INTRODUCTION

Acute lymphoblastic leukaemia (ALL), is a malignant disorder of lymphoid progenitor cells of the bone marrow, that affects both children and adults. In children, ALL represent 75% of all acute leukemias, with a peak incidence at 2 to 5 years of age. This percentage is much lower in adults, in whom acute myeloid leukemias (AMLs) and chronic lymphocytic leukemias are more common ***(Inaba et al., 2013)***.

Immunophenotyping of leukaemic lymphoblasts by flow cytometry is essential to establish the correct diagnosis and define cell lineage. The lineage of ALL established in this manner subdivides this disease into two categories: precursor B-cell ALL (B-ALL) and precursor T-cell ALL (T-ALL) ***(Bhojwani et al., 2013)***.

Cytogenetic analysis of leukemic cells is the cornerstone for the prognostic stratification of ALL patients at the onset of disease because they are independent factors in predicting clinical outcome of patients and determining the duration and type of treatment ***(Inaba et al., 2013)***.

Rearrangements involving the myeloid–lymphoid lineage (MLL) gene on chromosome band 11q23 are frequent cytogenetic events in both lymphoid and myeloid leukemia ***(Emerenciano et al., 2011)***. The most common partner gene is AF4 on chromosome 4q21; other common partner genes

include ENL on 19p13 and AF9 on chromosome 9p22. ALL with MLL gene rearrangement *(MLL-r)* is the most common leukemia in infants <1year of age accounting forapproximately 50 % of cases, it is less common in older children (2% of cases) and increases with age into adulthood (5-6% of cases) ***(Borowitz and Chan, 2008).***

Acute lymphoblastic leukemia with *MLL-r* is generally considered as high risk leukemia, characterized by hyperleucocytosis, CNS involvement, poor clinical outcome and post-remission treatment intensification with allogenic hematopoietc stem cell transplantation is indicated to improve the outcome ***(Hien et al., 2012).*** For this reason, the accurate detection of MLL-r is crucial when dealing with this aggressive type of leukemia to guide therapeutic decisions ***(Emerenciano et al., 2011)***.

Currently the used methods to detect MLL rearrangements include conventional Cytogenetics, fluorescence in situ hybridization (FISH) and reverse transcription-PCR (RT-PCR) analyses. Due to the fact that *MLL-r* detection using standard techniques can be costly and requires specialized skills; a rapid and cost-effective technique would be quite useful especially for developing countries and worldwide efforts have focused on achieving high sensitivity and specificity values using Flow cytometry to identify *MLL-r* ***(Emerenciano et al., 2011)***.

To identify cell-surface antigens of hematopoietic precursor and stromal cells, the monoclonal antibody (MoAb) 7.1 was generated to recognize a 220–240 kDa protein that shows a high degree of homology to the rat neuron-glial antigen 2 (NG2) chondroitin sulfate proteoglycan. NG2 has been first discovered in the mammalian central nervous system, and it is assumed that the majority of NG2-positive cells within the central nervous system are oligodendrocyte progenitors ***(Trotter et al., 2010).***

In human leukemia, NG2 expression was found in close association with chromosomal aberrations involving 11q23. Previous groups have reported that MLL-r may be predicted through the cell surface recognition of NG2 using the monoclonal antibody (MoAb) 7.1, these studies were performed using a small number of infants while the frequency of NG2 expression in adult ALL was yet unknown ***(Emerenciano et al., 2011)***.

Aim of the Work

This work aims to evaluate the role of NG2 as a tumor marker in ALL and its predictive value in MLL gene rearrangement

ACUTE LYMPHOBLASTIC LEUKEMIA

Definition

Acute lymphoblastic leukemia (ALL) is a neoplastic disease that results from multistep somatic mutations in a single lymphoid progenitor cell at one of several discrete stages of development. It is caused by accumulation of lymphoblasts in the bone marrow and is the most common malignancy of childhood ***(Pui, 2010).***

Incidence

ALL is the most common malignancy diagnosed in patients younger than age 15 years, accounting for 23% of all cancers and 76 % of all leukemias in this age group. Only 20 % of adult acute leukemia ***(Pui, 2010).***

- ***Age and sex***

Age-specific incidence patterns are characterized by a peak between the ages of 2 and 4 years, followed by falling rates during later childhood, adolescence, and young adulthood. Incidence rises again in the sixth decade and reaches a second, smaller peak in the elderly ***(Shah and Coleman, 2007).***

In general, ALL occurs slightly more often in males than in females, except that females have a slightly higher (1.5

times) incidence of leukemia in the first year of life ***(Le Viseur et al., 2008).***

- ***Race***

The incidence of ALL is substantially higher in white children than in black children, with a nearly threefold higher incidence from age 2 to 3 years in white children compared with black children ***(Shah and Coleman, 2007).***

Etiology and risk factors

The precise pathogenic events leading to development of ALL are unknown; however several theories and events are involved ***(Bunin, 2004; Lightfoot and Roman, 2004).***

A. Environmental Factors

Ionizing radiation and chemical mutagens have been implicated in the induction of leukemia but clear etiological factors for ALL can not be identified in nearly all cases ***(Pui et al., 2008).***

- **Radiation exposure**

In utero (but not postnatal) exposure to diagnostic x-rays confers a slightly increased risk of ALL, which correlates positively with the number of exposures ***(Carroll et al., 2006).***

- **Chemical exposure**

The role of toxic chemical exposure (e.g., benzene) in the development of childhood ALL is questionable. Other factors that could be involved in the development of ALL include besticide exposure (occupational or home use) and parental cigarette smoking before or during pregnancy, administration of vitamin K to neonates, maternal alcohol consumption during pregnancy, and increased consumption of dietary nitrites have each been suggested causes ***(Evans and Mcleod, 2003)***.

B. Immunodeficiency

HLA class I molecules serve the essential immunological function of presenting antigen to CD8+ T lymphocytes. Tumor cells may present tumor-specific antigen to T cells via these molecules, but many tumors show loss or down-regulation of HLA class I expression and this may serve as an immune escape mechanism ***(McEvoy et al., 2003)***.

Moreover, abnormally low serum immunoglobulin levels have been observed in 30% of newly diagnosed acute leukemia patients. It is unclear whether such abnormality precedes the development of leukemia or is a consequence of the disease ***(Broder et al., 1998).***

C. Infectious Agents

It has been reported that children diagnosed with ALL had significantly more clinically diagnosed infectious episodes in infancy than did controls; This difference was most apparent in the neonatal period, suggesting that a dysregulated immune response to infection in the first few months of life might promote transition to overt ALL ***(Campana et al., 2011).***

D. Inherited Predisposition and Hereditary Factors

The genetic aberrations leading to leukemia are not likely to be caused by a single factor but rather by an interaction of exposure to some factors with inherited genetic predisposition ***(Greaves, 2002).***

Many cases of ALL that develop in children have a prenatal origin. Evidence in support of this comes from observation that the immunoglobulin or T-cell receptor gene rearrangements that are unique to each patient's leukemia cells can be detected in blood samples obtained at birth. Similarly, ALL characterized by specific chromosomal abnormalities at the time of birth ***(Carroll et al., 2006).***

Genetic studies of identical twins with concordant leukemia further support the prenatal origin of some leukemias ***(Greaves et al., 2003).*** ALL is concordant in 25% of monozygotic twins within a year of the diagnosis of the first twin. Among dizygotic twins, there is a 4-fold increase in the risk of leukaemia compared with the general population ***(Faderl et al., 2003).***

Children with Down syndrome have an increased risk of developing both ALL and acute myeloid leukemia (AML), with a cumulative risk of developing leukemia of approximately 2.1% by age 5 years and 2.7% by age 30 years ***(Whitelock, 2006).*** Approximately one-half to two-thirds of cases of acute leukemia in children with Down syndrome are ALL ***(Maloney, 2010).***

Other less common pre-exiting chromosomal abnormalities have been linked to leukemia are klinefelter's syndrom, Bloom syndrom, and Fanconi's anemia. Lymphoid malignancies, with a predominance of T-ALL, have been reported in patients with ataxia telangiectasia (AT), an autosomal recessive disorder characterized by increased chromosomal fragility ***(Tartaglia et al., 2004).***

Leukemogenesis

In almost all cases of ALL, lymphoblasts have acquired genetic changes, three fourth of which have prognostic and therapeutic relevance ***(Pui et al., 2004).***

Changes include abnormalities in the number (ploidy) and structure of chromosomes. The later comprise translocations (the most frequent abnormality), inversions, deletions, point mutations and amplifications. Although the frequency of particular genetic subtypes differs between childhood and adult cases, as shown in figures 1 and 2, the general mechanisms underlying the induction are similar ***(Ferrando and Look, 2003).***

Mechanisms include the aberrant expression of proto-oncogenes, chromosomal translocations that create fusion genes encoding active kinases and altered transcription factors, and hyperdiploidy involving more than 50 chromosomes. These genetic alterations contribute to the leukemic transformation of hematopoietic stem cells or their committed progenitors by changing cellular functions. They alter key regulatory processes by maintaining or enhancing an unlimited capacity for self-renewal, subverting the controls of normal proliferation, blocking differentiation, and promoting resistance to death signals (apoptosis) ***(Pui et al., 2004).***

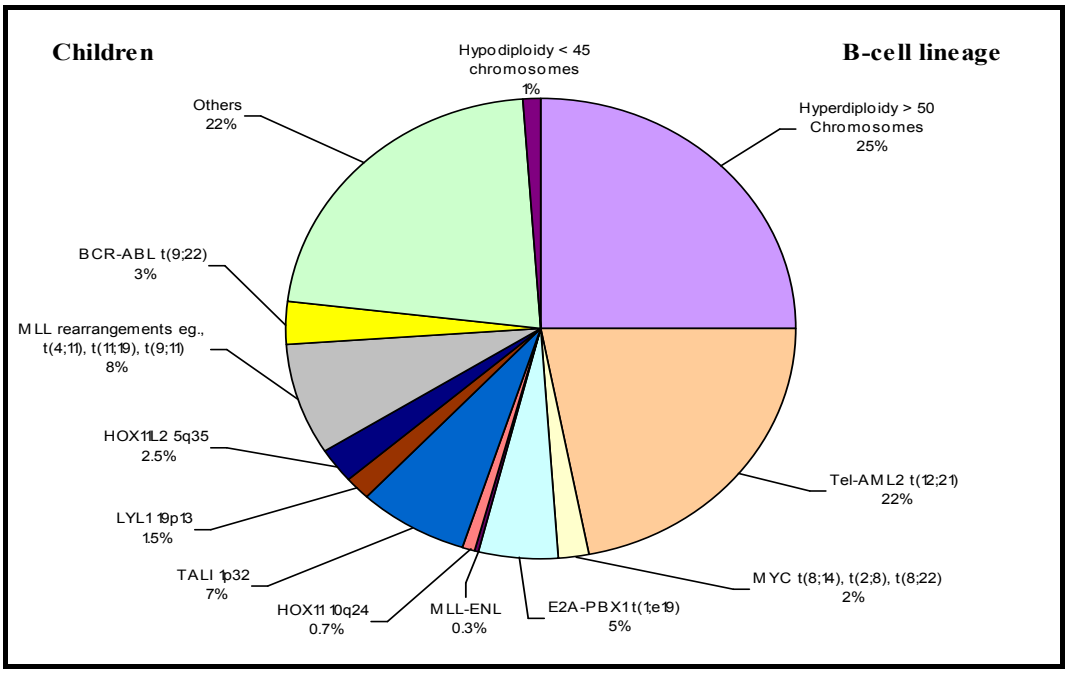

Figure (1): Estimated Frequency of Specific Genotypes of ALL in Children ***(Ferrando and Look, 2003).***

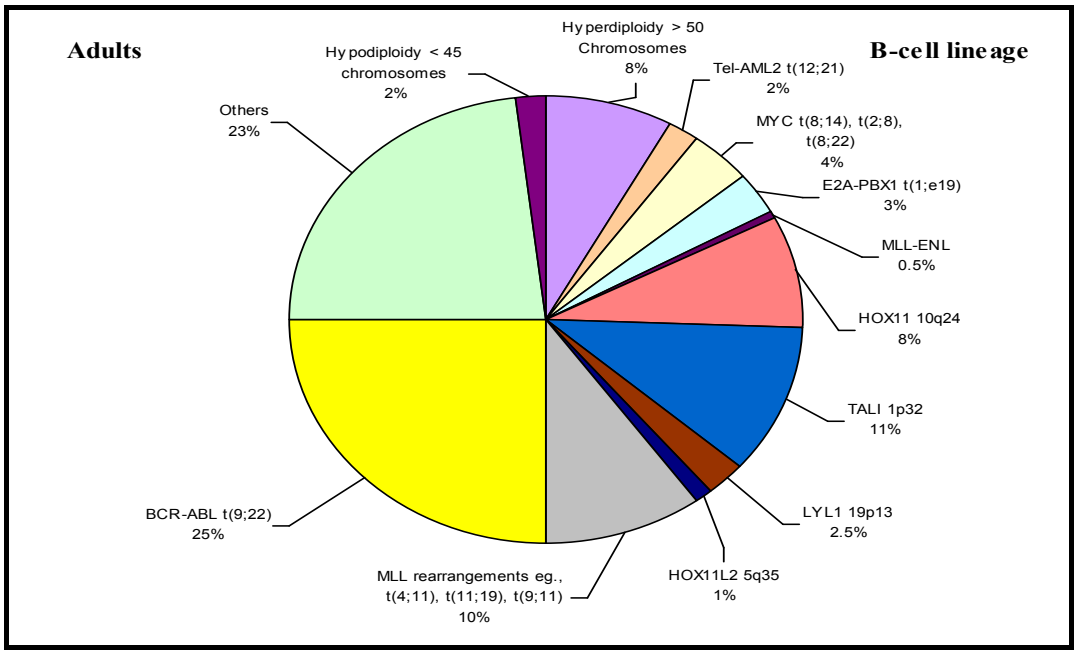

Figure (2): Estimated Frequency of Specific Genotypes of ALL in adults ***(Ferrando and Look, 2003).***

Another frequently altered regulatory network in ALL consists of the interrelated pathways controlled by the tumor suppressor genes such as the retinoblastoma protein (RB) and p53 which acts as molecular check points of cell cycle ***(Pui et al., 2004).***

The development of leukemia requires a hematopoietic stem cell or one of its committed progenitors to elude the normal mechanisms of homeostatic control that regulate growth-factor signaling, differentiation, apoptosis, and self-renewal. A common pathway targeted by translocation-generated chimeric transcription factors, such as MLL fusion

proteins, TEL-AML1, and E2A-PBX1, is the HOX gene–mediated transcriptional cascade ***(Pui et al., 2004).***

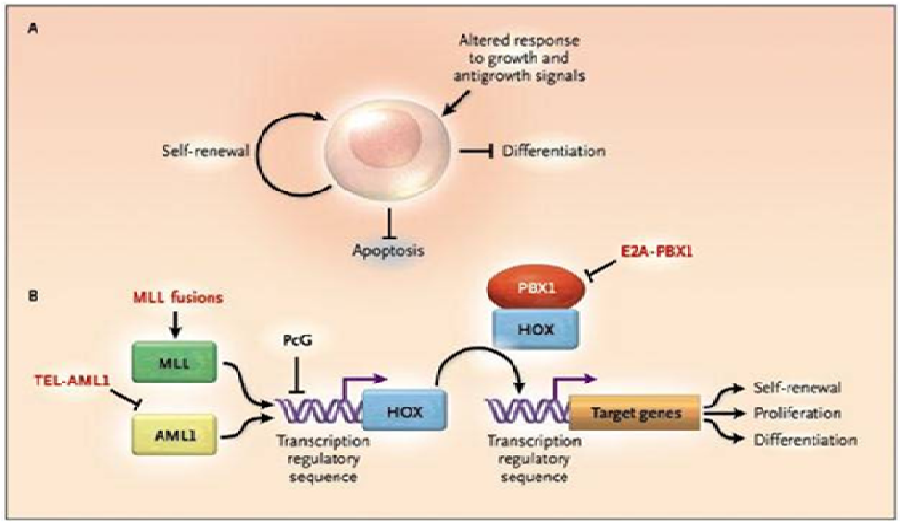

Figure (3): Transformation of Hematopoietic Cells in the Pathogenesis of ALL ***(Ferrando and Look, 2003)***

Classification

The French, American, and British (FAB) classification of ALL, which recognizes three subclasses of ALL (L1, L2, and L3), is based strictly on blast morphology and cytochemistry ***(Gökbuget and Hoelzer, 2011).*** The World Health Organization (WHO) classification scheme also incorporates immuno-phenotyping and cytogenetics ***(Borowitz and Chan, 2008).***

A. *FAB classification of ALL*

In the FAB system, three morphological sub-types of ALL were distinguished on the basis of cell size, nuclear shape, number and predominance of nucleoli, and the relative amount and appearance of the cytoplasm ***(Bennett et al., 1981).***

1. Acute lymphoblastic leukemia-L1

Morphology: L1- blasts are small and homogenous. The nuclei are round and regular with little clefting and indistinct or not visible nucleoli. Cytoplasm is scanty and usually without vacuoles (Figure 4A)**.**

Staining: myeloperoxidase (MPO) is always negative.

Maturation: most L1 ALLs are of pro B or pre B lineage. This subtype represents 25 to 30% of adult cases and 85% of pediatric cases ***(Whitlock and Gaynon, 2009).***

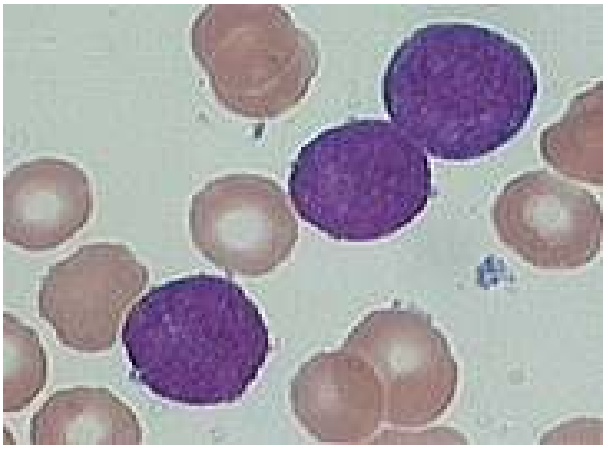

Figure (4A): FAB classification of ALL. Blasts morphology of L1 ***(Tkachuk and Hirshman, 2007).***

2. Acute lymphoblastic leukemia-L2

Morphology: L2 - blasts are large and heterogeneous. The nuclei are irregular and often clefted. One or more, usually large nucleoli are present. The volume of cytoplasm is variable, but often abundant and may contain vacuoles (Figure 4B).

Maturation: L2 ALLs may be of pro-B or pre-B lineage, but cases of T cell ALL are more likely to have an L2 than L1 morphology.

Staining: L2 - blasts may have granular or "chunky" periodic acid shiff (PAS) positivity with a negative cytoplasmic background (bright). Nonspecific esterase (NES) is usually negative. MPO is always negative. This is the most common subtype at 70% of adult cases but 14% of pediatric cases ***(Whitlock and Gaynon, 2009).***

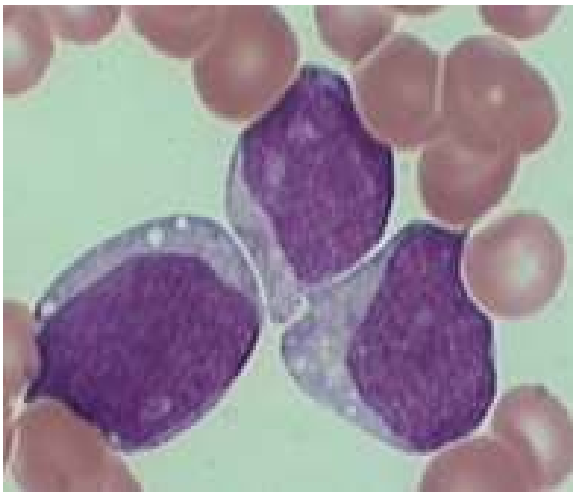

Figure (4B): FAB classification of ALL. Blasts morphology of L2 ***(Tkachuk and Hirshman, 2007).***

Acute lymphoblastic leukemia-L3 (Burkitt's leukemia/ Lymphoma)

Morphology: L3 blasts are moderate- large and homogeneous. The nuclei are regular and round- oval in shape. One or more prominent nucleoli are present. The volume of cytoplasm is moderate and contains prominent vacuoles. The cytoplasmic vacuoles are due to presence of lipid rather than glycogen (Figure 4C).

Staining: MPO is always negative. NSE is negative, but may show focal cytoplasmic positivity. The vacuoles are PAS negative, but are classically positive for the neutral lipid stain Oil Red O.

Maturation: ALL - L3 leukemias are surface immunoglobulin (SIg) positive and are of B cell lineage. This is just 1 to 2% of paediatric cases **(Whitlock and Gaynon, 2009).**

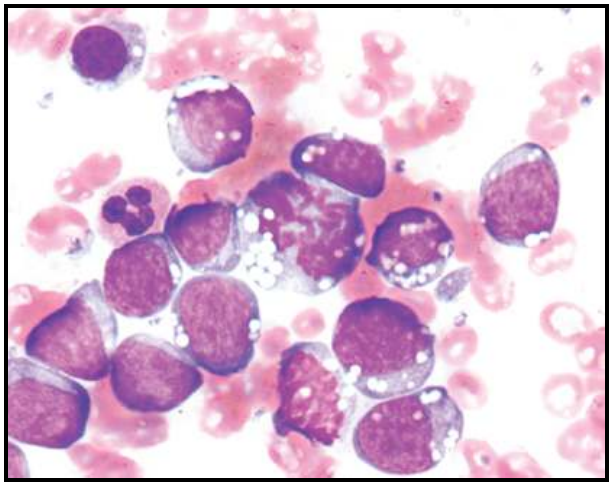

Figure (4C): FAB classification of ALL. Blasts morphology of L3 ***(Tkachuk and Hirshman, 2007).***