SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL

BIOCHEMISTRY

Evaluation and characterization of tumor lysis syndrome before and after chemotherapy among pediatric oncology patients in Tikur Anbessa Specialized Hospital

Haileleul Micho, Bsc in medical laboratory technology

A Master's thesis submitted to the department of medical Biochemistry, school of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of medical Biochemistry

August, 2017

ADDIS ABABA, ETHIOPIA

Addis Ababa University

School of Graduate Studies

This is to certify that the dissertation prepared by Haileleul Micho, entitled: ***Evaluation and characterization of tumor lysis syndrome among pediatric oncology patients in Tikur Anbessa Specialized Hospital before and after chemotherapy*****,** 2016/17 and Submitted in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of medical Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner: Dr. Wondimagegn Tigeneh Signature……..Date…….

Advisors: Dr. Solomon Genet Signature……..Date……

Dr. Daniel Hailu Signature…….Date…….

_____

Chair of Department or Graduate Program Coordinator

Principal investigator:

Haileleul Micho

Addis Ababa University, School of Graduate Studies

Department of Biochemistry

E-mail: liulmicho@gmail.com

Mobile number: +251913573518

Advisors:

Solomon Genet (Ph.D., Assistant professor)

Addis Ababa University, School of Medicine

Department of Biochemistry

E-mail: solgen73@yahoo.com

Mobile number: +251933944457

Daniel Hailu (MD)

Addis Ababa University, School of

Medicine Department of Pediatrics and

Child Health

E-mail: danieldk.dh@gmail.com

Mobile number: +251911407949

Declaration

I declare that this research paper entitled: ***Evaluation and characterization of tumor lysis syndrome among pediatric oncology patients in Tikur Anbessa Specialized Hospital before and after chemotherapy*****,*** 2016/17 is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research have duly been acknowledged.

Haileleul Micho

Signature …….....

Date …….

ACKNOWLEDGMENT

First for most, Glory to almighty GOD and his mother virgin saint Mary; for his unreserved blessing and endless care and support in every part of my life and success. GOD has a reason and every reason of him is for better success of me. My acknowledgement goes to Addis Ababa University Department of Biochemistry for giving me this opportunity and financial assistance to undergo this research. My gratitude also goes to Dilla University for sponsoring my study and financial support for my living expenditure.

I would like to express my special deep heartfelt gratitude to my research advisors and live mentors Dr. Solomon Genet and Dr. Daniel Hailu for their encouraging advice and for they have been engaged in this study from the conception of research title to the end of this thesis write up with unwavering support, guidance and vital supervision.

My special gratitude goes to Dr. Ayalew and Dr. Workabeba for their supportive and warm welcome at the first contact and for their timely and invaluable comments during proposal development and approval.

I would like to take this opportunity to give my warm gratitude to the Addis Ababa University medical faculty department of pediatrics and child health staff members and the study participants and their family for their cooperation and support during the study period.

Last but not least, I would like to thank my family and my beloved mother and all dear friends.

Contents

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

AKI	Acute Kidney Injury
ALL	Acute Lymphoid Leukemia
AML	Acute Myeloid Leukemia
ARF	Acute Renal Failure
BL	Burkitt's Lymphoma
BUN	Blood Urea Nitrogen
CKD	Chronic Kidney Disease
CLL	Chronic Lymphoid Leukemia
CML	Chronic Myeloid Leukemia
Co_2	Carbon Dioxide
CTLS	Clinical Tumorlysis Syndrome
DHBS	Dichloro Hydroxyl Benzexsulfonic Acid
ECG	Electrocardiogram
FDA	Food and Drug Administration
GLDH	Glutamate Dehydrogenase
H_2O_2	Hydrogen Peroxide
IQR	Inter Quartile Range
ISE	Ion Selective Electrode
IVF	Intra Venous Fluid
LDH	Lactate Dehydrogenase
LMIC	Low and Middle Income Countries
LTLS	Laboratory Tumorlysis Syndrome
MCP-1	Chemo Attractant Protein-1
MIF	Migration Inhibition Factor
NAD	Nicotinamide Adenine Dinucleotide
NH_3	Amonia
NHL	Non-Hodgkin Lymphoma
OAT	Organic Anion Transporter
pH	Power of Hydrogen
POD	Peroxidase

RFT	Renal Function Test
SNNPR	Southern Nation Nationality Peoples' Region
TASH	Tikur Anbessa Specialized Hospital
TLS	Tumor Lysis Syndrome
ULN	Upper Limit of Normal
URAT-1	Urate Transporter 1
WBC	White Blood Cell Count

ABSTRACT

Background: *Tumor lysis syndrome (TLS) is a life-threatening emergency disorder, caused by an abrupt release of intracellular metabolites after tumor cell death. It is characterized by a series of metabolic manifestations, especially hyperuricemia, hyperkalemia, hyperphosphatemia and hypocalcemia.*

Objective: *The aim of the present study was to evaluate and characterize the incidence of tumor lysis syndrome in pediatric oncology patients before and after treatment in Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.*

Materials and Methods: *Hospital based prospective cohort study was conducted in 61 newly diagnosed and admitted pediatric oncology patients in TASH in six moth duration. Purposive and convenient sampling technique was employed for the selection of health facility and study participants respectively. Socio-demographic data was collected by interview administered questionnaire. The patients were followed and the physical diagnosis, imaging and laboratory results were interpreted by senior physicians. Data was entered to and analyzed by SPSS version 23.*

Results: *Out of 61 pediatric oncology patients 39(63.9%) were males. The mean (±SD) age of the pediatric patients was 6.39* (±3.67) *years ranging from 2 months to 14 years. Total of 29.5% of patients were found to have TLS. There were 11.5% and 18.0% of laboratory TLS (LTLS) and clinical TLS (CTLS) cases respectively. There were 72.2% spontaneous and 27.8% treatment induced TLS. There was 23% and 21.3% cases of hyperuricemia and 4.9% and 6.6% cases of hyperkalemia incidence before and after treatment respectively. Overall two patients died, in the study period, because of TLS.*

Conclusion: *The pediatric oncology patients in this study had a high incidence of TLS. TLS occurred irrespective of socio-demographic variability in the study participants. This suggests that every child with cancer is at a risk of developing TLS. As TLS is a life-threatening complication of malignancies, early identification of patients at risk and reducing morbidity and mortality is of crucial importance.*

Key words: *tumor lysis syndrome, pediatric oncology, metabolic derangement, cell death*

1. INTRODUCTION

1.1. Back Ground

Cancer represents a group of heterogeneous diseases that affect humans with high frequency and contribute in significant manner to overall morbidity and mortality. Globally the prevalence and incidence of cancer is currently increasing at an alarming rate. Around 14.1 million people were affected by cancer and 8.2 million died as a result of cancer and cancer related complication in 2012 (Ferlay *et al*., 2015). Globally, the number of new cancer cases in all age groups will increase from 12.7 million in 2008 to 22.2 million by 2030 (Rodriguez-Galindo *et al*., 2015).

Globally, there are around 250,000 new cases of cancer diagnosed annually, in children under 15 years of age, but only about 20–30% of patients, mostly residing in high income countries, are adequately diagnosed and treated (Ribeiro *et al*., 2008). This significant gap in diagnosis and treatment exists despite the important advances in pediatric oncology that have produced dramatic improvements in survival rates in developed nations. Currently, more than 80% of children with cancer who are treated with modern multidisciplinary treatments in developed countries are cured (Rodriguez-Galindo *et al*., 2015).

However, in low and middle income countries (LMIC), notably those in sub-Saharan Africa, cure rates lag considerably, with often less than 25% of the children surviving (Ribeiro *et al*., 2008, Kellie *et al*., 2008). Many challenges exist in treating cancer effectively in LMIC including the lack of availability of common chemotherapeutic agents, cost of treatment, late stage at presentation, and limited radiotherapy and surgical resources making oncology care ultimately ineffective in achieving cure (Ndom *et al*., 2008).

The situation in Ethiopia, as observed from extrapolation of clinical records of the Tikur Anbessa Specialized Hospital (TASH) Radiotherapy Centre estimates is, that there are around 120,500 new cancer cases/year, although GLOBOCAN estimates are much lower (i.e, 51,000 per year) (Shad *et al*., 2013). Most of the patients present with advanced disease and there is a high rate of defaulting in the Ethiopian scenario.

Tumor lysis syndrome (TLS) is one of the most common cancer therapy related complication, first described by Bedrna and Polcák in 1929. It is a life-threatening condition with high morbidity and mortality, caused by an abrupt release of intracellular metabolites after tumor cell lysis. This leads to series of metabolic manifestations, especially hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcaemia. Besides seizures and cardiac arrhythmias, acute kidney injury (AKI) is the hallmark of TLS, which determines the clinical outcome (Alakel *et al.*, 2017).

While tumor lysis syndrome may occur spontaneously before treatment, but it usually develops shortly after the initiation of cytotoxic chemotherapy (Edeani, A. and Shirali, A., 2016). It usually occurs a few hours to a few days after commencing cytotoxic chemotherapy for tumors with a high percentage of proliferating and drug-sensitive cells. Cell death leads to the release of potassium, phosphate, uric acid, proteins and other purine metabolites into the systemic circulation. These factors will overtax the body's homeostatic mechanisms and overwhelm the capacity for normal excretion of these materials. When the renal clearance of these chemical moieties is overwhelmed, hyperkalemia, hyperuricemia, hyperphosphatemia and secondary hypocalcemia will result. Serum lactate dehydrogenase (LDH) levels are also often elevated concurrently (Cairo *et al.*, 2004).

Uncontrolled TLS progresses to lactic acidosis and acute renal failure (ARF). Clinically, this results in multi organ effects such as acute kidney injury (AKI), cardiac arrhythmias, and seizures or sudden deaths that require intensive care. TLS is an oncologic emergency, and without prompt recognition and early therapeutic intervention, morbidity and mortality is high (Gupta *et al.*, 2015).

TLS has been reported in association with a wide variety of tumors. It is most commonly seen in hematologic malignancies with large disease burden and high cell turn over and /or high leukocyte counts (Altman *et al.*, 2001). Patients at highest risk for TLS often are diagnosed with bulky, rapidly proliferating hematologic tumors, such as acute leukemia and non- Hodgkin lymphoma (Kaplow *et al*., 2007). Patients with elevated lactic

dehydrogenase (LDH), dehydration, and renal insufficiency are at greatest risk for developing TLS (McGraw *et al.,* 2008).

Hande and Garrow (1993) first initiated a definition of the clinical and pathologic characteristics of patients at risk for developing TLS. They classified TLS as laboratory TLS (LTLS) or clinical TLS (CTLS). Cairo and Bishop modified these criteria to formulate a commonly used classification system for TLS. This system defines LTLS when two or more of the following abnormalities are met within 3 days before or 7 days after the initiation of chemotherapy in the face of adequate hydration and use of uric acid lowering agent:

1) 25% decrease from baseline in serum calcium, and/or

2) 25% increase from baseline in the serum values of uric acid, potassium, or phosphorous.

Table 1: Cairo-Bishop definition of laboratory tumor lysis syndrome and clinical tumor lysis syndrome

Laboratory tumor lysis syndrome	
Metabolite or electrolyte	Criterion for diagnosis
Uric acid	≥8 mg/dL or 25% increase from baseline
Potassium	≥6 mEq/L or 25% increase from baseline
Phosphorus	≥6.5 mg/dL (children), ≥4.5 mg/dL (adults), or 25% increase from baseline
Calcium	≥25% decrease from baseline
Clinical tumor lysis syndrome	
LTLS and one or more of the following: 1) creatinine ≥1.5x ULN (age>12 years of age or age adjusted); 2) cardiac arrhythmia or sudden death; 3) seizure	

CTLS is defined as LTLS accompanied by one or more clinical manifestations such as cardiac arrhythmia, AKI, seizure, or death with an elevated serum creatinine >1.5ULN (upper limit of normal) (Sevinir *et al.*,2011).

1.2. Literature Review

1.2.1. Epidemiology and Etiology of TLS

TLS was first reported almost 90 years ago, but its incidence remains ill defined (Davidson *et al*., 2004). Reasons for the inability to precisely define TLS incidence was predicted by Cairo *et al*. This includes variations in defining the syndrome, variations in anticipating and studying its development in selected patient populations, and failure to report all occurrences (Gupta *et al*., 2015).

There are also other reasons which might explain the inability to precisely define TLS incidence. These include the variability of patient populations analyzed in TLS studies and case reports. Further, the prevalence varies among different malignancies, types of anticancer therapies used, and prophylactic procedures followed. Explained by the high rate of cell turnover and sensitivity to cytotoxic therapies, the incidence is higher among hematologic malignancies (Coiffier *et al*., 2008).

Hande and Garrow undertook a retrospective analysis of 102 adult patients with high grade non-Hodgkin's lymphoma. They reported the incidence of TLS identified through serial measurements of laboratory values. The incidence of laboratory TLS was found to be 42%, where as the incidence of clinically significant TLS was only 6% in the same population (Hande and Garrow, 1993). In another study Wössman *et al* reported the incidence of TLS to be 26.4% in children with B cell acute lymphoblastic leukemia (Wössman *et al*., 2003).

A descriptive study conducted by Fauzia *et.al.,* at Liaquat to determine frequency of tumor lysis syndrome on hematological malignancies revealed that out of 50 patients 10 fulfilled the criteria for TLS, six patients developed laboratory tumor lysis syndrome (LTLS), whereas four developed clinical tumor lysis syndrome. In the same study it was reported that acute renal failure was observed in four out of ten patients and Overall three patients died because of TLS (Wasim *et al*., 2012).

A retrospective study done in Turkey on 327 hematologically malignant children (113 NHL and 214 ALL) showed that hyperuricemia occurred in 26.5% and 12.6% of the

patients with NHL and ALL respectively. The corresponding figures for TLS were found to be 15.9% and 0.47% (Sevinir *et al*.,2011). Another review of 755 patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and non-hodgkin lymphoma (NHL) found that 18.9% developed hyperuricemia, 5.3% developed TLS and 0.9% Tumor lysis related mortality (Annemans *et al*., 2003).

A Prospective multicentre cohort study conducted by Darmon *et al*, that included 153 consecutive patients with malignancies at high risk for TLS reported that Laboratory TLS developed in 11.1% of patients and clinical TLS with AKI in 19.6% of patients. In this study, TLS occurred in 30.7% of high-risk patients. One third of all patients experienced AKI, for which TLS was an independent risk factor. TLS was associated with increased mortality, indicating a need for interventional studies aimed at decreasing early TLS-related deaths (Darmon *et al*., 2013). In an observational study of 772 patients with AML conducted by Montesinos *et al*, prevalence of TLS was 17% and was considered the major cause of death in 2% of patients (Montesinos *et al*., 2008).

TLS is usually therapy induced and associated primarily with chemotherapy, immunotherapy, and corticosteroids. Other stimuli and clinical conditions, such as acidic urine, azotemia, dehydration, oliguria, preexisting renal dysfunction, treatment-induced acute renal failure, large tumor burden, lactate dehydrogenase >1500 IU/L, mediastinal mass, Organomegaly and White blood cell count >50,000 cells/mm^3 can predispose patients to TLS. Certain treatments, such as excessive urine alkalinization that causes calcium and phosphate to precipitate in the renal tubules can also predispose a patient to the complications of TLS (Navolanic *et al*., 2003). TLS may also occur in other tumour types, especially tumors sensitive to cytotoxic treatment, that have a high proliferative rate or have a large tumuor size or burden (Cairo *et al*., 2010).

1.2.2. Risk stratification of TLS

Risk factors for TLS include cancer and patient-specific factors (Tosi *et al.,*2008). Increased tumor burden is the most cancer-specific risk factor and is demonstrated by elevated LDH, white blood cell count ≥50,000/mm^3, massive liver metastasis, bone marrow involvement, cancer stage, proliferation rate of cancer cells, and cell sensitivity

to cytotoxic therapy. Patient-related factors include age, volume depletion, preexisting CKD, hyperuricemia, and hyponatremia (Mirrakhimov *et al.,* 2014). Recognition of these high-risk factors is an important step in the management of TLS.

Other factors that have been shown to be predictive of TLS include male sex and presence of splenomegaly (Mato *et al*., 2006). Certain cytogenetic shifts may also portend greater risk for TLS. Specifically, MYCN gene mutation in neuroblastoma (Kushner *et al.,* 2003), t(8;14)(q24;q32) in L3 type of acute lymphoblastic leukemia, and inv(16)(p13;q22) in acute myelocytic leukemia (Seftel *et al*., 2002). are all linked to more aggressive disease and greater risk for TLS.

1.2.3. Pathophysiology and clinical manifestation of TLS

1.2.3.1. Hyperuricemia

The nucleic acids adenine and guanine are metabolized to xanthine, which is further metabolized by xanthine oxidase to the water-insoluble uric acid (Wilson *et al*., 2012) (Figure 1). Because humans lack a functional gene for urate oxidase (uricase), which further metabolizes uric acid to the freely soluble and excretable allantoin, patients with high-risk malignancy are susceptible to rapid increases in serum uric acid. Uric acid is freely filtered at the glomerulus, and handling in the renal proximal tubule is a combination of reabsorption and secretion via the luminal urate/anion exchanger urate transporter 1 (URAT-1) and the basolateral organic anion transporter (OAT). It is critical in regulating urate levels and is targeted by uricosuric and antiuricosuric agents (Enomoto *et al*., 2002).

When the capacity to transport luminal uric acid is overwhelmed, there is a potential for uric acid to crystallize within the tubular lumen. Uric acid crystals can cause tubular injury by obstruction, by induction of chemokine-mediated inflammation from monocyte chemo attractant protein-1 (MCP-1) and macrophage migration inhibition factor (MIF) (Umekawa *et al.,* 2003). There are also crystal-independent mechanisms which target hemodynamics, which include: increased peritubular capillary pressures, increased vasoconstriction, and decreased blood flow (Kang *et al*., 2005, Feig *et al.,* 2008). Uric acid may also prevent recovery from AKI in TLS, as it has been shown to inhibit proximal

tubule cell proliferation (Han *et al.,* 2007). These diverse mechanisms are united in their propensity to cause AKI.

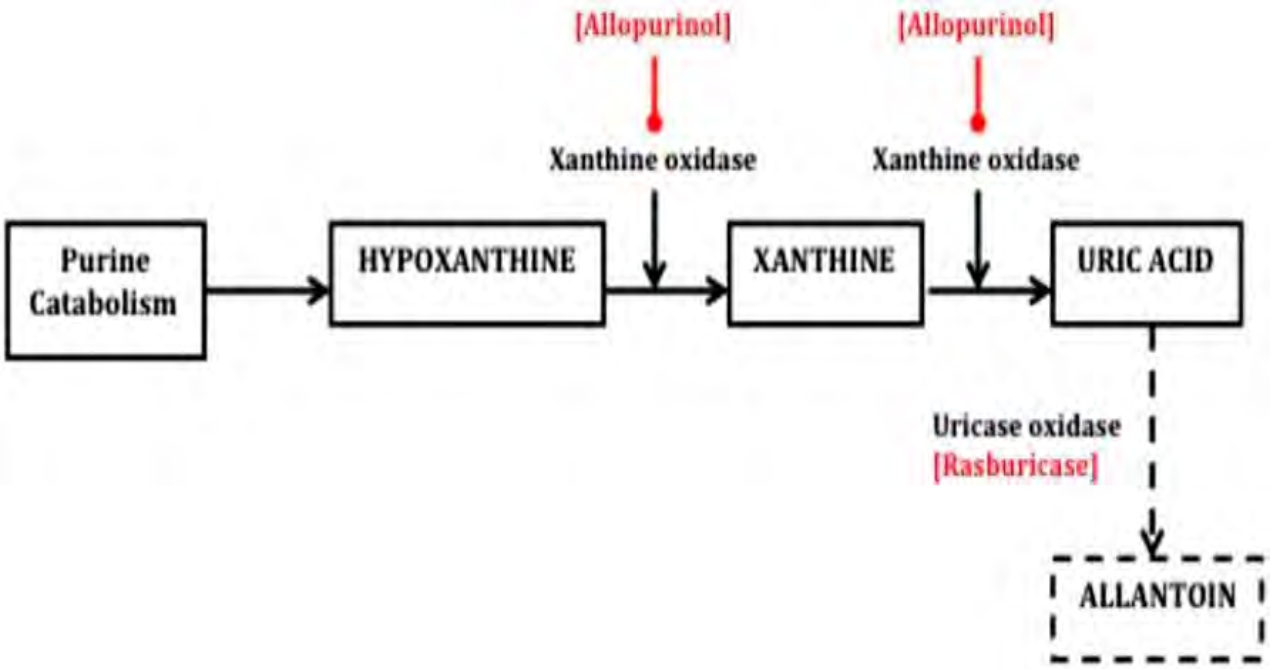

Figure 1: Schematic of purine metabolism. Allopurinol acts as an inhibitor of xanthine oxidase via its active metabolite, oxypurinol. Dashed arrow and box indicate arm of metabolism not constitutively present in humans; this conversion of uric acid to water-soluble allantoin is stimulated clinically by the administration of rasburicase (recombinant urate oxidase). Black arrows denote enzyme stimulation; red lines denote inhibition. (Edeani A. and Shirali A. 2016)

1.2.3.2. Hyperkalemia

Hyperkalemia is defined as a serum potassium level which is above 6.0 mmol/L and occurs due to excessive potassium release from destructed tumor cells. If the patient is given potassium support by intravenous fluid, iatrogenic hyperpotassemia develops (Celkan *et al.,* 2013). Therefore; potassium should not be used in fluids, when TLS is suspected.

The clinical findings of hyperpotassemia include nausea, malaise, vomiting, diarrhea, muscle weakness, cramps, paresthesia and paralysis. Cardiac side effects include peaked T wave on electrocardiogram (ECG), prolongation of PR interval, enlargement in QRS complex, asystole, ventricular tachycardia or fibrillation, syncope and sudden death (Rampello *et al.,* 2006).

Hyperkalemia is often a first sign of TLS, because potassium may start to leave dying cancer cells before they lyse. This electrolyte imbalance can impair normal cardiac function and cause lethal dysrhythmias. The kidneys, overwhelmed by excess potassium in the bloodstream, may be unable to excrete enough potassium to compensate for the hyperkalemia (Held-Warmkessel, 2010).

1.2.3.3. Hyperphosphatemia and hypocalcemia

Hyperphosphataemia results from the rapid release of intracellular phosphorous from malignant cells, which may contain as much as four times the amount of organic and inorganic phosphorous as compared to normal cells (Cairo *et al.,* 2007). Because phosphorus excretion is tied to kidney function, hyperphosphatemia occurs when the kidney's excretory capacity is overwhelmed.

Hyperphosphatemia may cause nausea, vomiting, diarrhea, or lethargy, but it exerts its predominant toxicity by binding to calcium ions. This results in secondary hypocalcemia and its downstream neuromuscular and cardiovascular effects such as cramps, hypotension, tetany, and arrhythmias. When the phosphate concentration is raised it will lead to precipitation of calcium phosphate crystals. These crystals in turn will lead to the development of nephrocalcinosis, urinary obstruction and tissue deposits (Darmon *et al*., 2013).

1.2.3.4. Acute kidney injury (AKI)

AKI in TLS may be either due to the aforementioned effects of acute urate nephropathy or hyperphosphatemic nephrocalcinosis affecting the renal tubulointerstitium or a

combination of the two. The association between AKI and TLS has been demonstrated across various populations and tumor subtypes (Wilson *et al*., 2012).

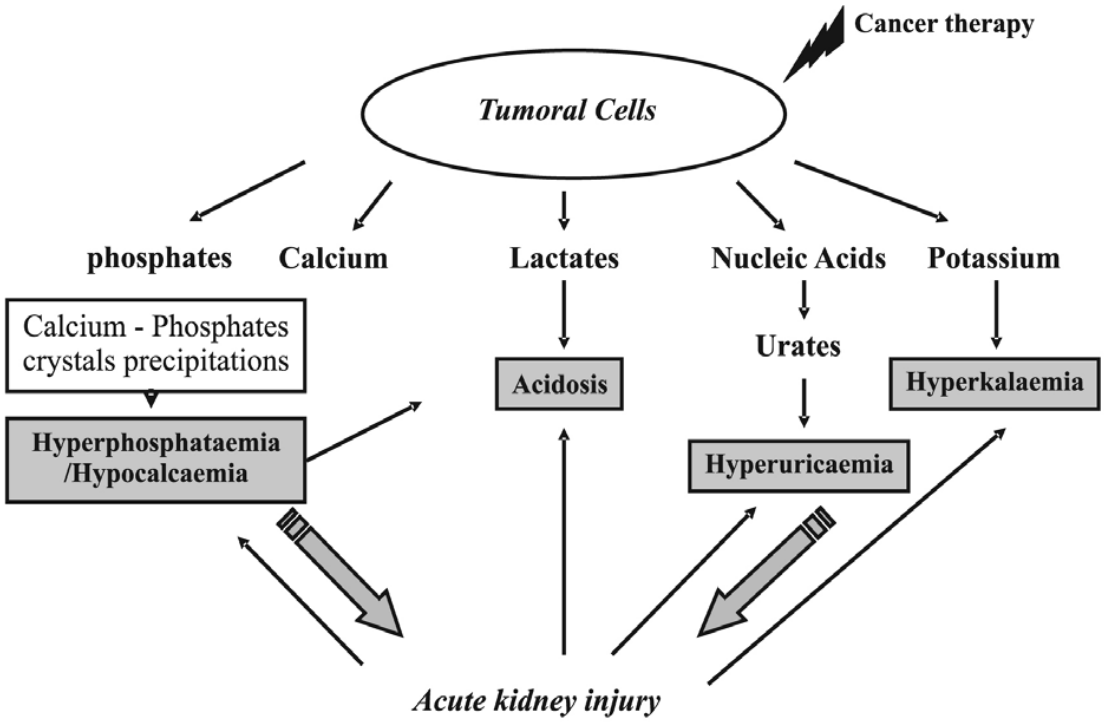

Figure 2: Pathophysiology of the tumor lysis syndrome (Michael Darmon, 2008)

1.2.4. Overview of Clinical Management of TLS

1.2.4.1. Prophylaxis and monitoring

Several key considerations and specific tasks are fundamental in the management of TLS. These include risk assessment, fluid management for TLS prophylaxis, and appropriate drug therapy for prophylaxis and TLS treatment (McBride *et al.,* 2012). Prevention of TLS begins with recognition of risk factors and close laboratory and clinical monitoring. Patients at highest risk of developing TLS require intensified monitoring with more frequent electrolyte checks. Patients with high-risk disease may be prone to lactic acidosis from massive tumor cell necrosis. Because acidosis inhibits uric acid excretion,

prompt recognition and correction of acidosis may prevent or ameliorate uric acid nephropathy (Ansari *et al.*, 2012).

1.2.4.2. Volume expansion

Delivery of crystalloid intravenous fluids (IVFs) is recommended for all patients and is essential for those with higher TLS risk. Volume expansion supports adequate intravascular volume and renal blood flow, which maintain glomerular filtration. This is the cornerstone of uric acid, potassium, and phosphate excretion and may delay and prevent the need for renal replacement measures (Coiffier *et al.*, 2008).

1.2.4.3. Allopurinol

Allopurinol is converted in vivo to oxypurinol and as a xanthine analog acts as a competitive inhibitor of xanthine oxidase and blocks the conversion of purines to uric acid (Figure 3). This prevents hyperuricemia but does not treat preexisting hyperuricemia. Furthermore, because oxypurinol also inhibits the conversion of xanthine to uric acid, serum and urine xanthine levels may rise and precipitate xanthine crystal deposition in the renal tubules and xanthine induced obstructive nephropathy (LaRosa *et al.*, 2007, Smalley *et al.*, 2000).

1.2.4.4. Rasburicase

Rasburicase(Elitek) is an Aspergillus-derived recombinant urate oxidase approved by the US Food and Drug Administration (FDA) in 2002 for the initial management of hyperuricemia in pediatric patients with leukemia, lymphoma, and solid tumor malignancies receiving anticancer therapy. It was subsequently approved for use in adults in 2009 (Elitek(Rasburicase) package lable). Rasburicase catalyzes the conversion of uric acid to allantoin, carbon dioxide, and hydrogen peroxide (Figure 3). Allantoin is 5- to 10-fold more soluble than uric acid and is readily excreted. Prior to FDA approval, 1,069 adult and pediatric patients received rasburicase on a compassionate use basis (Jeha *et al.*, 2005). Decreased serum uric acid levels were observed in 99% of children and 100% of adults.

In a study of 131 patients with newly diagnosed leukemia or lymphoma, Pui et al. reported a decrease in plasma uric acid concentrations from 9.7 to 1 mg/dL in 65 patients who presented with hyperuricemia and a decrease from 4.3 to 0.5 mg/dL in the remaining patients. There was negligible toxicity, and no patients required dialysis (Cortes *et al.*, 2010).

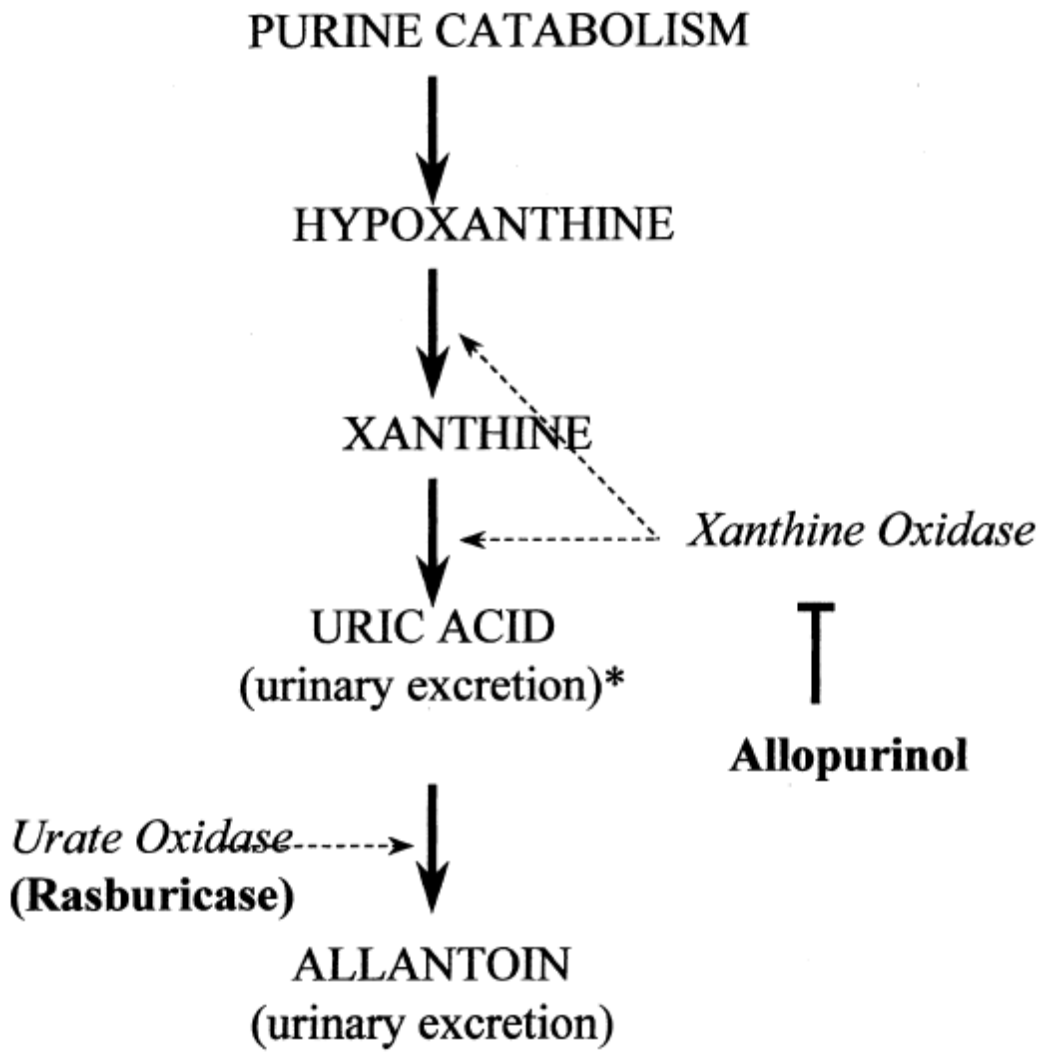

Figure 3: Mechanism of action: rasburicase and allopurinol. Depicted is the pathway of purine catabolism. Rasburicase is a recombinant form of urate oxidase, an enzyme that converts uric acid to allantoin. In comparison, allopurinol acts by inhibiting the endogenous enzyme xanthine oxidase, thereby inhibiting formation of uric acid. (Cairo, MS. and Bishop, M. 2004)

1.3. Statement of the problem

Over recent decades, substantial advances in the treatment of cancer led to an improvement in patient outcome. Due to significant knowledge about tumor biology, several novel agents are now available for a more targeted therapy. Further, established treatments were continuously optimized. However, therapy-related complications remain a challenge in cancer treatment despite large improvements in supportive care leading to therapy-related mortality being responsible for a high proportion of deaths in cancer patients (DeSantis *et al.,* 2014).

TLS is the most prominent onco-metabolic emergency resulting from rapid cell death and contributes a lot to a poor prognosis of cancer patients if not diagnosed and managed properly (Hörl, 2005). Thus, it requires emergent recognition and management by health professionals to minimize its devastating outcome.

Incidence of TLS varies among different malignancies and there is no age or sex predilection. The risk is influenced by a number of characteristics including the type of malignancy, tumor burden, serum lactate dehydrogenase (LDH) levels, degree of involvement of the bone marrow and sensitivity of tumor to chemotherapy (Sharma *et al.,* 2005).

Recently, increased incidence of TLS in patients with several forms of malignancies has been reported (Okamoto *et al*., 2015, Briton *et al*., 2015 and Saleh *et al*., 2015). A reason for this is the establishment of targeted therapies with high efficacy. However, targeted therapies are not available in our country and the incidence of TLS is not known.

TLS is considered to be the main factor responsible for the development of an acute kidney injury which will cause substantial morbidity and mortality in cancer patients (Matnitz *et al*., 2002). So, health professionals who are in direct contact with the treatment and management of cancer and its complications need to be aware of the factors which will lead to high morbidity and mortality to give the highest possible benefit to their patients.

TLS is a life threatening oncological emergency that typically follows administration of chemotherapy or may be spontaneous. Early identification of high risk patients and initiation of preventive measures are more rewarding than the painstaking management of established TLS (Rajendran *et al*., 2013).

Different studies report wide variations in the rates of TLS occurrence. Among the highest was 42%, reported in a study of adults with acute, high-grade non-Hodgkin lymphoma (Hande and Garrow, 1993). In another study the incidence of TLS was reported to be 26.4% in children with B cell acute lymphoblastic leukemia (Wössman *et al*., 2003). Wasim et al reported a 20% incidence of TLS in pediatric hematologic malignancy in Liaquat (Wasim *et al*., 2012).

As far as our knowledge goes there is no single published study on TLS in Ethiopia. So, in this study we have tried to give baseline information about TLS incidence and treatment outcome, with special emphasis given to laboratory parameters that can predict the onset and progress of TLS. These include: uric acid, electrolytes (potassium and calcium), lactate dehydrogenase (LDH) and renal function test panel (blood urea nitrogen/BUN and creatinine) in different pediatric oncology, like acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoid leukemia (ALL), non-Hodgkin lymphoma (NHL), Burkitt's lymphoma (BL) and some solid tumors in pediatric patients in TASH.

1.4. Rationale and significance of the study

TLS is the mainstay for the complication and unfavorable outcome of cancer treatments. Specifically cancers with high proliferation and high sensitivity to chemotherapy are highly susceptible to TLS and its complications. In the era of novel agents, there is greater concern that the incidence of TLS in pediatric oncology may occur more frequently. The problem is disturbing and causing a major concern in low income countries like Ethiopia. Unfortunately, due to socio-economical and other factors, patients come to health facilities at advanced stage of cancer in Ethiopia. To the best of our knowledge the incidence of TLS has not been specifically addressed in Ethiopia.

Therefore, determining the frequency, clinical course and outcome of TLS in pediatric oncology patients in our setup will significantly be important to help shape clinical as well as public health care of the patients and the population. Findings from this work can help TLS diagnosis and improve treatment strategies of carcinomas. In addition, the results obtained from this study is expected to pave the way for further related studies to be broadly and extensively done.

2. OBJECTIVES

2.1. General objectives

To assess the magnitude and management outcome of tumor lysis syndrome among newly admitted pediatric oncology patients at Tikur Anbessa Specialized Hospital, Ethiopia, from January 2017 - July 2017.

2.2. Specific objectives

- To assess the magnitude of TLS in pediatric oncologic patients.
- To assess and compare spontaneous and treatment induced TLS among pediatric oncologic patients.
- To measure and compare the level of biochemical parameters in pediatric oncologic patients before and after treatment.
- To assess the management outcome of pediatric oncologic patients with TLS.

3. Materials and Methods

3.1. Study area and period

The study was conducted from October, 2016 - July, 2017 at pediatric hematology /oncology unit of TASH. TASH is a large referral teaching hospital of Addis Ababa University, located in Addis Ababa, Ethiopia. The hospital has about 800 beds and gives diagnostic and treatment services for about 370,000-400,000 patients per year. The pediatric hematology/oncology unit of TASH is under the department of pediatrics and child health and gives out patient and in patient services. There are about 500-600 pediatric oncology patients visiting TASH annually. Professionally, the unit has hemato-oncologists, hemato-pathologists, residents, and nurses.

3.2. Study design

Hospital based prospective cohort study was conducted.

3.3. Population

3.3.1. Source population

The source population for this study was all pediatric oncology patients attending Tikur Anbessa Specialized Hospital (TASH).

3.3.2. Study population

The study population for this study was all newly admitted pediatric patients with hematologic and solid malignancy during the study period in TASH.

3.4. The inclusion and exclusion criteria

3.4.1. Inclusion criteria

- All newly admitted pediatric oncology patients in the study period
- Those pediatric patients who assented to participate in the research

3.4.2. Exclusion criteria

- Patients with chronic kidney diseases
- Patients who are already on chemotherapy

3.5. The sampling technique and sample size determination

3.5.1. The sampling technique

While purposive sampling technique was implemented to select the health care facility, convenient sampling technique was used to select study participants who met the inclusion criteria during the study period.

3.5.2. Sample size determination

All pediatric oncology patients admitted in the six month study period to Tikur Anbessa Specialized Hospital pediatric hemato-oncology unit were included in the study.

3.6. Variables

3.6.1. Dependent variables

Measured values of:

- uric acid
- Potassium
- Calcium
- Creatinine
- BUN
- WBC
- LDH

3.6.2. Independent variables

- Age
- Sex
- Residence
- Family income

3.7. Blood samples and data collection procedures

After the study participants had been asked for their assent, the demographic data was collected by using questionnaires which was translated into the local language Amharic (optional). Chief complaints and vital sign assessments, physical diagnosis and preliminary and definitive diagnosis were done by qualified physicians. About 5ml of blood sample collected in serum separator tube was allowed to stand for 10-20 minutes at room temperature to allow complete clotting and clot retraction. Samples were then centrifuged at 3500 rpm for 10 min to extract serum. The serum extracted was then used to determine the levels of renal profile parameters (BUN and creatinine), serum levels of electrolytes, serum uric acid concentration, and serum LDH. About 2ml of blood was collected in EDTA coated tubes for determination of total WBC. All the parameters were measured in all patients before the initiation of and during chemotherapy. Safety precautions were taken while handling blood and disposing it. Management (i.e hydration and allopurinol), to counteract TLS occurrence was done for all of the patients. The patients were followed up by a physician (data collector) from admission up to ten days, a period in which TLS has high probability to occur.

3.8. Test Principles of Laboratory Analyses

3.8.1. Total WBC counting principle

The Coulter principle relies on electrical impedance to count and sort cells. Cells are nonconductive and produce measurable changes in electrical resistance in a conducting solution. To count and sort white cells, red blood cell lysing agents are added to the sample. Next, the specimen is diluted in an electrolyte solution, such as isotonic saline. This solution is conductive and also preserves cell size and shape. A low voltage direct current is passed through the liquid. The cell suspension is drawn through an aperture positioned between two electrodes. As cells pass through the aperture, changes in electrical resistance are measured as a voltage pulse. The number of pulses corresponds to the number of cells, and the height of each pulse is proportional to the volume of the cell. The data collected are plotted on a histogram showing the number of cells and their

volumes. Thresholds can be set for exclusion of pulses above or below the desired amplitude range (Chabot-Richards and George, 2015)

3.8.2. Serum LDH concentration

LDH catalyzes the reversible oxidation of L-lactate to pyruvate with the concurrent reduction of β-nicotinamide adenine dinucleotide (NAD) to NADH.The enzyme may be assayed using either material as a substrate; however, the enzyme activities obtained by the two methods are not directly comparable. The forward reaction does not require pre incubation to exhaust endogenous α-keto acids and displays linearity over a wider range of activity in patient samples. Thus, this method utilizes the forward reaction. Lactate and NAD+ are converted to pyruvate and NADH by the action of LDH. NADH strongly absorbs light at 340 nm, whereas NAD+ does not. The rate of increase in absorbance at 340 nm is directly proportional to the LDH activity in the sample (Tietz, 2008).

Chemical reaction scheme:

$$\text{L-Lactate} + NAD^+ \xrightleftharpoons{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

3.8.3. Serum uric acid concentration

The nucleic acids adenine and guanine are metabolized to xanthine, which is further metabolized by xanthine oxidase to the water-insoluble uric acid. The principle of serum uric acid determination is based on uricase and peroxidase (POD) enzyme. Uricase transforms uric acid in the sample into allantoin, carbon dioxide (CO_2) and hydrogen peroxide (H_2O_2). By the action of peroxidase and in the presence of phenol-derivative, 3,5-Dichloro-2-hidroxy-benzenesulfonic acid (DHBS) and 4-Aminoantipyrine, hydrogen peroxide gives a coloured indicator reaction which can be measured at 520 nm. The increasing in absorbance is proportional to the uric acid concentration of the sample (Trivedi *et al*., 1978).

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\textit{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DHBS} \xrightarrow{\textit{POD}} \text{Red quinone} + 4H_2O$$

3.8.4. Serum creatinine concentration

Creatinine is produced as a waste product of creatine and phosphocreatine. Because much of the creatinine is produced in muscle, the amount of creatinine that is measured in blood is proportional to the patient's lean muscle mass. The waste product creatinine, enters the blood supply and is excreted in the urine. The measurement of creatinine is used to aid in the determination of renal function. Increased creatinine levels are found in acute kidney failure, chronic kidney insufficiency and hypoperfusion of the kidneys. The principle of the measurement of creatinine is based on the Jaffe reaction. That is, under alkaline conditions creatinine reacts directly with picric ions forming a reddish complex, the absorbance of which can be measured at 520 nm. The absorbance at 520nm is directly proportional to serum creatinine concentration(Lustgarten and Wenk, 1972).

3.8.5. Serum BUN concentration

Urea is hydrolyzed in the presence of urease enzyme and water to yield ammonia and carbon dioxide. The ammonia reacts with α-ketoglutaric acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and oxidized nicotinamide adenine dinucleotide (NAD^+). The rate of oxidation of NADH to NAD^+ is measured at 340nm over a limited urea concentration range and limited time period, and a decrease in the absorbance of NADH is proportional to the concentration of urea in the sample(Edmund *et al*., 2008).

$$\text{Urea} + H_2O \xrightarrow{\underline{UREASE}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-Ketoglutaric Acid} + NADH + H^+ \xrightarrow{\underline{GLDH}} \text{Glutamic Acid} + NAD^+ + H_2O$$

3.8.6. Principle of Serum electrolyte concentration

The principle of electrolyte analysis is based on ion selective electrodes (ISEs). Membrane potentials are caused by the permeability of certain types of membranes to selected anions or cations. Such membranes are used to fabricate ISEs that selectively interact with a single ionic species. The potential produced at the membrane sample solution interface is proportional to the logarithm of the ionic activity or concentration of the ion in question (D'Orazio and Meyerhoff, 2008).

3.9. Data quality control and management

- The data collection questionnaire was reviewed and checked for completeness, accuracy and consistency by supervisors and investigator and all variables were filled on the data extraction format daily.
- A data collector was given half day training about the objectives of the study and all the physical and clinical diagnosis was done by a qualified physician.
- All the laboratory procedures were handled by professional laboratory technologists.
- All the tests were standardized and automated.

3.9.1. Data processing, analysis and interpretation

Data was checked, cleaned, coded and entered to SPSS version 23 for analysis. Simple descriptive statistics were used to present the socio-demographic and clinical characteristics of the study subjects. Data distribution was checked by using Kolmogrov-Smirnov and Shapiro Wilk test. Paired sample *t* test was used for parametric data and wilcoxon signed rank test for the nonparametric data. Data was described by the use of means, standard deviation, median, inter quartile range (IQR) and percentage. While chi-square test was used to compare categorical variables, Pearson and Spearman rho correlation was applied for continuous variables. P-value < 0.05 at 95% confidence interval was considered as statistically significant in all the analysis.

3.9.2. Result Dissemination

The result was reported and utilized for the purpose of patient care. The final research paper will be submitted to Addis Ababa University School of Medicine Department of Biochemistry, Department of Pediatrics and Child Health, TASH and will be published in reputable journal. It will also be presented at seminars and annual research conferences. Thesis will be bound and submitted to the Department of Biochemistry, the School of Graduate Studies and the University Library.

3.9.3. Ethical consideration

Before starting data collection and preliminary study, ethical clearance letter with reference number SOM/DRERC/BCHM046/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Collaboration letter for data collection was also obtained from Addis Ababa University, Medical Faculty Department of Pediatrics and Child Health. The objective of the study was briefly clarified and explained for each participants' parent or guardian. Informed written consent from the study participants' family or guardian and assent from study participants was obtained after a clear explanation about the purpose and aims of the study to the participants' parents or guardian. Samples and data were collected after informed consent/assent had been obtained. Confidentiality, anonymity, neutrality, accountability and academic honesty was maintained throughout the study.

3.10. Operational definitions

Tumor Lysis Syndrome (TLS): is a serious metabolic disturbance caused by the death of cancer cells during cancer treatment and the release of their intracellular components into the blood stream. TLS is characterized by the rapid development of hyperkalemia, hyperphosphatemia, hypocalcemia, and hyperuricemia, and is potentially life-threatening.TLS is classified into two: laboratory TLS (LTLS) and clinical TLS (CTLS).

Laboratory TLS (LTLS): is a condition when two or more of the following abnormalities are met within 3 days before or 7 days after the initiation of chemotherapy: 1) 25% decrease from baseline in serum calcium, and/or 2) 25% increase from baseline in the serum values of uric acid, potassium, or phosphorous.

Clinical TLS (CTLS): CTLS is a condition when LTLS is accompanied by one or more clinical manifestations such as cardiac arrhythmia, death, seizure, or AKI with an elevated serum creatinine ≥1.5 times upper limit of normal.

Spontaneous TLS: refers to manifestations of TLS in patients who have not received cytotoxic therapy.

Before treatment: is the time in which the patients did not start any treatment for a cancer

After treatment: is the time after the patients started a treatment for the cancer, and it may not necessarily mean after the end of the treatment.

4. Results

4.1. Socio-demographic Characteristics of the study participants

This study enrolled 61 sample pediatric oncology patients, of which 39(63.9%) were males. The mean (±SD) age of the pediatric patients was 6.39 (±3.67) years ranging from 2 months to 14 years. Male to female ration was 1.77:1. Twenty eight (45.9%) of pediatric patients were found within the age group of 5 to 9 years. Seventeen (27.9%) of the children were the third babies of their family. Around 95.1% of the study participants were in low income (<1842.5 birr per month per individual) strata.

Table 2: Socio-demographic characteristics of pediatric oncology patients at Tikur Anbessa Specialized Hospital, Ethiopia, January - July 2017

Variables		Frequency	Percent (%)
Sex	Male	39	63.9
	Female	22	36.1
Age	0 – 4	19	31.1
	5 – 9	28	45.9
	10 – 14	14	23.0
N^{th} child of the parent	First	15	24.6
	Second	14	23.0
	Third	17	27.9
	Fourth	5	8.2
	Fifth	5	8.2
	Others [a]	5	8.2
Region	Addis Ababa	8	13.1
	Oromia	26	42.6
	Amhara	10	16.4
	SNNPR	12	19.7
	Others [b]	5	8.2
Residence	Urban	17	27.9
	Rural	44	72.1
Time of arrival in the hospital after the onset of sign and symptom	Within <72hrs	2	3.3
	72hrs – 7days	4	6.6
	After 7 days	55	90.2
History of cancer in the family	Yes	0	0.0
	No	61	100.0
	Total	100	100.0

[a], the sixth, seventh, ninth and tenth babies of the families are labeled as “others”.

[b], Tigray and Somali regions are labeled as “others”.

Twenty six (42.6%) of the children were from Oromia, followed by Southern Nation Nationality and People Region, 12(19.7%). Forty four (72.1%) of the study participants were from the rural area of the country.

Fifty five (90.2%) of the patients arrived late at the Hospital; after 7 days from the onset of sign and symptoms. None of the patients reported history of cancer in their family (**Table 2**).

4.2. Type of malignancy and Magnitude of tumor lysis syndrome in the study population

Out of 61 pediatric oncology patients, 65.9% (n=40) had hematologic malignancy while the rest 34.4% (n=21) had solid malignancy. Majority of the hematologic malignancies in our study were ALL, 34.4% (n=21) followed by NHL, 13.1% (n=8), AML, 11.5% (n=7), BL, 4.9% (n=3) and CML, 1.6% (n=1). Types of malignancies and their corresponding magnitude of TLS are shown in **Figure 4** below.

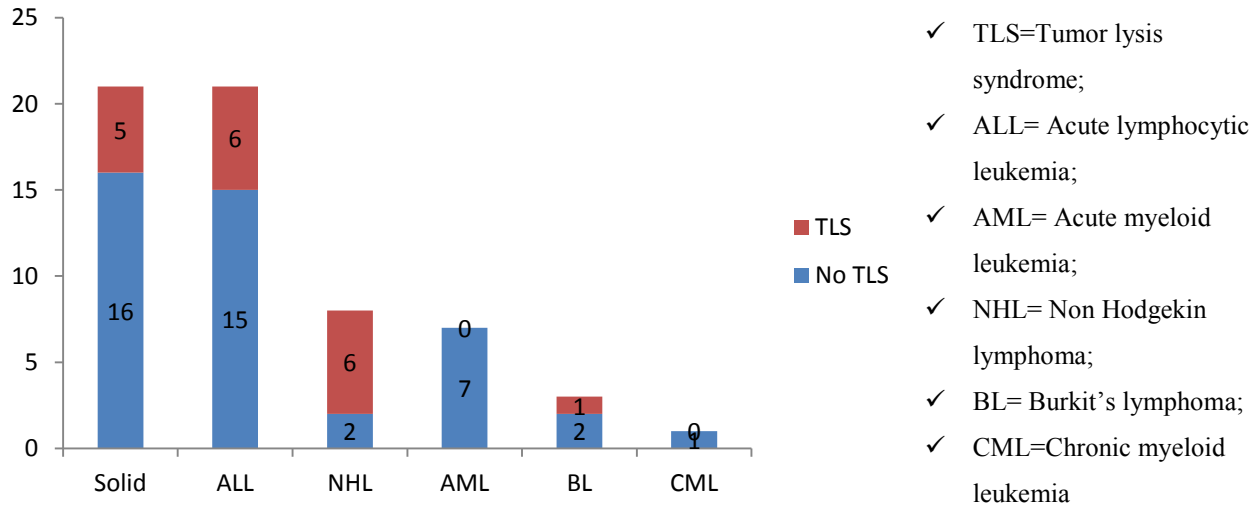

Figure 4: Frequency of TLS among solid and various types of hematological malignancies in pediatric oncology patients in TASH, Ethiopia, January - July 2017

A total of 18 (29.5%) patients were found to have TLS. Seven (11.5%) cases had LTLS whereas the rest 11 (18.0%) had CTLS (**Figure 5**). Out of the 18 TLS cases, 13 (72.2%) developed spontaneous TLS while the remaining 5 (27.8%) had treatment induced TLS.

Sixteen (88.9%) of the TLS cases in the study population resolved while 2 (11.1%) cases did not. Around 44.4% of the patients resolved from TLS within 72 hours of management with adequate hydration and uric acid lowering agent, allopurinol. There were two death reports (11.1%) considered to be caused by TLS, during the study period (**Table 3**).

Acute kidney injury was observed in 9 (14.8%) of patients, while cardiac arrhythmia and seizure was observed in single patients each.

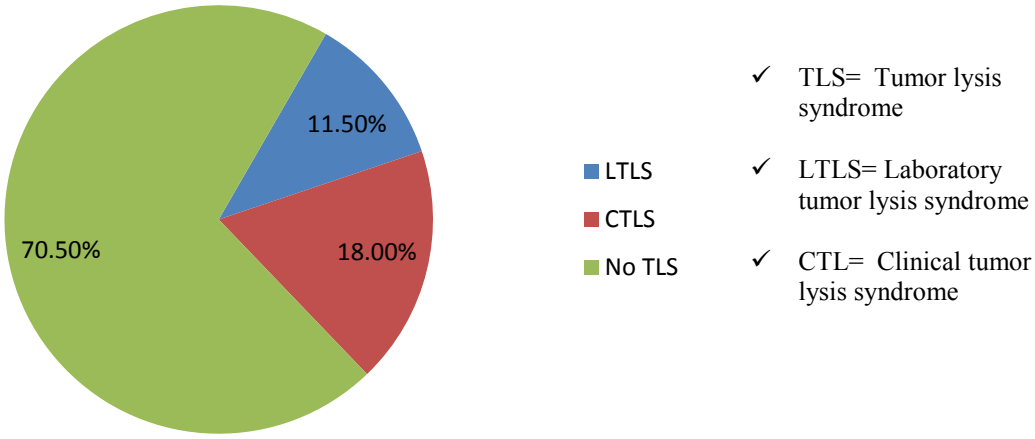

Figure 5: The incidence of LTLS and CTLS in pediatric oncology patients in TASH, Ethiopia, January - July 2017

4.3. Physical diagnosis and imaging results of the study participants

Easy fatigability, abdominal pain and prolonged fever were the most prevalent chief complaints by the study participants. There was also a high prevalence of swelling on different parts of the body (jaw, facial, neck, thigh and lower leg) of the study participants. Some of the patients also complained having headache, cough and bleeding (nasal and gum).

Out of 44 patients who underwent chest x-ray imaging, 33 were normal, four showed pleural effusion, four had lung opacity, two had cardiomegally and one showed solitary calcified nodule.

Ultra sound (US) and physical diagnosis results showed that there were 23 (37.7%) patients with hepatomegally, 20 (32.8%) with splenomegally, 6 (9.8%) with pleural effusion, 33 (54.1%) with lymph node enlargement in different site of their body and 9 (14.8%) with edema. In addition palmar and conjuctival pallor were observed in 47 (77%) of the study participants.

Table 3: Management and treatment outcome of TLS in pediatric oncology patients in TASH, Ethiopia, January - July 2017 (n=18)

Variables		Frequency	Percent (%)
Resolution	Yes	16	88.9
	No	2	11.1
	Total	18	100
Timing of resolution	Within 72hrs	8	44.4
	Within 72 – 120hrs	5	27.8
	Above 120hrs	3	16.7
	Death	2	11.1

4.4. Levels of biochemical parameters in the study participants before and after treatment

The average levels of biochemical parameters before and after treatment are shown in **Table 4**. A Wilcoxon signed rank test showed statistically significant decrease in total WBC count and serum levels of LDH and calcium after treatment ($p<0.05$). The study also showed that there is a statistically significant increase in the serum levels of uric acid, BUN and creatinine after treatment ($p<0.05$). There is no statistically significant difference ($p>0.05$) in serum levels of sodium and chloride before and after treatment in the study participants.

A two tailed paired sample *t* test revealed that there is a difference between potassium level before treatment ($\overline{x}=4.03$, SD=0.74) and after treatment ($\overline{x}=4.40$, SD=0.50) in the study participants, and this is statistically significant, $t(60) = -4.37$, $p<0.0001$.

Table 4: Laboratory values of biochemical parameters before and after treatment in pediatric oncology patients in TASH, Ethiopia, January - July 2017

Parameters	Before treatment Median(IQR)	After treatment Median(IQR)	P value
Potassium(mmol/L)[a]	4.03 ± 0.74	4.40 ± 0.50	**0.0001**
WBC(x10^3/μl)	12.50(8.400 – 37.700)	10.00(6.250 – 18.825)	**0.0001**
LDH(IU/L)	749.00(556.95 – 1274.00)	524.00(371.5 – 863.5)	**0.0001**
Uric acid(mg/dl)	4.94(3.15 – 6.90)	6.20(4.69 – 6.90)	**0.0001**
BUN(mg/dl)	17.00(12.50 – 25.50)	19.00(13.00 – 28.25)	**0.006**
Creatinine(mg/dl)	0.70(0.60 – 0.80)	0.89(0.80 – 1.00)	**0.0001**
Sodium (mmol/l)	139.0(137.0– 140.9)	139.0(138.0 – 141.0)	0.719
Calcium (mmol/l)	2.01(1.23 – 2.30)	1.21(1.09 – 1.90)	**0.0001**
Chloride (mmol/l)	105.0(101.20 – 109.00)	105.0(100.1 – 108.0)	0.671

[a] Potassium is normally distributed, expressed as mean ± standard deviation, the rest of the variables are not normally distributed, expressed as median and IQR

The incidence of hyperuricemia in this study was found to be 23% before treatment and 21.3% after treatment, while the incidence of hyperkalemia is 4.9% and 6.6% before and after treatment respectively. The study also revealed two cases of hypocalcemia both before and after treatment.

4.5. Socio-demographic characteristics and dependent variables

Bivariate Pearson correlation analysis showed that age was weakly and negatively associated with serum potassium level both before (r=-0.043, p=0.742) and after (r=-0.020, p=0.877) treatment in the pediatric oncology patients, and this is not statistically significant.

Spearman's rho correlation analysis was also used to see correlation between age and some of the dependent variables both before and after treatment in the study participants (**Table 5**).

Table 5: spearman's rho correlation between age and some of the dependent variables before and after treatment in pediatric oncology patients in TASH, Ethiopia, January - July 2017

Variable		WBC^1	WBC^2	LDH^1	LDH^2	UA^1	UA^2	BUN^1	BUN^2	Cr^1	Cr^2	Ca^1	Ca^2
AGE	**r**	-.05	-.08	-.07	-.06	-.11	-.04	.14	.20	.27*	.15	-.15	-.14
	p	.68	.55	.61	.66	.38	.75	.29	.13	.04	.27	.25	.28

[1]=before treatment; [2]=after treatment; UA=uric acid; Cr=creatinine; Ca=calcium

* Correlation is significant at the 0.05 level (2-tailed)

Chi-square (x^2) test was implemented to check for the association between the categorical independent variables (gender, residence and family income) and the outcome variable (TLS). The analysis revealed that none of the independent variables, gender (x^2=1.378, p=0.240), residence (x^2=0.105, p=0.746) and family income (x^2=1.625, p=0.202) showed significant association with the occurrence of TLS in the study participants (**Table 6)**.

Table 6: Chi-square test and cross tabulation between independent variables (gender, residence and family income) and TLS in pediatric oncology patients in TASH, Ethiopia, January - July 2017

Variables		None TLS, No(%)	TLS, No(%)	X^2	P-Value
Gender	male	30(49.2)	9(14.8)	1.378	0.204
	female	13(21.3)	9(14.8)		
Residence	urban	13(21.3)	4(6.6)	0.105	0.746
	rural	30(49.2)	14(22.9)		
Family income	<1228 birr/month/individual	33(54.1)	17(27.9)	1.625	0.202
	>1228 birr/month/individual	10(16.4)	1(1.6)		

5. Discussion

The present study evaluated serum levels uric acid, electrolytes (potassium, sodium, chloride and calcium), LDH, renal function test parameters (blood urea nitrogen and creatinine) and total WBC before and after treatment in newly diagnosed pediatric oncology patients admitted to TASH, Addis Ababa, Ethiopia; to evaluate and characterize the incidence of tumor lysis syndrome. We also used physical assessment and some imaging diagnostic tools to diagnose and classify the patients. Our study group demonstrated a marked male predominance. Significantly large proportion of the patients were found to have elevated levels of serum uric acid, potassium, creatinine and BUN after treatment. However some biochemical parameters such as total WBC count, serum LDH and calcium showed moderate decrements after treatment.

This study discusses the incidence of TLS and the abnormal biochemical result findings as well as socio-demographic factors and their implication with respect to tumor lysis syndrome in the pediatric oncology patients.

5.1. Incidence of TLS in the study participants

According to the result of this prospective study the incidence of TLS is 29.5%. Even though there is a wide variation in TLS occurrence across the world, this result is comparable with results reported by Wössmann *et al* 2003 and Darmon *et al* 2013 who reported 26.5% and 30.7% incidence of TLS respectively (Wössmann *et al* 2003 and Darmon *et al*., 2013).

In this study LTLS were found to be 11.5% whereas the CTLS were 18.0%. This result is in concordance with a previous result of 11.1% LTLS and 19.6% CTLS reported from a prospective multicenter cohort study by Darmon *et al* 2013 (Darmon *et al*., 2013). But the result of this research is not in agreement with a previous research result which reported a lower incidence of CTLS (2.9%) in Iran and 6.7% in Saudi Arabia (Esfahani, H., 2015 and Al Bagshi, M., 2013). Higher incidence of CTLS in our study compared with the previous studies can be result of advanced stage of the disease at time of diagnosis, late arrival of patients to a concerned specialty, lack of awareness on the part

of referring as well as treating clinician, underlying malignancy, and pre- existing renal insufficiency.

In our study we found that out of 18 TLS cases, 13 (72.2%) had spontaneous TLS while the remaining 27.8% developed treatment induced TLS. This result is contradictory with a previous research result reported as 21.9% spontaneous TLS versus 78.1% therapy induced TLS in Saudi Arabia (Al Bagshi, M., 2013). This result also contradicts with a result from another descriptive study which reported 20% spontaneous versus 80% chemotherapy induced TLS (Wasim et al., 2012).

Our result of spontaneous and therapy induced TLS is in disagreement both with previous researches and hypotheses. TLS usually occurs a few hours to a few days after commencing cytotoxic chemotherapy for tumors with a high percentage of proliferating and drug-sensitive cells, though sometimes it occurs spontaneously.

Difference in incidence rates reported here in our study can be attributed to several factors, such as application of slightly different criteria to recognize TLS, difference in study population, age, underlying malignancy, late presentation of the patients to the health facility and stage of disease at the time of diagnosis.

The other reason for a lower proportion of chemotherapy induced TLS than spontaneous TLS in our study may be explained by the type of anti-cancer drugs used in our facility. For instance highly myelo-suppressive chemotherapies such as high dose methotrexate and cytarabine are used less frequently in our setup. The employment of prophylactic managements (i.e. hydration and allopurinol) done for all admitted patients with malignancies and especially those having intermediate and high risk for the development of TLS may also attribute for a relative less incidence of chemo induced TLS. But this result needs further study to accurately decide on the causes of this discordance.

5.2. Levels of biochemical parameters in the study participants before and after treatment

The result of this study revealed that there were statistically significant decrease in total WBC count and serum levels of LDH after treatment ($p<0.05$). As the main target of cancer treatment is to eliminate or reduce cancerous cells, and a cancer load is mainly manifested by total WBC count (in hematologic malignancies) and serum LDH level, a decrease of this parameters during treatment sounds good outcome of treatment as suggested by Mirrakhimov *et al.,* 2014.

Cancer cells undergo anaerobic glycolysis at exceeding rate to meet their energy demand by extending the reactions up to the level of lactate production using LDH. This helps regeneration of NAD that feeds the glycolytic pathway. This is explained by the Warburg effect. The decrease in the serum concentration of LDH after treatment is may be due to a decrease in the tumor load that was thought to be responsible for high LDH before treatment.

The research finding also revealed that there was statistically significant decrease in the serum level of calcium after treatment ($p<0.05$). This result is in agreement with scientific assumptions. Because, as a cytotoxic therapy kills tumor cells there will be an increased release of the intracellular component of a cell into the extracellular environment. Hyperphosphataemia is one of the results from the rapid release of intracellular phosphorous from malignant cells. It was reported that cancer cells may contain as much as four times the amount of organic and inorganic phosphorous as compared to normal cells (Cairo *et al.,* 2007). When the phosphate concentration is raised it will lead to precipitation of calcium phosphate crystals, resulting in a decrease in serum calcium level (hypocalcemia) (Darmon *et al*., 2013).

In contrast to WBC, LDH and calcium this study showed a statistically significant increase in the serum level of uric acid, potassium, BUN and creatinine after treatment ($p<0.05$). As cancer treatment is intended to eliminate or reduce cancerous cells, and when the cells are killed their intracellular contents will leak out. Thus, there will be an

increase in the concentration of these intracellular components and their metabolites in the serum.

Uric acid is the end product of purine breakdown. When uric acid is in excess in the serum it results in hyperuricemia. The incidence of hyperuricemia in this study was 23% before treatment and 21.3% after treatment. The finding shows that the patients have already developed hyperuricemia before treatment. This result finding is in agreement with a result reported from a retrospective study in Turkey which showed a hyperuricemia of 26.5% (Sevinir *et al*.,2011). But our result is not in line with a result, 40% hyperuricemia, reported from a descriptive study conducted at Liaquat (Wasim et al., 2012). This variation in the incidence of hyperuricemia may be due to the variation in the types of cancers under study and their response to treatment. It might also be due to late presentation of the patients to the clinics, treatment protocol and study design.

Our study also revealed a 4.9% and 6.6% incidence of hyperkalemia in the study participants before and after treatment respectively. This result is comparable with a research report from Iran, which reported a 2.9% and 5.8% incidence of hyperkalemia before and after treatment respectively (Esfahani, H., 2015). However, our finding is not in line with another research report which reported a 23% incidence of hyperkalemia and 12% hypokalemia (Al Bagshi, M., 2013). A sample size difference may contribute for a difference in the hyperkalemia incidence. There was no hypokalemia in our case.

The result of this research also showed a significant increase in the serum levels of BUN and creatinine after treatment ($p<0.05$). This may be caused by a decrease in the capacity of the kidney to clear the waste products. Because as treatment is given to a patient to kill cancerous cells there will be an increase in waste and cell debris production, which leads to an increase in the concentration of waste materials in the serum of the patient. As a result the kidney will be overwhelmed to clear these waste products, and thereby an increase in the concentration of waste materials, creatinine and BUN, in the serum of the patients.

5.3. Socio-demographic characteristics and dependent variables

The majority of the pediatric patients were found within the age group of 5 to 9 years. This is not in line with a previous study done in Egypt in which the majority of the patients were in age group between 0 to 4 years (Rahman et al., 2015). In addition, the result of this study revealed that age is weakly and negatively associated with serum levels of potassium ($r=-0.043$, $p>0.05$ and $r=-0.020$, $p>0.05$), calcium ($r=-0.15$, $p>0.05$ and $r=-0.14$, $p>0.05$), uric acid ($r=-0.11$, $p>0.05$ and $r=-0.04$, $p>0.05$), LDH ($r=-0.07$, $P>0.05$ and $r=-0.06$, $p>0.05$), and total WBC count ($r=-0.05$, $p>0.05$ and $r=-0.08$, $p>0.05$) respectively before and after treatment, and none of this were statistically significant.

But at the same time the result of this analysis showed that age was weakly and positively associated with serum BUN ($r=0.14$, $p>0.05$ and $r=0.20$, $p>0.05$) and creatinine ($r=0.27$, $p<0.05$, significant and $r=0.15$, $p>0.05$) respectively before and after treatment. The significant association between age and creatinine may be due to muscle mass increase. There is a natural tendency that as age increases the muscle mass tends to increase, and also during cancer there is increased muscle wasting leading to a proportioned increase in creatinine level as a catabolic product of amino acids.

As can be observed from the result, age did not show strong association with most of the independent variables. This may be because we studied patient of almost similar age group, i.e., children from 2 months to 14 years. In previous studies age greater than 60 years were considered as a risk factor for TLS (Mirrakhimov *et al.,* 2014). But in our study, as our study groups were only children we could not compare age with outcome variable (TLS).

The result of our study also showed that gender does not have statistically significant association with the occurrence of TLS ($X^2= 1.378$, $P>0.240$). This finding is not in line with previous study, which says being a male is a risk factor for TLS (Mato *et al*., 2006). There is no significant gender relation in the outcome of TLS in our case, but this again may require a further elaborated study with a larger sample size and gender balance.

In the present study residence ($X^2=0.105$, $p>0.05$) and family income ($X^2=1.625$, $p>0.202$) did not show statistically significant association with the occurrence of TLS in

the study participants. But in previous studies children from low and middle income countries were shown to be at a greater risk of developing TLS (Ribeiro *et al*., 2008, Kellie *et al*., 2008). In our case almost all the study participants were in low income stratification, and still there was no significant variation within the group with respect to TLS occurrence.

5.4. Management and treatment outcome of TLS in pediatric oncology patients

A prophylactic management with intensive hydration, 3000ml/m^2/24hr, and a uric acid lowering agent, allopurinol, along with their respective treatments was done for the patients. Hydration was used to increase urine output, thereby to increase the rate of clearance of waste materials by the renal system. Allopurinol was used to reduce or inhibit the formation of uric acid by competitively binding and inhibiting xanthine oxidase, an enzyme responsible for uric acid production. Allopurinol has no role on the already formed uric acid. The already formed uric acid would have been managed by using a recombinant urate oxidase (rasburicase), but none of our patients received this agent, because it is not available in our set up.

Result of our study revealed that 88.9% of the TLS patients recovered from TLS during the follow up period. There were two (11.1%) death incidence as a consequence of TLS complication. There is a variation in death incidence report due to TLS across different research reports. For instance, a research from Saudi Arabia on incidence and outcome of TLS on children on acute leukemia reported no death incidence (Al Bagshi et al., 2013). At the same time another study from elsewhere reported 3 death cases out of 10 TLS patients from 50 pediatric patients with hematologic malignancies (Wasim et al., 2012).

The variation in the incidence of death report may have different reasons. One of these may be the difference in the treatment protocols and the managements taken, and also the variation in the study population and the nature of the underlying malignancies.

6. Conclusions

In our study we found a comparable incidence of TLS among our patients in comparison with previous studies. In this study TLS occurred irrespective of gender, residence and family income. This suggests that every child with cancer is at risk of developing TLS. Tumor lysis syndrome is a life-threatening complication of malignancy. Early identification of patients at risk and prevention is of crucial importance. In our study spontaneous TLS was more prevalent than the drug induced one. This may indicate that patients do not come to health facility early before complication and have advanced malignancy with high tumor burden.

The biochemical parameters studied in this research and in other studies have shown strong association with the occurrence of TLS. Thus, they need to be considered in TLS prediction, diagnosis and prognosis. TLS incidence in this study was high irrespective of intensive prophylactic managements (i,e., hydration and allopurinol). A protracted better management strategy shall be adapted in the clinic and valid and efficient diagnostic tests shall be used for better diagnosis and prognosis of cancer and TLS.

7. Strengths and Limitations of the Study

One strength of this study is that it includes several demographic, physical, clinical and biochemical parameters claimed to be associated with the variable under study. In addition, the study also considered a homogenous patient population, with respect to age. As TASH is the only cancer center in the country, and we used patients who came to this center, the result of this research can be considered as representative of the country's TLS incidence in pediatric oncology patients.

In addition, as a prospective cohort study design the research followed the patients for specific period of time and reported their outcome. As there is no published study data concerning TLS in our country, this study can serve as a baseline for future studies.

Despite the aforementioned strengths, this study has several limitations. These include:

- Small sample size
- Non homogenous types of cancer diseases, with small sample size in each, which makes comparison difficult
- Phosphorus analysis was not included as a laboratory parameter

8. Recommendations

The following ideas are recommended to further investigate and evaluate the incidence of TLS and its outcomes using biochemical parameters in pediatric oncology patients and to reduce the incidence rate and improve outcome of TLS management.

- Further studies should be conducted with a larger sample size and by considering specific type of cancer.
- Future studies need to consider the nutritional status of the study participants as most of the parameters under investigation can be affected by nutrition.
- Advanced laboratory techniques, such as flow cytometery and molecular techniques are required to link TLS incidence with cancer subtypes.
- Concerning our hospital, TASH, it should have a well designed and controlled laboratory, specially for urgent electrolyte analysis.
- An awareness campaign of cancer and early screening strategies shall be designed by the concerned authorities.
- Expansion of cancer diagnostic and treatment centers in different regions in the country can help know the extent of prevalence of different types of cancer and promote the provision of health services to the needy.

9. References

Al Bagshi, M., 2013. Tumor lysis syndrome in children with acute leukemia: Incidence and outcome. *Journal of Applied Hematology*, *4*(3), 100.

Alakel, N., Middeke, J.M., Schetelig, J. and Bornhäuser, M., 2017. Prevention and treatment of tumor lysis syndrome, and the efficacy and role of rasburicase. *OncoTargets and therapy*, *10*, 597.

Altman, A., 2001, April. Acute tumor lysis syndrome. In *Seminars in oncology*, 28, 3-8.

Annemans, L., Moeremans, K., Lamotte, M., Garcia Conde, J., Van Den Berg, H., Myint, H., Pieters, R. and Uyttebroeck, A., 2003. Pan-European multicentre economic evaluation of recombinant urate oxidase (rasburicase) in prevention and treatment of hyperuricaemia and tumour lysis syndrome in haematological cancer patients. *Supportive care in cancer*, *11*(4), 249-257.

Ansari, M.A., 2012. Tumour Lysis Syndrome in Haematological Malignancies. *JLUMHS*, *11*(02), 84.

Burtis CA, and Ashwood ER,. *Tietz Textbook of Clinical Chemistry*, 6th ed. Philadelphia, PA: WB Saunders; 2008:339–342.

Cairo, M.S. and Bishop, M., 2004. Tumour lysis syndrome: new therapeutic strategies and classification. *British journal of haematology*, *127*(1), 3-11.

Cairo, M.S., Coiffier, B., Reiter, A. and Younes, A., 2010. Recommendations for the evaluation of risk and prophylaxis of tumour lysis syndrome (TLS) in adults and children with malignant diseases: an expert TLS panel consensus. *British journal of haematology*, *149*(4), 578-586.

Cairo, M.S., Gerrard, M., Sposto, R., Auperin, A., Pinkerton, C.R., Michon, J., Weston, C., Perkins, S.L., Raphael, M., McCarthy, K. and Patte, C., 2007. Results of a randomized international study of high-risk central nervous system B non-Hodgkin lymphoma and B acute lymphoblastic leukemia in children and adolescents. *Blood*, *109*(7), 2736-2743.

Celkan, T. and Tüysüz, G., 2013. Tumourlysis syndrome; new approaches in diagnosis, follow-up and treatment. *Turk Arch Ped, 188-194*

Chabot-Richards, D.S. and George, T.I., 2015. White blood cell counts: reference methodology. *Clinics in laboratory medicine*, *35*(1), 11-24.

Coiffier, B., Altman, A., Pui, C.H., Younes, A. and Cairo, M.S., 2008. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. *Journal of Clinical Oncology*, *26*(16), 2767-2778.

Cortes, J., Moore, J.O., Maziarz, R.T., Wetzler, M., Craig, M., Matous, J., Luger, S., Dey, B.R., Schiller, G.J., Pham, D. and Abboud, C.N., 2010. Control of plasma uric acid in adults at risk for tumor Lysis syndrome: efficacy and safety of rasburicase alone and rasburicase followed by allopurinol compared with allopurinol alone—results of a multicenter phase III study. *Journal of clinical oncology*, *28*(27), 4207-4213.

D'Orazio, P. and Meyerhoff, M.E. Electrochemistry and chemical sensors. In: Burits, C.A., Ashwood, E.R. and Bruns, D.E., 6th ed. Fundamental of clinical chemistry. Philadelphia: Saunders, 2008: 87

Darmon, M., Thiery, G. and Azoulay, E., 2007. Preventing acute renal failure is crucial during acute tumor lysis syndrome. *Indian Journal of Critical Care Medicine*, *11*(1), 29.

Darmon, M., Vincent, F., Camous, L., Canet, E., Bonmati, C., Braun, T., Caillot, D., Cornillon, J., Dimicoli, S., Etienne, A. and Galicier, L., 2013. Tumour lysis syndrome and acute kidney injury in high-risk haematology patients in the rasburicase era. A prospective multicentre study from the Groupe de Recherche en Réanimation Respiratoire et Onco-Hématologique. *British journal of haematology*, *162*(4), 489-497.

Davidson, M.B., Thakkar, S., Hix, J.K., Bhandarkar, N.D., Wong, A. and Schreiber, M.J., 2004. Pathophysiology, clinical consequences, and treatment of tumor lysis syndrome. *The American journal of medicine*, *116*(8), 546-554.

DeSantis, C.E., Lin, C.C., Mariotto, A.B., Siegel, R.L., Stein, K.D., Kramer, J.L., Alteri, R., Robbins, A.S. and Jemal, A., 2014. Cancer treatment and survivorship statistics, 2014. *CA: a cancer journal for clinicians*, *64*(4), 252-271.

Edeani, A. and Shirali, A., 2016. Tumor Lysis Syndrome. *American Society of Nephrology*.

Edmund J.L. and Christopher P. *Tietz Textbook of Clinical Chemistry*, 6th ed. Philadelphia, PA: WB Saunders; 2008:385–8.

Elitek (Rasburicase) Package Label. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/103946s5083lbl.pdf.

Enomoto, A., Kimura, H., Chairoungdua, A., Shigeta, Y., Jutabha, P., Cha, S.H., Hosoyamada, M., Takeda, M., Sekine, T., Igarashi, T. and Matsuo, H., 2002. Molecular identification

of a renal urate–anion exchanger that regulates blood urate levels. *Nature*, *417*(6887), 447-452.

Esfahani, H., 2015. The Prevalence of Tumor Lysis Syndrome in Children and Adolescents with Cancer in Hamedan Province, Iran. *IJBC, 7(2): 97-99*

Feig, D.I., Kang, D.H. and Johnson, R.J., 2008. Uric acid and cardiovascular risk. *New England Journal of Medicine*, *359*(17), 1811-1821.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D. and Bray, F., 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer*, *136*(5).

Gupta, S., Howard, S.C., Hunger, S.P., Antillon, F.G., Metzger, M.L., Israels, T., Harif, M. and Rodriguez-Galindo, C., 2015. Treating childhood cancer in low-and middle-income countries. *Disease Control Priorities. Third Edition*, p.121. Han, H.J., Lim, M.J., Lee, Y.J., Lee, J.H., Yang, I.S. and Taub, M., 2007. Uric acid inhibits renal proximal tubule cell proliferation via at least two signaling pathways involving PKC, MAPK, cPLA 2, and NF-κB. *American Journal of Physiology-Renal Physiology*, *292*(1), 373-381.

Hande, K.R. and Garrow, G.C., 1993. Acute tumor lysis syndrome in patients with high-grade non-Hodgkin's lymphoma. *The American journal of medicine*, *94*(2), 133-139.

Held-Warmkessel, J., 2010. Preventing & Managing Tumor Lysis Syndrome.

Hörl, W.H., 2005. Tumor lysis syndrome: risk factors and treatment. *Wiener klinische Wochenschrift*, *117*(1), 7-17.

Jeha, S., Kantarjian, H., Irwin, D., Shen, V., Shenoy, S., Blaney, S., Camitta, B. and Pui, C.H., 2005. Efficacy and safety of rasburicase, a recombinant urate oxidase (Elitek™), in the management of malignancy-associated hyperuricemia in pediatric and adult patients: final results of a multicenter compassionate use trial. *Leukemia*, *19*(1), 34-38.

Kang, D.H., Park, S.K., Lee, I.K. and Johnson, R.J., 2005. Uric acid–induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. *Journal of the American Society of Nephrology*, *16*(12), 3553-3562.

Kaplow, R. and Hardin, S.R., 2007. *Critical care nursing: synergy for optimal outcomes*. Jones & Bartlett Learning.

Kellie, S.J. and Howard, S.C., 2008. Global child health priorities: what role for paediatric oncologists?. *European Journal of Cancer*, *44*(16), 2388-2396.

Kushner, B.H., LaQuaglia, M.P., Modak, S. and Cheung, N.K.V., 2003. Tumor lysis syndrome, neuroblastoma, and correlation between serum lactate dehydrogenase levels and MYCN-amplification. *Pediatric Blood & Cancer*, *41*(1), 80-82.

LaRosa, C., McMullen, L., Bakdash, S., Ellis, D., Krishnamurti, L., Wu, H.Y. and Moritz, M.L., 2007. Acute renal failure from xanthine nephropathy during management of acute leukemia. *Pediatric Nephrology*, *22*(1), 132-135.

Lustgarten, J.A. and Wenk, R.E., 1972. Simple, rapid, kinetic method for serum creatinine measurement. *Clinical Chemistry*, *18*(11), 1419-1422.

Mato, A.R., Riccio, B.E., Qin, L., Heitjan, D.F., Carroll, M., Loren, A., Porter, D.L., Perl, A., Stadtmauer, E., Tsai, D. and Gewirtz, A., 2006. A predictive model for the detection of tumor lysis syndrome during AML induction therapy. *Leukemia & lymphoma*, *47*(5), 877-883.

McBride, A. and Westervelt, P., 2012. Recognizing and managing the expanded risk of tumor lysis syndrome in hematologic and solid malignancies. *Journal of hematology & oncology*, *5*(1), 75.

McGraw, B., 2008. At an increased risk: tumor lysis syndrome. *Clin J Oncol Nurs*, *12*(4), 563-565.

Metnitz, P.G., Krenn, C.G., Steltzer, H., Lang, T., Ploder, J., Lenz, K., Le Gall, J.R. and Druml, W., 2002. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Critical care medicine*, *30*(9), 2051-2058.

Mirrakhimov, A.E., Ali, A.M., Khan, M. and Barbaryan, A., 2014. Tumor lysis syndrome in solid tumors: an up to date review of the literature. *Rare Tumors*, *6*(2).

Montesinos, P., Lorenzo, I., Martín, G., Sanz, J., Pérez-Sirvent, M.L., Martínez, D., Ortí, G., Algarra, L., Martínez, J., Moscardó, F. and de la Rubia, J., 2008. Tumor lysis syndrome in patients with acute myeloid leukemia: identification of risk factors and development of a predictive model. *Haematologica*, *93*(1), 67-74.

Navolanic, P.M., Pui, C.H., Larson, R.A., Bishop, M.R., Pearce, T.E., Cairo, M.S., Goldman, S.C., Jeha, S.C., Shanholtz, C.B., Leonard, J.P. and McCubrey, J.A., 2003. Elitek™–rasburicase: an effective means to prevent and treat hyperuricemia associated with tumor

lysis syndrome, a Meeting Report, Dallas, Texas, January 2002. *Leukemia*, *17*(3), 499-514.

Ndom, P., 2008. Challenges of anticancer chemotherapy in Africa. *The Canadian journal of urology*, *15*(1), 3909-3911.

Okamoto, K., Kinoshita, T., Shimizu, M., Okura, I., Kawada, A., Mizobuchi, K. and Ando, M., 2015. A case of spontaneous tumor lysis syndrome in a patient with ovarian cancer. *Case reports in obstetrics and gynecology*, *2015*.

Rahman, H.A., Moussa, E., Sedky, M., Gouda, I., El Wakeel, M. and Hassanein, O., 2015. Pediatric mature B-cell non Hodgkin lymphoma treatment with LMB-96 protocol. The Children Cancer Hospital Egypt experience. *International Journal of Cancer Therapy and Oncology*, *3*(2).

Rajendran A, Bansal D, Marwaha RK, Singhi SC., 2013. Tumor lysis syndrome. The Indian Journal of Pediatrics, 80(1), 50-4.

Rampello, E., Fricia, T. andMalaguarnera, M., 2006. The management of tumor lysis syndrome. Nature Clinical Practice Oncology, 3(8), 438-47.

Ribeiro, R.C., Steliarova-Foucher, E., Magrath, I., Lemerle, J., Eden, T., Forget, C., Mortara, I., Tabah-Fisch, I., Divino, J.J., Miklavec, T. and Howard, S.C., 2008. Baseline status of paediatric oncology care in ten low-income or mid-income countries receiving My Child Matters support: a descriptive study. *The lancet oncology*, *9*(8), 721-729.

Rodriguez-Galindo, C., Friedrich, P., Alcasabas, P., Antillon, F., Banavali, S., Castillo, L., Israels, T., Jeha, S., Harif, M., Sullivan, M.J. and Quah, T.C., 2015. Toward the cure of all children with cancer through collaborative efforts: pediatric oncology as a global challenge. *Journal of Clinical Oncology*, *33*(27), 3065-3073.

Saleh, R.R., Rodrigues, J. and Lee, T.C., 2015. A tumour lysis syndrome in a chemotherapy naive patient with metastatic pancreatic adenocarcinoma. *BMJ case reports*, *2015*, p.bcr2014207748.

Seftel, M.D., Bruyere, H., Copland, M., Hogge, D.E., Horsman, D.E., Nantel, S.H., Shepherd, J.D., Lavoie, J.C., Le, A., Sutherland, H.J. and Toze, C.L., 2002. Fulminant tumour lysis syndrome in acute myelogenous leukaemia with inv (16)(p13; q22). *European journal of haematology*, *69*(4), 193-199.

Sevinir, B., Demirkaya, M., Baytan, B. and Güneş, A.M., 2011. Hyperuricemia and tumor lysis syndrome in children with non-Hodgkin's lymphoma and acute lymphoblastic leukemia. *Turkish Journal of Hematology*, *28*(1).

Shad, A., Challinor, J. and Cohen, M.L., 2013. Paediatric oncology in Ethiopia: an inctr-USA and George Town University Hospital twinning initiative with Tikur Anbessa Specialized Hospital. *Cancer Control*, 108-12.

Sharma, S.K., Malhotra, P., Kumar, M., Sharma, A., Varma, N. and Singh, S., 2005. Spontaneous tumor lysis syndrome in acute lymphoblastic leukemia. *The Journal of the Association of Physicians of India*, *53*, 828.

Smalley, R.V., Guaspari, A., Haase-Statz, S., Anderson, S.A., Cederberg, D. and Hohneker, J.A., 2000. Allopurinol: intravenous use for prevention and treatment of hyperuricemia. *Journal of Clinical Oncology*, *18*(8), 1758-1763.

Suhag, V., Sunita, S.S., Nimbrian, V.K. and Dalal, A., 2005. JleocaecalBurkitt's lymphoma in a 14 year old male: a case report and review of literature. *Pak J Med Sci*, *21*, 78-81.

Tosi, P., Barosi, G., Lazzaro, C., Liso, V., Marchetti, M., Morra, E., Pession, A., Rosti, G., Santoro, A., Zinzani, P.L. and Tura, S., 2008. Consensus conference on the management of tumor lysis syndrome. *haematologica*, *93*(12), 1877-1885.

Trivedi, R.C., Rebar, L., Berta, E. and Stong, L., 1978. New enzymatic method for serum uric acid at 500 nm. *Clinical Chemistry*, *24*(11), 1908-1911.

Umekawa, T., Chegini, N. and Khan, S.R., 2003. Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate, phosphate and uric acid crystals. *Nephrology Dialysis Transplantation*, *18*(4), 664-669.

Wasim, F., Khaskheli, AM., Siddiqui, A.A., Tariq, O. and Ansari, M.A., 2012. Tumour Lysis Syndrome in Haematological Malignancies JLUMHS, (11):02

Wilson, F.P. and Berns, J.S., 2012. Onco-nephrology: tumor lysis syndrome. *Clinical Journal of the American Society of Nephrology*, *7*(10), 1730-1739.

Wössmann, W., Schrappe, M., Meyer, U., Zimmermann, M. and Reiter, A., 2003. Incidence of tumor lysis syndrome in children with advanced stage Burkitt's lymphoma/leukemia before and after introduction of prophylactic use of urate oxidase. *Annals of hematology*, *82*(3), 160-165.

10. ANNEXES

Annex I: Information sheet for participants' family (English version)

Patient ID _____ **Code Number** _____

Dear Research Participant's family

My name is Haileleul Micho, and I am an Assistant Lecturer at Dilla University health science college. I am doing a research study with my colleague Dr. Yasin Mohammed and my advisors Dr. Daniel Hailu and Dr. Solomon Genet on "Evaluation and characterization of tumor lysis syndrome among pediatric oncology patients in Tikur Anbessa Specialized Hospital before and after chemotherapy". I would like to tell you about this study and ask if you will permit your child take part in it.

We are doing this study to assess the prevalence and the associated risk factors of tumor lysis syndrome in pediatric patients. The finding of this research believed to have some positive contribution in the management of hematologic and solid malignancy of children.

So, we are requesting you to permit your child to take part in this research because our topic is mainly concerned with pediatric oncology. As a procedure we are going to use the findings obtained from your child, like physical diagnosis results, laboratory results and imaging results for a research purpose. You will be also required to fill some questionnaires.

The information and test results obtained from your child will be kept confidential and used only for the purpose of this research. You can give your informed consent/permission freely and voluntarily. The results of the research study may be published; no individual responses will be reported. There may not be direct benefit you or your child will get from the research.

You can contact us if you have questions about the study, or if you decide you don't want your child to be in the study any more.

My phone number is 09-13-57-35-18 THANK YOU !!!

Informed consent/permission form

Please Give Your Informed Consent Below

I have read the information regarding this research and I have been informed about all aspects of the study. I understand that there will be no effect if I agree to participate in this study and give permission to the researcher to use the information obtained from my child in understanding that the data obtained will be confidential and I and my child will not be identified in any way. I was also assured that I have full right to withdraw from this study anytime

I _____ give my informed consent to participate in the above study and permit the researcher to use information and test results obtained from my child.

Signature: _____ Date: _____

_____ _____

Name and Signature of Investigator/Person Obtaining consent Date

Annex II: Information sheet for participants' family (Amharic version)

የጥናቱ ተሳታፊዎች ቤተሰብ የመረጃ ቅጽ

ውድ የጥናቱ ተሳታፊ ወላጆች

ስሜ ሃይለልዑል ሚኾ ይባላል፡፡ በዲላ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ በባዮኬሚስትሪ ትምህርት ክፍል ረዳት ሌክቸረር ነኝ፡፡ የሁለተኛ ዲግሪ የመመረቂያ ጥናቴን ከስራ ባልደረባዬ ዶ/ር ያሲን መሐመድ እና አማካሪዎቼ ዶ/ር ደንኤል ሃይሉና ዶ/ር ሰለሞን ገነት ጋር "**Evaluation and Characterization of tumor lysis Syndrome among Pediatric oncology Patients in Tikur Anbesa Specialized Hospital before and after Chemotherapy**" በሚል ርዕስ ላይ እየሰራሁ እገኛለሁ ስለ ጥናቱ ነግሬአችሁ ጥናቱ ላይ ልጆቻችሁ እንዲሳተፉ ፈቃደኝነታችሁን ለመጠየቅ እፈልጋለሁ፡፡

የዚህ ጥናት ዋና አላማ ቱመር ላይሲስ ሲንድረም የሚባለውን በሽታ መጠንና ተዛማጅ ጉዳቶቹ በህፃናት ላይ ምን ያህል መሆኑን ለማወቅ ነው፡፡ ከጥናቱ የሚገኘ ውጤት ደግሞ የህፃናትን የደም ካንሰር እና ተዛማጅ የሆኑ በሽታዎችን ለማከምና ለመቆጣጠር ይረዳል ተብሎ ይታሰባል፡፡

በዚህ ጥናት ውስጥ የእርስዎ ልጅ እንዲካተት የተፈለገበት ዋናው ምክንያት የበሽታው ተጠቂ ስለሆነ/ች እና በተፈለገው የእድሜ ክልል ውስጥ ስለሆነ/ች ብቻ ነው፡፡ለዚህ ጥናት ከእርስዎ የምንፈልገው ትብብር አንደኛ የእርስዎን ልጅየህክ ምናምርመራ ውጤት ለጥናቱ እንድንጠቀምበት እንዲፈቅዱልንና ሁለተኛ ደግሞ የተወሰኑ ጥያቄዎች ስላሉን የመጠይቅ ፎርሙን እንትሞሉልን በትህትና እንጠይቃለን፡፡

ከእርሶና ከልጆ የሚገኘው መረጃና የምርመራውጤት ሚስጢራዊነቱ የተጠበቀ ነው፡፡ ከእርሶ የምናገኘውን መረጃና የምርመራ ውጤት ከዚህ ጥናት ውጪ ለሌላ ተግባር እንደማይውል ቃል እንገባለን፡፡ በጥናቱ ውስጥ የእርስዎ ልጅ እንዲሳተፍ/እንድትሳተፍ ፍቃደኝነትዎን በነፃነት የመግለጽና በፈለጉት ሰዓት ልጆትን ከጥናቱ የማግለል ሙሉ መብት እንዳልዎት ልናረጋግጥልዎት እንወዳለን፡፡አጠቃላይ የምርምሩ ውጤት ሊታተም ይችላል እንጂ ማንኛውም ግላዊ የሆኑ ውጤቶች ወይም መረጃዎች ለማንም ሪፖርት አይደረጉም፡፡

ከዚህ ጥናት እርስዎ ወይም የእርስዎ ልጅ ቀጥተኛ ተጠቃሚ የሚሆኑበት ዕድል እጅጉን ያነሰ ነው፡፡በጥናቱ ዙሪያ ጥያቄ ካልዎት በማንኛውም ሰዓት መጠየቅ ይችላሉ፡፡

ከምስጋና ጋር

ሃይለልዑል ሚኾ ባዮኬሚስትሪ ትምህርት ክፍል ጤና ሳይንስ ኮሌጅ አዲስ አበባ ዩኒቨርሲቲ

ስልክ 09-13-57-35-18

የፍቃደኝነት መጠየቂያ ቅፅ

እባክዎን ፈቃደኝነትዎን ከዚህበታች ይግለፁ

ስለጥናቱ የተገለፀውን መረጃ ሙሉውን አንብቤ ተረድቼዋለሁ፡፡ በተጨማሪም ስለጥናቱ በቂና ግልጽ የሆነ ማብራሪያ ተደርጎልኛል፡፡በዚህ ጥናት ምክንያት ምንም አይነት ጉዳት በልጄ ላይ እንደማይደርስ ተረድቼ ፈቃደኛ መሆኔን እገልጻለሁ፡፡ከእኔም ሆነ ከልጄ የሚወሰደው መረጃ ሚስጢራዊነቱ የተጠበቀ እንደሆነና በፈለኩት ሰዓት ልጄን ከጥናቱ ማግለል እንደምችልም በበቂ ሁኔታ ተገልፆልኛል፡፡

እኔ_____ከላይ በተጠቀሰው ጥናት ለመሳተፍና የልጄን የህክምና ውጤት እንዲወስድ በመፍቀድ ተስማምቻለሁ፡፡

ፊርማ_____ ቀን_____

የምስክር ስምና ፊርማ_____ ቀን_____

የመረጃ ሰብሳቢ ስምና ፊርማ_____ ቀን_____

Annex III: Information sheet for the research participants (English version)

Patient ID _____ Code Number _____

Dear research participants

My name is Haileleul Micho, and I am an Assistant Lecturer at Dilla University, health science college. I am doing a research study with my colleague Dr Yasin Mohammed and my advisors Dr. Daniel Hailu and Dr. Solomon Genet on *"***Evaluation and characterization of tumor lysis syndrome among pediatric oncology patients in Tikur Anbessa Specialized Hospital before and after chemotherapy"**. I would like to tell you about this study and ask if you will take part in it.

We are doing this study to determine the incidence and the associated risk factors of tumor lysis syndrome in pediatric patients, like you. The finding of this research believed to have some positive contribution in the management of cancer of children.

We are inviting you to take part in this research because you are in the age group and with the condition we are interested in (hematologic malignancy and solid tumor).We are requesting you to use the findings obtained from you like physical diagnosis results, laboratory results and imaging results for a research purpose. You will be also required to fill some questionnaires.

The information and the test results obtained from you will be kept confidential and used only for the purpose of this research. You can give your informed assent freely and voluntarily and you have full right to refuse giving information or to withdraw from participation in study at any stage. The results of the research study may be published; no individual responses will be reported. You may not have direct benefit from the research.

You can contact us if you have questions about the study, or if you decide you don't want to be in the study any more. You can talk to me, or your parents, or someone else at any time during the study.

My phone number is 09-13-57-35-18 THANK YOU !!!

Assent form to Participate in a Research Study

Please Give Your Informed assent Below

I have read the information regarding this research and I have been informed about all aspects of the study. I understand that there will be no negative effect if I agree to participate in this study and I give my assent to the researcher to use test results obtained from me in understanding that the data obtained will be confidential and I will not be identified in any way. I was also assured that I have full right to withdraw from this study anytime.

I _____ give my informed assent to participate in the above study and permit the researcher to use information and test results obtained from me.

Child's Signature: _____ Date: _____

Witness name and sign----------Date----------

_____ _____

Name and Signature of Investigator/Person Obtaining Assent Date

Annex IV: Information sheet for the research participants (Amharic version)

የጥናቱ ተሳታፊዎች የመረጃ ቅጽ

ውድ የጥናቱ ተሳታፊዎች

ስሜ ሃይለልዑል ሚኾ ይባላል፡፡ በዲላ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ በባዮኬሚስትሪ ትምህርት ክፍል ረዳት ሌክቸረር ነኝ፡፡ የሁለኛ ዲግሪ የመመረቂያ ጥናቴን ከስራ ባልደረባዬ ዶ/ር ያሲን መሐመድ እና አማካሪዎቼ ዶ/ር ደንኤል ሃይሉና ዶ/ር ሰለሞን ገነት ጋር “Evaluation and Characterization of tumor lysis Syndrome Among Pediatric oncology Patients in Tikur Anbesa Specialized Hospital before and after Chemotherapy” በሚል ርዕስ ላይ እሰራሁ እገኛለሁ፡፡ ስለጥናቱ ልነግራችሁና ጥናቱ ላይ ለመሳተፍ ፈቃደኝነታችሁን ለመጠየቅ እፈልጋለሁ፡፡

የዚህ ጥናት ዋና አላማ ቱመር ላይሲስ ሲንድረም የሚባለውን በሽታ መጠንና ተዛማጅ ጉዳቶቹ በህፃናት ላይ ምንያህል መሆኑን ለማወቅ ነው፡፡ ከጥናቱ የሚገኘውን ውጤት ደግሞ የህፃናትን የደም ካንሰር እና ተዛማጅ የሆኑ በሽታዎችን ለማከምና ለመቆጣጠር ይረዳል ተብሎ ይታሰባል፡፡በዚህ ጥናት ውስጥ እንድትሳፍ/እንድትሳተፊ የተፈለገበት ዋናው ምክንያት የበሽታው ተጠቂ ስለሆንክ/ሽ እና በተፈለገው የእድሜ ክልል ውስጥ ስለሆንክ/ሽ ብቻ ነው፡፡

ለዚህ ጥናት ከእርስዎ የምንፈልገው ትብብር አንደኛ የእርስዎን የህክምና ምርመራ ውጤት ለጥናቱ እንድንጠቀምበት እንዲፈቅዱልንና ሁለተኛ ደግሞ የተወሰኑ ጥያቄዎች ስላሉን የመጠይቅ ፎርሙን እንድትሞሉልን በትህትና እንጠይቃለን፡፡

ከአንተ/ከአንቺ የምናገኘው መረጃና የምርመራ ውጤት ሚስጢራዊነቱ የተጠበቀ ነው፡፡የምርመራውም ውጤት ከዚህ ጥናት ውጪ ለሌላ ተግባር እንደማይውል ቃል እንገባለን፡፡በጥናቱ ውስጥ ለመሳተፍ ያለህን/ያለሽን ፍቃደኝነት በፃነት የመግለጽና በፈለከው/በፈለግሽው ሰዓት ከጥናቱ እራስህን/እራስሽን የማግለል ሙሉ መብት እንዳልዎት ልናረጋግጥልዎት እንወዳለን፡፡ አጠቃላይ የምርምሩ ውጤት ሊታተም ይችላል እንጂ ማንኛውም ግላዊ የሆኑ ውጤቶች ወይም መረጃዎች ለማንም ሪፖርት አይደረጉም፡፡

አንተ/አንቺ ከዚህ ጥናት ቀጥተኛ ተጠቃሚ የሚሆኑበት ዕድል እጅጉን ያነሰ ነው፡፡ በጥናቱ ዙሪያ ጥያቄ ካልዎት በማንኛውም ሰዓት መጠየቅ ይችላሉ፡፡

ከምስጋና ጋር ስልክ 09-13-57-35-18

የፍቃደኝነት መጠየቂያ ቅፅ

እባክዎን ፈቃደኝነትዎን ከዚህ በታች ይግለፁ

ስለጥናቱ የተገለፀው መረጃ ሙሉውን አንብቤ ተረድቼዋለሁ፡፡ በተጨማሪም ስለጥናቱ በቂና ግልጽ የሆነ ማብራሪያ ተደርጎልኛል፡፡በዚህ ጥናት ምክንያት ምንም አይነት ጉዳት እንደማይደርስብኝ ተረድቼ በጥናቱ ለመሳተፍ ፈቃደኛ መሆኔን እገልጻለሁ፡፡ከእኔ የሚወሰደው መረጃ ሚስጢራዊነቱ የተጠበቀ እንደሆነና በፈለኩት ሰዓት እራሴን ከጥናቱ ማግለል እንደምችልም በበቂ ሁኔታ ተገልፆልኛል፡፡

እኔ_____ከላይ በተጠቀሰው ጥናት ላይ ለመሳተፍና የሚያስፈልገውን መረጃ ለመስጠት ተስማምቻለሁ፡፡

ፊርማ_____ ቀን_____

የምስክር ስምና ፊርማ_____ ቀን_____

የመረጃ ሰብሳቢ ስምና ፊርማ_____ ቀን_____

Annex V: questionnaire

Please answer every question in the questionnaire by circling the letters or filling the necessary information.

Patient ID_____ **Code NO**_____

Part 1: Socio-Demographic characteristics

1.1. Age_____

1.2. Sex _____

1.3. He/she is your _____child. (first, second, third, or specify…….)

1.4. Address: Region _____Zone _____ wereda _____phone No_____

1.5. Residence area: A. Rural B. Urban

1.6. Literacy of the parents:

1.6.1. **Mother;** A. illiterate B. can write and read C. primary D. secondary E. college F. graduate and above(specify)

1.6.2. **Father;** A. illiterate B. can write and read C. primary D. secondary E. college F. graduate and above(specify)

1.7. Occupation of parents:

1.7.1. **Mother;** A. Farmer B. Merchant C. Government employee(specify)…..... D. Private employee(specify)……. E. House wife

1.7.2. **Father;** A. Farmer B. Merchant C. Government employee(specify)…… D. Private employee(specify)…….

1.8. Family income _____

1.9. Family size _____

1.10. Time of arrival at the hospital after the onset of sign and symptom of the disease:

A. within <72 hours B. between 72hours and 7days C. After 7 days

1.11. Is there any history of cancer in your family? A. YES B. NO

1.12. If yes for question 1.11, please specify type of cancer and who was affected.

_____

Part 2: Clinical/laboratory data to be filled by a physician/lab personnel

1. Chief compliant _____
2. Preliminary diagnosis: stage (Risk) _____

A. High risk for TLS B. Intermediate risk for TLS C. Low risk for TLS

3. Definitive diagnosis: stage (Risk) _____

A. High risk for TLS B. Intermediate risk for TLS C. Low risk for TLS

4. Vital sign: Temperature _____
   Blood pressure _____
   Respiratory rate _____
   SPO_2 (oxygen saturation) _____
   Pulse rate _____
5. Anthropometry: Weight _____
   Height _____
   MUAC_____
   Weight /height_____
6. Physical finding: Lymph node enlargement (specify) _____
   Pleural effusion (specify) _____
   Pallor _____
   Abnormal enlargement:
   Hepatomegaly (specify) _____
   Splenomegaly (specify) _____
   Edema (grade) _____
7. Laboratory analysis :

Parameters	Before chemotherapy (test 1)	After chemotherapy (test 2)
WBC		
LDH		
Uric acid		
RFT: BUN		
Creatinine		
Electrolytes: K^+		
Na^+		
Cl^-		

Ca^{2+}		

8. AKI: A. No B. Yes(grade) _____
9. Imaging results:
   9.1. CXR_____
   9.2. U/S(specify)_____
   9.3. CT scan(specify)_____
   9.4. MRI report(specify) _____
10. FNA(specify) _____
11. Biopsy (specify)_____
12. Bone marrow biopsy_____
13. BMA _____
14. Type of malignancy? A. solid B. hematologic
15. If hematologic malignancy, please specify which type?
    A. AML B. ALL C. CML D. NHL E. BL F. other
16. Does the patient has cardiac arrhythmia? A. yes B. No
17. Does the patient has seizure? A. yes B. No
18. If yes for question 17, please specify the type of seizure. _____
19. Does the patient has TLS? A. yes B. No
20. If yes for question 19, what type of TLS is it? A. LTLS B. CTLS
21. Management (specify time initiated after admission)/how long
    21.1. Hydration (specify amount per/m^2/hr) _____
    21.2. Allopurinol _____
    21.3. Rasburicase _____
22. Outcome: Resolution A. Yes B. No (specify)
23. Timing of resolution : A. 72 hours B. 72 – 120 hours C. Other (specify)

Name of investigator _____

Signature _____ Date _____

Annex VI: Standard Operating Procedure (SOP) for blood sample collection

Blood must be collected with care and adequate safety precautions to ensure test results are reliable, contamination of the sample is avoided and infection from blood transmissible pathogens is prevented. Protective gloves should be worn when collecting and handling blood samples. Lancets, needles, and syringes must be sterile, and dry, and blood collecting materials must be discarded safely to avoid injury from needles and lancets.

Technique for collecting venous blood

Laboratory staff must not collect venous blood unless they have been adequately trained in the procedure. Newly qualified staff must be supervised until they have gained sufficient experience. When venous blood is required from infants, this should be collected by a medical officer. Do not collect blood for haematological tests from intravenous lines.

1. Select a sterile, dry, preferably plastic syringe of the capacity required, e.g. 2.5 ml, 5 ml, or 10 ml. Attach to it a 19 or 20 SWG needle (preferably a disposable one). If the patient is a child or adult with small veins, use a 23 SWG needle.

Note: When not using a disposable syringe or needle, check the syringe for good suction and the needle for any blockage, directing the syringe and needle *safely away from the patient*. Ensure all air is expelled from the syringe. *Whenever possible use a disposable needle and syringe.*

2. Apply a soft tubing tourniquet or velcro fastening arm band to the upper arm of the patient to enable the veins to be seen *and felt*. Do not apply the tourniquet too tightly or for longer than 2 minutes. Ask the patient to make a tight fist which will make the veins more prominent.
3. Using the index finger, feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt.
4. Cleanse the puncture site with 70% ethanol and allow to dry. Do not re-touch the cleansed area.
5. With the thumb of the left hand holding down the skin below the puncture site, make the venepuncture with the bevel of the needle directed upwards in the line

of the vein. Steadily withdraw the plunger of the syringe at the speed it is taking the vein to fill*. Avoid moving the needle in the vein.

*If the plunger is withdrawn too quickly this can cause haemolysis of the blood and the collapse of a small vein.

6. When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a piece of dry cotton wool. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.
7. Remove the needle from the syringe and carefully fill the container(s) with the required volume of blood. Discard the needle safely. *Do not* attempt to re-sheath it because this can result in needle-stick injury.

Important: Do not fill a container with the needle attached to the syringe. Forcing the blood through the needle can cause haemolysis.

8. Mix immediately the blood in an EDTA or citrate anticoagulated container. When required, make a thick blood film from the blood remaining in the syringe. Immediately label carefully all the blood samples.
9. Check that bleeding from the venepuncture site has stopped. Cover the area with a small dressing.

Avoiding haematoma: *when collecting venous blood* bleeding from a vein into the surrounding tissue (haematoma) can cause unnecessary distress to a patient and result in subsequent bruising. Haematoma can be avoided by ensuring an appropriate vein is selected and the needle is well positioned in it and not accidentally pulled out of the vein when withdrawing the plunger of the syringe. When removing the needle, always release the tourniquet *first* and apply pressure immediately to the puncture site, maintaining it until the bleeding has stopped completely (*always* check).

Avoiding haemolysis of blood samples

The haemolysis (rupture) of red cells can be a serious source of unreliable test results. If red cells are haemolyzed, substances from the cells are released into the serum or plasma leading to a false increase in the concentration of analytes, e.g. potassium. Haemolysis also interferes with many chemical reactions.

Haemolysis can be avoided by:

- Checking that the syringe and needle are dry and that the barrel and plunger of the syringe fit well.
- Not using a needle with too fine a bore.
- Not withdrawing the blood too rapidly or moving the needle once it is in the vein.
- Removing the needle from the syringe before dispensing the blood into the specimen container and allowing the blood to run gently down the inside wall of the container.
- Adding the correct amount of blood to anticoagulant. Do not shake the blood but gently mix it with the anticoagulant.
- Using clean dry glass tubes or bottles for blood from which serum is required. Allow sufficient time for the blood to clot *and* clot retraction to take place. Red cells are very easily haemolyzed by the rough use of an applicator stick to dislodge a clot.
- Centrifuging blood samples for a minimum period of time. Centrifuging for 5 minutes at about 1000 g is adequate to obtain serum or plasma. Not storing whole blood samples in, or next to, the freezing compartment of a refrigerator.

Annex VII: Standard operating procedure (SOP) for serum preparation:

Aim: Effective Separation of blood products

Purpose: To standardize separating procedures so that research samples will be uniform in quality

1. Select test tube with no anticoagulant, serum separator tube (SST)
2. Draw enough amount of blood (5ml) from the patient

3. Allow to stand for 20-30min for clot formation at room temperature before spinning and separating. A delay in centrifugation may have a detrimental effect on the sample quality and may result inaccurate results. Avoid hemolysis
4. Centrifuge the sample to speed separation and affect a greater packing of cells. Clot and cells will separate from clean serum and settle to the bottom of the vessel.

The supernatant is the serum which can be now collected by dropper or pipette for testing purposes or stored (-20C to -80C) for subsequent analysis or use.