Master Program in Clinical Laboratory Science

Cytogenetic Abnormalities in Palestinian Patients with Hematologic Malignancies in the Occupied West Bank (Including Jerusalem)

الاختلالات السيتوجينية لدى المرضى المصابين بسرطانات الدّم من الفلسطينيين في الضفة الغربية المحتلة (والقدس)

By

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Dedication

I dedicate this work to my dear father Moham'd and my beloved mother Rihab for inspiring me to continuously seek knowledge and struggle for improvement.

A special word of gratitude to my dear family members; my borhters and my sisters for their support, endurance, and all kinds of help they offered to me during the time I spent to complete this work. I am also grateful to my dear friend Dr Mazin for his ongoing huge support and encouragement.

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Abbreviations

DNMT	DNA methyl transferases
MALT	the mucosa-associated lymphoid tissue
ABL	Abelson murine leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myelogenous leukemia
APL	Acute promyelocytic leukemia
ASXL1	Additional sex combs-like 1 gene
ATM	Ataxia-telangiectasia mutated
ATO	Arsenic trioxide
ATRA	All-trans retinoic acid
BCR	Break point cluster region
BER	Base excision repair
BM	Bone marrow
CML	Chronic myelogenous leukemia
CMML	Chronic myelomonocytic leukemia
DCBA	Dual-color break-apart
DCDF	Dual-color dual-fusion
DI	DNA index
DLBCL	Aggressive diffuse large B-cell lymphoma
DNMTase's	DNA methyl transferases
EORTC	European organization for research and treatment of cancer
FAB	French American British
FISH	Fluorescence in situ hybridization
FL	FLT3 ligand
FLT3	Fms-like tyrosine kinase 3
FNA	Fine needle aspiration
HCL	Hairy cell leukemia
HD	Hyperdiploid
HIP1R	Huntingtin interacting protein 1 related

HL	Hodgkin lymphoma
HM	Haematologic malignancies
Hox	Homeobox
HRR	Homologous recombinational repair
HRS	Hodgkin and Reed-Sternberg
IARC	International Agency for Research on Cancer
IGH	Immunoglobulin heavy locus
IGHV	unmutated immunoglobulin heavy chain
ISCN	International System for Human Cytogenetic Nomenclature
ITD	Internal tandem duplication
IWF	International working formulation
JM	Juxtamembrane
LMO1 & 2	LIM domain only gene -1 & 2
LPD	Lymphoproliferative disorders
LYL1	Lymphoblastic leukemia-derived gene -1
MDR	Minimal deleted region
MDS	Myelodysplastic syndrome
MDS	Myelodysplastic syndromes
MDS-U	Myelodysplastic syndrome – unclassified
M-FISH	Multiple-FISH
MGUS	Monoclonal gammopathy of undetermined significance
MLL	Mixed–lineage leukemia
MM	Multiple Myeloma
MMR	Mismatch repair
MMSET	Multiple myeloma SET domain
MNC	Modal number of chromosomes
MPD	Myelo proliferative disorders
MPN	Myelo proliferative neoplasms
MYF6	Myogenic factor 6
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining

NHL	Non-Hodgkin's lymphoma
NOS	Not otherwise specified
NOTCH	Notch homolog 1
PI3K	phosphoinositide 3-kinase
PLL	Prolymphocytic leukemia
PML	Promyelocytic leukemia
PML-NB's	PML- nuclear bodies
PT3K	Phosphatidylinositol-3 kinase
RAEB-1	Refractory anemia with excess blasts-1
RAEB-2	Refractory anemia with excess blasts-2
RARA	Retinoic acid receptor, alpha
RARS	Refractory anemia with ring sideroblasts
RB1	Retinoblastoma-1
RCMD	Refractory cytopenia with multilineage dysplasia
RCUD	Refractory cytopenia with unilineage dysplasia
RTK	Receptor tyrosine kinase
SKY	Spectral karyotype
TAL1	T-cell lymphocytic leukemia
t-MDS	Therapy-related MDS
TNM	Tumor, node, metastasis
T-PLL	T cell prolymphocytic leukemia
TRIP	Tripartite
WCP	Whole-chromosome paint
WHO	International Agency for Research on Cancer
XPC	Xeroderma pigmentosum complementation

List of tables

List of figures

I. Contents

الملخّص

إنّ الاختلالات الجينية والسيتوجينية المسببة لسرطانات الدم مهمّة للتشخيص، وتقييم الخطورة، وتحديد العلاج، والمستقبل المرضي، ولكشف أسرار الفيسيولوجيا المرضية لسرطانات الدّم. بسبب هذه الأدوار المهمة فإن مسالة سرطانات الدّم ازدادت أهميتها عالميا وفي فلسطين، غير أنّه إلى الآن لا تتوفر المعلومات الكافية حول سرطانات الدّم عندنا في فلسطين.

لقد راجعنا باختصار في الجزء المخصص بالأدبيات العلمية المعارف الحالية حول سرطان الدم الليمفاوي الحاد ALL و ابيضاض الدم النقوي الحاد AML و سرطان الدم الليمفاوي المزمن CLL و ابيضاض الدم النقوي المزمن CML وسرطان الغدد الليمفاوية Lymphoma و المايلوما المتعددة MM وخلل التنسج النخاعي MDS . وقد اظهرت مراجعتنا لأدبيات الموضوع شحّ المعرفة وعدم كفايتها عن سرطانات الدّم المذكورة في فلسطين. في هذه الرسالة بدأنا بجمع الحالات المصابة بسرطانات الدّم في فلسطين (تحديدا منطقة الضفة الغربية والقدس) وقمنا بدراسة الاختلالات السيتوجينية المكتشفة فيها لتكون دراستنا نقطة انطلاق للبحث المستقبلي في هذا المجال ومن أجل اطلاق عملية جديّة للرّصد والتوثيق والبحث لهذه الحالات في فلسطين.

لقد قدمنا تحليلا سيتوجينيا للحالات وكشفنا بعض الاختلالات الكروموسومية كما أضفنا فحوصات بتقنية FISH لبعض الحالات من AML وCML بهدف الكشف عن اختلالات أدقّ متوقعين من هذه الدراسة ان يكون لها أثر ايجابي على واقع سرطانات الدم في فلسطين وأن تقدّم تبصرة أوّلية حولها.

لقد أظهرت نتائج الدراسة تشابها عامّا في واقع سرطانات الدّم في فلسطين مع دول العالم الأخرى. بخصوص ALL، كان لدينا 74 حالة وتمكنا من الكسف عن اختلالات في 31.1% من الحالات. أغلب الحالات 70.3% كانت من أصول-B. وجدنا حالتين منقوصتي الضعفانية hypodiploid ذات نمط نووي معقد، و7 حالات بنمط نووي شبه ضعفاني pseudodiploid ؛ 3 منها مثلت اختلالات تركيبية تشمل (q23;p13)(1:19) و t(4:12) بعض الحالات من مجموعة الضعفانية ,(47-50) و مرتفع الضيغة الضعفانية hyperdiploidهي (51=<) مثلت 25-30% من مجموع حالات ALL.

أما AML، حصلنا على 35 حالة 28.6% من الحالات كانت بنمط نووي مختلّ. وقد وجدنا حالات ذات مستقبل مرضي محبّذ وفيها t(8;21)(q22;q22) وt(15;17)(q22;q12) و inv(16)(p13q22) لم نحصل على التصنيف FAB لمعظم الحالات بسبب محدودية التشخيص المناعي لدى المشفى المحوّل.

اما CML فحصلنا على 34 حالة. 41.2% منها كانت ذات نمط نووي مختل وهي الحالات الموجبة لكروموسوم فيلادلفيا. كانت لدينا 4 حالات من CLL حالتان منهما بنمط نووي معقد بما فيها , del(22) و del(11). وثلاث حالات كانت هي ما حصلنا عليه من MDS حيث وجد نمط نوويا معقدا في حالة واحدة منها ذات مستقبل مرضي سيّء. المايلوما المتعددة كان لدينا منها 33 حالة، حالة واحدة كانت ذات نمط خلوي غير طبيعي معقد وذات مستقبل مرضي سيّء. الليمفوما كان لدينا منها 12 حالة. لم نعثر فيها على اختلالات في النمط النووي.

نستنتج ان الدراسات عن سرطانات الدم في فلسطين تحتاج جهود الأطباء والأخصائيين الجينيّين، وأخصائيي الوبائيات، والعاملين الآخرين في المجال الصّحي وهذه الدراسات هي بالغة الأهمية من أجل التعامل السليم مع المرضى وتقديم أفضل رعاية صحية لهم.

Abstract

Genetic and cytogenetic abnormalities underlying hematologic malignancies (HM's) are important for the diagnosis, risk stratification, treatment determination, prognosis, and demystifying the pathophysiology of HM's. Because of these important roles, HM had become a raising issue in Palestine and the whole world. But there is still no enough knowledge about these abnormalities in Palestine. The literature part reviewed briefly the current knowledge about ALL, AML, CLL, CML, Lymphoma, MM, and MDS and showed the lack of satisfying knowledge about these HM's cases in Palestine. In this thesis, we started to collect cases with HMs in Palestine (West Bank and Jerusalem) and studied the involved cytogenetic abnormalities in order to create a start point for future research in this field and start a serious reporting process and research on these cases in Palestine. We introduced Karyotyping analysis for these cases and detected some of the chromosomal abnormalities, we also introduced FISH test for some AML and CML cases to detect smaller abnormalities. We expect this study to have positive impacts on the HMs status in Palestine, and to show the first insights about them.

The results of this study showed a general similarity in the status of HMs in Palestine with other countries in the world.

For ALL, we had 74 cases, and we could detect abnormalities in (31.1%) of the cases. Most of the cases (70.3%) were of B-Cell origin. We found 2 cases with a hypodiploid complex karyotype, and 7 cases with pseudodiploid karyotype, three of them represented structural translocations including t(1:19)(q23;p13) and t(4:12). Some cases in ploidy group (47-50), and hyperdiploidy groups (>=51) representing 25-30% of all ALL cases. For AML, we had 35 cases. (28.6%) of the cases were found with various abnormal karyotypes. We found cases with favorable cytogenetic prognosis including cases with t(8;21)(q22;q22), t(15;17)(q22;q12), and inv(16)(p13q22). We did not have the FAB classification for most of the cases since we had limited immunophenotyping at the referring hospital. For CML, we had 34 cases. 41.2% of them were found with abnormal karyotypes; Ph positive results. For CLL, we had four cases. Two of them had abnormal complex karyotypes including del(11) and del(22).

For Myelodysplasia, we had 3 cases. Only one showed an abnormal complex karyotype with poor prognosis. For Multiple Myeloma, we had 33 cases. Only one case showed an abnormal complex karyotype associated with poor prognosis. For Lymphoma, we had 12 cases and no karyotypic abnormalities were found.

We conclude that studies on HM's in Palestine need efforts of physicians, geneticists, epidemiologists, and other health workers and are critical to properly manage patients and provide them with the best health care possible.

II. Introduction

The World Health Organization introduced molecular and cytogenetic markers as keys to the identification of Hematologic malignancies [1, 2, 3]. Chromosomal aberrations, which might be structural, numerical, or both are encountered in leukemia and myelodysplasia patients as well as lymphoma and multiple myeloma. Cytogenetic analysis for these patients is crucial in monitoring disease progression, treatment response, and prognosis. Molecular cytogenetic techniques including fluorescence in situ hybridization (FISH), and RT-PCR are increasingly becoming invaluable to patients management [4].

Investigations of haematologic malignancies (HM) in Palestine were limited in the past by lack of local resources. Many patients were referred to 'Israeli' or Jordanian hospitals. At these hospitals, Palestinian patients are diagnosed among other patients and the data was rarely collected identifying types of aberrations seen in Palestinians. Our study utilizing cytogenetics would provide significant information about the cytogenetic background of HM's in this population. Data from neighboring populations are also scarce. One study was conducted in Jordan but limited to AML patients [5]. In that study, Dr Ayesh and colleagues from Jordan university of Science and Technology, reviewed 35 patients with de novo AML, and analyzed the cytogenetic categories of patients when possible. Another study was conducted on the Egyptian population of the Nile Delta [6]. In this study, the researchers tried to investigate the status of HM's in different urban and rural areas. They found that the majority of the cases were in patients who were residents in rural areas. They found male predominance in both populations of urban and rural areas. Such studies were pioneering in our region but much more work needs to be done.

A. Hematologic Malignancies

1. Definition

Hematologic malignancies (HM) are neoplasm's located in the blood and blood-forming tissue (the bone marrow and lymphatic tissue). The forms of HM's include various types of leukemia's, lymphoma, and the myelodysplastic syndromes. HM are a heterogeneous group of diseases in terms of etiology, incidence, and outcome. HM's may be broadly categorized into Hodgkin versus non-Hodgkin lymphoma, chronic versus acute and lymphatic versus myeloid leukemia [7]. Over the last decades many classifications for HM's were established and refined by the refinement and discovery of novel diagnostic techniques.

The last wide review of the classification of HM's was the WHO 2008 update, which is a modification on the previous WHO 2001 classification [2]. The WHO 2008 classification tries to group the different HM's into distinctively characterized entities on the clinical and the biological levels. This sorting of HM's incorporates clinical features, morphology, immunophenotyping, and cytogenetic/molecular genetic data seems inevitably necessary by the increased usage of molecular methods in the routine practice for the optimization of treatment to approach the individual level. The WHO 2008 classification groups these HM's into; First; myeloid neoplasm's, including acute myeloid leukemia/Myeloid sarcoma, Myelodysplastic syndromes, and some myeloroliferative neoplasm's; second; Lymphoid neoplasm's, including the Non-hodgkin, and the Hodgkin lymphomas, Third; Histiocytic and Dendritic cell neoplasm's; Fourth; Hematologic neoplasm's with propensity for bony involvement [2, 8].

2. History of discovery of HM's

The early days of leukemia detection go back to 1844, when Alfred Donné detected arrest in maturation of the white blood cells. A year later the name leukocythemia was introduced by John Bennett based on microscopic observation of accumulated purulent leucocytes. The first to diagnose a leukemic case in a living person was Henry Fuller. In 1847, Rudolf Virchow introduced the disease as Leukemia as a reversed white and red cells balance [9]. Lymphoma was identified in the early 20th century (1932), by Thomas Hodgkin after whom the disease was named later [10].

The early classification was established by Nicolas Friedreich, in 1857 who described the "acute leukemia". Paul Ehrlich in 1877 classified leukemia further into the myeloid and the lymphoid types. Leukemia was further classified into the four well known types (ALL, AML, CML, & CLL) in 1913 [11].

3. Epidemiology of hematologic malignancies

HM's constitute a major health burden worldwide. The International Agency for Research on Cancer (IARC), in its 2008 GLOBOCAN series, published an update for the cancer estimates of incidence and mortality risk from 182 countries. Leukemia cases worldwide were estimated to be 351,000 with higher cumulative (0 – 74 years) risk in males than females (0.6 vs 0.4) and with an annual estimated 257,000 deaths. Non-Hodgkin Lymphoma came next with 355,000 annual cases, 191,000 deaths, with males being at higher risk to develop the disease (0.6 vs 0.4). Multiple myeloma incidence was 102,000 with 72,000 deaths, and males showed higher risk for the disease (0.2 vs 0.1). Hodgkin Lymphoma incidence was 67,000 with 30,000 deaths, and the disease showed no sex preference. Cumulatively these results reflect that HM's have an annual worldwide mortality of 550,000 or 7.26% of the total mortality by cancer, constituting the 5^{th} most common cause of cancer death after lung, stomach, liver, and colorectal cancers [12].

Leukemia world incidence is roughly one per 100,000 per year and constitutes 25% of childhood cancers [13]. Myelodysplasia is a disease of the elderly, with incidence of 20 per 100,000 of more than 70 years age [14].

According to the Palestinian Ministry of Health 2012 report, Cancer mortality in the west bank increased from 10.3% in 2007 to 12.4% of the total mortality in 2011 to become the second leading cause of death next to cardiovascular diseases. Lung, colon, and breast cancers ranked the top three most common cancers. Leukemia was ranked the 10th most common cancer (other HM's not included), and represented 1.9% of the cancer cases in the west bank in 2011 [15]. This is clearly an underestimate of the actual picture as data remain spotty and unrepresentative of true morbidity and mortality.

B. Molecular bases of hematologic malignancies

Malignancies arise from transformation of a normal cell into an immortal uncontrolled growing cell with altered function [16]. The pathophysiology of carcinogenesis has been under the focus of scientists over decades.

All body cells need to regulate their growth and differentiation through multiple steps. For a normal cell to transform to cancer, many separate events are needed to surpass the control mechanisms. We now know that there is a multistep process that involves accumulation of mutations either positively or negatively affecting the cell cycle control processes including proliferation, growth, genomic stability and apoptosis [17].

Mutated cells can acquire advantageous proliferation and survival over normal cells and undergo clonal expansion and proliferation leading to formation of tumor.

1. Mutagenic agents

Mutations involved in cancerogenesis are caused by genetic errors that escape endogenous corrective mechanisms. Such errors could be environmentally induced and in some cases inherited (familial mutations in tumor suppressor genes). Tobacco consumption, genotoxic exposure, UV exposure, unhealthy diets, and infections with some parasites , bacteria and viruses are environmental insults that increase cancer risk significantly [18].

Tobacco smoking is a major factor in increasing the risk of cancer if different body tissues, especially lung cancer. And it contributes to more than 30% of cancers around the world. Cigarettes contain small amounts of carcinogenic agents such as polycyclic aromatic hydrocarbons (PAHs) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). These agents don't directly cause cancer, because the need metabolic activation in order to show their carcinogenic effect. However, they increase the risk of developing it especially if the body has problems with his detoxification pathways [19].

Obesity, diet, and exercise are also risk factors for cancer, especially colon and esophagus cancers. Obesity is related to 14–20% of all cancer cases. And physical inactivity was also found to be related to cancer development because it causes overweight and suppression for the immune system [20].

Radiations, such as X ray and Gamma rays and UV light are also carcinogenic agents that affect body tissues specially the skin. These radiations can directly damage the DNA in cells and alter the normal genome and normal cellular activities, affecting the apoptosis process and leading to uncontrolled growth in the cells. For examples, UV light can cause damage for the DNA by producing DNA lesions that form bends or kinks in the structure of the DNA and affect the replication process. These lesions include cyclobutane pyrimidine dimmers, and 6-4 photoproducts [21].

Many chemical agents can also cause cancer, such as Dioxins, Benzine and Nitric oxide (NO). NO has a genotoxic effect and was found to play a role in different stages of cancer development. NO can cause DNA lesions by forming carcinogenic nitrosamines that cause breaks in DNA strands. Some chemotherapy drugs also can initiate secondary malignancies, such as alkylating agents, including Mechlorethamine and Chlorambucil that can cause leukemia and MDS. Alkylating agents usually bind to the DNA molecule by their alkyl group and initiate apoptosis as a treatment for cancer progression. However, during this process, a further DNA damage might occur and the cell lose the balance between apoptosis and growth pathways leading to the initiation of secondary malignancies [22, 23].

Around 15-20% of cancer cases around the world are due to infectious agents, such as viruses, including HIV, HBV, and HCV, and bacteria such as the H. Pylori bacteria which is a major cause for gastric cancer [24].

Human immunodeficiency virus (HIV) is one of the major viruses associated with high risk of cancer. Many HIV cases were associated with other conditions such as preleukemia, myelofibrosis, and myeloid hyperplasia [25].

Studies have shown that HIV cases are also associated with leukemia, myeloma, and lymphoma, especially the B-Cell non-Hodgkin Lymphoma which is considered the second most common cancer in HIV patients. HIV can stimulate the over growth of B-Cells and cause their accumulation in the blood and bone marrow. HIV also can target the CD4-T cells, suppressing the immune system and the apoptotic pathways. This results enhances the growth of the leukemic clones [26].

Human papilloma virus (HPV) contributes to 90% of cervical carcinomas in women. HPV genome interferes with the cellular genome as oncogenic genetic sequences. The HPV genome includes the E6 and the E7 genes that play a major role in increasing the proliferation of cells and inducing cancer. The E6 associate protein usually binds with p53 and causes its degradation. This results in suppression of apoptosis and increased proliferation [27].

Hematologic malignancies, T-Cell Lymphoma in specific was found to be associated with Human T-cell lymphotropic virus type 1, which is a retrovirus that was classified in the same class with the HIV virus. This virus was shown to have a causative relationship with adult T-cell leukemia/lymphoma (ATLL); around 2%-5% of patients who are infected with T-cell lymphotropic virus type 1 also develop adult T-cell leukemia/lymphoma (ATLL) [28].

Bacteria have been reported as a causative agent for cancer such as the H. Pylori which is the first leading for gastric cancer and also associated with pancreatic and colorectal cancers [29]. Bacterial mechanism of causing cancer is indirect unlike the viral mechanism; bacteria can cause chronic inflammations and suppress the immune system in body tissues. Furthermore, some studies showed that bacteria have a natural ability for homing tumors and thus inducing more replication and over growth of tissues [30].

For instance, the Non- Hodgkin Lymphoma (NHL) is also related to infectious agents, such as lymphocyte-transforming viruses, such as the Epstein Barr virus (EBV) and the HIV virus. (NHL) can also be caused by Hepatitis C virus (HCV), which chronically stimulate the immune system and produce mixed cryoglobulinemia, a B lymphocyte proliferative disorder, and a low-grade lymphoproliferative disorder that can progress to NHL [31] .

Parasites and yeast are also possible causes of cancer. The parasite S. haematobium that causes Schistosomiasis is related to bladder and colorectal cancers. S. haematobium can trigger inflammation response that affect the squamous cells of the bladder and other organs, and thus can initiate cancer in these tissues [32].

A study from the United States suggested an association between malaria and brain tumors [33]. Infection with malaria was found to be associated with Burkitt's Lymphoma, the functional genetic variety of malaria provides it with more resistance and helps in the development of tumors, especially when the patient also have Epstein Barr Virus (EBV) [34]. Other studies suggested that Candida and fungal infections could also be causative agents for cancer. Candida plays a role in weakening the body by producing toxins and activating the immune system for a long time to fight the infections. Candida produces Ethanol in the body tissues, which cause general fatigue and produce free radicals that damage the DNA of the cells[35].

2. Role of DNA repair systems in HM's

Carcinogenetic mutations can arise by DNA replication errors such as by mismatch or proofreading errors [18]. Homologous recombinational repair (HRR), non-homologous end joining (NHEJ), nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR) are the five DNA repair systems which can all be impacted by aberrations that increase susceptibility for neoplasia [36].

Many genes of the DNA repairing system are known to be involved in hematologic malignancies. Mismatch repair (MMR) genes are one example. MMR genes like MSH2, MSH6, MLH3, MSH3 MLH1, PMS2 are found to play role in cancerogenesis including blood cell neoplasias [37, 38]. MMR are known to correct single base-pair mismatches and small deletion-insertion loops produced during replication. Cellular processes in which such events may take place include Immunoglobulin class switching, and cellular damages by many agents [39, 40].

X-ray cross complementing gene XRCC3 is an important example of DNA double strand breaks repair by homologous recombination repair pathway. XRCC3 Thr241Met polymorphism has been found to be associated with leukemia in Asiatic patients,[41]. It is thought that the substitution might influences the enzymatic function by affecting the phosphorylation site of this enzyme involved in HRR [42].

Findings about NHEJ show a complex role in cancerogenesis. It seems that both hyperactivity and underactivity of NHEJ related genes could contribute to genomic instability and DNA repair infidelity leading to cancer, as seen in Bloom's syndrome and myeloid leukemia [43, 44, 45].

The Xeroderma pigmentosum complementation, group C (XPC) gene is an example of nucleotide excision repair (NER) genes known to be involved in cancerogenesis. It is well established that the Xeroderma pigmentosum characterized by increased risk of UV-induced skin cancer and extreme sensitivity to sunlight is associated with defects with NER genes called XP genes ranging from XPA to XPG [46]. XPD and XPC mutations among AML patients showed to decrease survival compared to AML patients of the same

risk group with wild type for XPD and XPC genes. This suggests that some variants of NER genes may modulate the outcome in AML patients [47].

Functionally speaking, mutations that lead to cancer can affect either cancer promoting genes called protooncogenes or cancer inhibitor genes called tumor suppressors. Yet, a gene may be considered as an oncogene in one context and as a tumor suppressor in another depending on the developmental stage and the type of tissue where it is expressed [48, 49].

3. Role of Protooncogenes and tumor suppressor genes in HM's

Protooncogenes usually encode proteins stimulating growth, differentiation, and inhibition of apoptosis. To develop cancer, these genes can acquire gain of function mutations with dominant expression, of which the mutated forms are called oncogenes. Oncogenes overexpression can result in enhanced cellular growth, decreased differentiation, and halted cellular death. Oncogenic proteins may be growth factors, or their receptors, as well as other proteins downstream their signal transduction pathway, and transcription factors [50].

Tumor suppressor genes encode proteins that inhibit proliferation or act as brakes to the cell cycle. Mutations in tumor suppressor genes are usually loss of function mutations acquired in homozygous form. These mutations inactivate an inhibitory signals produced by the corresponding proteins and this contributes to the process of carcinogenesis [51]. Another form of tumor suppressor genes is "caretaker genes" whose responsibility is DNA repair, defects in such genes even a single mutated copy render cells more prone to mutations in other genes and that can lead to cancer [52] . Here next we review the role of some common oncogenes and tumor suppressor genes in cancerogenesis and the therapeutic implications of the understanding of their molecular details.

a) FLT3

Fms-like tyrosine kinase 3 (FLT3) is a class III receptor tyrosine kinase (RTK) that has an essential role in signaling for haematopoiesis which is known as common protooncogene. High levels of FLT3 expression are found in various hematologic malignancies including AML (25% of patients), and also in other HM's like the B-cell ALL [53].

The chromosomal location of FLT3 gene is 13q12 which encompasses 24 exons. The gene encodes two isoforms ; a membrane-bound glycosylated protein of 993 amino acids with a molecular weight of 158-160 kDa, and a non-glycosylated protein of 130-143 kDa that is not associated with the plasma membrane [54, 55].on the other hand, the gene for the FLT3 ligand (FL) gene encodes for a 235 amino acids composing a type 1 trans membrane protein containing an amino acid terminal signaling peptide, four extracellular helical domains, spacer and tether regions, a transmembrane domain and a small cytoplasmic domain, [56]. The FL is expressed in most body tissues including hematopoietic organs with highest expression in peripheral blood mononuclear cells. However FLT3 is mainly found in early hematopoietic progenitor cells from where its key position among the protooncogenes involved in HM's [57].

It is by interaction of FLT3 and its ligand that FLT3 plays its important role in the survival, proliferation and differentiation of hematopoietic cells as well as leukemia cells. FLT3 [58].

Mutations discovered first of all as internal tandem duplication (ITD) of the juxtamembrane (JM) domain-coding sequence. This type of mutations the ITD-mutations is found mainly in AML and associated with increased leukocyte count and poor prognosis [59]. The FLT3-ITD mutations causes an uncontrolled receptor activation, manifesting as ligand-independent receptor dimerization, with constitutive FLT3 signaling and consequent activation of STAT5 and of the RAS/MAPK and PI3 K pathways downstream signaling of the FLT3 precipitating in increased proliferation [60]. A second type of mutations involved in FLT3 are the mutations on the level of the activation loop of the second kinase domain causing constitutive activation of the receptor FLT3. This type of mutations groups small deletions, substitutions, and insertions, [61]. A recently discovered type of FLT3 mutations involves a group of point

mutations in the juxta membrane domain on amino acid residues such as 579, 590, and 594 [62]. FLT3 mutations are the subject of intensive research with efforts focusing on development of FLT3 inhibitors. Many inhibitors are generated with various degrees specificities for the FLT3, some of these inhibitors are now in phase III clinical trials with ongoing exploration of their role in conjunction with conventional chemotherapy [55]. Quizartinib is an example as a promising potent inhibitor of the tyrosine kinase activity in FLT3-ITD positive AML patients [63].

b) PML-RARα fusion gene

The promyelocytic leukemia gene (PML) belongs to the gene family called ring-type zinc fingers and the gene family tripartite motif-containing (TRIP). This gene is located on 15q 22. It is usually involved in acute promyelocytic leukemia with t(15:17) that also involves the retinoic acid receptor alpha on chromosome 17 [64, 65].

The PML gene is considered as a tumor suppressor gene producing the PML protein necessary for the formation of the macromolecular dynamic structures called PML-nuclear bodies (PML-NB's). The PML shuttling between nucleus and cytoplasm and interacting with different proteins contributes to different pathways with its two cytoplasmic and nuclear isoforms. The PML-NB structures seem to interact with other proteins involved in apoptosis, control of cellular growth and differentiation. When this function is altered as it is the case in APL, it represents a step in leukemogenesis by loss of the PML functions precipitating in proliferation of cells arrested in the promyelocytic stage [66, 67].

On the other hand, retinoic acid receptor, alpha (RARA) is a nuclear receptor that in absence retinoic acid "its ligand" represses the transcription of target genes by the help of other co-repressors. RARA in the presence of physiological levels of its ligand RA in converted from repressor into activator of its target genes involved in myeloid differentiation [68].

PML-RARA fusion protein produces limited transcriptional activation by RARA, now fused to PML, under physiologic levels of retinoic acid. RARA in the resulting fusion

protein PML-RARA is unable to block cell proliferation or induce apoptosis [69]. The second important result is that PML-RARA heterodimer counteracts the formation of nuclear bodies necessary for regulating other proteins interacting with them like in P53 pathway [68].

Promyelocytic leukemia induced by the PML-RARA fusion protein was one of the most severe forms of leukemia before the discovery of the all-trans retinoic acid (ATRA) role in treatment of this disease. ATRA the active form of vitamin A, at higher than physiologic doses induced remission by restoring the normal differentiation of arrested promyelocytes into functional granulocytes. Thus, doing so, ATRA, represented a paradigm for "differentiation therapy" in cancer [70, 71].

Another treatment was also introduced and enhanced the outcome among patients with PML-RARA fusion it is arsenic trioxide (ATO) that is thought to target PML moiety of the fusion protein. It induced partial differentiation at low concentrations, while at high concentrations the ATO induces apoptosis of the APL cells, Thus APL was made curable disease by the introduction of combined therapy with ATRA and ATO optimized regimens [72, 73].

*c) **P53***

TP53 is a tumor suppressor gene that produces a protein called P53 that works as a transcription factor targeting other genes to arrest cell cycle, induce apoptosis, or senescence in response to diverse processes [74]. the gene is mapped to chromosomal position 17p13.1-p12 [75].

The tumor suppressive activity of P53 is estimated to be inactivated in around 27 million people living with tumors, corresponding to around the half of human cancer cases. It is also estimated that half of the estimated number of tumors with inactivated p53 have the p53 pathway altered rather than the gene itself [76]

The expression of P53 is found mainly decreased due to the increased negative regulation exerted by the murine double minute gene human homologues the MDM2, and MDM4

regulators that may be either over expressed or amplified. MDM2 negative regulator is found to be amplified or over expressed in more than 10% of human cancers, and the MDM4 in 10-20% of human cancers including lung , breast, stomach, and colon [77, 78].

Therapeutic approaches focus on restoring the tumor suppressor function by disrupting the P53 interaction with its negative regulators MDM2, and MDM4. Some inhibitors for the negative regulators of P53 were successful in 'in vitro' proof-of-principle experiments. These approaches included the utilization of small peptide molecules targeting the 3 N-terminal amino acids in P53 protein in order to disrupt the P53-MDM2 interaction. Another approach is the utilization of molecules that mimic the P53 boxI peptides that represent a highly conserved MDM2 domain necessary for the interaction with P53 during the negative regulation [79, 80].

4. Role of epigenetics in the development of HM's

Epigenetic contribution to cancerogenesis is being more and more elucidated recently. We know now that epigenetics changes in the structure and packaging of the DNA such as via methylation while not changing DNA sequence effects gene expressions in such a way to induce untimely expression or repression of cancer genes [81].

The concept of "Epimutations" and altered expression of the proteins involved in the chromatin structure functioning in reading and writing of the epigenetic code are the key mechanisms behind epigenetic role in diseases including cancer. Epigenetic role in cancerogenesis includes silencing of tumor suppressor genes and reactivation of the silenced protooncogenic regions. Epigenetic effects can act as part of other genetic multistep events to precipitate in malignant cell transformation [82, 83].

MicroRNA's also play role to the epigenetics of cancer, by playing part in transcriptional regulation affecting both tumor suppressors and protooncogenes [84, 85].

a) DNA methylation 'focus on the epigenetic role of DNMT'

The addition of a methyl group (-CH3) to Cytosine in the dinucleotide (CpG) referred to as DNA methylation is one of the most important epigenetic control tools of gene

expression. Most of the (CpG) dinucleotides in our genome are methylated, It is only the (CpG) islands in the promoter regions of the genes that are non-methylated,[86, 87]. Non-methylated promoters are accessible to transcription factors, while methylated are not, and thus they are not expressed 'turned off', [88]. It is also thought that DNA methylated CpG regions recruit repressor complexes that also interact with Histone deacetylases to produce less mobile deacetylated histones. This results in wrapping of the DNA and inhibiting transcription of the resultant more condensed chromatin, [89, 90].

There are several isoforms of DNA methyl transferases DNMTase's responsible of epigenetic DNA methylation. Examples of these DNMT's include DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT1 which is responsible of the most part of the maintenance and the de novo methylation in vertebrates somatic cells [91, 92]. The DNMT's gene is located on chromosomal position 19p13.3-p13.2 [93].

Methylation of CpG islands was experimentally proved to be principal tool for transcriptional silencing of the genes by means of methyltransferase inhibitors. Inhibitors include (antisense RNA's and siRNA's), and 5-aza-2-prime-deoxycytidine. It was showed that tumor suppressor genes activity in cancer cells was restored when using such methyltranferase inhibitors against DNMT1 but not DNMT3a, and B,[94]. This is now believed that DNMT1 is the isoform responsible of the faithful maintenance of the pattern of genomic DNA methylation in cancer cells which is necessary for their survival and proliferation [95].

An example of epigenetic implication of DNA methylation in cancer development is the loss of function of the SHP-1 phosphatase in T cell lymphoma. SHP-1 phosphatase is an inhibitor regulator of cell signaling. It was demonstrated that DNMT1, and other cooperating molecules form complexes that play role in SHP-1 gene silencing. Therapeutic treatment with anti-sense RNA for the DNMT1 is found to take part in an effective anticancer approach targeting epigenetic silencers of tumor suppressor genes [95].

b) Role of microRNA's (miRNA's) in the epigenetics of HM's

Micro RNAs are small endogenous non coding RNA acting as genomic regulators on post transcriptional level by being complementary to their target messenger RNA's. one miRNA molecule can regulate the expression of many target genes and conversely many miRNA's can simultaneously work on one target gene, [96, 97]. MiRNA's function in two ways: binding the 3' untranslated region (3' UTR) of their target mRNA and causing its suppression, or targeting mRNA causing its degradation [98]. There may be around 1000 miRNA genes in human genome controlling one third of human protein-coding genes. These miRNA genes contribute in creating the specific miRNA milieu specific to each cell type in the human body [99].

It is thought that the interactions of miRNA's with their target mRNA's contribute to many biological processes including cell division, apotptosis, development, differentiation, and control of tumorigenesis [100]. An example of the cancerogenic role of miRNA's is the deletion 13q14 in more than half of the B-CLL. This 13q14 deletion is common in CLL, and mantle cell lymphoma, but also found in multiple myeloma, and prostate cancers. It may be in hemizygous and/or homozygous forms. Calin and colleagues found that two miRNA genes (miR15, and miR16) are found to be important contributors in b-CLL with del(13q14). They also showed that these genes are either deleted or down regulated in the majority of CLLcases [101]. Other studies demonstrated that miR15, and miR16 expressions are inversely correlated with B cell lymphoma 2 gene (BCL2). BCL2 gene is known to inhibit cell apoptosis, and seemingly the BCL2 overexpression results from the down regulation of the miR15, and miR16 tumor suppressor genes. This finding makes the miR15, and miR16 a therapeutic target of leukemias and cancers overexpressing the BCL2 gene [102].

C. Acute Lymphoblastic Leukemia (ALL)

ALL is characterized by the accumulation of malignant, immature lymphoid cells in the bone marrow and, in most cases, also in peripheral blood. These cells morphologically and immunophenotypically resemble the B and T lineage precursors [103, 104].
Clinical presentation depends on the degree of bone marrow replacement by the leukemic blasts and the resulting cytopenias. Symptoms include mainly fever, fatigue and lethargy, bone and joint pain, and bleeding tendency [103].
ALL is classified by the last update of the WHO classification in 2008 as follows:

Precursor lymphoid neoplasms.

B-lymphoblastic leukemia/lymphoma, NOS (not otherwise specified).

B-lymphoblastic leukemia/lymphoma, with recurrent genetic abnormalities.

B-lymphoblastic leukemia /lymphoma, with t(9;22)(q34;q11.2);BCR-ABL.

B-lymphoblastic leukemia /lymphoma, with t(v;11q23); MLL rearranged.

B-lymphoblastic leukemia /lymphoma, with t(12;21)(p13;q22);TEL-AML1 (ETV6-RUNX1).

B-lymphoblastic leukemia /lymphoma, with hyperdiploidy.

B-lymphoblastic leukemia /lymphoma, with hypodiploidy.

B-lymphoblastic leukemia /lymphoma, with t(5;14)(q31;q32);IL3-IGH.

B-lymphoblastic leukemia/lymphoma, with t(1;19)(q23;p13.3); E2A-PBX1 (TCF3-PBX1).

T-lymphoblastic leukemia/lymphoma.

Mature B-cell neoplasms, including the mature-cell B-ALL which is not listed as a distinct entity [2].

ALL ranks as the most common among pediatric malignancies (with 25%). The total incidence of ALL in childhood is 34 per 100,000 per year, while for adults it is less than 1 per 100,000 [105, 106]**.** The B Cell Precursor-ALL, representing most of the ALL cases, is primarily a disease of childhood with 75% of patients are under the age of 6 years. On the other side mature B-cell ALL is rare and the T-cell ALL represents approximately 15% of childhood and 25% of adult ALL. The T-cell ALL is also considered as a high-risk malignancy [107].

The different recurrent cytogenetic/molecular abnormalities as used in the WHO classification correspond to different risk categories. Philadelphia positive ALL with the t(9;22)(q34;q11.2), BCR-ABL is of poor prognosis, with poor response to most chemotherapy combinations, short remission and reduced survival rates. Tyrosine kinases when introduced for treatment they ameliorated survival, but imatinib monotherapy though it produces high response rate in Ph+ALL, it is transient with recurrence in months, when resistance develops and relapse occurs. Imatinib when used with intensive chemotherapy produces a survival of 3 years in children and adolescents with Ph+ALL [108, 109, 110].

T(11q23) represents another abnormality defining an ALL distinct entity. This rearrangement of the mixed–lineage leukemia gene (MLL) presents a poor prognosis ALL entity [111]. MLL is known to regulate the Homeobox (Hox) gene expression through promoter binding and histone modification. The MLL alterations including fusions, partial tandem duplications, and amplifications result in upregulation of the Hox oncogene which apparently results in block of hematopoitic differentiation [112].

Another genetic abnormality that may be found in some ALL is the t(12:21)(p13;q22), TEL-AML1 (ETV6-RUNX1). This translocation involves an in frame fusion between the ETS-related gene (ETV6) and the AML1 (RUNX1) locus producing a fusion gene participating in the development of a precursor B-cell ALL with favorable prognosis under conventional therapeutic strategies, [113]. The pathogenic implication of this fusion gene remains unclear; this may be in part because animal murine model with

RUNX1 knockout does not survive. But AML1/RUNX1in vivo studies by inducible gene targeting methods shows that AML1 deficient bone marrow results in defective B and T cell development, as well as for megakaryocytes [114]. It seems that TEL/AML1 protein functions as a transcriptional repressor recruiting other corepressors such as NCoR, Sin3A, and histone deacetylase inhibiting AML1 target genes which seems to hinder maturation [115, 116].

Hyperdiploidy is clinically characterized by favorable outcome. It is the most prevalent cytogenetic abnormality in pediatric precursor B-cell ALL. It is usually associated with nonrandom gains of chromosomes X, 4, 6, 10, 14, 17, 18, and 21 [117]. Hyperdiploidy has slightly variable but favorable outcome and variable cytogenetic aberrations associated with the variable modal number of chromosomes (MNC). For MNC (57-60) +8, +5, +11, +12 add to the previous series of chromosomes (X, 4, 6, 10, 14, 17, 18, and 21) that appear at lower MNC. For the chromosomes 2, 3, 9, 16, and 22 they are observed among the highest MNC (63-67) [118, 119]. The European organization for research and treatment of cancer (EORTC) has studied prospectively the factors contributing to the outcome among hyperdiploid (HD) patients (>50 chromosomes), they found that the strongest factor of good prognosis was the MNC with 6-years event free survival among 51-53, 54-57, and 58-66 MNC groups was 80%, 89%, and 99% respectively. They found also that ploidy determined by karyotype was a better indicator than DNA index (DI) assessed by flow cytometric estimation of cellular DNA [120].

Hypodiploidy is the presence of (<46) chromosomes. It can be further classified into three subgroups based on MNC including near-haploid (23-29 chromosomes), low hypodiploid (33-39 chromosomes) and high hypodiploid (42-45 chromosomes). The outcome is poor and the lower the MNC (near haploid and low hypodiploid) the worse the outcome. The high hypodiploid subgroup has more complex karyotypes involving mainly 7, 9, and 12 [121]. By the introduction of microarray profiling and sequencing many recurring genetic alterations in hypodiploid ALL have been identified. Such alterations include the TP53 (present in 91.2% of childhood low hypodiploid ALL). The Retinoblastoma-1 (RB1) and IKZF2 alterations are also hallmarks of hypodiploid ALL. Other alterations target receptor tyrosine kinase signaling (phosphoinositide 3-kinase

(PI3K)) and Ras signaling, suggesting that drugs like PI3K inhibitors should be explored as new strategies for the treatment of this poor outcome ALL [122].

T-cell ALL represents 15%-25% of pediatric and adult ALL. This T-cell lineage ALL is in general considered to have a worse outcome than that of the B-lineage [123]. For the significance of the cytogenetic abnormalities, the high MNC expects a favorable prognosis for the T-cell ALL as well as the pre B-cell lineage, but the pseudodiploid karyotypes more associated with the T-lineage phenotype are more associated with the poor outcome [124].

The T-cell ALL cytogenetic structural abnormalities include t(11;14)(p13p15;q11), t(11;14)(q24;q11), t(7;19)(q35;p13), and many other translocations [125]. Such structural abnormalities mainly target the T-cell receptor genes. An example of such translocations in T-cell ALL is the t(5;14)(q35;q32) which is present in 20% of pediatric T-cell ALL and 10% of adult T-cell ALL. This translocation causes transcriptional activation of the HOX11L2 gene on chromosome 5 responsible by the influence of the commonly highly expressed during T-cell development CTIP transcriptional regulation elements present on chromosome 14. This gives evidence of the role of HOX11L2 in T-cell ALL leukemogenesis [126, 127].

Micro array expression profiling in case of absence of chromosomal abnormalities allowed the identification of five molecular subgroups corresponding to the altered oncogenes, Homeobox gene -11 (HOX11), T-cell lymphocytic leukemia gene -1 (TAL1), Lymphoblastic leukemia-derived sequence -1 (LYL1), LIM domain only gene -1 and 2 (LMO1, and LMO2). In which HOX11 expression has a favorable outcome, while the TAL1, and LYL1 activation confers a worse response to treatment [128].

D. Acute Myeloid leukemia (AML)

AML is a group of clonal myeloid neoplasm with transformation of early myeloid precursor cells. Different types of AML correspond to the differentiation stages of myeloid progenitors with maturation arrest at different points of myelopoiesis, leading to the accumulation of myeloblasts in bone marrow and or blood [129, 130] .
The general AML symptomatology is related to the decreased normal hematopoiesis, and organ infiltrations by the leukemic clones. Patient complaints usually include a viral-like illness characterized by malaise and fatigue. Progressive infections after skin scratches are also common. Bone pain and tenderness may also be present in long bones and ribs [131].

The 2008 WHO classification update states that myeloblasts must comprise at least 20% of nucleated cells in bone marrow or blood to establish a diagnosis of AML. Several exceptions for the 20% are considered in the classification including cases with t(15;17), inv(16)/t(16;16), or t(8;21) and other phenotypic features where the diagnosis is made irrespective to the blast percentage [129].
AML is considered a disease of adults with median age at diagnosis over 65 years. Its incidence is one per 100,000 per year but the incidence increases to become 15 per 100,000 on ages over than 75 year [132]. It is among elderly where the disease is more frequent that it shows increased intrinsic resistance to chemotherapy, as well as reduced survival rates [133].AML is considered the most lethal neoplasia between the ages of 30 to 40 years of age. AML is considered as the most frequent form of leukemia, representing around 25% of all leukemia in adults in recent studies among western patients [134].
The original French American British (FAB) classification of AML included the following categories: M1 (Acute myeloid leukemia without maturation), M2 (Acute myeloid leukemia with maturation), (M2Baso: with basophilia < 2%. M3 (Acute promyelocytic leukemia). Subtype “M3v”: microgranular variant. M4 (Acute myelomonocytic leukemia). M5 “Acute monoblastic leukemia”. (Subtype “M5a”) :

immature monoblasts, “M5b” monoblasts with maturation. M6 (Acute erythroleukemia), and M7 (Acute megakaryoblastic leukemia) [135].

Structural chromosomal aberrations in AML include t(8;21), t(15;17), inv(16), t(11q23;n), t(3;5), t(3;3), t(8;16), t(6;9), t(1;3), t(9;22). Numeric aberrations are represented mainly by deletions -5, -7, 5q-, 7q-, 20q- or 12p-, Trisomies +8, +13, +21[125].

While the FAB classification depends on morphologic and immunophenotypic features, the WHO classification takes in account genetics and clinical features to define classes of clinical relevance. Chromosome abnormalities that occur in MDS and MPD also occur in AML, although but with different proportions. Some abnormalities occur only in AML, and rarely occur in other disorder. Such abnormalities include t(8;21)(q22;q22), t(15;17)(q24;q21), and inv(16)(p13q22). From phenotypic perspective, it is of importance to notice that aberrations confined to granulocytic cells are usually associated with a good prognosis, while aberrations in the other clones of the myeloid lineage are broadly associated with a poorer prognosis [136].

The Last update of the WHO classification of AML Included fourteen types of AML with the first one being acute myeloid leukemia with recurrent genetic abnormalities. This type of AML malignancies includes the following:

AML with t(8;21)(q22;q22); RUNX1-RUNX1T1

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11

APL with t(15;17)(q22;q12); PML-RARA

AML with t(9;11)(p22;q23); MLLT3-MLL

AML with t(6;9)(p23;q34); DEK-NUP214

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1

AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1

Provisional entity: AML with mutated NPM1

Provisional entity: AML with mutated CEBPA

The rest of AML types seem to be based on immunophenotypic as well as cytologic and clinical features [137].

Some cytogenetic abnormalities are considered to be more associated with some AML-FAB classes. Starting with M1, There is no specific cytogenetic abnormality, although

trisomy 13 is most common in M0 and M1 and can be associated with a poor prognosis, [138]. The most common abnormality in M2 is t(8;21)(q22;q22). It is associated with a relatively good prognosis with intensive chemotherapy. Loss of a sex chromosome (X in females or Y in males) may be associated with this translocation [139].
In M3 it is the t(15;17)(q24;q21) that is most specific. It is of bad prognosis if untreated, but treatment with all-trans retinoic acid (ATRA) ameliorates the prognosis to long term survival in most of treated patients [140]. A variant translocation involving RARA is t(11;17)(q23;q21) [141].
In M4, as in M2 t(8;21)(q22;q22) occurs with lower incidence [139]. (M4 eo) is associated with inv(16)(p13q22) and t(16;16)(p13;q22), which have a relatively good prognosis. A del(16)(q22) also occurs and its prognostic relevance is considered normal when it is a variant of the previously mentioned inversion. But it's considered of poor prognosis when it comes in the context of MDS [142, 143].
A t(8;16)(p11;p13) takes place in both M4 and M5. M5a is associated with t(9;11)(p21-22;q23) which may be easily missed since it is very subtle [144].
M6**:** Erythroleukemia: no specific cytogenetic abnormality, but about 25% of all occurrences of t(3;5)(q21-25;q31-35) are found in M6. M7 leukemia risk is increased among trisomy 21 patients, and abnormalities like 3q21 and/or 3q26 are more common. In infants , t(1;22)(p22;q13) occurs in M7 patients [145, 146].
Treatments are highly dependent on subtyping of AML. Subtyping now involves not only patient history (age, previous MDS etc) but increasingly dependent on these cytogenetic endpoints [139, 147] and some mutations (NPM1, FLT3, CEBPA). Appropriate diagnosis of the involved chromosome/molecular changes allows for effective targeted therapy [129, 148].

In Western (US and UK) patients, AML associated with t(8;21), t(15;17) or inv(16) predicted a relatively favorable outcome while patients lacking these favorable changes, having complex karyotype, −5, del(5q), −7, or abnormalities of 3q defined a group with relatively poor prognosis, [149]. For instance, among western patients, AML associated with t(8;21), t(15;17) or inv(16) predicted a relatively favorable outcome while patients lacking these favorable changes, having complex karyotype, −5, del(5q), −7, or abnormalities of 3q defined a group with relatively poor prognosis [149].

To shed some light on molecular bases of AML subtypes we go briefly over some common mutations. T(8;21)(q22;q22) corresponds to separate AML entity with recurrent genetic abnormalities. It is one of the most frequent karyotypic abnormalities in AML, and considered as the most common abnormality in M2 subtype [150]. This translocation involves chromosomal rearrangements between the RUNX1(also named AML1) gene on 8q22 and the RUNX1T1 (also named ETO) gene on 21q22 [151, 152]. This form of fusion is found in about 5-12% of all AML patients and usually associated with good prognosis [153]. RUNX family has many members, including the AML1 member, they are needed to regulate the transcription of genes needed in hematopoietic process [154]. RUNX1 protein is a DNA-binding transcription factor in which the "runt" domain mediates the binding process. The transcription of many genes necessary for the myeloid lineage growth factor signaling is mediated by the RUNX1 transcription factor, such genes include IL-3, GM-CSF, the M-CSF receptor, and c-Mpl targeted genes [154]. The RUNX1-RUNX1T1 fusion gene, t(8;21)(q22;q22) usually needs a second action in order to cause AML [154]. In general it is the core binding function that is disrupted during the translocation, [155]. It is thought that the translocation disturbs the transcriptional regulatory function mediated by the RUNX1 gene since it contains the only the amino-terminal DNA binding domain. On the other hand It contains most of the ETO gene that is responsible in recruiting molecules like mSin3 and nuclear hormone co-repressors, N-CoR, and histone deacetylases, that also inhibit transcription of RUNX1 target genes [155].

Inv (16)(p13.1q22) is also important to study. It is found in M4/M4Eo subtype of some AML cases. This inversion results from a breakpoint near the end of the core binding factor CBF beta (also known as PEBP2) on (16q) and another breakpoint on smooth muscle myosin heavy chain (SMMHC) gene (also known as MYH11) on 16p. This inversion creates a CBFB/MYH11 fusion gene [156]. Studies showed that the CBFB/MYH11 fusion has favorable prognosis with high rate of remission compared to other forms of AML, [157]. Several mutations coexisting with CBF AML involve genes like the KIT gene, the NRAS and KRAS genes, and the FLT3 gene. With KIT mutation involving exon 8 mutations and exon 17 are detected in around 30% of CBF AML which may associate a higher incidence of relapse in this context [158].

Acute promyelocytic leukemia (APL; FAB M3) results from the translocation t(15;17) leading to the formation of a fusion gene between the rentinoic acid receptor alpha (RARA) gene on chromosome 17 and the PML gene on chromosome 15 which represented a bad prognosis before the introduction of the targeting all trans retinoic acid discussed earlier [159].

Another common abnormality associated with AML is t(9;11)(p22;q23); *MLLT3-MLL* also called AF9-MLL. This translocation is observed in both acute lymphoid leukemia and acute myeloid leukemia. And it is specifically observed in M5 as well as in (AMML-M4) [160]. The MLL gene located on 11q23 (also named ALL-1, Htrx, HRX,) recombines with around 40 different chromosomal regions as well as with itself, with the most frequent translocations being t(6;11) and t(11;19) detected in AML, and the t(4;11) and t(11;19) found primarily in ALL patients, for which chemotherapy with topoisomerase II inhibitors have been developed, [161]. On the other hand MLLT3 (also called AF9, LTG9) located on 9p22 with functions still under study. The fusion product seems to play important role in keukemogenesis by resulting in aberrant proliferation [162].

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M6**:** Erythroleukemia: no specific cytogenetic abnormality, but about 25% of all occurrences of t(3;5)(q21-25;q31-35) are found in M6. M7 leukemia risk is increased among trisomy 21 patients, and abnormalities like 3q21 and/or 3q26 are more common. In infants , t(1;22)(p22;q13) occurs in M7 patients [145, 146].

E. Chronic Lymphocytic Leukemia (CLL)

CLL is a low-grade leukemic lymphoma with clonal proliferation and accumulation of morphologically mature but immunologically incompetent lymphocytes of the B-cell lineage, and represents the most common type of leukemia among adults [163]. According to the WHO CLL is invariably a B cell neoplasia whereas the formerly named T-cell CLL is referred to as T cell prolymphocytic leukemia (T-PLL), [164]. The general underlying event is a progressive accumulation of neoplastic mature B lymphocytes that undergo transformation due to genetic and epigenetic events in the bone marrow, blood, and lymphatic tissue [165]. These genetic events underlying the transformation of B cells are thought to be more related to apoptotic deficiency rather than increased proliferation, with the outstanding irregularities of CLL clone being the CD5 positivity and almost undetectable surface immunoglobulins [166].

CLL is considered as a disease of heterogeneous nature with patients ranging from those with very stable disease with favorable outcome to patients with rapidly progressive disease and decreased life expectancy [167]. CLL belongs to the wider class of hematologic malignancies the lymphoproliferative disorders (LPD), that include in addition to CLL, the prolymphocytic leukemia (PLL), and hairy cell leukemia (HCL). CLL Incidence is 2–3 cases/100,000 population/year representing 30 % of all leukemia in western nations, and 5% in Asia. Median age at diagnosis is 55 years. Only 10 % of patients are under 50 years of age [125]. Patients with CLL usually present with enlarged lymph nodes, sometimes other symptoms may manifest as hepatosplenomegaly, pallor, petechiae and/or mucocutaneous bleedings [168].

Detailed cytogenetic analysis of cells in CLL patients is not easily obtained since CLL cells are poorly dividing and inducing their division is not easily obtained by traditional mitotic agents. FISH techniques were developed assay for things like the trisomy 12 that is common in Western Patients with CLL [169] are now used routinely in diagnosis and prognosis [170]. Novel technologies for division induction in CLL make use of CD40 ligand, IL2, or a combination of CD40 ligand with CpG-oligodeoxynucleotides. These mitotic agents produce more division and ameliorate the culture outcome [171].

Del 17p is considered the most important risk factor. It is also better detected by immunohistochemistry and FISH techniques [172]. With estimated overall survival of 3 years [173]. This abnormality represents the strongest predictor of poor survival among CLL patients and may be considered as the only abnormality with independent prognostic value [174]**.** The mutation affecting TP53 gene (discussed earlier) represents a major clinical challenge, with disagreement on the optimal treatment approach in such cases [175].

Del 11q is found to be also associated with adverse clinical course, with survival of 6 to 7 years. It is identified by FISH in around 20% of CLL, and frequently encountered with bulky lymphadenopathy and bad outcome, genomic profiling allowed to map the minimal deleted region (MDR) in CLL with del 11q to include the (ataxia-telangiectasia mutated) gene which was the first blamed for the pathogenesis. ATM represented a candidate target responsible in part for the CLL since the defect in ATM results in impaired cellular response to DNA damage, as well as it is found in 30-40% of patients with 11q deletion. On the other hand another possibly interfering gene is BIRC3 which is a negative regulator of noncanonical NFκB signaling located at 11q22 [176]. ATM is a member of the phosphatidylinositol-3 kinase (PT3K) gene family [177]. The nuclear serine/threonine kinase ATM protein activities are induced by endogenous chromosomal double-strand breaks or caused by damaging agents by exposure to ionizing radiations or drugs [178]. The ATM gene is considered the principal activator of P53 gene in case of double strand breaks, this seems to alter apoptotic response hypothesized to play important role in the pathogenesis of CLL with impaired over-all treatment free survival, [179].

On the other hand isolated del 13q is associated with a favorable outcome with survival reaching 11years [180]. While isolated del 13 has probably a favorable outcome, CLL patients with del 13 as well as the TP53 disruption, c-MYC abnormalities, unmutated immunoglobulin heavy chain (IGHV) are all high risk groups to develop Richter's syndrome 'Richter's transformation'. Richter's syndrome is associated with very poor outcome of 11 months and it refers to the development of aggressive diffuse large B-cell lymphoma (DLBCL) during the course of CLL [181].

Trisomy 12 is considered of intermediate outcome, with median survival 114 months when it is the sole abnormality, but the outcome becomes worse if this condition is associated with *NOTCH1* (Notch homolog 1) mutations [182]. Two genes were found to be overexpressed in CLL patients with trisomy 12; huntingtin interacting protein 1 related (HIP1R) and the myogenic factor 6 (MYF6) [183]. Two other genes the P2RY14, and the CD200 located on another chromosome (3q21-q25), (3q12-q13) are interestingly found to be underexpressed in CLL with trisomy 12 but located on another chromosome (3q21-q25), and (3q12-q13) [184].

Other karyotypic abnormalities recurrent in CLL include del 6q, +8q, and translocations involving the 14q32. CLL cases with complex abnormalities (3 or more abnormalities) are usually associated with adverse prognoses [185]. What we aim to find out is if the nature and prognostic impact of rearrangements in the Palestinian areas are similar to what was reported elsewhere (e.g. see an interesting study of CLL in Taiwan [186].

F. Chronic Myeloid Leukemia (CML)

Chronic myelogenous leukemia (CML) is a myeloproliferative neoplasm (MPN) originating from a clonal, pluripotent hematopoietic stem cell defined by the presence of the *BCR-ABL1* fusion gene, usually as a result of t(9;22)(q34;q11.2) [187] . Although the term CML is synonymous with chronic granulocytic leukemia, the term CML includes also some rare disorders such as the chronic neutrophilic, eosinophilic, and basophilic leukemias, juvenile chronic myeloid leukemia; and chronic myelomonocytic leukemia [136].CML represents approximately 20% of adult leukemias, with annual incidence of 1 per 100,000. It has a peak incidence in the 5^{th} and 6^{th} decade, and it rarely affects people under age 20 years.

The classic translocation t(9;22) is detected in approximately 90% of all patients by standard cytogenetic methods, with variant and cryptic translocations found in >5% of patients [125]. Patients with CML may progress through chronic phase to the accelerated phase CML and CML with blast crisis. Disease progression may mean additional secondary chromosomal and molecular abnormalities including +8, doubling of the Ph chromosome, i(17q), +19, or –Y [4].

Various combinations of the secondary abnormalities are found to be associated, this association may be called positive when the clone acquiring the first abnormality excerts a positive selective pressure for the development of the second abnormality, thes following couples of abnormalities are examples of such positively associated abnormalities (+ 8 and i(l7q), + *8* and + 19, and + 19 and + Ph), there is also negatively associated abnormalities as in the following (i(I7q) and + 19 and i(17q) and + Ph), [105]. CML may show some abnormalities also found in myelodysplastic syndrome including +8, -7, 7q-, -5, 5q-, and 20q- [188].

t(9;22) translocation creates a fusion of human homologue of the Abelson murine leukemia virus (ABL) on 9q34 with break point cluster region (BCR) gene on 22q11, [189, 190]. ABL is a non-receptor tyrosine kinase that is expressed universally in all

cells. It shuttles between the nuclear and the cytoplasmic compartments and has responsibilities to transduce signals from cell surface growth factor and adhesion molecules [191]. ABL has also DNA-binding site which is regulated by differential phosphorylation levels during interphase and mitosis suggesting a cell cycle function for ABL [192]. The breakpoint of the 9q34 in CML is only 13kb from the beginning of the ABL oncogene [193].

On the other hand, the BCR gene encodes a phosphoprotein with serine/threonine kinase activity, while the breakpoint cluster region refers to a 5.8 kb fragment that is situated within a central region of the gene on the 22q11, which is disrupted in Philadelphia positive patients, [194]. Depending on the variable breakpoint on the BCR gene two BCR-ABL chimeric proteins are found. The two proteins with constitutive tyrosine kinase activity are $p210^{BCR-ABL}$ protein that characterizes a chronic phenotype of leukemia (CML), and a $p190^{BCR-ABL}$ protein that is found in acute either ALL or less often AML [194].

Because of the essential role of the tyrosine kinase function of the chimeric protein product of the BCR-ABL mutation, treatment options include blocking the kinase function. Druker et al were the first who gave evidence of the effectiveness of the treatment with such tyrosine kinase inhibitor using ST1571 (Gleevec) escalating doses in trial on CML patients for whom the treatment with interferon alpha failed. He proved that Gleevec was antileukemic and produced complete hematologic response with little side effects [195].

G. Myelodysplastic Syndrome (MDS)

MDS is defined as a heterogeneous group of myeloid disorders characterized by peripheral blood cytopenias and high risk of transformation to AML[196]. These disorders have the hallmark of ineffective haemapoiesis leading to a hypercellular bone

marrow with cytopenia in peripheral blood. Blood smears present with dysplastic changes in myeloid cells with abnormal proliferation and differentiation of one or more blood cell lineages [197, 198].

The WHO classification of MDS includes the following heterogeneous groups:

Refractory cytopenia with unilineage dysplasia (RCUD)

Refractory anemia with ring sideroblasts (RARS)

Refractory cytopenia with multilineage dysplasia (RCMD)

Refractory anemia with excess blasts-1 (RAEB-1)

Refractory anemia with excess blasts-2 (RAEB-2)

Myelodysplastic syndrome – unclassified (MDS-U):

MDS associated with isolated del(5q)

Therapy-related MDS (t-MDS) [137]

MDS is considered as a disease of the elderly with 20 per 100,000 incidence, and a prevalence of 7-35 per 100,000 people of more than 60 years age patients. MDS also has male preference over females, and increased frequency in people with history of cytotoxic therapy [14, 199]. Disorders in MDS are mainly secondary to prior cytotoxic therapies or exposures, although they may be primary disorders. Abnormalities can be both structural, or numerical with monosomy 7, -7,q-, 20q-; trisomy 8, 14, or 19; complex anomalies, sex chromosome loss being the most common [125]**.** MDS primary cytogenetic abnormalities were classified by Schanz et al. (2012) [200] into five prognostic subgroups, See (figure.1). The subgroups include very good (with median OS of 61 months), good (49 months), intermediate (26 months), poor (16 months), and very poor (6 months).

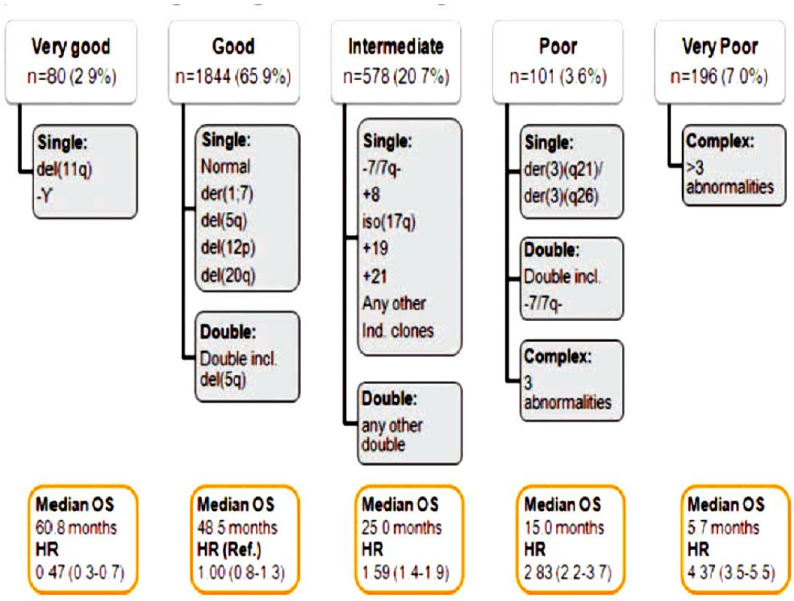


Figure 1. New cytogenetic classification of MDS. Adapted from Schanz et al. (Ref. 7). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure1. Cytogenetic classification of Myelodysplasia from Schanz et al**. [200].**

On the molecular level, we mention the example of the ASXL1 gene (additional sex combs-like 1 gene) as somatic mutations in this gene mapping chromosomal position 20q11 which is still incompletely understood with conflicting literature on how it participates in inducing malignancies [201]. Mutations on this gene are found in MDS as well as other myeloid neoplasms as the AML, myeloprliferative neoplasms (MPN), and chronic myelomonocytic leukemia (CMML)[202]. This gene is located close DNMT3B (mentioned earlier), and contains 12 exons with the twelfth exon being most of the time the site of detected mutations [203]. The product encoded by the ASXL1 gene is a nuclear protein of 1084 residues with functions in regulating transcription and epigenetic control through interaction with polycomb complex proteins and various regulators and repressors of transcription [204, 205].

Mutations involving the gene have just recently been reported and still poorly understood. They include missense and frame shift mutations probably resulting in truncated protein [203]. Though the mutations are usually heterozygous incdicating the role of insufficiency in the pathogenesis, some literature suggested a dominant negative role of the mutation resulting in the loss of the ASXL1 protein and thus a loss of function

mechanism [206]. Such mutations are usually associated with poor outcome and may help well in prognosis assessment in clinical practice [207].

Many other mutations we mention as other examples that may be identified in MDS including GATA binding protein 2 gene (GATA2) which is composed of 7 exons and localized on 3q21.3 chromosomal position, known to be involved in gene expression regulation in the hematopoietic cells [208, 209]. This is thought to be a loss of function mutation resulting in loss of the GATA2 gene that predisposes to familial myelodysplastic syndromes (MDS)/acute myeloid leukemia (AML); and Emberger syndrome (primary lymphedema with MDS) [210].

H. Multiple Myeloma (MM)

Multiple myeloma is a neoplastic plasma-cell disorder that is characterized by clonal proliferation of malignant plasma cells in the bone marrow microenvironment, monoclonal protein in the blood or urine, and associated organ dysfunction, [211]. It begins as asymptomatic premalignant proliferation of abnormal plasma cells. Multiple genetic changes and microenvironment changes are reported to be involved in transformation from monoclonal gammopathy of undetermined significance (MGUS) to smoldering myeloma and to symptomatic myeloma (Fig. 2). In some cases this transformation becomes extramedullary myeloma which represents the severe and extreme case of the disease [211, 212, 213].

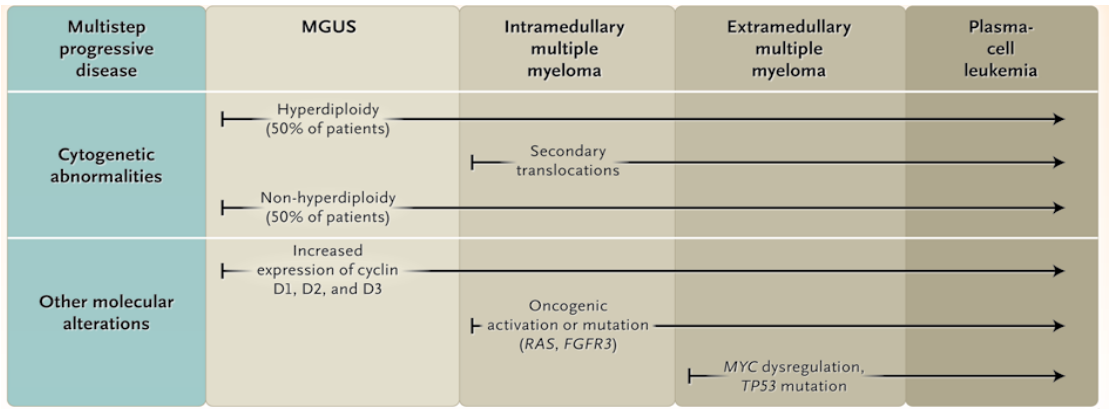


Figure. 2. The multiple steps of genetics changes and environmental change in myeloma and related forms from Palumbo and Anderson, 2011 [211].

To diagnose multiple myeloma at least 10% clonal plasma cells are needed in the bone marrow or the presence of plasmacytoma in biopsy, with evidence of end-organ damage (lytic bone lesions, hypercalcemia, or renal failure) attributed to the existing plasma cell disorder[214].

Detection methods for MM include flow cytometry and conventional karyotyping. Karyotyping shows abnormalities in 20-30% of MM cases because of limited proliferative nature of MM cells in culture. Fluorescence in situ hybridization (FISH) can detect chromosomal aberration in 40-70% of cases (Sawyer, 2011). Identifying genetic markers via cytogenetics (G-banding and FISH) and/or via other molecular methods is important for disease management and risk stratification [212, 215, 216].

Multiple myeloma can be broadly divided into hyperdiploid and non-hyperdiploid subtypes. The latter represents cases with IgH translocations that are usually associated with more aggressive clinical features and shorter survival. The three essential translocations targeting IgH in myeloma are the t(11;14)(q13;q32), t(4;14)(p16;q32) and t(14;16)(q32;q23). The hyperdiploid and trisomies subtype on the other hand characterizes a more indolent form [217, 218].

The main prognostic markers include deletions in chromosome 17 and 13 and 1p deletion as well as 1p amplification. Other important molecular defects include mutations resulting in factor-B activation and aberrant control of the cyclin-dependent pathways,

[219].Del 17p13 harboring the locus of the tumor suppressor gene P53 (discussed earlier) is the most important prognostic factor in multiple myeloma. This deletion is known to confer very poor prognosis with shortened survival and increased potential to develop extramedullary disease [217].

Monosomy 13 as well as deletions of the long arm of chromosome 13 are found in around 50% of MM cases and represent a bad prognostic marker with poor survival [220, 221]. Abnormalities in chromosome 13 are mainly a monosomy 13 or large deletions in the long arm and rarely interstitial deletions [220]. Screening for possibly involved genes in MM patients with del 13 via expression profiling showed that RB1 (retinoblastoma 1) was the most under expressed among other genes which points towards a possible role of the RB1 in the pathogenesis of MM. but yet the relevance for disease progression has not been obviated [222]. Many genetic abnormalities usually coexist with del 13 like del17p , and t(4;14) and others. Del 13 has not yet been studied well alone so that its significance on survival cannot be conferred to itself rather than being a surrogate for other coexisting markers [223]. This dissociation with bad prognosis was found when Del 13 was found in patients who lack t(4;14) and del(17p) the prognostic value using FISH analysis disappeared which supports the idea that the role is dependent on other coexisting lesions [224].

Translocations involving the immunoglobulin heavy chain locus on chromosome 14q32 are frequent in MM in around 40% of patients with the most common translocations involving the loci 11q13, 4p16 , and 16q23 corresponding to (CCND1*)* in 15%, (FGFR3/MMSET) in 15%, and (MAF) in 5% respectively. some less frequent loci may be involved include 6p21 (CCND3) and 20q11 (MAFB) [225]. Scientists have demonstrated that t(4;14)(p16;q32) is associated with poor survival regardless of the treatment applied,[226]. This t(4;14) causes the simultaneous upregulation of FGFR3 (fibroblast growth factor receptor 3) and MMSET (multiple myeloma SET domain) both of which with protooncogenic properties that are brought next to the IGH enhancers. Since the poor prognosis is maintained in the absence of the FGFR3 expression while the MMSET is always overexpressed in t(14;14) which gives more importance to its role [227]. The exact mechanism by which the MMSET is subject of intensive research and

recently scientists demonstated the major role of MMSET in regulation of chromatin structure and transcription in t(4;14) +MM patients [228].

Upregulation of the MMSET gene as seen in t(4;14)+ MM seems to produces a specific histone methylation that results in an opened chromatin structure which facilitates transcription. It is also found to interact with different downstream pathways like the p53 pathway, the cell cycle regulation pathways, and integrin signaling which all require the histone methyltransferase activity of MMSET [228]. Another important role played by the MMSET which is the focus of future therapeutic manipulations is the regulation of miR-126 which is predicted to target the c-MYC mRNA inhibiting its translation and decreasing the level of c-MYC protein [229].

I. Lymphoma

The malignant lymphomas constitute a group of neoplasms of varying degrees of malignancy, derived from the basic cells of lymphoid tissue, the lymphocyte, and histiocytes in any of their developmental stages [230]. The broad histological classification of lymphoma includes the presence or the absence of Hodgkin and Reed-Sternberg (HRS) cells to distinguish between Hodgkin versus Non-Hodgkin lymphomas [231]. Lymphoma are usually diagnosed by histologic evaluation samples taken by Fine needle aspiration (FNA) procedure, then correlating the findings with the clinical context [232]. Hodgkin's Lymphoma was classified into five subtypes: Lymphocyte depleted,

Lymphocyte rich, nodular sclerosing, mixed cellularity, and nodular lymphocyte-predominant. In general it is considered curable lymphoma [233].

Cotsworld classification of HL, is a staging system other than the TNM (Tumor, node, metastasis) which is also a clinical staging system but only applicable to solid tumors not to lymphomas. This Cotsworld classification is anatomical based on the preceding classification of Ann Arbor that depended on the site and number of lymph nodes affected to risk categorize HL patients, but the Cotsworld classification added information about the presence of bulky disease (adverse risk factor corresponding to widening of the mediastinum by more than one third, or the presence of a nodal mass measuring more than 10 cm in any dimension). Such classifications are based to guide treatement and estimate prognosis [234, 235]. The WHO 2008 classification included the next categories [137]:

Nodular lymphocyte predominant Hodgkin lymphoma

Classical Hodgkin lymphoma;

- Nodular sclerosis classical Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma
- Mixed cellularity classical Hodgkin lymphoma
- Lymphocyte-depleted classical Hodgkin lymphoma

Chromosomal abnormalities detected in FNA specimens of HL patients show gains of 17q, 2p, 12q, 17p, 22q, 9p, 14q, and 16p while frequent losses in HL include 13q, 6q, 11q, and 4q [236].

Non-hodgkin's Lymphomas are also heterogeneous group of lymphoproliferative malignancies with different responses to treatment [237]. Historically different classifications were implemented for NHL. The IWF (International working formulation) introduced a classification in 1981, it was based on natural history and survival patterns [238].The IWF classification divided NHL into three grades each containing different subtypes; Low grade, Intermediate grade, and high grade lymphomas based on natural history and survival. The Revised European-American Lymphoma (REAL) was introduced in 1994. REAL was based on clinical and pathologic findings as well as immunophenotypic features of NHL subtypes [239, 240]. In 2008 The WHO updated the

REAL classification integrating cytogenetics, and molecular genetics providing better reproducibility and practicality by dividing Lymphoma to more homogeneous entities. The WHO classification with the 2008 updates categorized NHL subtypes under the B-cell Lymphomas into many categories explained in the literature, see next reference [137] and [241]. The most common chromosomal abnormalities are t(14;18) in Follicular lymphoma involving the BCL2 gene interfering with apoptosis regulation ider(3) in DLBCL involving BCL6 gene with transcription regulation function [242]; del(13q) in B-cell CLL/SLL involving BCL1 gene a cell cycle regulator; t(11;14) in Mantle cell lymphoma involving the BCL1cell cycle regulator; t(1;14) in MALT lymphoma involving BCL10 gene with apoptosis regulation activity; and t(8;14) in Burkitt's lymphoma involving c-myc gene involved in cell proliferation and growth [243].
The bcl-2 (B-cell leukemia/lymphoma 2) gene functions as protooncogene since it is an important inhibitor regulators of programmed cell death [244].

BCL2over expression correlates with adverse clinical outcome and may be caused by t(14;18)(q32;q21) in which the translocation activates the BCL2 constitutive expression in B-cells when linked to the IGH locus [245]. It may be also over expressed in BCL2 gene amplification in DLBCL, as well as mutations in the open reading frame may affect the protein interaction with other co acting proteins [246].

Mutations may come together in two or three recurrent chromosome translocations; MYC/8q24 loci, usually in combination with the t (14; 18) (q32; q21) bcl-2 gene or/and BCL6/3q27 chromosomal translocation. This is called the double hit lymphoma or the triple hit lymphoma which represent a rare type of lymphomas known to have a poor prognosis [247].

The T-cell NHL on the other hand, is uncommon representing 12% of all Lymphoma. It has different subtypes with different clinical outcomes which makes its systematic studies quiet difficult [248, 249]. The following chromosomal breaks were associated with worse prognosis: 1p, ap22, 2q, 3q, 14q, and 14q32. A gain in 1q was also of bad prognosis, while 7q was associated with favorable prognosis [249].

J. Aims and objectives

Our study aims to evaluate the status of HM's among Palestinians living in the West Bank based on our cases referred during the period 2012, 2013, and the early 2014.

Our specific aims include the following

1- To evaluate the cytogenetic features in ALL, AML, CML, and CLL patients
2- To evaluate the cytogenetic features in preleukemic (MDS) patients
3- To evaluate the cytogenetic features in MM patients
4- To evaluate the cytogenetic features in Lymphoma patients
5- To determine the nature of existing cytogenetic abnormalities in each of the malignancies.
6- Shed some light on the epidemiology of each of the malignancies.
7- Open the door for future work on HM's related to etiologies and adapted therapies among our people.

III. Materials and Methods

A. Research Design

We evaluated different cytogenetic abnormalities encountered in various patients diagnosed with leukemia (including ALL, AML, CML, and CLL), multiple myeloma, lymphoma, and myelodysplasia. We included a total of 241 samples from patients already sent for clinical diagnosis. There was no material requested specifically for research and since patient anonymity was obtained by studying archived material, we did not ask for patient consent specifically for this study (Patient consent was obtained for all cases to take the sample for the clinical diagnosis). The study fulfilled the ethics review guidelines of both Bethlehem and Birzeit Universities. . The samples after diagnosis are

normally discarded (slides are kept for quality assurance). In this case, we obtained samples from the archives of SiParadigm- Bethlehem University laboratory, and those collected and received during the study in our laboratory.

Already existing slides were G-banded from the archives of Bethlehem University cytogenetics laboratory and some more slides were made from archived cell pellets, in addition those received during the study. Our samples were bone marrow samples received in Sodium Heparin tubes. A confirmatory FISH study was also applied to some patient with AML, and CML leukemia subtypes. Patients' history and their diagnosis were available from laboratory records received with the submitted clinical samples. The out coming various chromosomal aberrations of patients were sorted and tabulated with respect for each hematologic malignancy.

B. Study population

Patients were residents of the West Bank (including Jerusalm) who were referred to the laboratory as likely cases of hematologic malignancy. Both those who started treatment or still in first diagnosis were included. Age, sex, and other epidemiologic and demographic information about patients were used when available from the medical records but patient names and other private information were kept confidential. Usually diagnosis via flow cytometry was done chronologically parallel with cytogenetic studies.

C. Cytogenetic methods

All cases are set-up for routine cytogenetic testing that have now become standardized (see for example [136]. For prospective samples, bone marrow (BM) was cultured in RPMI 1640 with fetal bovine serum, and harvested at 24 and 48 hour cultures. B-cell neoplasms including B-CLL, B-lymphomas and multiple myelomas were also set-up using stimulated 5 day cultures with lipopolysachcharide containing medium (B-cell mitogen). The rare T-cell neoplasms (T-ALL, T-cell lymphoma) received a three day

culture with phytahaemagglutinin as a mitogen. After incubation, Colcemid was added at a final concentration of 0.1 µg/ml for 30-45 minutes. Then the cells are treated with hypotonic solution (Potassium chloride 0.075 M) for 18 minutes and fixed with Carnoys fixative (methanol/acetic acid in 3:1) for three times and slides made per the usual methods. Metaphase chromosomes were banded using the conventional GTG banding technique and karyotyped with the karyotype described according to the International System for Human Cytogenetic Nomenclature (ISCN 2009), [250]. For each sample twenty metaphases are studied to define the nature of the aberrations for each of the samples.

For retrospective studies, samples studied in our clinical laboratory have been evaluated as per the above procedure for prospective studies and we reexamined them to validate findings and record data. Over 200 cases have been assayed for hematologic malignancies by routine G-banding in our laboratory. Cell pellets were kept on these cases which allowed us to reevaluate and in some cases, slides made for FISH studies.

1. G-banding (Giemsa Banding) and screening

G-Banding is a major cytogenetic technique that can detect wide range of abnormalities in hematologic malignancies. This technique plays an essential role in the diagnosis of leukemia. Bone marrow samples are cultured, and then cells are arrested in metaphase stage, where chromosomes are in the most condensed state. Then the Giemsa stain is introduced allowing the formation of banding patterns that distinguishes each chromosome from the others. Thus it becomes possible to notice most of the structural and numeric abnormalities in the cells. The analysis of cells in their metaphase stage also helps to detect inversions and aneuploidies, which are common in AML, ALL and CML [251, 252].

G-banding technique has limitations due to the over condensation and poor chromosomal morphology, or due to problems in the dividing process of cells. Therefore, FISH

technique was introduced for further and more accurate detection for the translocations and deletions and other abnormalities related to hematologic malignancies [253].

For G-banding, aged slides of metaphase chromosomes were placed for a few seconds in HBSS (Gibco), trypsinized for 60 seconds (6.25) mg/ml trypsin in disodium phosphate buffer (pH 7.0) and stained with 2% Giemsa stain. Each slide was screened for well banded metaphase spreads and the positions for at least 20 good spreads were recorded using an England finder. Well spread chromosomes were screened and observed with an x100 objective under oil and phase contrast microscope [254].

a) Reagents

1. Trypsin l0X (Sigma T-4549)

2. Sodium Chloride (Sigma S-9625)

3. 0.4%Giemsa Stain (Sigma G-3032)

4. Cover Slips 24 X 60 mm (Sigma C-9056)

5. Buffer (can use one of the following, see note on step 4 Working Solutions)

10mM potassium phosphate buffer

Add 20 mM potassium phosphate, monobasic salt (Sigma P-5379, 2.72 g KH_2PO_4 in 1L H20) to 20 mM potassium phosphate, dibasic salt (Sigma P-8281, 3.48g K_2HPO_4 in 1L H20) until the correct pH is reached

6. Mounting Medium: Fluka 03989, Eukitt quick-hardening mounting medium.

b) Solutions needed

1. Trypsin working solution
Take 0.5 mls of 10X trypsin stock solution and dissolve into 49.5 mls of a 0.9 % Sodium Chloride solution.
2. Buffer pH 6.8: stored at room temperature.

3. Buffer pH 7.0: stored at room temperature.

4. Giemsa working solution: Mix 5 mls of Giemsa stock solution with 45 mls of 6.8 Buffer solution.

c) Procedure

1. Place slide into coplin jar containing 50 mls of Trypsin working solution for 10-25 seconds.
2. Immediately rinse slide in coplin jar containing 50 mls of pH 7.0 Buffer solution for 5 seconds.
3. Place slide in coplin jar containing a 50 mls of pH 7.0 Buffer solution for 1 minute.
4. Transfer slide to a coplin jar with the Giemsa working solution for 5 minutes.
5. Air dry the slide.
6. Using Mounting Medium, cover slip the slide.

D. Fluorescence in situ hybridization (FISH)

The development of fluorescence in situ hybridization (FISH) in the 1980s [255] has resulted in revolutionary advances in clinical diagnostics. Hundreds of papers showing the importance of this technique were published in the early 1990s. Today there are panels of FISH probes used routinely in patients in Western countries see [256, 257]. FISH is used in Western Countries for the following [258, 259]:

1) To detect abnormalities that could be detected by G-banding especially for follow-up to detect remission and relapse. This is because routine G-banding is limited by presence of metaphases and their analyzability (usually we analyze 20 metaphases). Using FISH scoring while specific for the translocation or other abnormality allows for scanning of 100-500 interphase nuclei very quickly. This is used for example in follow-up to treatment by Gleevec of cases of Chronic Myeloid Leukemia with t(9;22). In AML, FISH is used for t(8;21), inv(16), trisomy 8, deletion 5, deletion 7, and deletion 20. In ALL it is used to detect translocation involving 11q23 (MLL), t(1;19), and t(9;22).

2) To detect chromosome changes that are subtle and are usually missed by routine G-banding. This include t(12;21) in B- ALL and t(5;14) in T-ALL.
3) To detect chromosome rearrangements when proliferating normal cells dominate and cancer cells do not proliferate in vitro. This is especially an issue with multiple myeloma and CLL. Testing is done by probes such as those specific for deletion 13q, t(14q), del(17p) (p53), and trisomy 12 (in CLL only).
4) To investigate cases where G-banding fails because of lack of metaphases
5) To clarify complex abnormalities seen by G-banding.

Fluorescence in situ Hybridization (FISH) involves the preparation of two main components: the DNA probe and the target DNA to which the probe will be hybridized. The DNA probe typically comes from cloned sources such as plasmids, cosmids, PACs, YACs, or BACs; where the insert may contain a specific gene or originate from a specific chromosomal locus. Whole-chromosome paints may also be used but are usually applicable to metaphase preparations. The purified DNA can then be labeled and detected indirectly using haptens, or labeled directly using fluorochrome or dye-conjugated nucleotides. Labeling strategies are also variable, employing standard nick translation or PCR labeling methods. The target DNA can take the form of chromosomes spreads or interphase nuclei. The sources of interphase targets may come from cytogenetic preparations or from paraffin-embedded tissues. Both the labeled DNA probe and DNA target are denatured to a single-stranded state and permitted to hybridize to each other. Post-hybridization washes and fluorescently-labeled antibody incubations follow the 24-hour hybridization, and the specimen is ready for visualization by fluorescent microscopy. Successful interpretation of FISH experiments is dependent on the quality of the starting materials, hybridization efficiencies, and stringency of post-hybridization washes and antibody detections [260].

Nowadays, chromosome analysis is usually performed by interphase FISH using a probe set that, according to our current knowledge, covers regions often involved in numerical or structural rearrangements [171].

The FISH analysis is used to detect abnormalities that could not be detected by G-banding especially for follow-up to detect remission and relapse. This is because routine G-banding is limited by presence of metaphases and their analyzability (usually we analyze 20 metaphases). And to detect chromosome rearrangements when proliferating normal cells dominate and cancer cells do not proliferate in vitro. This is especially an issue with multiple myeloma and CLL. Testing here is done by probes such as those specific for deletion 13q, t(14q), del(17p) (p53), and trisomy 12 (in CLL only).
For the FISH analysis, chromosomal spreads (from stored retrospective cases or from cell pellet of new cases) are subjected to drying, dehydration, then co-denaturation at 73 C for five minutes on hotplate with the probe DNA in hybridization buffer. Hybridization is done at 37 C overnight. The sealed coverslip is gently removed and the slides are washed in 70% formamide in 2X SSC at 39 C and counterstained with DAPI counterstain (the 4',6-diamidino-2-phenyl indole). Then slides are visualized by immunofluorescece microscope and nuclei are photographed and analyzed.

There are three major types of probes used in clinical FISH testing: Locus specific probes, Centromeric specific probes, and whole-chromosome paint (WCP) probes.

Locus specific probes bind to single-copy DNA sequences of a chromosome or gene. The best application for this probe is to identify structural abnormalities, including chromosome inversions, and gene deletion or amplification. This probe is also useful for detecting the BCR and ABL1 genes for the t(9;22) in CML and ALL, and the break-apart MYC probes for MYC abnormalities in high-grade B cell lymphoma.

Centromeric specific probes are suitable for observing chromosomes in interphase and metaphase cells. This type of probes gives a large and clear signal easily distinguished. This type is useful for determining extra copies of chromosomes such as trisomy 21 in Down syndrome.
Whole-chromosome paint (WCP) probes are actually multiple probes that use multiple fluorescent dyes and work together to recognize different regions in the chromosome. These probes are useful for the identification of marker chromosomes and the detection of cryptic translocations.

1. Reagents

2X-SSC

NP-40

Ethanol (100%, 85% and 70%)

Acetic acid (Glacial)

Methanol (acetone free)

KCl (0.075M)

Formamide

Probe mixture

Protease

NaCl

NaSCN

10% buffered formalin

DPBS

HCL

NaOH

2. Equipment

Incubator, 37°C

Freezer, -8~-20 °C

Pipetter and appropriate sterile tips (0.1 -10 ul, 30-200 ul)

Automatic pipette aid

Slide warmer

10 mL, 5 mL and 1 mL sterile graduated pipettes

Biohazard Disposal Containers

15 mL sterile conical centrifuge tubes

Slanted Rack for 15 mL tubes

Gauze and/or paper towels

Humidity chamber

Labels for reagent bottles

Labels for culture tubes

Laboratory permanent markers

Glass cover slips

HyBrite

Rubber cement

Phase microscope

Lab coat, Gloves and Goggles

3. Procedure

a) Slide preparation

-Spin the cell suspension for 10 minutes at 1000 rpm and aspirate the suspension before and then re-suspend pellet "should be white at this stage" in a small volume of fixative 0.5~1 mL depending on pellet size "should appear cloudy". Evaluate slides made: More fix can be added if too concentrated or spin down & re-suspend in smaller volume if too diluted. Use a new transfer pipette for each culture tube.

-Drop slides from one case at a time.

-Place a paper towel flat on the counter top make it wet by spraying water on it. Keep the towel wet throughout the dropping procedure.

-Take a clean slide from the slide box; dip it into water at room temperature.

-While holding the slide at a slight angle drop a small amount of the cell suspension on it.

The slide is placed on the humid wet towel for 50~90 seconds (depending on the room humidity).

-The back of the slide is wiped, and the slide is immediately placed on a warm plate (40~46°C) until completely dry.

-Prepare the slides per as needed.

-Label the slides as follows: case number, patient name and probe name.

b) Prehybridization

Make sure that all supplies are there within the reach of the operator.

Turn on the HYBRIT® allowing it to reach the desirable temperature (90°C)

Bake the slides at 90°C for 10min

After baking allow the slides to settle down at room temperature for 1~2 min

c) Slide pretreatment

-Soak slides 10~20 minutes in 2X SSC, pH 7.0 at 37˚C.

-Dehydrate 70%, 85%, 100%, ETOH 1.0 min each at room temp.

-Dry the slides using the slide warmer (37~42°C)

d) Hybridization

-Readjust the HYBRIT® to 75°C

- Apply the probe to its corresponding position on the slides

- Cover slip the slide using the appropriate glass cover slip

Incubate the slide(s) using the HYBRIT® at 75°C for 3 min in order to Co-denature the DNA "Specimen/Probe".

-Apply rubber cement to ensure complete enclosure of the probe within the glass cover slip area.

-Place the slide to the humidity chamber at 37°C over night (6~16 hours).

e) Post Hybridization

-Remove carefully the rubber cement, and use the following washing method:

-Place the slides in 50% Formamide (40 mL: 20 mL formamide, 4 mL 20X

-SSC & 16 mL H2O) at 45 °C for 10~15 minutes.

-Shake the slide gently every 5 min

-Wash the slides in 2XSSC at 37°C for 10~15 minutes.

-Shake the slide gently every 5 min

-Allow the slides to stand at room temperature for 2 min in order to apply the counter stain (DAPI-II)

-Cover with medium sized cover slip.

-Keep the slides in freezer till the time of scanning.

4. FISH Analysis

The FISH analysis is used to detect abnormalities that could not be detected by G-banding especially for follow-up to detect remission and relapse. This is because routine G-banding is limited by presence of metaphases and their analyzability (usually we analyze 20 metaphases). And to detect chromosome rearrangements when proliferating normal cells dominate and cancer cells do not proliferate in vitro.

We analyzed 24 samples with FISH technique using the following two probes; For AML: PML-RARA. And for CML: BCR-ABL. Then slides are visualized by immunofluorescece

microscope and nuclei are photographed and analyzed**.** We were able to minimize the resource needed for the FISH technique by applying four samples per slide in an optimized procedure that we developed during our study.

IV. Results

A. ALL results

We had 74 ALL cases, with ages from 2 months to 59 years (median age 10 years). Frequency of ALL differs over ages, it peaked on (2 to 6 years) 29.8%, (14-17 years) 12.3%, and (49-53 years) 9.5%. The disease was mostly among children with those six years or less representing 37.8% of our ALL patients. The disease showed some male preference with a male to female ratio (M:F ratio) of 1.24:1.

We had 74 cases of ALL of all ages. ALL of B cell linage (B-cell and pre-B-cell ALL's) predominated with 53 of the 74 (71.6%) cases compared to 13 (17.6%) of ALL of T cell phenotype (8 cases were not sub classified).

We did not notice subclass preference among sexes, but we noticed that T-cell subclass was less frequent among children less than 6 years compared to older patients (10% vs 20%). This means that childhood ALL is more of B cell than T cell linage.

A normal karyotype was noted in 47 cases (67.1%) and an abnormal karyotype in 23 cases (32.9%) the karyotype of 4 cases was not obtainable.

Case Number	**Age**	**Karyotype**
		B-Cell ALL
SB-12-037	6	59-62,XY,+X,+3,+4,+6,+8,+10,+11,+13,add(14)(q32),+20,+21,+2-,3mar[cp10] / 46,XY[8]
SB-12-045	4	55,XX, del(1)(q32)+6,+8,add(9) (p24), +10, +11, +18, +21,+21, +2, -3mar[cp20]
SB-12-057	2	58-59,XY, +X,+Y,+4,+5,+6,+8,+13,+14,+15,+21,+21 [10] / 46,XY[10]
SB-12-096	1	54,XY, +6,+8,+10,+18,+21,+22,+mar(3) / 46,XY[17]
SB-12-116	4	46,XY, del(5)(p12) [20]

SB-12-117	19	45,XX,-5,t(7;14)(q11;q32),add(11)(p12),der(12)?inv(12),-13,-16,-17,-20,+4mar(19) / 46,XX[1]
SB-12-119	16	46, XX, add(14)(q32)[4] / 46,XX[16]
SB-12-152	19	45,XX, del(5)(q22q33),der(7;15)(q10;p10),?9,add(14)(q32),-15,add(16)(p13.3),?18,-22,+1-2mar[15] / 46,XX [4]
SB-12-166	16	47-48,XX, add(7) (p13), +8, der(19)t(19;21)(q23;p13),+21[10] / 46,XX[10]
SB-13-018	3	50,XY, t(1:19)(q23;p13), +5,+8,+11,,der(19)t(1;19),+22[12] / 46,XY[2]
SB-13-056	14	56-58,XY, +X+4+6+8+8,t(9;22)(q34;q11.2),+10,+13,+15,+2mar,+other[cp18] /46,XY[2]
SB13-138	5	54,XY, +4+6+8+10+18+21+1-6MAR [cp16] /46, XY [2]
SB13-174	1	70-72,XY, +2,+3, del(3),+4,+6,+8,+10,+11,+12
SB13-174	1	46,XY, t(1:19)
		pre-B cell ALL
SB-12-047	2	55-57,XY, +4, +8, +10, +14, I (17) (q10), +18, +21, +4-5mar, (cp4) / 46, XY[20]
SB-12-054	4	46,XX, t(4;12)(q12;p13) [2] / 46,XX[18]
SB13-191	4	46,XY, t(1:19)(q23;p13)
SB13-137	21	49-60, XY,+3+4+6+8+10+18+1-6 mar [cp12]
SB13-269	0.2	58,XY,+X+Y+4+6+7+10+13+15+16+18+21 / 46XY[15]
		T-cell ALL
SB-12-048	17	46,XY, del(6), (q21q27),[18]
SB-12-142	8	47,XX, +19(3) / 46,XX[17]
SB13-227	4	46,XX, -7, add(9), add(21) t(7;9;21)

Table.1. Abnormal karyotypes found in different ALL subtypes in our series

Here in the next Figure 3. We show the result of a B-cell ALL patient with case nmber SB-12-037.

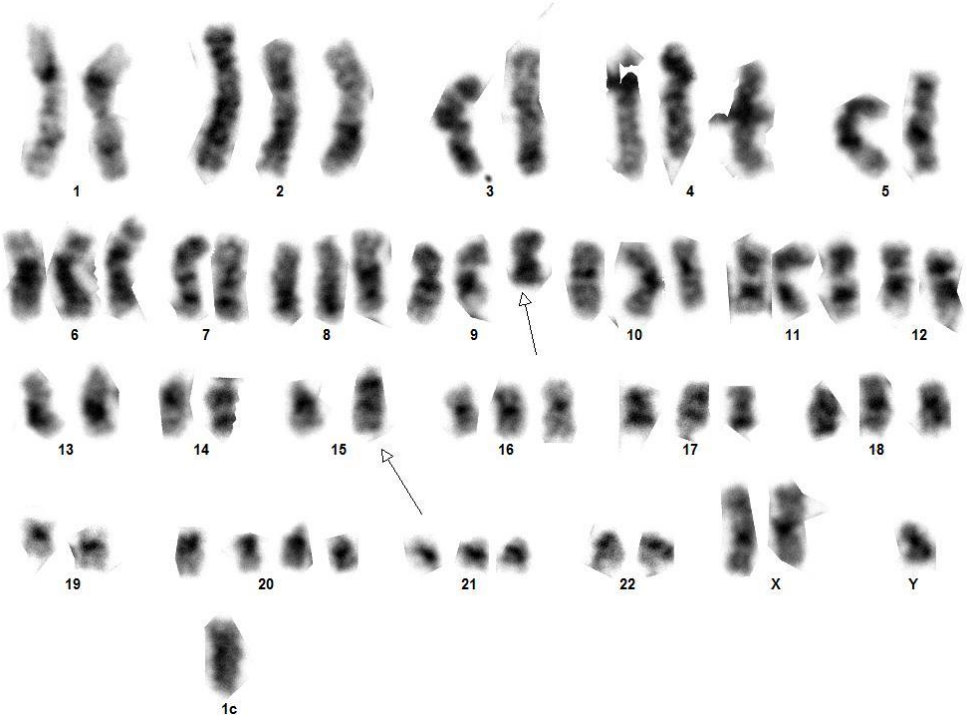


Figure 3. The karyotype of the sample SB-12-037 for a 6 year old patient with hyperdiploid B-cell ALL**.**

B. AML results

We had 35 AML cases. The median age of patients was 35.5 years, with 79.4% of cases over 18 years old. 24 out of 35 were males, presenting a M:F ratio of 2.18:1.

Comparing age-gender distribution for AML, we found that among AML patients less <18 years old , M:F ratio was 6:1 which may mean that male are much more susceptible to AML than female children but the female risk increases after puberty. We did not have the FAB classification for most of the cases since we had limited immunophenotyping at the referring hospital. A normal karyotype was found in 25 (71.4%) cases compared to 10 (28.6%) with various abnormal karyotypes (**Table 2).**

Case number	**Age**	**Karyotype**
SB-12-051	16	46,XY,t(4;9)(q34;q13),t(8;21)(q21;q21),del(11)(q13)(10)

		/ 46,XY[10]
SB-12-075	17	46,XY, t(15;17)(q22;q22) [10] / 46,XY[10]
SB-13-125	20	46,XX,inv(16)
SB-13-200	3	47,XY, +mar? der(22)
SB-12-087	21	46,XX, del(20)(q12) [10] / 46,XX[10]
SB-12-095	60	46,XY,del(20)(q12) [5] / 46,XY[15]
SB-13-103	29	46,XY,t(8:21)(q22;q22)[15] / 46, XY
SB-13-134	30	46,XY, del(2)(p13), t(15:17)(q22;q21)[15] / 46, XY,[5]
SB-13-141	46	46-49,XY,del(1)(p22),-5,?14,-19,?der(19)t(1:19),+3,-4 mar [cp19] / 46, XY[1]

Table.2. Abnormal karyotypes found in AML cases in our series

Here next we show the karyotype of AML patient with a case number SB-13-103, see figre.4.

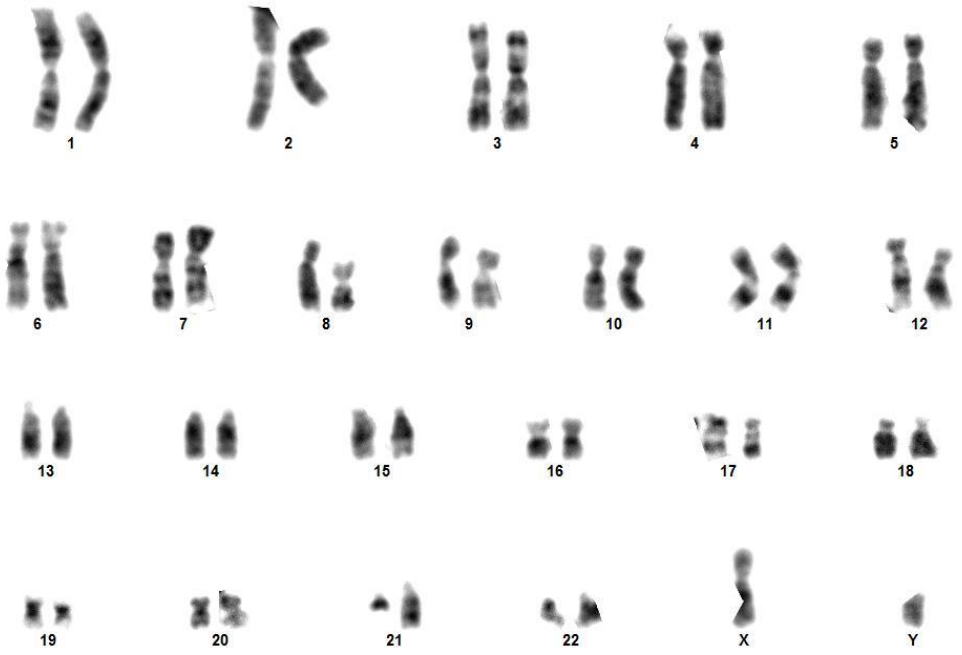


Figure.4 The karyotype of the sample SB-13-103 for a 29 year old patient with t(8;21) AML.

C. CML and FISH Results

We had 34 cases of CML. The disease occurred almost at all ages but 58.8% of the cases ranged from (18-45 years) with median age of patients 38 years. Gender distribution showed 20 females (58.8%) versus 14 males (41.2%) with M:F ratio of 0.7:1. We 19 females (63.3%) versus 11 males (36.7%) among CML patients aged >=18 years while only four patients were <18 years, 3 (75%) were males versus one female (25%). A normal karyotype was found in 20 (58.8%) cases versus 14(41.2%) with abnormal karyotypes. Abnormal karyotypes are shown here, **see table.3.**

Case Number	**Age**	**Karyotype**
SB-12-034	15	46, XY, t(9;22)(q34;q11.2) [20]
SB-12-003	26	46,XX, t(9;22)(q34;q11.2) [20]
SB-12-036	32	46,XY, t(9;22)(q34;q11.2) [20]
SB-12-046	26	46,XY, t(9,22), (q34;q11.2)
SB-12-067	28	46,XX, t(9;22)(q34;q11.2) [20]
SB-12-079	54	46,XX,t(9;22)(q34;q11.2)[17]/ 46,idem,i(17)(q10)[30]
SB-12-151	43	46,XX,t(9;22) (q34;q11.2)[20]
SB-13-042	21	46XX, t(9:22)(q34;q11.2)[1]
SB-13-072	38	46XX, t(9:22)(q34;q11.2)[9]
SB-13-146	51	46, XX, t(9:22)(q34;q11.2)[2]
SB-13-172	50	46, XX, t(9:22)/46XX[17]
SB-13-189	56	46, XY, t(9:22)
SB-12-125	20	46, XX, t(9;22)(q34;q11.2)[20]

Table.3. Abnormal karyotypes found in CML cases in our series

Here next we show the karyotype of a sample with t(9;22) obtained in or laboratory see figre.5.

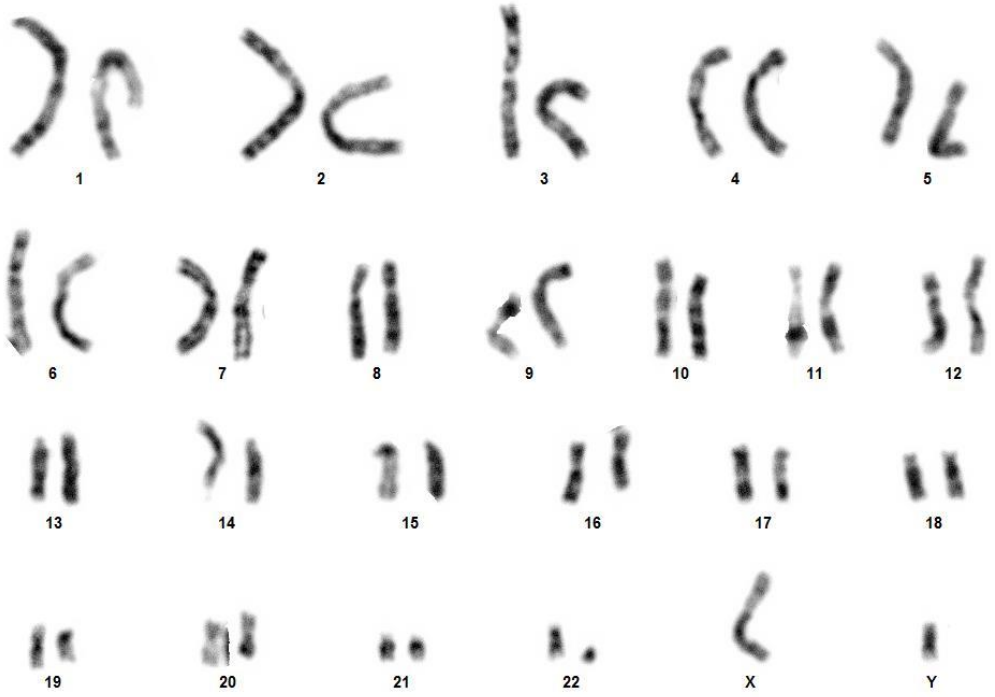


Figure.5 The karyotype of the sample SB-12-034 for a 15 year old patient with t(9;22) CML.

Results of FISH analysis applied to some of the CML and AML cases as a double check testing are shown here, see **table.4**.

Case number	CML 9;22	FISH	Case number	AML 15;17	FISH
SB12-130	46,XY	Neg	SB12-095	46,XY,	Neg
SB12-046	46,XY,(9,22)(q34;q11.2)	Pos	SB 13-149	del(20)(q12) [5]/ 46,XY [15]	Neg
SB13-172	46,XX,t(9:22)/46XX[17]	Pos	SB13-198	46,XY	Neg
SB13-47	46,XY,[20]	Neg	SB13-194	46,XY	Neg
SB13-139	46,XY,t(9:22)(q34;q11.2)	Pos			Neg

SB13-189	46,XY,t(9:22)	Pos	SB13-141	46,XY	Neg
SB13-164	46,XX[20]	Neg	SB12-149	46-49,XY,del(1)(p22), -5, ?14, -19, ? der(19) t(1:19), +3, -4 mar [cp19]	Neg
SB13-205	46,X,[20]	Neg			
SB13-146	46,XX,t(9:22)(q34;q11.2) [2]	Pos			
SB12-003	46,XX,t(9;22)(q34;q11.2)[20]	Pos	SB12-160	46,XX	Neg
SB12-34	46,XY,t(9;22)(q34;q11.2)[20]	Pos			
SB12-46	46,XY,(9,22)(q34;q11.2)	Pos			
SB13-179	46,XX,[20]	Neg			
SB12-161	46,XX	Neg			

Table.4. FISH study results for some AML and CML cases of our series

D. CLL results

We had four cases of CLL. The mean age was sixty years, ranging from fifty nine to seventy six. We had 3 male cases versus one female ; M:F (3:1) ratio. Two cases had abnormal karyotype versus two normal karyotypes (Table 4).

Case Number	**Age**	**Karyoype**
SB-13-009	59	46,XX, del(11)q23,add(12)(p13),14q,+other [cp2] /46,XX [18]
SB-13-123	52	47,XY, der(12),del(22) +mar [cp3]
SB-13-208	53	46,XY,[5]
SB-13-226	76	46,XY,[20]

Table.5. Abnormal karyotypes found in CLL cases in our series

E. Myelodysplasia

We had 3 patients suspected or referred to rule out Myelodysplasia. The first was male of one year age, the second was MDS/CLL, was also male of 74 years, both with normal male karyotype. The third AML/MDS was a male of 52 years, with the following karyotype: 47,XY,+21(2)/46,XY[18] abnormal male karyotype.

F. Multiple Myeloma and Lymphoma

We had 33 cases of MM. Patients ages ranged from 35 to 77 years with median age 59 years. Gender distribution showed 10 females (30.3%) versus 23 males (69.7%) with M:F ratio of 2.3:1. All the patients presented a normal karyotype except one male patient aged 46 years who had the following karyotype: 45-46,XY,der(9)t(9:?),inv(19)(p13q13)[cp8].

We had 12 cases of Lymphoma. Ages ranged from (4 to 59 years) with median age around 31 years. Two thirds of our patients aged >=18 years. M:F ratio was 2.66:1. All the patients presented a normal karyotype.

V. Discussion

This study revealed that acute leukemias 74.2% are more prevalent than chronic leukemias 25.8%. This result is similar to published results in some countries like in Pakistan [261]. In our study, we found that males contributed to 55.8% of leukemia patients, while females contributed to 44.2% of the patients giving a ratio of 1.26:1. Actually, studies show and confirm that leukemia prevalence in males is higher than females. This is already noticed based on huge epidemiologic studies that confirmed the male preponderance of leukemia and many other types of hematologic malignancies [262, 263]. The possible explanation of this maybe that males are more exposed to occupational and environmental carcinogenic agents [264]. Another explanation may be more fit that speculates the existing sex based difference in immune system regulation may contribute to such a difference [265].

In general the leading hematologic malignancies were ALL, AML, and MM as shown in Figure.6.

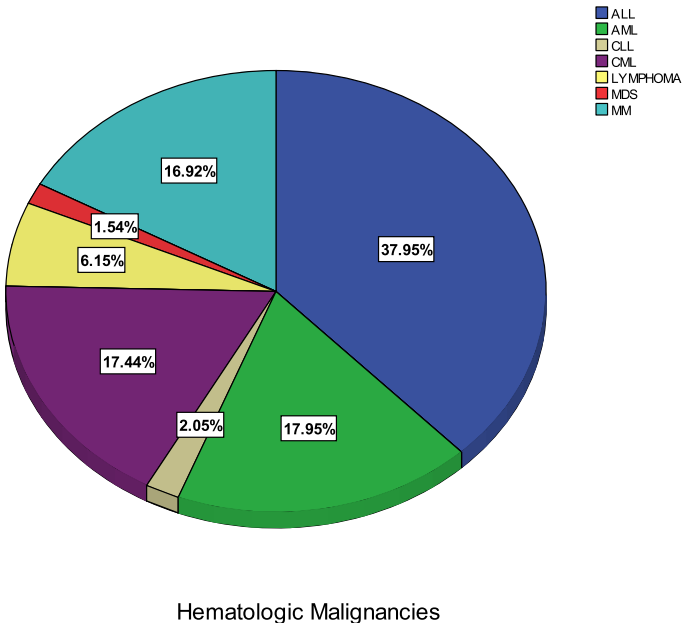


Figure .6. Distribution of different HM's among patients in our series

A. ALL

This study revealed that 71.6% of ALL cases are of B-Cell linage while the estimated contribution of B cell linage in ALL is around 80% in other studies [266].

Most of our ALL patients 47 (67.1%) were 18 years old and less which reflects the early childhood and young age preponderance of ALL. We also noted ALL age distribution was tri-modal with three peaks of incidence; one from (2 to 6) which was remarkable, a second from (14-17), and a third from (49-53). A bimodal incidence distribution was suggested in literature as in the USA population based study conducted by Dores et al, who found a first peak of incidence among infants <1 year followed by a decrease in childhood and then an exponential rise beginning in young adulthood and advancing with age with a slight peak around 60 years [267]. This heterogeneity in age of incidence may reflect the etiological heterogeneity among our patients who present with various types of abnormalities with different outcomes.

Chromosomal abnormalities are usually found in 60-70% of ALL cases [266, 268]. However, in our study the majority of ALL cases 67.1% demonstrated a normal karyotype, and only 32.9% of the cases demonstrated an abnormal karyotype, either numeric abnormalities 16.2% or structural changes 4.1% such as translocations, inversions, or deletions.

Type of numerical change	**Number of patients**	**Age range**	**Chromosome Gains or losses (their frequency)**	**Translocations**
I. Hyopodiploid (<46), and Pseudodiploid	9	1-19	+1, -5, del5q, add11p, der(12), inv(12), -13, -16,-17, -20, -15, add14q, add16p, der19, -20,-22, -7.	t(7:14), t(4:12), t(1:19),
Hyperdiploid	4	3-16	Add7p,+8,+11,der19,+21,	t(19:21),

47-50			+22, +1,+3,+4,+6,+10,+18,+19	t(1:19)
Hyperdiploid 51-60	4	0.2-21	+X,+Y,+7, +2,+3,+4,+6,+8,+10,+11 +13, add14q, Add9p,del(1),+5,+14,+15 , +1, +20,+21,+18,	t(9:22)
Hyperdiploid (>60)	1	3	+2, del3, +3,+4, +6, +8, +10, +11, +12,	

Table 6. Karyotypic abnormalities distribution over different ploidy groups of ALL cases in our series.

For the 63.5% of the cases that appeared with normal karyotype, this high percentage of normal karyotypes can be explained by different factors. We were unable to exclude cases with prior treatment, this would have decreased the abnormal karyotypes due to patients in induced remission. Also cryptic aberrations seem to give an important contribution to pathogenesis of our cases. Usvasalo et al. (2009) used comparative genomic hybridization CGH to detect copy number variations among adolescent ALL patients with normal karyotypes detecting aberrations in 85% of cases [269]. Thus ALL cases of normal karyotype by routine cytogenetics may underestimate clonal aberrations. This interpretation should encourage recruiting further molecular techniques in the process of diagnosis and risk stratification.

The use of sophisticated techniques for diagnosis and risk stratification has shown to have important advantages. For example, Yeoh et al (2002) used microarray technique for expression profiling for pediatric ALL patients, and produced enough evidence that their work provided and enhanced an accurate risk stratification of the ALL patients. For ease of analysis, we grouped the abnormal karyotypes into five categories: hypodiploid (<46), pseudodiploid, hyperdiploid (47-50), hyperdiploid (51-60), hyperdiploid (>60).

1. The hypodiploid (<46) & pseudodiploid categories

Hypodiploidy is already known to be associated with poorer outcome and the karyotypic feature of hypodiploidy is considered unfavorable in ALL conferring high risk of treatment failure [121, 270]. Our results showed two cases with a hypodiploid karyotype that has complex feature with translocation and numerical aberrations. Deletions and whole chromosome losses commonly seen were (-20, -22, -17,-16, -15, -13, -5, del5). Additions and whole chromosome gains commonly seen were (+1, add11, add14, add16). Inversions included inv12. t(7:14) and a der(7:15) were also found as well as various numbers of marker chromosomes.

For the chromosomal losses, deletion of chromosome 20 has a favorable outcome when it is the sole abnormality in all diseases, while it confers a bad prognosis in a complex cytogenetic context which was the female case of 19 years old with complex karyotype thar contained also t(7;14)(q11;q32) for which we didn't figure out the significance in this context [271]. The other case of the 19 years female harbored another complex karyotype with del(5)(q22q33), -15, -22, and others. Del5q is usually found in MDS and represents a distinct subtype in MDS with indolent course that rarely develops to acute leukemia. This del5q is also rarely found in de novo acute B-cell leukemia but in the context of MDS when the del5q is not the sole abnormality it will be less likely to achieve complete response and the potential to transform into acute leukemia is raised [272, 273].

We had seven cases with pseudiploid karyotype, three of them represented structural translocations included two male cases with t(1:19)(q23;p13) aged 1 year and another with 4 years, and a 4 years female with t(4:12). T(1;19) involves the E2A gene mapping on 19p13.3-p13.2, with the PBX1 (also named PRL gene 'pre-B cell leukemia gene') on 19p13.3 to form E2A-PRL fusion encoding for a chimeric protein which is a consistent feature of this translocation [274, 275]. This translocation in the context of pseudodiploidy confers a poor outcome in pre-B cell ALL as expected in our 4 years old male [276]. However, this translocation is associated with a favorable prognosis when it is found in hyperdiploid ALL [277]. For the female with t(4:12) it is a rare translocation which was reported by Harada et al [278] who found it to be associated with

characteristic unique B lymphoid immunophenotype. It was found that adults who had this characteristic translocation had difficulty to induce complete remission with conventional therapy while children presented much better response. It is noteworthy that some of the rare reported cases with this translocation presented with history of exposure to mutagenic agents [278] which raises alarms on the possible mutagenic exposures among our people. Deletions and chromosomes losses included -7, del6q, and del5p. Deletions of the long arm of chromosome 6 in childhood ALL is not associated with high risk in pediatric ALL, and patients are found to be more likely to have T-cell phenotype with event free survival around 6 years under conventional treatment [279]. Monosomy 7 and del7q are cytogenetic features found in AML and MDS [280].

In general, for the pseudodiploid group children are expected to have a good outcome despite the poor prognostic impact of the cytogenetic features. This became possible by the development of appropriately intensified regimens with multidrugs that nullify the effect of the poor impact of the aberrations with the exception for t(9;22) or t(4;1 l) [281]. The role of identification of features of either good or bad prognosis is to tailor the intensity of treatment to get the best cure rates with the least toxicity [282].

2. For the ploidy group (47-50)

We found common numerical abnormalities including (+21, +18, +8, +10) and others. Similar findings were encountered in other studies, for example see the study by Raimondi et al [283]. The study showed how the presence or absence of chromosomal translocations or the gains of specific chromosomes did not show significant difference on event free survival when the patients were treated with the multiagent chemotherapy [283].

3. For hyperdiploidy groups of more than fifty chromosomes (>=51)

Representing 25-30% of all ALL cases, Both groups (51-60) and (>60) are considered to be of a favorable prognosis, whereas the presence of certain translocations; Ph chromosome, and t(4:11) predicts a bad outcome [284]. Among our cases we had some favorable chromosomal gains according to the literatures [117, 285], including non random gain of chromosomes X, 6, 14, 17, 18, 21, 4 and 10. The latter gains of

chromosomes 4, and 10 when present together in progenitor-B ALL are easily cured with antimetabolites chemotherapy, this finding was also replicated in nearby Egyptian population [286, 287]. We found a case with trisomy 5, which is a rare feature usually associated with bad prognosis when it represents the sole abnormality, the condition that was fortunately not satisfied for the karyotype of our patient [288].

B. AML

AML is considered as the most frequent type of leukemia, it accounts for approximately 25% of all leukemias in adults in the Western world [289]. In this study we found that AML contributes to 23.8% of our leukemia cases. We also share similar results with worldwide populations in terms of having the highest prevalence of AML in adults' age group compared to a low prevalence in children and infants.

But to compare the median age at diagnosis which was 35.5 years to the median in the US which is 69 years [290]. We find a wide difference which raises questions about the exposure to mutagenic agents earlier in life or more frequently among our population to accumulate enough genetic background precipitating in AML production. This is why this result may warn us to investigate for the possibly increased risk among our population for AML which is known to be mainly enhanced by exposures to mutagens like ionizing radiations from different sources, occupational exposures to chemicals, smoking, diets, and infections [291]. In our study we observed a male preponderance in AML patients which is also in accordance with the previous studies [292].

The majority of our cases showed a normal karyotype, and around one third of the cases were found to have abnormalities in their chromosomes. We found cases with favorable cytogenetic prognosis including cases with t(8;21)(q22;q22), t(15;17)(q22;q12), and inv(16)(p13q22). Other abnormalities we found included del(11q) which has been designated as MDS related cytogenetic abnormality in the WHO 2008 classification, [2, 153].

This study found Two pre-pubertal and one adult male cases with t(8:21)(q22;q22) abnormality. This abnormality is associated with good prognosis and good response to chemotherapy, and usually coincides to be FAB subtype 2. One of these three cases had also other translocations such as t(4;9)(q34;q13) and del(11)(q13)(10) combined with

t(8:21)(q22;q22). In a study conducted by Nishii et al they compared t(8;21) AML as sole abnormality to t(8;21) AML with other abnormalities harboring del(9)(q13q32), and trsiomy 4 as well as other abnormalities they found no difference in clinical outcome for the additional abnormalities. The exception was for the trisomy 4 which had a distinctive poor outcome with different clinical features [293].

Two Cases were found with t(15;17)(q22;q22) abnormality, one of them belongs to the pre-pubertal age group and the other is adult and has also del(2)(p13) combined with t(15;17)(q22;q22). As we explained earlier, this translocation usually coincides to be FAB subtype 3, and associated with favorable prognosis with ATRA treatment in addition to chemotherapy.

One female patient belongs to the children age group was found with inv(16) (p13.1q22). This abnormality is also associated with good prognosis and good response to chemotherapy, and usually corresponds to M4eo by the FAB classification. Patients with this abnormality usually have abnormal eosinophil component in their bone marrow [294]. Del(20q) was also found a the sole abnormality in two cases; male and female patients. This abnormality is found in myeloid malignancies including MDS, Myeloproliferative disorders, and AML. It is thought to be of favorable prognosis in MDS when it is the sole abnormality, [295, 296]. We had only one case of a 46 years male with complex karyotype harboring eight abnormalities. This karyotypic feature is known to be associated with bad prognosis and poor response to chemotherapy but with the introduction of stem cell transplantation four years survival became possible in 61% of AML patients with complex karyotype [297].

C. CML with FISH results

CML patients in this study represent 22.4% of all leukemia patients. This result is close to the results in other studies occupying 20-25% of all leukemia [289, 298].

Philadelphia chromosome presence is the hallmark for the cytogenetic diagnosis of CML, representing most likely a unique condition in HM that one translocation has shown to be necessary and enough to produce the transformed phenotype of CML cells [299].

For the Male to female ratio it was 0.7 which is much less than what we find in the literature in the western and Asiatic populations >1 [300, 301]. This result may be happening because of the small number of cases.

In our study the median age at diagnosis was younger 38 years contrasting with median age at diagnosis in western countries which is 65 years[300]. Our result is similar to that found in India which has median age for CML diagnosis from 38-40 years [302]. This seems reflect increased risk to cancer and mutagenic exposures in developing countries population who develop the disease at earlier ages similarly to our findings in AML.

Ph positivity is usually found in around 90% of the CML patients [2]. However, in this study only 42.4% of the patients were Ph positive, this might be explained by the fact that some patients were received after being exposed to chemotherapy in the hospital or misidentified by the initial screen for CML. Cytogenetic analysis is essential in CML, but In case of CML phenotype negative for the Ph chromosome further molecular testing is required using fluorescence in situ hybridization (FISH), and RT-PCR to detect the fusion, [2].

For the FISH confirmatory testing we found no difference in results between FISH and the karyotyping results in the tested CML and AML samples for t(9;22) and t(15;17) respectively. This may be interpreted in part by the mall sample number and the fact that these translocation were evident by karyotyping which produced similar sensitivity as FISH here. The disadvantage that FISH cannot cover all the karyotypic abnormalities, which is remarkable for the AML sample with del1, makes us say that FISH though its high sensitivity should always be used as adjunct to karyotyping which remains the reference for the detection of chromosomal abnormalities unless chromosomal painting is applied[303].

D. CLL

Four cases of CLL were found in this study representing 2.7% of all leukemias. This result is not unexpected, since similar results in Asiatic countries revealed similar percentage of around 2.5%, unlike western countries where CLL contributes to 20% of all leukemias [304]. This strike difference may be due to increased survival with better diagnosis and access to treatment in European countries as well as possibly some underlying biological mechanisms, related to still unknown factors associated with CLL pathogenesis. The mean age at diagnosis is 60 years with 2:1 M:F ratio.

The strike difference in gender distribution is not well understood, but when analysing the gender based distribution of FISH molecular defects in CLL, by Eduardo and his colleagues, molecular abnormalities mainly 11q (ATM ene loss) as well as some other abnormalities were also skewed in the same direction with nearly (2.5:1) M:F ratio, which made them hypothesize that sex chromosomes may provide the genetic basis for the male preponderance of CLL [305].

One female elderly case was detected with del(11)q23 abnormality, which is the second most common abnormality in CLL and associated with good prognosis. The detection of this deletion becomes easier as the disease develops more. Another male elderly was detected with der(12) abnormality combined with del(22). These complex abnormalities are thought to affect the prognosis of this case negatively and results in worse response to chemotherapy [306].

E. MDS

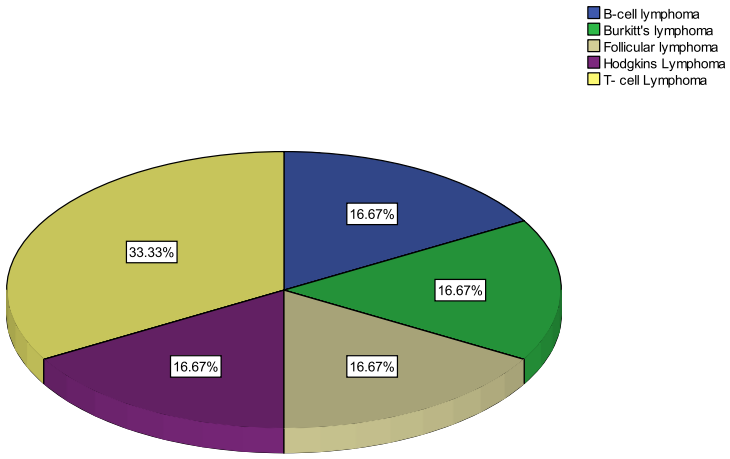
We had only three cases of myelodysplasia, two of them were of tentative diagnosis. They were all males, and had ages 1, 74, and 52 years. Two of them had a normal male karyotype, and the third case of 52 years had an extra chromosome (+21) in the abnormal blood lineage. This abnormality (+21) is considered the second most common

abnormality in AML, and MDS, and it is considered of intermediate prognostic impact in MDS patients, [200]. The male preponderance as well as the old age are noticed. These characteristics are similar to other findings in similar studies, see for eg [307], who found the median age to be (76 years), and the gender difference in incidence where males had higher incidence than females (4.5 vs 2.7 per 100,000 per year).

F. MM and Lymphoma

MM ranked third among hematologic malignancies, with 16.9% contribution. Similar results were seen in a recent study by Smith and yong [308], they found that MM contributed to 10-15% of the HM in the US. In HEMACARE project,[7] recent data from 44 European countries showed that MM incidence risk among males was 5.44 compared to 5.06 per 100,000. The USA M:F ratio corresponds to 1.4:1 ratio. Our study showed significantly different result with 2.3:1 ratio. Only one case was noted to have an abnormal karyotype among our MM patients. Abnormal karyotypes were found in 30-50% of US cases [309].

Lymphoma cases represented (6.15%) of all HM. The case reports didn't provide us enough information to analyze lymphoma types, we had the subtypes for only six samples as explained in the figure.



Phenotypes available for Lymphoma patients

Figure 4. Distribtion of Lymphoma according to phenotype in our series

The ages for the cases of pooled types of lymphoma ranged from 4 to 59 years, two thirds aged eighteen or over. Male to female ratio was 2.66. All the patients presented a normal karyotype which implies that those cases likely did not have bone marrow involvement or had undetectable BM involvement.

VI. Conclusions

ALL was predominated by B cell subtype, with remarkable three age peaks in early childhood, around the pubertal age, and elderly. Cytogenetically Abnormal ALL karyotypes were less than in other studies. The numerical abnormalities were the most frequent. We had important number of patients with hypo and pseudodiploid ALL karyotypes compared to other favorable ploidy categories. AML similar to data from other populations was the most important HM in adults, less frequent in children and showed a male predominance. One third of the cases showed abnormal karyotypes. Frequent favorable karyotypic abnormalities were found. CML was present in around one fifth of the leukemia cases, because of the fact that some of our participating patients were undergoing chemotherapy the abnormal karyotypes were much less than in other studies. CLL had a low incidence risk similar to other Asiatic countries and unlike European results where high incidence risk is reported. This may be due to lack of reporting and poor diagnosis in developing countries, increased risk in European countries for still unidentified causes as well as their increased life expectancy while the disease occurs at old ages. MDS was also rare with male and old age preference. MM was the third most common HM, our group of patients had an important male preference to catch the disease compared to other studies. Apparently normal karyotypes were the most common with rare abnormal one. Lack of further in depth data about subtypes of Lymphoma precluded adequate analysis. All the cases were karyotypically normal with male and adult age preference.

Our work pioneered the cytogenetic investigations of HM's in the West Bank and is clearly deficient in many ways. We think to avoid the pitfalls in our study more strict selection criteria should be adopted in other studies which should better be applied on de novo diagnosed cases as well as complete immunophenotype, detailed clinical data, results of genetic testing of important common mutations for each HM, survival data, and treatment regimens should all be combined in future studies.

Specific recommendations:

For ALL, AML, CML, lymphoma, MM, and MDS we found low percentages for abnormal karyotypes. This is due to the selection criteria and limited techniques used in this study. Many small genetic lesions could be detected by applying more advanced techniques including FISH analysis, and PCR. We recommend screening for the ALL patients at least for the most important genetic determinants of outcome in ALL patients as for eg t(9;22)(q34;q11)/ BCR-ABL1, t(4;11)(q21;q23)/ MLL-AFF1 and near-haploidy/low hypodiploidy for high risk stratification and, to a lesser extent, t(12;21)(p13;q22)/ ETV6-RUNX1 and high hyperdiploidy for good risk management and so on [312].

Further studies could be done for immunophenotype of AML in specific, in order to reach a full picture and accurate classification for AML patients to help better establish clinically important genotype phenotype correlation studies. More advanced techniques including FISH and PCR could also be introduced to detect cytogenetic abnormalities that our study couldn’t detect.

ALL and AML cases revealed a decreased age of diagnosis; this might be explained by the persistent and increased exposure to carcinogenic factors during the early life of the patients, such as the exposure to smoking and environmental pollution, in addition to the decreased lifespan of people in Palestine which may in part interpret the relatively low age at diagnosis. However, the exact underlying reasons for the decreased age of diagnosis are still not clear; we recommend further studies to be done in Palestine and better analysis for clinical data in order to help promote the health status of our people.

We recommend future studies that focus on mapping of new genetic lesions that we couldn't detect in order to identify their prognostic value, to reach better management, diagnosis, support, and treatment for HM cases in Palestine.

We recommend also a better cooperation with physicians in specialized cancer hospitals for conducting prospective studies with follow up after treatment to evaluate event free survival after different treatment regimens for various genetic risk categories of HM's under studies in order to optimize treatment to best fit the biology of the disease and to come as possible closer to personalized treatment of patients.

Finally we recommend authorities to start establishing a cancer registry that collects detailed data on all types of cancers in Palestine, invests and implements research recruiting the efforts of many people, including physicians, researchers, epidemiologists, public health planners, legislators, medical students, and others.

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