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Title of the project:

Hepatotoxicity Incidence And Risk Factors Among Adults and Pediatrics Acute Lymphoid Leukemia Patients On Chemotherapy Induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia, 2019

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This is to certify that the thesis prepared by Ayale Tsegaye, entitled:

"Hepatotoxicity incidence and risk factors among adults and pediatrics acute lymphoid leukemia patients on chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia'' complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviation

ALL	Acute Lymphoblastic Leukemia
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATP	Adeno-Tri-Phosphatase
BLSH	Black Lion Specialized Hospital
BLI-D	Direct Bilirubin
BLI-T	Total Bilirubin
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CLD	Chronic Liver Disease
DILI	Drug Induced Liver Injury
EPHI	Ethiopian Public Health Institute
ETB	Ethiopian Birr
LFT	Liver Function Test
NCI	National Cancer Institute
SPSS	Statistical Package for Social Science
US	United States
ULN	Upper Limit of Normal range
WHO	World Health Organization

Operational definition

Acute lymphoid leukemia: isa malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, peripheral blood and extra medullary sites.

Cases: are Leukemic patients who are confirmed to have acute lymphoid leukemia

Elevated aminotransferase: Increase level of liver enzymes (AST and ALT) above the reference level (0-39IU/L for AST) and (0-40 IU/L for ALT).

Hepatotoxicity: is caused by hepatotoxins, could be due to environmental exposure for chemicals, dietary sources or pharmaceutical drugs, which results an increase in ≥3 folds of ALT from the normal level.

Hyperbilirubinemia: is higher than normal level of bilirubin in the blood this is any level above 1.2 mg/dl and critical hayperblilirubinmia12 mg/dl.

Induction therapy: the first in series of therapeutic measures taken to treat disease typically a cancer.

L –Asparaginase: treatment options during induction therapy for ALL

Over-the-counter (OTC) Drugs: Are in contrast to prescription drugs that require a doctor's order.

Obese: is a medical condition in which excess body fat has accumulated to an extent that it may have a negative effect on health. People are generally considered obese when their body mass index (BMI) is over 30 kg/m2.

Weight loss: reduction of the total body mass, due to a mean loss of fluid, body fat or adipose tissue or lean mass, namely bone mineral deposits, muscle, tendon, and other connective tissue.

Abstract

Chemotherapy-induced hepatotoxicity is a common cause of abnormal liver function test in patients with ALL. L-asparginas is a drug for ALL patients as induction therapy. The majority of adverse effects are hypersensitivity reactions, but serious liver and other organ related injury may occur. Incidence rate and associated risk factors is not well document in our setting.

Objective: To assess hepatotoxicity incidence and risk factors among adults and pediatrics ALL patients on chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia from January 2019 – September, 2019.

Methodology: Hospital based prospective cohort study design was conducted from February 1, 2019– September 30, 2019 at BLSH hematology unit. A total of 40 admitted and consenting participants were included in the study using convenient sampling technique. A relevant clinical and demographic data and biochemical profile of liver function & pancreatic organ function tests were computed using appropriate statistical tools. Descriptive statistics was used for most variables, repeated measurement of ANOVA, paired T test and binary logistic regression was used to test the association among various variables. P-value of < 0.05 was used as a measure of statistical significance.

Result : From the total (40) ALL cases, an overall increased level of serum AST, ALT, ALP, total bilirubin, direct bilirubin, amylase, lipase was found in 16(40%), 18 (45%), 19(47.5%), 19(47.5%), 27(67.5%) ,10(25%), and 9(22.5%) cases after induction therapy, respectively. The overall hepatotoxicity among all the subjects was5% (2/40). The mean value of serum alkaline phosphatase, total bilirubin, and bilirubin direct were significantly increased in ALL patients after induction therapy as compared with before induction therapy. After induction therapy blirubin total was significantly associated with alcohol intake and ALP was significantly associated with weight loose. AST, ALT, blirubin direct, amylase and lipase were not associated with weight loose, alcohol intake, age, gender and blood transfusion.

Conclusion: The finding of this study concluded that chemotherapy drugs like L-asparginase, vincristine, Predensolon, Doxourobin and others cause significant alterations of clinical chemistry tests like liver and pancreatic enzymes. These alterations could be contributed to liver and pancreatic dysfunction on ALL patients. Patients more susceptible to hepatotoxicity such as those with malnutrition or alcoholism should be followed up more closely. And also, pediatrics has acute pancreatitis so they should have followed them more frequently.

Keywords: Liver function Test, pancreatic test, acute lymphoid leukemia

I.

1. Introduction

1.1 Background Information

Leukemia is cancer of the blood forming cells; it occurs when immature or mature cells proliferate uncontrollably and infiltrates into systemic circulation and thereby accumulate in bone marrow and other organs. It has different criteria of classifications, according to cellular origin to lymphoid or myeloid and either to acute (rapidly progressing disease) with a predominance of highly immature cells, which infiltrate bone marrow, blood and other organs, or chronic, which denotes slowly progressing disease with greater numbers of more mature cells (1).

The exact cause of leukemia is unknown. A combination of genetic factors and environmental (non-inherited) factors are believed to play a role. Risk factors include smoking, ionizing radiation, some chemicals (such as benzene), prior chemotherapy, and Down syndrome. People with a family history of leukemia are also at higher risk. There are four main types of leukemia- acute lymphoblastic leukemia, acute myeloid leukemia , chronic lymphocytic leukemia and chronic myeloid leukemia . Acute lymphoblastic leukemia is a type of leukemia which is characterized by 20% or more lymphoblast in the bone marrow and/or the blood. It is a rapidly developing, abnormal growth of the cells that are precursors of lymphoblasts. ALL is the second most common acute leukemia in adults, with an incidence of over 6500 cases per year in the United States alone. The hallmark of ALL is chromosomal abnormalities and genetic alterations involved in differentiation and proliferation of lymphoid precursor cells (2, 3, 4).

L -Asparaginase is commonly used in combination chemotherapy of both pediatric and adult acute lymphoblastic leukemia. The majority of adverse effects are hypersensitivity reactions, but serious liver injury may also occur. Its mechanism of action is inhibition of protein synthesis for lymphoid cells by hydrolyzing L -asparagine to L -aspartate. L –Asparagine is an essential amino acid for lymphoid cells, since these cells lack asparagine synthetase which is needed to synthesize their own asparagine from glutamate-dependent transamination.

Adverse effects of L-asparaginase include mainly hypersensitivity reactions, acute pancreatitis, coagulation disorders, immune suppression, and liver injury.Asparaginase is directly toxic to hepatocytes resulting in inhibition of protein synthesis and export of lipoproteins and lipids, with resultant steatosis and hepatic dysfunction (8, 5, 9).

The clinical patterns of liver injury are defined as hepatocellular, with a predominant initial elevation of the alanine aminotransferase level (ALT), cholestatic, in which the serum alkaline phosphatase concentrations are increased, or mixed, if both enzymes are elevated. An ALT level of more than three times the upper limit of normal values and a total bilirubin concentration of more than twice the upper limit are used to define clinically significant abnormalities on liver test. Elevation in serum enzyme levels is taken as indicator of liver injury, whereas increases in bilirrubin levels, albumin concentration and the prothrombin time are measures of overall liver function. Many over-the-counter (OTC), prescription medications, chemotherapyandheavy drinking over many years are possible causes of liver toxicity (5, 6, 7).

When the liver is badly damaged by high intake of alcohol, it becomes swollen. This swelling blocks the removal of bilirubin, and bilirubin levels then rise in the blood. A raised bilirubin level indicates serious long term damage to your liver. Drug induced liver injury (DILI) represents an insult to the liver by various compounds ranging from medications to herbal supplements. Though not exceedingly common, DILI represents a significant concern in medical practice. Medications cause 85% of DILI cases. Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by exposure to a drug. Serum hepatitis can be serious complication of blood transfusion and it is possible that even mild attack may lead to cirrhosis which causes elevated liver function test. An ALT level of more than three times the upper limit of normal values used to define clinically significant abnormalities on liver test (10, 9, 11, 12).

The physiological changes associated with ageing, along with increasing co-morbidities and polypharmacy, mean that older people are more likely to have test results that fall outside of the normal reference range. An example of the effect of age, sex and other variables on interpretation of laboratory results is serum alkaline phosphatase (ALP), which may be requested as part of liver function tests. The upper reference limit is markedly increased during puberty as this is the time of maximum bone remodeling. The issue of whether or not liver function is compromised in the elderly population remains unresolved. Numerous age-related changes in hepatic structure and function have been described, but many of these observations are qualitative, were made under suboptimal experimental conditions, or are simply contradictory. Changes in hepatocellular structural parameters, e.g., increased

hepatocyte size, increase in the number of binucleated cells, altered mitochondria, and endoplasmic reticulum, and have been reported for age related liver function.A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth (23, 24, and 27).

Risk factors that have increased the damage of liver in addition to the chemotherapy drugs are taking a medication or over-the-counter pain reliever, serious liver disorder such as cirrhosis, nonalcoholic fatty liver disease, chronic infection with a hepatitis virus, age, sex, drinking alcohol while taking medications.

The study has to give some information for the hepatotoxicity of drugs (chemotherapy) induced for acute leukemia patients. And also knowing risk factors that increase toxicity of liver. So this study will give some clue about risk of hepatotoxicity among patients on acute lymphoid leukemia.

1.2 Statement of problem

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extra medullary sites. While 80% of ALL occurs in children, it represents a devastating disease when it occurs in adults. Within the United States, the incidence of ALL is estimated at 1.6 per 100 000 population. In 2016 alone, an estimated 6590 new cases were diagnosed, with over 1400 deaths due to ALL. The incidence of ALL follows a bimodal distribution, with the first peak occurring in childhood and a second peak occurring around the age of 50.2 while dose intensification strategies have led to a significant improvement in outcomes for pediatric patients, prognosis for the elderly remains very poor. Despite a high rate of response to induction chemotherapy, only 30–40% of adult patients with ALL will achieve long-term remission. The toxicity of chemotherapy is a common cause of morbidity and mortality in cancer patients, as well as a frequent source of sequelae at mid-long term (4, 9).

In a recent analysis, however, Mayo Clinic researchers showed that liver disease-related mortality in the US has been underestimated during the past two decades, and the figure was closer to 66,000 deaths annually. Current, but probably undervalued, worldwide estimations show that 844 million people have CLDs, with a mortality rate of 2 million deaths per year (13, 14).

During the treatment for acute leukemia (AL) a patient may experience a wide variety of complications that mainly have three possible origins, namely the disease itself (leukemic infiltration), peripheral blood cell depression (because of hemorrhagic or infectious processes) and toxicity induced by chemotherapy. The toxicity of chemotherapy is a common cause of morbidity and mortality in cancer patients, as well as a frequent source of sequelae at mid-long term (9).

Taking a medication or over-the-counter pain reliever that carries a risk of liver damage increases risk of toxic hepatitis. This is especially true if multiple medications or more than the recommended dose of medication are taken. Having a serious liver disorder such as cirrhosis or non-alcoholic fatty liver disease makes much more susceptible to the effects of toxins. Chronic infection with a hepatitis virus (hepatitis B, hepatitis C, or one of the other extremely rare

hepatitis viruses that may persist in the body) makes liver more vulnerable. As age, liver breaks down harmful substances more slowly. This means that toxins and their byproducts stay in body longer. Drinking alcohol while taking medications increases the risk of toxicity. Because women seem to metabolize certain toxins more slowly than men do, their livers are exposed to higher blood concentrations of harmful substances for a longer time. Abnormal liver function may be due to multiple causes in patients with AL. Leukemic infiltration usually causes mild to moderate hepatomegaly with limited impact on serum transaminase levels. Transfusions increase the likelihood of viral hepatitis. Inheriting certain genetic mutations that affect the production and action of the liver enzymes that break down toxins may make more susceptible to toxic hepatitis. Obesity is a particularly significant problem among ALL survivors, which can intensify cardiovascular outcomes and place these individuals at greater risk for other chronic health conditions. ALL patients are at increased risk of overweight/obesity, hepatic dysfunction in the form of elevated liver enzymes, bilirubin levels, and C viral hepatitis (15, 16 and 9).

Asparaginase-associated pancreatitis (AAP) is acute pancreatitis in patients that are receiving L-asparaginase treatment at the time of onset of acute pancreatitis.Asparaginase is an essential agent used to treat ALL and some mast cell tumor types, but it has been linked as a principal cause of acute pancreatitis in ALL patients. This often painful and sometimes life-threatening condition occurs in approximately 2-18% of patients, complicating their treatment and threatening their overall chances of being cured. In the present study an increased level of serum amylase has been found in the L-Asparaginase treated patients (17).

1.3 Significance of the study

Chemotherapy-induced hepatotoxicity is a common cause of abnormal liver function test in patients with ALL. Asparaginase is one of the treatment options during induction therapy for ALL. According to literature search, the incidence & pattern of liver, and other organ injury related to the L-Asparaginase is very diversified. Assessing the overall incidence among different age groups (Pediatric vs Adult), and gender and other demographic dependent risk factors could provide insight for clinicians treating ALL with LAsparaginase. As to our knowledge, there is no such data in our setting.

It helps to implement appropriate preventive interventions on liver and other organ related damages associated with acute lymphoid leukemia. This finding will also be use as reference for other researchers.

2. Literature Review

Abnormalities of liver test results occur in almost all patients treated with chemotherapy drugs. Serum aminotransferase elevations during asparaginase therapy are mild-to-moderate in severity (2 to 10 times the upper limit of normal) and self-limiting. The abnormalities typically arise after 2 to 3 weeks of therapy and resolve within 2 to 4 weeks of stopping. The frequency of this clinically apparent liver injury after asparginase therapy is estimated to be 15% to 20% in adults but less than 5% in children (18).

In one study the ALL patient's blood sample were taken before chemotherapy and the second blood sample was taken after one month of induction phase of therapy. In this study a moderate increase of ALT & AST was observed at initial presentation of ALL, which may be due to hepatic injury from Leukemic infiltrates. Chemotherapy also causes liver damage and the level of LFT enzymes increased significantly after chemotherapy. The study shows a significant increased blood level of bilirubin, ALT and Alkaline phosphatase of ALL patients after chemotherapy as compared to before chemotherapy (22).

Retrospective study conducted at the Mount Sinai Hospital in 2013, the cumulative incidence ofDrug induced liver injury in the total study population was 6.1% (17/284), and in the population who had appropriate liver test performed it increased to 18.9% (17/90). Chemotherapeutic agents such as, L-asparginase, vincristine and others were determined to be the cause of DILI in 82 % (14/17) of patients, and the treatment plans were changed in 59% (10/17) of patients (10).

Chemotherapy drugs produce a hepatocellular injury pattern. Serum bilirubin levels are usually between 3 and 7 mg/dl, with moderate elevations in aminotransferases and alkaline phosphatase. Most episodes of jaundice occur more than 30 days after the initiation of therapy. The study's showed that, 14 of 40 patients developed hepatotoxicity with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values above 150 U/l. Hepatoxicity occurred in 13% of subjects given dactinomycin on five consecutive days (19).

Prospective study conducted atKarachi, Pakistan in 2015was performed on 20 ALL patients that shows chemotherapy had liver toxicity. The total bilirubin, direct bilirubin, Alanine amino transferase, alkaline phosphatase and γ-glutamyl levels were increased as compared to controls

level and the level before chemotherapy. In this there was significant increase in the blood level of Bilirubin, Alanine aminotransferase and alkaline phosphatase in patients after chemotherapy (Vincristine, L-asparaginase, Daunomycin, and Methotrexate) as compared to patients before therapy (20).The study shows a significant increased blood level of bilirubin, ALT and Alkaline phosphatase of ALL patients after chemotherapy as compared to before chemotherapy. The study's showed the results before and after chemotherapy; bilirubin –T (0.61 ± 0.08 toμ 1.55 ± 0.32, bilirubin –D (0.21 ± 0.05 to 0.61 ± 0.15), ALT (33.95 ± 5.2 to 61.9 ± 4.63) and ALP (333.7 ± 26.18 to 536.56 ± 56.44). So, these elevated biochemical parameters were due to hepatotoxicity induced by chemotherapy after induction phase of treatment with L-Asparaginase, vincristine drugs (20).

In a retrospective analysis conducted at Princess Margaret Hospital analyzed(USA) March 2017 on 162 ALL patients, hemorrhage and hepatotoxicity attributable to chemotherapy drugs administration occurred in 12% and 13% of patients, respectively. During induction, a total of 17 deaths occurred. Causes of death included hemorrhage in seven cases (five cerebral) and uncontrolled infection in six. In this study, during the intensification phase, more than half of patients developed hepatic toxicity with ten developing hyperbilirubinemia and one death as a result of liver failure (21).

In cross-sectional analyses, when the cohort was divided into quartiles of age, higher baseline serum bilirubin levels were associated with older age in analyses adjusted for sex, which shows Serum bilirubin levels gradually increase with age in older adults. A research conducted in turkey, pancreatic tissue, trypsin, protease, and lipase activities increased with age due to high fat diets on older peoples (25, 26).

Acute pancreatitis (AP) is a complication in children with acute lymphoblastic leukemia (ALL) receiving chemotherapy and has often been reported associated with L-asparaginase therapy. Retrospective cohort study was conducted by reviewing the data of total 192 pediatric ALL patients, of total incidence of AP in children with ALL and L-asp-associated AP was 8.3% and 7.3%, respectively. The mortality rate of AP group was significantly higher than the patients without AP (43.8% vs. 19.3%, respectively) (22).

In general, the pancreatic enzymes (amylase and lipase) has shown to increase after development of L- aspraginase induced AP on acute lymphoid leukemia patients. Not only pancreatic enzymes but also the liver enzymes increase with 2-4 folds of the upper limit of normal range (ULN) on ALL patients. Hepatotoxicity has increase with other risk factors such as chronic disease, obesity, taking other medication and others.

2.1 Conceptual frame work

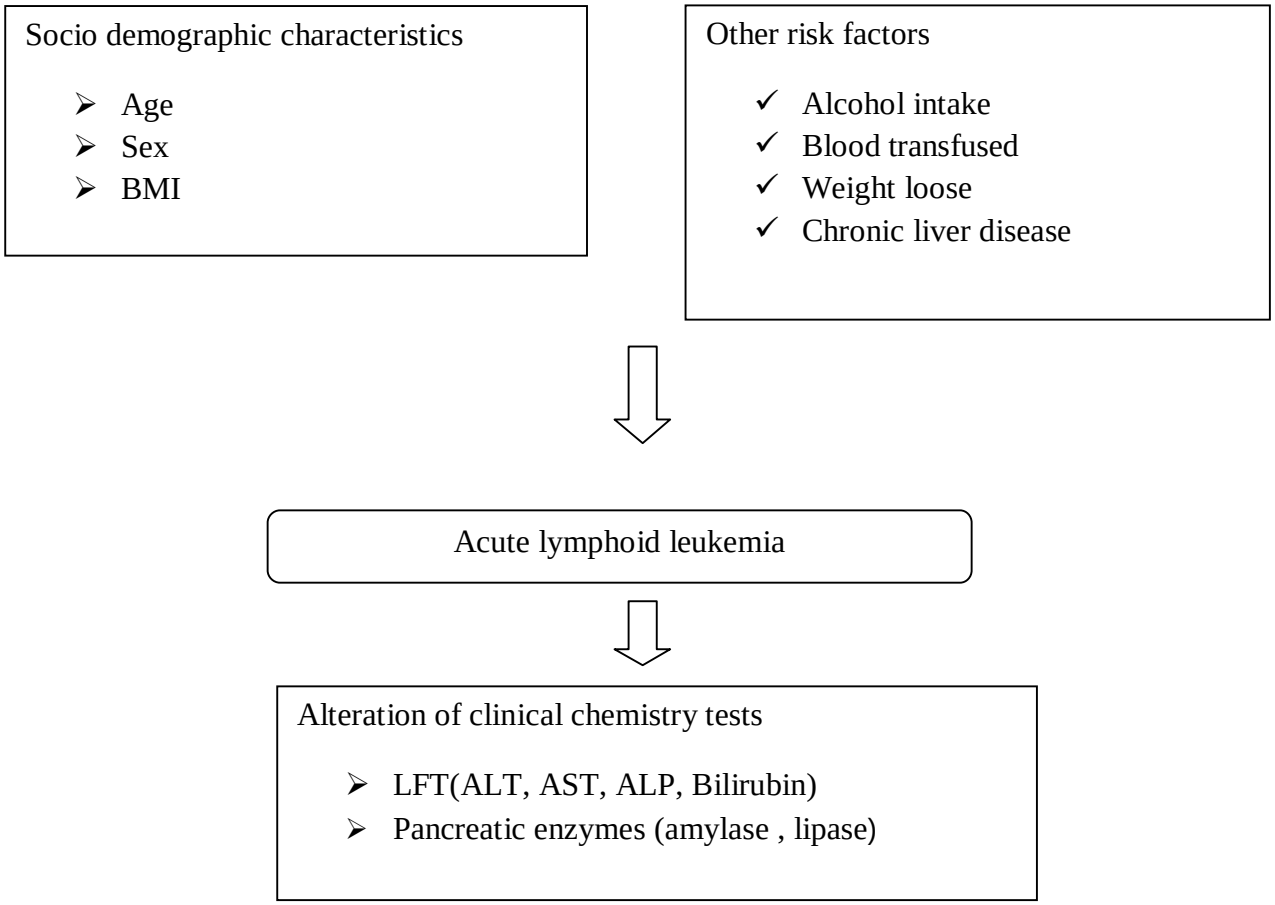


Figure: 1Conceptual frame works

3. Objective

3.1 General objective

To assess drug related liverand other organ injury's incidence and associated risk factors among adults and pediatrics ALL patients on chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia

3.2 Specific objectives

- To assess the liver and pancreatic enzymes before and after induction therapy among admitted ALL patients.
- To compare the effect of chemotherapy on liver injury selected clinical chemistry tests between adults and pediatrics.
- To identify the risk factors associated with selected clinical chemistry test alteration on ALL patients.

4. Hypothesis

Ho= There is no significance difference of liver and pancreatic tests before and after Chemotherapy induction on ALL.

5. Materials and methods

5.1 Study Area

This study was conducted in Black Lion specialized hospital, located in the capital Addis Ababa, Ethiopia. It is Ethiopia's largest specialized public tertiary referral Hospital and one of University Hospitals in the country where patients from all over the country get referral service. Black Lion is a very large referral hospital and sees approximately 370,000- 400,000 patients a year. The hospital has 800 beds, with 169 specialists, 65 non-teaching doctors. It got eight major operating theatre rooms. Black Lion Specialized Hospital is affiliated with the Addis Ababa University's School of Medicine. It is the training center for fellows, postgraduate, undergraduate, medical students, dentists, nurses, Radiographers and laboratory technicians.

5.2 Study design and periods

Hospital based cohort study design was conducted from January, 2019 – September, 2019 to assess **hepatotoxicity incidence and risk factors among ALL patients before and after chemotherapy induction on adults and pediatrics.**

5.3 Population

5.3.1 Source population

The source population of this study was all patients with ALL diseases who visited BLSH.

5.3.2 Study population

The study population of this study were all consenting patients with particularly induction therapy ALL disease who are admitted at the hematology clinic during the study period.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

- ✓ Individuals having chemo drugs on ALL disease patients and those admitted and planned to stay for minimum of one month period.

5.4.2 Exclusion criteria

- Patients who are on other treatment other than chemotherapy drugs
- Patients who were not admitted for minimum of one month during the data collection period
- Patient who were already on sever hepatotoxicity condition before the treatment

5.5 Study variables

5.5.1 Dependent variables

- AST, ALT, ALP, Bilirubin –D , Bilirubin –T, Amylase and Lipase

5.5.2 Independent variables

- Demographic data and Clinical data (Age, Sex, BMI, etc...)
- Duration of Treatment

5.6 Sample size and sampling techniques

5.6.1 Sample size determination

The sample size was calculated by using the mean value of ALT from the previousstudy which gives the highest sample size (20).

Table1: Mean and SD of ALL patients for sample size calculation from others study

Tests	Mean ± SD of ALL Before chemo	Mean ± SD After chemo	N(sample size)
ALP	333.7±26.18	536.56±56	2
ALT	33.95±5.2	61.9±4.63	17
BLI-D	0.21±0.05	0.61±0.15	3
BLI-T	0.61±0.08	1.55±0.32	2

From the previous conducted study mean value of ALT among ALL patients (33.95±5.2; 61.9±4.63) was used to calculate sample size using the following formula:

$n = (s_1^2+s_2^2)/d^2*(Z\alpha+Z\beta)^2$ where n= desired sample size, s1= standard deviation of before chemo drugs =5.2, s2= standard deviation of after chemo drugs =4.63 from previous study, $Z\alpha$=1.96, $Z\beta$ =power = 0.84, d= difference between two means = 61.9 - 33.95= 27.95 from previous study conducted.

$$n = \frac{(0.05)^2 + (0.15)^2 * (1.96+0.84)^2}{(0.4)^2} = 17.9 \text{ round up to } 18$$

10%non-response rate = 0.1*16= 1.7 therefore the minimum sample size was 16 +1.6= 18.7 Increasing sample size can give greater power to detect difference between adult and pediatric ALL case. Therefore 40 cases were studied in this study depending on time and cost.

5.6.2 Sampling method

Convenient sampling technique was employed to select study participants.

5.7 Data and sample collection procedures

5.7.1 Data collection procedures

After a brief explanation, the patient's consent was asked for participation. Then structured data collection format were used to collect baseline data. It was included information's about social demographic characteristics, and the medical record of the patient. This data was collected by interviewing and evaluating the patient and from clinical records.

5.7.2 Blood sample collection and processing

Venous blood sample was collected from the participant into 5ml serum separator tubes. After keeping the tube for 20 minutes to clot, the specimen was centrifuged at 1500 rpm for 5 min and the serum was separated and put at 4^0C until assay is conducted. Liver function tests and other necessary tests were analyzed from serum at EPHI (**Roche - COBAS** Integra® 400) clinical chemistry laboratory.

5.7.3Test analysis

Liver function and pancreatic enzymes test includes measurement of the concentration of alanine transaminase, aspartate transaminase, alkaline phosphatase, direct bilirubin, total bilirubin, amylase and lipase in the serum. The analysis was done by the principle of spectrophotometry for measuring the absorption spectrum of the analyt at each wave length. **Roche - COBAS** Integra® 400 automated chemistry analyzer was used. All the tests were performed based on the manufactures protocol.

5.8 Data quality assurance

5.8.1Pre-analytical phase

Thequestionnaires were pre tested on 5% of the study population one week before the actual data collection to ensure clarity, length, logical sequence and skip patterns of the questions.

To maintain the quality of data obtained through face to face interview of the participants'. The well-prepared questionnaires was translated into Amharic version and cross checked with English version. Data collection formats were checked for completeness and consistence.

In order to maintain the quality of blood sample standard operating procedures were followed at every step of specimen collection and processing Samples was stored in appropriate refrigerator temperature 2-8°C until analysis in BLSH. Samples with incomplete information were rejected.

5.8.2 Analytical phase

There was quality control (percicontrol clinical chemistry level 1with 25025400 lot number & percicontrol clinical chemistry level 2 with 34827300 lot number) sample which were run daily in the morning before the actual sample running to check the performance of clinical chemistry analyzers.

5.8.3 Post analytical phase

The results wererecorded into SPSS version 23 database sheet; data completeness was checked.

5.9 Data analysis and interpretation

SPSS (Statistical package for social science) software was used to analyze the obtained data. The data was entered in to SPSS version 23 software and analyzed accordingly. Descriptive statistics was used and the descriptive data was expressed in number and percentage in the form of tables and figures. Binary logistic regression was used to check the significant association risk factors with abnormal values of tests. Repeated measurement of ANOVA and paired T test was used to analyze the results of adult and pediatric ALL cases. The P value <0.05 with corresponding 97.5% percentiles was considered as significant.

5.10 Ethical considerations

Written consent was obtained from Medical Laboratory Department research and ethical review committee and was given to the concerned bodies. Name of the patient was not included to preserve privacy of the respondents. Furthermore, the consent of each participant was asked after a brief explanation of the objective of the study.

6. Workflow

Fulfill Assessment of criteria Not Fulfill

Excluded

Included

Patients/cases

Interviewed using structured data collection format

Medical history and additional information will be taken from medical record

3-5 ml of venous bloodwas collected

Centrifuged at 1500 rpm for 5 min

LFT and other analysis at EPHI chemistry laboratory

The result was taken as a secondary data

Fig: 2 Workflow notifications for prospective study on hepatotoxicity incidence and risk factor among adults and pediatrics ALL patients on chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia from January2019 – September, 2019.

7. Result

7.1 Socio demographic Characteristics of study participants

This study included 40 ALL confirmed patients (22 of them were males). The median (2.5^{th}-97.5^{th} range) was 14(2-51), with twenty(50%) of them pediatrics (Table2).

Table 2: Socio-demographic characteristics of ALL patients at Black Lion Specialized Hospital Addis Ababa, Ethiopia from January 2019 to September 2019 (n=40)

Sociodemographic variables	ALL cases (n=40)
Gender	
Male n (%)	22(55%)
Female n (%)	18(45%)
Total	40(100%)
Age in year n (%)	
1-10	18(45%)
11-20	9(22.5%)
21-30	10(25%)
31-40	0(0%)
≥41	3(7.5%)
Total	40(100%)
Median age (years) (2.5-97.5 percentile)	14(2-51)

7.2 Results of Adults and Pediatric acute lymphoid leukemia

Without adjusting for gender, the overall Mean value of BLI-T, Amylase, and lipase were significantly higher in pediatricspatients (0.98±0.60, 94.8±33.5, 42.1±23.7, respectively) than adults (1.30±0.94,79.4±38.6, 35.8±18.5,respectively) ($P<0.05$).The mean value of serum ALP and was significantly increased ($P<0.05$) in adults than pediatrics ALL patients. Pancreatic enzymes (amylase and lipase) were significantly increased on pediatrics. However mean value of AST, ALT were higher in both adult and pediatric patients but not significantly increased(Table3). Mean value of bilirubindirect (0.93±0.93 and0.53±0.34, $p<0.05$) was significantly increase in both adult and pediatric respectively (Table3).Mean value of AST, ALT, ALP, amylase and lipase was 27.4±21.5, 36.0±24.8, 174.7±81.2, 75.5±37.4, 37.2±19.4 and 35.1±24.9, 25.4±20.3, 329.9±137.5, 63.1±33.3, 23.4±9.8 for pediatrics and adults before induction therapy

was not significantly increased respectively (Table 4).Mean value of bilirubin total and bilirubin direct (0.91±0.41, 0.28±0.20 P <0.005) was significantly increase in pediatrics and adults respectively before treatment.

Table 3: Comparison of clinical chemistry tests among adult and pediatric ALL casesafter induction therapy at Black Lion Specialized Hospital Addis Ababa, Ethiopiafrom January 2019 to September 2019 (n=40)

	ALL cases (n=40)							
Parameters	Adults				Pediatrics			
	Before treatment Mean±SD	After treatment Mean±SD			Before Mean±SD	After treatment Mean±SD		
		First	Second	*p-v		First	Second	*p-v
AST	27.4±21.5	31.6±21.6	38.1±29.8	0.265	35.1±24.9	38.7±18.9	38.9±25.2	0.812
ALT	36.0±24.8	40.7±18.9	52.6±38.1	0.235	25.4±20.3	36.8±28	37.4±23.7	0.069
ALP	174.7±81.2	209.1±91.0	262.6±151.6	***0.035***	329.9±137.5	381.1±198.3	430.2±274.3	0.188
BIL-T	0.91±0.41	1.05±0.80	1.30±0.94	0.089	0.53±0.64	0.83±0.60	0.98±0.60	***0.004***
BIL-D	0.46±0.66	0.80±0.64	0.93±0.93	***0.021***	0.28±0.20	0.44±0.33	0.53±0.34	***0.005***
Amylase	75.5±37.4	79.5±38.5	79.4±38.6	0.915	63.1±33.3	74.6±28.0	94.8±33.5	***0.007***
Lipase	37.2±19.4	34.1±18.9	35.8±18.5	0.685	23.4±9.8	31.0±14.4	42.1±23.7	***0.002***

- *Repeated measurement of ANOVA was used for statics analysis
- N –number , P-v -p value ,AST-Aspartate transaminase ,ALT- alanine transaminase ,BLI-D –blirubin direct ,BLI-T, blirubin – total, ALP- alkalinphosphatas

7.3 Results of Liver function test and pancreatic tests on gender and age adjusted acute lymphoid leukemia cases

The mean value of lipase activity on the age group (1-10) were show some increment at the post treatment acute lymphoid leukemia cases as compared to base line, (43.2±24.6vurses24.4±9.9) respectively(Table 4, 6) .The mean value of bilirubin total were shows significantly different values on acute lymphoid leukemia patients in both gender at the base line and only ALT was significantly increase only on males on ALL cases at the post treatment, $p<0.05$ (Table 4).The mean value of male AST, ALP and bilirubin direct were have no significantly differenc value on different age groups at the base line, $p>0.05$ (Table 4), HoweverBLT and BLI-D were have significantly increase on males on post treatmentALL cases (Table 5). The mean value of ALP was increased on the age group (1-10) as compared to others age group at base line (Table 4).

Table4: Baselines (before treatment) mean±SD of clinical chemistry tests among adults and pediatrics; Age and Gender adjusted values at Black Lion Specialized Hospital Addis Ababa, Ethiopia from January 2019 to September 2019 (n=40)

Age(year) and sex	AST(IU/L)Mean±SD	ALT(IU/L) Mean±SD	ALP (IU/L) Mean±SD	BILT(mg//l) Mean±SD	BILD(mg/l) Mean±SD	Amylase(IU/L) Mean±SD	Lipase(IU/L)
1-10(n=18)	37.5±25.1	25.8± 21.3	346.8±132.6	0.57±0.66	0.23± 0.22	62.7±34.9	24.4±9.9
Male(n=11)	39.9±28.9	22.6±12.8	360.6±107.8	0.70± 0.82	0.34±0.24	57.4±30.8	22.0±9.8
Female(n=7)	33.9± 19.1	30.7±31.1	325.1±172.0	0.37± 0.21	0.23± 0.096	71.1±41.6	28.1±9.6
***p-value*[a]**	0.502	0.215	0.475	0.329	0.374	0.364	0.314
11-20(n=11)	22.9±18.8	26.6± 24.4	203.7±106	0.67±0.58	0.34±0.49	61.8±32.9	32.6±24.4
Male (n=6)	23.3± 16.0	15± 6.6	192.5± 104.0	0.33± 0.29	0.1± 0.05	65.5±18.4	28.0±25.5
Female (n=5)	22.6± 22.7	35.8± 30.4	212.6± 118.9	0.93± 0.64	0.53±0.60	58.8±43.3	36.3±25.8
***p-value*[a]**	0.808	0.155	0.793	0.247	0.321	0.568	0.272
21-30 (n=8)	30.3±24.5	45.7± 23.2	155.7±53.5	0.91± 0.28	0.24± 0.1	88.7±39.1	39.1±16.2
Male (n=6)	36.2± 29.9	46.0±27.3	149.8± 37.0	0.87± 0.35	0.23± 0.1	87.2±37.8	36.6±16.5
Female (n= 2)	22.5± 16.3	33.0±17.0	138.5± 20.5	0.83± 0.02	0.19± 0.04	110.5±70.0	28.9±10.3
***p-value*[a]**	0.679	0.681	0.075	0.949	0.823	0.628	0.335
31--- (n=3)	22.0± 18.4	23.3± 13.5	153.3±54.6	1.2±0.1	1.4± 1.3	67±24.5	29..7±17.6
Male (n=1)	43.0	37	155.0	1.1	0.16	45.0	49.1
Female (n= 2)	11.5±3.5	16.5±9.2	152.5± 77.1	1.2± 0.1	1.96± 1.1	78.0±21.2	20.1±7.4
***p-value*[a]**							
***p-value*[a]**							
Both sex	0.134	0.405	0.738	***0.042***	0.200	0.990	0.251
***p-value*[b]Male**	0.070	***0.001***	0.071	0.825	0.918	0.307	0.085
Female	0.917	0.979	0.459	0.266	0.148	0.753	0.761

[a]*paired sample T test; to seemean difference gender*

[b]*one way ANOVA; to see gender difference on adjusted age group*

Table5 : Post treatment T1mean±SD of clinical chemistry tests among adults and pediatrics; Age and Gender adjusted values at Black Lion Specialized Hospital Addis Ababa, Ethiopia from January 2019 to September 2019 (n=40)

Age and sex	**AST(IU/L)Mean±SD**	**ALT(IU/L) Mean±SD**	**ALP (IU/L) Mean±SD**	**BILT(mg//l) Mean±SD**	**BILD(mg/l) Mean ±SD**	**Amylase(IU/L) Mean ±SD**	**Lipase(IU/L) Mean ±SD**
1-10(n=18)	40.8±18.5	38.9± 28.7	403.9±195.4	0.86±0.62	0.46±0.34	75.4±27.2	32.1±14.8
Male(n=11)	40.6± 17.8	37.5± 30.2	448.0± 195.7	0.96± 0.71	0.50± 0.37	72.4±27.6	25.7±9.5
Female(n=7)	41.1± 21.0	41.0± 28.6	334.7± 187.7	0.70± 0.43	0.4± 03	80.3±28.1	42.2±16.7
p-value[a]	0.988	0.456	0.174	0..459	0.576	0.4320.059	
11-20(n=9)	27.7±19.9	33.8±20.9	202.3±108.7	0.66±0.46	0.45±0.47	82.8±39.2	30.8±11.2
Male (n=4)	27.5± 26.8	35.5± 25.0	184.5± 42.9	0.38± 0.21	0.21± 0.06	94.5±50.1	30.7±11.8
Female (n=5)	27.8± 16.0	32.4± 19.9	216.6± 147.3	0.89± 0.49	0.63± 0.58	73.4±30.6	30.9±12.1
p-value[a]	0.0.869	0.833	0.793	***0.021***	0.324	0.614	987
21-30 (n=8)	35.0±24.1	44.6±18.1	203.9±64.6	1.0±0.57	0.9±0.64	80.1±39.4	38.4±22.6
Male (n=6)	41.7± 28.3	43.7± 20.1	203.2± 57.0	0.86± 0.48	0.85± 0.69	81.8±37.4	41.6±28.5
Female (n= 2)	23± 5.7	37.0± 2.8	151.0± 70.7	1.2±1.1	0.62± 0.72	84.0±52.0	29.7±15.5
p-value[a]	0.723	0.934	0.074	0.411	0.575	0.928	0.978
31--- (n=3)	24.7±17.7	33.7±17.6	225.0±123.6	2.0±1.4	1.2±0.79	59.7±39.8	21.2±18.1
Male (n=1)	13	23.0	142	0.42	0.30	78.0	21.2
Female (n= 2)	30.5± 20.5	39.0± 21.2	266.5	2.8± 0.11	1.67± 0.14	50.5±51.6	21.1±25.5
p-value[a]							
p-value[a] **Both sex**	0.291	0.122	0.653	0.778	0.6770.333		0.958
p-value[b]**Male**	0.350	0.988	0.633	***0.044***	***0.033***	0.404	0.554
Female	0.051	0.454	0.540	0.074	0.067	0.081	0.125

[a]*paired sample T test; to seemean difference on gender*

[b]*one way ANOVA; to see gender difference on adjusted age group*

-T-test

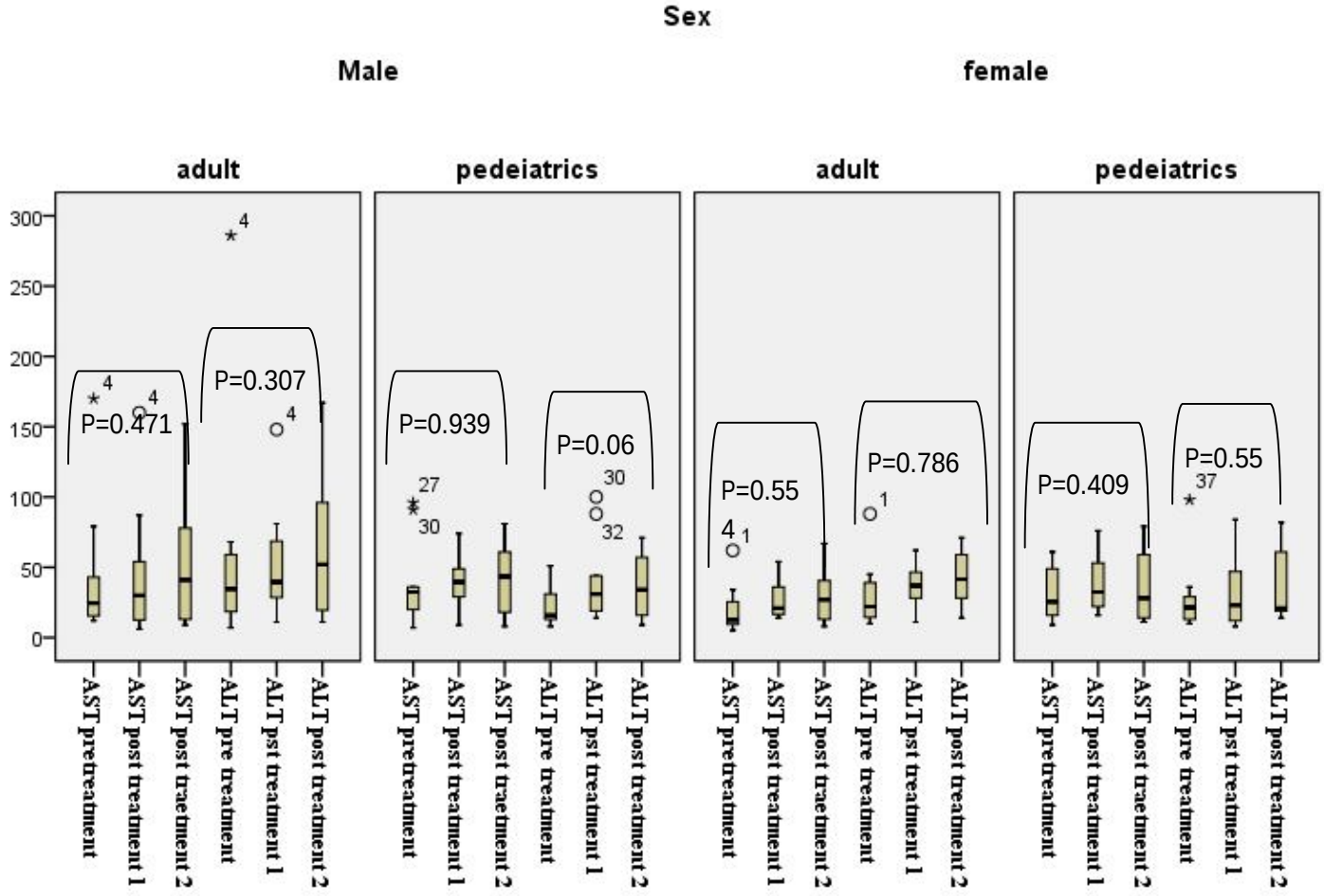
Table6 : Post treatment T2 mean ±SD of clinical chemistry tests among adults and pediatrics; Age and Gender adjusted values at Black Lion Specialized Hospital Addis Ababa, Ethiopia from January 2019 to September 2019 (n=40)

Age and sex	AST(IU/L)Mean±SD	ALT(IU/L) Mean±SD	ALP (IU/L) Mean±SD	BILT(mg//l) Mean±SD	BILD(mg/l) Mean ±SD	Amylase(IU/L) Mean ±SD	Lipase(IU/L) Mean ±SD
1-10(n=18)	41.6±25.1	39.7±23.9	458.8±274.3	1.01±0.62	0.55±0.34	95.5±34.7	43.2±24.6
Male(n=11)	39.0±25.8	36.4± 22.8	482.9± 268.9	1.1± 0.70	0.54± 0.33	86.7±28.7	40.8±22.6
Female(n=7)	45.7± 25.4	44.9± 26.5	421.0± 299.8	0.93±0.49	0.56± 0.39	109.3±40.9	47.1±29.0
***p-value*[a]**	0.416	0.393	0.667	0.523	0.844	0.086	0.878
11-20(n=9)	34.6±36.5	50.9±50.9	220.4±117.7	0.82±0.65	0.49± 0.50	86.4±40	34.0±9.3
Male (n=4)	53.0±50.2	71.5± 73.3	216.0± 31.4	0.64± 0.3	0.31± 0.1	100±59.3	30.7±6.9
Female (n=5)	19±12.8	34.4± 19.8	224.0± 164.1	0.96± 0.85	0.63± 0.66	75.6±15.7	36.7±10.9
***p-value*[a]**	0.255	0.317	0.495	0.899	0.569	0.499	0.467
21-30 (n=10)	38.9±23.8	51.0±28.3	299.6±170.1	1.4±0.79	0.91±0.80	76.3±40.1	37.0±23.0
Male (n=6)	41.3± 28.5	43.0± 28.0	290.7± 212.4	1.3± 0.71	0.73± 0.77	84.7±36.4	38.4±26.2
Female (n= 2)	34.0± 15.5	45.5± 16.3	234.5± 95.5	1.4± 1.5	1.14± 1.23	72.1±72.1	36.5±32.0
***p-value*[a]**	0.921	0. 934	0.611	0.442	0.446	0.921	0.937
31--- (n=3)	30.7±31.7	39.3±27.9	206±151.6	1.8±1.66	1.89±1.55	74.9±26.1	34.8±24.6
Male (n=1)	2.99.0	18	96.0	0.15	0.09	83.0	18.2
Female (n= 2)	41.5± 36.1	50.0±29.7	261.0± 166.9	2.7± 0.99	2.78±0.13	70.9±35.6	43.2±28.1
***p-value*[a]**							
Both sex	0.185	0.147	0.700	0.778	0.326	0.692	0.968
***p-value*[b]Male**	0.065	0.507	0.704	***0.048***	0.088	0.073	0.061
Female	0.033	0.086	0.649	0.578	0.454	0.158	0.344

[a]*paired sample T test; to seemean difference of gender*

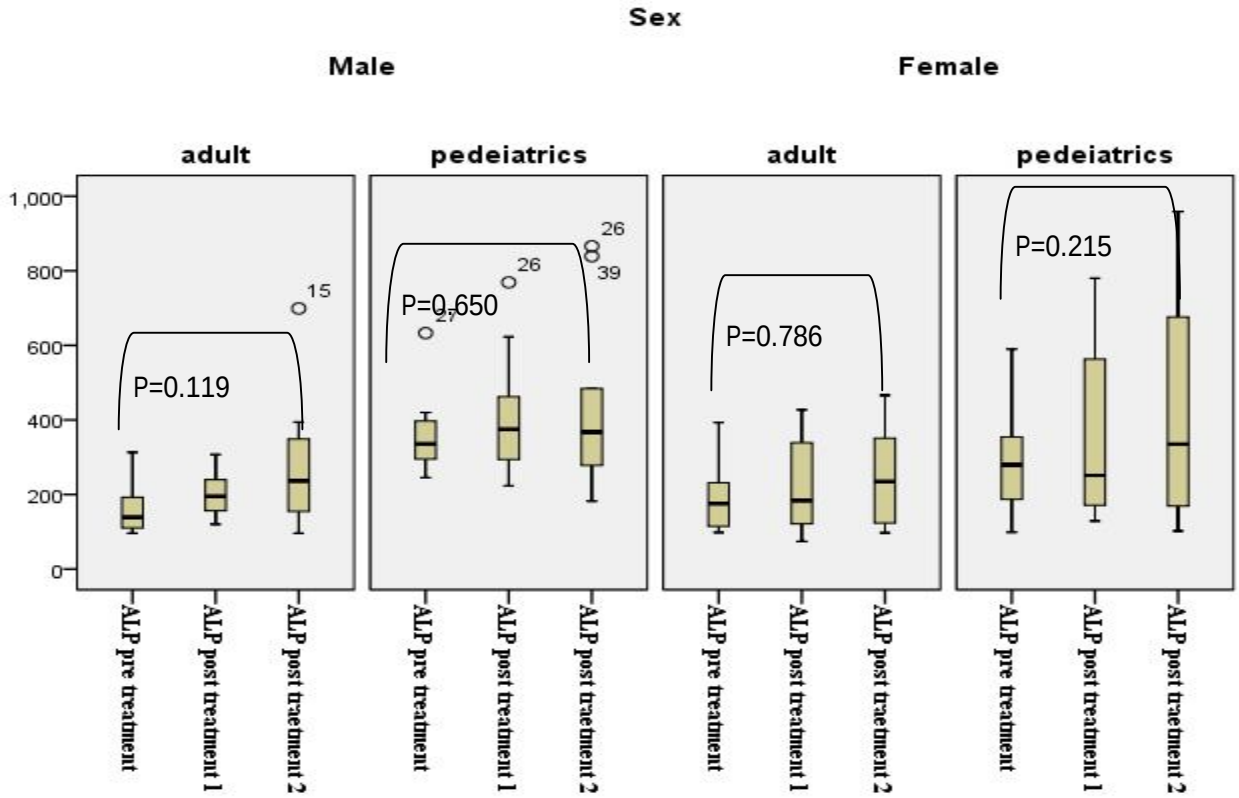
[b]*one way ANOVA; to see gender difference on all adjusted age group*

Figure 3. Box-whisker of gender specific between adult and paediatrics at all measurements
figure 3a. Box-whisker of gender specific ALT and AST activity between adult and paediatrics



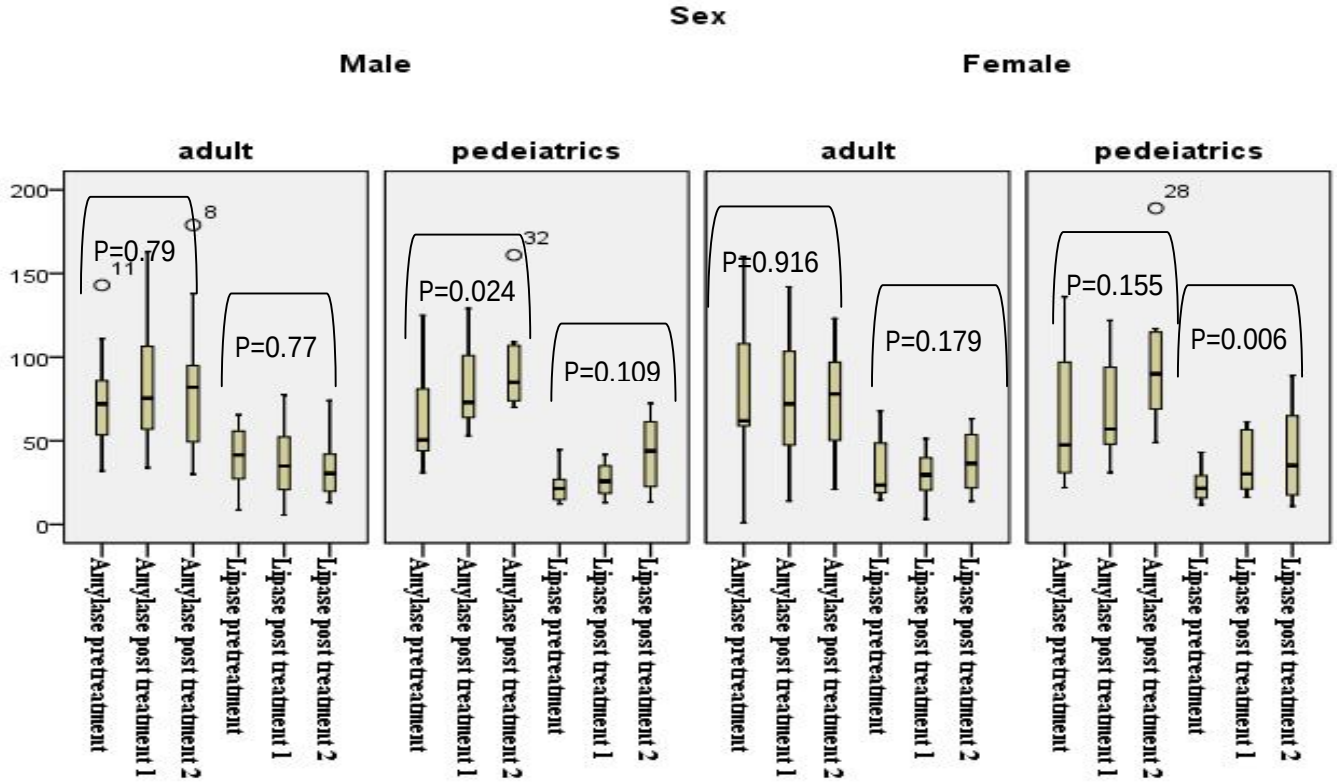
- *Repeated measurement of ANOVA was used for p-value*

Figure3b.Box-whisker of gender specific ALP activity between adult and paediatrics at all measurements



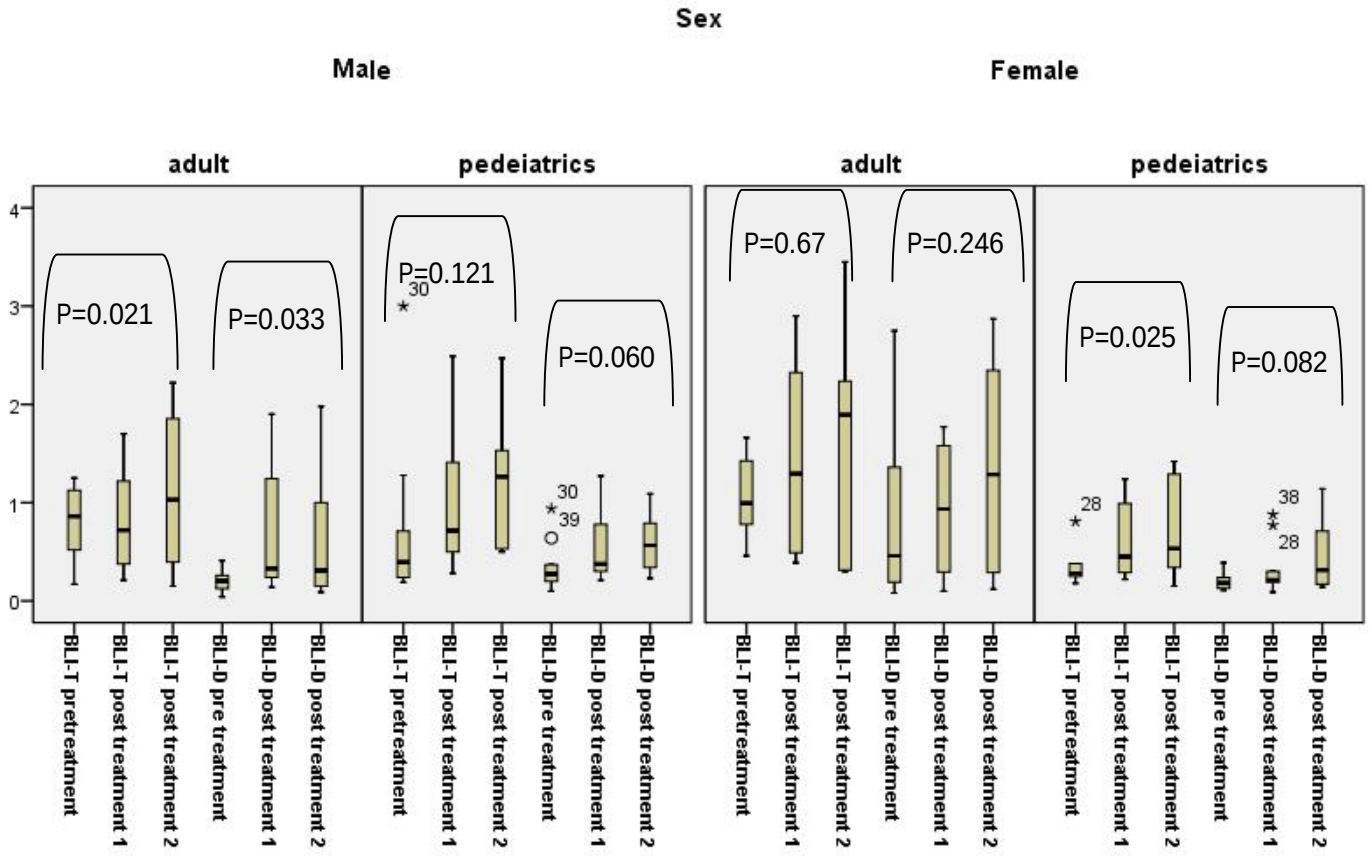
- *Repeated measurement of ANOVA was used for p-value*

Figure3c.Box-whisker of gender specific amylase and lipase activity between adult and paediatrics at all measurements



- *Repeated measurement of ANOVA was used for p-value*

Figure 3d. Box-whisker of gender specific BLI-D and BLI-T activity between adult and paediatrics at all measurement

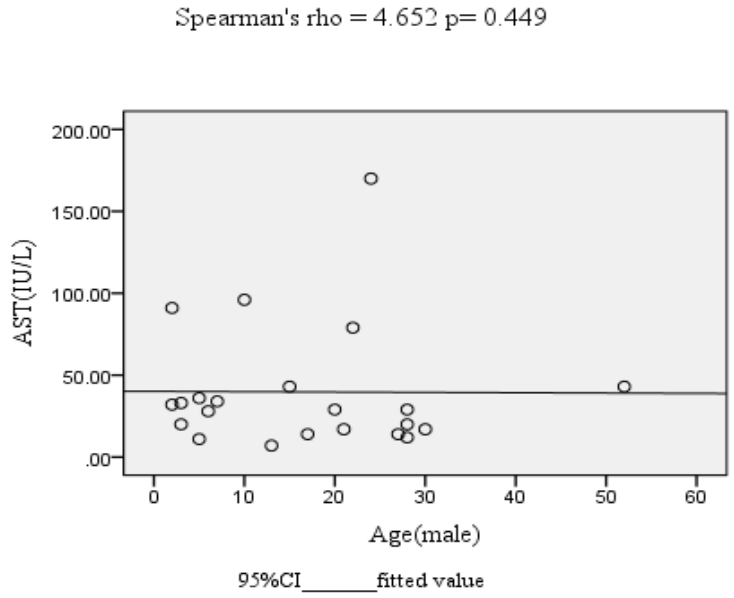


- *Repeated measurement of ANOVA was used for p-value*

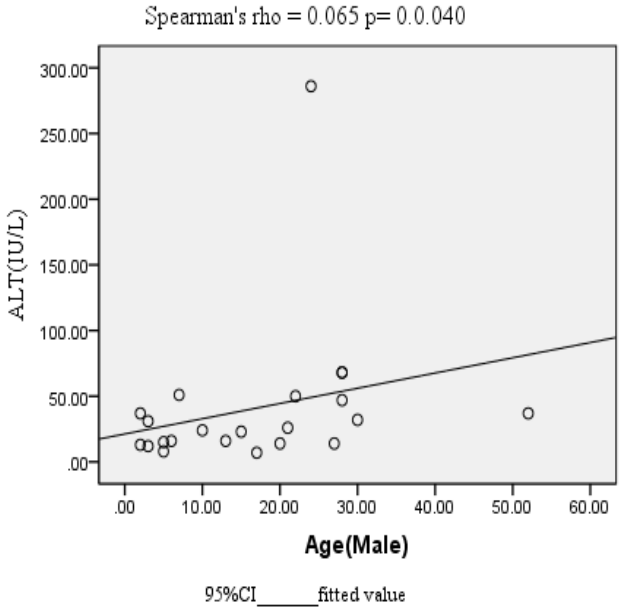
Figure 4: Regression fit of age versus analyst activity of male and female study participantsbefore treatment

Figure 4.1: Regression fit of age versus analyst activity of Male study participants before treatment.

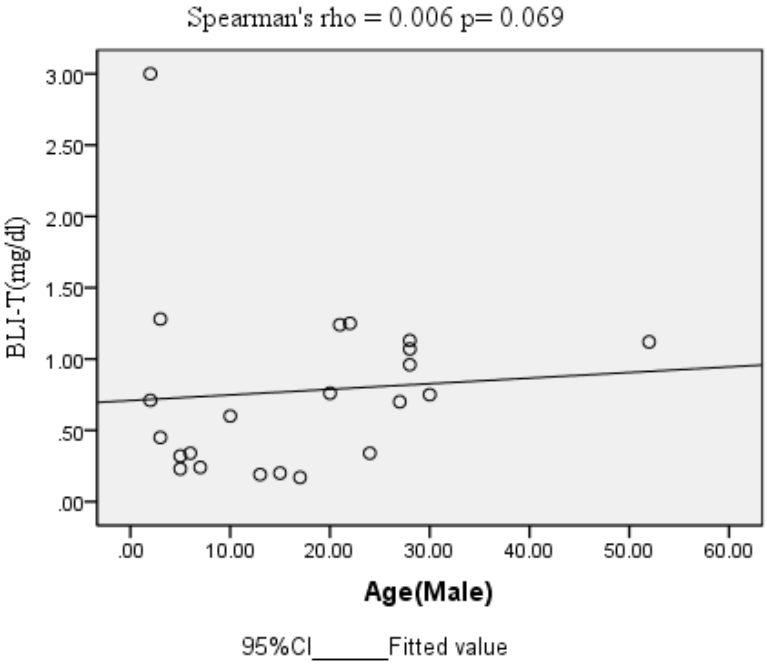
A. Regression fit age versus AST before treatment



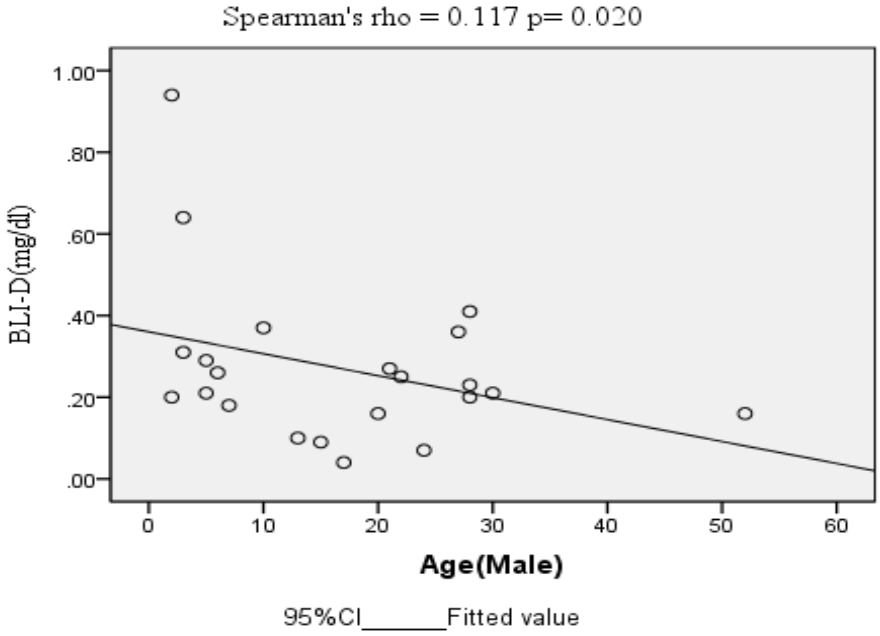
B. Regression fit age versus ALT before treatment



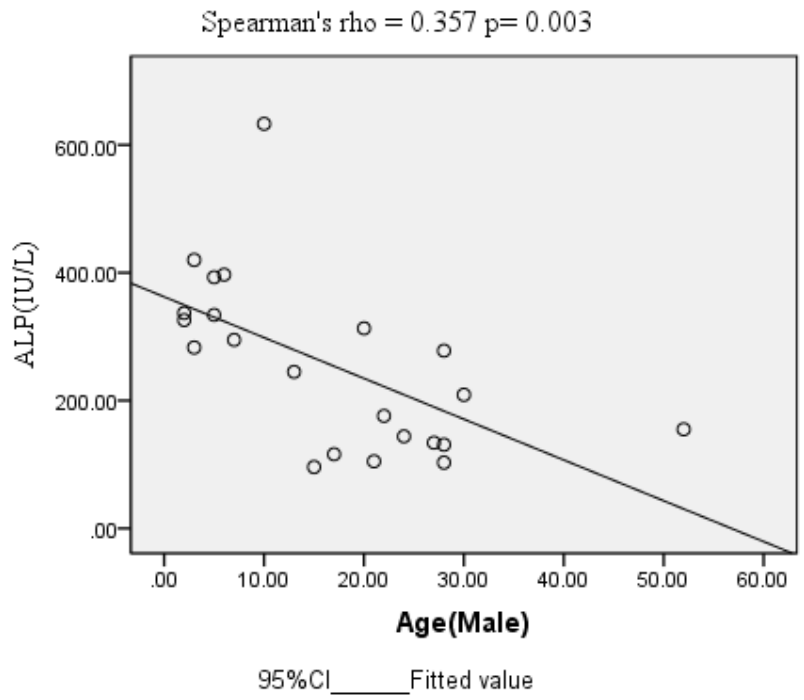
C. Regression fit age versus BLI-T before treatment



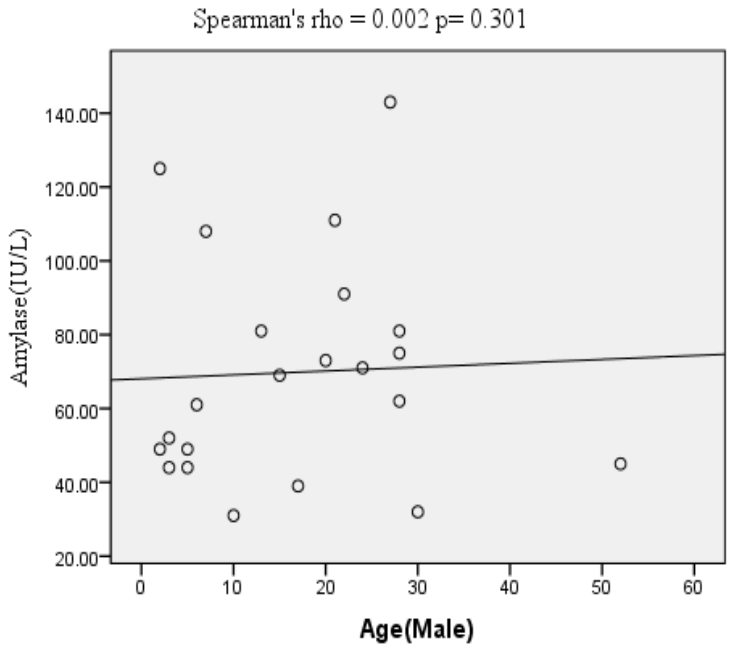
D. Regression fit age versus BLI-D before treatment



E. Regression fit age versus ALP before treatment



F. Regression fit age versus amylase before treatment



G. Regression fit age versus lipase before treatment

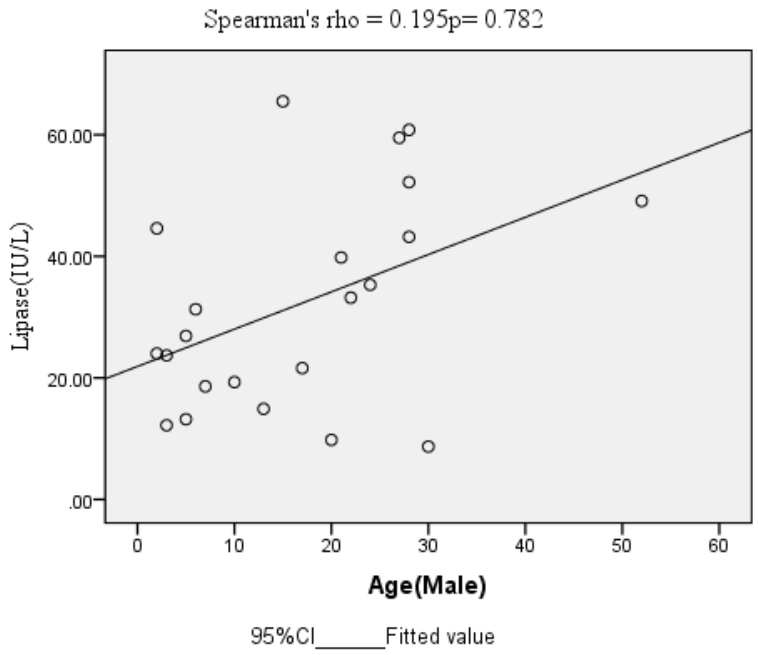
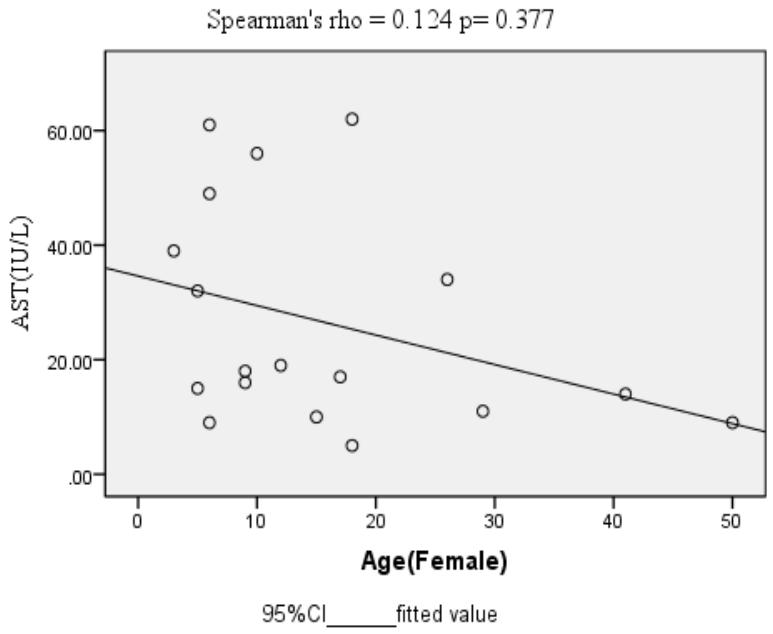
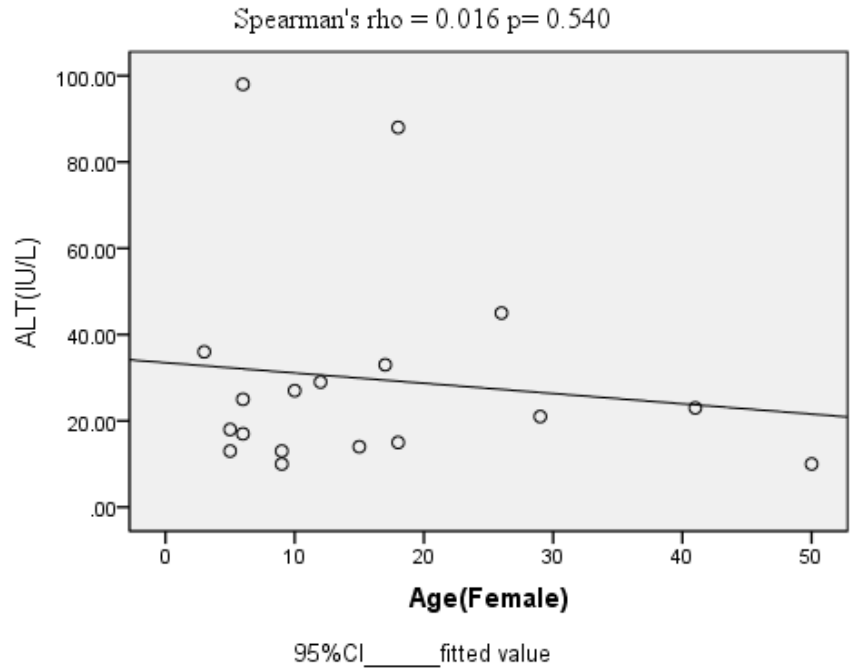


Figure 4.2: Regression fit of age versus analyst activity of female study participants before treatment.

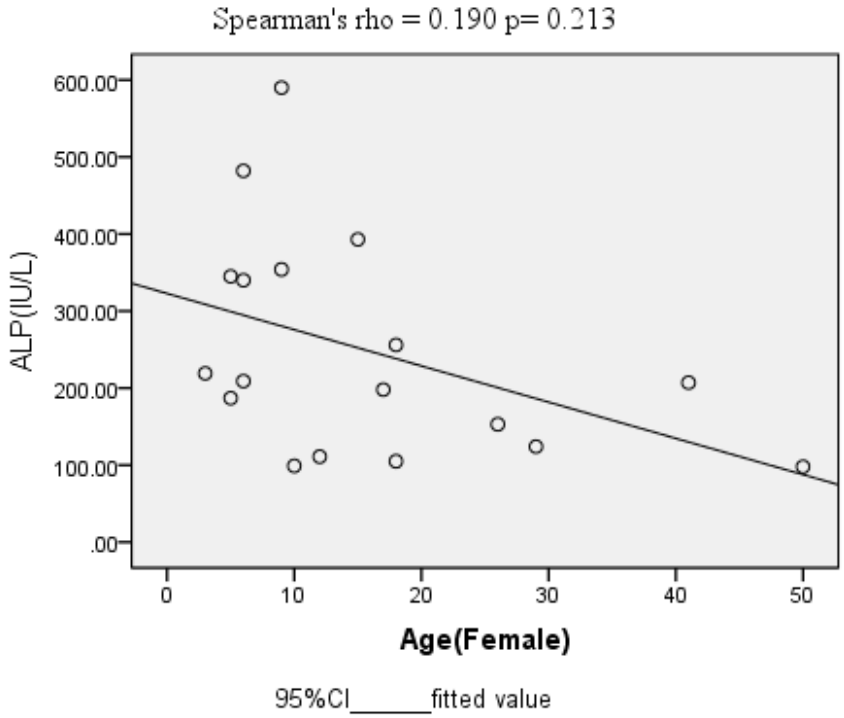
A. Regression fit age versus AST before treatment



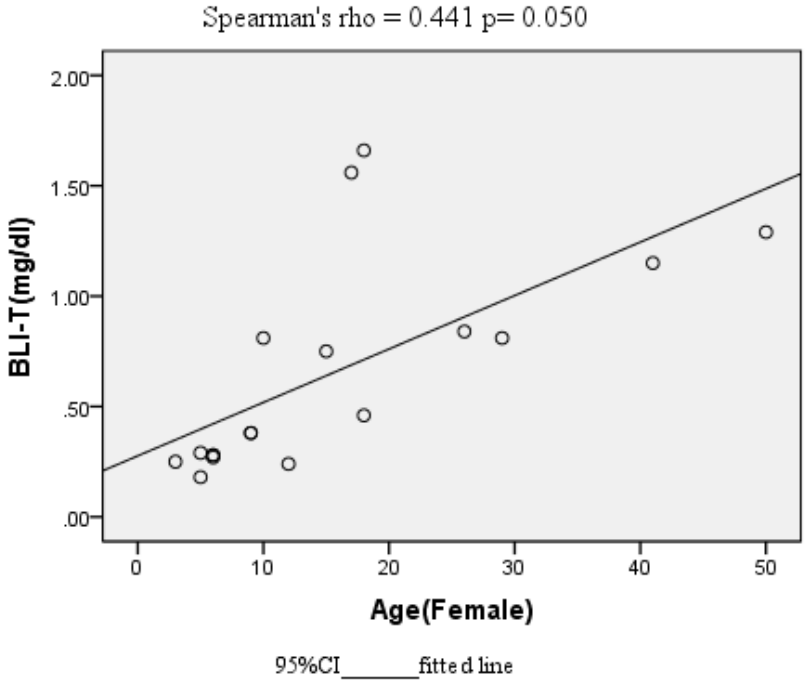
B. Regression fit age versus ALT before treatment



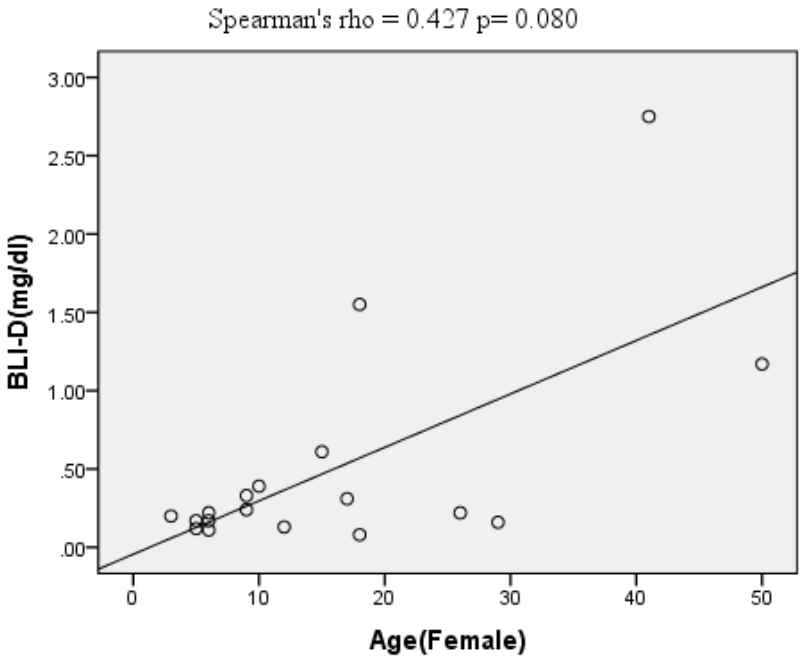
C. Regression fit age versus ALP before treatment



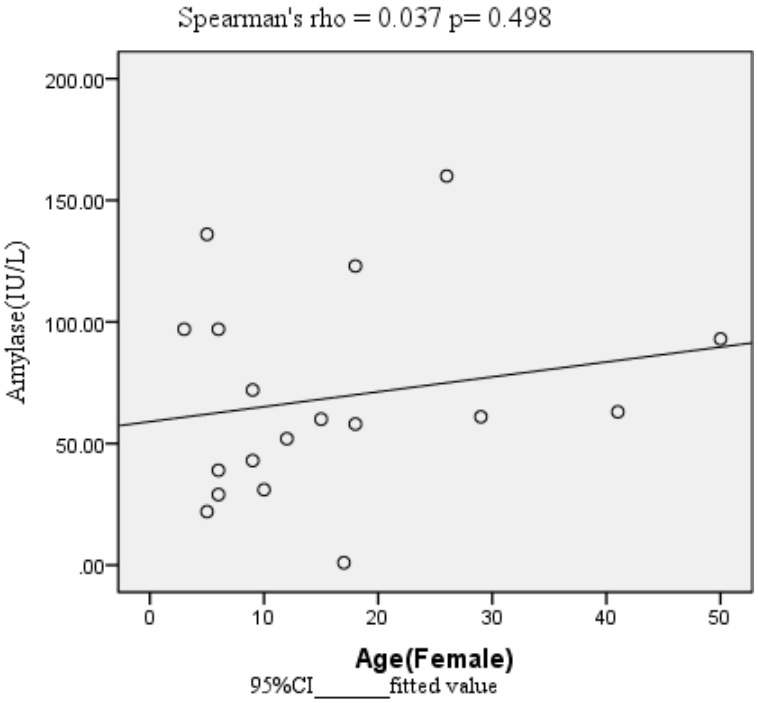
D. Regression fit age versus BLI-T before treatment



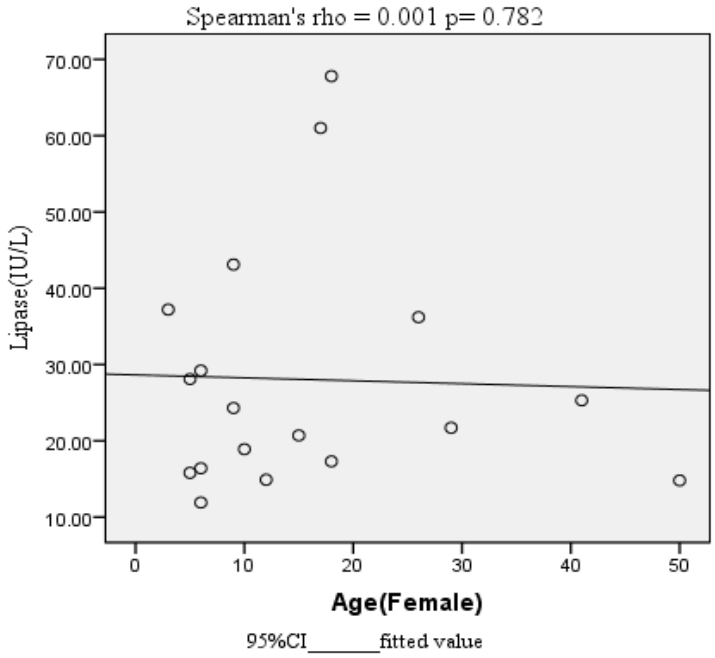
E. Regression fit age versus BLI-D before treatment



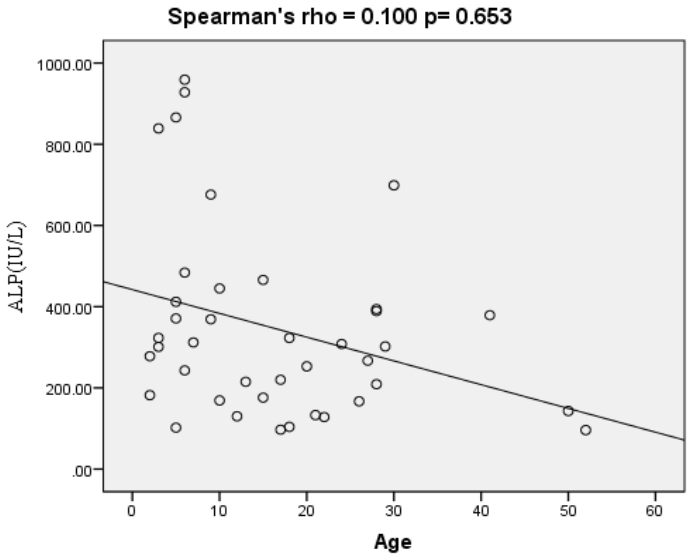
F. Regression fit age versus amylase before treatment



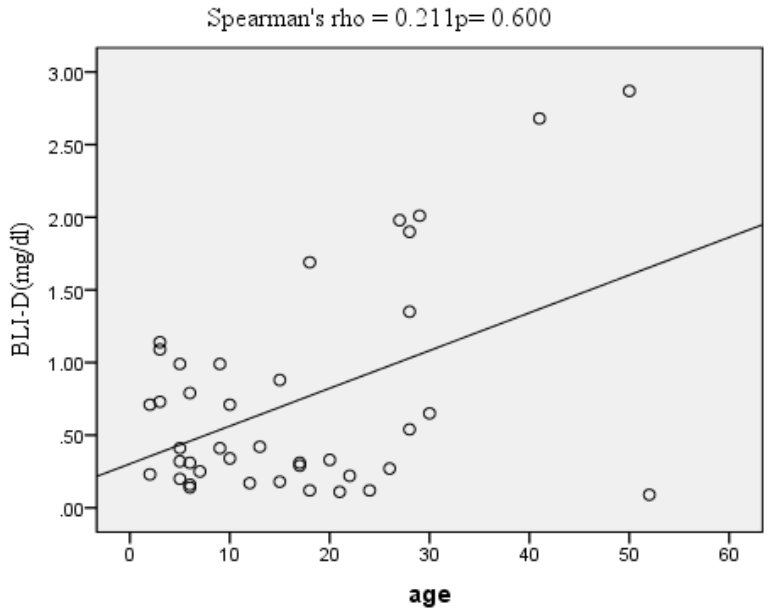
G. Regression fit age versus lipase before treatment



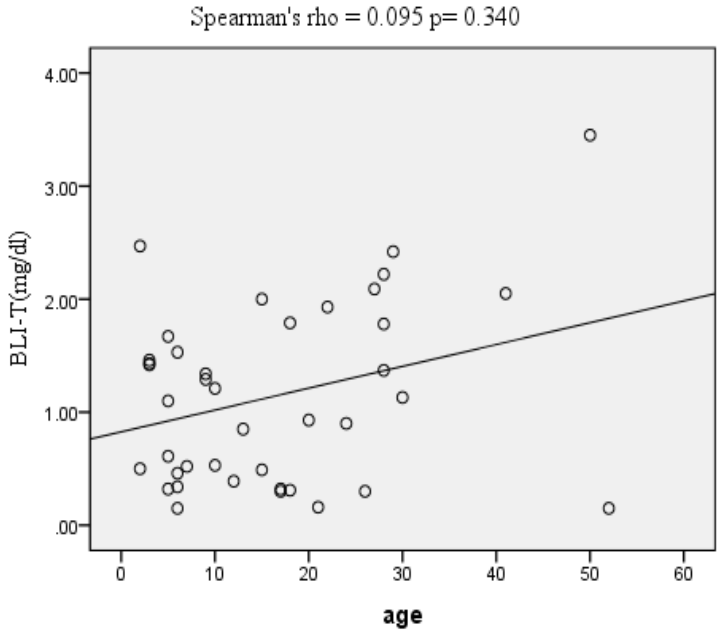
H. Regression fit age versus ALP post treatment two.



I. Regression fit age versus BLI-D post treatment two



J. Regression fit age versus BLI-T post treatment two



7.3 Selected factors for ALL patients and abnormal clinical chemistry test results

Risk factors presented at the initial evaluation of ALL cases were: Transfused 35 (87.5%), alcohol intake 5(25%), underweight 7 (17.5%). An increased value of ALP was significantly associated with weight loose (AOR=1.24) with P<0.05. ALL patients with weight loose would have 4.9 times more likely to have higher ALP, Table (7). The increased value of total bilirubin was significantly associated with alcohol intake with (AOR= 1.5) with P<0.05, Table (8). ALL patients with alcohol intake would have 1.5 times more likely to have higher total bilirubin. However there was no statistical significant association of AST, BLI-D, lipase and amylase with alcohol intake, weight loose, gender, blood transfused and age, p>0.05.

Table 7: The association of ALP with risk factors of ALL cases at Black Lion Specialized Hospital Addis Ababa, Ethiopia from January 2019 to September 2019 (n=40)

Factor		Alkaline phosphatase			
		H(n)	N(n)	p-value	AOR9(95%CI)
Alcohol intakes	Yes	4	1	0.0157	1.385(1.04-1.844)
	No	5	10	1	1
Blood transfused	Yes	4	5	0.91	1.2(1.09-1.3)
	No	0	31	1	1
Weight loose	Yes	4	3	0.045	1.24(1.06-1.452)
	No	4	29	1	1
Gender	M	1	21	0.21	0.23(0.023-2.5)
	F	3	15	1	1

Table 8: The association of BLI-T with risk factors of ALL casesat Black Lion Specialized Hospital Addis Ababa, Ethiopiafrom January 2019 to September 2019 (n=40)

Factor		Bilirubin-total			
		H(n)	N(n)	p-v	AOR(95%CI)
Alcohol intake	Yes	4	1	0.025	1.5(1.05-2.1)
	No	5	10	1	1
Blood transfused	Yes	8	27	0.47	0.44(0.63-3.1)
	No	2	3	1	1
Weight loose	Yes	3	7	0.29	2.7(0.50-15.4)
	No	4	26	1	1
Gender	M	4	18	0.27	0.44(0.10-1.9)
	F	6	12	1	1

7.4 Results of Liver and pancreatic test on acute lymphoid leukemia patients

From 40 ALL cases an increased level of serum AST, ALT, ALP, direct bilirubin, total bilirubin, amylase, lipase was found in 12(30%), 9 (22.5%), 14(355%), 16(40%), 7(17.5%) ,7(17.5%), and 4(10%) cases at base line respectively(Table 9). Meanwhile after chemotherapy induction there were an increment of AST, ALT, ALP bilirubin direct, bilirubin total, amylase and lipase with the result of 16(40%), 18(45%),19(47.5%),19(47.5%), 27(67.5%), 10(25%) , 9(22.5%) respectively(Table 9). From all study acute lymphoid leukemia cases 5% have hepatotoxicity with elevated serum ALT value greater than 3 folds of the normal value (Table 9).

Table 9: Results of clinical chemistry test on ALL cases at Black Lion Specialized Hospital Addis Ababa, Ethiopiafrom January 2019 to September 2019 (n=40)

Parameter					
	Before chemotherapy induction		After chemotherapy induction		
	High	Normal	High	Normal	ALL Cases N (%)
	N(%)	N (%)	N(%)	N(%)	
AST	12 (30)	28 (70)	16(40)	24(60)	
ALT	9 (22.5)	31(77.5)	18(45)	22(55)	
ALP	14 (35)	26(65)	19(47.5)	21(52.5)	
BIL-T	7 (17.5)	33(82.5)	19(47.5)	21(52.5)	
BIL-D	16 (40)	24(60)	27(67.5)	13(32.5)	
amylase	7 (17.5)	33(82.5)	10(25)	30(75)	
Lipase	4 (10)	36 (80)	9(22.5)	31(77.5)	
Hepatotoxicity level					2(5)

N= number,AST-Aspartate transaminase, ALT- alanine transaminase ,BLI-D –blirubin direct ,BLI-T, blirubin –total, ALP- alkalinphosphatas

7.5 Selected abnormal clinical chemistry test results of ALL patients

The mean value of ALP, bilirubin total, direct andamylase was significantly higher in ALL patients after induction therapy than before with means ± SD was (252.3±136.4 versus 346.4±234.6, P=0.008), (0.72±0.56 versus 1.14±0.8, P=0.004), (0.28±0.20versus 0.53±0.34, P=0.005) and(73.5±49.4 versus 87.1±36.5, p=0.045) before and after respectively.However the mean value AST, ALT and lipase were not significantly increased. Chemotherapy drugs increased the liver function tests and other clinical chemistry analyte on acute lymphoid leukemia patients.

Table 10: Abnormal clinical chemistry test on ALL cases in at Black Lion Specialized Hospital Addis Ababa, Ethiopiafrom January 2019 to September 2019 (n=40)

	ALL cases (N=40)			
Parameters	Before Mean ±SD	After chemo drug intake		*p-value
		First Mean ±SD	Second Mean ±SD	
AST	33.8±31.5	37.7±28.2	41.0±32.6	0.384
ALT	35.7±45.7	41.3±29.2	47.5±36.8	0.201
ALP	252.3±136.4	295.1±175.4	346.4±234.6	0.008
BLI-T	0.72±0.56	0.94±0.70	1.14±0.8	0.004
BLI-D	0.28±0.20	0.44±0.33	0.53±0.34	0.005
amylase	73.5±49.4	77.1±33.3	87.1±36.5	0.045
lipase	37.7±35.3	32.5±16.6	39.0±21.3	0.124

- *N – number ,SD- standareddevation ,AST-Aspartate transaminase ,ALT- alanine transaminase ,BLI-D –blirubin direct ,BLI-T- blirubin –total, ALP- alkalinphosphatas*
- **-Repeated measurement of ANOVA was used for statics analysis*

8. Discussion

Acute lymphoblastic leukemia is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extra medullary sites. While 80% of ALL occurs in children, it represents a devastating disease when it occurs in adults **(**4**).**

Hepatotoxicity is an injury to the liver that is associated with impaired liver function caused by exposure to a drug. An ALT level of more than three times the upper limit of normal values used to define clinically significant abnormalities on liver test(7).

The mean value of direct bilirubin and total bilirubin showed increment in acute lymphoid leukemia cases. However, only mean value of direct bilirubin was significantly higher in acute lymphoid leukemia patients as compared to the level before chemotherapy. This finding is in line with the study conducted in other area which shows that serum bilirubin was significantly higher in acute lymphoid leukemia. In addition, different case reports conducted in different areas showed that elevated total and direct bilirubin in acute lymphoid leukemia patients (20, 21).

According to this study chemotherapy drugs has significant effect on the function of liver and pancreas changes in acute lymphoid leukemia patients. The mean value of ALP, total and direct bilirubin was significantly higher in acute lymphoid leukemia patients. However, there was no statistical significant effect of acute lymphoid leukemia on the mean of AST, ALT, amylase and lipase.

The present study shows a significant increase blood levels of ALP and blirubin-directon acute lymphoid leukemia adult patients after chemotherapy as compared to before chemotherapy while a significant increase blood levels of bilirubin-total, direct ,amylase and lipase were seen on acute lymphoid leukemia pediatric patients. So, this elevated biochemical parameter was due to liver injury induced by chemotherapy after induction phase of treatment with drugs, weconcluded that chemotherapy drugs like Vincristine, L-asparaginase, predensolonand others cause liver damage and also produces acute pancreatic (10).

In the present study the mean of serum ALP, total and direct bilirubin was significantly higher in acute lymphoid leukemia cases. This finding is in line with the previous study conducted in other place (20) which reported significantly higher value of ALP, total and direct bilirubin in acute lymphoid leukemia patients. Chemotherapy induction has effects on adults and pediatrics acute lymphoid leukemia cases, elevation of LFT and pancreatic enzymes. Furthermore, different case reports support the present study and reported liver dysfunction with raised value of serum AST, ALT and bilirubin, ALP from acute lymphoid leukemia patients (21, 20). This may be attributed toleukemic infiltration, peripheral blood cell depression (because of hemorrhagic or infectious processes) and toxicity induced by chemotherapy (9).

The mean value of AST, ALT, amylase and lipase showed slight increment in acute lymphoid leukemia cases. Different case reports conducted in different places showed that elevated AST, ALT, amylase and lipase in acute lymphoid leukemia patients (19, 17). This may attribute to the chemotherapy induction drugs and associated risk factors.

In this study amylase and lipase were significantly increase only on pediatric acute lymphoid leukemia. This attribute to L-asparginase drug which causes acute pancreatitis on pediatric patients. Induction therapies have effect on liver profiles. The mean value of AST, ALT, ALP, bilirubin total and bilirubin direct was higher as compared to before induction therapy in the present study. This finding is in line with the previous study conducted in different areas (20) which reported higher value of AST, ALT, and ALP, bilirubin total and direct.

In the present study assessed the effect of gender, age, blood transfusion, weight loose and alcohol intake on clinical chemistry test alterations of acute lymphoid leukemia patients. ALP and total bilirubin were significantly associated with weight loose and alcohol intake. However, the abnormal value of AST, ALT and direct bilirubin has no significant association with gender, age, blood transfusion, weight loose and alcohol intake in the present study.The present study also assessed the association of alcohol intake of acute lymphoid leukemia with the alteration of clinical chemistry tests. The value of bilirubin total was significantly associated with alcohol intake. This may be attributed to alcohol causes swollen of liver, which blocks the removal of bilirubin (13). The value of ALP was also significantly associated with weight loose. This also might be attributed to malnutrtioned cause deficiency of protein, calcium, magnesium, which causes abnormal value of ALP (11).

In this study only 5% hepatotoxicity which have 3 times ALT level were elevated. The present study shows lower level of hepatotoxicity as compared with other study which shows 13% (19).This is might be genetical and other associated risk factors (11).

In this study some analytes like lipase and bilirubin results have increase before treatments which is in line with study conducted at turkey and other places this is attribute to as the age increase activity of some enzymes like lipase was increase. Bilirubin direct and blirubin total have significantly increased on male but only ALT was significantly increased on females in acute lymphoid leukemiaon post treatment. This attribute to the physiological difference of male and female(23, 25), with the chemotherapy drugs, which have effects on liver function tests. ALP was increase at the age group(1-10 years) this is due to increased osteoblast activity following accelerated bone growth was happen at this stage of age group(26,27).

9. Strengthens and limitations of the study

9.1 Strengthens of the study

Up to the knowledge of principal investigator and advisors this study is the first in Ethiopia and it provides baseline information for further study and policy makers.

9.2 Limitations of the Study

Different tests like coagulation profile, protein, albumin gamma glutamate, and electrolyte were not done in this study due to lack of budget and time.

The literatures for this study were limited and this makes difficult to discuss our result with other findings**.**

10. Conclusion

Acute lymphoid leukemia patients showed significant alterations of liver function and pancreatic enzyme tests. This may indicates that chemotherapy drugs were the determinant factor for developing of liver and pancreas disorder**.** So clinicians should assess not only liver enzymes but also pancreatic enzymes should be consideration because fatality rate might be not only due to liver it might be due to pancreatitis. The clinical chemistry tests like ALPand total bilirubinwere significantly associated with weight loose and alcohol intake. The hepatotoxicity level of this study was only 5% of from total study participants.

11. Recommendations

- It is better if health professionals and stake holders use all necessary clinical chemistry tests for management of liver dysfunction and pancreatic disorders of acute lymphoid leukemia patients to prevent the complications of the disease.
- Large scale cohort study is recommended to study the effect of chemotherapy drugs on different organs of the body to build a comprehensive picture on the risk of ALL.

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13. ANNEXS

13.1 ANNEX I: Information sheet in English Version

Title of the Research Project: Assessment of hepatotoxicity incidence and risk factors among adults and pediatrics ALL patients' chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia

Principal Investigator: Ayale Tsegaye (BSc, MSc candidate)

Name of the Organization: Addis Ababa University College of Health Sciences, Department of medical laboratory.

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams was include one principal investigator, two advisors; one from Addis Ababa University, Medical Laboratory department and one from hematology unit ,Black Lion Specialized Hospital. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we have been trying to asses' Hepatotoxicity among ALL patients before and after chemotherapy induction on adults and pediatrics.

Procedures

In order to perform the indicated study Black Lion Specialized Hospital, Addis Ababa, Ethiopia, you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by a principal investigator, nurses of hematology unit and laboratory technologist of the hospital laboratory. Then, you are requested to give your consent to the sample collector.

After consent, 5ml blood specimen will be collected from you by specimen collector and face to face interview for additional questions.

Potential risks and Discomforts

There will be minor discomfort during blood specimen collection. During collection of specimen from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. But based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for managing of ALL patients from liver abnormality and other related discomforts. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free.

Contact information

If you have any questions about this study you can contact the following principal investigators for further information.

PI: Ayale Tsegaye (BSc, MSc candidate)

Phone: 0921219900

E-mail:ayaltsegaye@gmail.com

13.2 Annex II: Information sheet in Amharic version

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

መግቢያ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎት ንማንኛዉም ሃሳብ ይጠይቁ፡፡

የጥናቱ ርዕስ፡Hepatotoxicity incidence and risk factors among adults and pediatrics ALL patients on chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia

የጥናቱባለቢት፡Ayale Tsegaye (BSc, MSc candidate)

የጥናቱ አላማ፡ Hepatotoxicity incidence and risk factors among adults and pediatrics ALL patients chemotherapy induction at Black Lion Specialized Hospital,AddisAbaba, Ethiopia **እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቀሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡**

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪሰዎች ብቻ ናቸው። ከዚያም በላይ ስለእርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመሳፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚነዎት የእርሶዎ ተሳትፎ የእርስዎን ናየወገንዎትን የጉበት የምርመራ ውጤት ለመከታተል ከፍተኛ ጥቅም ይኖረዋል።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት። የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገርግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚከተለው አድራሻ ይጠቀሙ።

Ayale Tsegaye

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13.3 Annex III: Informed consent form in English version

Code.…..……

Name of principal investigator: Ayale Tsegaye , Department of medical laboratory; AAU

Advisors/Co-investigators: Mr. Samuel Kinde, Department of medical laboratory; AAU, Dr. Fisehatsion Tadess, Black lion specialized hospital, AAU and Dr. Daniel Hailu, Black lion specialized hospital, AAU

Name of institute: AAU and BLSH

Funded by: AAU

Reviewed by: Departmental Research and Ethics Review Committee (AAU),

Research Title: Hepatotoxicity incidence and risk factors among adults and pediatrics ALL patients chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia

I had been informed that the objective of this study. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood sample and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

Signature_____ Date_____

Thank you for consenting to take part in the study

13.4 Annex IV: Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ Hepatotoxicity incidence and risk factors among adults and pediatrics ALL patients on chemotherapy induction at Black Lion Specialized Hospital, Addis Ababa, Ethiopia.

"ጥናት ላይ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል። የጥናቱንም አላማዎችም ተረድቻለሁ። በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል። ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳት ናበፍጹም ፍቃደኝነት ነው። በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉን ምምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ---------- **ቀን** ----/---/----------

13.5 Annex V: Structured questionnairefor acute lymphoid leukemia patients

A. Englishversion

Instruction: Please try to complete all information

Part I: Socio-demographic characteristics

1. Code. _____

2. Age (in yrs)._____

3. Sex: Male ☐ Female ☐

4. Have you ever consumed alcohol products?

A. Yes B. No

5. Do you have another chronic disease problem before this drug taken?

A. Yes B. No

6. If yes for Q5, what type of disease you have

A. chronic liver disease B. Diabetic C. Hypertension D. others, specify

7. Have you transfused blood for the last one month?

A. Yes B. No

Checklist to record anthropometric data

S. No	Variables	Value	Remark
1.	Weight (Kg)		
2.	Height (m)		
3.	BMI (Kg/m^2)		

Checklist to record clinical data and current medication

S. No	Variables	Value			Remark
1	Type of clinical diagnosis				
2	Type of current medication	1.	2.	3.	
3	Date of Rx started				
4.	Date of first sample collected				
5.	Date of second sample collected				
6.	Date of third sample collected				

Checklist to record laboratory findings

S. No.	Tests	Results	Remark
1	AST		
2	ALT		
3	ALP		
4	Bilirubin -T		
5	Bilirubin -D		
6.	Amylase		
7.	Lipase		

B. Amharic version

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ት/ክፍል

መመሪያ፡ እባክዎ ሁሉንም መረጃ ለመምላት ይሞክሩ

I: **የግል መረጃና ከጉበት ህመም ጋር ተያያዥነት ያላቸው ጥያቄዎች**

1. **ኮድ** _____

2. ***ዕድሜዎት***_____

3. **ጾታ ወ ☐ ☐ ሴ**

4. **የአልኮል ውጤቶች (አረቂ,ቢራ, ጠጅ, ጠላ)ይጠቀማሉ?**

   ሀ. **አዎ ለ. አልጠቀምም**

5. **ይህን መድሃኒት ከመጀመርው በፊት ሌላ የቆየ ህመም ነበረብዎት?**

ሀ. ***ዐዎ*** **ለ. የለብኝም**

6. **ለ5ኛ ጥያቄ መልስዎ አዎ ከሆነ ምን ዐይነት ህመም ነበረበዎት?**

ሀ. **የጉበት ለ. የስኩር ሐ. የግፊት መ.ሌላ ካለ…….**

7. **ደም ወስደዋል መዲሃንቱን ከወሰዱ በሀዋላ?**

   ሀ. **አዎ ለ. አልወሰድኩም**

ለትብብርዎ እናመሰግናለን፡፡

13.6 Annex VI: Standard operating procedure (SOP)

A. Pre-analytical phase

Materials and equipment for serum preparation

- ✓ Human blood sample
- ✓ SST
- ✓ Serological pipette of appropriate volume
- ✓ Centrifuge
- ✓ Nunc tube

First there will have Proper patient identification and then fasting blood samples will be taken from the anti-cubital vein of the arm by using 5-cc syringes after proper antisepsis with alcohol. Then the blood from each participant will be transferred to serum separator tube (SST) and allowed to stand for 30 minutes till clotted. Then serum will be separated by centrifugation at 1500 rpm for 15 minutes.

Procedure for serum separation

1. 4 ml sample whole blood will be drawn into SST containing no anticoagulant.
2. Then it will be incubate in an upright position at room temperature for 30-40 minutes (no longer than 60 minutes s) to allow clotting
3. It will centrifuged for 15 minutes (1000-2000rpm)
4. Then it will be inspected for turbidity. Turbidity sample should be centrifuged and aspirate again to remove remaining insoluble matter.
5. Aliquot into nunc tubes and stored at –20 °C. The nunc tubes will be labeled with patient identification number.

B. Analytical phase

1. Liver function tests

I. Alanine Aminotransferase

Test Method

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+. The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm.

Test procedure

Pipette into cuvte	at37 □	25□-30□
Working reagent	1000µl	1000µl
Sample	100µl	200µl
Mix, read the absorbance after 1 minutes and at the same time start the stop watch ,read the absorbance again exactly after 1, 2, and 3 minutes. Measure the change absorbance (ΔA) of the sample . Then calculate concentration based on conversion factor.		

Clinical significance

The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have uncomplicated myocardial infarction.

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity.

In patients with vitamin B6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme.

II. Aspartate Aminotransferase

Test method

This assay follows the recommendations of the IFCC, but was optimized for performance and stability. AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Test procedure

Pipette into curvet	At37	25℃-30□
Working reagent	1000µl	1000µl
Sample	100µl	200µl
Mix, read the absorbance after 1 minutes and at the same time start the stop watch ,read the absorbance again exactly after 1, 2, and 3 minutes. Measure the change absorbance (ΔA) of the sample . Then calculate concentration based on conversion factor.		

Clinical significance

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in disease involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak 2 days after onset.

In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of apoenzyme to holoenzyme. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the serum of patients with coronary and hepatobiliary disease.

III. Alkaline phosphatase

Principle

Colorimetric assay in accordance with a standardized method. In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Procedure

Pipette into curvet	. At 25□ , 30□and 37□
Working reagent	1000µl
Sample	20µl
Mix, read the absorbance after 1 minutes and at the same time start the stop watch ,read the absorbance again exactly after 1, 2, and 3 minutes. Measure the change absorbance (ΔA) of the sample. Then calculate concentration based on conversion factor.	

Clinical significance

Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type, and the variant from the germ cells. It occurs in osteoblasts, hepatocytes, leukocytes, the kidneys, spleen, placenta, prostate, and the small intestine. The liver-bone-kidney type is particularly important.

A rise in alkaline phosphatase occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget's disease, hyperparathyroidism, rickets, and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.

IV. Bilirubin –T

Test method

Colorimetric diazo method. Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium. The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

Test procedure

Pipette into cuvettes	Reagent blank	Sample /STD
Sample /CAL	-	20µL
Dist. Water	20µL	-
RGT1	1000µL	1000µL
Mix gently and incubate for exactly 5 min at 37□. Read absorbance one (A1)		
RGT2	250µL	250µL
Mix gently and incubate for exactly 5 min at 37□. Read absorbance two (A2). ΔA=A2-A1 Then calculate the concentration based on correction factor .		

Clinical significance

Measurement of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder blockage. Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

V. Bilirubin –D

Test method

Acidified sodium nitrite produces nitrous acid, which reacts with sulfanilic acid (in acidic solution) to form a diazonium salt. The diazotized sulfanilic acid then reacts with bilirubin to form isomers of azobilirubin. In the direct bilirubin assay, only conjugated bilirubin is converted by the diazotized sulfanilic acid. The intensity of the red color of azobilirubin is measured photometrically and is proportional to the direct (conjugated) bilirubin concentration.

Test procedure

Pipette into cuvettes	Reagent blank	Sample /STD
Sample /CAL	-	100µL
Dist. Water	100µL	-
RGT1	1000µL	1000µL
Mix gently and incubate for exactly 2-5 min at 37□. Read absorbance one (A1)		
RGT2	250µL	250µL
Mix gently and incubate for exactly 5 min at 37□. Read absorbance two (A2). ΔA=A2-A1 Then calculate the concentration based on correction factor.		

Clinical significance

Bilirubin is an organic compound formed by the reticuloendothelial system during the normal and abnormal destruction of red blood cells. The heme portion from hemoglobin and from other heme containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Measurements of bilirubin are used in the diagnosis of liver disease, in the detection of hemolytic anemia, and to evaluate degrees of jaundice. Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure

causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

2. Pancreatic enzyme test

I. a-amylase

Test method

Enzymatic colorimetric assay acc. to IFCC. Defined oligosaccharides such as 4, 6-ethylidene-(G7) p-nitro phenyl-(G1)-α-D-maltoheptaoside (ethylidene-G7 PNP) are cleaved under the catalytic action of α-amylases. The G2PNP, G3PNP and G4PNP fragments so formed are completely hydrolyzed to p-nitro phenol and glucose by α-glucosidase. The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity. It is determined by measuring the increase in absorbance.

Test procedure

	Blank	Sample
Calibrator /Sample	-	20µL
Distilled water	20 µL	-
Buffer	1000 µL	1000 µL
Mix carefully; incubate for 5 min. Start reaction by adding SUB.		
SUB.	250µL	250µL
Mix , Incubate 2 min. at 37□ read absorbance and start stop watch . After exactly 1 and 2 min. read absorbance again and then calculate ΔA/min.		

Clinical significance

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin, and glycogen by cleaving 1,4-α-glucosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously. Maltotriose, the smallest such unit, is converted into maltose and glucose, albeit very slowly. Two types of α-amylases can be distinguished, the pancreatic type (P-type) and the salivary type (S-type). Whereas the P-type can be attributed almost exclusively to the pancreas and is therefore organ-specific, the S-type can originate from a number of sites. As well as appearing in the salivary glands it can also be found in tears, sweat, human milk, amniotic fluid, the lungs, testes, and the epithelium of the fallopian tube.

Because of the sparsity of specific clinical symptoms of pancreatic diseases, α-amylase determinations are of considerable importance in pancreatic diagnostics. They are mainly used in the diagnosis and monitoring of acute pancreatitis. Hyperamylasemia does not, however, only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions, or in the case of macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme – lipase also be determined.

II. Lipase

Test method

The chromogenic lipase substrate 1,2-O-dilauryl-rac-glyero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-racglycerol and an unstable intermediate, glutaric acid (6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. Addition of detergent and colipase increases the specificity of the assay for pancreatic lipase. Virtually no lipase activity is detected in the absence of colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholatesen sure that the esterases present in the serum do not react with the chromogenic substrate due to highly negative surface charge.

a. 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester + Lipase---------->
1,2-O-dilauryl-rac-glycerol + glutaric acid-(6-methylresorufin) ester

b.Glutaric acid-(6-methylresorufin) ester ---->glutaric acid + methylresorufin

The color intensity of the red dye formed is directly proportional to the lipase activity. It is determined by measuring the increase in absorbance at 583 nm.

Test procedure

	Blank	Sample
Calibrator /Sample	-	20µL
Distilled water	20 µL	-
Buffer	1000 µL	1000 µL
Mix carefully, incubate for 5 min. Start reaction by adding SUB.		
SUB.	250µL	250µL
Mix, Incubate 2 min. at 37□ read absorbance and start stop watch . After exactly 1 and 2 min. read absorbance again and then calculate ΔA/min.		

Clinical significant

Pancreatic lipases the most important clinical chemistry parameters for the differential diagnosis of diseases of the pancreas. The lipase activity determination has gained increasing international recognition because of its high specificity and rapid response. After acute pancreatitis the lipase activity increases within 4-8 hours, reaches a peak after 24 hours and decreases after 8-14 days. However, there is no correlation between the lipase activity determined in serum and the extent of damage to the pancreas. Numerous methods have been described for the determination of lipase which determine the decrease in substrate turbid metrically or nephelometrically or determine degradation products.

Signed Declaration

I, the undersigned declare that this thesis is my work and it has not been presented for a degree or some other purpose in my university, college or institution and that all sources of material used for the thesis have been dully acknowledged.

MSC candidate: Ayale Tesegaye

Signature _____

Date of Submission: 2020

This thesis has been submitted for examination with my approval as university Advisors.

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