INTRODUCTION

β-Chronic lymphocytic leukemia (β-CLL) is the most common leukemia in adults. Though modern treatment are highly effective in most CLL, a challenging subgroup of patients shows poor response to standard regimens and a survival of less than two years ***(Rossi et al., 2011).***

Identifying chemorefractory patient early, ideally before treatment and designing therapeutic strategies tailored to overcome chemorefractory remain the issues toward an optimized management ***(Hallek et al., 2008).***

Early studies of the molecular genetics of CLL revealed that deletions of cytobands 17p13 and 11q22-q23 are major determinants of chemorefractoriness in this leukemia ***(Stilgenbauer and Zenz, 2010).***

Deletions of 11q23-q23 almost invariably include the ATM (Ataxia Teleangiectasia) mutated gene tumor ***(Stilgenbauer and Zenz, 2010).***

ATM is a large gene that consists of 66 exons spanning 146 kb of genomic DNA, and encodes a 370 kD nuclear phosphoprotein sharing homology with phosphatidylinositol 3-kinase (PI-3-K). Similar to other PI-3-K related proteins, ATM functions in controlling the integrity of DNA repair and recombination and regulates cell cycle progression ***(Negrini et al., 2010).***

ATM and TP53, cancer genes recurrently affected by mutations in high-risk CLL now also include NOTCH1 and SF3B1. Mutations of all these genes predict poor prognosis in consecutive CLL series, mainly because of refractoriness to standard treatment ***(Fabbri et al., 2011).***

In the case of ATM disruption, ATM mutant cells exhibit an impaired DNA double strand break repair. Inhibition of poly (ADP-ribose) polymerase (PARP) imposes the requirement for DNA double strand break repair, and selectively sensitizes ATM-deficient tumor cells of killing. On these grounds, PARP inhibitors have been proposed as appropriate agents for treating refractory ATM mutant lymphoid malignancies ***(Weston et al., 2010).***

Biological Characteristics of the Leukemia cells, such as the mutational status of immunoglobulin heavy chain variable gene. Chromosome aberration ***(Kor et al., 2002).*** CD38 and ZAP-70 expression ***(Rassenti et al., 2004)*** and P53 dysfunction bear an important prognostic value and have allowed patient to be stratify intorisk categories. These parameters are in fact important independent predictors of disease progression and survival.

The ATM protein is a pleiotropic molecules that protect the integrity of genome by regulating the cell-cycle arrest at G1/S and G2/M to prevent processing of damaged DNA,and activating DNA-repair pathways and inducing apoptosis if the DNA damage cannot be repaired ***(Quillette et al., 2010).***

Aim of the Work

The aim of this study is to investigate the role of some genetic alteration in B-CLL patients and their value in predicting disease progression.

CHRONIC LYMPHOCYTIC LEUKEMIA

I- Definition:

Chronic lymphocytic leukemia (CLL) is a clonal lymphoproliferative disorder characterized by progressive accumulation of mature lymphocytes in the blood, bone marrow, lymph node, and spleen. B-CLL cells express CD19, CD5 and CD23 with absence or low expression of surface CD22, CD79b and FMC7 ***(Hodgson et al., 2011).***

II- Epidemiology:

A) Incidence:

CLL is the most common adult leukemia in western countries that accounts for 25% to 30% of all adult leukemias, it is more frequent in males with a median age of 65 years and increases with age. It is 2.8 times higher for older men than older women and less than 10% of cases are seen in persons 40 years old or younger. For unknown reasons, the incidence of CLL is extremely low in Asian countries such as China and Japan, where it is estimated to occur at a frequency that is only 10% that of countries of western world. The incidence of CLL in Africa is not solow as in Asia ***(Eichhorst et al., 2011).***

B) Course:

CLL have different forms, some people have disease that is slow growing and with minimal changes in their blood cell

counts (an increase in the number of blood lymphocytes and little or no decrease in the number of red blood cells, normal neutrophil and platelet counts) may have stable disease for years. Other people with CLL have a faster-growing form of the disease (the CLL cells accumulate in the bone marrow and blood, and there is decrease in the numbers of red blood cells and platelets) ***(Louis, 2014).***

People with faster-growing CLL may have:

- Enlarged lymph nodes lead to compression of neighboring organs. For example, enlarged lymph nodes in the abdomen can interfere with the functions of the gastrointestinal tract
- A severe immunoglobulin deficiency, sometimes with a low neutrophil count, which can lead to recurrent infections.
- An enlarged spleen which can press on the stomach causing early fullness (satiety) while eating food and discomfort in the left upper part of the abdomen ***(Louis, 2014).***

Early CLL cannot treat it and late CLL can treat with chemotherapy and monoclonal antibodies. DNA analysis has distinguished two major types of CLL, with different survival times. CLL that is positive for the marker ZAP-70 has an average survival of 8 years, but CLL negative for ZAP-70 has an average survival of more than 25 years ***(Byrd, 2014).***

c) Predisposing factors:

1. Infections:

Antibodies specific for type C hepatitis virus (HCV) and/or viral DNA have been identified in some patients, suggesting a pathogenic role. Patients of CLL having higher incidence of infection just prior the diagnosis, suggesting role of infection in the etiology of this disease ***(Landgren et al., 2007).***

2. Environmental:

Environmental factors not take a role in the pathogenesis of CLL ***(Caligaris-Cappio, 2003).***

3. Occupational factors:

A higher incidence of CLL is seen in some people workes in the rubber industry, the chemicals that are linked to the development of CLL include carbon tetrachloride, carbon disulfide and acetone, the duration and level of exposure to these chemicals correlate with the risk of developing leukemia ***(Rai and Gupta, 2003).*** Specific agricultural exposures linked with elevated risk of CLL include Dichlorodiphenyltrichloroethane (DDT) and working in flour mills ***(Zheng et al., 2002).***

4. Hereditary and genetic Factors:

Family history of CLL is a strong risk factor. The genetic factors that contribute to the increased incidence of CLL in certain families are unknown but there is no linkage between

human leukocyte antigen (HLA) haplotype and disease susceptibility ***(Chiorazzi, 2012).***

d) Pathogenesis of chronic lymphocytic leukemia:

1. Resistance to apoptosis:

The basic defect in CLL is cellular accumulation rather than proliferation and relative resistance to physiological cell death (apoptosis) ***(Danilov et al., 2006).***

It has become clear that a defect in apoptosis, with the majority of being long-lived non cycling and with a small fraction of replicating cells in the lymph node and marrow is responsible for disease progression. Drug resistance can lead to defects in the apoptosis ***(Johnston et al., 2009).***

Among the most critical intracellular factors controlling the apoptotic response are the members of BCL-2 protein family (Table 1). All BCL-2 family members share conserved amino acid sequences and dimerize with themselves and with others ***(Schimmer et al., 2003).***

Table (1): Proteins of the BCL-2 family.

Inhibit apoptosis	Promote apoptosis
BCL-2	BAX
BCL-XL	BCL-XS
MCL-1	BAK
A-l	BIK
BhrF-l(Epstien Barr virus)	BAD
P53(Baculovirus)	
Ced-q(Nematode)	

(Schimmer et al., 2003)

The neoplastic B-cells of patients with CLL also characteristically express high levels of anti- apoptotic proteins, such as BCL-XL, MCL-1and high level of BCL-2. Indeed, BCL-2 over expression, which occur in 85% of cases, is not a consequence of any known chromosomal translocation, as is the case with the t(14;18) characteristic of follicular lymphoma ***(Chiorazzi, 2012)***.

In CLL possible mechanisms include hypomethylation of BCL-2 promotor region or product of basic fibroblast growth factor (bFGF) by the CLL cells themselves, which in turn induces BCL-2 expression. BCL-2 is a negative regulator of apoptosis although it is likely that the BCL-2: bax ratio is more critical in setting the precise threshold for physiological cell

death. In CLL, a high ratio correlates with progressive disease and resistance to treatment ***(Hallek et al., 2008).***

Other proteins play a key role in apoptosis and their expression associated by the chromosomal changes that occur in CLL such as the tumor suppressor gene p53 located on chromosome l7 which is a transcriptional activator and increases following DNA damage. The deletion or mutation of this gene in previously untreated CLL. The frequency of abnormal p53 function increases to nearly 50% of patient ***(Grever et al., 2007).*** P53 abnormalities have a strong prognostic impact and predict treatment failure with alkylation agent. P53 abnormalities are associated with high lymphocytic count and low survival ***(Del principle et al., 2004).***

2. Cytokines:

Such as tumor necrosis factors, Interleukin-4 (IL-4) and Transforming growth factor-β (TGF-β). They have a role in the pathogenesis of CLL, thus growth factors could be produced by CLL cells and have an autocrine function by stimulating the leukemic cells to proliferate, or by preventing them from undergoing spontaneous apoptosis. And they have a paracrine function and suppress the growth of normal lymphoid and marrow cells leading to immunesupression and myelosupression, that are typically seen in CLL ***(Hodgson et al., 2011).***

Classification of CLL:

A. WHO classification:

Table (2): WHO classification of mature B- cell neoplasms

2008 WHO classification	2016 revision	Comments
Chronic lymphocytic leukemia/ Small lymphocytic lymphoma	Chronic lymphocytic leukemia/ Small lymphocytic lymphoma	- Defined as >5 × $10^{[9]}$/L PB CLL cells - *TP53, NOTCH1, SF3B1,* and *BIRC3* mutations of potential clinical relevance
Monoclonal B-cell lymphocytosis	Monoclonal B-cell lymphocytosis "low count" and "high count"	- Low count MBL <0.5 × 10^{9}/L PB CLL cells - High count MBL >0.5 but <5 × $10^{[9]}$/L PB CLL cells
B-cell prolymphocytic leukemia	B-cell prolymphocytic leukemia	- No major changes
Splenic marginal zone lymphoma	Splenic marginal zone lymphoma	- No major changes
Hairy cell leukemia	Hairy cell leukemia	- Disease-defining mutation, *BRAF* V600E
Splenic diffuse red pulp small B-cell lymphoma	*Splenic diffuse red pulp small B-cell lymphoma*	- No major changes - Remains a provisional entity
Hairy cell leukemia-variant	*Hairy cell leukemia-variant*	- *MAP2K1* mutations in 50% the cases
Lymphoplasmacytic lymphoma/ Waldenström macroglobulinemia	Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia	- Disease-defining mutation, *MYD88* L265P - This mutation is not specific for LPL - ~50% of MGUS IgM carry this mutation
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	- No major changes
Nodal marginal zone lymphoma *-Pediatric marginal zone lymphoma*	Nodal marginal zone lymphoma *-Pediatric marginal zone lymphoma*	- No major changes
Follicular lymphoma (FL) *-in situ* follicular lymphoma	Follicular lymphoma (FL) - *in situ* follicular neoplasia - Duodenal-type FL - Predominantly diffuse FL with 1p36 deletion	- Molecular landscape better understood, *CREBBP*, *EZH2* and *KMT2D* (*MLL2*) possible early driver mutations. - Change in nomenclature. - Considered a precursor lesion with low risk of progression. - Localized variant with low risk of dissemination - New recognized variant, often localized inguinal mass, lacks *BCL2* rearrangement
- Pediatric FL (variant of FL)	Pediatric-type FL	- Change in nomenclature. - Usually occurs in children and young adults, rarely in older individuals. - Excellent prognosis even with conservative approach. - Recognized as a definite disease entity - Frequent *TNFRSF14* and *MAP2K1* mutations
	Large B-cell lymphoma with IRF4 rearrangement	- New provisional entity to distinguish from PTFL and DLBCL - Usually occurs in children and young adults - Involves mainly Waldeyer ring and cervical lymph nodes
Mantle cell lymphoma - classical MCL - *in situ* mantle cell lymphoma	Mantle cell lymphoma - Classical MCL - Leukemic non-nodal MCL - Cyclin D1- - *In situ* mantle cell neoplasia	- Two MCL subtypes are recognized - Mostly unmutated IGHV, mostly $SOX11^{+}$ - Mutated IGHV, $SOX11^{-}$, PB, BM, and spleen - *TP53* may occur and result in aggressive disease - ~ 50% *CCND2* rearrangements - Change in nomenclature

(WHO, 2016)

B. FAB classification:

The French-American-British classification system divides patients into 3 groups according to the percentage of abnormal cells ***(O'Brien and Keting, 2005).***

1- **Classical CLL (80% of patients):** more than 90% of lymphocytes are small with clumped chromatin and scanty cytoplasm.

2- **CLL/PLL:** when 11-54% of the cells are prolymphocytes.

3- **Atypical CLL:** when more than 15% of the lymphocytes are plasmoid or cleaved and less than 10% are prolymphocytes.

In the more aggressive disease the lymphocytes may be more pleomorphic or they may be small with scanty cytoplasm and contain cracked nuclei suggestive of follicular cell origin and often is identified by the term "Reider cells". Clefts or folded lymphocyte nuclei are present nearly in 10-15% of patients with B-CLL and this morphologic finding is associated with a uniformly unfavorable prognosis regardless of other staging scores ***(Rawstron et al., 2008).***

3. Clinical features:

A. General symptoms:

About 70% of CLL patients are asymptomatic and diagnosed incidentally with routine medical examination, while others may present with more severe symptoms as weight loss,

recurrent infections, bleeding and/or symptomatic anemia. However, night sweats and fever but may be uncommon ***(Chiorazzi, 2012).***

B. Lymphadenopathy:

Nearly 80% of all CLL patients have lymphadenopathy at diagnosis, enlargement of the cervical and supraclavicular lymph node can be more frequently than axillary or inguinal lymphadenopathy, the lymph nodes are usually discrete, freely movable and non-tender. Painful enlarged lymph nodes usually sugest superimposed infection ***(Byrd and Flynn, 2014)***.

C. Splenomegaly and hepatomegaly:

Approximately 50% of CLL patients can be present with mild or moderate splenomegaly. Splenomegaly may result in hypersplenism contributing to anemia and thrombocytopenia. However, in CLL, cytopenias are more commonly due to extensive bone marrow involvement with CLL or more rarely to autoimmune hemolysis. Hepatomegaly can occur less frequently than splenomegaly ***(Chiorazzi, 2012).***

D. Extranodal involvement:

Organ infiltration with leukemic cells is frequently detected at autopsy but not have a symptom. However, it may become symptomatic when it find in certain locations such as in the retro-orbit producing proptosis, pericardium or lung parenchyma producing nodular or miliary pulmonary infiltrates

that can be detected on chest x-ray film ***(Mauro and Foa, 2004).***

The gastrointestinal tract may be infiltrated by leukemic cells causing mucosal thickening, ulceration and may accompany by bleeding. Small bowel affection may causes intestinal malabsorption which makes megaloblastic anemia due to folate malabsorption. The finding of iron deficiency may take the physician to evaluate for gastrointestinal bleeding from mucosal ulcerations or secondary gastrointestinal malignancy ***(Mauro and Foa, 2004).***

Leukemic cell infiltration of the central nervous system is uncommon but may produce headache, meningitis, cranial nerve palsy, or coma. The development of neurologic changes in CLL may be caused by infections with unusual organisms, including Cryptococcus neoformans and Listeria monocytogenes ***(Byrd and Flynn, 2014).***

E. Immunological complications:

Patients with CLL have an increased risk for infection which is the major cause of death due to many factors such as hypogamma-globulinemia, low complement levels, functional defects in T cells and impaired granulocytic function ***(Francis et al., 2006).***

4. Laboratory findings:

A. Peripheral blood findings:

1- Lymphocytosis:

The diagnosis of CLL is the lymphocytosis greater than 5000/µl. At diagnosis, the median absolute lymphocyte count is 30,000/µl and in the most patients there is continuous increase in the lymphocytic count over time. The majority of lymphocytes are small with scanty, bluish cytoplasm, clumped nuclear chromatin, inconspicuous nucleolus, and absence of azurophilic granules in the cytoplasm (figure 1) ***(Amato et al., 2007).***

Smudge cells (basket cell or shadow cell of Gumperchet) are frequently seen in the blood smear and appear to be caused by decrease in vimentin causing fragility and distortion during preparation of the peripheral smear on the glass slide ***(Byrd and Flynn, 2014).***

The French American British (FAB) classification system can be divide patients into three groups depending on the percentage of abnormal cells. In typical CLL more than 90% of the cells are small; in CLL/PLL, 11% to 54% of the cells are prolymphocytes and in atypical CLL, there is heterogeneous morphology but less than 10% of the cells are prolymphocytes (figure 2) (table 3) ***(O'Brien and Keating, 2005).***

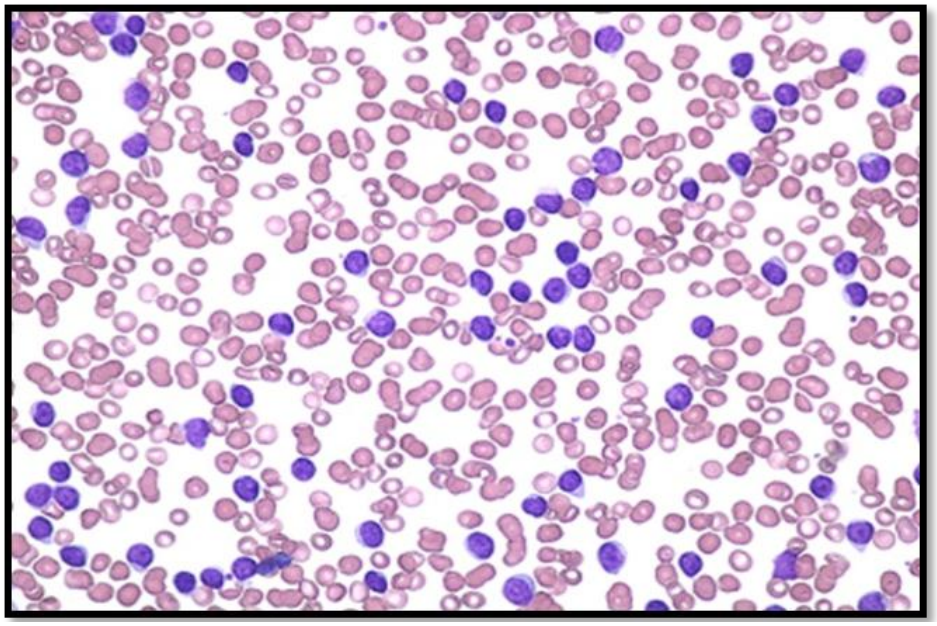

Figure (1): Peripheral smear showing the appearance of the lymphocyte morphology in CLL ***(Montsserat, 1999).***

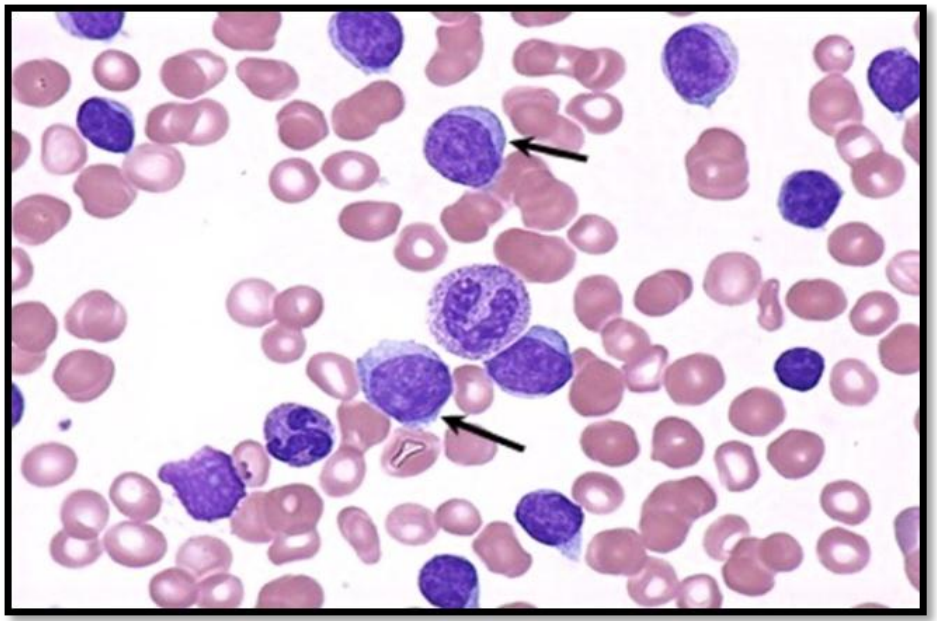

Figure (2): Prolymphocytes in a case of CLL ***(Montsserat, 1999).***