	12.5 µl	2X
3 -	Primer	2.5 µl	10 pmol/ml
4 -	Template DNA	2.0 µl	25 ng/ µl
Note:- Final volume = 25 µl			

Table (2 – 6): Amplification program (Xian *et al*., 2005):-

Stage	Cycle	Step	Temperature	Time (min.)	State
1	1	1	94^{o}C	2:00	initial denaturation

2	40	1	94°C	0:30	denaturation
		2	38°C	1:00	annealing
		3	72°C	2:00	extension
3	1	1	72°C	10:00	final extension

2.2.8 DNA methylation analysis:

Restriction digestion of genomic DNA with methylation specific enzymes *(Hpa*II and *Msp*I) were done according to manufactures' protocol (Promega) in a sterile 0.5 ml eppendorff tubes (table 2 - 7). The reaction mixture was mixed gently by pipetting, then the tube was centrifuged at 13,000 rpm for a few seconds in a microfuge and incubated at 37°C for four hours. 50 ng of digested DNA with either *Hpa*II or *Msp*I were then amplified with nine PCR primers which were used in RAPD PCR analysis.

Table (2 – 7): The protocol for restriction enzyme (*Hpa*II and *Msp*I) digestion of genomic DNA reagents.

Addition Order	Component	Volume	Concentration
1 -	RE Buffer	2 µl	10X
2 -	Acetylated BSA	0.2 µl	10 µg/µl
3 -	DNA	1 µl	1 µg/µl
4 -	Restriction Enzyme	0.5 µl	8-10 u/µl
5 -	Sterile, deionized water	16.3 µl	-
Note:- Final volume = 25 µl			

3.1 leukemia patients

In this study 65 leukemia patients (appendix1) were subjected to genomic analysis by RAPD-PCR, these patients were distributed into four groups, acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphoid leukemia (CLL). Table (3 – 1) showed the incidence of four types of leukemia (AML, ALL, CML, and CLL) in males and females, and in various age groups. The results showed that higher incidence was observed in ages (32 – 40) years and overall incidence in males is higher than in females. In this regard other investigators found that the incidence of leukemia in males was higher than that in females. This is being chiefly due to greater incidence of leukemia observed in young boys and middle age men (Borson and Leob, 1994; Hoffbrand *et al.*, 2002).

Table (3 - 1): Distribution of leukemia according to the age and gender.

Age (Year)	Gender	Case no.	Percentage incidence of leukemia %	Total
2 – 11	Females	2	3.1	7.7
	Males	3	4.6	
12 – 21	Females	3	4.6	13.8
	Males	6	9.2	
22 – 31	Females	6	9.2	18.4
	Males	6	9.2	
32 – 41	Females	6	9.2	20.0
	Males	7	10.8	
42 – 51	Females	6	9.2	12.3
	Males	2	3.1	
52 – 61	Females	4	6.2	13.9
	Males	5	7.7	
62 – 71	Females	2	3.1	9.3
	Males	4	6.2	
72 – 95	Females	1	1.5	4.6
	Males	2	3.1	
Total	-	65	-	100%

The results which were shown in table (3 - 2) demonstrated the incidence of ALL in the age groups for both females and males. The results presented in this table showed that there is a higher incidence for ages (12 – 21) years, and the incidence in male childhood is approximately equal to female childhood. Lowenberg *et al.*, 1999 indicated that the incidence of ALL was higher in childhood and equal in both sex.

Table (3 - 2) Distribution of Acute Lymphoid Leukemia according to the age and gender.

Age (Year)	Gender	Case no.	Percentage incidence of leukemia	Total
2 – 11	Females	2	14.3	35.7
	Males	3	21.4	
12 – 21	Females	3	21.4	42.8
	Males	3	21.4	
22 – 32	Females	1	7.2	21.5
	Males	2	14.3	
Total	-	14	-	100%

Table (3 – 3) showed the incidence of AML in various age groups. The observed results indicated that the incidence for ages (36 – 45) years was high, and the incidence for females is more than in males, similar results were reported by (Mukeef, 1999). In this respect other investigators reported the incidence of AML in males was slightly more than females and it occurred in all age groups (Lowenberg *et al.*, 1999).

Table (3 - 3) Distribution of Acute Myeloid Leukemia according to the age and gender.

Age (Year)	Gender	Case number	Percentage incidence of leukemia	Total
16 – 25	Females	0	-	11.8
	Males	2	11.8	
26 – 35	Females	3	17.6	17.6
	Males	0	-	
36 – 45	Females	3	17.6	29.4
	Males	2	11.8	
46 – 55	Females	2	11.8	11.8
	Males	0	-	
56 – 65	Females	1	5.9	17.7
	Males	2	11.8	
66 –75	Females	1	5.8	11.6
	Males	1	5.8	
Total	-	17	-	100%

The results presented in table (3 – 4) showed the incidence of CLL in various age groups. The results showed that the CLL incidence for ages (67 – 76) years was high in males in comparison with other age groups, and the

incidence of males is more than females, similar result reported by (Kipps, 2000).

Table (3 - 4): Distribution of Chronic Lymphoid Leukemia according to the age and gender.

Age (Year)	Gender	Case number	Percentage incidence of leukemia	Total
27 – 36	Females	0	-	10.0
	Males	1	10.0	
37 – 46	Females	1	10.0	20.0
	Males	1	10.0	
47 – 56	Females	1	10.0	20.0
	Males	1	10.0	
57 – 66	Females	0	-	20.0
	Males	2	20.0	
67 – 76	Females	0	-	30.0
	Males	3	30.0	
Total	-	10	-	100%

The results for CML patients were shown in table (3 – 5), revealed that the higher age group frequency was (31 – 45) years, with equal incidence of CML in female and males. Other investigator reported that the ratio of incidence of

male to female is 1.4:1 and it occurs more frequently in middle age (Hoffbrand *et al.,* 2002).

Table (3 - 5) Distribution of Chronic Myeloid Leukemia according to the age and gender.

Age (Year)	Gender	Case number	Percentage incidence of leukemia	Total
21 – 30	Females	2	8.3	16.6
	Males	2	8.3	
31 – 40	Females	4	16.6	45.8
	Males	7	29.2	
41 – 50	Females	3	12.5	16.7
	Males	1	4.2	
51-60	Females	1	4.2	12.5
	Males	2	8.3	
61 – 70	Females	1	4.2	4.2
	Males	0	-	
71 – 95	Females	1	4.2	4.2
	Males	0	-	
Total	-	24	-	100%

In general, the reported results in this study showed that acute leukemia occurred at any age mainly in early childhood and a progressive rise in incidence was observed with age. Over all incidences, AML occurs in all age groups, while ALL is the most common leukemia of childhood. CML and CLL are rarely found in childhood, but occurred in adolescents and adults.

In this study we had shown that the frequency of occurrence of leukemia patients were in the following order: CML, AML, ALL and CLL.

3.2 Molecular genetic analysis

This study included molecular genetic analysis of genomic DNA extracted from peripheral blood of 65 patients with various types of leukemia (ALL, AML, CLL and CML) and ten normal individuals (five males and five females) as a control with leukemia patients.

3.2.1 Genomic DNA isolation from blood cells

Two methods were used for isolation of genomic DNA from human whole blood of leukemia patients. The first one (rapid isolation method of genomic mammalian DNA) was described by Sambrooke and Russell, (2001). The other authors indicated that DNA extracted by this method is suitable for use as a template in PCR experiment. In this study, the quantities of DNA which were obtained from 600 µl of whole blood of normal individuals and leukemia patients were equal or less than 50 µg, and the purity of the prepared DNA was less than 1.0. DNA preparation with such quality might be suitable for specific PCR assays (Saiki *et al.*, 1990). Although, RAPD-PCR technique does not require large quantities of DNA (Rafalski, 1997), but this assay and restriction enzyme experiments required highly purified DNA (Strauss, 2002). Hence, the genomic DNA obtained by this method was found not suitable for

the purpose of experimental work designed in this research. Therefore another method of DNA extraction was followed, namely by using extraction kit.

The second method for extraction of genomic DNA was carried out with wizard genomic DNA purification kit obtained from Promega Company. In this method suitable quantities of DNA approximately (100 – 600 µg) were obtained from 300 µl of whole blood. The purity of isolated DNA ranged from (1.6 - 1.9). Fig. (3 - 1) illustrated the differences in DNA yield between the two methods used for DNA isolation from the same blood samples.

According to these results it was found that the second method is more suitable method for DNA isolation from whole blood for RAPD-PCR analysis and restriction enzyme experiments, other investigators reported similar results (Neri *et al.*, 2004; Yu *et al.*, 2006; Tan *et al.*, 2006).

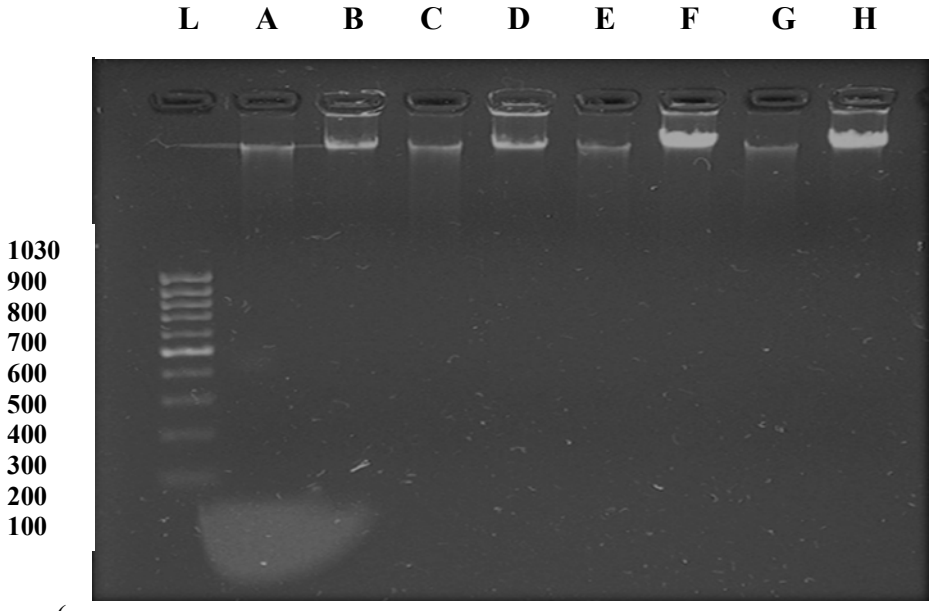


Fig (3 – 1): Agarose gel (1%) electrophoresis of the DNA samples isolated from four patients, with run 3volt/cm for two hours. The lanes A, C, E, and G represent the DNA bands of leukemia patients obtained with Sambrook's method lanes B, D, F, and H represent the DNA bands of leukemia patients obtained with wizard genomic DNA isolation kit method. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

Various obstacles faced extraction of genomic DNA from several leukemia patients. These problems could be summarized as following :

1- The two methods which were used for DNA extraction in this study were found not suitable for DNA extraction from clotted blood.
2- The low count of W.B.C which result in low genomic DNA yield.

Figure (3 - 2) illustrated the number of leukemia cases that were studied for DNA extraction considering blood samples characteristic, i.e clotted blood, low and high W.B.C count. Only 45 patients of the total number of leukemia cases gave good yield of DNA. The rest of cases were distributed between ten cases of clotted blood, and ten cases showed low W.B.C count with low DNA concentration.

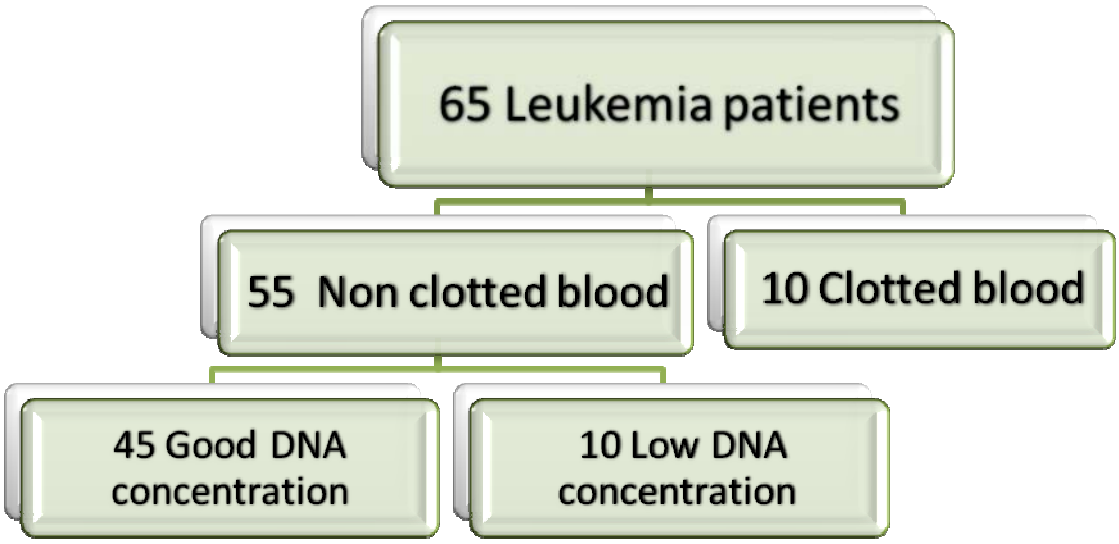


Figure (3 – 2): The characteristics of blood samples and DNA yield from leukemia patients.

3.2.2 RAPD-PCR Analysis

In this study (22) different arbitrary primers of decamer oligonucleotides were screened for RAPD analysis using genomic DNA isolated from (45) patients

of different types of leukemia (ALL, AML, CLL and CML). The control RAPD experiments were carried out by isolation of genomic DNA from ten normal individuals. DNA pooling procedure was used for normal male and female individuals (Sham *et al.*, 2002). RAPD technique is known to be sensitive to the reaction conditions (Penner *et al.*, 1993). Therefore, a number of preliminary experiments were carried out. The first amplification resulted products with long smeary forms. Therefore, the results could not be analyzed because no clear and discrete bands could be detected. This smearness of RAPD product is generally contributed to several reaction condition factors such as: DNA concentration, primer quality and concentration, the Taq polymerase and dNTPs concentration, and the number of the cycles in the program (Rafalski, 1997). Therefore, in later experiments, attempts were made to empirically optimize these factors. These included the retesting of genomic DNA concentration prepared, accuracy of pipetting, well mixing of the reaction contents, and changing the cycles number required for amplification (Benter *et al.*, 1995). After optimizing these conditions, successful results were obtained using the same primers. After the optimization of RAPD-PCR reaction, the obtained results classified the 22 different primers into two categories:

In the first category, no amplified products were detected. In spite of repeating a number of experiments, similar results were obtained using 13 different primers. These included OPA-01, OPA-11, OPA-12, OPA-14, OPB-11, OPB-12, OPC-01, OPF-18, OPI-18, OPJ-01, OPK-08, OPY-10 and OPZ-19. The failure of these primers to amplify the genomic DNA of normal persons and leukemia patients may be attributed to the absence of suitable priming sites for these primers in the genomes of tested leukemia patients and normal individuals. In other words, there are no complementary sequences for these primers in leukemia patients and normal individual's genomes. Similar

results have been reported by other investigators. Papadopoulos and his coworkers identified a number of RAPD primers that didn't show amplification products in breast cancer and uveal melanoma (Papadopoulos *et al.*, 2002).

Whereas primers in the second category were found to produce amplified products, this category included nine primers, namely OPA-09, OPA-13, OPB-15, OPB-17, OPB-18, OPE-05, OPJ-05, OPW-17 and OPZ-02. When using these primers, a reasonable degree of DNA polymorphism was detected between normal person and leukemia patients. According to RAPD analysis, DNA polymorphisms among individuals are generally revealed and detected in three forms (Dil-Afroze *et al*., 1998; Papadopoulos *et al.*, 2002; Wang *et al*., 2002):

1. The presence or absence of DNA bands.
2. Differences in the molecular weight of bands.
3. Number of amplified bands.

In this study nine primers were used to investigate four different types of leukemia, and each primer had shown various number and pattern of bands. Thus, a detailed survey of RAPD results observed in each type of leukemia when using each of these nine primers will be described.

3.2.2.1 Primer OPA-09

This primer which has the sequence of (GGGTAACGCC) gave various numbers and patterns of bands across the lanes for four types of leukemia as seen in figure (3 – 3, a, b, c and d). Amplified bands were detected ranged (2 - 4) across the lanes. The molecular weights of these bands ranged (1000 -

3162) bp. Although, this primer amplified detectable and discrete bands, there were loss of amplified products in several lanes (lane 2 in fig. 3 – 4, lanes 2, 5, 6, 8, 9, 11, 13 in fig. 3 – 5) which represent CML1, AML1, AML4, AML5, AML7, AML8, AML9 and AML11 patients respectively. The loss of amplified products in these lanes may be caused by the absence of binding sites for this primer in the genome of these patients (Mahenthiralingam *et al.*, 1996). The results which were shown in figures (3 – 3, 3 – 4, 3 – 5 and 3 - 6) demonstrated that the DNA polymorphism which can be easily detected by primer OPA-09 among the leukemia patients. Loss or gain of amplified band(s) in leukemia patient in comparison with normal individual is clearly detectable. For example the band number one with molecular weight 3162 bp lost in ALL1 , ALL2 , ALL3 , ALL4 , ALL6 , ALL7 , ALL9, ALL10, ALL11, AML2, AML3 , AML6 , AML10, CLL1 , CLL2 , CLL3 , CLL4 , CLL5 , CLL6, CLL8, CML2, CML3, CML4, CML5, CML6, CML7, CML10, CML11, CML12, CML13 and CML15, while this band was present in normal individuals and other leukemia patients.

The bands number two and three with molecular weight 2187 and 1660 bp respectively were present in all normal samples and in all four types' leukemia patients, except in patient CLL 9, band number two was lost.

The band number four with molecular weight 1000 bp was gained in ALL2 , ALL3 , ALL4 , ALL6 , ALL7, AML3, CML4 and CML9 while this band was absent in normal persons and other patients.

3.2.2.2 The primer OPA- 13

RAPD analysis with primer OPA-13 which has the sequence (CAGCACCCAC) resulted in amplified bands ranging between (5 – 13) bands across the lanes as shown in fig (3 – 7, 3 – 8, 3 – 9 and 3 - 10). The molecular weight of these bands ranged between (250 - 1260) bp.

The bands number one and two with molecular weight 1260 and 910 bp respectively were present in most of leukemia patients and normal individuals, but were absent only in AML5 patient. The band number one was absent in CLL2 and CLL3 patients.

The band number three with molecular weight 810 bp was absent in AML5, CLL2, CLL3, CLL4, CLL5, CML1 and CML7 patients and it was present in normal individuals and in other leukemia patients.

The band number four with molecular weight 760 bp was present in normal individuals and in the majority of leukemia patients, however it was absent in AML5, CLL1, CLL5, CLL6, CLL7, CML1, CML2, CML3, CML4, CML5, CML6, CML7, CML10, CML11 and CML12 patients.

The band number five with molecular weight 740 bp was absent in ALL1, AML5, AML11, CML7 and in CLL patients, on the other hand, it was present in other leukemia patients and normal male individuals.

The bands number six, seven, nine and 12 with molecular weights 730, 600, 510 and 300 bp respectively were present in all leukemia patients and normal individuals.

The band number eight with molecular weight 580 bp was present in ALL7, ALL8, ALL10, ALL11, AML6, AML7, AML8, CLL3, CLL4, CLL5, CLL5, CLL6, CLL7 and CLL8, while this band was absent in normal individuals and in other leukemia patients.

The band number ten with molecular weight 490 bp was absent in ALL1, ALL4, ALL5, ALL7, ALL9, ALL10, ALL11, AML3, AML11, CLL3, CLL5, CLL6, CML1, CML3, CML4, CML5, CML6, CML7, CML10 and CML15. However this band was present in normal individuals and other leukemia patients.

The band number 11 with molecular weight 350 bp was absent in normal individuals, ALL1, ALL4, ALL5, ALL6, ALL9, ALL11, AML3, AML4, AML7, AML8, AML11, CLL3, CLL4, CLL5, CML5, CML6 and CML9. On other hand this band was present in other leukemia patients.

The last band (number 12) with molecular weight 250 bp was absent in normal individuals and in most leukemia patients, while it was present in ALL2, ALL3, ALL4, ALL5, ALL6, ALL7, ALL8, AML1, AML2, CLL2, CLL3, CLL7 and CML9.

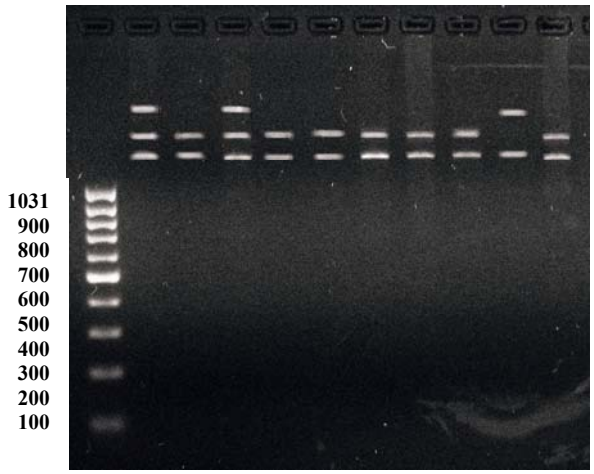


Figure (3 – 3): RAPD-PCR analysis of band patterns of CLL patients obtained with OPA-09 primer. Electrophoresis was performed on (1.5%)

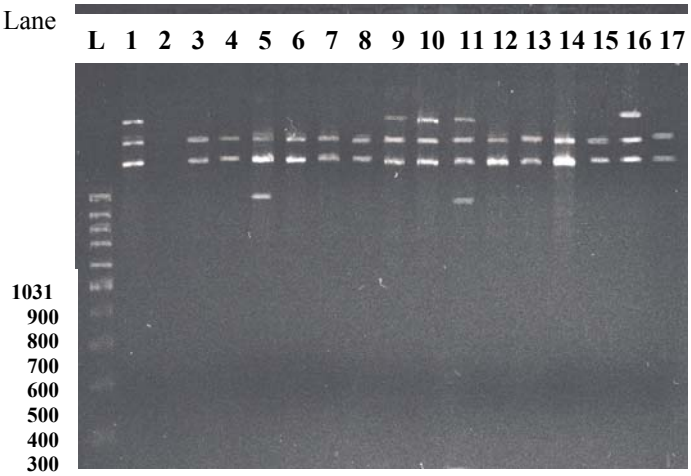


Figure (3 – 4): RAPD-PCR analysis of band patterns of CML patients obtained with OPA-09 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males,

males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

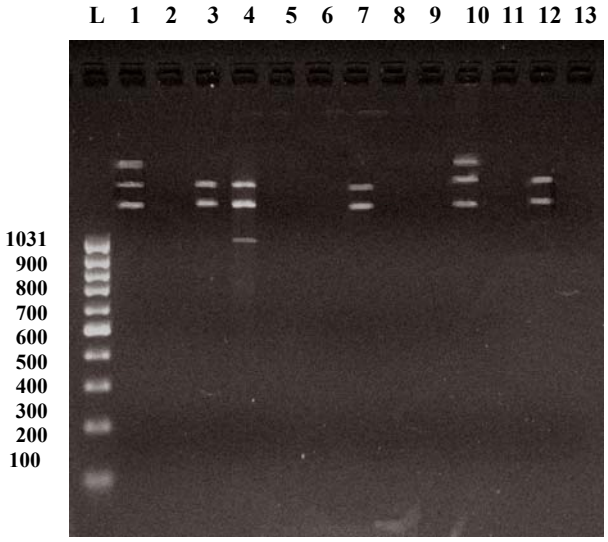


Figure (3 – 5): RAPD-PCR analysis of band patterns of AML patients obtained with OPA-09 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

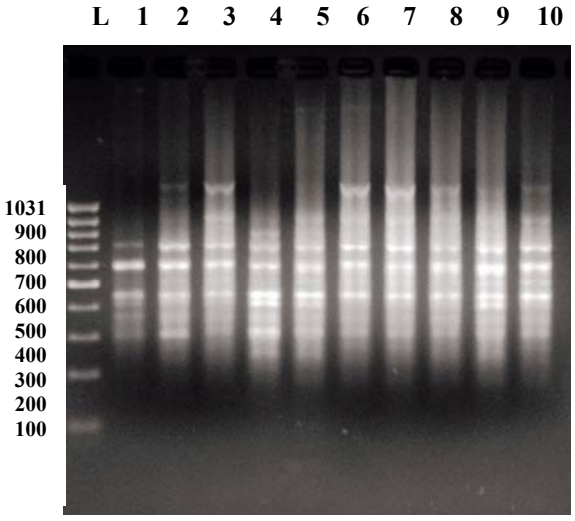


Figure (3 – 7): RAPD-PCR analysis of band patterns of CLL patients obtained with OPA-13 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL

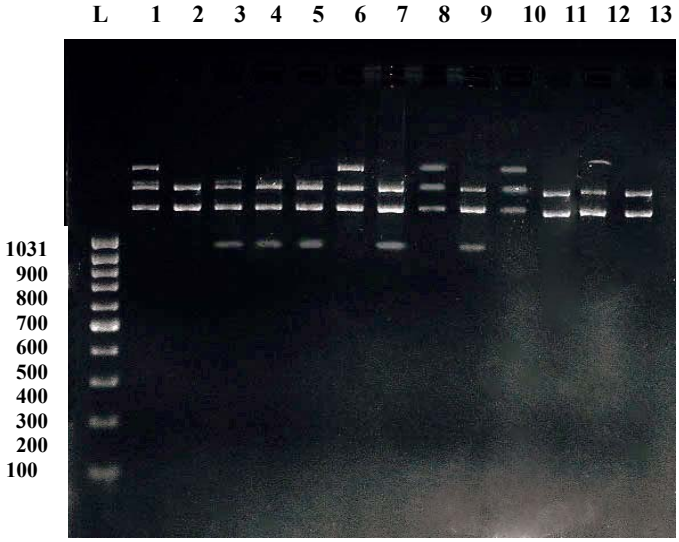


Figure (3 – 6): RAPD-PCR analysis of band patterns of ALL patients obtained with OPA-09 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

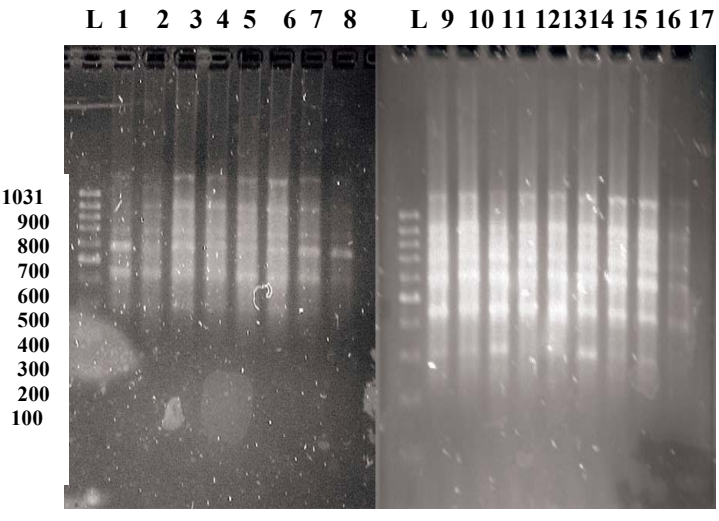


Figure (3 – 8): RAPD-PCR analysis of band patterns of CML patients obtained with OPA-13 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

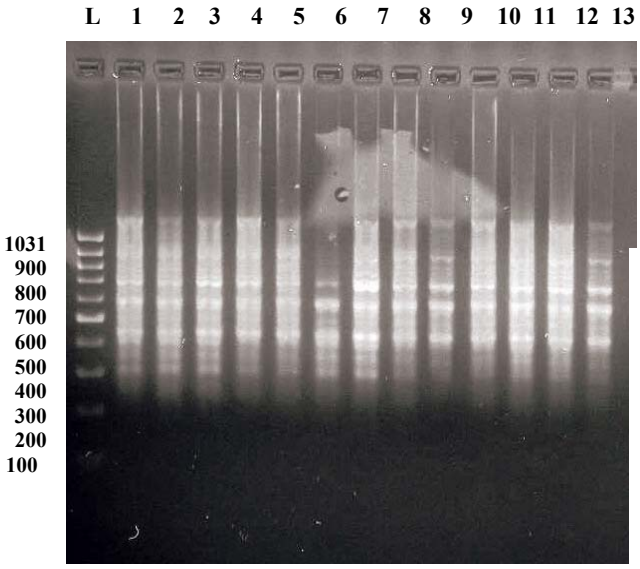


Figure (3 – 9) : RAPD-PCR analysis of band patterns of AML patients obtained with OPA-13 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

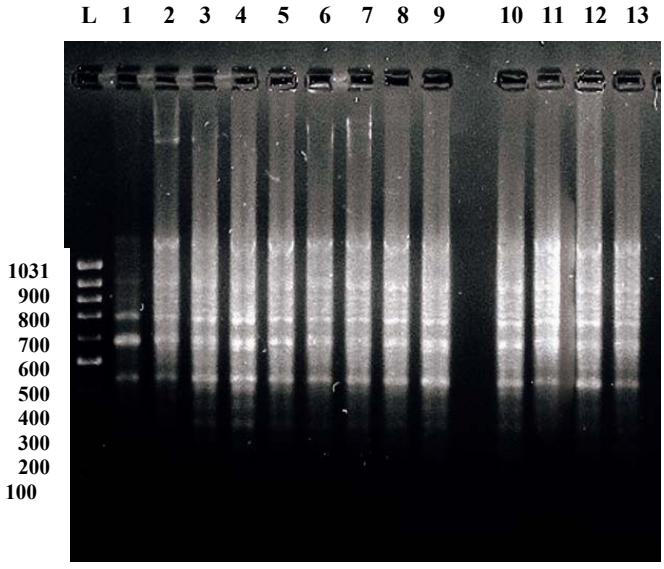


Figure (3 – 10) : RAPD-PCR analysis of band patterns of ALL patients obtained with OPA-13 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

3.2.2.3 The primer OPB-15

RAPD analysis using this primer which has the sequence of (GGAGGGTGTT) gave a total number of 433 bands. These bands ranged between (2 - 13) across the lanes as seen in Figure (3 – 11, 3 – 12, 3 – 13 and 3 - 14). This primer gave bands with molecular weights ranged between (95 - 2560) bp. The band number one with molecular weight 2560 bp was present in ALL2, ALL3, ALL4, ALL5, ALL6, ALL7, ALL8, ALL9, ALL10, ALL11, AML3, AML9, AML10, CLL1, CLL4, CLL5, CLL6, CML8, CML14 and CML15. On the other hand this band was absent in normal individuals and other leukemia patients.

The bands number two, three and four with molecular weights 2300, 2100 and 1950 bp respectively were present only in ALL 2, ALL3, ALL4, ALL5, ALL6, ALL8, ALL9, ALL10, ALL11, CLL1, CLL4, CLL5, CLL6 and CLL7. Bands number two and three only were present in AML 3. Bands number two and four were present only in CLL2 and CLL3. Bands number three and four were present only in CML2, CML3, CML4, CML5, CML6, CML8, CML11, CML12, CML14, CML15. However these bands were absent in normal individuals and other leukemia patients.

The bands number five and six with molecular weight 1080 and 900 bp respectively were present in normal individuals and most of leukemia patients, other patient had the band shifting of these bands. The band shifting of band number five with molecular weight 1140 bp. was present in ALL1, AML1, CML1, CML2, CML3, CML4, CML5, CML6 and CML7. The band shifting of band number six with molecular weight 950 bp was present in ALL1, AML1, AML5 and AML7.

The band number seven with molecular weight 760 bp was absent in AML1, AML4, AML5, AML6, AML7, AML8 and present in normal individuals and other leukemia patients. It is noteworthy to mention that the band with molecular weight 800 bp (probably band shift of band number seven) was present in ALL1 and AML3.

The band number eight with molecular weight 690 bp was present in normal individuals, ALL2, ALL3, ALL4, ALL5, ALL6, ALL7, ALL8, ALL9, ALL10, ALL11, AML patients and CLL2. Here band shifting also was observed, the band shift of band number seven with molecular weight 710 bp was present in ALL1.

The band number nine with molecular weight 580 bp was present in normal individuals and most of leukemia patients except in AML1, AML2, AML4, AML5, AML6, AML7, AML8, AML9 and AML10 was lost.

The bands number ten and 11 with molecular weights 480 and 460 bp respectively were present in normal individuals, ALL2, ALL7, ALL8, ALL9, ALL10, ALL11, AML2, AML3, AML9, CLL patients, CML2, CML4, CML5, CML6, CML8, CML9, CML11, CML12 and CML14. Band number 11 was present in ALL3, ALL4, ALL5 and ALL6. These bands were absent in other leukemia patients.

The band number 12 with molecular weight 360 bp was present in normal individuals, ALL2, ALL3, ALL4, ALL5, ALL6, ALL7, ALL8, ALL9, ALL10, ALL11, CLL2, CLL3, CLL6 and CLL7. This band was absent in other leukemia patients.

The band number 13 with molecular weight 290 bp was present in normal individuals and in ALL, CLL and CML leukemia patients, but was absent in AML patients.

The band number 14 with molecular weight 95 bp was present in normal individuals and in all leukemia patients.

3.2.2.4 The primer OPB-17

A total number of 255 amplified bands were detected when using this primer that has the sequence of (AGGGAACGAG). This primer amplified bands ranging between (3 - 11) across the lanes as seen in Figure (3 – 15, 3 – 16, 3 - 17 and 3 - 18). The molecular weights of these bands ranged between 250 - 1770 bp. The bands number one and two with molecular weight 1770 bp and 1620 bp respectively were absent in normal individuals and most leukemia patients except ALL2, ALL3, ALL4, ALL5, ALL7, ALL10, AML2, AML3, AML6, AML9, AML10, CLL5, CLL6, CML1, CML2 and CML4, thus these two bands were considered gain bands. On the other hand band number one was considered a gain band in following patients: ALL 8, ALL9, ALL11, AML4, AML5 and AML7.

The band number three with molecular weight 1240 bp was present in ALL2, ALL4, ALL5, ALL8, ALL11, AML2, AML3, AML4, AML5, AML6, AML9, AML10, CLL3, CLL5, CLL6, CLL7. The band shifting of this band was observed with molecular weight of 1.18 kbp which was present in ALL10. However this band was absent in normal individuals and other leukemia patients.

The band number four with molecular weight 1020 bp was present in ALL2, ALL3, ALL4, ALL5, ALL7, ALL8, ALL9, ALL10, ALL11, AML2, AML3,

AML6 and CML4. It was absent in normal individuals and other leukemia patients.

The band number five with molecular weight 960 bp was present in normal individuals, ALL3, ALL8, ALL9, AML1, AML4, AML5, AML7, AML8, CLL3, CLL6 and CLL8. There were two bands shifting of this band, first band shifting with molecular weight 940 bp was present in ALL7, ALL10, AML2, AML3, AML6, AML9, AML10, AML11, CLL1, CLL4, CLL5, CLL7, CML1, CML2, CML3, CML4, CML5, CML6, CML7, CML8, CML9, CML12, CML13, CML14 and CML15. The second band shifting with molecular weight 980 bp was present in ALL1, ALL2, ALL4, ALL5, ALL6, ALL11. This band was absent in CLL2, CML10 and CML11.

The band number six with molecular weight 860 bp was present in normal individuals, ALL3, ALL8, ALL9, ALL11, AML1, AML4, AML5, AML7, AML8, CLL and CML patients. There were two bands shifting of this band, first with molecular weight 830 bp was present in ALL7, ALL10, AML2, AML3, AML6, AML9, AML10 and AML11. The second band shifting with molecular weight 880 bp was present in ALL1, ALL2, ALL4, ALL5 and ALL6.

The band number seven with molecular weight 660 bp was present in normal individuals, ALL1, ALL6, ALL7, ALL8, ALL9, ALL10, ALL11, AML2, AML3, AML5, AML6, CLL patients, CML1, CML2, CML4, CML8, CML9, CML13, CML14 and CML15. The band shifting of this band with molecular weight 680 bp was present in ALL2, ALL3, ALL4 and ALL5. In other hand this band was absent in other leukemia patients.

The band number eight with molecular weight 560 bp was present in AML patients. This band was absent in normal individuals and other leukemia patients. The band number nine with molecular weight 500 bp was present in normal individuals, ALL2, ALL3, ALL4, ALL5, ALL7, ALL9, ALL10, ALL11, CLL1, CLL5, CML1, CML2, CML4, CML5, CML6, CML7, CML8, CML10, CML11, CML12, CML13, CML14 and CML15. This band was absent in other leukemia patients.

The band number ten with molecular weight 430 bp was present in normal individuals, ALL2, ALL3, ALL7, ALL9, ALL10, AML2, AML3, AML5, AML6, AML9, AML10, CLL1, CLL4, CLL5 and CLL7. However, this band was lost in other leukemia patients.

The band number 11 with molecular weight 390 bp was absent in normal individuals and most leukemia patients, but was gained in AML2, AML3 and AML6.

The band number 12 with molecular weight 250 bp was absent in normal individuals and most leukemia patients, but was present in AML1, AML2, AML3, AML6, AML9 and AML10.

L 1 2 3 4 5 6 7 8 9 10 **L 1 2 3 4 5 6 7 8 L 9 10 11 1213 14 15 16 17**

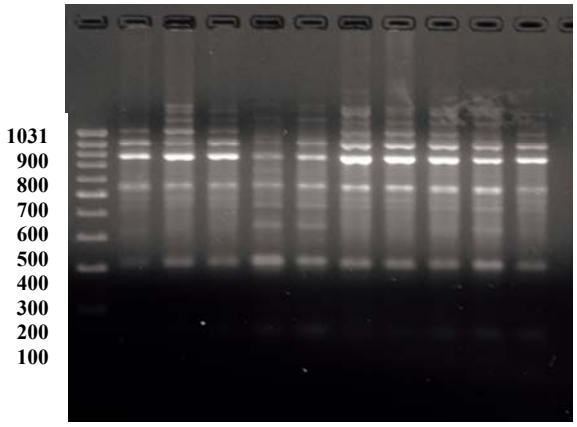


Figure (3 – 11) : RAPD-PCR analysis of band patterns of CLL patients obtained with OPB-15 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patients, lane 4 represent normal males, lanes from 3 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

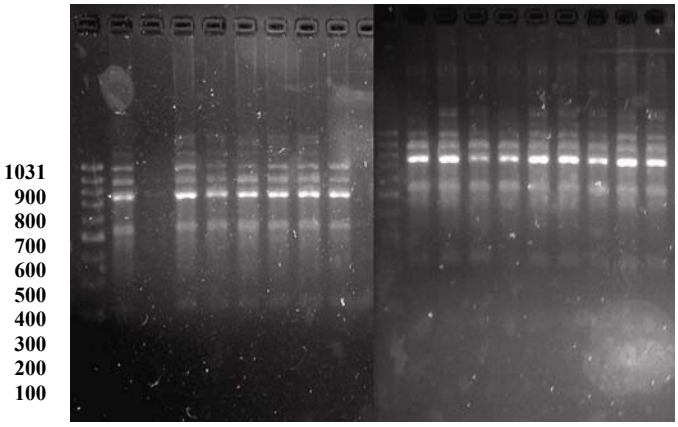


Figure (3 – 12) : RAPD-PCR analysis of band patterns of CML patients obtained with OPB-15 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

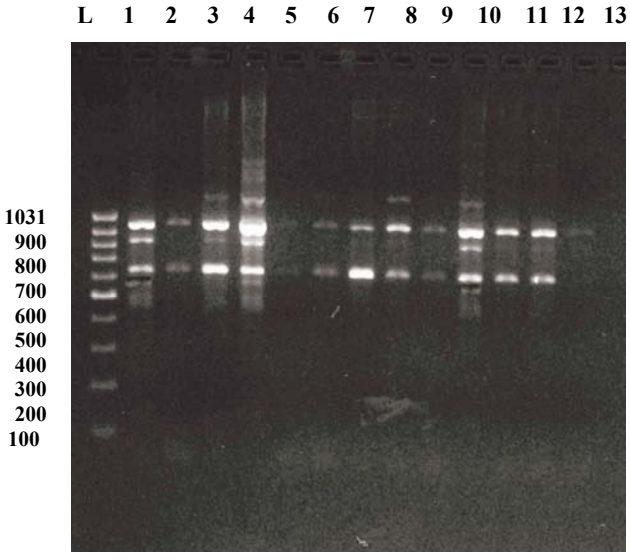


Figure (3 – 13) : RAPD-PCR analysis of band patterns of AML patients obtained with OPB-15 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patients, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

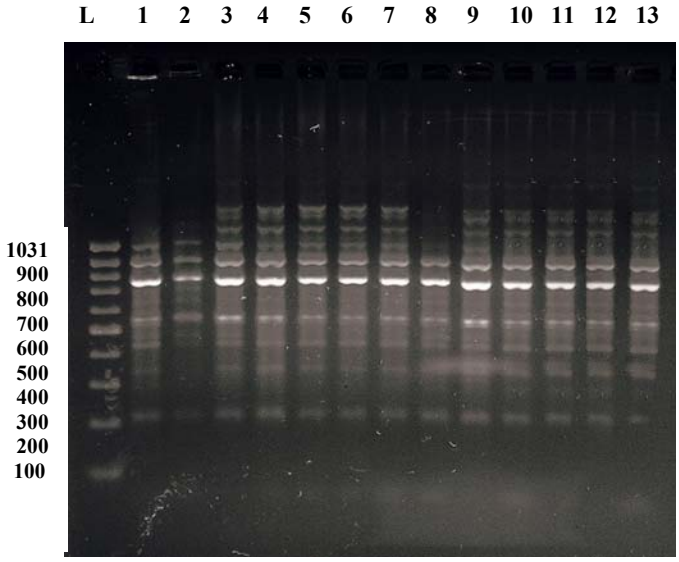


Figure (3 – 14) : RAPD-PCR analysis of band patterns of ALL patients obtained with OPB-15 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 7 represent the ALL female patients, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031bp).

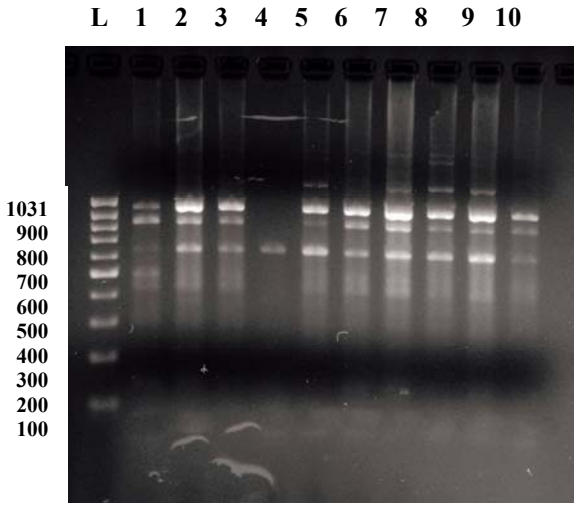


Figure (3 – 15) : RAPD-PCR analysis of band patterns of CLL patients obtained with OP-B17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

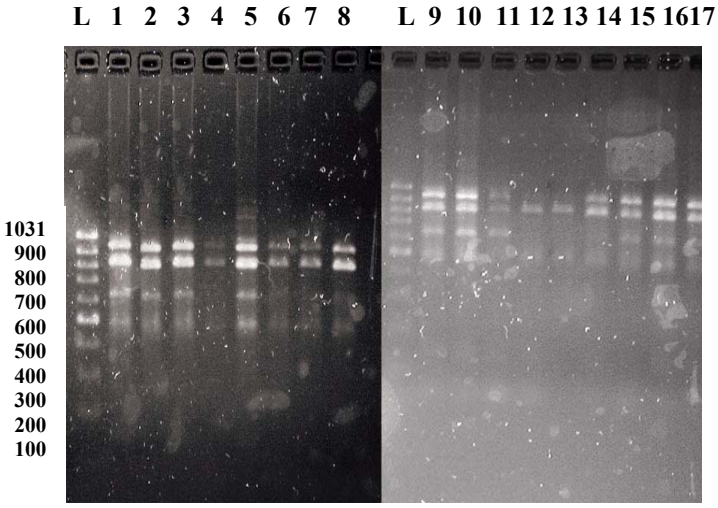


Figure (3 – 16): RAPD-PCR analysis of band patterns of CML patients obtained with OPB-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

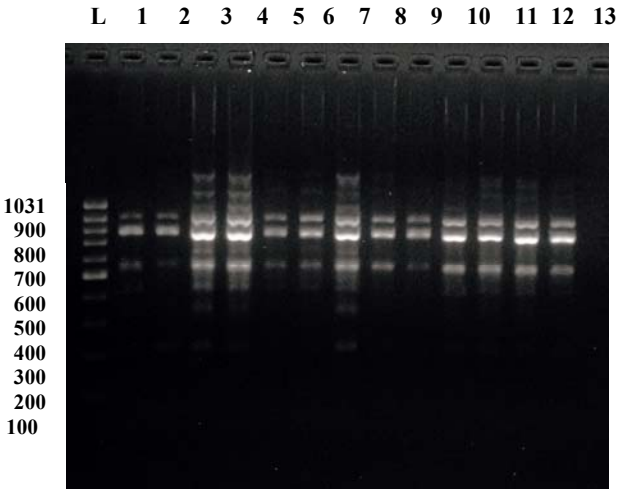


Figure (3 – 17): RAPD-PCR analysis of band patterns of AML patients obtained with OPB-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder.

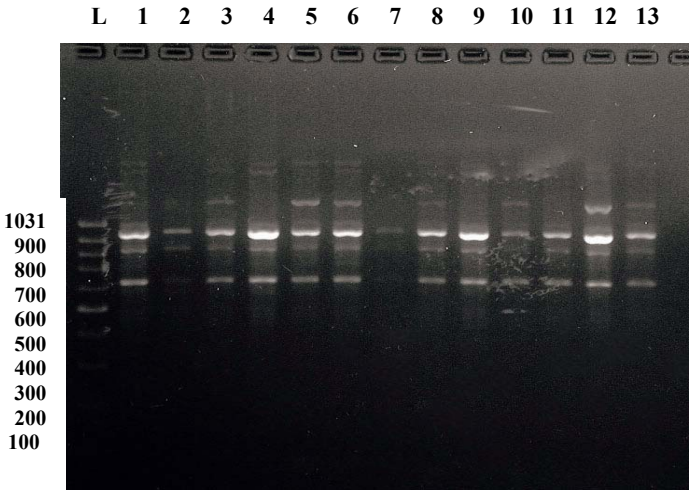


Figure (3 – 18): RAPD-PCR analysis of band patterns of ALL patients obtained with OPB-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder.

3.2.2.5 The primer OPB- 18

When using this primer which has the sequence of (AGGTGACCGT), a total number of 314 amplified bands were detected and they ranged between (1 - 10) across the lanes as seen in Figures (3 – 19, 3 - 20, 3 - 21 and 3 - 22). The molecular weights of these bands ranged between (220 - 1820) bp. The band number one with molecular weight 1820 bp was present only in CML2, CML3 and CML8 patients and was lost in other normal individuals and leukemia patients.

The band number two with molecular weight 1610 bp was lost in ALL1, ALL5, ALL9, ALL11, AML1, AML4, AML5, AML8, AML9, AML10, AML11, CLL2, CLL3, CLL7, CML1, CML6 and CML10. However, it was present in normal individuals and in other leukemia patients.

The band number three with molecular weight 1520 bp was lost in ALL1, ALL9, ALL11, AML4, CLL2, CLL3, CLL6, CLL7, CML1, CML6 and CML10. However, it was present in normal individuals and other leukemia patients. The band shifting of this band with molecular weight 1500 kbp was present in AML2, AML3 and AML6.

The band number four with molecular weight 1250 bp was present in ALL10, CML2 and CML8, but it was absent in normal individuals and other leukemia patients.

The bands number five with molecular weight 1150 bp was present in normal individuals, ALL6, ALL7, CLL1, CLL2, CLL3, CLL6, CLL7, CLL8, CML2, CML3, CML4, CML5, CML7, CML8, CML9, CML13 and CML15. There were two bands shifting of this band, the first band's shift with molecular weight 1172 bp was present in ALL2, ALL3, ALL4, AML1, AML4, AML5, AML7, AML8, AML9, AML10 and AML11. The second band's shift with

molecular weight 1138 bp was present in ALL10, AML2, AML3, AML6, CLL4 and CLL5.

The band number six with molecular weight 850 bp was gained in ALL7, ALL8, AML5, AML6, CML2, CML3 and CML9, but was absent in normal individuals and other leukemia patients.

The band number seven with molecular weight 740 bp was present in normal individuals, ALL2, ALL7, ALL8, ALL9, ALL10, AML1, AML2, AML4, AML5, AML6, CLL2, CML2, CML3, CML4, CML8, CML9, CML10 and CML15. Band shifting of this band with molecular weight 700 bp was present in ALL3. On other hand this band was absent in other leukemia patients.

The band number eight with molecular weight 640 bp was present in normal individuals, ALL2, ALL3, ALL4, ALL6, ALL7, ALL8, ALL10, AML1, AML2, AML3, AML5, AML6, AML7, AML9, AML10, AML11, CLL2, CLL3, CLL4, CLL5, CLL6, CLL7, CLL8, CML2, CML8, CML9 and CML10, but was absent in and other leukemia patients.

The band number nine with molecular weight 430 bp was present in ALL2, ALL3, ALL7, ALL8, CLL2, CLL3, CLL4, CLL5, CLL7, CML2, CML3, CML9 and CML10, but was absent in normal individuals and other leukemia patients.

The bands number ten, 11 and 12 with molecular weight 380, 350, 280 bp respectively were present in most leukemia patients except the following cases: in patients ALL7, ALL10, CLL2 band number ten was absent;

whereas, band number 11 was absent in ALL 9, ALL11, CML14, in addition band number 12 was absent in ALL11, CML6 and CML7.

The band number 13 with molecular weight 220 bp was present in normal individuals, AML4, AML6, AML7. It was absent in other leukemia patients.

3.2.2.6 The primer OPE- 05

This primer has the sequence of (TCAGGGAGGT). It amplified a total number of 384 bands ranging between (2 - 10) across the lanes. The molecular weights of these bands ranged between (450 - 2270) bp as seen in Figures (3 – 23, 3 - 24, 3 - 25 and 3 - 26).

The bands number one and band number two with molecular weights 2270 and 2110 bp respectively were absent in ALL1, ALL3, ALL4, AML1, AML4, AML5, AML6, AML7, AML8, AML10, AML11, CLL2, CLL3, CLL7, CML2, CML9 and CML10. On the other hand these bands were present in normal individuals and other leukemia patients.

The bands number three, four and five with molecular weights 1580, 1440 and 1240 bp respectively were present in normal individuals and in most leukemia patients, except in patients ALL1, ALL3, ALL4, ALL9, bands number three and four were absent and in AML1, AML8, CLL2, CLL3, CLL4 and CLL7 band number three was absent. Band number five was absent in AML8.

The bands number six and seven with molecular weights 1070 and 960 bp respectively were present in normal individuals and in most leukemia patients, except in ALL1, ALL3, ALL4, ALL9, AML1, AML2, AML8, CLL1, CLL7, CLL8 band number six was absent, and in AML1, AML2, AML8, CML2, CML9 and CML10 band number seven was absent.

The bands number eight and nine with molecular weights 820 and 750 bp respectively were present in normal individuals and in most leukemia patients, except in ALL9, AML1, AML5, AML8, CLL1, CLL2, CLL3, CLL4, CLL7, CLL8, CML2, CML9 and CML10, the two bands were absent .

The bands number ten, 11 and 12 with molecular weights 660, 500 and 430 bp respectively were present in normal individuals, ALL2, ALL5, ALL6, ALL7, ALL8, ALL10, ALL11. Band number ten was present only in CLL patients. Band number 11 was present only in CML2, CML9 and CML10.

L 1 2 3 4 5 6 7 8 9 L 10 11 12 13 14 15 16 17 18

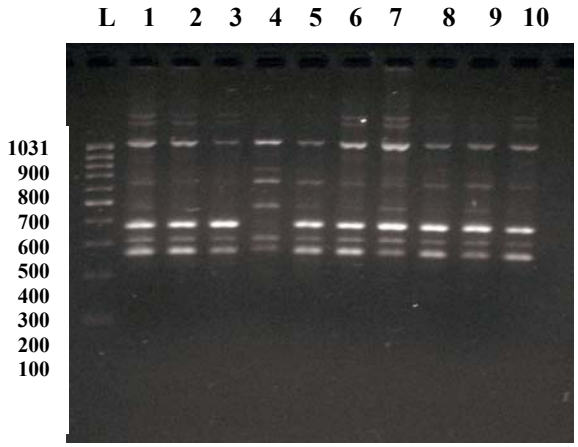


Figure (3 – 19) : RAPD-PCR analysis of band patterns of CLL patients obtained with OP-B18 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

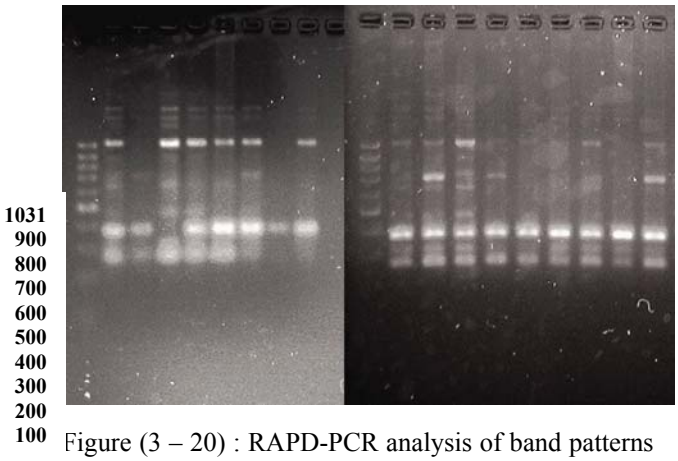


Figure (3 – 20) : RAPD-PCR analysis of band patterns of CML patients obtained with OP-B18 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

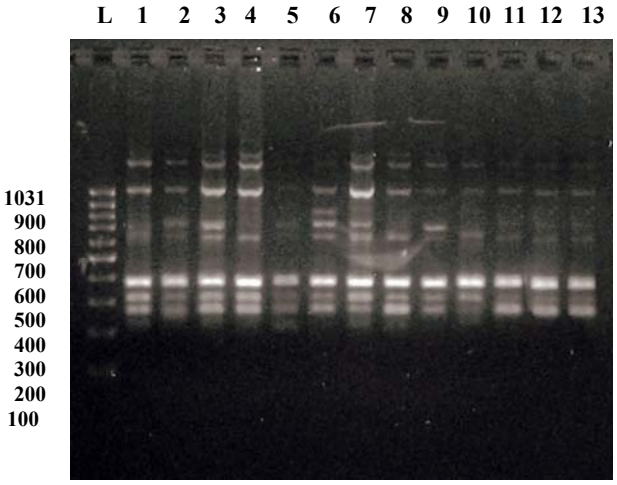


Figure (3 – 21) : RAPD-PCR analysis of band patterns of AML patients obtained with OP-B18 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

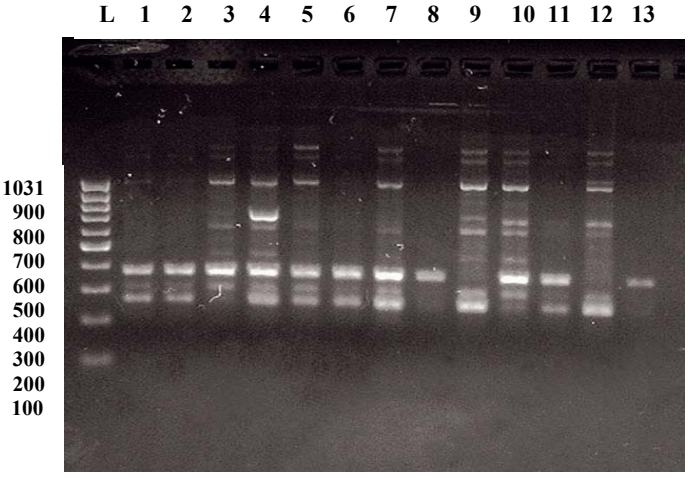


Figure (3 – 22) : RAPD-PCR analysis of band patterns of ALL patients obtained with OP-B18 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

L 1 2 3 4 5 6 7 8 9 10

L 1 2 3 4 5 6 7 8 L 9 10 11 12 13 14 15 16 17

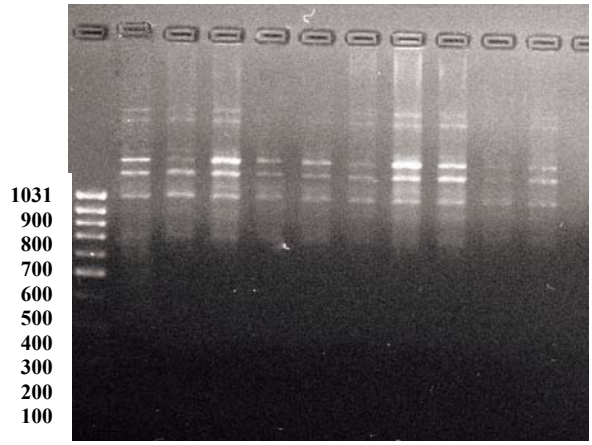


Figure (3 – 23) : RAPD-PCR analysis of band patterns of CLL patients obtained with OPE-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

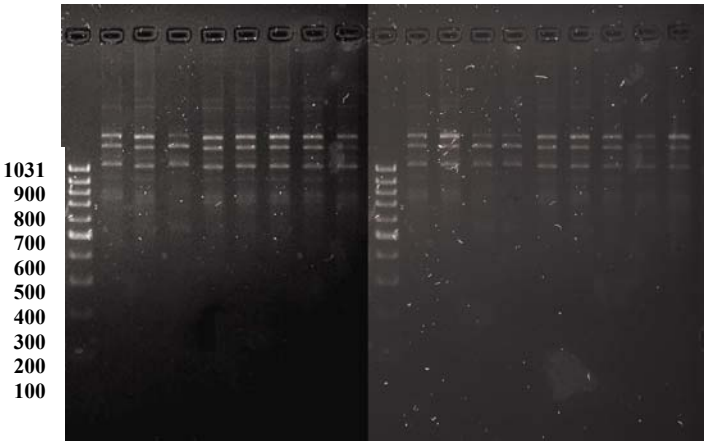


Figure (3 – 24) : RAPD-PCR analysis of band patterns of CML patients obtained with OPE-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

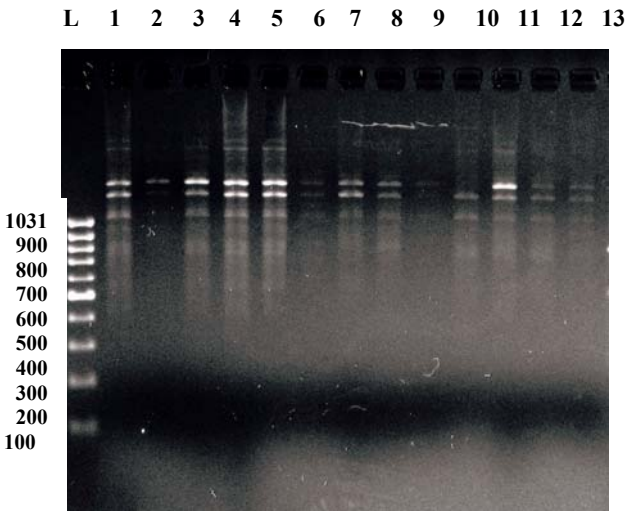


Figure (3 – 25) : RAPD-PCR analysis of band patterns of AML patients obtained with OPE-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

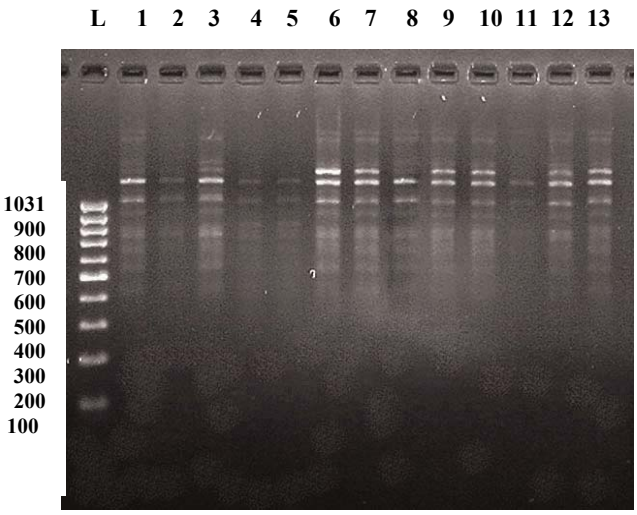


Figure (3 – 26) : RAPD-PCR analysis of band patterns of ALL patients obtained with OPE-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

3.2.2.7 The primer OPJ- 05

This primer has the sequence of (CTCCATGGGG). It amplified a total number of 511 bands ranging between (4 - 15) across the lanes. The molecular weights of these bands ranged between (95 - 1570) bp as seen in Figures (3 – 27, 3 - 28, 3 - 29 and 3 - 30).

The bands number one and two with molecular weights 1570 and 1460 bp respectively were present in ALL1, ALL2, ALL3, ALL4, ALL5, ALL7, ALL8, ALL9, AML3, AML6, AML10, CLL1, CLL3, CLL5, CLL6, CML1, CML2, CML3, CML4, CML5, CML11, CML12 and CML13. On the other hand this band was absent in normal individuals and other leukemia patients.

The bands number three and four with molecular weights 1240 and 1120 bp respectively were present in normal individuals and most leukemia patients, except in CLL2 and CML9 were absent. Band number three was absent in ALL10, ALL11, CLL7 and CML10.

The band number five with molecular weight 960 bp was present in normal individuals and all leukemia patients.

The bands number six and seven with molecular weights 760 and 730 bp respectively were present in normal individuals and most leukemia patients, except in CML8 and CML9 were absent, whereas in ALL10 and ALL11 the band number seven was absent.

The band number eight with molecular weight 670 bp was gained in ALL2, ALL3, ALL4, ALL7, ALL8, ALL9, AML3, AML4, CLL1, CLL2, CLL5 and

CLL6. However it was absent in normal individuals and other leukemia patients.

The band number nine with molecular weight 620 bp was present in normal individuals and most of leukemia patients. It was lost in ALL10, ALL11, CLL2, CLL4, CLL7, CML5, CML6, CML7, CML8, CML9, CML10, CML11, CML12, CML14 and CML15.

The band number ten with molecular weight 530 bp was present in normal individuals and all leukemia patients.

The band number 11 with molecular weight 460 bp was absent in normal female individuals and other leukemia patients. On the other hand this band was present in normal male individuals, ALL7, ALL8, ALL9, ALL10, AML9, AML10, CLL2, CLL3, CLL4, CLL5, CLL6, CLL8, CML4, CML8, CML9, CML10, CML11, CML12, CML13, CML14 and CML15.

The bands number 12, 13 and 14 with molecular weights 350, 230 and 190 bp respectively were present in normal individuals and most leukemia patients, except in ALL11 and CLL7 were lost. Bands number 13 and 14 were lost in ALL10, CLL4, CLL7 and CLL8. Band number 12 was lost in AML7, AML8 and CML8.

The band number 15 with molecular weight 95 bp was absent in normal individuals and most leukemia patients except in AML2, AML4, AML5, CLL2, CLL3, CLL7 and CLL8, this band was gained.

3.2.2.8 The primer OPW- 17

When using this primer that has the sequence of (GTCCTGGGTT), a total number of 372 bands were obtained as seen in figure (3 – 32, 3 - 33, 3 - 34 and 3 - 35). The molecular weights of these bands ranged between (197 - 1883) bp. Amplified bands were ranged between (3 - 12) across the lanes.
The bands number one and two with molecular weights 1883 and 1743 bp respectively were absent in normal individual and most leukemia patients. On the other hand this band was gained only in AML9 and CLL5.

The bands number three, four and five with molecular weights 1560, 1500 and 1400 bp respectively were gained in AML1, AML3, AML4, AML5, AML7, AML8. Band number four and five was gained also in AML2, AML9, AML10 and AML11. However, this band was absent in normal individuals and other leukemia patents.

The bands number six and seven with molecular weights 1380 and 1310 bp were present in normal individuals and most leukemia patient, except in ALL7, CML11 and all AML patients were lost.

The bands number eight and nine with molecular weights 1070 and 970 bp were present in normal individuals and in majority of leukemia patients, except in CML11 were lost. Whereas in ALL7 band number eight was lost.

The band number ten with molecular weight 870 bp was absent in normal individuals, and in ALL, CLL and CML patients. This band was present in AML patients; however, the band shifting of this band with molecular weight 800 bp was present in AML2, AML3, AML6, AML9, AML10 and AML11

The band number 11 with molecular weight 740 bp was absent in normal individuals and ALL, CLL and CML patients, however it was present in most AML patients except in AML4, AML6 and AML8 was lost.

The band number 12 with molecular weight 700 bp was present in AML4, AML5 and AML7. It was absent in normal individuals and other leukemia patients.

The bands number 13 and 14 with molecular weights 640 and 600 bp were present in normal individuals and most leukemia patients, except in AML6, AML7 and AML8 were absent. Band number 13 was absent in AML4, AML5. Band number 14 was absent in ALL9, ALL10, ALL11.

The band number 15 with molecular weight 520 bp was present in normal individuals and all leukemia patients.

The band number 16 with molecular weight 420 bp was present in normal individuals and in ALL, CLL, CML patients. However, this band was absent in AML patients.

The band number 17 with molecular weight 290 bp was present in AML, CLL patients. It was absent in normal individuals and other leukemia patients.

The band number 18 with molecular weight 190 bp was present in AML1, AML2, AML3, AML5, AML6, AML7, AML8, AML9, AML10, CLL4, CLL5 and CLL6. It was absent in normal individuals and other leukemia patients.

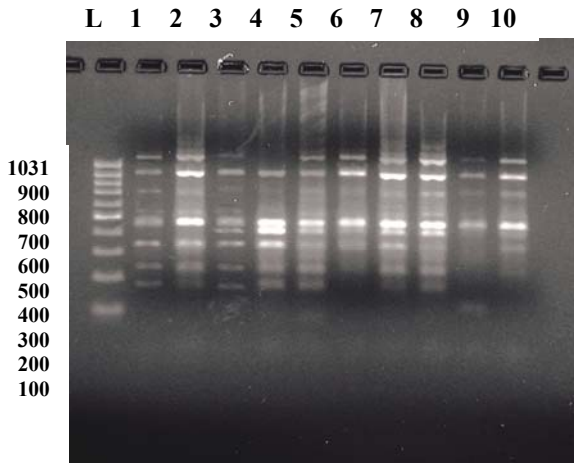


Figure (3 – 28) : RAPD-PCR analysis of band patterns of CLL patients obtained with OPJ-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

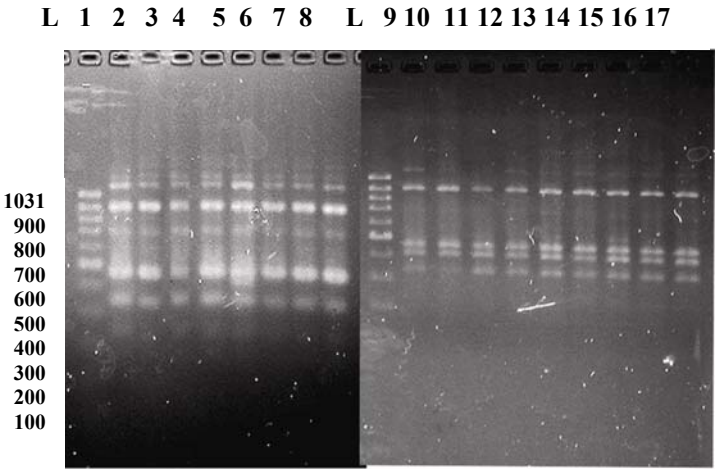


Figure (3 – 29) : RAPD-PCR analysis of band patterns of CML patients obtained with OPJ-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder 100 – 1031 bp).

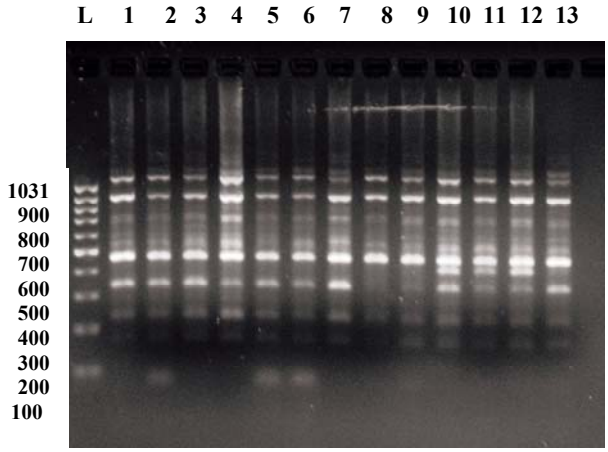


Figure (3 – 30) : RAPD-PCR analysis of band patterns of AML patients obtained with OPJ-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

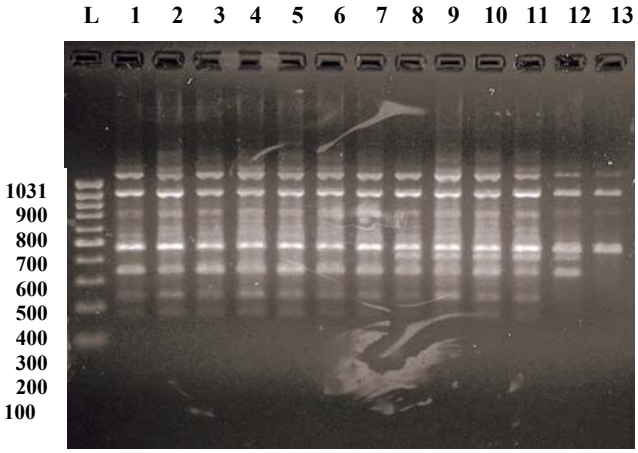


Figure (3 – 31) : RAPD-PCR analysis of band patterns of ALL patients obtained with OPJ-05 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

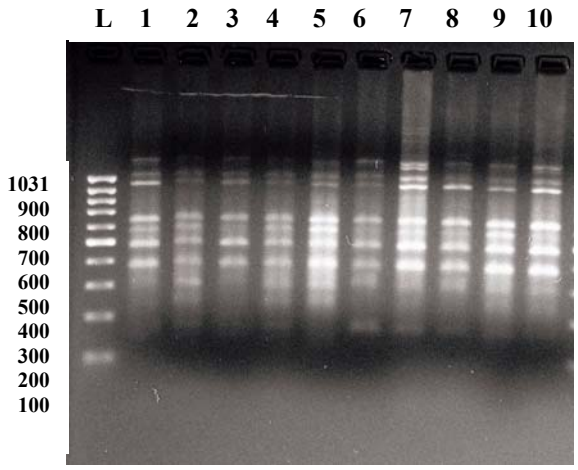


Figure (3 – 32) : RAPD-PCR analysis of band patterns of CLL patients obtained with OPW-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

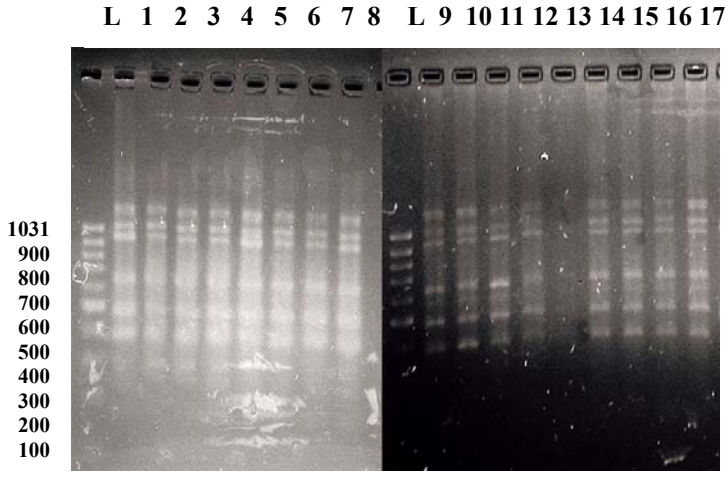


Figure (3 – 33) : RAPD-PCR analysis of band patterns of CML patients obtained with OPW-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

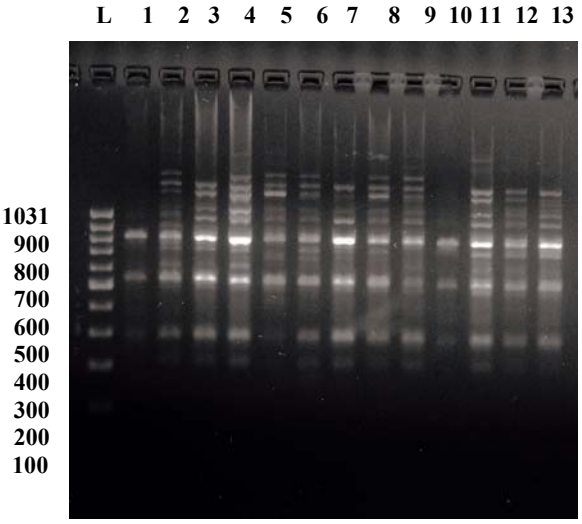


Figure (3 – 34) : RAPD-PCR analysis of band patterns of AML patients obtained with OPW-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

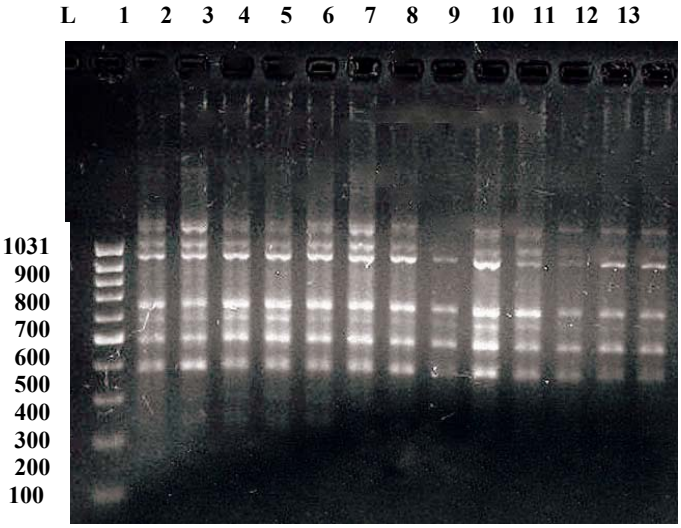


Figure (3 – 35) : RAPD-PCR analysis of band patterns of ALL patients obtained with OPW-17 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

3.2.2.9 The primer OPZ- 02

This primer which has the sequence of (CCTACGGGGA) gave a total number of (376) bands. The molecular weights of these bands ranged between (370 - 1734) bp. The amplified bands ranged between (1 - 11) across the lanes (Figures 3 – 36, 3 - 37, 3 - 38 and 3 - 39).

The band number one with molecular weight 1743 bp was present in normal individuals, ALL2, ALL3, ALL4, ALL5, ALL7, ALL8, ALL9, ALL10, ALL11, AML1, AML2, AML3, AML4, AML9, AML10, CLL1, CLL5, CLL6, CML3, CML4, CML5 and CML6 patients. On the other hand this band was absent in other leukemia patients.

The bands number two and three with molecular weights 1428 and 1167 bp respectively were lost in ALL1, AML7, AML11, CLL2, CLL8, CML1, CML2, CML7, CML10, CML11 and CML14. Band number two was also absent in CLL3 and CML11. However these bands were present in other leukemia patients.

The band number four with molecular weight 1143 bp was present in normal individuals, ALL1, AML1, AML2, AML4, AML5, AML7, AML8, AML10, AML11, CLL1, CLL2, CLL3, CLL7, CLL8 and CML patients, except in CML1 and CML2 was lost. Band shifting of this band with molecular weight 1.126 kbp was present in other leukemia patients.

The band number five with molecular weight 960 bp was absent in ALL1, CML1, CML2 and CML7. It was present in normal individuals and other leukemia patients.

The band number six with molecular weight 850 bp was lost in ALL1, ALL9, AML2, AML6, AML7, AML8, CML1, CML2, CML4, CML5, CML6, CML7, CML8, CML11, CML14 and CML15. However it was present in normal individuals and other leukemia patients.

The band number seven with molecular weight 820 bp was present in normal individuals, ALL4, ALL5, ALL6, ALL10, ALL11, AML and CLL patients, CML9, CML10, CML12 and CML13. The band's shift of this band with molecular weight 790 bp was present in ALL2, ALL3, ALL7 and ALL8. It was absent in other leukemia patients.

The band number eight with molecular weight 670 bp was present in normal individuals, and in most leukemia patients but lost in ALL1, AML1, AML2, AML3, AML4, AML5, AML7, AML8, AML11, CLL1, CLL8, CML2, CML5, CML6, CML7 and CML10.

The band number nine with molecular weight 570 bp was present in normal individuals, and in all leukemia patients except in CML2 was absent.

The band number 10 with molecular weight 430 bp was present in normal individuals, ALL2, ALL3, ALL4, ALL5, ALL6, ALL7, ALL8, ALL9, ALL10, ALL11, AML1, AML5, AML6, CLL1, CLL2, CLL3, CML8, CML9 and CML15. In other hand this band was absent in other leukemia patients.

The band number 11 with molecular weight 370 bp was present in normal individuals, and in all leukemia patients except in CML patients which was absent.

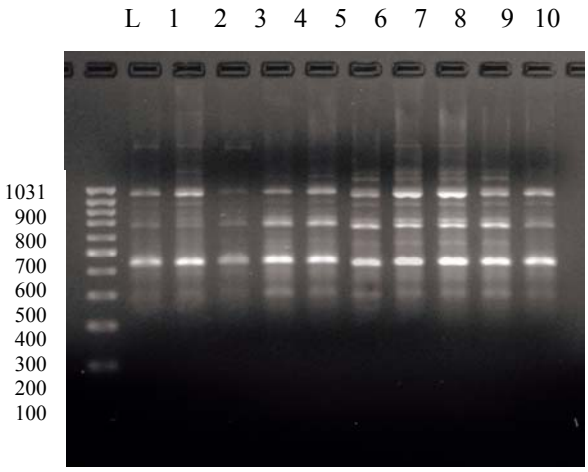


Figure (3 – 36) : RAPD-PCR analysis of band patterns of CLL patients obtained with OPZ-02 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lane 2 represent the CLL female patient, lane 3 represent normal males, lanes from 4 to 10 represent CLL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

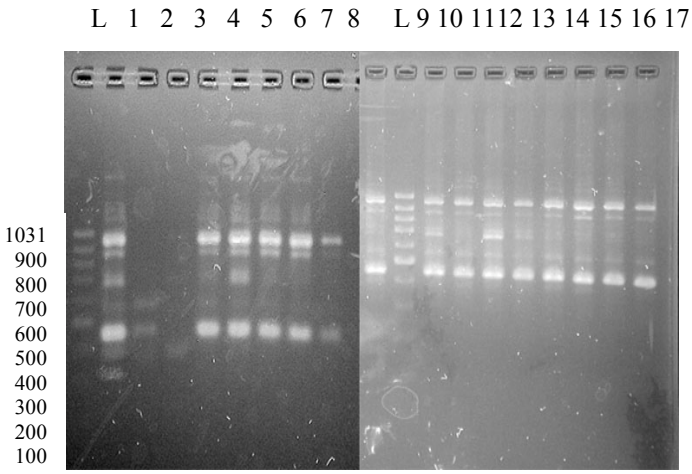


Figure (3 – 37) : RAPD-PCR analysis of band patterns of CML patients obtained with OPZ-02 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm. The lane 1 represent normal females, lanes from 2 to 8 represent the CML female patients, lane 9 represent normal males, lanes from 10 to 17 represent CML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

L 1 2 3 4 5 6 7 8 9 10 11 12 13

L 1 2 3 4 5 6 7 8 9 10 11 12 13

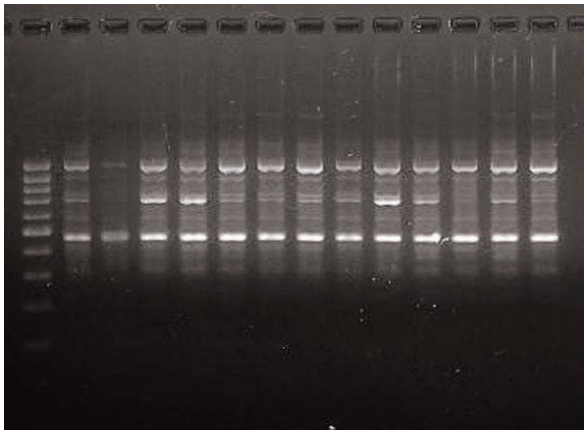
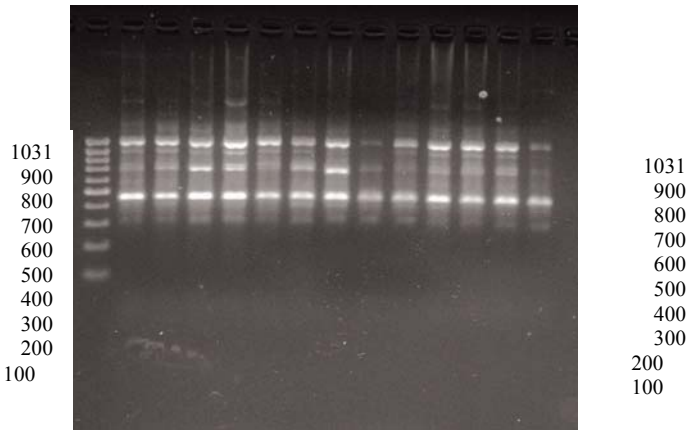


Figure (3 – 38) : RAPD-PCR analysis of band patterns of AML patients obtained with OPZ-02 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to 9 represent the AML female patient, lane 10 represent normal males, lanes from 11 to 13 represent AML males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).

Figure (3 – 39) : RAPD-PCR analysis of band patterns of ALL patients obtained with OPZ-02 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours. The lane 1 represent normal females, lanes from 2 to7 represent the ALL female patient, lane 8 represent normal males, lanes from 9 to 11 represent ALL males patients. Lane L indicates the λ DNA as a ladder (100 – 1031 bp).



In this study, the results of comparative RAPD-PCR analysis of nine primers had shown DNA polymorphism in genomic DNA obtained from normal individuals and leukemia patients. This was revealed in patients as well as in normal individuals; these polymorphisms were either in number or in the molecular weight of amplified bands. The reported results in this study had shown different patterns in respect of bands' profiles obtained by each primer. It was possible to observe the variation in bands' numbers, for example one band was observed when using primer OPB-18 and OPZ-02 in one patient , on the other hand the highest number of bands (15 bands) were

observed when using primer OPJ-05, other primers showed bands' number in the range > 1 and < 15.

The results of RAPD-PCR analysis of leukemia patients and normal individuals had shown differences in the molecular weights of amplified bands, the lowest molecular weight of amplified band was 95 bp when using primers OPB-15 and OPJ-05, and the highest molecular weight of amplified band was 3162 bp which was obtained with primer OPA-09. The polymorphism in the molecular weight of amplified bands demonstrated the differences in the length between the primer binding sites and the genomic DNA (Papadopoulos *et al.,* 2002).

The occurrence of DNA polymorphisms in leukemia patients might due to nucleotides sequence of primer in question and on the genotype of leukemia patients. In other words the number of compatible sites of primer in genomic DNA of leukemia patients which is effected by different types of mutations and translocations, this will affect the primer-template interaction sites and will result in the loss or gain of a band. Mutations such as (insertion or deletion or substitution) and translocations will result in adding or deleting primers binding sites, as a result this will lead to differences in number of amplified bands. The polymorphism also includes the differences in molecular weights of amplified bands which resulted from mutation occurred between the primer-template interaction, resulted in a mobility shift of bands (Wang *et al*., 2001; Wang *et al*., 2002; Xian *et al.*, 2005; Papadopoulos *et al.,* 2002; Ong *et al.,* 1998).

In addition, it was possible to observe a decrease or increase in the relative intensities of the bands obtained in the RAPD analysis of genomic DNA of leukemia patients in comparison with normal individuals. In this respect other investigators were able to observe such variations in the intensities of the

amplified bands (Dil-Afroze *et al.*, 1998; Maeda *et al.*, 1999; Xian *et al.*, 2005).

It was possible (from the reported results in this work) to group the polymorphism of genomic DNA according to results of RAPD primers, in this respect the first group which represents 37.8% of patients which included (ALL7, ALL9, AML1, AML2, AML3, AML4, AML5, AML7, AML9, AML10, CLL1, CLL2, CLL3, CLL5, CLL7, CML1, CML2 and CML10) gave different pattern of DNA polymorphism with nine primers used in this study. Second group which represents 48.8% of patients included (ALL10, ALL11, AML6, AML8, AML11, CLL4, CLL8, CML3, CML4, CML5, CML6, CML7, CML11, CML12, CML13, CML14 and CML15) polymorphisms were detected by eight primes. Third group which represents 8.9% of patients and included (ALL8, CLL6, CML8 and CML9) polymorphisms were detected by seven primers. Fourth group which represents 4.5% of patients and included (ALL5 and ALL6) polymorphisms were detected by six primers.

The observed polymorphisms in patients varied widely, some showed highest polymorphism this may be due to the patients who has not yet taken any treatment or does not show any response to the treatment, or may be the patients were in the last stages of disease. Other patients who revealed less polymorphism, this may be due to number of reasons such as the patient is in the early stages of disease or may be the patient has shown good response to the treatment (Wang *et al*., 2001; Wang *et al*., 2002).

In this study the efficiency of using primers in amplification reaction was measured by dividing the number of polymorphic bands on the total number of amplified bands for each primer (Hassan, 2002). Thus it was possible to evaluate the ability to detect genetic alteration by each primer. Table (3 - 6) showed that the highest value of efficiency was belonging to primer OP-B17

(0.059) and primer OPE-05 being the lowest (0.016). The range of values for primer efficiency could demonstrate the ability of a primer to give highest ratio of polymorphic bands as compared to total number of amplified bands. The efficient primer was not the primer which could amplify highest number of amplified bands, but the ability to show the polymorphism between normal individuals and leukemia patients. So we observed that among (255) amplified bands detected by using primer OPB-17, the number of polymorphic bands were (15); while for primer OPE-05 among (348) observed amplified bands, the number of polymorphic bands were (6) bands. This indicates that some loci in the genomic DNA were apt to changes of nucleotides sequence like the sequence of primer OPB-17, and the sequence of primer OPE-05, this might suggest that the genomic DNA of leukemia patients tend to remain stable in the evolution of the disease. In this respect several investigators were able to detect such stable in genomic changes in various type of cancer (Papadopoulos *et al.,* 2002; Ong *et al.,* 1998; Wang *et al*., 2001; Wang *et al*., 2002).

Some primers were able to amplify a band in certain type of leukemia, such band was absent in normal persons and some types of leukemia patients, e.g. when using primer OPB-17, the band number eight with molecular weight 560 bp was present in AML patients, however this band was absent in normal individuals and other leukemia patients. The result of primer OPW-17 showed that the band number ten with molecular weight 870 bp was absent in normal individuals, and in ALL, CLL and CML patients, however it was present in AML patients; the band number 17 with molecular weight 290 bp was present in AML, CLL patients. However, it was absent in normal individuals and other leukemia patients.

Band`s profile of some primers showed that certain band was present in

normal persons and some types of leukemia patients, however it was absent in other types of leukemia, e.g. the results of primer OPA-09 showed that the band number one with molecular weight 3162 bp was lost in 82% of ALL patients and 87% of CLL patients, while this band was present in normal individuals and some leukemia patients. The result of primer OPB-15, the band number 12 with molecular weight 360 bp was present in normal individuals and majority of ALL and CLL patients. However, it was absent in AML and CML patients. The band number 13 with molecular weight 290 bp was present in normal individuals and in ALL, CLL and CML leukemia patients, but was absent in AML patients. The result of primer OPB-17 showed that the band number nine with molecular weight 500 bp was present in normal individuals and was found in variable frequency in ALL, CLL and CML patients, however it was absent in AML patients. The band number ten with molecular weight 430 bp was present in normal individuals and some patients. However, this band was lost in CML patients. Band`s profile of primer OPW-17 showed that the bands number six and seven with molecular weights 1380 and 1310 bp were present in normal individuals and most leukemia patients, except in AML patients which were lost. The band number 16 with molecular weight 420 bp was present in normal individuals and in ALL, CLL, CML patients. However, this band was absent in AML patients. The result of primer OPZ-02 showed that the band number 11 with molecular weight 370 bp was present in normal individuals, and in all leukemia patients except in CML patients which was absent.

Moreover it was to observe a primer which could amplify certain band in normal male individuals but it could not amplify this band in normal female individuals e.g. band`s profile of primer OPJ-05 showed that the band number

11 with molecular weight 460 bp was absent in normal female individuals. On the other hand this band was present in normal male individuals.

Table (3 - 6): Analysis of RAPD-PCR results of leukemia patients.

primer	The range of no. of amplified bands	The range of molecular weights of amplified bands	No. of polymorphic bands	Total no. of amplified bands	efficiency
OPA-09	2 - 4	1000 - 3162 bp	3	93	0.032
OPA-13	5 – 13	300 - 1260 bp	10	467	0.021
OPB-15	2 - 13	95 - 2560 bp	14	433	0.032
OPB-17	3 - 11	250 – 1770 bp	15	255	0.059
OPB-18	1 - 10	220 - 1820 bp	17	314	0.054
OPE-05	2 - 10	430 - 2270 bp	6	384	0.016
OPJ-05	4 - 15	95 - 1570 bp	12	511	0.023
OPW-17	3 - 12	197 - 1880 bp	18	372	0.048
OPZ-02	1 - 11	370 - 1734 bp	13	376	0.034

3.2.3 DNA Methylation analysis

The aberration of DNA methylation is well established in genomic DNA of cancer patients. For example, global hypomethylation leads to oncogene activation and chromosomal rearrangement; in addition, hypermethylation is

observed in cancer and is associated with tumor suppressor gene silencing (Nephew and Huang, 2003). The differential detection of methylated and unmethylated genomic DNA can be accomplished through digestion with methylation sensitive restriction enzymes (Liang *et al*., 2002; Gonzalgo *et al*., 1997). Thus, DNA methylation content of genomic DNA can be studied on the basis of discordant patterns of digestion by isoschizomeric restriction enzymes that differed only in their digestion sensitivity to methylated cytosine in DNA sequence. In this respect, restriction enzymes *Msp*I and *Hpa*II can digest unmethylated CCGG sequence, but *Hpa*II cannot digest methylated cytosine in aforementioned sequence, whereas *Msp*I can. Then, RAPD-PCR analysis of digested genomic DNA with *Msp*I and *Hpa*II will reveal the difference in methylation patterns among leukemia patients and normal individuals.

In this study, genomic DNA of CML patients and normal individuals of selected age group (forty years old individuals) were digested with *Msp*I and *Hpa*II, then, the digested genomic DNA was amplified with 9 primers which were selected depending on our earlier work on amplification of genomic DNA reported previously in this study (section 3.2.2). Genetic alteration were identified as band loss, gain, intensity change and shift in the RAPD-PCR amplified fragments of normal or CML genomic DNA. Clear variations in bands patterns were observed when using each of nine primers to amplify digested genomic DNA by two enzymes for normal individuals and CML patients. Earlier study showed comparable results when studying the DNA methylation of aging human ovaries (Ibrahim *et al.,* 2004).

In this study the obtained results from the electrophoresis of digestion patterns of eight normal individuals and eight CML patients were shown in figures (3 - 39), (3 – 40), (3 – 41), (3 – 42), (3 – 43), (3 – 44), (3 – 45), (3 – 46), (3 – 47), (3 – 48), (3 – 49), (3 – 50), (3 – 51), (3 – 52), (3 – 53), (3 – 54), (3 – 55),

(3 – 56), (3 – 57), (3 – 58), (3 – 59), (3 – 60), (3 – 61), (3 - 62), (3 – 63), (3 – 64) and (3 – 65). The normal females are represented by: 1N, 2N, 3N and 4N; whereas, CML patients females were: 1P, 2P, 3P and 4P. The normal males are represented by: 5N, 6N, 7N and 8N; whereas, CML patients males were: 5P, 6P, 7P and 8P. DNA samples were digested with *Hpa*II (H), *Msp*I (M). Digested and non digested samples were amplified with nine primers (OPA-09, OPA-13, OPB-15, OPB-17, OPB-18, OPE-05, OPJ-05, OPW-17 and OPZ-02).

The obtained results of the RAPD-PCR bands generated following digestion of genomic DNA of normal individuals by both enzymes, indicated that most primers showed variation in band patterns. This is expected, since DNA methylation is effected by age, sex, health and environment. However, it is worth to mention that primer OPB-17 showed similar banding pattern for amplified products of digested and undigested genomic DNA extracted from normal female individuals (fig. 3 - 49), this might due to specific binding of the primer sequence to complementary genomic sequence.

On the other hand, RAPD-PCR analysis of digested and undigested of CML genomic DNA showed clear indication of variation in genomic methylation as compared with normal individuals.

The reported results which were shown in the present work, demonstrated the possible variation in methylation patterns of CML genomic DNA of various patients. Moreover, the RAPD-PCR bands obtained as a result of digestion of genomic DNA of CML patients indicated that primer J-05 gave lower number of bands for CML genomic DNA predigested with *Msp*I. This could be explained by suggestion that the complementary sequence for primer used have several restriction sites sensitive to *Msp*I, so the amplification was not possible, or the amplification products were of low molecular weight and could not be detected.

It is possible to conclude from the obtained results reported in this study that digestion of genomic DNA of normal individuals with *Hpa*II and *Msp*I showed lower degree of differences in RAPD-DNA patterns, indicating normal methylation patterns. However, different picture was shown when studying RAPD-DNA bands profile of CML genomic DNA following digestion with restriction *Hpa*II and *Msp*I.

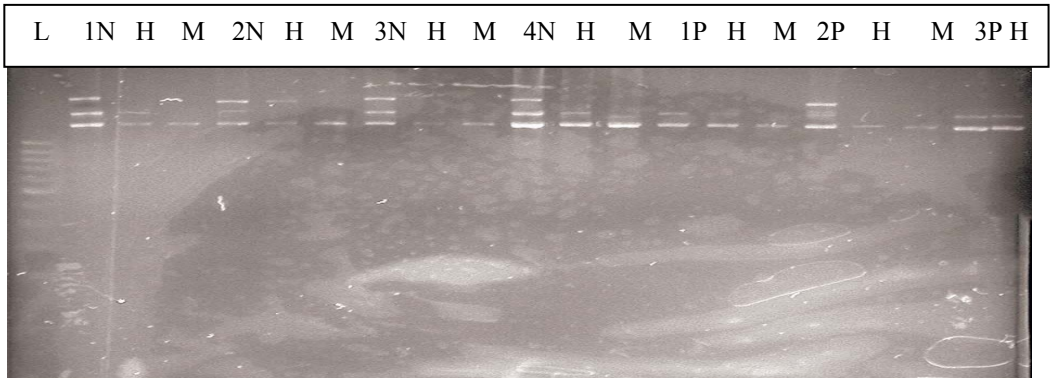
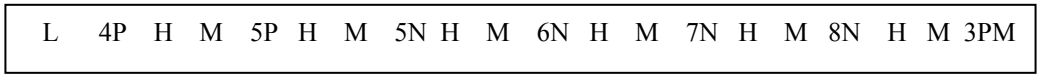


Figure (3 – 39): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPA-09 before and after digestion with *HpaII* and *MspI*. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P, 2P and 3P:Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031bp).



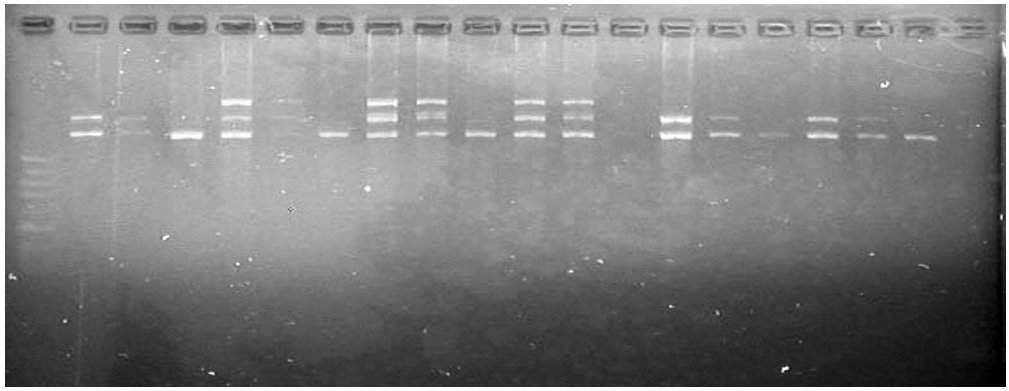


Figure (3 – 40): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPA-09 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5P : Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

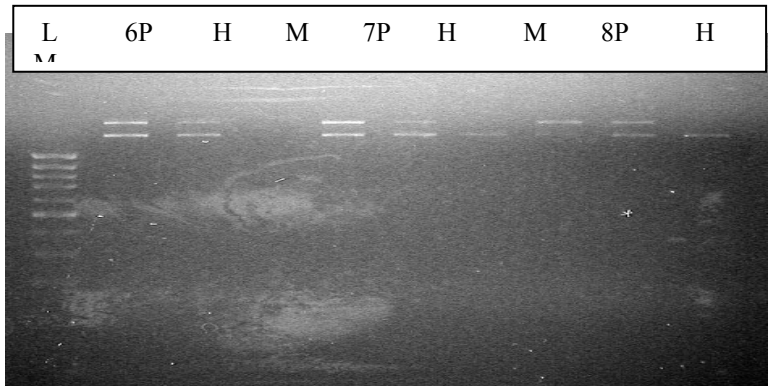
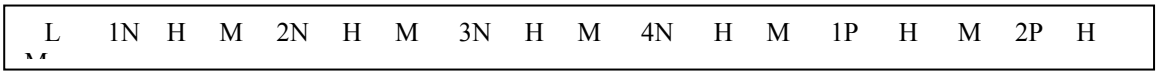


Figure (3 – 41): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPA-09 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



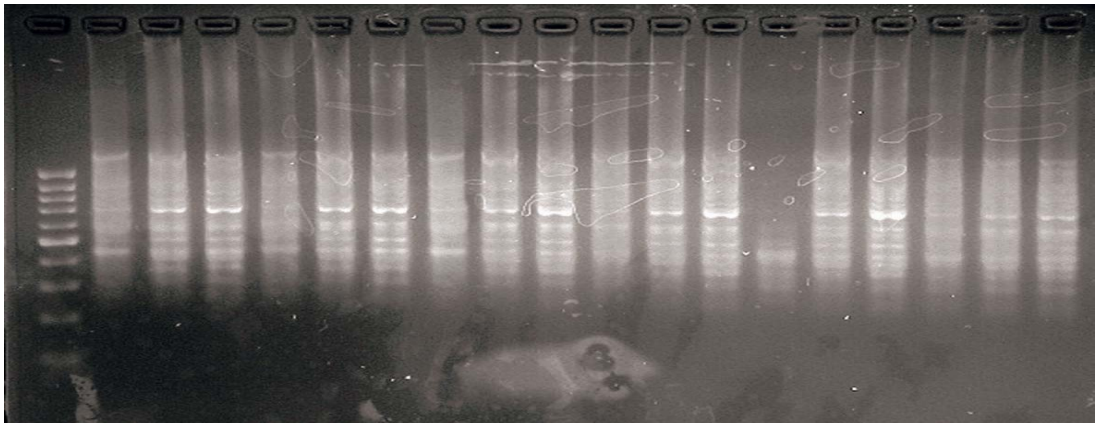


Figure (3 – 42): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPA-13 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P, 2P and 3P:Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031bp).

L	3P	H	M	4P	H	M	5N	H	M	6N	H	M	7N	H	M	8N	H

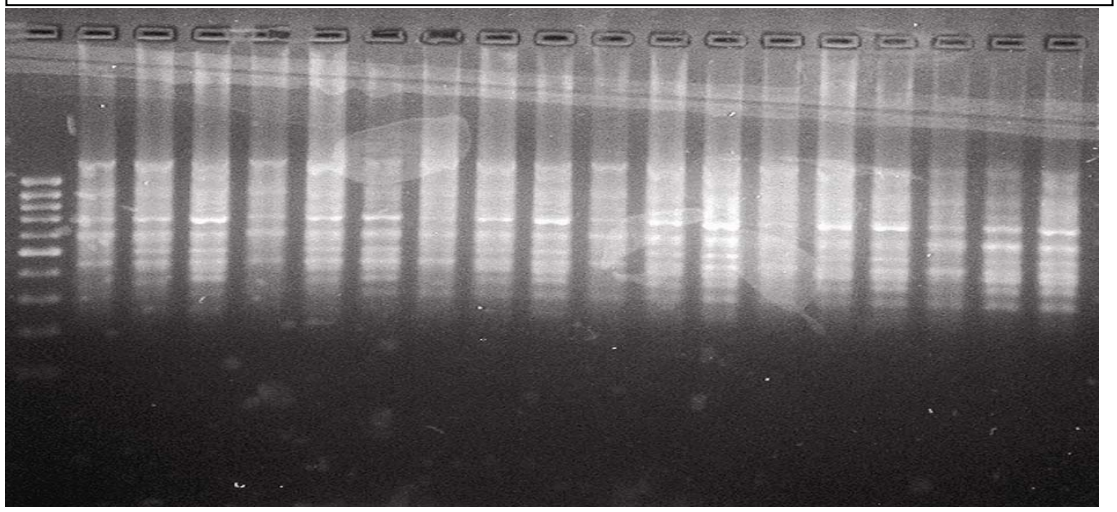


Figure (3 – 43): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPA-13 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P and 4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp)

L	5P	H	M	6P	H	M	7P	H	M	L	8P	H	M

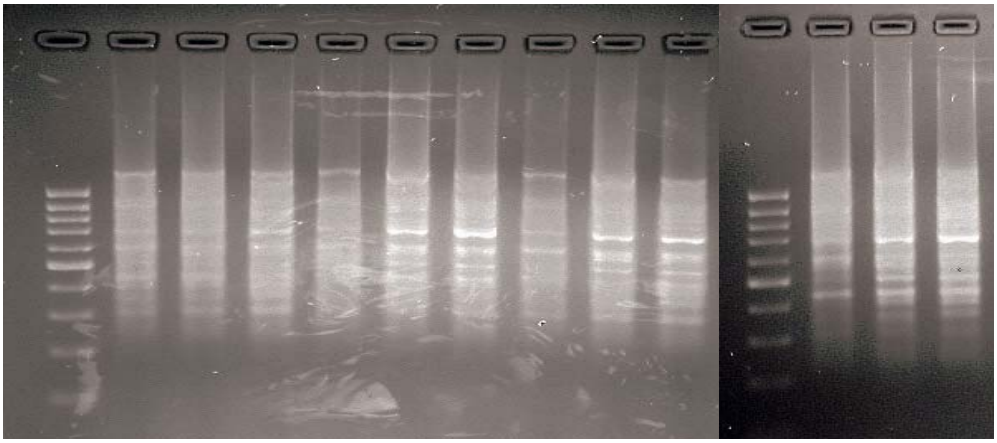


Figure (3 – 44): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPA-13 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5P, 6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

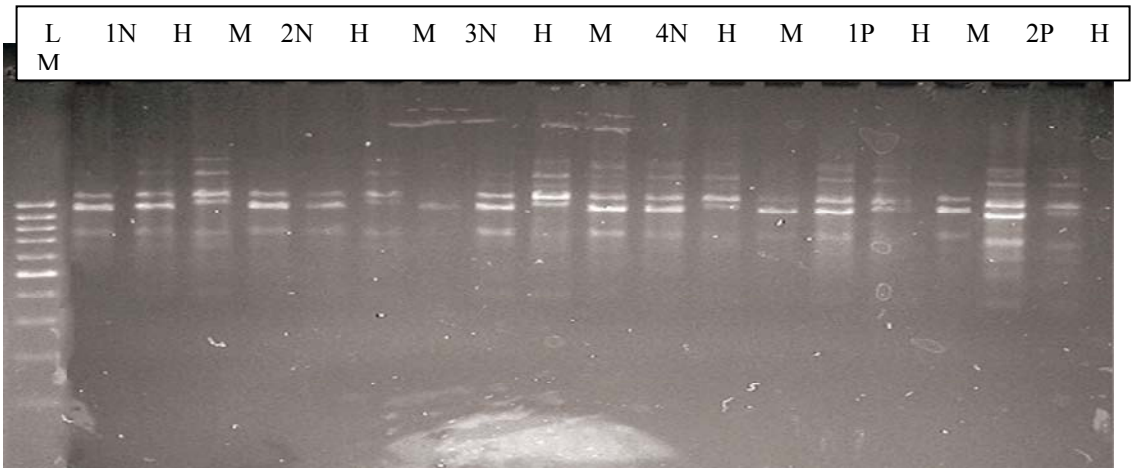
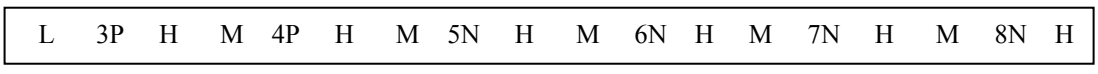


Figure (3 – 45): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPB-15 before and after digestion with *HpaII* and *MspI*. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P :Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



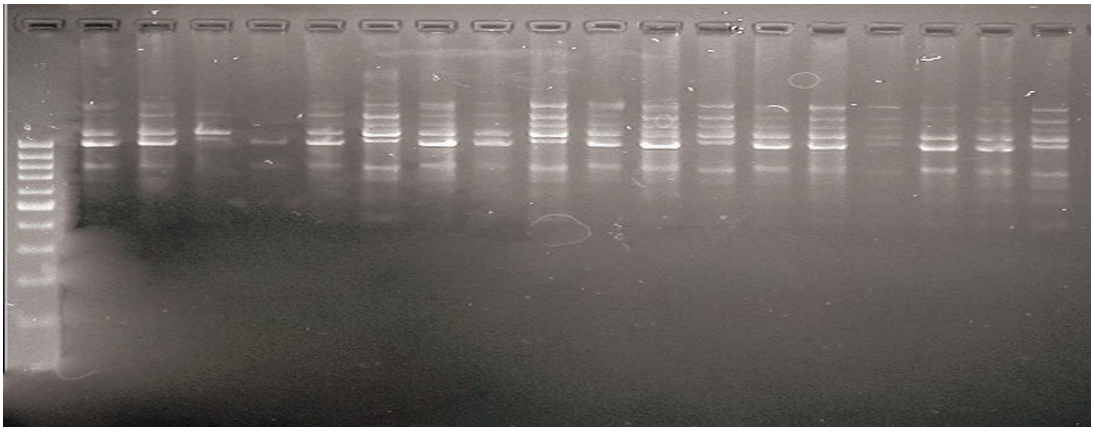


Figure (3 – 46): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPB-15 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P, 4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

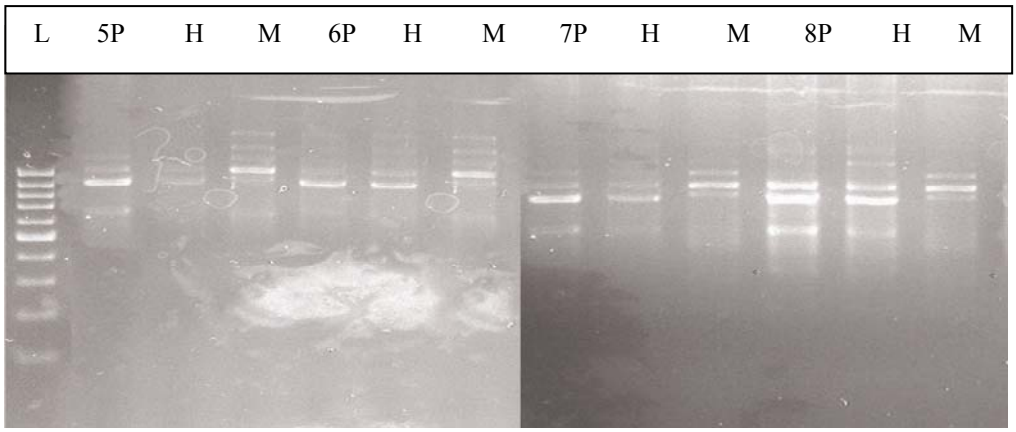
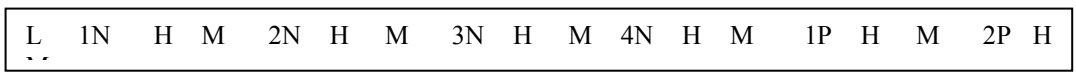


Figure (3 – 47): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPB-15 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5P, 6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



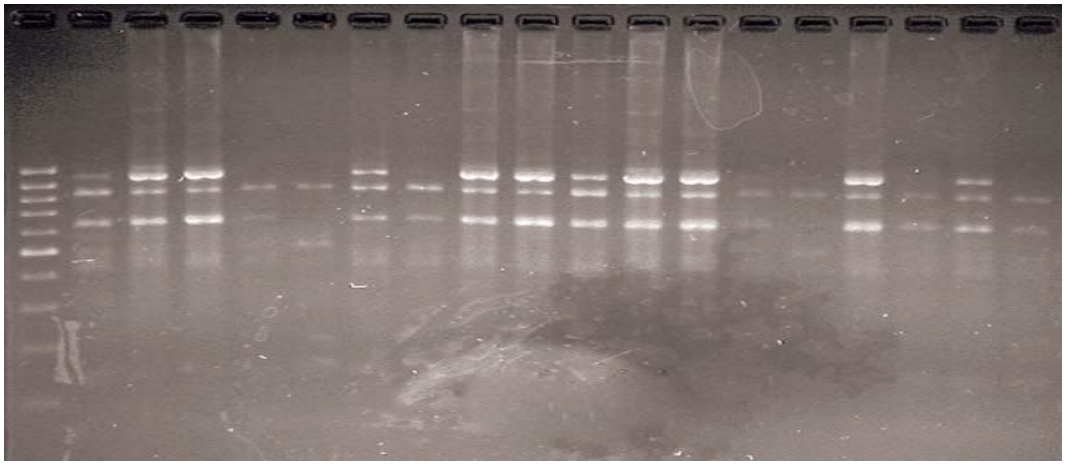


Figure (3 – 48): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPB-17 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P :Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

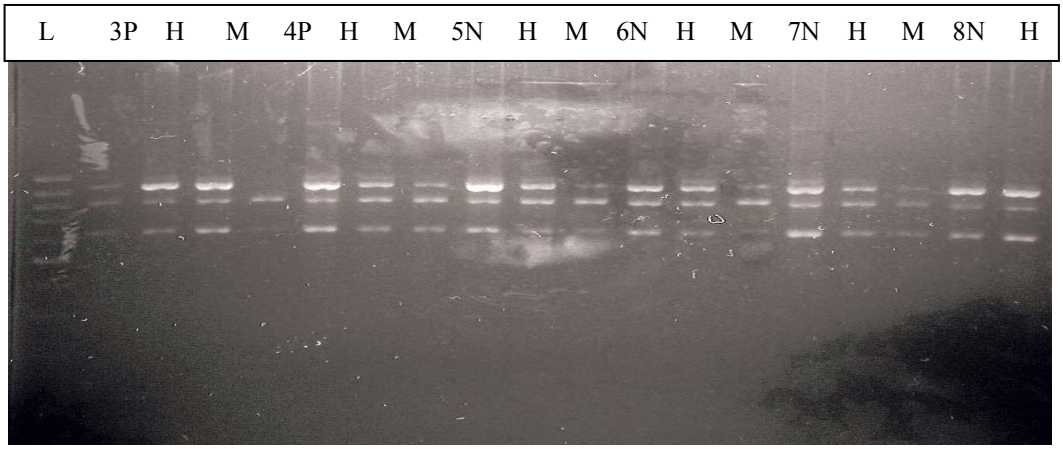
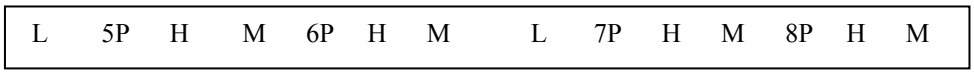


Figure (3 – 49): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPB-17 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P, 4P and 5P: Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



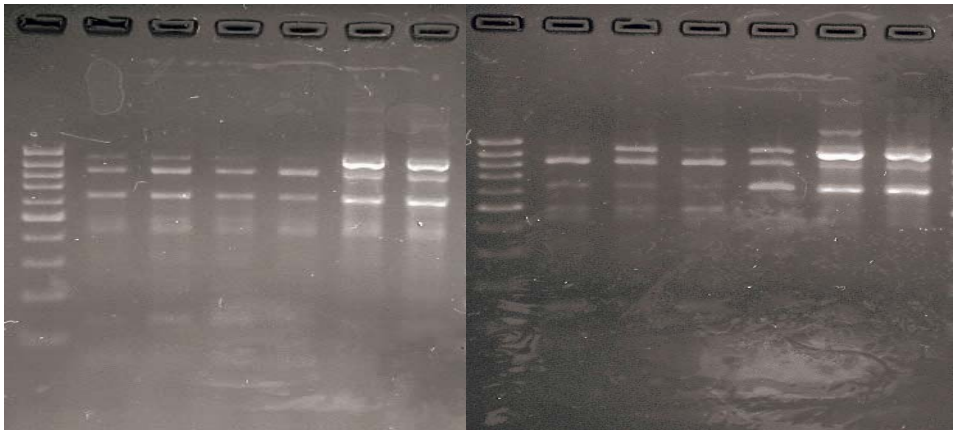


Figure (3 – 50): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPB-17 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5P, 6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

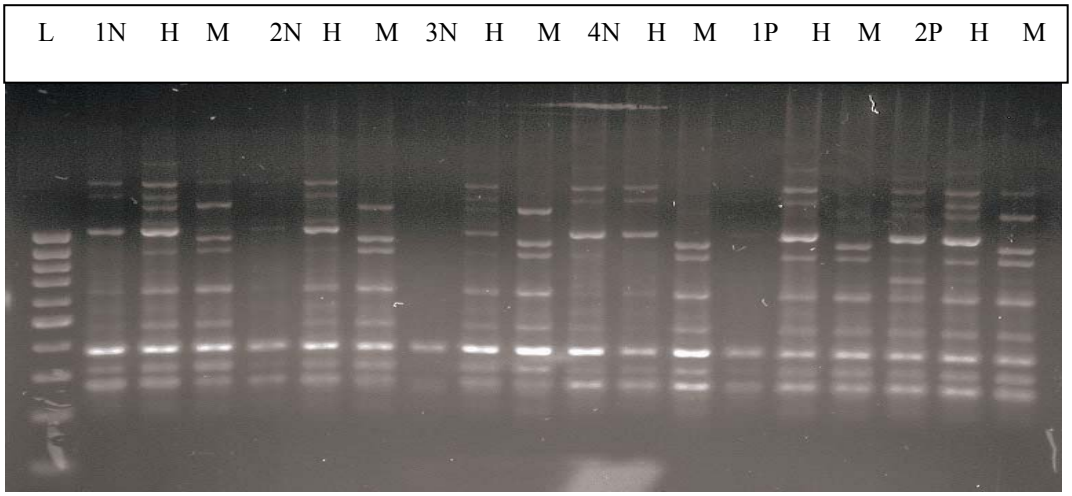
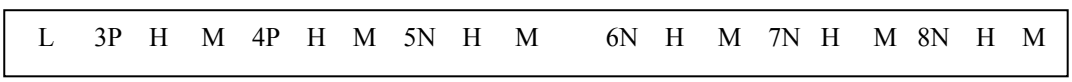


Figure (3 – 51): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with with OPB-18 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P :Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



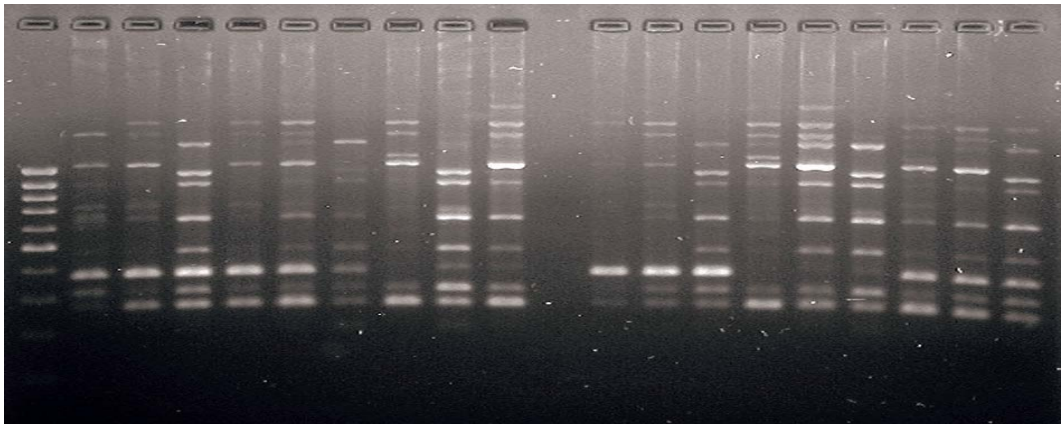


Figure (3 – 52): RAPD-PCR patterns of genomic DNA CML patients females and normal males CML patients obtained with OPB-18 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P and 4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

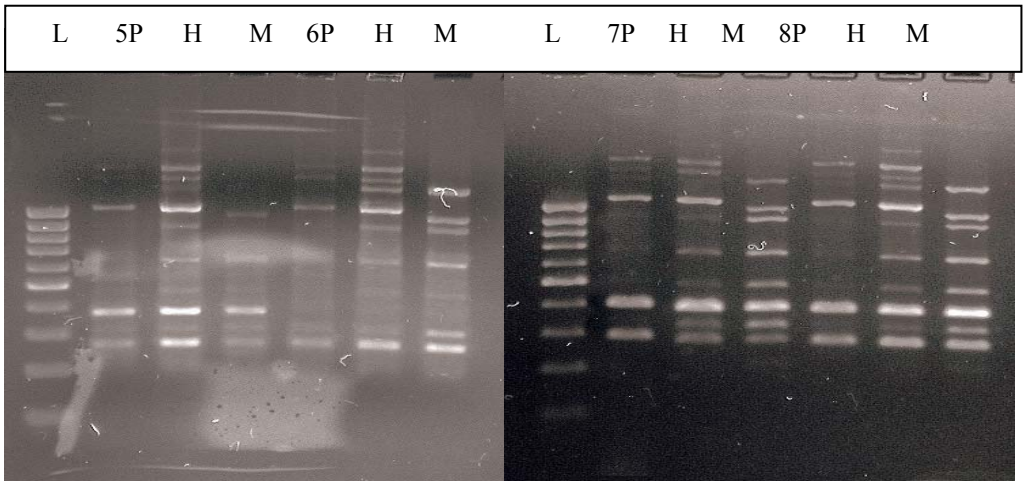
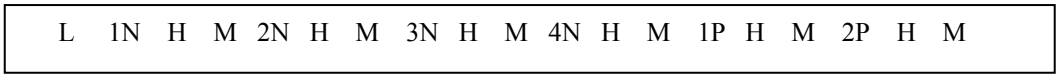


Figure (3 – 53): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPB-18 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



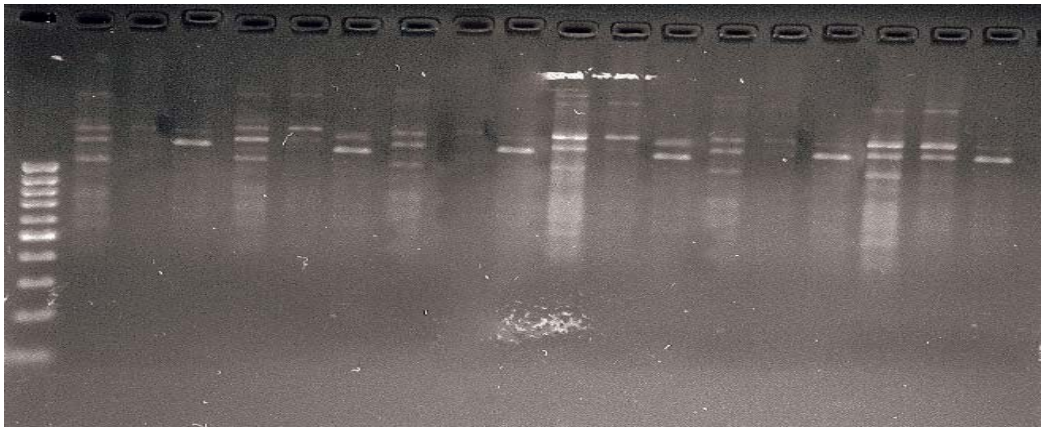


Figure (3 – 54): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPE-05 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P: Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

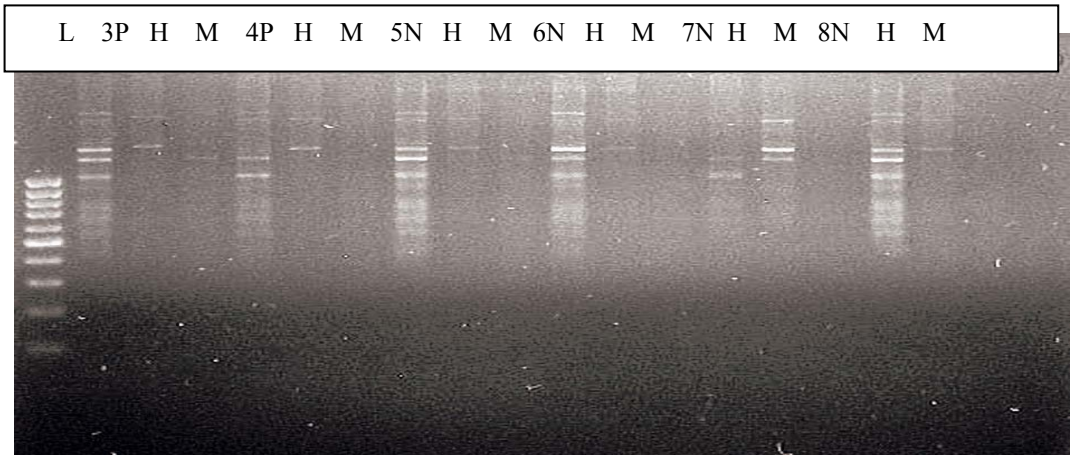
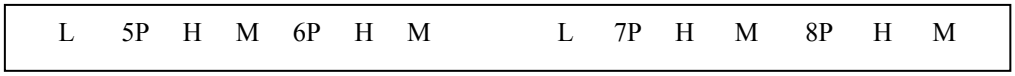


Figure (3 – 55): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPE-05 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5P : Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



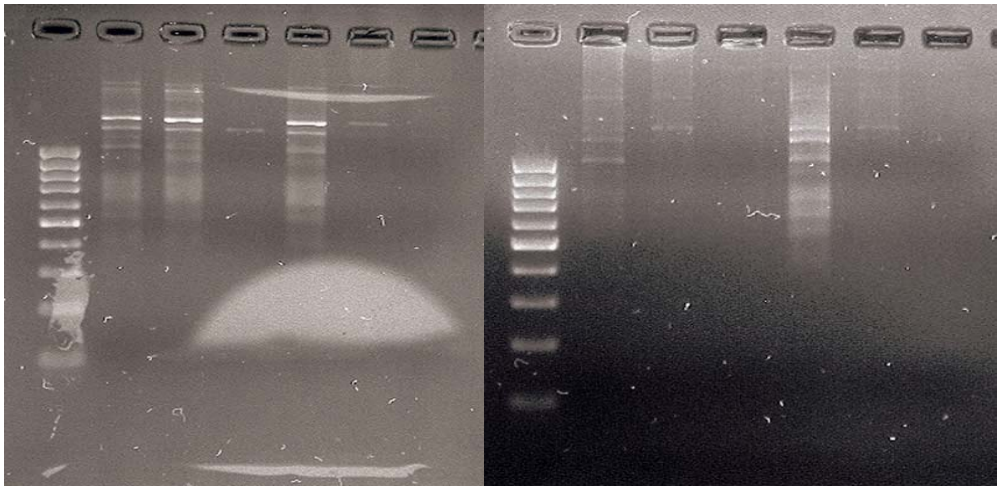


Figure (3 – 56): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPE-05 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5P, 6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

L 1N H M 2N H M 3N H M 4N H M 1P H M 2P H M

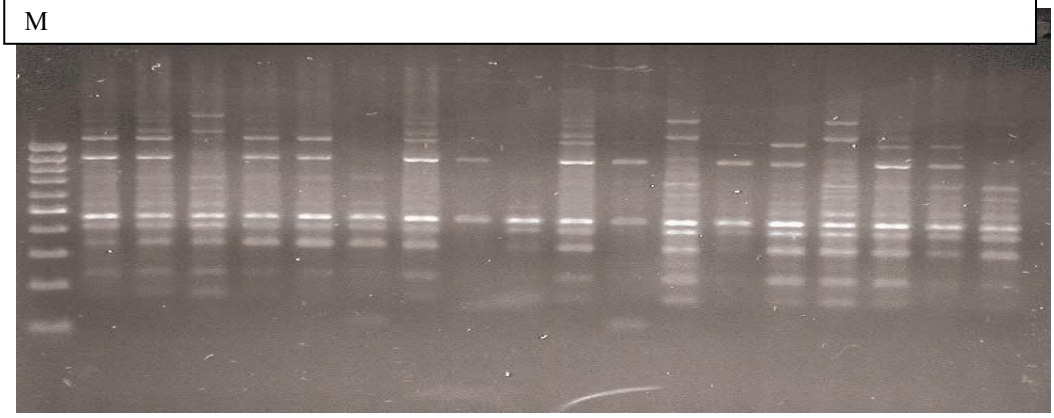


Figure (3 – 57): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPJ-05 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P:Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

L 3P H M 4P H M 5N H M 6N H M 7N H M 8N H M

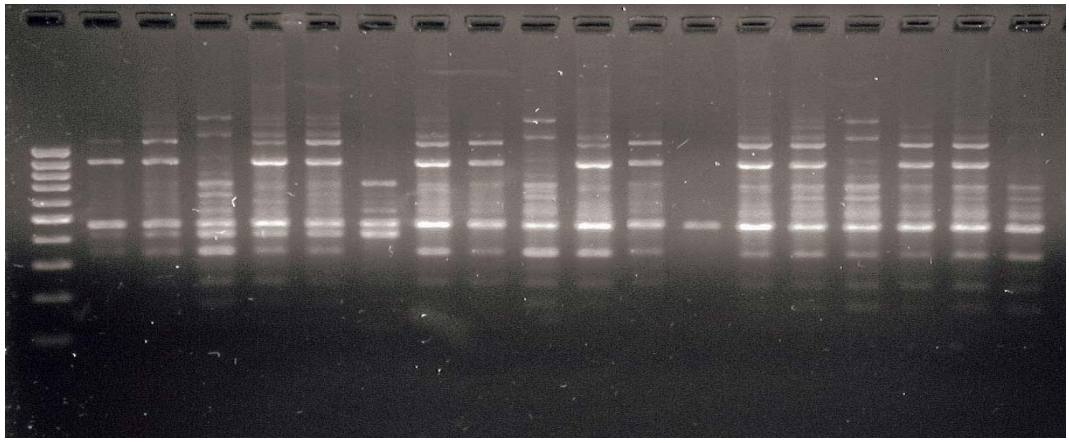


Figure (3 – 58): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPJ-05 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P, 4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

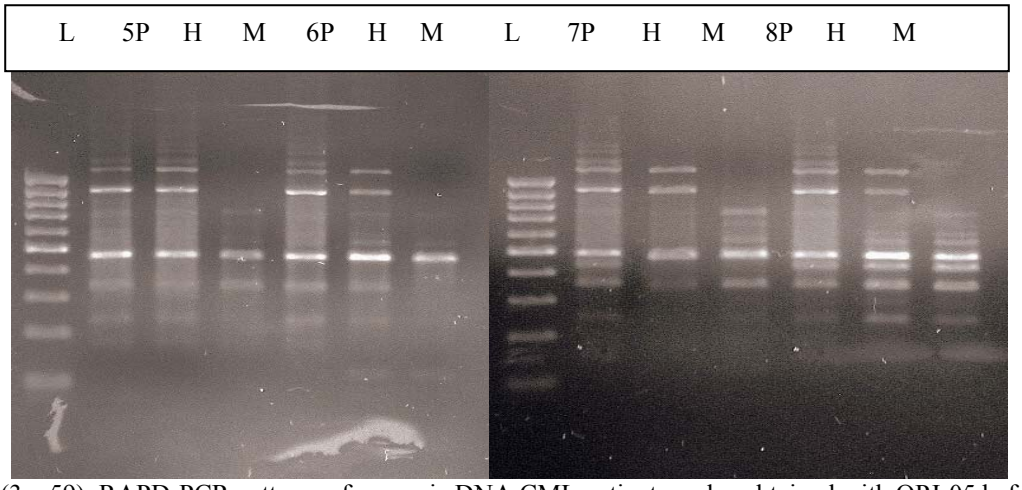
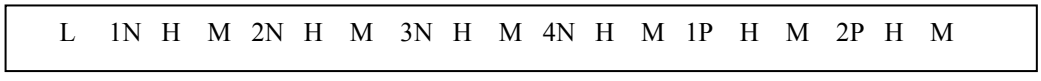


Figure (3 – 59): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPJ-05 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



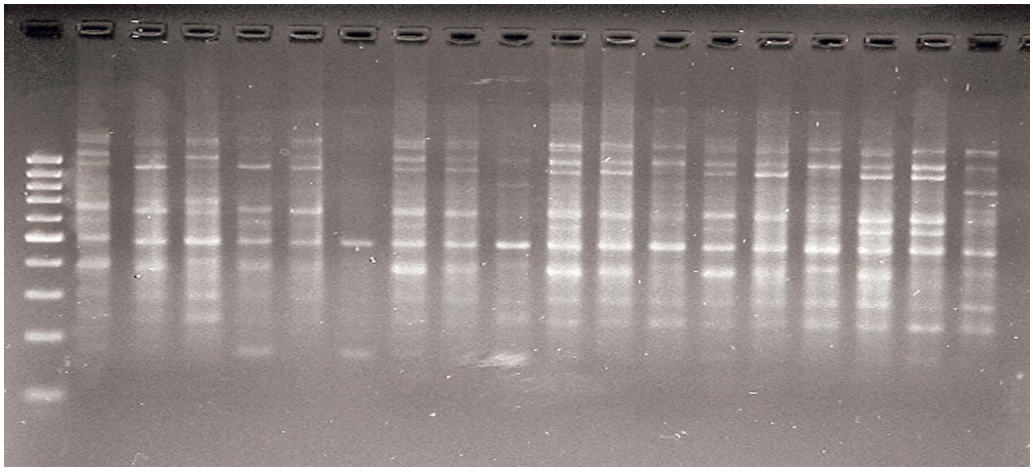


Figure (3 – 60): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPW-17 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P:Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

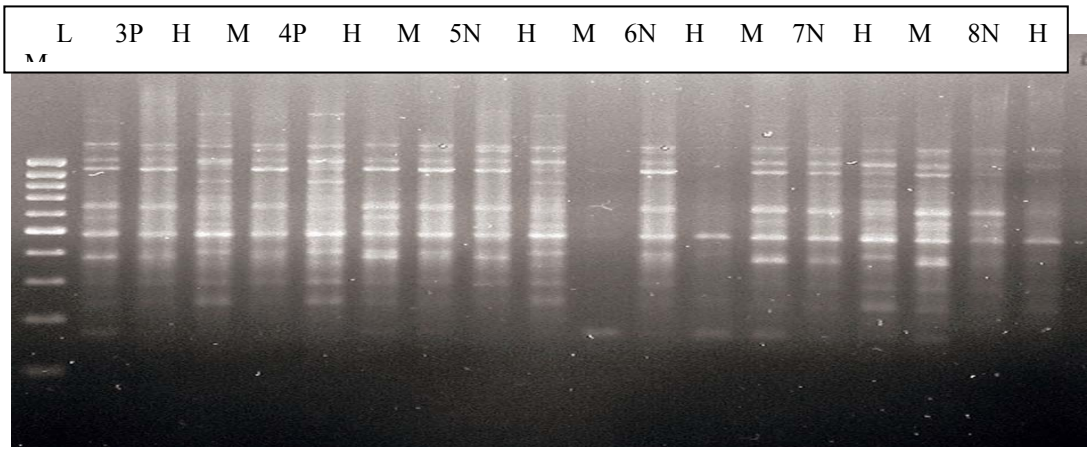
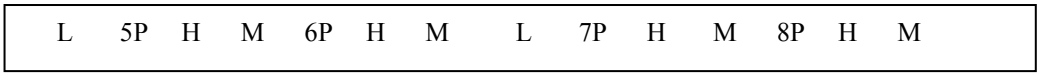


Figure (3 – 61): RAPD-PCR patterns of genomic DNA CML patients females and normal malesobtained with OPW-17 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P, 4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).



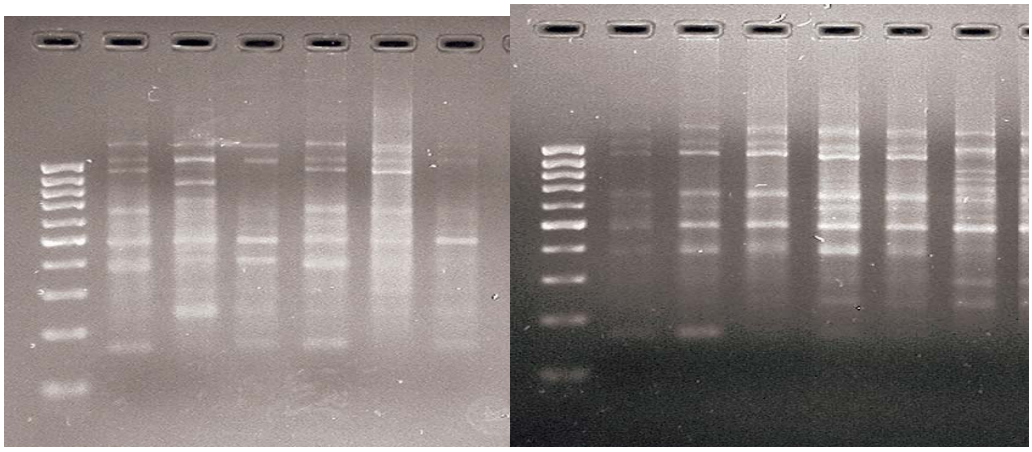


Figure (3 – 62): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPW-17 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5P, 6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

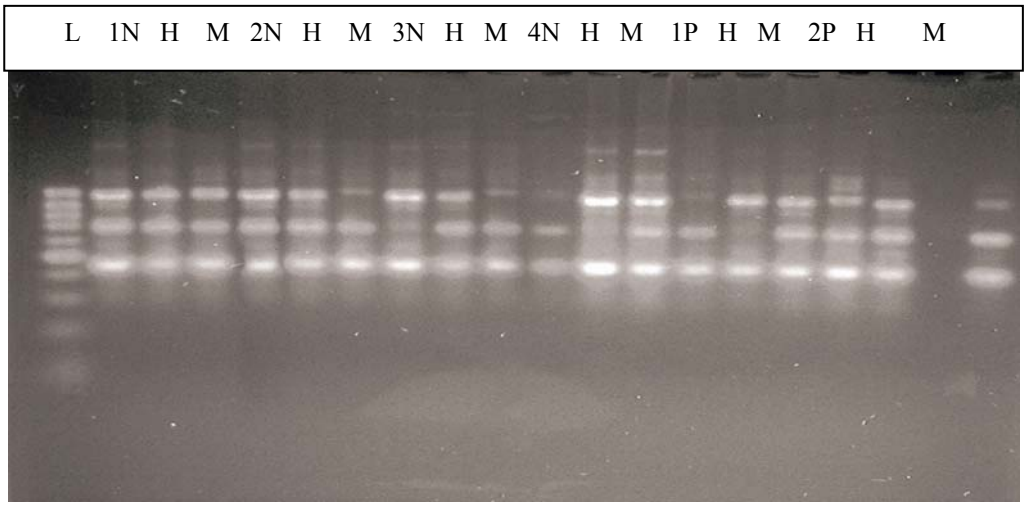


Figure (3 – 63): RAPD-PCR patterns of genomic DNA normal females and CML patients females obtained with OPZ-02 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

1N, 2N, 3N and 4N: Amplified products of undigested genomic DNA extracted from normal females.
1P and 2P:Amplified products of undigested genomic DNA extracted from CML patients females.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

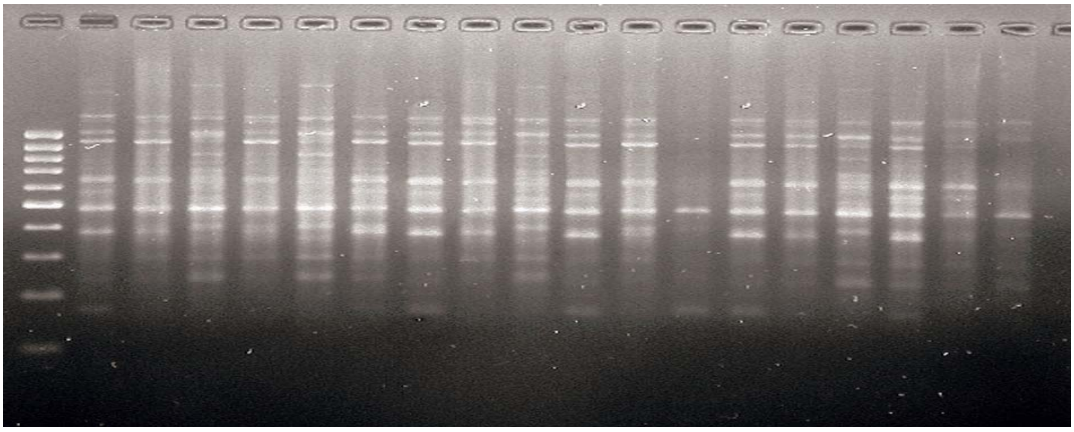


Figure (3 – 64): RAPD-PCR patterns of genomic DNA CML patients females and normal males obtained with OPZ-02 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

3P and 4P : Amplified products of undigested genomic DNA extracted from CML patients females.
5N, 6N, 7N and 8N: Amplified products of undigested genomic DNA extracted from normal males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

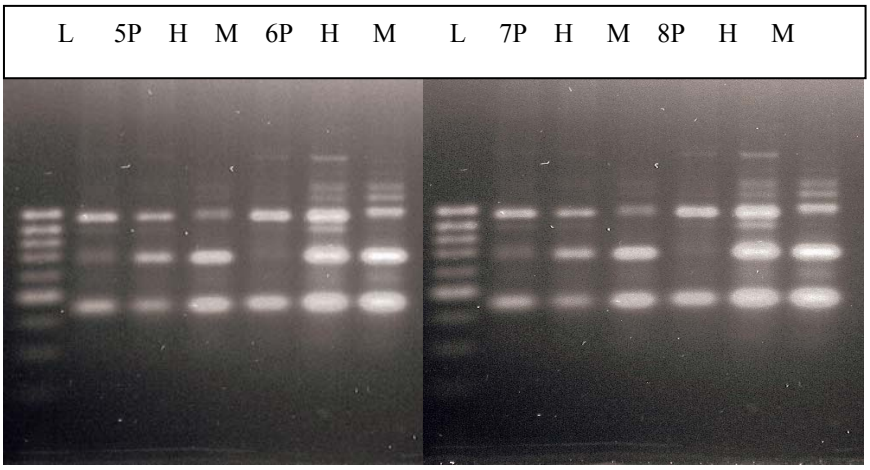


Figure (3 – 65): RAPD-PCR patterns of genomic DNA CML patients males obtained with OPZ-02 before and after digestion with *Hpa*II and *Msp*I. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt/cm for two hours.

5p, 6P, 7P and 8P:Amplified products of undigested genomic DNA extracted from CML patients males.
H : Amplified products of digested genomic DNA with *Hpa*II.
M : Amplified products of digested genomic DNA with *Msp*I.
L : Lambda DNA (100 – 1031 bp).

Conclusions

1- Genomic DNA purification kit was found more suitable for DNA isolation from blood of leukemia patients and normal individuals in this work.

2- 22 different arbitrary primers were used to detect genetic polymorphism. 13 RAPD primers which include (OPA-01, OPA-11, OPA-12, OPA-14, OPB-11, OPB-12, OPC-01, OPF-18, OPI-18, OPJ-01, OPK-08, OPY-10 and OPZ-19) showed no amplified genomic products, whereas nine primers which include (OPA-09, OPA-13, OPB-15, OPB-17, OPB-18, OPE-05, OPJ-05, OPW-17 and OPZ-02) were found to produce amplified products.

3- DNA polymorphisms were detected in normal individuals and leukemia patients. The DNA polymorphisms were detected as loss of a normal band, gain of a new band, band shifting and change in band intensities. Some RAPD primers showed interesting DNA fragment profiles in leukemia patients in comparison with that obtained in normal individuals.

4- The percentages of detected polymorphism among leukemia patients was variable. 37.8% of leukemia patients showed the highest DNA polymorphism because they were detected by nine primers, 48.8% of patients showed detected polymorphism by eight primers, 8.9% of patients revealed DNA polymorphism as detected by seven primers and 4.5% of patients showed DNA polymorphism as detected by six primers.

5- The study indicated that there were significant differences in the ability to detect genetic alteration by various primers, primer OPB-17 showed the highest value of detection efficiency (0.059), followed by OPB-18 (0.054) and OPW-17 (0.048), whereas primer OPE-05 showed the lowest (0.016). Thus, RAPD primers might be used for selection of DNA marker in leukemia patients.

6- Digestion of genomic DNA by (*Msp*I and *Hpa*II) showed variable methylation patterns in normal individuals and leukemia patients.

Recommendations

1. Further work is required to investigate and screen the available RAPD primers to ascertain their suitability for detection of DNA polymorphism in leukemia patients.

2. Performing sequence analysis of DNA fragments which showed unique patterns.

3. Future study might be required for development of the sequences of RAPD primers which showed promising results in detection of genomic polymorphism between normal individuals and leukemia patients.

4. Further studies are required for validation of restriction analysis of DNA methylation in leukemia patients.

A

Alanne, M.; Salomaa, V.; Saarela, J.; Peltonen, L. and Perola, M. (2004). DNA extraction yield is associated with several phenotypic characteristics: results from two large population surveys. J. Thromb Haemost., 2: 2069–2071.

Albinger-Hegyi, A.; Hochreutener, B.; Abdou, M.T.; Hegyi, I.; Dours-Zimmermann, M.M.; Kurrer, M.O.; Heitz, P.U. and Zimmermann, D.R. (2002). High Frequency of t(14;18)-translocation breakpoints outside of major breakpoint and minor cluster regions in follicular lymphomas : Improved polymerase chain reaction protocols for their detection. Am. J. Pathol., 160: 823-832.

Arribas, R.; Capella G.; Tortola, S.; Masramon, L.; Grizzle, W.E.; Perucho, M. and Peinado, M.A. (1997). Assessment of genomic damage in colorectal cancer by DNA fingerprinting: prognostic applications. J. Clin. Oncol., 15: 3230–3240.

Atienzar, F.A. and Jha, A.N. (2006) .The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat. Res., 613 (2-3): 76-102.

Atienzar, F.A.; Venier, P.; Jha, A.N. and Depledge, M.H. (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutation. Mutat. Res., 521 (1-2): 151-163.

B

Bagasra, O.; Seshamma, T. and Pomerantz, R.J. (1993). Polymerase chain reaction *in situ*: intracellular amplification and detection of HIV-1 proviral DNA and other gene sequences. J. Immunol. Meth., 158: 131–145.

Ballabio, A.; Gibbs, R. and Casky, C.T. (1990). PCR test for cystic fibrosis detection. Nature, 434: 20.

Benter, T.; Papadopoulos, S.; Pape, M.; Manns, M. and Poliwoda, A. (1995). Optimization and reproducibility of random amplified polymorphic DNA in human. Analytical Biochemistry, 230: 92- 100.

Bernard, P.S. and Wittwer, C.T. (2002). Real-time PCR technology for cancer diagnostics. Clin. Chem., 48: 1178-1185.

Bird, A. (2002). DNA methylation patterns and epigenetic memory. Genes Dev., 16:6-21.

Bishop, J.M. (1987). The Molecular genetics of cancer. Science, 235 :305-311.

Blin, N. and Stafford, D.W. (1976). A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acid Res., 3: 2303-2308.

Boom, R. ; Sol, C.; Weel, J.; Gerrits, Y.; de Boer, M. and Wertheim, D. P. (1999). A highly sensitive assay for detection and quantitation of human cytomegalovirus DNA in serum and plasma by PCR. J. Clin. Microbiol., 37:1489-1497.

Borson, R. and Loeb, V. (1994). Acute and chronic leukemias in adults. Cancer J. Clin., 44: 323-352.

Bos, J.L.; Fearon, E.R.; Hamilton, M.; Vries, M.V.; Boom, J.H.; Eb, A.J. and Volgelstein, B. (1987). Prevalence of *ras* gene mutations in human colorectal cancers . Nature, 327: 293.

Boultwood, J. and Fidler, C. (2002). Methods in Molecular Medicine, vol. 68: Molecular Analysis of Cancer. Humana Press Inc., Totowa, New Jersey.

Brechtbuehl, K.; Whalley, S. A.; Dusheiko, G. M. and Saunders, N. A. (2001). A rapid real-time quantitative polymerase chain reaction for hepatitis B virus. J. Virol. Methods, 93: 105-113.

C

Caetano-Annoles, G.; Bassam, B.J. and Gresshoff, P.M. (1991). DNA Amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology, 9: 553-557.

Cardinez, C.J.; Foster, S.; German, R.R.; Jamison, P.M.; King, J.B.; Stewart, S.L.; Thompson, T.D.; Weir, H.K.; Wilson, R.J.; Edwards, B.K.; Reichman, M.E.; Howe, H.L.;Kohler, B.A.; Anderson, R.N. (2006). United States Cancer Statistics: 2003 Incidence and Mortality. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute.

Cave, H.C.; Bosch, J.W.; Stefan Suciu, S.; Guidal, C.; Waterkeyn, C.; Otten, J.; Bakkus, M.; Thielemans, K.; M.D., Grandchamp, B.; Vilmer, E.; Nelken, B.; Fournier, M.; Boutard, P.; Lebrun, E.; Méchinaud, F.; Garand, R.; Robert, A.; Dastugue, N.; Plouvier, E.; Racadot, E.; Ferster,

A.; Gyselinck, J.; Fenneteau, O.; Duval, M.; Solbu, G. and Manel, A.M. (1998). Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. New Engl. J. of Med., 339 (9): 591-598.

Cenis, J.L. (1992). Rapid extraction of fungal DNA for PCR amplification. Nucleic Acid Res., 20 (9): 2380.

Chappro, J.; Wilcox, P.; Gratta Paglia, D.; Mallev, D.O.; McCord, S.; Sederoff, R.; Mclntyre, L. and Whtten, R. (1992). Genetic Mapping of Pine Using RAPD Markers: Construction of 191 Marker Map and Development of Half-Genetic. Advances in Gene Technology: Feeding World in 21St Century. Miami Winter Symposium Florida.

Chou, Q.; Russell, M.; Birch, D.E.; Raymond, J. and Bloch, W. *(1992). Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Res., 20: 1717-1723.*

Clark, S. J.; Harrison, J.; Paul, C. L. and Frommer, M. (1994). High sensitivity mapping of methylated cytosines. Nucleic Acids Res., 22: 2990–2997.

Croce, C.M. (2008). Oncogenes and cancer. N. Engl. J. Med., 358 (5): 502-511.

Costa, C.; Pissard, S.; Girodon, E.; Huot, D. and Goossens, M. (2003). A one-step real-time PCR assay for rapid prenatal diagnosis of sickle cell disease and detection of maternal contamination. Mol. Diagn., 7: 45-48.

D

Davies, H; Bignell, G.R.; Cox, C.; Philip Stephens, p.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M.J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray, K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B.A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Maitland, N.; Chenevix-Trench, G.; Riggins, G.J.; Bigner, D.D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J.W.C.; Leung, S.Y.; Yuen, S.T.; Weber, B.L.; Seigler, H.F.; Darrow, T.L.; Paterson, H.; Marais, R.; Marshall, C.J.; Wooster, R.; Stratton, M.R. and Futreal, P.A. (2002). Mutations of the BRAF gene in human cancer. Nature, 417: 949-954.

Desjardin, L.E.; Chen, Y.; Perkins, M.D.; Teixeira, L.; Cave, M.D. and Eisenach, K.D. (1998). Comparison of the ABI 7700 system (TaqMan) and competitive PCR for quantification of IS6110 DNA in sputum during treatment of tuberculosis. J. Clin. Microbiol., 36: 1964-1968.

Dil-Afroze; Misra, A.; Sulaiman, I.M.; Sinha, S.; Sarkar, C.; Mahapatra, A.K. and Hasnain, S.E. (1998). Genetic alterations in brain tumors identified by RAPD analysis. Gene, 206: 45-48.

Ding, C. and Cantor, C.R. (2004). Quantitative analysis of nucleic acids--the last few years of progress. Journal of Biochemistry and Molecular Biology, 37 (1): 1–10.

Diss, T.C. and Pan, L. (1997). Polymerase chain reaction in the assessment of lymphomas. Cancer Surv., 30: 21–44.

Dohner, H.; Genbauer, S.S.; Benner, A.; Leuolt, E.; Kroper, A.; Bullinger, L.; Dohner, K.; Bentz, M. and Lichter, P. (2000). Genomic

aberrations and survival in chronic lymphocytic leukemia. N. Engl. J. Med., 343: 1910-1916.

Don, R.H.; Cox, P.T.; Wainwright, B.J.; Baker, K. and Mattick, J.S. *(1991).* Touchdown PCR to circumvent spurious priming during gene amplification. *Nucl. Acids Res., 19: 4008.*

Ducasse, M. and Brown, M.A. (2006). Epigenetic aberrations and cancer. Molecular Cancer, 5: 60-69.

E

Elgort, M. G.; Herrmann, M. G.; Erali, M.; Durtschi, J. D.; Voelkerding, K. V. and Smith, R. E. (2004). Extraction and amplification of genomic DNA from human blood on nano porous aluminum oxide membranes .Clinical Chemistry, 50: 1817-1819.

Enrietto, P.J.; Payane, L.N. and Hayman, M.J. (1983). A recover avian myelocytomatosis virus that induces lymphomas in chickens and their molecular basis. Cell, 35: 369-379.

Esteller, M. (2008). Epigenetics in Cancer. N. Engl. J. Med., 358 :1148-1159.

F

Faderl, S.; Talpaz, M.; Estrov, Z.; Usan, O.S.; Razelle, K. and Hagopm, K. (1999). The biology of chronic myeloid leukemia. N. Eng. J. Med., 341 (3): 164-172.

Fearon, E.R. and Vogelstein, B. (2000). Tumor Suppressor Gene Defects in Human Cancer. In: Bast Jr., R.C.; Kufe, D.W.; Pollock, R.E.; Weichselbaum, R.R.; Holland, J.F. and Frei, E.; Cancer Medicine. 5th ed. B.C. Decker Inc., Hamilton, London.

Folgueira, L.; Delgado, R.; Palenque, E.; Aguado, J. M. and Noriega, A. R. (1996). Rapid diagnosis of *Mycobacterium tuberculosis* bacteremia by PCR. J. Clin. Microbiol., 34: 512–515.

Friedland, L. R.; Menon, A.G.; Reising, S.F.; Ruddy, R.M. and Hassett, D.J. (1994). Development of a polymerase chain reaction assay to detect the presence of *Streptococcus pneumoniae* DNA. Diag. Microbiol. Infect. Dis. 20: 187-193.

Friedman, J.M. (1996). Cancer and Genetics .In Friedman, J.M.; Dill, F.J; Haydcn, M.R. and Mc Gillivrary, B.C. (Eds). Genies 2nd Williams Wilkins, London, 136-161.

G

Garms, C.; Buys, T.PH. and Lam, W. (2004). Genetic alteration and gene expression modulation during cancer progression. Mol. Cancer, 3 (9): 1-26.

Garnis, C.; Coe, B.P.; Ishkanian, A.; Zhang, L.; Rosin, M.P.; and Lam, W.L. (2004). Novel regions of amplification on 8q distinct from the MYC locus and frequently altered in oral dysplasia and cancer. Genes, Chromosomes Cancer, 39: 93–98.

Goldman, J.; Greaves, M.F. and Nau, M. (1999). Understanding of leukemia and related cases, edited by Williams, J.W.editor.

Goldman, J.M. and Melo, J.V. (2003). Chronic myeloid leukemia — advances in biology and new approaches to treatment. N. Engl. J. Med., 349 (15): 1451-1464.

Gonzalgo, M.L.; Liang, G.; Spruck, C.H.; Zingg, J.M.; Rideout, W.M. and Jones, P.A. (1997). Identification and characterization of differentially methylated regions of genomic DNA by methylation-sensitive arbitrarily primed PCR. Cancer Res, 57: 594-599.

Grimberg, J.; Nawoschik, S.; Belluscio, L.; McKee, R.; Turck A. and Eisenberg, A. (1989). A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acids Res., 17 (20): 8390.

Gross-Berllard, M.; Oudet, P. and Chambon, P. (1973). Isolation of high molecular-weight DNA from mammalian cells. Eur.J. Biochem., 36: 32-8.

Gunnarsson, C.; Ahnstrom, M.; Kirschner, K.; Olsson, B.; Nordenskjold, B.; Rutqvist, L. E.; Skoog, L. and Stal, O. (2003). Amplification of HSD17B1 and ERBB2 in primary breast cancer. Oncogene, 22: 34-40.

Gustafson, S.; Proper, J.A.; Bowie, E.J. and Sommer, S.S. (1987). Parameters affecting the yield of DNA from human blood. Anal. Biochem., 165: 294-299.

H

Haase, A.T.; Retzel, E.F. and Staskus, K.A. (1990). Amplification and detection of lentiviral DNA inside cells. Proc. Natl. Acad. Sci. U.S.A., 87: 4971–5.

Hahn, W.C. and Weinberg, R.A. (2003). Rules for making human tumors cells. N. Eng. J. Med., 347 (20): 1593-1603.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell, 100: 57–70.

Hall, A. (1984). Oncogenes - implications for human cancer. Journal of the Royal Society of Medicine, 77 (5): 410-416.

Harrison, C.J. (2000). The managements of patients with leukemia: the role of cytogenetics in this molecular. Br. J. Haematol., 108: 19-30.

Hassan, S.A. (2002). Epidemiology study of *Pseudomonas aeruginosa* by conventional bacterial markers and DNA polymerase chain reaction. Ph.D. Thesis. College of Science, Baghdad University.

He, Q.; Wang, J.P.; Osato, M. and Lachman, L.B. (2002). Real-time quantitative PCR for detection of *Helicobacter pylori*. J. Clin. Microbiol., 40: 3720-3728.

Herman, J.G.; Graff, J.R.; Myöhänen, S.; Nelkin, B.D. and Baylin, S.B. *(1996). Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc. Natl. Acad. Sci. U.S.A., 93 (13): 9821-26.*

Herman, J.G. and Baylin, S.B. (2003). Gene silencing in cancer in association with promoter hypermethylation. N. Engl. J. Med., 349 (21): 2042-2054.

Hoffbrand, A.V.; Pettit, J.E. and Moss, P.A.H. (2002). Essential Haematology, 4th ed., Blackwell Scientific Publications, Oxford.

I

Ibrahim, M.A.K.; Dhahi, M.A.; Rabee, M.B.; Baker, F.A. and Jober, A. (2004). DNA Methylation polymorphism of aging human ovaries. Selected Res. Papers, 4: 42-49.

J

Jeanpierre, M. (1987). A rapid method for the purification of DNA from blood. Nucl. Acid Res., 15 (22): 9611.

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

Johns, M.J. and Paulus-Thomas, J.E. (1989). Purification of human genomic DNA from whole blood using sodium perchlorate in place of phenol. Anal. Biochem., 180 (2):276-8.

Jones, N.; Ougham, H. and Thomas, H. (1997). Marker and mapping. New phyto. 137: 165-177.

Jones, P.A. and Baylin, S.B. (2002). The fundamental role of epigenetic events in cancer. Nat. Rev. Genet., 3: 415-428.

K

Kanai, N.; Fujii, T.; Saito, K. and Yokoyama, T. (1994). Rapid and simple method for preparation of genomic DNA from easily obtainable clotted blood. Clin. Patho., 47: 1043-1044.

Keating, M.J. (1999). Chronic lymphocytic leukemia. Semin. Oncol., 26: 107-14.

Kieser, T. (1995). Preparation and Analysis of Genomic and Plasmid DNA. John Innes. Center , Nerwich, NR47UH, U.K.

Kipps, T.J. (2000). Chronic lymphocytic leukemia. Curr. Opin. Hematol., 7: 223-234.

Kirby, K.S. (1957). A new method for the isolation of deoxyribonucleic acids: Evidence on the nature of bonds between deoxyribonucleic acid and protein. Biochem. J., 66: 495-504.

Klein-Lankhorst, R.M.; Vermunt, A.; Weide, R.; Liharska, T. and Zable, P. (1991). Isolation of molecular markers for tomato (*Lycorpericon esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Appl. Genet., 83: 110-114.

Knudson, A.G. (2001). Two genetic hits (more or less) to cancer. Nat. Rev. Cancer, 1: 157-162.

Kolomietz, E.; Marrano, P.; Yee, K .; Thai1, B.; Braude, I.; Kolomietz, A.; ,Chun, K.; Minkin, S.; Kamel-Reid, K.; Minden, M. and Squire, J.A. (2003). Quantitative PCR identifies a minimal deleted region of 120 kb extending from the Philadelphia chromosome ABL translocation breakpoint in chronic myeloid leukemia with poor outcome. Leukemia, 17: 1313–1323.

Kösters, K., Riffelmann, M., and Wirsing von Konig, C.H. (2001). Evaluation of a real-time PCR assay for detection of *Bordetellapertussis* and *B. parapertussis* in clinical samples. J. Med. Microbiol., 50: 436-440.

Krug, U.; Ganser, A. and Koeffler, H.P. (2002). Tumor suppressor genes in normal and malignant hematopoiesis .Oncogene, 21 : 3475- 3495.

Kurzrock, R.; Gutterman, J.U.; Talpaz, M. (1988). The molecular genetics of Philadelphia chromosome-positive leukemias. N. Engl. J. Med., 319: 990-8.

L

Lahiri, D. and Nurnberger, J. (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res., 19: 5444.

Lahiri, D.K. and Schnabel, B. (1993). DNA isolation by a rapid method from human blood samples: effects of $MgCl_2$, EDTA, storage time, and temperature on DNA yield and quality. Biochem. Genet., 31 (7-8): 321-328.

Laird, P.W.; Zijderveld, A.; Linders, K.; Rudnicki, M.A.; Jaenisch, R. and Berns, A. (1991). Simplified mammalian DNA isolation procedure. Nucleic Acid Res., 19 (15): 4293.

Lam, N.Y.L.; Rainer, T.H.; Chiu, R.W.K. and Dennis Lo., Y.M. (2004). EDTA is a better anticoagulant than heparin or citrate for delayed blood processing for plasma DNA analysis. Clinical Chemistry, 50: 265-257.

Lawyer, F.C.; Stoffel, S.; Saiki, R.K.; Myambo, K.; Drummond, R. and Gelfand, D.H. (1989). Isolation, characterization and expression in *Escherichia coli* of the DNA polymerase gene from *Thermus aquaticus*. Journal of Biol. Chem., 264: 6427-6437.

Lee, M.S.; Chang, K.S.; Cabanillas, F.; Freireich, E.; Trujillo, J. and Stass, S. (1987). Detection of minimal residual cells carrying the t(14;18) by DNA sequence amplification. Science, 237: 175–178.

Lee, W.I.; Kantarjian, H.; Glassman, A.; Talpaz, M. and Lee, M.S. (2002). Quantitative measurement of BCR/abl transcripts using real-time polymerase chain reaction. Annals of Oncology, 13: 781–788.

Levine, A.J. (1993). The tumor suppressor genes. Annu. Rev. Biochem., 62: 623-625.

Liang, G.; Gonzalgo, M.L.; Salem, C. and Jones, P.A. (2002). Identification of DNA methylation differences during tumorigenesis by

methylation-sensitive arbitrarily primed polymerase chain reaction. Methods, 27: 150-155.

Lisby, G. (1997). Application of Nucleic Acid Amplification in Clinical Microbiology. In: Meltzer, S.J. (ed). PCR in Bioanalysis. Humana Press, Totowa, New Jersy. Pp. 1-29.

Liu, H.X.; Zhu, P.; Zhang, Y.; Wang, H.X.; DU, J.W.; Liu, J.; Gu, J.Y. and Ou, Y. (2007). Real-time PCR array for simultaneous detection of 37 kinds of fusion genes in leukemia. Zhonghua Yi Xue Za Zhi., 87 (8): 526-632.

Li , Z.G.; Wu, M.Y.; Zhao, W.; Li, B.; Yang, J.; Zhu, P. and Hu, Y.M. (2003). Detection of 29 types of fusion gene in leukemia by multiplex RT-PCR. Zhonghua Xue Ye Xue Za Zhi., 24 (5): 256-258.

Lodish, H.; Berk, A.; Matsudaira, P.; Kaiser, C.A.; Krieger, M.; Scott, M.P.; Zipursky, S.L. and Darnell, J. (2003). Molecular Cell Biology. 5th Ed. New York: W. H. Freeman and Co. pp: 935- 972.

Loeb, L. A. (2001). A Mutator phenotype in cancer. Cancer Res., 61: 3230–3239.

Loeb, L.A. (1991). Mutator phenotype may be required for multistage Carcinogenesis. Cancer Res., 51: 3075-3079.

London, K.W.; Burnie, J.P.; Coke, A.P. and Matthews, R.C. (1993). Application of polymerase chain reaction to fingerprinting *Aspergillus fumigates* by random amplified polymorphic DNA. J. Clin. Microbiol., 31: 1117-1121.

Longmire, J.L.; Albright, K.L.; Lewis, A.K.; Meincke, L.J. and Hildebrand, C.E. (1987). A rapid and simple method for the isolation of high molecular weight cellular and chromosomespecific DNA in solution without the use of organic solvents. Nucl. Acid. Res., 15 (2): 859

Lowe, A.J.; Hanotte, O. and Guarino, L. (1996). Standardization of Molecular Genetic Techniques for the Characterization of Germplasm Collection: The Case of Random Amplified Polymorphic DNA (RAPD). Plant Genetic Resources Newsletter, 107: 50-54.

Lowenberg, B.; Downing, J.R. and Burnett, A. (1999). Acute myeloid leukemia. N. Eng. J. Med., 341 (14): 1051-1062.

Lukas, J.; Niu, N. and Press, M.F. (2000). p53 mutations and expression in breast carcinoma *in situ*. Am. J. Pathol., 156: 183–191.

Lund, A.H. and Lohuizen, M.V. (2004). Epigenetics and cancer. Genes & Dev. 18: 2315-2335.

Luo, L.; Li, B. and Pretlow, T.P. (2003). DNA alterations in human aberrant crypt foci and colon cancers by random primed polymerase chain reaction. Cancer Res., 63: 6166-6169.

M

Mackie, M.J.; Ludlam, C.A. and Hayness, A.P. (1999). Disease of the Blood. In: Haslett, C.; Chilvers, R.E.; Hunter, A. and Boon, A.N. Davidson's Principle and Practice of Medicine 18th ed. Churchil Living Ston, New York.

Maeda, T.; Jikko, A.; Hiranuma, H. and Fuchihata, H. (1999). Analysis of genomic instability in squamous cell carcinoma of the head and neck using the random amplified polymorphic DNA method. Cancer. Lett., 138: 183-188.

Mahenthiralingam, E.; Campbell, E.; Foster, J.; Lam, J.S. and Speert, D.P. (1996). Random amplified polymorphic DNA typing of P. aeruginosa isolated recovered from patients with cystic fibrosis. J. Clin. Microbiol., 34 (5): 1129-1135.

Manchester, K.L. (1995). Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. Biotechniques, 19 : 208–210.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol., 3: 208-218.

Martein, P.M.; Schasion, H.P. and Tanzer, J. (1999). Chronic myeloid leukemia: No rearrangement of the break point cluster region. Cancer Genet. Cytogenet., 36: 227-229.

McCarty, M. (1985). The transforming principle: Discovering that genes are made of DNA, Ist edition, pp. 83-85. W.W. Norton, New York.

McCormack, E.; Bruserud, O. and Gjertsen, B.T. (2008). Genetic models of acute myeloid leukaemia. Oncogene, 27: 3765–3779.

McManus, E.J. and Alessi, D.R. (2004). Cancer, oncogenes and signal transduction. Genome Biology, 5: 332-334.

Mehrotra, R.; Metz, P. and Kohlhepp, S. (2002). Comparison of in-house polymerase chain reaction method with the Roche Amplicortrade mark

technique for detection of *Mycobacterium tuberculosis* in cytological specimens. Diagn. Cytopathol., 26: 262–265.

Melo, J.V. (1996). The molecular biology of chronic myeloid leukemia. Leukemia, 10: 751-756.

Miller, S.A.; Dykes, D.D. and Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucl. Acids Res., 16: 1215.

Mirelman, D.; Brancha, R.; Rozenblatt, S. and Grafmke, L.I. (1991). Repetitive DNA elements characteristic of pathogenic *Entamoeba histoliytica* can be also detected after polymerase chain reaction in cloned non-pathogenic strain. Inf. Immum., 58: 166016-63.

Moore, D.D. and Dowhan, D. (2002). Purification and Concentration of DNA. In: Ausubel, F.M.; Brent, R.; Kingston, R.E.; Moore, D.D.; Seidman, J.G.; Smith, J.A. and Struhl, K. Current Protocols in Molecular Biology. Vol. 5. John Wiley and Sons, Inc. New York. Pp: 2.1.1-2.1.10

Moss, T.J. and Wallrath, L.L. (2007). Connections between Epigenetic Gene Silencing and Human Disease. Mutat Res., 618 (1-2): 163–174.

Mukeef, F.J. (1999). Cytogenetic study on leukemia patients. M.Sc. Thesis., College of Science, Al-Nahrain University.

Mullis, K.; Faloona, F.; Scharf, S.; Saiki, R.; Horn, G. and Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol., 51: 263-273.

Murray, M.G. and Thompson, W.E. (1991). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res., 8: 4321-4325.

Myers, T.W. and Gelfand, D.H. (1991). Reverse transcription and DNA amplification by a *Thermus thermophilus* DNA polymerase. Biochemistry, 30: 7661–7666.

N

Nasedkina, T.V.; Zharinov, V.S.; saeva, E.A.; Mityaeva, O.N.; Yurasov, R.N.; Surzhikov, S.A.; Turigin, A.Y.; Rubina, A.Y.; Karachunskii, A.I.; Gartenhaus, R.B. and Mirzabekov, A.D. (2003). Clinical screening of gene rearrangements in childhood leukemia by using a multiplex polymerase chain reaction-microarray approach. Clinical Cancer Research, 9: 5620-5629.

Navarro, J.M. and Jorcano, J.L. (1999). The use of arbitrarily primed polymerase chain reaction in cancer research. Electrophoresis, 20: 283–290.

Nephew, K.P. and Huang, T.H. (2003). Epigenetic gene silencing in cancer initiation and progression. Cancer Lett., 190: 125–133.

Neri, Q.V.; Tanaka, N.; Wang, A.; Katagiri, Y.; Takeuchi, T.; Rosenwaks, Z. and Palermo, G.D. (2004). Intracytoplasmic sperm injection. Accomplishments and qualms. Minerva Ginecol., 56 (3): 189-196.

Newton, C.R. and Graham, A. (1997). PCR 2nd ed. Bios Scientific Publishers Ltd., Oxford, U.K.

Novak, K. (2004). Epigenetics changes in cancer cells. Med. Gen. Med., 6 (4): 17.

O

Odero, M.D.; Soto, J.L.; Matutes, E.; Martin-Subero, J.I.; Zudaire, I.; Rao, P.H.; Cigudosa, J.C.; Ardanaz, M.T.; Chaganti, R.S.; Perucho, M. and Calasanz, M.J. (2001). Comparative genomic hybridization and amplotyping by arbitrarily primed PCR in stage A B-CLL. Cancer Genet. Cytogenetic, 130: 8–13.

Ong, T.M.; Song, B.; Qian, H.W.; Qian, T.W.; Wu, Z.L. and Whong, W.Z. (1998). Detection of genomic instability in lung cancer tissues by random amplified polymorphic DNA analysis. Carcinogenesis, 19: 233-235.

Osumi, K.; Fukui, T.; Kiyoi, H.; Kasai, M.; Kodera, Y.; Kudo, K.; Kato, K.; Matsuyama, T.; Naito, K; Tanimoto, M.; Hirai, H.; Saito, H.; Ohno, R. and Naoe, T. (2002). Rapid screening of leukemia fusion transcripts in acute leukemia by real-time PCR. Leukemia and Lymphoma, 43 (12): 2291–2299.

Ou, C.Y.; Kwok, S.; Mitchell, S.W.; Mack, D.H.; Sninsky, J.J.; Rrebs, J.W.; Feorino, P.; Warfield, D. and Schochetman, G. (1988). DNA of amplification for direct detection of HIV-1 in DNA peripheral blood Mononuclear Cells Science, 239: 295-297.

P

Palmiter, R.D. (1974). Magnesium precipitation of ribonucleoprotein complexes. Expedient techniques for the isolation of undegraded polysomes and messenger ribonucleic acid. Biochemistry, 13: 3606-3615.

Papadopoulos, S.; Benter, T.; Anastassiou, G.; Pape, M.; Gerhard, S.; Bornfeld, N.; Ludwig, W.D. and Dorken, B. (2002). Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. Int. J. Cancer, 99: 193–200.

Parish, J.H. (1972). Isolation I: Principles and Practice of Experiment with Nucleic Acids, pp.105-125. Longman, London.

Parzer, S. and Mannhalter, C. (1991). A rapid method for the isolation of genomic DNA from citrated whole blood. Biochem. J., 273: 229-231.

Payne, S.R. and Kemp, C.J. (2005). Tumor suppressor genetics. Carcinogenesis, 26 (12): 2031–2045.

Pedraza-Fariña, L.G. (2006). Mechanisms of oncogenic cooperation in cancer initiation and metastasis. Yale J. of Biology and Medicine, 79 (3-4): 95-103.

Penman, S. (1966). RNA metabolism in the HeLa cell nucleus. J. Mol. Biol., 17: 117-130.

Penner, G.A.; Bush, A.; Wiser, R.; Kim, W.; Domier, L.; Kashar, K.; Laroche, A.; Scoles, G.; Molnar, S.J. and Fedak, Q. (1993).

Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Appli., 2: 341 - 45.

Pui, C.; Relling, M.V.; Pharm, D. and James, R.D. (2004). Acute lymphoblastic leukemia. N. Engl. J. Med., 350 (15): 1535-1548.

Q

Qin, Y.Z.; Li, J.L.; Zhu, H.H.; Li, L.D.; Chang, Y.; Le, H.; Ruan, G.R.; Liu, Y.R.; Huang, X.J. and Chen, S.S. (2007). Detection of common fusion transcript levels in untreated leukemia patients by real-time quantitative RT-PCR technique. Zhonghua Xue Ye Xue Za Zhi., 28 (7): 433-437.

Queipo-Ortuño, M. I.; Morata, P.; Ocón, P.; Manchado, P. and Colmenero, J. D. (1997). Rapid diagnosis of human brucellosis by peripheral blood PCR assay. J. Clin. Microbiol., 35: 2927–2930.

R

Rafalski, J.A. (1997). Randomly Amplified Polymorphic DNA (RAPD) Analysis. In: Caetano-Anolles, G. and Gresshoff, P.M. DNA Markers: Protocols, Application, and Overview. Wiley-Liss, Inc., New York. pp: 75-83.

Rafalski, J.A. and Tingey, S.V. (1993). Genetic diagnostics in plant breeding RAPD, microsatellites and machines. Genetics, 9: 275-280.

Richardson, A.J.; Narendran, N.; Guymer, R.H.; Vu, H. and Baird, P.N. (2006). Blood storage at 4 degrees C-factors involved in DNA yield and quality. J. Lab. Clin. Med., 147 (6): 290-294.

Reischl, U.; Linde, H.J.; Lehn, N.; Landt, O.; Barratt, K. and Wellinghausen, N. (2002). Direct detection and differentiation of *Legionella* spp. and *Legionella pneumophila* in clinical specimens by dual-color real-time PCR and melting curve analysis. J. Clin. Microbiol., 40: 3814-3817.

Robertson, M. (1983). Oncogenes and the origins of human cancer. B.M.J., 286 (6359): 81-82.

Rozaman, C. and Montserrat, E. (1995). Chronic lymophcytic leukemia. N. Eng. J. Med., 333(16): 1052-1057.

S

Saiki, R.K.; Gelfand, D.H.; Stoffel, S.; Schoef, S.J.; Higuchi, R.; Horn, G.T.; Mullis, K.B. and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239: 487-490.

Saiki, R.K.; *Scharf, S.; Faloona, F.; Mullis, K.B.; Horn, G.T.; Erlich, H.A. and Arnheim, N.* (1985). Enzymatic Amplification of ß-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science, 230: 1350-1354.

Saiki, R.K. (1990). The Design and Optimization of the PCR. In: Erlich, H.A., (Ed.). PCR Technolgy, Principle and Application for DNA Amplification. Stockton Press, New York, U.S.A. pp.7-16.

Sambrook, J. and Russell, D.W. (2001). Molecular Cloning. A laboratory manual. 3rd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sawyers, C.L. (1999). Chronic myeloid leukemia. N. Eng. J. Med., 340 (17): 1330-40.

Scarpa, A.; Moore, P.S.; Rigaud, G. and Menestrina, F. (2001). Genetic alterations in primary mediastinal B-cell lymphoma: an update. Leukemia and Lymphoma, 41: 47–53.

Settin, A.S.; Al-Haggar, M.M.; Neamatallah, M.; Al-Said, A.M. and Hafez, M.M. (2006). Detection of beta-thalassaemia mutations using primer-specific amplification compared to reversed dot blot hybridization technique in Egyptian cases. Haema., 9 (3): 401-409.

Sham, P.; Bader, J.S.; Craig, I.; O'Donovan, M. and Owen, M. (2002). DNA Pooling: A tool for large scale association studies. Nature Rev. Genet, 3: 862-871.

Sharkey, D.J.; Scalice, E.R.; Christy Jr., K.G.; Atwood, S.M. and Daiss, J.L. (1994). Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. Bio/Technology, 12: 506-509.

Sherr, C. (2004). Principles of tumor suppression. Cell, 116 (2): 235-246.

Shovlin, C. L.; Lamb, J.R. and Haslett, C. (1999).The molecular and cellular of disease. In Haslett, C.; Chilvers, R.E.; Hunter, A.J. and Boon, A.N. Davidson's principles and practice of medicine 18th ed. Churchil Living Ston, New York.

Sieber, O.M.; Heinimann, K. and Tomlinson, I.P.M. (2003). Genomic instability — the engine of tumorigenesis? .Nature, 3: 701-708.

Sinclair, P.B.; Nachera, E.P. and Green, A.R. (2000). Chronic myeloid leukemia with ph cell. Hematology, 17: 25-30.

Singer, E.; Kuenzle, C.; Thomann, P.E. and Hubscher, U. (1988). DNA finger printing : improved DNA extraction from small blood samples. Nucleic Acid Res., 16 (15): 7738.

Singer-Sam, J.; Grant, M.; LeBon, J.M.; Okuyama, K.; Chapman, V.; Monk, M. and Riggs, A.D. (1990). Use of a *Hpa*II-polymerase chain reaction assay to study DNA methylation in the Pgk-1 CpG island of mouse embryos at the time of X-chromosome inactivation. Mol. Cell. Biol., 10: 4987–4989.

Singh, K.P. and Roy, D. (2001). Identification of novel breast tumor-specific mutation(s) in the q11.2 region of chromosome 17 by RAPD/AP-PCR fingerprinting. Gene, 269: 33-43.

Specchia, G.; Mininni, D.; Guerrasio, A.; Palumbo, G.; Pastore, D. and Liso, V. (1995). Ph positive acute lymphoblastic leukemia in adults: molecular and clinical studies. Leuk. Lymphoma, 18 (1): 37-42.

Sood, A.K. and Buller, R.E. (1996). Genomic instability in ovarian cancer: a reassessment using an arbitrarily primed polymerase chain reaction. Oncogene, 13 : 2499-2504.

Strauss, W.M. (2002). Preparation of Genomic DNA from Mammalian Tissue. In: Ausubel, F.M.; Brent, R.; Kingston, R.E.; Moore, D.D.; Seidman, J.G.; Smith, J.A. and Struhl, K. Current Protocols in Molecular Biology. Vol. 5. John Wiley and Sons, Inc. New York. Pp: 2.2.1-2.2.3.

T

Tan, A.; Westerman, D.; McArthur, G.A.; Lynch, K.; Waring, P. and Dobrovic, A. (2006). Sensitive detection of KIT D816V in patients with mastocytosis. Clin. Chem., 52: 2250–2257 .

Thomas, S.M.; Moreno, R.F. and Tilzer, L.L. (1989). DNA extraction with organic solvents in gel barrier tubes. Nucleic Acid Res., 17 (13): 5411.

Thomas, X. (2006). Highlights in the history of leukaemia - a historical review from the beginnings to current therapeutic developments. Haema., 9 (2): 191-211.

Tilzer, L.; Thamas, S.; Moreno, R.F. (1989). Using of silica gel polymer for DNA extraction with organic solvent. Anal. Biochem., 183: 1021-1027.

Tingey, S.V.; Rafalski, J.A.; Hanafy, M.K. (1994). Genetic Analysis with RAPD Markers, in: Plant Molecular Biology Eds. by Goruzzi, G. and Puigdomenech, P. vol. H81. Spring-Verlage Berlin. pp. 491-500.

Truong, T.L.; Menard, C.; Mouton, C. and Trahan, L. (2000). Identification of mutants and other oral Streptococci by random amplified polymorphic DNA analysis. J. Med .Microbiol., 49: 63-71.

Troutt, A.B.; McHeyzer-Williams, M.G.; Pulendran, B. and Nossal, G.J.V. (1992). Ligation-anchored PCR:A simple amplification technique with single-sided specificity. Proc. Natl. Acad. Sci. U.S.A., 89: 9823–5.

V

Van-Reenen, C.A.; Dicks, L.M.T. (1996). Evaluation of numerical analysis of random amplified polymorphic DNA RAPD-PCR as method to differentiate *Lactobacillus plantarum* and *Lactobacillus pentosus*. Current Microbiology, 32: 183-87.

Velculescui, V.E. and Eldeiry, W.S. (1996). Biological and clinical importance of the p53 tumor suppressor gene. Clinical Chemistry, 42 (6): 858-868.

Vrettou, C.; Traeger-Synodinos, J.; Tzetis, M.; Malamis, G. and Kanavakis, E. (2003). Rapid screening of multiple beta-globin gene mutations by real-time PCR on the Light Cycler: application to carrier screening and prenatal diagnosis of thalassemia syndromes. Clin. Chem., 49: 769-776.

W

Wang, J.; Wang, Q.W.; He, Z.W. and Ye, F. (2001). Genetic instability in cancer tissues analyzed by RAPD PCR. Shi Yan Sheng Wue Bao., 34 (2): 151-155.

Wang, J. ;Wang, Q.W. and Ye, F. (2002). Genetic instability in cancer tissues analyzed by random amplified polymorphic DNA PCR. C.M.J., 115 (3): 430-432.

Wang-Johanning, F.; Lu, D.W.; Wang, Y.; Johnson, M.R.; Johanning, G.L. (2002). Quantitation of human papillomavirus 16 E6 and E7 DNA and RNA in residual material from ThinPrep Papanicolaou tests using real-time polymerase chain reaction analysis. Cancer, 94: 2199–2210.

Weber, J. and McClure, M. (1987). Oncogenes and cancer. B.M.J., 294 (6582): 1246-1248.

Weinberg, R. A. (1989). Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis. Cancer Res., 49 : 3713-3721.

Weinberg, R.A. (1991). Tumor suppressor genes. Science, 254: 1138-1146.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213–7218.

William, D. and Foulkes, W.D. (2007). P53 — Master and Commander. N. Engl. J. Med., 357 (25): 2539-2541.

Williams, J.G.; Hanafey, M.K.; Rafalski, J.A. Tingey, S.V. (1993). Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzymology, 218: 704-740.

Williams, J.G.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531–6535.

X

Xian, Z.H.; Cong, W.M.; Zhang, S.H. and Wu, H.C. (2005). Genetic alteration of hepatocellular carcinoma by random amplified polymorphic DNA analysis and cloning sequencing of tumor differential DNA fragment. World J. Gastro., 11 (26): 4102-4107.

Xinias, I.; Demertzidou, V.; Kardaras, P.; Tsiouri, H.; Papachristou, F.; Tsiouris, I. (2005). Diagnostic difficulties in paediatric acute lymphoblastic leukaemia: a case report. Haema., 8 (2): 278-282.

Y

Yeang, C.H.; Ramaswamy, S. and Tamayo, P. (2001). Molecular classification of multiple tumor types. Bioinformatics, 17 (1): S316-S322.

Yu, Z.; Loehr, C.V.; Fischer, K.A.; Louderback, M.A.; Krueger, S.K.; Dashwood, R.H.; Kerkvliet, N.I.; Pereira, C.B.; Jennings-Gee, J.E.;

Dance, S.T.; Miller, M.S.; Bailey, G.S. and Williams, D.E. (2006). In utero exposure of mice to dibenzo[a,l]pyrene produces lymphoma in the offspring: role of the aryl hydrocarbon receptor. Cancer Res., 66: 755–762.

Z

Zhang, S.H.; Cong, W.M.; Xian, Z.H.; Dong, H. and Wu, M.C. (2004). Genomic instability in hepatocelluar carcinoma revealed by using random amplified polymorphic DNA method. J. Cancer Res. Clin. Oncol., 130 (12): 757-761.

Zhang, Y.; Rajagopalan, M.; Brwon, B.A. and Wallace, R.J. (1997). Random amplified polymorphic DNA PCR for comparison of *Mycobacterium abscessus* strains from nosocomial outbreaks. J. of Clinical Microbiology, 35: 3132-3139.

Zhu, Y.L.; Zhang, Y.; Zhu, P.; Yang, Y.; Du, J.W. and Liu, J. (2005). Role of molecular screening for common fusion genes in the diagnosis and classification of leukemia. Beijing Da Xue Xue Bao., 37 (3): 236-239.

Zietkiewicz, E.; Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics, 20 (2): 176-183.

Appendix 1: Gender and age of patients, blood characteristic and results of genomic DNA yield isolated for 65 leukemia patients.

Patient no.	Gender	Age	Type of leukemia	Blood characteristic	DNA Concentration
1	♀	32	ALL	Non-clotted	100 µg
2	♂	56	CLL	Non-clotted	100 µg
3	♀	39	AML	Non-clotted	200 µg
4	♀	49	CML	Non-clotted	100 µg
5	♂	37	CML	Non-clotted	300 µg
6	♂	32	CML	Clotted blood	-
7	♂	13	ALL	Non-clotted	> 100 µg
8	♀	63	CML	Non-clotted	>100 µg
9	♂	31	CML	Non-clotted	>100 µg
10	♂	44	CLL	Non-clotted	100 µg
11	♀	34	CML	Non-clotted	100 µg
12	♂	33	CML	Non-clotted	200 µg
13	♀	18	ALL	Non-clotted	100 µg
14	♀	53	AML	Non-clotted	100 µg
15	♂	56	CML	Non-clotted	200 µg
16	♂	28	ALL	Non-clotted	300 µg
17	♀	51	AML	Non-clotted	100 µg
18	♂	57	CML	Non-clotted	100 µg
19	♀	30	CML	Non-clotted	100 µg

20	♀	41	CML	Non-clotted	>100 µg
21	♂	19	AML	Non-clotted	400 µg
22	♂	16	AML	Non-clotted	100 µg
23	♀	67	AML	Non-clotted	100 µg
24	♀	43	AML	Non-clotted	>100 µg
25	♀	95	CML	Non-clotted	600 µg
26	♂	22	CML	Non-clotted	100 µg
27	♂	31	CML	Non-clotted	300 µg
28	♀	46	CLL	Non-clotted	100 µg
29	♂	50	CML	Non-clotted	100 µg
30	♂	67	CLL	Non-clotted	>100 µg
31	♀	31	CML	Non-clotted	>100 µg
32	♀	30	CML	Clotted blood	-
33	♀	45	AML	Non-clotted	200 µg
34	♂	7	ALL	Non-clotted	100 µg
35	♂	22	ALL	Non-clotted	100 µg
36	♀	56	AML	Non-clotted	>100 µg
37	♀	16	ALL	Non-clotted	100 µg
38	♂	65	CLL	Non-clotted	100 µg
39	♀	26	AML	Non-clotted	300 µg
40	♀	5	ALL	Non-clotted	100 µg
41	♂	6	ALL	Non-clotted	100 µg
42	♂	20	ALL	Clotted blood	-

43	♂	21	ALL	Non-clotted	200 µg
44	♂	27	CLL	Non-clotted	300 µg
45	♂	67	CLL	Non-clotted	100 µg
46	♂	40	CML	Clotted blood	-
47	♂	62	CLL	Non-clotted	100 µg
48	♂	37	AML	Non-clotted	Low
49	♂	21	CML	Non-clotted	100 µg
50	♂	73	AML	Non-clotted	100 µg
51	♂	2	ALL	Non-clotted	>100 µg
52	♀	40	CML	Non-clotted	300 µg
53	♂	37	CML	Non-clotted	100 µg
54	♀	37	CML	Non-clotted	100 µg
55	♂	15	ALL	Non-clotted	100 µg
56	♂	26	AML	Non-clotted	200 µg
57	♀	4	ALL	Non-clotted	100 µg
58	♀	51	CML	Clotted blood	-
59	♂	76	CLL	Non-clotted	100 µg
60	♀	47	CML	Clotted blood	-
61	♀	29	AML	Non-clotted	100 µg
62	♂	37	AML	Clotted blood	-
63	♀	53	CLL	Clotted blood	-
64	♂	59	AML	Clotted blood	-
65	♂	61	AML	Clotted blood	-

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

دراسة وراثية جزيئية لمرضى أبيضاض الدم

رسالة

مقدمة إلى كلية العلوم / جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير علوم في التقانة الاحيائية

من قبل

نهى محمد صالح شبيب

أب / 2008 شعبان/ 1429

الخلاصة

تضمن البحث اجراء دراسة وراثية جزيئية لمرضى ابيضاض الدم بأنواعه الأربعة (ابيضاض الدم اللمفوي الحاد, ابيضاض الدم النخاعي الحاد, ابيضاض الدم اللمفاوي المزمن وابيضاض الدم النخاعي المزمن). إذ أجريت فحوصات الوراثة الجزيئية لـ 65 مريضا انحدروا من مناطق جغرافية مختلفة من سوريا تمت أحالتهم الى مستشفى البيروني الجامعي ومستشفى الأسد الجامعي و شخصت إصابتهم بالمرض بأستخدام الفحص السريري للدم ونخاع العظم.

أوضحت النتائج أن كميات الدنا المعزول من الأشخاص الطبيعيين ولمرضى ابيضاض تتراوح بين (100- 600) مايكروغرام لكل 300 مليليتر وبنقاوة تتراوح بين (1,7-1,9) عند أستعمال العدة المجهزة من شركة Promega وفي حالة أستخلاص الدنا بطريقة Sambrook and Russell, 2001 تم الحصول على أقل من 100 مايكروغرام لكل مليلتر وبنقاوة <1.0. ومن جانب أخر أوضحت النتائج من أن 45 مريضا فقط من مجموع المرضى اعطوا كميات دنا جيدة. اما بقية الحالات التي لم تعطي كميات جيدة من الدنا فقد توزعت ما بين 10 حالات دم متخثر و10 حالات تميزت بأنخفاض تعداد كريات الدم البيض.

في هذه الدراسة استخدم اثنان وعشرون بادئاً لتقييمها في الكشف عن التغاييرات الوراثية على مستوى الدنا بين الاشخاص الطبيعيين والمرضى. وهدفت التجارب في بداية الامر الى تحديد الظروف المثلى لتفاعلات ال (RAPD-PCR) كتركيز قالب الدنا والبادئات وبرنامج التضاعف وغيرها وكانت النتائج كالتالي:

أ- أن ثلاثة عشر باديئ لم تعطي نواتج تضاعف في كل الطرز الوراثية للأشخاص الطبيعيين وكذلك لمرضى ابيضاض الدم.

ب- تبين أن تسعة بادئات كانت لها قدرة متباينة في تضاعف حزم الدنا ضمن الاشخاص الطبيعيين والمرضى. وأوضحت النتائج وجود تباينات في الدنا المجيني بين مرضى ابيضاض الدم بالمقارنة مع الاشخاص الطبيعيين المشمولين بهذه الدراسة. وقد شملت هذه التباينات اختلافات في عدد الحزم المتضاعفة من خلال وجودها او غيابها او الاختلاف في الوزن الجزيئي ضمن الاشخاص الطبيعيين والمرضى حيث تباين العدد الكلي للحزم المضخمة ما بين (1- 15) وأوزانها الجزيئية تراوحت بين (95 – 3162) زوج قاعدة تبعا للباديء المستخدم. حيث لوحظت حزمة واحدة عند استخدام البادئات OPZ-02, OPB-18 بينما ظهرت (15) حزمة مع الباديء OPJ-05. و لوحظ أن أقل حجم جزيئي كان 95 زوج قاعدة قد ظهر مع الباديء OPJ-05 ,OPB-15 وان اعلى وزن جزيئي للقطع المتضاعفة كان 3162 زوج قاعدة بأستخدام الباديء OPA-09.

ت- لوحظ ايضا ان هناك تغيير في شدة تألق حزم الدنا لمرضى ابيضاض الدم بالمقارنة مع الاشخاص الطبيعيين.

ث- بينت الدراسة من ان 37,8% من بين مرضى ابيضاض الدم قد اظهروا اعلى نسبة تباينات في الدنا المجيني بالمقارنة مع الاشخاص الطبيعيين حيث حددت هذه التباينات في جميع البادئات المستخدمة في هذه الدراسة, حيث بلغت 48,8% من المرضى بأستعمال ثمانية بادئات. و حددت التباينات في 8,9% من المرضى في سبعة بادئات. وأخيرا حددت التباينات في 4,5% من المرضى في ستة بادئات.

ج- كما قيمت في هذه الدراسة ايضا مدى كفاءة البادئات المستخدمة في اظهار التباين الوراثي. حيث وجد ان أعلى قيمة للكفاءة تم استحصالها مع الباديء OPB-17 بقيمة (0,059) وأن اقل قيمة للكفاءة كانت (0,016) حيث تم الحصول عليها مع الباديء OPE-05.

ح- أظهرت الدراسة وجود تغييرات وراثية متكررة في الدنا المجيني لمرضى ابيضاض الدم ويمكن الكشف عنها باستخدام بادئات مناسبة في تقنية (RAPD-PCR) ومثال على ذلك الباديء-OPA 09 اذ يمكن استخدامه في الكشف عن مرضى ابيضاض الدم المفاوي الحاد وابيضاض الدم اللمفاوي المزمن. والبادئان OPB-17 و OPW-17 كانت لهم القابلية على اظهار حزمة معينة موجودة في مرضى ابيضاض الدم النخاعي الحاد في حين لم تظهر هذه الحزمة في الاشخاص الطبيعيين ولا في بقية مرضى انواع ابيضاض الدم الاخرى. ومن جانب اخر اظهر الباديء

OPW-17القابلية على اظهار حزمة معينة في مرضى ابيضاض الدم النخاعي الحاد ومرضى ابيضاض الدم اللمفاوي المزمن في حين لم تظهر هذه الحزمة في الاشخاص الطبيعيين ولا في بقية مرضى انواع ابيضاض الدم الاخرى. أما في حالة استخدام البادئان OPW-17, OPB-17 فقد تم فقدان حزمة معينة في مرضى ابيضاض الدم النخاعي الحاد في حين تواجدت هذه الحزمة في الأشخاص الطبيعيين وبقية مرضى انواع ابيضاض الدم الاخرى. وكذلك ايضا اظهرت النتائج عند استخدام البادیء OPZ-02 انه تم فقدان حزمة معينة في مرضى ابيضاض الدم النخاعي المزمن في حين تواجدت هذه الحزمة في الأشخاص الطبيعيين وبقية مرضى انواع ابيضاض الدم الاخرى.

خ- ومن الجدير بالملاحظة ان البادیء OPJ-05 كانت له القابلية على تضخيم حزمة معينه في الرجال الطبيعيين ولكن هذه الحزمة فقدت في الاناث الطبيعيين.

استخدمت تقنية التضاعف العشوائي متعدد الاشكال (RAPD-PCR) للتحري عن التنوع الوراثي في طرز مثيلة الدنا المجيني المستخاص من دم مرضى ابيضاض الدم النخاعي المزمن و أشخاص طبيعيين لفئة الاربيعينيات العمرية. بينت نتائج التضاعف العشوائي متعدد الاشكال للدنا المجيني المهضوم بأنزيمات التقييد الخاصة بالكشف عن طرز المثيلة (*Msp*I , *Hpa* II) وجود اختلافات في طرز مثيلة الدنا المجيني لمرضى ابيضاض الدم بالمقارنة مع الاشخاص الطبيعيين مما يشير الى وجود تنوع في طرز مثيلة المواقع الحساسة للمثيلة (CGs)، و تم تحليل النتائج اعتماداً على الاختلافات في طرز الحزم المتضاعفة بفقدان الحزم او / و تغير في شدة تألق الحزم و تغير في الحجم الجزيئي للحزم .