



*I am deeply thankful to "**Allah**" by the grace of whom, this work was possible.*

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To my **Father**, to whom I will never find adequate words to express my gratitude for his help and support.

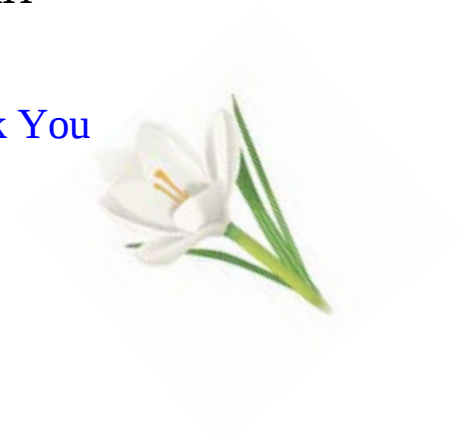
To my **Mother**, for her continuous support and encouragement during this work.

To my **Husband**, for his kind and loving help.

To my **Brother**, for his great help and support.

And finally to my **Daughters**, my small angels and the gift of ALLAH

I Love You All & Thank You



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَأَنْزَلَ اللَّهُ عَلَيْكَ
الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ تَكُنْ
تَعْلَمُ وَكَانَ فَضْلُ
اللَّهِ عَلَيْكَ عَظِيمًا

صدق الله العظيم

سورة النساء الآية
(١١٢)

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates from single B or T lymphoid cells at an early stage of proliferation with accumulation of blast cells in the marrow result in suppression of the hematopoiesis and thereafter the presence of anemia, thrombocytopenia and neutropenia(**Sherry, 2007andJalali et al., 2012**).

Thrombo-embolism (TE) is a well recognized serious complication associated with ALL with potential impact on overall outcome regarding mortality and morbidity and subsequently quality of life (**Rau et al., 2007and Jalali et al., 2012**).

Acute lymphoblastic leukemia is the most common childhood malignancy as associated with various coagulation abnormalities such as hemorrhage and thrombosis. The reported incidence of TE in childhood ALL varies from 1.1% to 36.7% (**Stefana et al., 2011**). The wide variation in the reported incidence of TE seems to be related to the definition of TE (symptomatic versus asymptomatic), diagnostic method used for detection of TE, study design (prospective versus retrospective) and treatment protocol (**Ulrike et al., 2009**).

The majority of symptomatic TE in children with ALL is venous and 5% of patients were reported to have multiple sites involved. Thromboembolism of CNS occurs in 66% of cases, DVT

in 16%, while right atrium and pulmonary embolism in 2% of patients (**Stefana et al., 2011**).

The pathogenesis and mechanism of the coagulation abnormalities is a complex one. The most important of these relate to the leukemic cells circulating in the blood which may contain procoagulants, fibrinolysis activators or proteases (**Maria et al., 2010**). These substances can be released into the blood from disintegrating cells during cytostatic treatment (**Paul et al., 2009**). Coagulation disorders may also occur due to leukemia associated complications such as sepsis or hepatic impairment (**Pui et al., 2008**).

For understanding the underlying pathogenesis causing coagulation abnormalities in ALL, information concerning the physiological elements involved in the coagulation process (endothelium, anticoagulants, platelets, fibrinolysis, genetics, homocysteinemia, lipoprotein(a), interleukins) are essential, in this study we specially focusing on the distribution pattern of plasma anticoagulant proteins activity to find a possible correlation with prevalent phenomenon of hypercoagulation in ALL patients (**Jalali et al., 2012**).

Protein C is a plasma vitamin K dependent protein, a plasma serine protease zymogene which can be converted to an active serine protease by thrombin (**Jalali et al., 2012**). This glycoprotein

is activated by thrombin and thrombomodulin act as an inhibitor of blood coagulation through a selective inactivation of factors Va and VIIIa (**Payne and Vora, 2007, Jalali et al., 2012**).

Antithrombin III (ATIII) is a glycoprotein produced by the liver, a member of family of serine protease inhibitor (SERPIN). It is the major physiological anticoagulant that neutralizes thrombin and factors Xa, 1Xa and X1a irreversibly by forming complexes in reactions accelerated by heparin or by heparin sulphate on endothelial surfaces (**Lipay et al., 2012**).

AIM OF THE WORK

The objective of this study is to determine the plasma activity level of protein C and antithrombin III in children with acute lymphoblastic leukemia in order to find possible correlation between these parameters and the prevalent phenomenon of hypercoagulation.

CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates from single B or T lymphoid cells at an early stage of proliferation with accumulation of blast cells in the marrow result in suppression of the hematopoiesis and thereafter the presence of anemia, thrombocytopenia and neutropenia (*Jalali et al., 2012*).

Epidemiology

The incidence of childhood cancer is around 120-150/million/year in children 0-14 years old and the number of new cases/year worldwide is approximately 250,000(*Pui et al., 2008*).

Leukemia is the most common malignancy in children accounting for one-third of all childhood cancers (*Onciu, 2009*). Approximately three fourths of all cases of childhood leukemia are ALL, which is the most common childhood malignancy accounting for 30% of childhood cancers (*Chen et al., 2010*).

The annual incidence of childhood ALL is 26 per million person-year. About 3000 children in the United States and 5,000 children in Europe are diagnosed with ALL each year (*Ni et al., 2011*).

The peak incidence of ALL occurs between the age of 2 and 5 years followed by falling rates during latter childhood and adolescence. The incidence is slightly higher among boys than girls

except during infancy where there is a slight female predominance **(Mrozek et al., 2009)**.

Geographic variation in the incidence of ALL is noted with highest rates have been reported among Hispanics, Filipinos and Chinese and the lowest rates among Africans the reason for this difference is unexplained **(Chen et al., 2010)**.

Moreover, childhood ALL has a peak incidence approximately 80 per million among white children with less peak incidence approximately 35 per million in black children **(Bhayat et al., 2009)**.

Etiology of Acute Lymphoblastic Leukemia (ALL).

A- Pathogenesis.

Acute lymphoblastic leukemia is a malignant proliferation of lymphoid cells that are blocked at an early stage of differentiation **(Faderl et al., 2010)**.

Cancer is usually caused if there is an alteration or mutation of the DNA, the changes in the DNA cause the normal blood progenitors that are committed to differentiate into either T-cell or B-cell pathway to change its behavior toward this normal pathway of differentiation and become leukemia cells **(Li et al., 2010)**. Leukemia as a cancer occurs when the balance between the oncogenes and tumor suppressor genes is altered **(Szczepanski et al., 2010)**.

Acute lymphoblastic leukemia is thought to originate from various important genetic lesions in hematopoietic stem cell possessing multilineage developmental capacity (**Faderl et al., 2010**). The genetic abnormalities (mutation) lead to clonal rearrangement in their immunoglobulin or T-cell receptor genes and express antigen receptor molecules and other differentiation linked cell surface glycoproteins that largely recapitulate those of immature lymphoid progenitor cells within the early developmental stages of normal T and B lymphocytes (**Andreson et al., 2011**).

Acquired genetic changes are considered to be central to the development of leukemia. These changes affect the number (ploidy) and/or the structure of chromosomes such as translocations, inversion, deletion, point mutation and amplification. Transformation of hemopoietic stem cell requires subversion of controls of normal proliferation, a block in differentiation, resistance to death signals (apoptosis) and enhanced self-renewal (**Campana and Pui, 2011**).

Translocations are common errors (mutations) of genes (DNA) that form the basis of leukemia (**Shaffer et al., 2009**). Translocation means that the DNA from one chromosome breaks off and becomes attached to another chromosome (**Ravandi and Kebriaei, 2009**). This break off at chromosome can turn on oncogenes or turn off tumor suppressor genes leading to leukemia with uncontrolled wide capability cell division and multiplication (**Gastier-foster, 2010**). There may be other types of genetic abnormalities (mutations) e.g. deletion where part of the

chromosome is lost or inversion where segment of the chromosome is rearranged and placed wrongly, so chromosomal mutation may be concerned with pathogenesis of ALL (**Douer, 2012**).

In many cases of ALL, the gene changes that lead to the leukemia are not known. Usually the mutation related to DNA occurs during the person's lifetime rather than having been inherited before birth. They may result from exposure to radiation or cancer creating chemicals but in most cases the reason for its occurrence is not known (**Tallen et al, 2010**).

B. Predisposing factors:

1. Congenital disorders

A small proportion (< 5%) of patients with childhood ALL have underlying hereditary genetic abnormalities, several congenital disorders with chromosomal abnormalities are associated with an increased risk of leukemia (**Gaikward et al, 2009**). Children with trisomy 21 (Down's syndrome) are up to 15 times more likely to develop leukemia than normal children (**Guazzarotti et al., 2009**). The greatest risk for leukemia in children with Down's syndrome is before the age of 5 years (**Maloney, 2011**), other less common chromosomal abnormalities have been linked to leukemia are Klinefelter's syndrome, Bloom's syndrome and Fanconi's anemia, lymphoid malignancies with predominance of T-ALL have been reported in patients with ataxia telangiectasia, an abnormal recessive disorder characterized by increased chromosomal fragility (**Alvarnas et al., 2012**).

2. Radiation exposure:

Exposure to high level of radiation is a risk factor for both ALL and acute myeloid leukemia (AML). Japanese bomb survivors had greatly increased risk of acute leukemia usually within 6 to 8 years after exposure **(Pui et al., 2010)**. The possible risk of leukemia from exposure to lower level of radiation such as radiation therapy or medical imaging test (such as X-ray) are not well known **(Zuurbier et al., 2010)**.

Exposure of the fetus to radiation within the first months of development may carry on increased risk of leukemia but the extent of the risk is not known **(Clappier et al., 2010)**.

3. Chemical exposure:

The risk of ALL may be increased by exposure to certain chemotherapy drugs and certain chemical including cigarette smoke, detergents and benzene which is a solvent used in the rubber industry, oil refiners, shoe manufacturing, in some glues, **(Dahl et al., 2009)**. However, chemical exposure is more strongly linked to an increased risk of AML than ALL **(Pui et al., 2011)**.

4. Infection exposure:

Several hypothesis have been proposed to suggest a role for infection, particularly in utero or during early infancy **(Zuurbier et al., 2010)**.

The age distribution of ALL at diagnosis corresponds with a time when the immune system is developing and is perhaps more

vulnerable to the oncogenic effects of some viruses. Some authors have suggested an increased risk of ALL in children born to mother infected recently with influenza, varicella or other viruses but no definitive link between parental viral exposure and leukemia risk has been confirmed. The only link is the association between Epstein – Barr Virus (EBV) and Burkitt's leukemia (**Goldstein and Li, 2009**).

5. Identical twins:

Fraternal twins of patients have a 2 to 4 folds increased risk of ALL during first decade of life compared with that of unrelated children. The increased risk among identical twins may be due to leukemia cells being passed from one fetus to the other while still in the uterus via the shared placental circulation (**Onciu, 2009**).

6. Uncertain risk factors:

Other factors that have been studied for possible risk to ALL (**Faderl et al., 2010**) including:

1. Exposure to electromagnetic field such as living near power lines or using cell phones.
2. Work place exposure to diesel pesticides
3. Smoking
4. Exposure to hair dyes

However, none of these factors has been linked conclusively to ALL, research in these areas continues (**Shaffer et al., 2009**).

Diagnosis of ALL

A- Clinical Presentation

The onset of ALL maybe insidious and slow over weeks to months or acute and explosive (**Pui et al., 2009**). The duration of symptoms in children presenting with ALL may vary from days to weeks, in general the more indolent the onset of symptoms the better the outcome (**Ensor et al., 2011**).

Symptoms and signs of ALL are consequences of bone marrow failure or involvement of medullary and extra medullary sites by leukemic cells (**Clappier et al., 2010**). The first symptoms are usually nonspecific and include anorexia, irritability and weight loss (**Pui et al., 2009**). Progressive bone marrow failure leads to anemia in 60%- 70% of patients in the form of pallor, dizziness, easy fatigability and shortness of breath. Bruises, petechiae and bleeding gum or epistaxis in 60% of ALL patients due to thrombocytopenia, recurring infections and fever due to neutropenia (**Veerman et al., 2009**).

Fever is common finding occurring in approximately 60% of patients with no infection basis is found in most cases especially if the neutrophil count $>0.2 \times 10^9/L$, but infection is presumed and treated parentally with broad spectrum antibiotics (**Lehrnbecher et al., 2009**).

Over one third of patients may present with bone pain, arthralgia, and tenderness over sternum or limping due to leukemia infiltration of periosteum, bone, joints or due to the expansion of the bone marrow cavity by leukemic cells (**Sanz et al., 2008**).

At the initial diagnosis 60-70% of children have enlarged organs hepatomegaly and splenomegaly, usually asymptomatic **(Ensor et al., 2011)**, lymphadenopathy occurs in 60% of children with ALL, usually painless, localized or generalized. Involvement of the mediastinal lymph nodes can lead to life threatening respiratory distress **(Vangemnt et al., 2007)**.

Central nervous system (CNS) involvement by leukemia usually asymptomatic occurs in 2 to 3% of patients with ALL at diagnosis however symptoms when present include headache, vomiting or cranial nerve palsies **(Borowit and Chan, 2008)**. Males may rarely have painless enlarged or irregular testes and testicular involvement except for infants, overt testicular infiltration carries no adverse prognosis. Testicular biopsy is discouraged at presentation as it is likely to be positive even in patient with normal testicular examination **(Berntjens, 2012)**.

The T-cell subtype of ALL often affects the thymus an enlarged thymus can press on the trachea causing coughing or trouble breathing and it may press on superior vena cava (SVC) causing SVC syndrome with swelling in the face, neck, arms and upper chest **(Szizepanski et al., 2010)**.

Less common presentation includes subcutaneous nodules (Leukemia-cutis), enlarged salivary glands and priapism. Finally in some patients infiltration of tonsils, adenoids, appendix or mesenteric lymph nodes leads to surgical intervention before leukemia is diagnosed **(Campana and Pui, 2011)**.