Introduction

Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease consisting of monoclonal small B-cell lymphocytes, caused by both increased proliferation and accumulation of cells with an increased lifespan ***(Wilkins et al., 2010).***

Angiogenesis is involved in the pathogenesis of B cell chronic lymphocytic leukemia (CLL), and high microvascular density has been found in CLL to be associated with a poor prognosis. Several angiogenic growth factors are involved in the pathogenesis of CLL ***(Kayne et al., 2009).***

Serum vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF- 2) were elevated in CLL patients, and high VEGF levels also predicted disease progression in early stage CLL ***(Molica et al., 2008).***

Hypoxia-inducible factor-1alpha (HIF-1alpha) is an oxygen-dependent transcriptional activator, which plays crucial roles in the angiogenesis of tumors and mammalian development. The stability and activity of HIF-1alpha are regulated by various post-translational modifications, hydroxylation, acetylation, and phosphorylation. Therefore, HIF-1alpha interacts with several protein factors including PHD, pVHL, ARD-1, and p300/CBP ***(Jeong et al., 2006).***

The target genes of HIF-1alpha are especially related to angiogenesis, cell proliferation/survival, and glucose/iron metabolism. Moreover, it was reported that the activation of HIF-1alpha is closely associated with a variety of tumors and oncogenic pathways ***(Makino et al., 2009).***

The blocking of HIF-1a itself or HIF-1alpha interacting proteins inhibit tumor growth. Based on these findings, HIF-1alpha can be a prime target for anticancer therapies ***(Semenza et al., 2008).***

Aim of the Work

The aim of this work is to measure the level of HIF-1 alpha in CLL patients using flowcytometry and to correlate it with the staging system, LDH and CD 38.

B-Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative malignancy with clonal accumulation of CD5+CD19+B cells ***(Bockstaele et al., 2008).***

These B cells are arrested in the differentiation pathway at some intermediate stage between pre-B cell and mature B cell. Morphologically, cells resemble mature lymphocytes in the normal peripheral blood ***(Capalbo et al., 2000).***

A. Incidence:

B-cell chronic lymphocytic leukemia is the most common leukemia in adults ***(Ottaggio et al., 2003).***

It affects twice as many males as females, with a peak incidence between 60 and 80 years**.** It is rarely diagnosed below the age of 40 years ***(Catovsky et al., 2005).***

B. Etiology:

The etiology of CLL is unknown but the following factors may have a role:-

1. Hereditary factors:

Although most cases of CLL are sporadic, first degree relatives of patients with CLL are more than three times at risk

for having this disorder or other lymphoid neoplasms than for the general population and often present at a younger age ***(Sgambati et al., 2001).***

The genetic factors that contribute to the increased incidence of CLL in certain families are unknown, with no apparent association between HLA haplotype and disease susceptibility ***(Kipps, 2006).***

2. Environmental factors:

Environmental factors do not appear to play a role in the pathogenesis of B-CLL ***(Hamblin, 2007).***

3. Occupational factors:

A higher incidence of CLL is seen in some groups of workers in the rubber industry. Some chemicals used are linked to the development of CLL including carbon tetra-chloride, carbon disulfide, acetone and ethylacetate. The duration and level of exposure to these chemicals appear to correlate with the risk of developing leukemia ***(Rai and Gupta, 2003).*** Benzene exposure is associated with an increased incidence of CLL ***(Zhang et al., 2007).***

C. Pathogenesis:

The molecular pathogenesis of CLL is unknown; but several recurrent genetic defects have been found in CLL cells ***(Patel et al., 2005).***

1. Defective apoptosis:

Chronic lymphocytic leukemia is characterized by accumulation of long lived CD5+ B-lymphocytes,***(Caligaris-Cappio & Ghia, 2007).***Which are not because they are dividing more rapidly than normal, but because they are surviving too long ,as this disease represents the best example of human malignancy caused by failed programmed cell death (apoptosis) (Figure 1) ***(Sakai et al., 2000).***

Many factors have been described to determine this abnormal extended life span due to defective apoptosis of the B-CLL cells, among them bcl-2 and bcl-xL genes over expression, increased serum levels of interferon gamma, interleukin-2,4,6 and 13, interleukin-8, free iC3b (the ligand for beta2 integrins), and interferon alpha. B-CLL cells have a down-regulated expression of the apoptosis inducer CD95 (Fas), a disrupted CD95-dependent apoptotic pathway and a perturbed T-cell /B-cell interaction ***(Cioca and Kitano, 2002).***

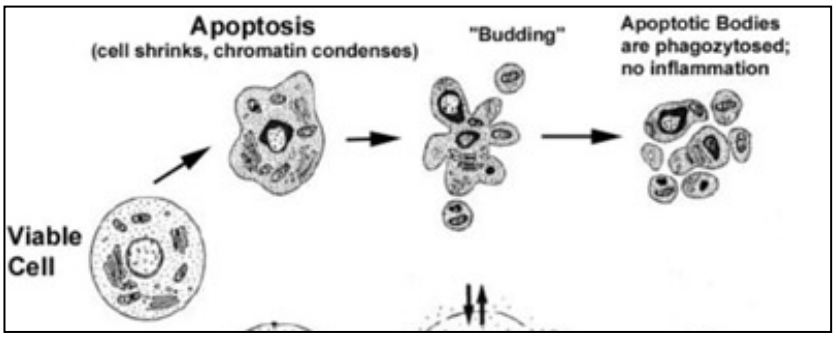

Figure 1: Morphological features of apoptosis ***(Gewies et al., 2003).***

Indeed, bcl-2 overexpression, which occurs in 85% of cases, is not a consequence of any known chromosomal translocations. However, possible mechanisms include hypomethylation of the bcl-2 promoter region or production of basic fibroblast growth factor by the CLL cells themselves, which in turn induces bcl-2 expression. It is likely that the bcl-2: bax ratio is more critical in setting the precise threshold for programmed cell death ***(Cavenagh and Lister, 2003).***

2. Genetic aberrations:

Although there is increasing knowledge about the cytogeneic abnormalities that occur in CLL, their precise pathogenetic molecular consequences are largely unknown ***(Cavenagh and Lister, 2003).***

Genomic aberrations and the mutation status of the variable segments of immunoglobulin variable region heavy chain gene (IgVH) of B-cell receptor (BCR) is involved in BCR signaling and influences cell cycle ***(Danilov et al., 2006)***.

Predictive factors, such as tyrosine kinase Zap-70 and soluble factors found in patient sera , may be associated with B-cell receptor signal transduction. Interestingly, an alteration of these factors fits closely, with the absence of somatic mutations in IgVH genes, suggesting that the latter may be due to either epigenetic events leading to an unstable genome or to an inherited defect in the immune response of malignant B-cells ***(Bouley et al., 2006)***.

3. Cytokines:

It has been suggested that cytokines may play a role in the pathogenesis and progression of CLL such as, tumor necrosis factor (TNF) and interleukin 8 (IL-8) which are produced and released by CLL cells, as well as IL-2, which is produced by T lymphocytes ***(Kalil and Cheson, 1999)***. Tumor necrosis factor alpha (TNF alpha) may act as an autocrine factor for B-CLL cells growth and survival ***(Rosati et al., 2005)***.

The expression of CXCR4 correlates significantly with Rai staging. Patients with Rai stage IV disease has a significantly higher expression of CXCR4 compared with patients with Rai stage 0 disease ***(Ghobrial et al., 2004).***

There is association between the under expression of the CXCR4 chemokine receptor and survival and this assosiation is consistent with its involvement in the trafficking and homing of B-CLL cells to bone marrow ***(Ishibe et al., 2002).***

D. Clinical Presentation:

1. General manifestations:

In approximately 50% of patients, the disease is diagnosed by chance following blood examination. In others, presentation is prompted by symptoms of anemia or systemic symptoms such as pyrexia, sweating or weight loss ***(Catovsky et al., 2005).***

2. Lymphadenopathy:

Painless lymph node enlargement which is symmetrical and usually involves the neck is the most common presenting symptom ***(Catovsky et al., 2005).***

Lymph node enlargement is more common in the cervical and subclavicular lymph nodes followed by axillary or inguinal lymph nodes ***(Johnston, 2004).***

3. Organomegaly:

Approximately 50% of CLL patients present with mild to moderate splenomegaly which may result in hypersplenism contributing to anemia and thrombocytopenia ***(Johnston, 2004).***

Less frequently, patients develop hepatomegaly secondary to leukemic cell infiltration to the liver ***(Kipps, 2006).***

4. Extanodal infiltrations:

Extranodal infiltrations occur in different organs including pleura leading to pleural effusion, lung causing nodular infiltrates, skin, central nervous system and rarely gastrointestinal tract ***(Kipps, 2006).***

E. Laboratory Findings:

1. Complete blood count:

a) WBCs:

The diagnosis of CLL requires lymphocytosis greater than $5x10^9$/L. Morphologically, the lymphocytes are small and show scanty cytoplasm and a characteristic pattern of nuclear chromation clumping; the nucleolus is inconspicuous and azurophil granules are seen only in a minority of normal T cells (Figure 2) ***(Catovsky et al., 2005)*** **.**

The presence of smear cells, which correlates with the level of WBC, is of diagnostic value. It is also called smudge (basket) cells, the so-called Gumprecht phenomenon. This occurs due to an artifact that is probably related to the abnormal membrane fluidity of CLL cells ***(Skarin, 1991).***

A proportion of prolymphocytes (1-5%) are nearly seen with counts > $30x10^9$/L (Figure 3) ***(Catovsky et al., 2005).***

Some patients have a mixed pattern of small and large cells and others have lymphoplasmacytoid features or even cells with nuclear clefts. These are often associates with other atypical features ***(Catovsky et al., 2005).***

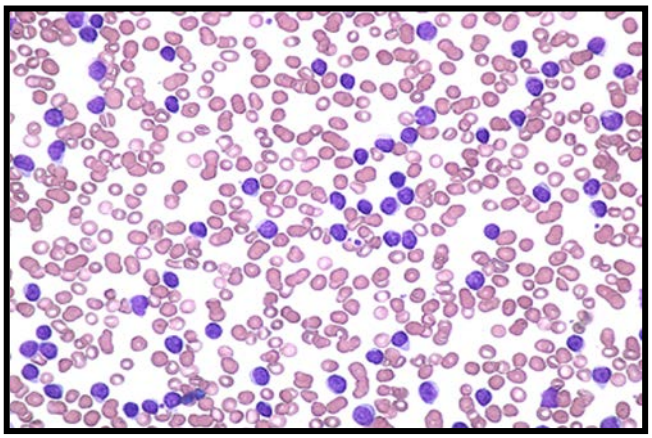

Figure 2 : Peripheral smear showing the appearance of the lymphocyte morphology in CLL ***(ASH image Bank, 2003).***

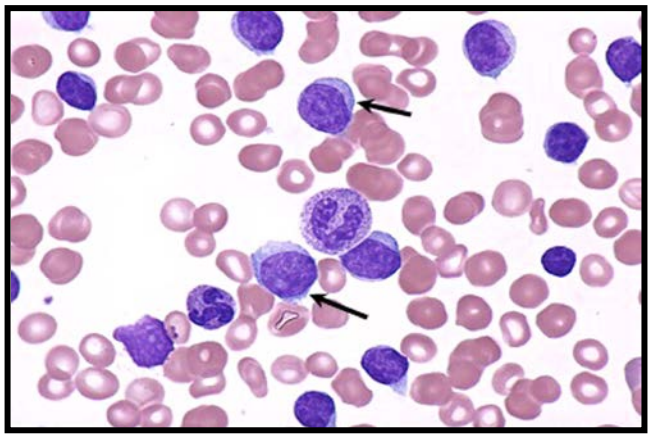

Figure 3: Prolymphocytes in a case of CLL ***(ASH Image Bank, 2003).***

Two subtypes of mixed cell type CLL are proposed in the FAB system:

- Chronic lymphocytic leukemia/prolymphocytic leukemia (CLL/PLL), having a dimorphic population of small lymphocytes and prolymphocytes in the peripheral blood, the prolymphocyte population constituting more than 10% and fewer than 55% of the circulating lymphocytes.

- Spectrum of small to large lymphocytes with fewer than 10% prolymphocytes in the peripheral blood ***(Frater et al., 2001)***.

b) RBCs:

Patients with CLL may develop anemia, about 15% of the CLL patients present with normocytic normochromic anemia ***(Kipps, 2006).*** It occurs in CLL patients due to several factors including bone marrow failure caused by leukemic infiltration, which is by far the most common cause, autoimmune hemolytic anemia, which is more frequent in active CLL, hypersplenism, pure red cell aplasia, post chemotherapy and coincident hematinic deficiency which occurs in advanced CLL stage III (RAI & BINET) ***(Mauro et al., 2000)***.

c) Platelets:

Platelet count below 100,000/cmm is a feature of poor prognosis and is due to marrow replacement or hypersplenism. However, at any stage patients can develop immune thrombocytopenia due to antiplatelet antibodies ***(Montserrat, 1999).***

2. Bone marrow examination:

Bone marrow aspiration is useful to confirm the cell morphology, to assess residual haempopoiesis and to as certain any myelodysplastic features ***(Catovsky et al., 2005).***

The criteria for positive involvement is the presence of more than 30% lymphocytes and/or the presence of atypical lymphocytes or blastoid cells, even if the proportion was lower than 30 % (Figure 4) ***(*Drayson *et al., 2005).***

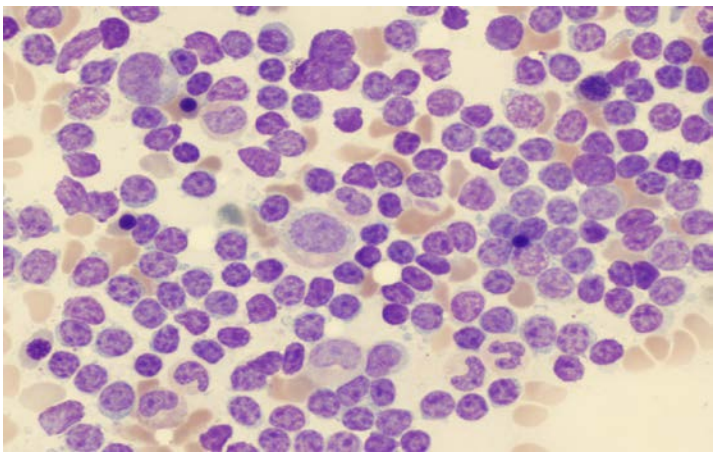

Figure 4: Lymphoid infiltration of bone marrow in CLL, Leishman stain ***(*Drayson *et al., 2005).***

B-CLL is characterized by the occurrence of three different cellular components: small lymphocytes, pro-lymphocytes, and paraimmunoblasts.

Small lymphocytes have a scanty amount of cytoplasm and round nucleus with coarsely dispersed chromatin. Pro-lymphocytes are larger than small lymphocytes and display a moderate amount of acidophilic cytoplasm and round nucleus, with dispersed chromatin and a small but evident nucleolus. Paraimmunoblasts have a wide rim of cytoplasm that stains greyish at Giemsa, and round-oval nucleus with fine chromatin and a prominent central nucleolus (Figure 5) ***(Pileri et al., 2004).***

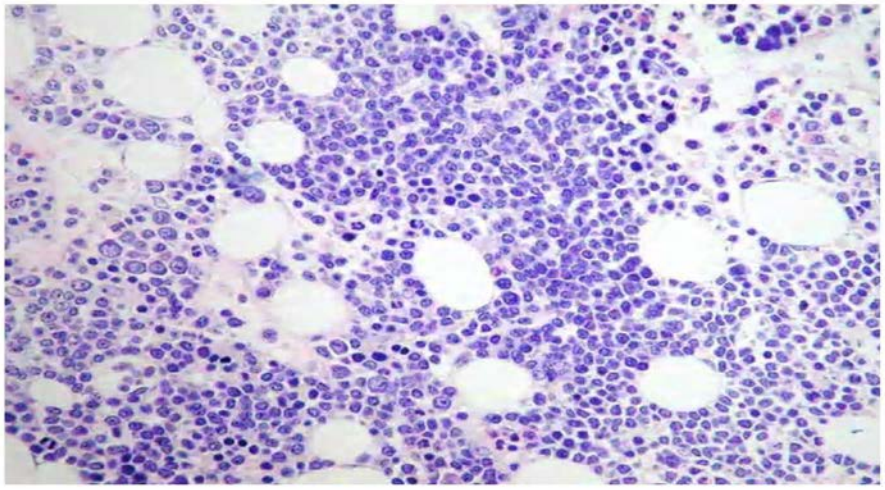

Figure 5: Types of cells in CLL, B.M: small lymphocytes (predominating), pro-lymphocytes, and paraimmunoblasts, Giemsa stain 500x ***(Pileri et al., 2004).***

Occasionally, B-CLL reveals plasmacytoid differentiation, these cases have a more aggressive clinical course ***(Pileri et al., 2004).***

3. Immunophenotyping:

In B-CLL, the cells have a number of distinct markers that help in its diagnosis and differentiation from other disorders (Table 1). Their immunophenotype typically shows co expression CD5/CD19,CD20,CD21,CD24,CD23 & K or λ light-chain restriction in addition to low expression of surface membrane immunoglobulin (sIg) usually of IgM type or associated with IgD type, Lack of expression of FMC7, CD11c and CD25 is also seen in CLL cases ***(Cavenagh and Lister, 2003).***

A scoring system has been proposed in which a score of 4 or 5 could distinguish CLL from other B-cell chronic lymphoid leukemias in 90% of cases (Table 2). The highest scores in CLL are found in those cases with more typical morphology ***(Matutes et al., 1994).*** Modified scoring system have been introduced in which the antibody CD79b replaces CD22, a score of 3 or above correctly diagnoses CLL in 96.8% of cases ***(Goldman and Mughal, 2005).***

Table (1): The immunophenotype of CLL: tests used as basis for a scoring system.

Marker	Score
Weak sIgM	1
CD5(+)	1
CD23(+)	1
FMC7(-)	1
CD79b (- or -/+)	1
Total	**5**

(Goldman and Mughal, 2005)