INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is now established as a standard therapeutic modality for a variety of malignant and non-malignant diseases. The first successful allogeneic HSCT was done with bone marrow (BM) as the source of hematopoietic stem cells in 1968. Nowadays transplant physicians are faced with 3 viable choices of stem cells for allogeneic HSCT, namely BM, prephiral blood stem cells (PBSC) and cord blood (CB) and clinicians have to face the challenges of selecting the optimal stem cell source. Although all 3 sources of stem cells are capable of reconstituting the hematopoietic system in recipient after transplant, they have many inherent differences in cellular constituents and biological and immunological properties ***(Cheuk, 2013).***

Granulocyte colony stimulating factor (G-CSF) - mobilized PBSC are increasingly used instead of BM cells for allogeneic transplantation because they provide faster engraftment and better survival in recipients with poor-risk disease. Important difference among the sources of stem cell is the amount of mature T cells present. PBSC usually contains a lot more mature T cells compared to BM, which in turn contains more T cells compared to CB, and this partly explains the differences in the risk of graft rejection and graft-versus-host disease (GVHD). Depletion of T cells is associated with

increased risk of graft rejection and disease relapse, but lower risk of GVHD ***(Switzer et al., 2013).***

One of the main reasons for preferring PSC worldwide is the important advantages provided by this method to the donor. These advantages are avoidance of anesthesia, lack of the need for hospitalization or blood transfusion, and very low serious adverse event risk ***(Sirinoglu et al., 2012)***.

Most of the randomized controlled trials (RCTs) comparing matched related donor BM and PBSC transplantation for patients with hematological malignancies found no significant differences between the two stem cell source in important outcomes including overall survival, disease-free survival, transplant-related mortality, relapse, acute GVHD and chronic GVHD. However, all trials showed significantly faster neutrophil engraftment in PBSC transplants, and all but one trial showed significantly faster platelet engraftment in PBSC transplants, which may result in earlier hospital discharge for PBSC recipients and lower cost for PBSC transplantation. Lymphocyte recovery was also found to be better in the PBSC group in one trial ***(Powles et al., 2000).***

Some trials showed significantly higher probability of relapse in BM recipients than in PBSC recipients, which might translate into better disease-free survival in PBSC transplants compared with BM transplants ***(Mielcarek et al., 2012).***

The conditioning regimen has a key role in allogenic BMT, and has a significant effect on treatment outcome ***(Gupt et al., 2011)***. Only limited numbers of agents have both immunosuppressive and anti-tumor activities.

In Allogenic Hematopoietic stem cell transplantation (AHSCT), the goal of the conditioning regimen is not only to achieve anti-tumor activity but also to suppress the immunity of the recipient in order to prevent graft rejection. The great majority of myeloablative conditioning in children and adults includes either total Body Irradiation (TBI) or Busulfan (BU). These two conditionings were much compared in terms of efficacy and early toxicity.With increasing good results in HSCT, it became essential also to compare their late toxicity in a long-term comprehensive evaluation of both medical health status and quality of life (QoL). To our knowledge, this was never done before in a large cohort of patients ***(Ferry et al., 2003).***

To avoid or diminish the late complications of irradiation, especially in young children, regimens based on chemotherapy alone have been introduced by some researchers. The most commonly used chemotherapy regimens for acute leukemia have been the combination of busulfan (BU) and cyclophosphamide (CY). Significant advantages of BU-based regimen over TBI-based regimens aside from prevention of long-term morbidities associated with TBI are easier administration without any need for sedation or anesthesia particularly in small children, fewer secondary malignancy,

prevention of further irradiation and toxic exposure in patients who have received prior dose-limiting irradiation and overcoming practical limitations in the availability of irradiation at some institutions ***(Bunin et al., 2003).***

BU-CY regimen has some disadvantages which should not be neglected. The essential disadvantages of this regimen would be less potent antitumor effect in sanctuary site and unpredictable drug metabolism ***(Aschan et al., 2007).***

AIM OF THE WORK

The aim of the study is to assess outcome in Egyptian patients diagnosed with Acute Lymphoblastic Leukemia subjected to allogeneic hematopoietic stem cell transplantation using a uniform (BU/CY)based conditioning regimen. in the time period from December 1999 to May 2017.

- **Data Collection Regarding:**
  - Patient and donor characteristics.
  - Transplant-related mortality (TRM).
  - Disease-free survival (DFS).
  - Overall survival (OS).
  - Relapse post bone marrow transplant.

Chapter 1

ACUTE LYMPHOBLASTIC LEUKEMIA

- Definition

Is a cancer of the lymphoid line of blood cells, characterized by the development of large numbers of immature lymphocytes.

Symptoms may include feeling tired, pale skin color, fever, easy bleeding or bruising, enlarged lymph nodes, or bone pain. As an acute leukemia, Acute lymphoblastic leukemia (ALL) progresses rapidly and is typically fatal within weeks or months if left untreated.

- Epidemiology:

Incidence of ALL ranges from 0.4 to 2 per 100, 000 per year.

It occurs in both children and adults with highest rates seen between the ages 3-7 years. Around 75% of cases occur before the age of 6 with a secondary rise after the age of 40. ***(Hoffbrand, 2006).*** It is estimated to affect 1 in 1500 children ***(Boer, 2017).***

ALL represents approximately 20% of adult and 80% of childhood leukemias, making it the most common childhood cancer ***(Baljevic et al., 2017).*** Although 80 to 90% of children

will have a long term complete response with treatment, ***(Ginsburg et al., 2014)*** it remains the leading cause of cancer-related deaths among children ***(Ma et al., 2014).***

85% of cases are of B-cell lineage and have equal incidences in both males and females. The remaining 15% of T-cell lineage have a male predominance.

Globally ALL typically occurs more often in Caucasians, Hispanics, and Latin Americans than in Africans ***(Glader et al. 2013; Urayama and Manabe, 2014).*** In the US, ALL is more common in children from Caucasian (36 cases/million) and Hispanic (41 cases/million) descent when compared to those from African (15 cases/million) descent.

In 2016 there were a total of 53, 000 cases worldwide ***(Solomon et el., 2017).***

- Pathophysiology of Acute lymphoblastic leukemia:-

Several characteristic genetic changes lead to the creation of a leukemic lymphoblast. These changes include chromosomal translocations, intrachromosomal rearrangements, changes in the number of chromosomes in leukemic cells, and additional mutations in individual genes ***(Charles, 2015).***

Chromosomal translocations involve moving a large region of DNA from one chromosome to another. This move can result in placing a gene from one chromosome that promotes cell

division to a more actively transcribed area on another chromosome. The result is a cell that divides more often.

An example of this includes the translocation of C-MYC, a gene that encodes a transcription factor that leads to increased cell division, next to the immunoglobulin heavy- or light-chain gene enhancers, leading to increased C-MYC expression and increased cell division ***(Charles, 2015).***

Other large changes in chromosomal structure can result in placement of two genes directly next to each other. The result is the combination of two usually separate proteins into a new fusion protein. This protein can have a new function that promotes the development of cancer. Examples of this include the ETV6-RUNX1 fusion gene that combines two factors that promote blood cell development and the BCR-ABL1 fusion gene of the Philadelphia chromosome. BCR-ABL1 encodes an always-activated tyrosine kinase that causes frequent cell division. These mutations produce a cell that divides more often, even in the absence of growth factors ***(Charles, 2015).***

Other genetic changes in B-cell ALL include changes to the number of chromosomes within the leukemic cells. Gaining at least five additional chromosomes, called high hyperdiploidy, occurs more commonly. Less often, chromosomes are lost, called hypodiploidy, which is associated with a poorer prognosis. Additional common genetic changes in

B-cell ALL involve non-inherited mutations to PAX5 and IKZF1 ***(Charles, 2015).***

In T-cell ALL, LYL1, TAL1, TLX1, and TLX3 rearrangements can occur ***(Inaba, 2013).***

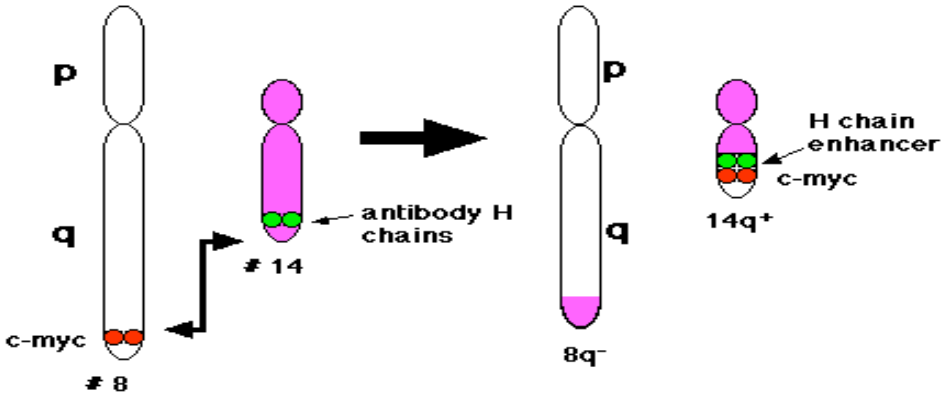

Figure (1): Cmyc mutation t(8:14).

ALL results when enough of these genetic changes are present in a single lymphoblast. In childhood ALL, for example, one fusion gene translocation is often found along with six to eight other ALL-related genetic changes ***(Inaba, 2013).*** The initial leukemic lymphoblast copies itself into an excessive number of new lymphoblasts, none of which can develop into functioning lymphocytes. These lymphoblasts build up in the bone marrow and may spread to other sites in the body, such as lymph nodes, the mediastinum, the spleen, the testicles, and the brain, leading to the common symptoms of disease ***(Charles, 2015).***

- Clinical diagnosis of ALL:-

A) Signs & symptomps:-Initial symptoms can be nonspecific, particularly in children. Over 50% of children with leukemia had one or more of five features: a liver one can feel (64%), a spleen one can feel (61%), pale complexion (54%), fever (53%), and bruising (52%) ***(Thompson et al., 2016).***

Additionally, recurrent infections, feeling tired, arm or leg pain, and enlarged lymph nodes can be prominent features.

The B symptoms, such as fever, night sweats, and weight loss, are often present as well.

Central nervous system (CNS) symptoms such cranial neuropathies due to meningeal infiltration are identified in less than 10% of adults and less than 5% of children, particularly mature B-cell ALL (Burkitt leukemia) at presentation. (***Seite, 2014***)

- **The signs and symptoms of ALL are variable and include**:-
  - Generalized weakness and feeling tired.
  - Anemia.
  - Dizziness.
  - Headache, vomiting, lethargy, nuchal rigidity, or cranial nerve palsies.(CNS involvement).

- Frequent or unexplained fever and infection.
- Weight loss and/or loss of appetite.
- Excessive and unexplained bruising.
- Bone pain, joint pain (caused by the spread of "blast" cells to the surface of the bone or into the joint from the marrow cavity).
- Breathlessness.
- Enlarged lymph nodes, liver and/or spleen.

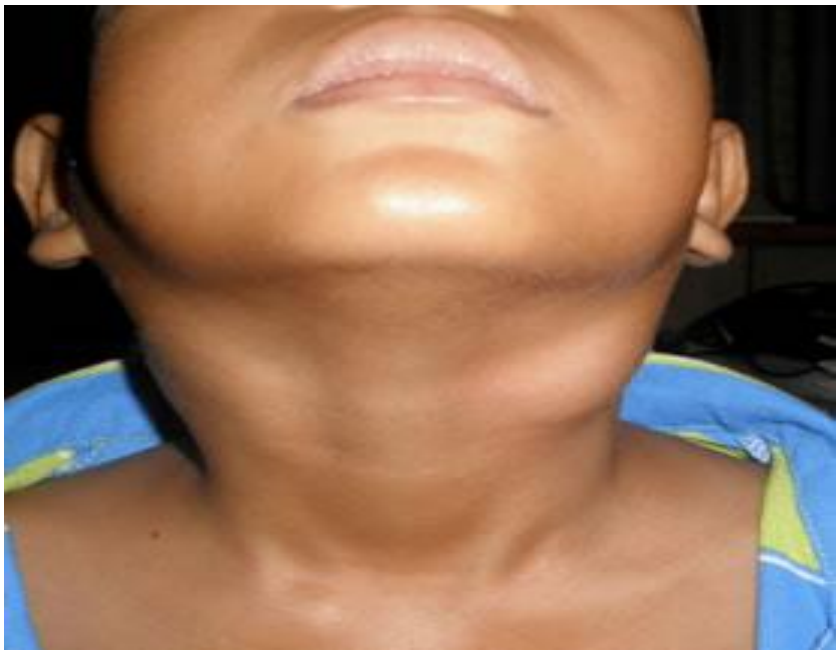

Figure (2): Lymph adenopathy.

- Pitting edema (swelling) in the lower limbs and/or abdomen.
- Petechiae, which are tiny red spots or lines in the skin due to low platelet levels.

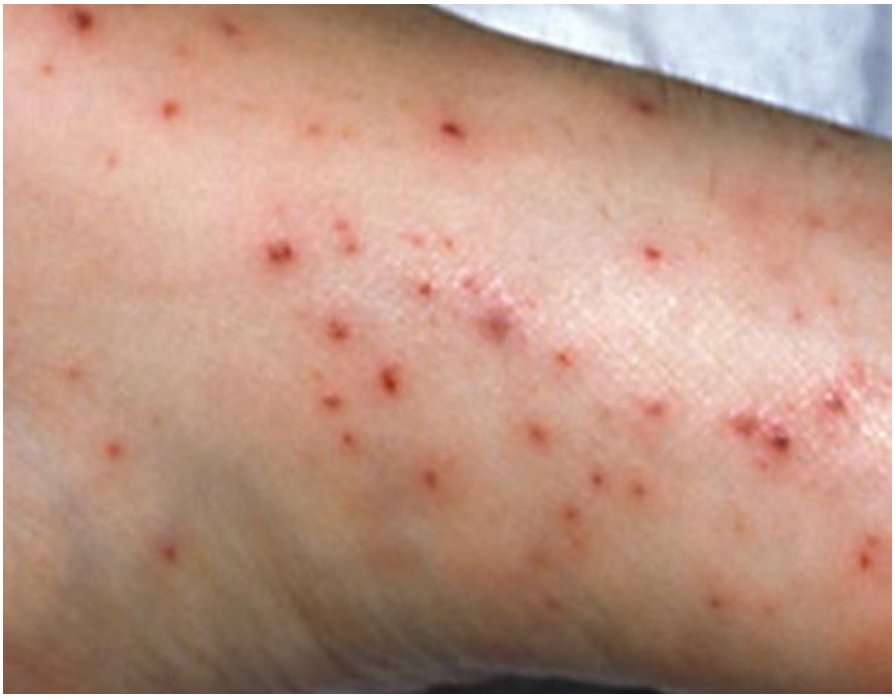

Figure (3): Petechaie.

- Testicular enlargement.
- Mediastinal mass.
- Diagnosing ALL begins with a thorough medical history, physical examination, complete blood count, and blood smears. While many symptoms of ALL can be found in common illnesses, persistent or unexplained symptoms raise suspicion of cancer. Because many features on the medical history and exam are not specific to ALL, further testing is often needed.
- A large number of white blood cells and lymphoblasts in the circulating blood can be suspicious for ALL because they indicate a rapid production of lymphoid cells in the

marrow. The higher these numbers typically points to a worse prognosis. While white blood cell counts at initial presentation can vary significantly, circulating lymphoblast cells are seen on peripheral blood smears in the majority of cases ***(Baljevic et al., 2017)***.

- A bone marrow biopsy provides conclusive proof of ALL, typically with >20% of all cells being leukemic lymphoblasts ***(Longo, 2011).***
- A lumbar puncture can determine whether the spinal column and brain have been invaded. Brain and spinal column involvement can be diagnosed either through confirmation of leukemic cells in the lumbar puncture or through clinical signs of CNS leukemia as described above. Laboratory tests that might show abnormalities include blood count, kidney function, electrolyte, and liver enzyme tests ***(Seiter, 2014).***
- Pathological examination, cytogenetics (in particular the presence of Philadelphia chromosome), and immunophenotyping establish whether the leukemic cells are myeloblastic (neutrophils, eosinophils, or basophils) or lymphoblastic (B lymphocytes or T lymphocytes). Cytogenetic testing on the marrow samples can help classify disease and predict how aggressive the disease course will be. Different mutations have been associated with shorter or longer survival ***(Mrozek, 2009)***.

- Immunohistochemical testing may reveal Terminal Deoxynucleotidyl Transferase (TdT) or Common Acute Lymphoblastic Leukemia Antigen (CALLA) on the surface of leukemic cells. TdT is a protein expressed early in the development of pre-T and pre-B cells, whereas CALLA is an antigen found in 80% of ALL cases and also in the "blast crisis" of CML ***(Devi, 2011).***
- Imaging (such as ultrasound or CT scanning) can find invasion of other organs commonly the lung, liver, spleen, lymph nodes, brain, kidneys, and reproductive organs ***(Rytting, 2013).***

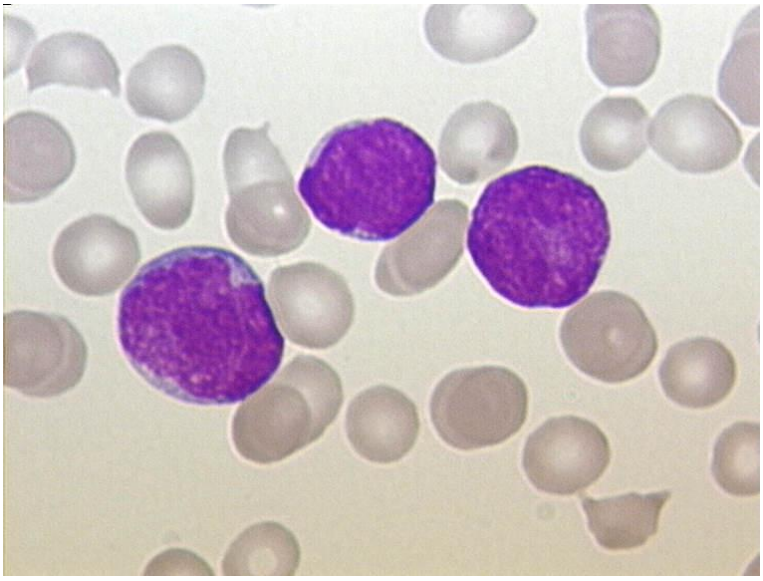

Figure (4): Acute lymphoblastic leukemia (ALL), peripheral blood of a child, Pappenheim stain, magnification x100.

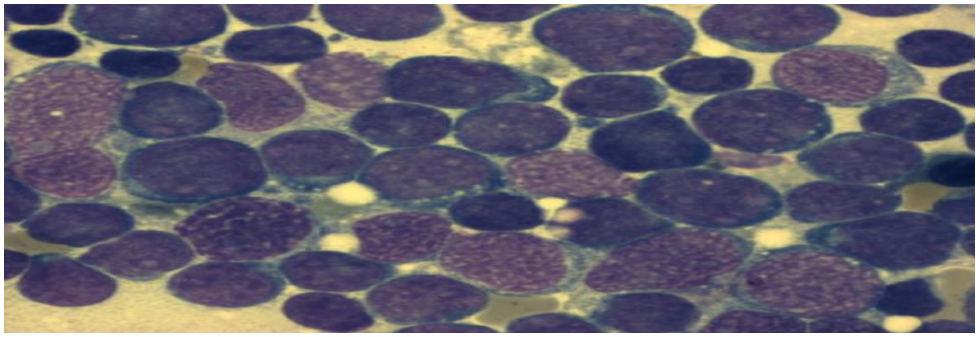

Figure (5): Bone marrow smear (large magnification) from a patient with acute lymphoblastic leukemia.

Immunophenotyping:

Table (1): Classification of ALL according to maturation ***(Baljevic et al., 2017).***

B cell Lineage	T cell Lineage
pre-pre-B ALL (pro-B-ALL)	precursor T- ALL
common ALL	mature T-cell ALL
pre-B ALL	
mature B-cell ALL (Burkitt leukemia - FAB L3)	

In addition to cell morphology, immunophenotyping, a laboratory technique used to identify proteins that are expressed on their cell surface, is a key component in the diagnosis of ALL.

The preferred method of immunophenotyping is through flowcytometry. In the malignant lymphoblasts of ALL, expression of terminal deoxynucleotidyl transferase (TdT) on