Nutritional Assessment of Leukemic Children and its Relationship with serum Selenium, Zinc and Copper

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Supervisor's Copy

Dedicated to My Beloved Parents

To Whom It May Concern

This is to certify that Khurshida Azad Siddiqua, student of M. Phil (Session 2015-16), Department of Biochemistry and Molecular Biology, University of Dhaka has completed her thesis work on **"**Nutritional assessment and serum zinc and copper concentration of Leukemic children in Bangladesh**"** as a part of M. Phil. course at Department of Biochemistry and Molecular Biology, University of Dhaka under my supervision.

I hereby, endorse his thesis to be submitted for evaluation.

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List of Abbreviations

ALL	Acute Lymphoblastic Leukemia
ALT	Alanine aminotransferase
aPPT	Activated Partial Phromboplastin Time
BCP	B cell precursor
BSMMU	Bangobondhu Shiekh Mujib Medical University
DNA	Deoxyribonucleic acid
LDH	Lactate Dehydrogenase
NCI	National Cancer Institute
NCHS	National centre for Health Statistics
PCR	Polymerase Chain Reaction
PT	Prothombin Time
WHO	World Health Organization
Mg	Miligram
dL	Deciliter
U/L	Unit per Liter

Abstract

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and accounts for 30 – 35% of all cancers in children. Only 50 years ago, the disease was uniformly fatal with an Overall Survival (OS) rate < 5%. In modern day, multi-drug chemotherapy is associated with an overall survival rate over 80%. When undergoing chemotherapy and/or radiotherapy, children with ALL may present important nutritional disorders because of the gastrointestinal toxicity of most chemotherapy agents or the effects of radiation on the organism. These patients may also present changes in their serum concentrations of trace elements such as zinc and copper. The present study aimed to follow anthropometric parameters and serum levels of zinc and copper in a group of children in Bangladesh suffering from ALL before chemotherapy in age, sex and related differences.

A case-control study on 101 children with ALL and 121 children without any cancer or chronic diseases as control subjects were conducted. Anthropometric parameters such as weight and height and the daily intakes were recorded at diagnosis, and serum levels of copper and zinc were analyzed afterwards. Serum albumin, total protein, Ca++, ALT & LDH levels were analyzed by spectrophotometer. The trace elements Zn^{2+} and Cu^{2+} were analyzed by atomic absorption spectroscopy.

Though Serum Ca^{+} was normal the Cu^{2+} and Zn^{2+} showed significantly ($p<0.001$, respectively) higher levels in ALL. There was significant difference in age between 1 to 5 years than control group ($p <0.01$). There were more male children than female between the two groups: 69.3% of cases versus 56.2% of controls were male. The difference in levels found between the groups may be associated with factors such as stage of disease, diet and drugs associated with treatment. The serum albumin was

significantly lower compared to control ($p < 0.05$). The serum ALT ($p < 0.001$), and LDH ($p < 0.001$), were significantly higher among ALL children compared to control while serum calcium ($p > 0.05$) level was non-significantly normal when compared to control.

Key words: Acute Lymphoblastic Leukemia (ALL), Atomic Absorption Spectroscopy, Multi-drug chemotherapy, Nutritional status, Zinc.

Chapter one

Introduction

Introduction

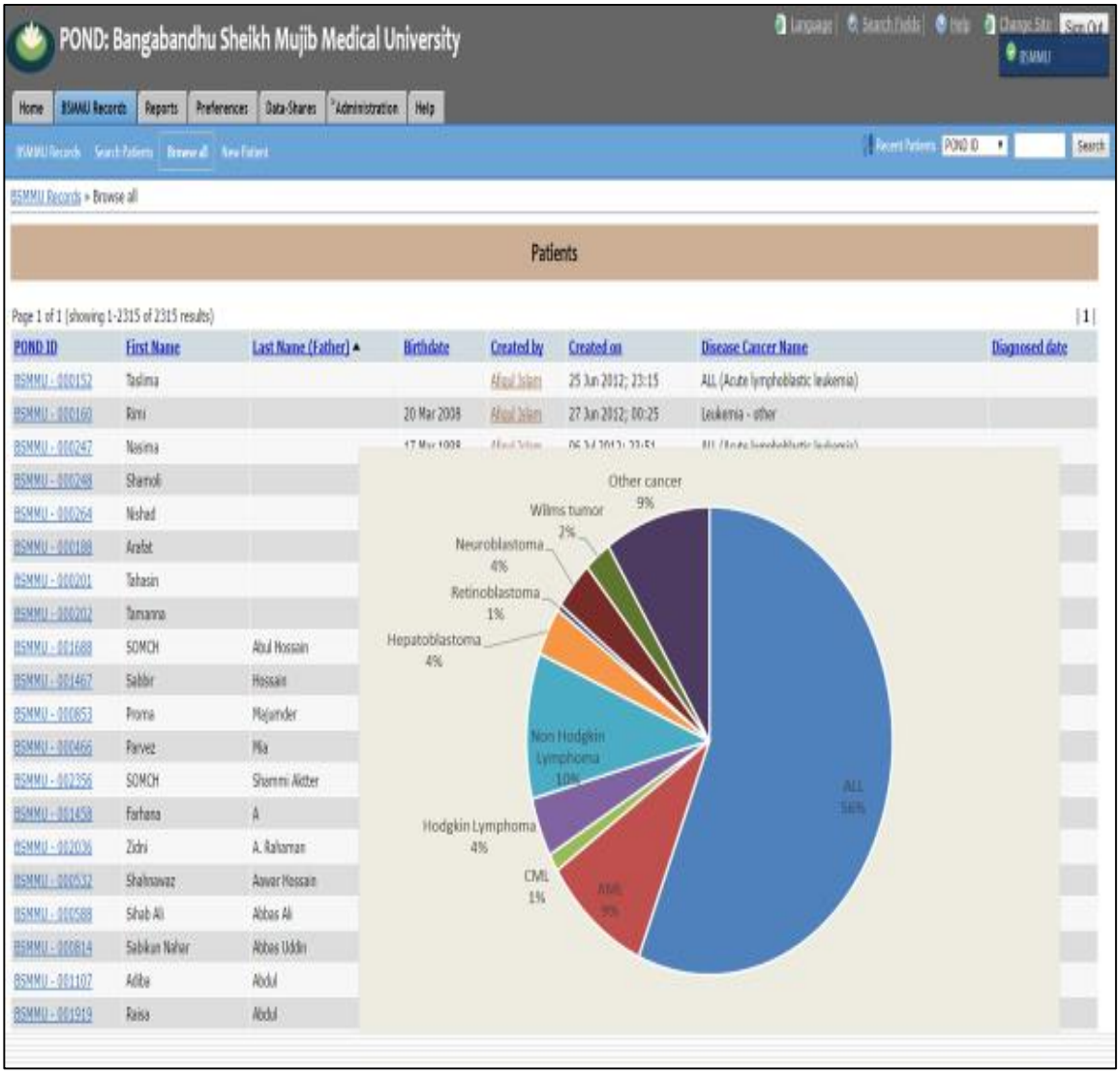
1.1 Cancer is a Disease of the Genome

Cancer, like other chronic diseases, can have an adverse effect on the nutrient balance because of a combination of factors (Pencharz PB et al.,1998). Children with cancer are at risk of suffering from under nutrition that, if severe, affects tolerance and may influence the patient's overall survival (Kien CL et al.,1981). Cancer or cancer therapy may result in anorexia, vomiting or maldigestion/malabsorption, with the net result of reductions in absorbed nutrient intake.

Trace elements have been extensively studied over recent years, to assess whether they have any modifying effects regarding the etiology of cancer. Different authors have tried to establish a relationship between trace elements, especially zinc and copper, and malignant diseases. Changes in blood zinc and copper have been found in lymphoproliferative disorders, as well as in breast, lung and gastrointestinal tumors.

The general trend towards slightly decreased zinc concentrations in malignant diseases supports the experimental results obtained by Brown et al.1980, thus suggesting that zinc deficiency is associated with the etiology of cancer.

Several studies (Shah I et al.,1983, Tessmer et al.,1972) have shown that serum copper levels in cases of malignant disease increase with increasing disease activity. Remission is usually associated with the return of copper levels to normal ranges. It has been suggested that serum copper would be a useful indicator for the extent of leukemia and malignant lymphoma and might be a predictor for chemotherapy response.



- Annual incidence of 4.5 cases per 100,000 children in USA.
- No definite study & incidence rate of acute Leukemia in our country is known. However, in BSMMU number of ALL admitted patient is 1135 out of 2315 total patient number.

  (From June 2012-September 2016)

1.2 Acute Lymphoblastic Leukemia

Leukemia or leukaemia (Greek leukos λευκός, "white"; aima αίμα, "blood") literally means “white blood” and is a cancer of the blood or bone marrow characterized by an abnormal increase of blood cells, usually leukocytes (white blood cells). It is part of the group of diseases categorized as hematological neoplasms. Depend on their progression, leukemias are usually divided into two major groups based on their clinical behavior. These are: 1) Acute Leukemia, which progresses quickly with many immature white cells;

and 2) Chronic Leukemia, which progresses slowly with more mature white cells (Pui, 2006).

Normally, the bone marrow contains self-renewing hematopoietic stem cells which develop into different types of mature blood cells including B lymphocytes, T lymphocytes, natural killer cells, various types of granulocytes, red blood cells, and platelets. ALL results from the clonal expansion of lymphoid cells arrested at an early stage of differentiation. ALL accounts for 75% of childhood leukemia cases with an incidence of 3-4 children per 100,000 individuals. Thus, between 2500 and 3000 children in the United States are diagnosed annually. ALL is the most common cancer in children representing about 25-35% of all childhood cancers and its diagnosis peaks between the ages of 2 and 5 years (Xie *et al*., 2003). The incidence of the disease for this age group is approximately 4-fold higher than that for infants and is close to 10-fold higher than that for adolescents (ages 16 – 21 years) (Ries *et al*., 2010). If leukemia is left untreated, it is uniformly fatal.

1.2.1 Etiology of ALL

A long list of often conflicting epidemiologic and environmental studies has attempted to elucidate the etiology of ALL. Two parallel infection-based hypotheses have been postulated based on abnormal responses to infection; a peak in ALL incidence at 2-5 years of age; increased prevalence of the disease in the developed countries; and the presence of some geographical case clustering. Kinlen (2004) predict s that excess cases of childhood leukemia result from exposure to common but low-pathologic infections after population-mixing with carriers occurs. This hypothesis was supported by a U.S study (Warten berg *et al*., 2004) through US SEER data. However, other studies have shown higher incidence of ALL in urban or high-density regions (Li *et al*., 1998; Hjalmars *et al*., 1999; Adelman *et al*., 2005). Greaves (2006) suggested a delayed infection hypothesis based on a two-hit model involving a delayed and dysregulated immune response to common infectious pathogens which leads to the transformation of pre-leukemic cells into acute lymphoblastic leukemia. So far, there is little evidence to support any role of viral transformation a s a cause. Furthermore, it does not appear that there is a single cause for childhood ALL – for most patients, a combination of factors appears necessary. There are conflicting reports of factors that pose an increased risk for ALL, including parental

occupation; maternal reproductive history; parental exposure to pesticides; and even exposures to high levels of residential electric and magnetic fields (Ahlbom, 2000; Buffler *et al*., 2005)

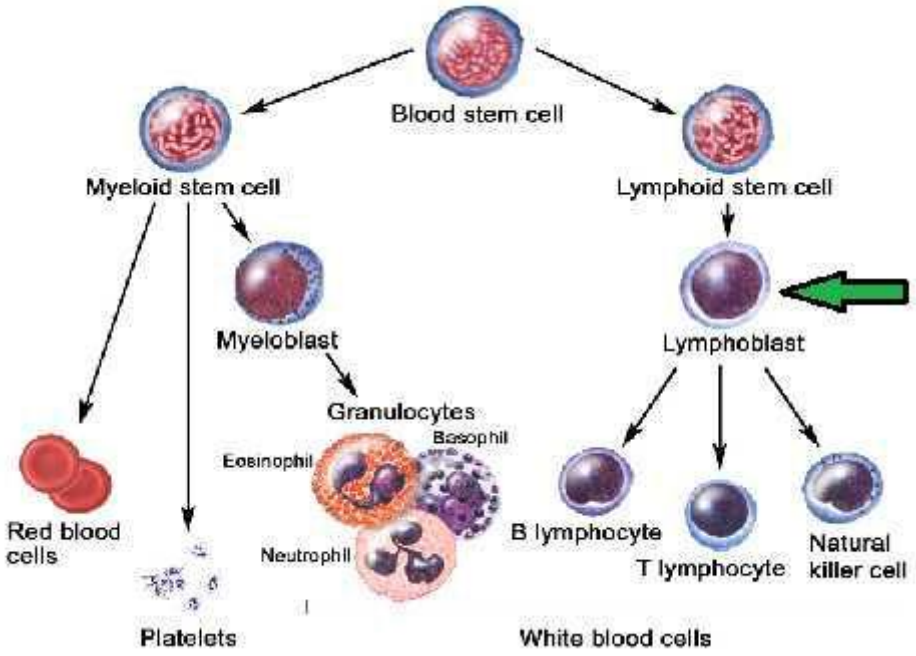


Figure 1.1: Blood cell development. Several steps are needed for a blood stem cell to become a red blood cell, platelet , or white blood cell.
(http://www.cancer.gov/cancertopics/pdq/treatment/childALL/HealthProfessional/)

Recurrent genetic abnormalities have also been observed in ALL patients, including chromosomal translocation that deregulate gene expression; chromosome copy number variations; and gene-specific mutations. The precise biological and genetic mechanisms that lead to the development of ALL, however, remain unknown. Based on phenotypic observation and experimental models, however, it likely involves genes that control lymphoid cells differentiation, resulting in a clonal neoplastic disorder of the hematopoietic system. Recently, studies suggest that the etiology of ALL may be linked to a variety of genetic lesions in blood progenitor cells that are committed to differentiation or in some cases to lesions that arise in a hematopoietic stem cell (HSC) that has multi-lineage differentiation capacity. The cellular micro environment also appears to have an impact on leukemic cell transformation (Armstrong SA *et al*., 2005; Wang *et al*., 2005; Barabe *et al*., 2007).

The importance of trace elements

Trace elements have some fundamental roles within human body like protection against cellular oxidative stress and synthesis and structural stabilization of proteins and nucleic acids. According to the etiology of neoplastic disease and alteration in antioxidants levels, many researches over recent years have studied if trace elements have any modifying effect in clinical outcome (Atieh *et al*.,2012).

Zinc:

Zinc is known as an essential micronutrient for human health because of its structural and biochemical functions, influencing growth and affecting multiple aspects of the immune system. Zinc has been extensively studied in neoplastic processes but its role in children with leukaemia still remains to be elucidated in several aspects (Consolo *et al.,* 2013).

Zinc is an essential component of more than 300 metalloenzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micro nutrients. At the cellular level, the function of zinc can be categorized into Catalytic, Structural and Regulatory. Various enzymes depend on zinc for their ability to catalyze vital chemical reactions within body. Zinc dependent enzymes can be found in all known classes of enzymes. Zinc plays an important role in the structure of proteins and cell membrane. The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function. Zinc finger proteins have been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signaling and has been found to influence hormone release and transmission of nerves impulse (Bimola *et al.,* 2014).

Zinc is an essential element whose significance to health is increasingly appreciated and whose deficiency may play an important role in the appearance of diseases. zinc deficiency can be induced by continued use of low mineral purified foods (minerals are lost during purification), foods containing additives with chelating activity. Gastrointestinal surgery, Crohn's disease, ulcerative colitis, short bowel syndrome, and other digestive diseases can all decrease zinc absorption and increase zinc loss from the body (Jayant *et al.,* 2013)

Copper:

Copper is an essential nutritional element. It is required for normal cell metabolism and is present in all human cells and tissues. The highest concentrations of copper are found in the liver, brain and hair (Gordon, *et al.,* 1997). It's also consider as an essential trace element that participates in metabolic pathways involving cellular respiration, peptide biogenesis, connective tissue biosynthesis and antioxidant defense. It is an acute phase reactant increasing in response to infection, injury and in chronic inflammatory conditions. Copper exists in the human body in both oxidation states, oxidized Cu (II) and reduced Cu (I) (Georgia *et al.*,2012). It serves as a co-factor of the antioxidant enzyme, superoxide dismutase which detoxifies the toxic superoxide radical. It is proven that measuring serum Cu level could be useful in assessing disease activity and response to treatment in some kinds of lymphoma and acute leukaemia (Atieh *et al*., 2012).

Copper is an essential trace element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron donor or acceptor. Conversely, exposure to high levels of copper can result in a number of adverse health effects. Acute copper toxicity is generally associated with accidental ingestion; however, some members of the population may be more susceptible to the adverse effects of high copper intake due to genetic predisposition or disease. Copper status has also been associated indirectly with a number of neurological disorders, including Alzheimer's disease and prion diseases. Depending on the source of the biological material, copper content ranges from parts per billion (ppb) to parts per million (ppm). Copper-deficient diet produces insufficient red blood cells (Bonnie *et al*., 2007).

1.2.2 Clinical Features

The clinical presentation of ALL is often acute, although a small percentage of cases evolve slowly over several months (Pui, 1998 and 2006). The common symptoms include fever, fatigue, bone or joint pain and bleeding. These presenting symptoms correlate with the uncontrolled growth of the malignant cell population invading the bone marrow, lymphoid organs, and extramedullary (outside of the bone marrow) sites. Anemia, neutropenia, thrombocytopenia, lymphadenopathy, splenomegaly, and hepatosplenomegaly are common clinical features. Bone pain is common among younger children with ALL, whose first symptom is often the onset of a limp or a refusal to walk.

These result from the infiltration of periosteum by leukemic cells, bone infarction, or expansion of the marrow cavity. Findings at initial physical examination are usually unremarkable. Asymptomatic lymphadenopathy and hepatosplenomegaly occur in more than half of patients. The most common affected extramedullary site is the central nervous system (CNS). Although CNS involvement at diagnosis is relatively uncommon, when symptoms do occur, the clinical features can include headache, nausea and vomiting, lethargy, and irritability.

Other symptoms include:

Fatigue, Paleness that is resulting from anemia caused by insufficient numbers of red blood cells, Recurrent minor infections due to insufficient numbers of healthy mature white blood cells to fight off infection, Fever without a known cause, Bruising- poor healing of minor cuts, uncontrolled bleeding due to insufficient numbers of platelets (thrombocytopenia).

1.3 Diagnosis

Several procedures can be employed to help confirm the diagnosis of ALL. These include a complete morphologic, immunologic, and genetic examination of the leukemic cells. This is essential to establish the diagnosis of acute lymphoblastic leukemia. The cellular hallmark of ALL, is the presence of lymphoblasts, a relatively undifferentiated cell with diffusely distributed nuclear chromatin, one or more nucleoli, and a basophilic cytoplasm. Accurate diagnosis and classification are the foundation for the successful treatment and biologic study of childhood acute leukemia. Modern classification of leukemia is based on the incorporation of morphologic findings, immunophenotype, and genetic abnormalies, in an attempt to define homogeneous disease subtypes that are clinically and biologically relevant.

1.3.1 Laboratory:

Clinical laboratory findings are usually unremarkable for ALL. The most common laboratory findings in ALL include anemia, thrombocytopenia, neutropenia, and leukocytosis. Other laboratory abnormalities include increased serum levels of uric acid, potassium, phosphorus, calcium, and lactate dehydrogenase (LDH). The degree of

abnormality reflects the leukemic cell burden and cell lysis. Coagulation studies including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen level, and D-dimer level are used to assess the degree of disseminated intravascular coagulation (Pui, 2006).

1.3.2 Pathology:

1.3.2.1 Morphology

The morphologic diagnosis of leukemia consists of two broad steps. First, is establishing a general diagnosis of leukemia and second is classifying the leukemic process. For most patients with ALL, examination of the peripheral blood smear is normally sufficient to reveal leukemic lymphoblasts. Definitive diagnosis, however, usually requires examination of the bone marrow. According to the WHO, at least 25% blast cells are required to render a final diagnosis of acute leukemia. More than three fourths of patients, however, have more than 50% lymphoblasts in the bone marrow at initial presentation.

The lymphoblasts in ALL are relatively uniform in appearance with round to oval to indented nuclei under a light microscope. The classic morphologic characteristics of ALL were established by the 1976 French-American-British (FAB) system, based on the microscopic features of the leukemia cells using Wright-Giemsa-stained smears. The FAB classification system groups ALL into three morphologic sub-groups designated L1, L2, and L3 (Bennett *et al*., 1981). In the most common subtype L1, the lymphoblasts appear small in size, with scant cytoplasm, homogeneous nuclear chromatin and indistinct or absent nucleoli. The less common L2 sub-type presents with large cell size, more heterogeneous nuclear chromatin, irregular/clefting nuclear shape and prominent nucleoli. In practice, the majority of ALLs show a morphologic spectrum between the L1 and L2 subtypes, making the distinction between these categories somewhat arbitrary. The L3 sub-type is rarely seen. It consists of large blasts, regular nuclear features, with an abundant deeply basophillic cytoplasm, finely stippled chromatin, and often prominent nucleoli. Subsequent studies involving the immunophenotype and molecular biology of Burkitt lymphoma, revealed the L3 subtype was the leukemic phase of non-Hodgkin's lymphoma with a mature B-cell immunophenotype (Hecht *et al*., 2000). Up to 10% of lymphoblast cells may depart from the characteristics of each morphology sub-type. The

FAB system lacks independent prognostic significance and thus is seldom used in current medical practice

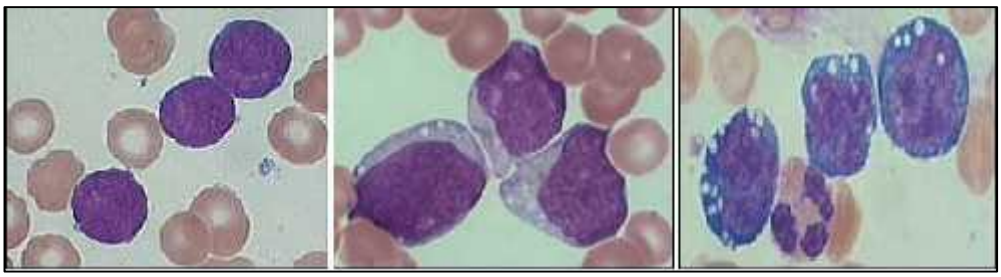


Figure 1.2 Morphological representation of lymphoblasts. L1, left; L2, middle; L3, right. (Source: Dept. of Pathology, The Children's Hospital, Auror a, CO.)

1.3.2.2 Immunophenotype

The diagnosis and treatment of ALL are depended on the recognition of a leukemic population, its lineage, and sometime its stage of maturation. Immunophenotypic studies are an essential part of the diagnostic workup of ALL; the results complement morphologic studies by establishing the leukemic cell lineage, determining the precise stage of differentiation, and often clonality. Unlike morphologic features, immunophenotyping divides acute leukemia into two broad, but clinically and biologically meaningful, categories: B-cell ALL (B-ALL) and T- cell ALL (T-ALL). Several hundred monoclonal antibodies have now been assigned to over 300 cluster differentiation (CD) groupings by the International Workshops on Leukocyte Differentiation Antigens (Manson *et al*., 2002). A panel of antibodies is used to establish the diagnosis of ALL by flow cytometry. This process includes at least one marker that is highly lineage specific. For example, CD 19 marks the B-lineage; CD7 marks the T-lineage; and CD 33 marks myeloid lineage cells. For the B-lineage, the panel often also includes PAX-5 (B-cell specific activator protein), CD20, CD22, and CD79a. A large percentage of B-ALL cases also show expression of CD10 (common acute lymphoblastic leukemia antigen). Other markers should be included for a diagnosis of pre-B cell ALL. These include CD34 and terminal deoxynucleotidyl transferase (TdT). The pre-B-cell group accounts for 80% of ALL cases and is subdivided on the basis of cytoplasmic immunoglobulin (Ig) expression into early pre-B-ALL (which lacks the Ig expression),

pre-B-ALL (with expression of cytoplasmic μ chains, but without Ig light chains), and transitional pre -B-ALL (with cytoplasmic and weak surface expression of μ chains, without Ig light chain expression) (Swerdlow *et al*., 2008; Li *et al*., 2003). T-ALL is characterized by the expression of the T-lineage-associated antigens CD2, CD3, CD4, CD5, CD7, CD8 as well as CD10, CD34, HLA-DR, and TdT (Swerdlow *et al*., 2008).

1.4 Genetic Features

Despite relatively homogeneous morphologic and immunophenotypic features, ALL displays significant heterogeneity at the genetic level. The genetic characteristics define disease subsets with distinct biologic behavior and prognostic implications. They are used in the risk stratification for most modern treatment protocols. Molecular techniques have contributed greatly to our understanding of the pathogenesis and prognosis of ALL through the discovery of various common genetic alterations that occur in leukemic cells. The general underlying mechanisms of ALL are similar. They consist of aberrant proto-oncogene expression; chromosomal translocations that generate fusion transcripts encoding active kinases and altered transcription factors; and hyper diploidy (≥ 50 chromosomes per cell). These genetic alterations are linked to essential changes in cellular regulation and function that support the leukemic transformation of hematopoietic stem cells. These genetic changes enhance the cell's capacity for self-renewal by altering the normal cell proliferation, blocking differentiation, and promoting resistance to programmed cell death (apoptosis).

1.4.1 Chromosomal abnormalities

ALL is associated with several chromosomal abnormalities with distinct biologic features that are critical for modern risk stratification. These abnormalities occur in 60– 80% cases of childhood ALL. Hyper diploidy occurs in almost 30% of ALL cases with favorable prognosis. Leukemia cells with hyper diploidy are more susceptible to the induction of apoptosis and the accumulation of high levels of chemotherapeutic agents or their metabolites. This may explain the favorable outcomes typically observed in these patients. Trisomy (extra copies of certain chromosomes) is another favorable prognostic factor observed in some ALL patients. Patients whose leukemic cells have extra copies of chromosome 4 and 10 appear have a particularly favorable outcome among

hyperdiploid ALL patients. The presence of trisomies 4 and 10 has been incorporated into current treatment protocols. Approximately 1% of childhood ALL cases have less than 45 chromosomes, a condition termed hypodiploidy. These patients are at high risk for treatment failure

1.4.2 Chromosomal translocations

Recurring chromosomal translocations can be detected in approximately 35 – 40% of childhood ALL patients. In some cases, this has prognostic significance. Currently, there is little evidence as to whether translocations are a product of errors in the DNA processing or are caused by external factors such as chemicals or viruses. However, most translocations are not sufficient to cause disease since they are more common (perhaps by up to 100-fold) in the general population than their associated leukemias (Eguchi-Ishimae *et al*., 2001; Mori *et al*., 2002). ETV6/RUNX1 [molecular counter part of t (12;21)] is most commonly observed genetic lesion in childhood B-ALL but it is only rarely seen in T-cell ALL. This genetic lesion is thought to arise in utero in a B precursor cell during fetal hematopoiesis to generate a pre-leukemia clone. It has been suggested, therefore, that this translocation is an initiating event in B-ALL (Greaves, 2006). Patients with ETV6/RUNX1 are generally diagnosed with B-ALL between the ages of 2 – 9 and have excellent treatment outcome even in the case of relapsed patients. The ETV6/RUNX1 fusion transcript probably inhibits the transcription of the normal RUNX1 gene involved in the proliferation and differentiation of hematopoietic cells.

BCR-ABL1 fusion transcripts [known as a Philadelphia chromosome, t (9;22)] are formed as a head-to-tail fusion of the breakpoint cluster region (BCR) gene on chromosome 22 with the Abelson gene (ABL1) on chromosome 9. BCR-ABL1 encodes two distinct BCR-ABL1 oncoproteins, termed p210 and p190, that arise from two different splice sites in the BCR gene (Heisterkamp *et al*., 1985). In chronic myeloid leukemia (CML), the BCR-ABL1 fusion transcript (p210) originates in HSC, whereas the p190 fusion in pediatric ALL appears to arise in a lymphoid lineage precursor. It (p190) is observed in approximately 3% of childhood ALL cases and it confers an unfavorable prognosis, especially when associated with a high White Blood Cell (WBC) count at the time of diagnosis. Philadelphia-positive ALL has higher frequency in older patients,

shows poor response to prednisone, and is associated with a higher level of residual disease after induction chemotherapy.

E2A-PBX1 fusion transcript [molecular counter part of t (1;19)] occurs in 3 – 6% of childhood ALL cases and exits as either a balanced or an unbalanced translocation. This rearrangement generates a fusion transcript that encodes a chimeric transcription factor from the amino -transactivation domain of E2A and the DNA-binding domain of PBX1 (Kamps *et al.*, 1990). E2A-PBX1 may contribute to leukemogenesis by binding and sequestering normal partners of the PBX protein, such as HOX proteins – thereby leading to uncontrolled cell-cycle progression (Aspland *et al.*, 2001). This is primarily observed in the pre-B ALL (i.e., cytoplasmic Ig positive). Some studies suggested that patients with a balanced t (1;19) translocation may do worse than patients with an unbalanced translocation, but this remains a point of debate due to inconsistencies between studies on the subject.

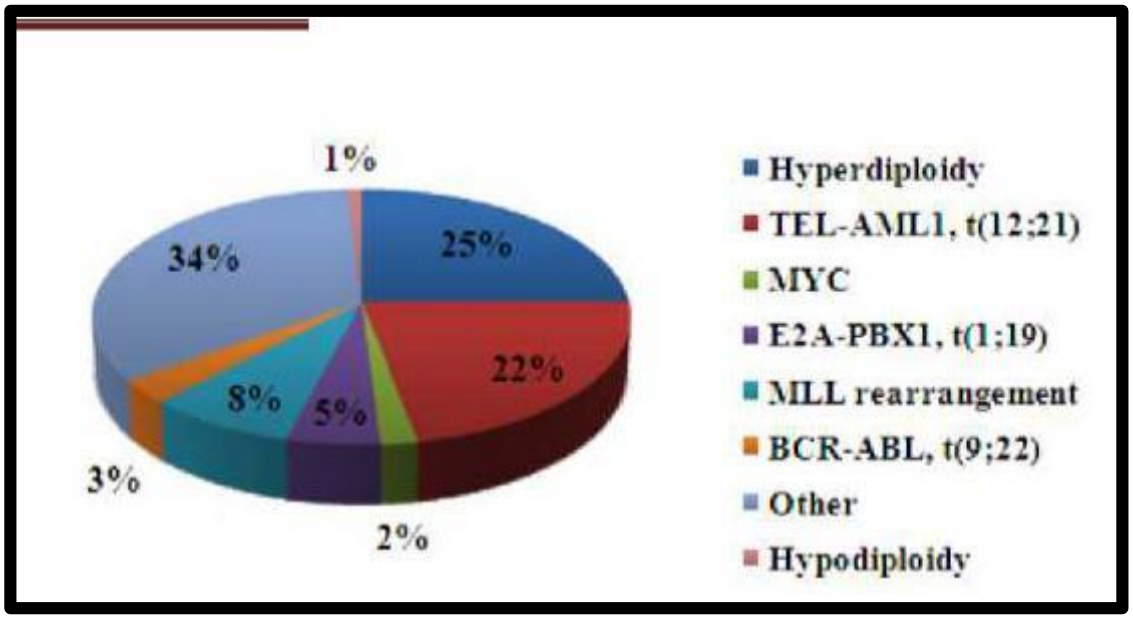


Figure 1.3: Distribution of chromosomal abnormalities in ALL. Data were modified from Pui and Downingg to exclude the T-cell genotypes (Pui *et al*, 2004)

1.5 Prognostic Groups

The National Cancer Institute (NCI) classifies childhood ALL patients into different treatment regimen groups. Patients between 1- 10 years old who have a white blood cell

count of less than 50,000/μl at diagnosis are classified as standard-risk ALL. The remaining patients are classified as high-risk ALL. The very high-risk group is defined by the presence of t (9;22) or hypodiploidy. The prognosis of childhood ALL has improved dramatically over last five decades as a result of adapting therapy to the level of risk of disease relapse and continual reconfiguration/optimization of existing chemotherapeutic drugs.

1.6 Treatment

Leukemia is a systemic disease the treatment for which is primarily based on the use of chemotherapy. The backbone of contemporary multi-drug chemotherapeutic regimens consists of three treatment stages: induction, consolidation/intensification, and maintenance. Successful treatment of children with ALL requires the control of systemic disease as well as treatment of extramedullary disease – particularly in the central nervous system (CNS) because systemically administered anti-leukemic drugs do not cross the blood-brain barrier. CNS prophylaxis therapy, therefore, is generally administrated during each treatment stage. The general aim of therapy in ALL is to cure the patient of the disease which includes: 1) to induce a clinical and hematologic remission; 2) to maintain remission by systemic chemotherapy and prophylactic CNS therapy, and 3) to treat any complications arising from the therapy or the disease. Chemotherapy is the main treatment for ALL. Usually, a combination of chemotherapy drugs and steroids is given according to a treatment plan (often called a protocol or regimen). The treatment is given in several phases, or 'blocks', which are explained below.

1.6.1 Induction

The goal in this treatment stage is to eradicate the signs and symptoms of the disease and to restore normal hematopoiesis. Success of this stage is marked by “clinical remission” (CR). A patient in CR must have no morphologic evidence of leukemia (i.e., <5% lymphoblasts) and a normal complete blood cell count (CBC). CR status also includes the absence of detectable CNS or extramedullary disease as evaluated by microscopic examination of the bone marrow and cerebrospinal fluid (CSF). The treatment regimen consists of a 3-drug (vincristine, steroids and asparaginase) or a more intense 4-drug (i.e., adding anthracycline to the previous mix) treatment combination. For patients who are at

standard-risk of treatment failure, the more intense 4-drug induction therapy appears not to be necessary. In general, more than 95% of ALL patients will achieve a complete remission within the first 4 weeks after diagnosis.

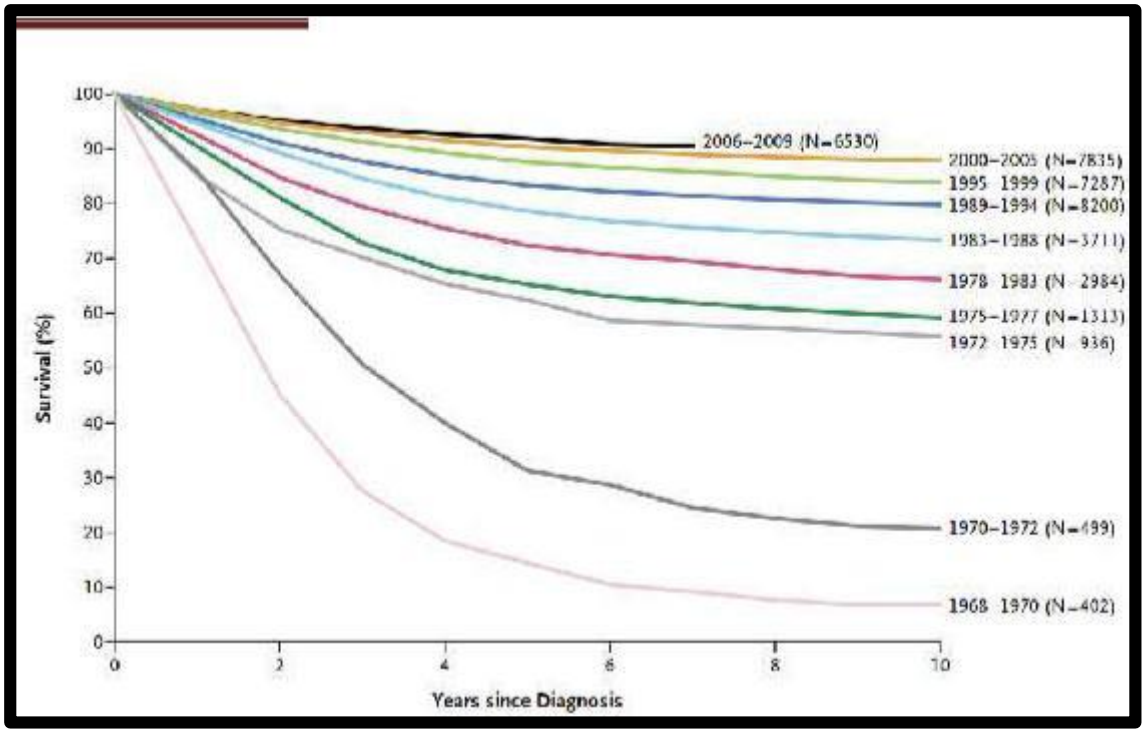


Figure 1.4: Overall Survival among Children with Acute Lymphoblastic Leukemia (ALL) Who Were Enrolled in Children's Cancer Group and Children's Oncology Group Clinical Trials, 1968–2009. (Stephen Hunger *et al*; 2015: The New England Journal of Medicine)

Day-28 bone marrow is evaluated morphologically for CR and its minimal residual disease (MRD) status. Those cases that show a very slow response to induction therapy typically receive an additional 2 weeks of treatment after which, a bone marrow sample is evaluated. Patients with induction failure (< 3%) and a high-risk status receive an additional 4-drug therapy. The early institution of adequate CNS prophylaxis therapy is critical for eliminating CNS disease and preventing CNS relapse in patients without clinical CNS involvement at diagnosis. Intrathecal chemotherapy may be the most effective form of presymptomatic CNS therapy Alternately, methotrexate alone, methotrexate in combination with cytarabine plus hydrocortisone and/or cranial radiation may be used. This regimen usually started at the beginning of induction, intensified during

the consolidation stage, and often continued throughout the maintenance stage. The CNS relapse rate is less than 5% for the standard-risk ALL patients treated with this regimen.

1.6.2 Consolidation

Early studies demonstrated that disease remission alone is insufficient to cure ALL. A significant amount of additional therapy is required to eradicate all malignant lymphoblasts – thus reducing the risk of relapse. Although the importance of this treatment phase is very clear, there is little consensus on the best regimens and duration of treatment. The most commonly used strategies include high-dose methotrexate plus mercaptopurine and reinduction with the same chemotherapeutic agents. The consolidation stage also includes systemic chemotherapy treatment in conjunction with additional CNS sanctuary therapy. The Children's Hospital of Denver belongs to the COG, and thus, its regimen consists of a combination of 6 mercaptopurine, vincristine, corticosteroids, and methotrexate with intrathecal therapy. This stage usually lasts 3 months. Afterwards, a delayed intensification phase (3 months) with asparaginase, cyclophosphamide, doxorubicin, and cytarabine is initiated. This approach has significantly improved the outcome in standard-risk ALL cases.

1.6.3 Maintenance

For reasons that are still poorly understood, patients with ALL require continuation treatment to effectively prevent relapse of the disease. Although approximately two-thirds of ALL patients can be treated successfully with only 12 months of therapy, prospectively identifying those patients with any certainty is impossible (Toyoda Y *et al*.,2000). Therefore, all patients receive additional therapy. The last stage (maintenance) of treatment for childhood ALL includes low dose daily oral mercaptopurine and weekly intravenous methotrexate administration. Maintenance therapy is the longest stage for ALL patients. It generally continues for 2- 3 years from the point of complete remission. In some protocols, additional pulses of vincristine and corticosteroids may be added. A COG randomized trial demonstrated an improved outcome for patients receiving vincristine/prednisone pulses. A meta-analysis of data from multiple clinical trials showed increased event-free survival as well. The CCG-1952 protocol illustrates the common approach used to treat standard-risk ALL Bone marrow treatment is only used for children with ALL that's likely to come back following standard chemotherapy. It can

also be used for children whose leukaemia has come back (recurred) following standard treatment. Some other therapies also may be applied to the patients given bellow.

1.6.4 Testicular radiotherapy

In some situations, it may be necessary for boys to have radiotherapy to their testicles. This is because leukaemia cells can survive in the testicles despite chemotherapy.

1.6.5 Immunotherapy

CD19 is a cell- surface antigen that is present at high density on most B- cell ALL cells. Several groups have developed strategies to transduce autologous T- cells with an anti-CD19 antibody fragment coupled to intracellular signaling domains of the T- cell receptor, thereby redirecting cytotoxic T lymphocytes to recognize and kill B- cell ALL cells. These chimeric antigen receptor–modified T cells provide a major new treatment option.

The successful treatment of childhood acute lymphoblastic leukemia represents one of the great medical accomplishments of the 20th century. Only five decades ago this disease was uniformly fatal. Since the introduction of multi-drug chemotherapy cure rate has risen to over 80%. In fact, the most recent results from the patients with ALL enrolled in Children's Oncology Group (COG) protocols showed an improvement in the 5-year overall survival rate from 83.6% to 90.3% over the last decade (Friedrich, 2009).

1.7 Aim of this thesis

The aim of this thesis work is to determine where there are any association between Child acute lymphoblastic leukemia and nutritional parameter & trace elements in Bangladeshi population. Still no study is done in Bangladeshi population about whether there is association or not. The thesis is divided into four parts. The first part 'Introduction' describes general information available about acute lymphoblastic leukemia, malnutrition and trace element (Zn & Cu) and their relationships. Second part 'Materials and Methods' describes the study design and procedures. Third part 'Result' describes the findings from the study. And fourth part 'Discussion' describes the validation of our result, comparison with other previous studies and success & limitations of the study.

The specific aims of the study is as follows.

- To describe the protein-energy nutritional status and serum zinc and copper of children with newly diagnosed leukemia.
- Find the association of biochemical parameters with onset of ALL in Bangladeshi population.
- These specific aims, taken together, represent a first step toward understanding the relationship between biochemical parameters and treatment outcome in this clinically defined "homogeneous" group of patients.

1.8 Significance of the study

Currently, the investments in nutrition in developed countries have allowed earlier nutritional intervention & treatment & probably because of this, the prevalence of cancer is lower in studies in those countries. It is therefore, vital to pursue an active investigation of nutritional parameters in children with cancer & interrelationship of protein energy nutritional status as well as role of trace elements like zinc & copper and response to treatment.

Chapter Two

Methods and Materials

Methods and Materials

2.1 Study design

The study was designed as a case-control study.

Case: Child with newly diagnosed Acute Lymphoblastic Leukemia (ALL) before chemotherapy between the ages of 1 and 10 years.

Control: Child without history of ALL or any other blood cancer and chronic diseases of similar age.

2.1.1 Study subjects

The study subjects were comprised of ALL (n=101) and control (n=121) groups, all having a Bengali ethnic background, were enrolled in this study. These ALL subjects were recruited from Pediatric Hematology and Oncology department of Bangabandhu Sheikh Mujib Medical University (BSMMU). The ALL subjects have been defined on the basis of clinical and immunophenotype data & 121 healthy child volunteer with no history of ALL or other chronic diseases has recruited as control.

2.2 Ethical Issue

All participants were given an explanation of the nature of the study. They were informed about their rights to withdraw from the study at any time. They were also informed that the identity of the individual and data obtained following analysis would not be disclosed. Available data would only be used for research purpose.

2.3 Questionnaire

All study subjects completed a questionnaire covering information on age, gender, height, weight, family history including occupation of the head of the family, monthly income, smoking habit etc.

2.4 Nutritional assessment by anthropometric methods

Standardized anthropometry measurements of body weight and height were performed by trained nurses, within the first 24 hours of admission. Waterlow's (Waterlow JC et al 1972;3:566-9) classification system of nutritional status, based on the concepts of height-for-age and weight-for-height, was used. Percentage-of-the-median values for each record were calculated from the reference median of the NCHS reference population (NCHS et al,1976).

2.5 Dietary intake history

Mean daily intakes were assessed through the 24-hour dietary recall method, for one day, performed together with the child's mother.

2.6 Collection and Storage of Blood Samples

Blood samples from the child ALL and the control subjects were collected under protocols approved by each participating institution. About 5 mL of venous blood was drawn from each individual with the help of a trained person, using a disposable syringe and in aseptic condition. the blood was transferred to empty tube (for serum). The tube containing blood was centrifuged for 15 min at 2,000 rpm by centrifuge machine (Digi system laboratory instrument, INC) and serum was collected with Pasteur pipette in eppendorf tubes and kept in an ice box for transportation to the laboratory and stores at -20^{o}C until further use.

2.7 Estimation of serum Albumin Method

Bromocresol green forms with albumin in citrate buffer a coloured complex. The absorbance of this complex is proportional to the albumin concentration in the sample.

Contents

RGT	4x100 or 1x1000 ml colour reagent	
	Citrate buffer (pH 4.2)	30 mmol/l
	Bromocresol green	260 µmol
STD	1 x 3 ml standard	
	Albumin	4 gm/dl or 40 gm/l
	Sodium azide	0.095 %

STD is standardized against the certified reference material CRM-DA470k/IFCC.

Reagent preparation

RGT and STD are ready for use.

Specimen

Serum or heparinized or EDTA-plasma.

Stability in serum: at 2-8 ºC up to 1 month

at 15- 25 ºC up to 1 week.

Assay

Wavelength: 578 nm

Optical path: 1 cm

Temperature: 20-25 ºC.

Measurement: Against reagent blank. Only one reagent blank per series is required.

Pipetting scheme

Pipette into cuvettes	Reagent blank	Sample or STD
Sample / STD	-----	10 µl
RGT	1000 µl	1000 µl

Mix, incubate for 5 min. at 20-25 ºC. Measure the absorbance of the sample and the standard against the reagent blank within 30 min.

Calculation

$$C = 4 \times \frac{\Delta A \text{ Sample} * C}{\Delta A \text{ Standard}} \text{ (g/dl)}$$

2.8 Estimation of serum Total Protein Biuret method

Cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

Contents

RGT 4 x 100 ml or 1 x 1000 ml colour reagent.

Sodium hydroxide	200 mmol/l
Potassium sodium tartrate	32 mmol/l
Copper sulfate	12 mmol/l
Potassium iodide	30 mmol/l

STD 1 x 3 ml standard

Protein	8 g/dl
Or	
Sodium azide	0.095 %

Reagent preparation & Stability

RGT and STD are ready for use.

They are stable even after opening up to the given expiry date when stored at 2-25 ºC.

Specimen

Serum or heparinized or EDTA-plasma.

Stability in serum

Up to 1 month at 2-8 ºC**,** up to 1 week at 15-25 ºC**.**

Assay

Wavelength: 520-580 nm

Optical path: 1 cm

Temperature: 20-25 ºC.

Measurement: Against reagent blank. Only one reagent blank per series is required.

Pipetting scheme

Pipette into cuvettes	Reagent blank	Sample or STD
Sample / STD	-----	20 µl
RGT	1000 µl	1000 µl

Mix, incubate for 10 min. at 20-25 ºC. Measure the absorbance of the sample and the standard against the reagent blank within 30 min.

Calculation

$$C = 8 \times \frac{\Delta A \text{ Sample} * C}{\Delta A \text{ Standard}} \text{ (g/dl)}$$

2.9 Estimation of serum Calcium

Principle

Calcium ions form a violet complex with O-Cresolphthaleincomplexone in an alkaline medium. The intensity of the color formed is directly proportional to the amount of calcium present in the sample. The 8-Hydroxyquinoline prevents Mg+ from interference up to 4 mmol/L (100 mg/L)

S.N.		Reagents	Concentrations	Suppliers
1	R1	Aminomethypropanol (pH 10.7)	3.5 mmol/L	EXIM GmbH
2	R2	Detergenzien		EXIM GmbH
		o-Cresolphthalein Complex (CPC)	0.16mmol/L	EXIM GmbH
		8-Hydroxychiloline	6.90 mmol/L	EXIM GmbH
		HCL (pH 1.1)	160 mmol/L	EXIM GmbH
Standard		Standard (Calcium ion)	10mg/dl (2.5mmol/L)	EXIM GmbH

Preparation

2 volumes of R1 was mixed with 1 volume of the R2.

Stability: 4 hours at 18-25ºC

20 hours at 2-8 ºC

Assay Procedure

Aliquot of 20 µL of serum sample, 20 µL of standard solution were taken into two separate eppendrof tubes designed as sample, and standard respectively. Then 1000 µL of working reagent was added to each tube. 1000 µL of working reagent was taken into another tube, used as blank. The contents were mixed well and incubated for 5-30 minutes at room temperature. Then reading was taken against blank at 565 nm using 1 cm light pathway cuvette.

Calculation

$$\text{Calcium (mg/dl)} = \frac{\Delta A \text{ Sample} * C}{\Delta A \text{ Standard}}$$

C = Standard concentration (mg/dl)

3.10 Alanine Aminotransferase Assay

Principle:

Alanine aminotransferase (ALT), also known as serum glutamic pyruvic transaminase (SGPT) is a transaminase enzyme. It is found in serum and in many other body tissues. Alanine aminotransferase in serum converts the L-alanine and alpha-keto-glutarate in the reaction to L-glutamate and pyruvate. Here, the pyruvate that is formed reacts with reduced Nicotinamide Adenine Dinucleotide (NADH) in the presence of lactate dehydrogenase (LDH) to form lactic acid and oxidized Nicotinamide Adenine Dinucleotide (NAD). And, the rate of conversion of the reduced cofactor to the cofactor can be determined by monitoring the decrease in absorbance spectrophotometrically at 340 nm. This rate of conversion from the reduced cofactor to the cofactor is a function of the activity of ALT.

Reagents used:

The reagent kit was purchased from "EXIM-GmbH, Germany" to perform the ALT assay in the samples. The kit contained two (2) reagent bottles (R1 and R2). The compositions of these R1 and R2 are as follows:

Reagent	Composition	Concentration
R1:	TRIS-Buffer (pH 7.5)	100 mmol/l
	L-Alanine	500 mmol/l
	(LDH) Lactate dehydrogenase	≥ 1200 U/l
R2:	2-Oxoglutarate	15 mmol/l
	NADH	0.18 mmol/l

Assay Procedure:

In eppendorf tube 100µl of samples were mixed with 1ml of R1 reagent. Then, after waiting for 1 min, 250µl of R2 reagent was mixed well. These mixtures were incubated for 1 min. Then the change of optical density per minute (ΔOD/min) were measured for 3 minutes.

Calculation:

The activity of ALT in the sample was calculated by the following equation-

Activity of ALT (U/L) =F×ΔOD/min; [F=calculation factor]

(Units: One international unit (IU) is the amount of enzyme that transforms 1µmol of substrate per minute, in standard condition).

2.11 Estimation of Copper, Zinc and Selenium by Atomic Absorption Spectroscopy (AAS)

Principle

The technique of graphite atomic absorption spectroscopy (AAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800ºC.

Digestion of Serum Samples

The serum samples (approximately 200 µL) were digested in Teflon vessels with 9.0 ml nitric acid (70% v/v, supra pure) and 1.0 mL hydrogen peroxides (50% v/v), using a closed microwave digestion system and heated 40 mints. Blank digestion was also performed to quantify possible contamination during sample preparation and analysis. After digestion, each sample was filtered to 50 ml volumetric flask and filled up to the mark with deionized water. This was performed at Bangladesh Council of Scientific and Industrial Research

(BCSIR). Teflon vessels and all glass wares used were previously treated for 24 h in diluted nitric acid and rinsed with double distilled deionized water prior to use.

Analysis of Cu, Zn and Se

The analytical quantification of elements in the serum samples such as (Hg, Pb, Cd, As Cu, Cr, Zn and Se) by graphite Atomic Absorption Spectrophotometry (AAS). Background corrections were applied whenever required during the analysis and the method of standard additions was used to compensate for matrix effects. Performance of the instrument was checked by analyzing the certified reference material (CRM, Japan) concurrently to check the precision of the instrument.

2.12 Statistical analysis of the data

Results were expressed as mean ± SEM (Standard error mean). Data analyses were carried out using the Statistical Package Graph Pad Prism (version 5.03, Graph Pad Software, Inc., California Corporation) for Windows. Odds ratios (OR), as measure of relative risk, at 95% confidence intervals (95% CI) were estimated using logistic regression models. The statistical method used was student's t-test (two tailed), Fisher exact test. Differences were considered significant with $p \leq 0.05$.

Chapter Three

Results

Results

The study was conducted with two groups of subjects: Healthy child control group (n =121) and Child ALL group (n = 101). Blood sample was collected from the study subjects. Serum Albumin, Total Protein, LDH, ALT, Calcium, Copper Zinc and Selenium were analyzed.

3.1: Characteristics of the Study Subject

Distribution of study subjects according to age, gender was summarized in Table 3.1. The Mean ±SEM age of ALL child patients and control subjects was 5.471 ± 0.2960 and 5.631 ± 0.3370 years respectively. As shown in Table 3.1, there were more male child than female between the two groups: 69.3% of cases versus 56.2% of controls were male and 30.7% of the cases versus 43.8% of the controls were female. There was no significant difference in age between these groups (p >0.05).

Child subjects were distributed in two age groups (Figure 3.1) age between 1 to 5 years & above 5 to 10 years. There was significant difference in age between 1 to 5 years than control group (p=.0053). But there is no significant difference in other age group compare to control groups.

Table 3.1: Characteristics of the study subject

Variable	Control (n= 121)	Patient (n =101)	p value*
Age (Year)			
1-5	65 (53.7)	56(55.4)	**.0053**
>5-10	56 (46.3)	45(44.6)	.4205
Mean age, (Years)	5.63 ±0.34	5.48 ± 0.30	
Gender			
Male Children	68(56.2)	70(69.3)	
Female Children	53(43.8)	31(30.7)	

*Results are expressed as number (percentage). Unpaired t-test was performed to calculate the statistical significance. p<0.05 was taken as level of significance.

3.2: Biochemical Parameters in different study groups

Serum Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Calcium, Albumin, Total Protein, Zinc and Copper activity was measured in laboratory and analyzed with the help of statistical data analysis tool.

Table 3.2 Biochemical Parameters in different study groups

Parameter	Patient	Control	P value
WFH<-2SD	50 (49.50%)	17 (14.05%)	**0.0369**
S .Albumin	3.91 ± 0.07	4.18 ± 0.09	**0.0162**
Total Protein	7.22 ± 0.12	7.22 ± 0.17	0.9876
S. Calcium	8.86 ± 0.16	9.12 ± 0.13	0.1962
ALT	61.17 ± 4.98	24.82 ± 0.80	**<0.0001**
LDH	1797.0 ± 119.7	815.6 ± 27.04	**<0.0001**
Zinc	102.5 ± 1.0	77.02 ± 1.54	**<0.0001**
Copper	148.4 ± 2.84	86.14 ± 0.98	**<0.0001**
Selenium	ND	ND	

*Data represented as mean ± SEM. Unpaired t-test was used to determine significant difference between the two groups. P <0.05 was taken as level of significance. ND; Not Detected (Bellow Detection Level)

3.2.1 Anthropometric measurement in different study

49.50% of the children in the case group was wasted (weight for height Z-score lie below -2 SD) as opposed 14.05% in the control group. A high prevalence of malnutrition was found by anthropometric criteria in ALL patients. The t test detected a significant difference (p =0.0369) in the mean weight for height value of ALL patients compared to control group [Table 3.2].

3.2.2 Serum Albumin level in different study group

Serum Albumin of children ALL and control subject shown in Figure 3.1. The mean serum albumin level was 3.905±.06967 (mg/dl) in ALL and 4.182±.08669 (mg/dl) in control group respectively. Significant difference was found in the albumin level of patient group (p =0.0162) compared to control group.

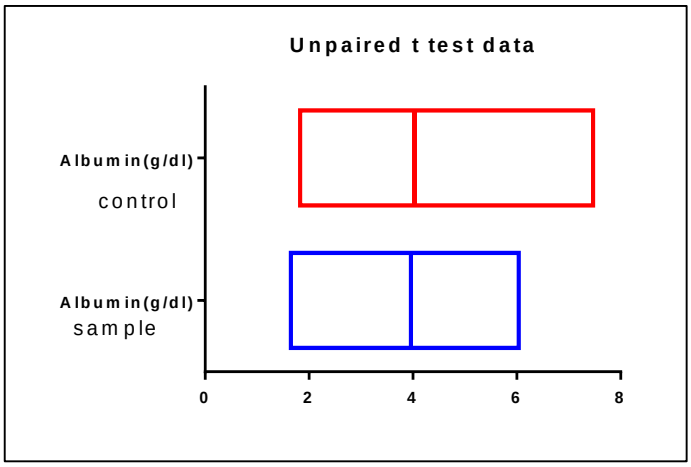


Figure 3.1 Serum Albumin level in different study group

3.2.3 Serum Calcium level in different study group

Serum calcium of children ALL and control subject shown in figure 3.2. The mean serum calcium level was 8.859 (mg/dl) in ALL and 9.118 (mg/dl) in control group respectively. No significant difference was found in the calcium level of patient group (p <0.1962) compared to control group.

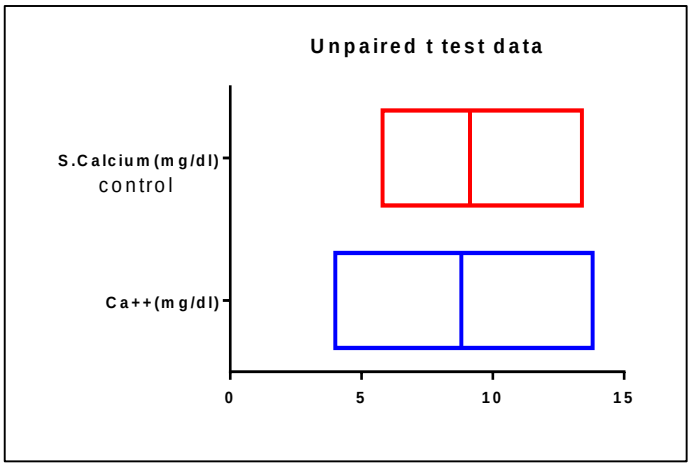


Figure 3.2 Serum Calcium level in different study group

3.2.4 Serum ALT level in different study group

Serum **ALT** of children ALL and control subject shown in figure 3.3. The mean serum **ALT** level was 61.17±4.983 u\l in ALL and 24.82±0.6973 u\l in control group respectively. Highly significant difference was found in the **ALT** level of patient group ($p < 0.0001$) compared to control group.

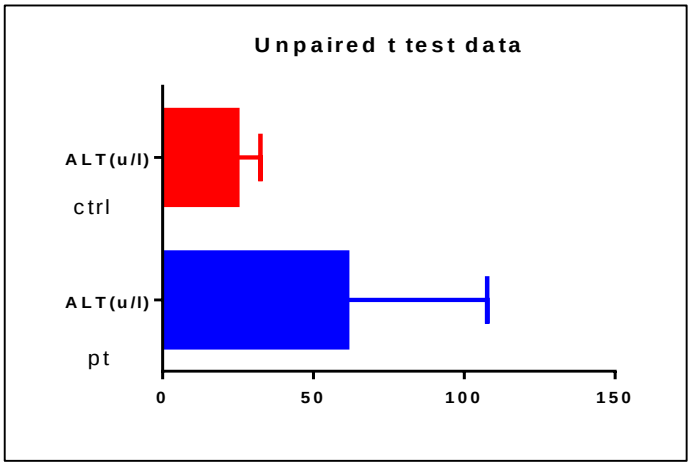


Figure 3.3 Serum ALT level in different study groups

3.2.5 Serum LDH level in different study groups

Serum LDH of child ALL and control subject shown in **figure 3.4**. The mean serum LDH level was 1797±119.7 U/L and 815.6±27.04 u/l in ALL and control group respectively. The LDH level of patient group was significantly higher ($p < 0.0001$) compared to control group**.**

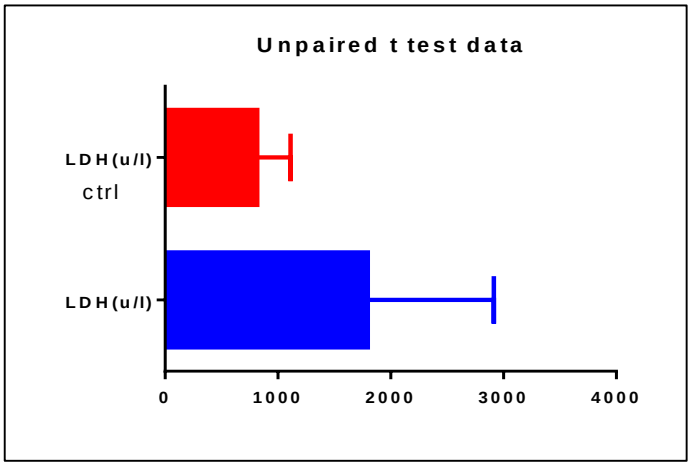


Figure 3.4 Serum LDH level in different study groups

3.2.6 Serum Copper level in different study groups

Serum Copper of child ALL and control subject shown in figure 3.4. The mean serum Copper level was 148.4 ± 2.84 µg/dL and 86.14 ± 0.98 µg/dL in ALL and control group respectively. The Copper level of patient group was significantly higher ($p < 0.0001$) compared to control group.

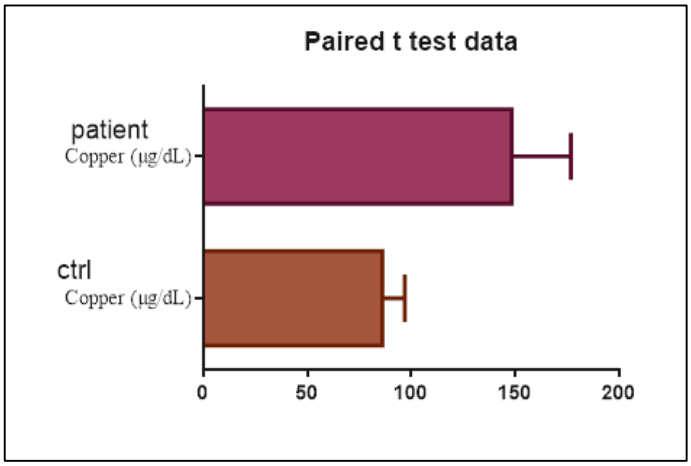


Figure 3.5 Serum Copper levels in different study groups

3.2.7 Serum Zn level in different study groups

Serum Zn of child ALL and control subject shown in figure 3.4. The mean serum Zn level was 102.5 ± 1.0nµg/dL and 77.02 ± 1.54 µg/dL in ALL and control group respectively. The Zn level of patient group was significantly higher ($p < 0.0001$) compared to control group.

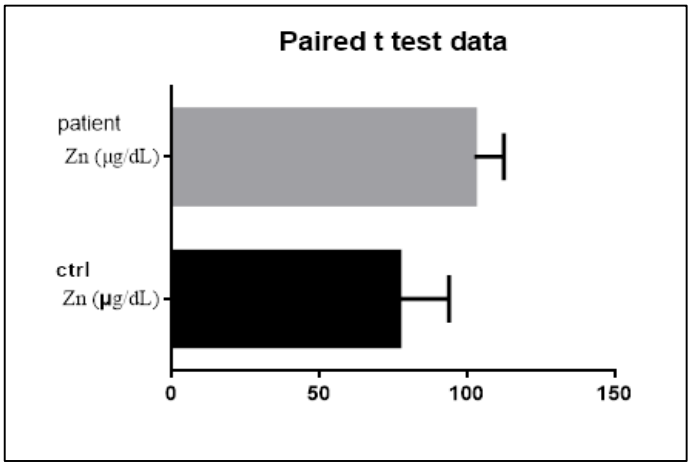


Figure 3.6 Serum Zinc levels in different study groups

3.3 Correlation analysis between biochemical parameters:

3.3.1 Serum Calcium and Albumin among ALL children

The correlation coefficient between serum Calcium and Albumin was r = 0.49 in child ALL group. This indicates that there was a high degree of positive correlation between this two variable in child ALL patient. The correlation was significant (p <0.001).

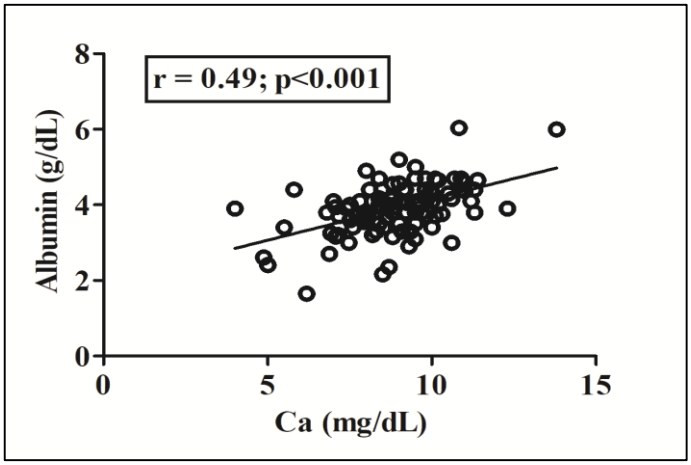


Figure 3.7 Correlation between Calcium and Albumin among ALL patients

3.3.2 Serum Calcium and ALT among ALL children

The correlation coefficient between serum Calcium and ALT was r = 0.30 n child ALL group. This indicates that there was a high degree of positive correlation between this two variable in child ALL patient. The correlation was significant (p<0.01).

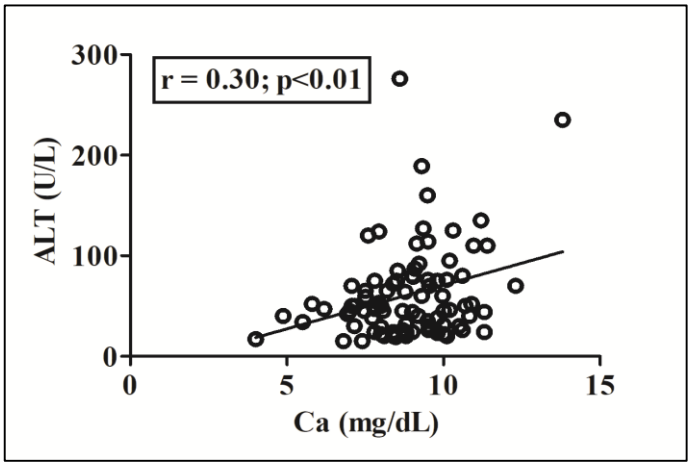


Figure 3.8 Correlation between Calcium and ALT among ALL patients

3.3.3 Serum Calcium and Total Protein among ALL children

The correlation coefficient between serum Calcium and Total Protein was r = 0.48 in ALL children group. This indicates that there was a positive correlation between this two variable in children with ALL and the correlation was significant statistically ($p < 0.001$).

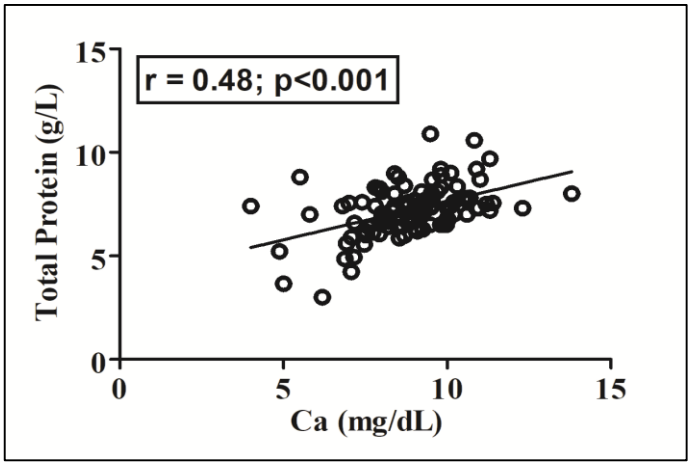


Figure 3.9 Correlation between Calcium and Total Protein among ALL patients

3.3.4 Serum ALT and LDH among ALL children

The correlation coefficient between serum LDH and ALT was r = 0.40 in child ALL group. This indicates that there was a high degree of positive correlation between this two variable in child ALL patient. The correlation was significant ($p < 0.001$).

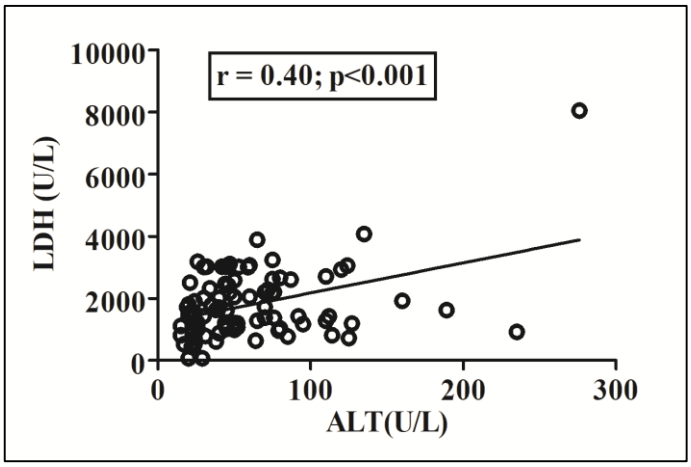


Figure 3.10 Correlation between ALT and LDH among ALL patients

3.3.5 Serum ALB and Total Protein among ALL children

The correlation coefficient between serum ALB and Total Protein was r = 0.59 in children ALL group. This indicates that there was a high degree of positive correlation between this two variable in children with ALL. The correlation was significant (p < 0.001).

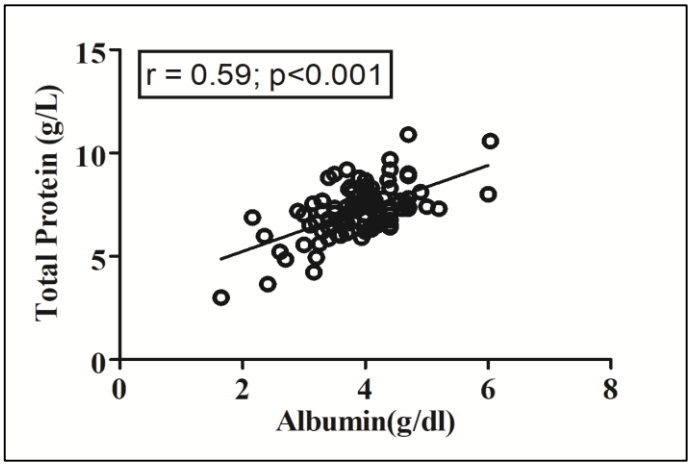


Figure 3.11 Correlation between ALB and Total Protein among ALL patients

3.3.6 Serum Cu and Zn among ALL children

The correlation coefficient between serum Cu and Zn was r = 0.41 in children ALL group. This indicates that there was a high degree of positive correlation between this two variable in children with ALL. The correlation was significant (p < 0.001).

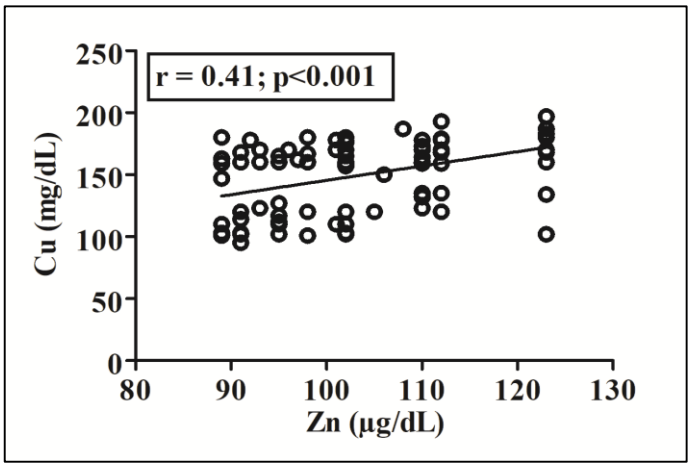


Figure 3.12 Correlation between Cu and Zn among ALL patients

Chapter Four

Discussion

DISCUSSION

Last few years, there has been great improvement in the survival of children with cancer. Hence, worldwide the emphasis now is not just on the longevity but also on the quality of life of these children. Although the association of malnutrition with malignancy has been well known, only recently it has been recognized that the nutritional status of a child with malignancy has a bearing on the treatment and survival.

Nutritional status is multidimensional and can be assessed by methods that are anthropometric, biochemical, dietary, clinical and functional. Weight, height and body mass index are used for most clinical purposes in pediatrics (Wright et al., 1994), and are particularly suitable for patients with ALL because height and weight are measured routinely and carefully (Weir et al., 1998).

The frequency of malnutrition at diagnosis in the present study (49.50% wasted) was significantly higher compared to control group (p=0.0369). Almost 50% ALL patients are malnourished. Viana et al., (1994) who found that 21.2% of children with newly diagnosed ALL had a weight-for-age score of less than – 2SD. In a similar study of ALL patients (Lahorra et al., 1989), the prevalence of malnutrition at the time of diagnosis was found to be 52% by WFA which rose to 88% when arm anthropometric parameters were also evaluated.

The higher number of malnourished children at diagnosis in present study might be that most of the children in BSMMU came from low socioeconomic status (52 ALL patient's family monthly incomes were equal or below 10,000 takas only) and remain usually malnourished to some extent which aggravated during leukemia.

Hypercalcemia is a rare metabolic complication in childhood with an incidence of 0.4% in pediatric oncology patients (Mckay et al., 1993). ALL accounts for approximately 50% of this metabolic complication pediatric age group (Hibi et al., 1997) and in half of these patients, Hypercalcemia is present at diagnosis.

The underlying mechanism of Hypercalcemia in lymphoproliferative disorder has been identified as increased osteoclastic activity mediated by cytokines produced by malignant

cells, as tumor necrosis factor, interleukin 1 and 6 and parathormone-related protein (Roodman et al., 1997).

In contrast reduce bone mineral (including calcium) density and increased fracture risk had reported in child with acute lymphoblastic leukemia (Guens et al., 2010). Decrease in serum calcium level was found associated with leukemia in many studies (Soem et al., 2014; Kaste et al., 2001, 2006 and 2009; Guens et al., 2010).

Our study found no significant difference in serum calcium level in patients compared to control (P=0.1962). We examined our patient's sample at early diagnosis. As because calcium level depends on the osteoclastic activity our study shown non-significant change in calcium level.

In our present study serum Albumin level in ALL patients was significantly lower compared to control (p=0.0162). But the mean level of serum albumin (3.905 mg/dl) was within normal limit in patient's sample. According to study done by Ali Esfahani et al., (2013) pre albumin was common indicator for chemotherapy related complication in patients with both ALL and AML. Early nutritional assessment can help to find patients with acute leukemia who needs nutritional support and it may contribute to better outcome and less toxicity.

Serum aminotransferase (ALT) elevation during and following treatment of childhood acute lymphoblastic leukemia is a common incidence. We investigated that serum ALT level are significantly higher in patients than control group ($p<0.0001$). This finding was in agreement with results of previous study done by Al-Hammami (2015), which reported that patients with ALL showed elevated ALT due to infiltration of leukemic cells. It also agreed with Segal et al. (2010) who reported that elevated transaminases are common at initial presentation of ALL and are likely due to hepatic injury from leukemic infiltrates.

Elevated level of S.LDH is most common in myocardial disease, anemia, leukemia including other malignant tumors. S.LDH rose when immature leukocytes increase in blood and bone marrow (Bierman et al., 1957). We found that serum LDH level are significantly higher in patients compared to control group ($p <0.0001$).The findings of Pui et al., (1985), Kornberg et al., (1980) and Erickson et al., (1961) supported our study. They found mean S.LDH level 5-10 times higher in leukemic patients compared to control .This study suggests that serum

LDH level estimation can be the most potential bio marker for ALL which is consistent with other studies (Hafiz et al., 2007; Kornberg et al., 1980).

Oxidative stress produces free radicals which are involved in the pathogenesis of many diseases including hematological malignancy (Blackburn et al., 1999). Although some possible mechanisms through which oxidative stress exerts a regulatory role in tumor growth and progression including genomic instability, oncogene activation and angiogenesis are known, several important questions remain unanswered (McEligot et al., 2005). Patients with cancer not only have excessive oxidative stress but also altered levels of antioxidants such as SOD, catalase, vitamin E and thiols (Gundogdu et al., 2007).

There are contradictory results regarding the alterations in serum or tissue trace elements and concentrations of serum antioxidant protein in various cancers. The mechanisms by which these alterations occur in certain cancers need to be elucidated. It is also obscure that whether these alterations are a cause or a consequence of the malignancy (Beguin et al., 1987).

In our study all serum trace elements like serum zinc and copper were higher in patients with leukemia than in healthy children and the increase was statistically significant ($P<0.001$).Results of another study showed that serum zinc concentrations were similar in patients with ALL and healthy individuals (Radhakrishnan et al., 2013). Results of another study on patients with lymphoblastic leukemia showed a significant increase in serum concentration of copper and a significant reduction in cell zinc compared to healthy controls (Carpentieri et al., 1986).

Serum concentrations of zinc and copper in healthy children reported in this study are similar to previously published values (Behrman et al., 1983). In addition the variations of serum copper in patients with acute lymphocytic leukemia have been described before (Delves et al., 1973; Tessmer et al., 1972; Andronikashvili et al., 1980). However, the changes in serum zinc concentration found in some leukemic patients (Tessmer et al., 1972; Andronikashvili et al., 1980) were not observed in ours. This discrepancy is possibly due to differences in diet and time of blood sampling since zinc is subject to a circadian rhythm and its concentration is sensitive to dietary intake (Markowitz et al., 1985). One reason for the significant increase in oral intake of copper and zinc could be that, based on our observations, with the onset of

disease, the dietary pattern of the patients becomes healthier, and the intake of nutrients such as nuts, fish, poultry, and dried fruits which are rich in zinc and copper increases.

The changes in ALL may represent either a characteristic of an abnormal clone population of lymphocytes or a phase of the maturation process of normal lymphoid elements. The high turnover of these cells (Norton et al., 1982) may explain the increased concentrations of minerals in the serum, although anemia and poor general nutrition, conditions frequent in acute lymphocytic leukemia, may be additional factors (Foster et al., 1980). Changes in metallothioneins should also be considered (Oestreicher et al., 1985).

In our study we found out that there was low degree of positive correlation between serum LDH and serum calcium (r =0.07408) level in child ALL patients and it was not statistically significant. We investigated that Serum LDH level had a high degree of positive correlation (r =-0.4033) with serum ALT and it was statistically significant. There were also high positive correlations between calcium and albumin (r=0.4933) and calcium and ALT (r=0.2979) and both were statistically significant.

Selenium is an essential trace element that is present in its inorganic forms (Selenate and Selenite) in soil and in its organic forms (Selenomethionine and Selenocysteine) in plants (NIH, 2019). Selenomethionine is also present in animal tissues. Se is a component of over 24 different Selenoproteins, which are important for reproduction, thyroid hormone metabolism, and DNA synthesis, as well as protection from infection and oxidative damage. There has been great interest in Se as a potential Chemopreventative agent against various types of cancers (Vinceti et al., 2014; He et al., 2017). Though many studies demonstrated an inverse association between serum Se levels and cancer incidence (Hurst et al., 2012; Cai et al., 2016), there have been conflicting results from randomized controlled trials with Se supplementation. . Human serum contains very low amount of Se and it’s difficult to measure Se by AAS when Se concentration is low. We have measured Se concentration in serum samples of the study subjects by AAs, but the Se level was bellow detection level.

Overall, in our population-based case control study, we observed statistically significant associations between malnutrition and child ALL risk. The LDH and ALT levels were significantly higher in patients while Calcium level was within normal limit.

CONCLUSION

The present population-based case-control study suggested that there is significant association between malnutrition and risk of developing child acute lymphoblastic leukemia (ALL). So it may be concluded that the nutritional assessment is an important determinant for acute lymphoblastic leukemia in Bangladeshi children. The LDH and ALT levels are significantly higher in patients while albumin level is significantly lower compared to control group. We can use these markers as diagnostic and prognostic tools for children with ALL. Our findings will be very helpful for the physician to treat ALL patients for supportive care modality that has associated with improved tolerance to chemotherapy, improved survival, increased quality of life and decreased risk of infection in children undergoing anticancer therapy. It will also be useful for introducing personalized medicine for Bangladeshi population. Our sample size was not large enough. Further study is needed with a larger sample size to have the concrete result.

Chapter Five

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