ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prognostic importance of Lactate dehydrogenase and Uric acid as compared to breakpoint cluster region-Abelson transcript level among chronic myeloid Leukemia patients in Tikur Anbessa Specialized teaching Hospital, Addis Ababa, Ethiopia.

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A Research thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical Chemistry track).

Jun, 2017

Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Temesgen Sisay Hailemariam entitled "**Prognostic importance of Lactate dehydrogenase and Uric acid as compared to breakpoint cluster region-Abelson transcript level among chronic myeloid Leukemia patients in Tikur Anbessa Specialized teaching Hospital in Addis Ababa, Ethiopia"** submitted in partial fulfillment of the requirements of the Degree of Masters of Sciences in Clinical Laboratory Sciences (Clinical chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Background: Chronic myeloid leukemia (CML) is characterized by the presence of Philadelphia (Ph) chromosome, which results from by the breakpoint cluster region-Abelson (BCR-ABL) fusion gene. Increasing levels of BCR-ABL are strongly predictive of cytogenetic and hematologic relapse. However, this molecular test for BCR-ABL is readily available in the developed world and rarely available in low and middle-income countries, which is also limited in access and very expensive to afford. As a result of this, serum LDH and uric acid estimations, which are easily available and cost effective, have gained considerable appreciation as valuable prognostic markers of CML.

Objectives: To evaluate prognostic importance of total LDH and uric acid as compared to breakpoint cluster region-Abelson (BCR-ABL) transcript level in chronic myeloid leukemia (CML) patients among treated and treatment naive patients.

Methods: A cross- sectional study was carried out from January to March 2017 at Tikur Anbessa specialized hospital in Addis Ababa. Convenient sampling method was used to include eighty one (81) CML patients from those who came for testing GeneXpert RT-PCR transcript level. We studied the correlation between LDH with BCR-ABL and hematological parameters using spearman correlation, Mann-Whitney U test and roc curve data analysis tool.

Result: A total of 81 CML patients were assayed 46(56.8%), medication treated group and the remaining 35 (43.2%) are treatment naive patients. Significant positive correlations were observed between LDH and BCR-ABL (r=0.79, P<0.001). The correlation coefficient value of (r=0.295, p<0.008) indicated that weak correlation exists between uric acid and BCR-ABL. Strong correlation were observed between LDH and hematological parameters: WBC, lymphocyte and Basophil (r=0.73, p<0.001), (r=-0.61<0.001), (r=0.75, p<0.001) respectively. No significant correlation of LDH and platelet count (r=0.24, p<0.069).There was statistically significant (p<0.001) difference in the median level of BCR-ABL and LDH among patients on treatment group (median=21%, 350U/L) and treatment naive group (median=57%, 1246U/L), respectively. For uric acid there was no statistically significant (p < 0.542) difference between the study group. AUC for LDH, Basophil and WBC 0.881, 0.889, and 0.748 respectively showed better performance for the follow-up of patients with CML compared with uric acid (0.695) and platelets (0.70).

Conclusion and recommendation: CML LDH value strongly correlated with BCR-ABL transcript level whereas uric acid was weakly correlated with BCR-ABL therefore; LDH could serve as an alternative cost effective prognostic biomarker to follow-up in those patients.

Keywords: Chronic Myeloid leukemia, LDH, Uric acid, BCR-ABL, WBC, platelets, RBC, hemoglobin

Acknowledgment

I would like to express my sincere gratitude to Department of Medical Laboratory Science, School of Allied Health Science, and Addis Ababa University for financing and allowing me to conduct this MSc work. My special thanks goes to Tikur Anbessa Specialized Hospital Laboratory for allowing me to do the laboratory experiment.

I would also like to express my sincere gratitude to my advisors Dr. Daniel Seifu, from Adis abeba University department of Biochemistry and Mr. Samuel Kinde and Mr. Abebe Edao from department of medical laboratory science for their valuable guidance they provided me throughout my work.

My sincere thanks goes to health professionals on the study site that helped me in analyzing blood samples from the study participants.

Last but not least, my sincere appreciation goes to all my study participants. Above all, I thank God for enabling me, for the strength, courage and wisdom he has given me throughout my study and I would like also to extend my gratitude to my families.

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Abbreviations

ABL	Abelson
ALL	Acute lymphocytic leukemia
ALP	Alkaline Phosphatase
AML	Acute myeloid leukemia
AUC	Area under the curve
BCR	Breakpoint cluster region
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
ELN	European Leukemia Net
IPI	International Prognostic Index
LDH	Lactate dehyderogenase
MDS	Myelodysplastic Syndrome
NAD	Nicotinamide adenine dinucleotide
NCCN	National Comprehensive Cancer Network
OS	Over all suvaival
PCC	Probe Check Control
RT-PCR	Real-time Polymerase Chain Reaction
SPSS	Statistical package for social science
UA	Uric acid
WHO	World Health Organization

Operational definitions

Breakpoint cluster region-Abelson (BCR-ABL) level means the number of copies of the Breakpoint cluster region-Abelson (BCR-ABL) gene found in the body by tests. It reflects the amount of leukemia or CML cells in the body.

International Scale (IS) is a standardized scale for measuring and reporting results of a very sensitive test that measures the number of cells that have the BCR-ABL gene

Prognosis: is the likely or expected course and outcome of a disease and assessments are made at 3, 6 and 12 months after beginning therapy.

Molecular responses are defined by RT-PCR for detection of BCR-ABL mRNA transcript levels.

A complete hematologic response is normalization in peripheral blood counts, including total leukocyte count of less than 10 $\times 10^9$/L, platelet count of less than 450$\times$ 10^9/L, absence of palpable splenomegaly, and absence of immature myeloid cells in the peripheral blood.

Complete hematologic response Normal CBC and differential, absence of palpable splenomegaly

Major molecular response BCR-ABL < 0.1% transcript level

Sensitivity of the test Ability of the test to be correctly positive among those who are known to have the disease

Specificity of the test Ability of the test to be correctly negative among those who are known

Positive predictivity of the test Ability of the test to correctly predict the presence of disease

Negative predictivity of the test Ability of the test to correctly predict the absence of disease

Inherent validity of the test Ability of the test to be correctly positive or correctly negative

Predictive validity of the test Combined ability to correctly predict presence or absence of disease

A complete absence of transcripts is defined as a complete molecular response (CMR) decrease or a reduction to 0.1% compared with the baseline level of BCR-ABL transcripts is defined as a major molecular response (MMR)

1. Introduction

1.1 Back ground

Leukemia's are malignant disorders of the hematopoietic stem cell compartment, characteristically associated with increased numbers of white cells in the bone marrow and peripheral blood. The word leukemia though literally means "white blood" but is used to describe a variety of cancers that begin in the blood forming cells of the bone marrow. Leukemia is caused by the mutation of the bone marrow pluripotent or most primitive stem cells and this neoplastic expansion results in abnormal leukemic cells and impaired production of normal red blood cells, neutrophils and platelets [1].

Leukemia is classified into four main groups according to cell type and rate of growth: acute lymphocytic (ALL), chronic lymphocytic (CLL), acute myeloid (AML), and chronic myeloid (CML) [2]. Chronic myelogenous leukemia (CML) is a myeloproliferative disorder affecting the hematopoietic stem cell compartment. Chronic means the leukemia grows and progresses slowly. Myelogenous means it starts in immature white blood cells called myeloid cells. It can occur in all age groups but is predominantly a disease of adults, accounting for 20% of adult leukemias [3].
CML is caused by the breakpoint cluster region-Abelson (BCR-ABL) fusion gene. This gene is not found in normal blood cells and is not passed down from parents to children. The BCR-ABL gene is formed by a translocation between parts of chromosome 9 and 22. The short bottom place of chromosome 9 has the Abelson (ABL) gene. The ABL gene codes for protein called a tyrosine kinase. The short top place of chromosome 22 has the breakpoint cluster region (BCR) gene. These short places attach to each other. As a result the two genes join (fuse) together and form the BCR-ABL fusion gene. This also result in a longer chromosome 9 and a shorter chromosome 22. The shorter chromosome 22 is called the Philadelphia chromosome. The Philadelphia chromosome is the hallmark of CML. It contains the BCR-ABL gene. Cells with this Philadelphia chromosome are different from normal blood cells in a few key ways. The change in their genes causes CML cells to make new cells that are not needed. The cells also live too long and do not work as they should [4]**.** The BCR-ABL gene makes the abnormal BCR-ABL protein that helps CML cells grow and survive. It is a type of protein called a tyrosine kinase**.** These proteins send signals that tell cells

when to grow and divide. The BCR-ABL protein is not normal. It is locked in the "on" position so that it is always sending signals for cells to grow and divide. This causes blood stem cells to make too many white blood cells called granulocytes. White blood cells made by the BCR-ABL protein aren't normal. They all are CML cells and contain the BCR-ABL gene. These cells do not mature in to healthy normal cells. They may make new cells too quickly. The cells also do not die when they should. Over time the CML cells can build up in the bone marrow. They can overcrowd the bone marrow so there is not room for healthy white blood cells, red blood cells, and platelets. Over months or years, the CML cells can spill out of bone marrow in to the bloodstream. Without treatment the CML cells can eventually reach and collect in other organs such as the spleen. This can damage organs and cause symptoms [4]. The fusion protein varies in size from 190 to 230 kDa, depending on the site of the breakpoint within the BCR gene. The majority of patients with CML express a 210-kDa BCR-ABL protein [5].

Hematological and bone marrow examination are the main parameters for the diagnosis of CML and also important indices for monitoring the prognosis of leukemia during and after treatment. In addition the level of leukemic inhibition after treatment can be measured by quantitative real-time PCR (RT-PCR) which has become the main molecular technique used to monitor BCR-ABL transcript levels in CML during treatment with kinase inhibitors. Increasing levels of BCR-ABL are strongly predictive of cytogenetic and hematologic relapse after allogeneic transplant **[6].** Patients who presented transcript in the peripheral blood samples also showed high lactate dehydrogenase (LDH) and uric acid concentration. As a result biochemical test such as serum LDH activity and uric acid concentration have gained importance in monitoring the prognosis of CML especially during the phase of treatment [7].

In this study, we have described serum LDH and uric acid estimations which are easily available and cost effective valuable prognostic markers of CML. So in order to evaluate prognostic value of serum LDH activity and uric acid concentrations we have tested GeneXpert RT-PCR transcript level.

1.2 Statement of the problem

Chronic Myeloid Leukemia (CML) is a global problem with an incidence of 1–2 cases per 100,000 adults, and accounts for 15% of newly diagnosed cases of leukemia in adults [8]. CML is most common in older adults and among men. Based on the cases reported between 2009 and 2013 in the world the number of new cases of CML was 1.8/100,000 individuals per year whereas the number of deaths was 0.3/100,000 individuals per year [9].

CML will affect over 100,000 patients worldwide every year and represent a significant global health burden. The incidence rate in the United States is roughly 1.6/100,000 [3]. Of all cancers occurring in sub-Saharan Africa, hematologic malignancies have emerged as a major cause of morbidity and mortality. Leukemia together accounted for 8.7% of incident cancer diagnoses and 9.9% of cancer deaths in 2008 [10].

When a patient presents with suspected chronic myeloid leukemia (CML) appropriate assessments are needed to confirm the diagnosis and stage of disease and to assign a risk score to that patient [11]. Regular molecular monitoring is crucial for assessing response to therapy as well as for the early identification of non adherence, treatment resistance, or treatment failure in patients with CML [12]. Therefore testing prior to treatment initiation is critical [3]. Molecular assessments are made at diagnosis and at 3, 6 and 12 months after beginning therapy [13].These molecular tests for BCR-ABL is readily available in the developed world but it is rarely available and very expensive (5500-6500 ETB) in low and middle-income countries like Ethiopia [14,15]. Studies indicated that Patients who presented transcript in the peripheral blood samples also showed high LDH and uric acid concentration. As a result serum LDH and uric acid estimations which are easily available and cost effective have gained considerable appreciation as valuable alternative prognostic markers of CML. As far as our knowledge there is no previous study conducted to evaluate Prognostic importance of lactate dehydrogenase and Uric acid as compared to BCR-ABL transcript in Ethiopia.

Therefore this study tried to confirm the prognostic importance of LDH and uric acid by evaluating them as compared to BCR-ABL.

1.3 Significance of the study

The BCR-ABL transcript level is the gold standard method to monitor CML during treatment. It supports in the process of appropriate assessments to confirm the diagnosis and prognosis of CML, and to assign a risk score to patients. However, this test is limited in access and very expensive to afford by Ethiopian CML patients. As a result the use of alternative test to monitor prognosis is indispensable. Therefore, the finding of this study may indicate LDH and uric acid tests as alternative prognostic markers of CML in terms of possible use as cheaper and easily available biochemical parameters of neoplastic activity in CML patients. And to initiate these tests to monitor treatment programs for their ability to appropriately treat patient and to ensure safe and effective drug administration. Moreover, the result of this study may be used by physicians, health policy planners or policy makers, and other concerned bodies to initiate the application of these tests along with other monitoring packages in the CML.

2. Literature Review

2.1 Biochemistry of Lactate Dehyderogenase

Lactate dehydrogenase (LDH) EC 1.1.1.27; L-lactate: NAD^+ (Nicotinamide adenine dinucleotide) oxidoreductase; LD) is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD^+as a hydrogen acceptor [16]. LDH is a tetrameric enzyme belonging to the 2-hydroxy acid oxidoreductase family, which increases the rate of the simultaneous inter-conversion of pyruvate to lactate and nicotinamide adenine dinucleotide (NAD)H to NAD+ by 14 orders of magnitude [17,18]. LDH is a pyridine-linked enzyme found virtually in all animal and human tissues, functions primarily in the metabolism of glucose, catalyzing the reduction of free pyruvate to lactate during the last step of glycolysis, as well as the conversion of lactate to pyruvate during gluconeogenesis. The reaction is reversible and its equilibrium strongly favors the reverse reaction namely the reduction of pyruvate to lactate. The optimal pH varies with the source of enzyme and depends on the temperature as well as on substrate and buffer concentrations [19].

Five isoforms of LDH have been identified as a result of the five different combinations of polypeptide subunits [20]. They catalyze the reversible conversion of pyruvate to lactate with concomitant regeneration of NAD^+, which is needed for the continuous generation of ATP to maintain glycolysis. Active LDH is a homo or heterotetamer assembled from two types of subunit with molecular weights of approximately 35,000 Da each. And a tetrameric enzyme consisting of two monomer types: H (for heart) and M (for muscle) that combine to yield five LDH isoezymes: HHHH (I1), HHHM (I2), HHMM (I3), HMMM (I4), and MMMM (I5). The structures of LD-M and LD-H are determined by loci on human chromosomes 11 and 12, respectively the subunit compositions of the five isoenzymes. Tissue-specific expression of the H and M genes determines the relative proportions of each subunit in different tissues. isoenzymes I1 predominates in heart tissue, and isoenzymes I5 in liver. Thus, tissue injury releases a characteristic pattern of LDH isoenzymes that can be separated by electrophoresis and detected using a coupled assay [21, 22].

2.2 Clinical application of lactate dehyderogenase

LDH is ubiquitous cytoplasmic enzyme that is widely distributed, found in all cells in man, but is specifically plentiful in cardiac and skeletal muscles, liver, kidney, red blood cells, brain, lung, lymph nodes and white blood cells [23].

Cellular enzymes in the extracellular space although of no further metabolic function in this space, are still of benefit because they serve as indicators suggestive of disturbance of cellular integrity induced by pathological conditions and is used to detect cell damage or cell death. Serum LDH is abnormal in a host of disorders, therefore the total serum LDH is highly sensitive but nonspecific test [24].

LDH level almost always increase, sometimes markedly, an event related to the number of white blood cells during remissions or relapses of the disease. On the other hand, it was suggested that elevated serum LDH may relate to total leukemia cell mass, although values did not correlate neither with initial white blood cells nor circulating lymphoblastic count [23].

In addition highest levels of total LDH are seen in pernicious anemia and hemolytic disorders. Intramedullary destruction of erythroblasts causes elevation as a result of the high concentration of LDH in erythrocytes. Liver disorders, such as viral hepatitis and cirrhosis, show slight elevations of two to three times' upper lower normal. Skeletal muscle disorders and some leukemias contribute to increased LDH levels. Marked elevations can be observed in most patients with acute lymphoblastic leukemia in particular [25].

Also elevated LDH levels are seen in cancer patients, and its prognostic value has been shown in several malignancies such as germ cell tumors, lymphoma, melanoma and renal cell carcinoma [26]. Ferreira et al, 2012 found that metabolic derangements may occur before the tumour becomes macroscopic, that is, before it is clinically detectable [27].

Malignant cells have a distinctive type of metabolism in which the glycolytic sequence and the tricarboxylic acid cycle are poorly integrated. Hence the cells tend to utilize from five to ten times as much glucose as do normal tissues, converting most of it into lactate. It is not clear whether the increased serum levels of LDH commonly found in cancer patients reflect greater production and release of the enzyme by malignant cells, is not clear. LDH exists in many different cell systems and subsequent to tissue or cell damage, serum LDH levels may be elevated [28].

So in the absence of liver muscle diseases and other factors high level of LDH is considered to be one of more specific indicator for different types of cancer [29].In order to optimize the diagnostic value, LDH isoenzymes can be measured. This can be further used as help in making decision, regarding the management strategies to improve the diagnosis and prognosis of leukemia and fetal outcome [24]. Despite all criticism cited, LDH seems to show a potential clinical role. It should be

important to try to validate the role of LDH in clinical practice. In fact, LDH could be considered an ideal biomarker, easily obtained in every laboratory, reproducible, and low costing.

Assay for Enzyme Activity of lactate dehyderogenase

LDH catalyzes the interconversion of lactic and pyruvic acids using the coenzyme NAD^{+}.
Pyruvate +NADH + H^{+}↔ Lactate + NAD^{+}
The reaction can proceed in either a forward (lactate [L]) or reverse (pyruvate [P]) direction. Both reactions have been used in clinical assays [16].

2.3 Uric Acid

Uric acid is the product of catabolism of the purine nucleic acids. Although it is filtered by the glomerulus and secreted by the distal tubules into the urine, most uric acid is reabsorbed in the proximal tubules and reused. Uric acid is relatively insoluble in plasma and, at high concentrations, can be deposited in the joints and tissue, causing painful inflammation [16].

Purines, such as adenosine and guanine from the breakdown of ingested nucleic acids or from tissue destruction, are converted into uric acid, primarily in the liver. Uric acid transported in the plasma from the liver to the kidney, where it is filtered by the glomerulus. Reabsorption of 98% to 100% of the uric acid from the glomerular filtrate occurs in the proximal tubules. Small amounts of uric acid secreted by the distal tubules into the urine. Renal excretion accounts for about 70% of uric acid elimination; the remainder passes into the gastrointestinal tract and is degraded by bacterial enzymes. Nearly all of the uric acid in plasma is present as monosodium urate. At the pH of plasma (pH 7), urate is relatively insoluble; at concentrations greater than 6.8 mg/dL, the plasma is saturated. As a result, urate crystals may form and precipitate in the tissues. In acidic urine (pH 5.75), uric acid is the predominant species and uric acid crystals may form [25].

2.4 Clinical Application

Uric acid is measured to assess inherited disorders of purine metabolism, to confirm diagnosis and monitor treatment of gout, to assist in the diagnosis of renal calculi, to prevent uric acid nephropathy during chemotherapeutic treatment, and to detect kidney dysfunction [25]. Serum uric acid is also raised in patients with haematopoietic malignancies due to increase in cell turnover of malignant cells [1].

Uric acid in serum is produced by the breakdown of the cellular nucleic acids of leukemia cells, and may be a marker of disease aggressiveness. A high rate of proliferation and a high tumor burden causes the rapid lysis of cancer cells. Cell lysis leads to the release of intracellular contents, which include electrolytes, proteins, and nucleic acids into the bloodstream. Because purine nucleic acids are subsequently catabolized to uric acid (UA), the serum UA concentration reflects the breakdown of cancer cells, and is a marker of increased cellular turnover [30].

2.5 Association of LDH and leukemia

The different metabolism of cancer compared with that of normal cells confers a selective advantage for their proliferation and survival. The use of lactate as an energy source requires the conversion of lactate into pyruvate as well as the transport of lactate into and out of tumor cells by specific transporters. The former pathway is regulated by lactate dehydrogenase (LDH). LDHs catalyze the conversion of pyruvate and lactate with concomitant conversion of NADH and NAD^+ [31].

Serum LDH levels usually reflect tumor burden in hematological or solid malignancies. This observation may suggest that high serum LDH levels may reflect high risk for disease relapse regardless with tumor burden. In a study performed by Ozenmis T.*et al*. they found that elevated serum LDH levels were correlated with treatment failure [32].

LDH levels have been evaluated as predictive marker in various malignancies including non-Hodgkin lymphomas, germ cell tumors and small cell lung cancer. Several different mechanisms may underlay an increase in LDH in hematological malignancies including Myelodysplastic Syndrome (MDS), lymphomas and leukemias. One possible factor may be the increased turnover and degradation of myeloid cells in the bone marrow, spleen, and other tissues. Among the other possible mechanisms; ineffective hematopoiesis could be encountered. Additional cofactors may be an infiltration of the liver and spleen by immature myeloid cells or iron overload and also serum LDH levels have been shown to indicate hypoxic status associated with tumor cells and as a result, high serum levels were found to be correlated with worse prognosis [8, 32].

2.6 Association of LDH and BCR-ABL transcript level in CML

In 2011, Tatakihara RI. *et al* conducted case control study survey of 22 patients and 56 healthy individuals in Brazil evaluate the concentration of LDH in plasma and the detection of the Bcr-Abl transcripts in patients with CML. They found that patients who presented transcript in the peripheral blood samples and whose LDH concentration were high. Lactate dehydrogenase (LDH) has been identified as a most suitable follow-up parameter in a screening program searching for dynamic prognostic determinants. Determination of LDH and Bcr-abl transcript in the follow-up of patients with CML could be considered a marker for therapy following [33].

A case report study conducted by Horvath A. in Targu Mureş (2011), Romanian treatment follow-up of chronic myelogenous leukemia monitoring the BCR-ABL transcript in peripheral blood with CML shows 140% of BCRABL gene. Blood chemistry is with high lactate dehydrogenase (LDH) level (2105 U/L). And the second patient RT-PCR examination from peripheral blood for BCR-ABL transcript detection was 100% and LDH was elevated (2010 U/L). This shows that the percent BCR-ABL transcript level and LDH value is increased simultaneously [34].

Another case Report conducted on three patients in Turkey in 2016 by Yilmaz, M *et al*. on Cases of chronic myeloid leukemia presenting with isolated thrombocytosis, exceptionally from isolated CML behavior they found that no splenomegaly was present in these patients, peripheral leukocyte numbers and formulae were normal, LDH and uric acid levels were lower than in classic CML, while hemoglobin levels were higher. BCR/ABL levels were similar. This report identifies potentially significant differences between patients with CML presenting with isolated thrombocytosis and patients with typical CML [35].

2.7 Lactate dehydrogenase and CML

Diagnostic criteria for myeloproliferative neoplasms (MPNs) are based on the 2008 World Health Organization (WHO) classification that includes clinical features; basic laboratory investigations for MPNs should include CBC, the peripheral blood smear, an erythropoietin (EPO) level, and biochemistry tests lactate dehydrogenase [LDH], uric acid, vitamin B12, and iron status. This report also mentioned patients with MPNs may have nonspecific abnormalities in a variety of biochemistry tests, including increased serum LDH [36].

Case control study conducted in India by Assam T. in 2016 indicated that serum LDH activity in leukemic patients before and after chemotherapy found that the difference between the mean of

serum LDH activity of leukemic patients and the corresponding mean of controls have been found to be significant at diagnosis and after one month of chemotherapy. The difference in serum LDH activity between CML and ALL patients before and after one month of chemotherapy was significant. Serum LDH activity increases in leukemic patients and there is a significant reduction after chemotherapy [37].

Another study conducted in India by Pujari NK. and his friends in 2012 on lactate dehydrogenase levels in leukemia's, they found that lactate dehydrogenase activity in the serum of leukemic patients were significantly high as compared to control. They suggest that increased cellular LDH activity reflects a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of malignant cells accompanied by a high turnover rate. These observations demonstrate that, evaluation of LDH level in patients with leukemia could represent an additional and useful parameter in determining the clinical and prognostic aspect of the disease [19].

Study that included 265 Indian leukemia patients by Mani R. *et al* in 2006 confirms that serum LDH plays an important role both in the diagnosis as well as prognosis of hematological malignancies. This finding suggests that serum LDH can be used as an important biomarker in diagnosis and prognosis of hematological malignancies. Increased values of serum LDH directly reflect the tumor mass in patients with CML. Hence it is suggested that serum LDH can be used as one of the important biomarker of survival in patients with hematological malignancies. Patients of NHL, CML, ALL, AML and multiple myeloma who have increased serum LDH levels than others had a 90% risk of death in the first five months. Abnormalities in total serum LDH levels have a good diagnostic significance apart from the prognosis [38].

Prospective study conducted on 7895 individuals in UK by Wulaningsih W. *et al* (2015) on Serum lactate dehydrogenase and survival following cancer diagnosis, convinced prognostic value of serum LDH has been suggested in several types of cancer, particularly haematological malignancies. Also high level of serum lactate dehydrogenase (LDH) is associated with poorer overall survival and greater risk of dying from cancer was seen with increasing LDH in those diagnosed with hematological cancer. Similar associations with survival have been established in chronic myeloid and lymphocytic leukaemias [39].

Also elevated levels of serum LDH are prognostic biomarkers for poor survival in multiple cancers, including hematological malignancies. The risk of death was increased almost six fold in patients

with LDH levels >2 ULN (upper limit of normal) compared those with levels <1 ULN. Furthermore, patients with LDH levels <1 ULN had the best overall survival (OS) [40].

2.8 Association of uric acid and CML

Study conducted in India carried out among 30 patients by Buzarbaruah L and Kishore PJ in 2016 on serum uric acid concentration in leukemic patients before and after chemotherapy indicates the difference between the means of serum uric acid concentration of leukemic patients and the corresponding means of controls have been found to be significant at diagnosis and after one month of chemotherapy. They have seen that in CML at diagnosis the mean serum uric acid was 8.98 mg/dl whereas the mean after chemotherapy was 6.91 mg/dl. A highly significant difference (lowering) in serum uric acid level has been observed between the CML patients at diagnosis and after chemotherapy. The biochemical alteration of serum uric acid concentration can play an important role in the prognostic aspect of the disease [41].

Another study conducted in India by Saharia GK and his friends in 2015 on utility of serum lactate dehydrogenase and uric acid concentrations as prognostic indices for leukemia patients under chemotherapy on thirty newly diagnosed cases of leukemia and equal number of controls, found that serum uric acid concentration and LDH activity were altered in leukemia patients after chemotherapy, 83.3% of cases with leukemia have serum uric acid concentration above the normal range with the mean uric acid level being 8.92 mg/dL and 16.6% of cases towards the higher normal range with the mean uric acid level being 6.16mg/dL at diagnosis. Maximum rise in serum uric acid concentration before chemotherapy was seen in AML followed by CML. As they indicated the probable cause of increased serum uric acid level in the study group is due to increased nucleic acid catabolism due to increased turnover of malignant cells resulting in increased purine catabolism [1].

3. Objectives

3.1 General objective

- To evaluate prognostic importance of LDH and uric acid as compared to BCR-ABL transcript level in CML patients. Palliative

3.2 Specific objectives

- To determine the correlation of total LDH activity and uric acid with BCR-ABL percent transcript level in chronic myeloid leukemia patients
- To compare the level of LDH and uric acid in patients on treatment and treatment naive with chronic myeloid leukemia's
- To determine the correlation of serum LDH activity with hematological parameters in leukemic patients.

4. Hypothesis

There is no association between lactate dehydrogenase and uric acid with BCR-ABL transcript level among chronic myeloid leukemia patients.

5. Materials and methods

5.1 Study area

This study was carried out at Tikur Anbessa Specialized hospital which is the largest referral hospital in the country, and located at the center of the Addis Ababa. This hospital was selected because it is the only hospital which provides service for leukemia patients in the country. Tikur Anbessa specialized hospital offers diagnosis and treatment for approximately 370,000- 400,000 patients a year. The hospital has 800 beds, with 130 specialists, 50 non-teaching doctors. The emergency department sees around 80,000 patients a year. The hematology clinic hosted approximately 1000 clients, of whom 250 were treatment naive and the rest 750 on treatment.

5.2 Study design and period

A Cross sectional study was conducted from January 2017 to March 2017 at Tikur Anbessa specialized hospital to determine the prognostic importance of lactate dehydrogenase and uric acid as compare to BCR-ABL transcript level.

5.3 Population

5.3.1 Source population

All CML positive patients who were visiting Tikur Anbesa specialized referral hospital hematology clinic from January 2017 to March 2017 were our source population.

5.3.2 Study Population

The study populations were CML patients those who come for testing Xpert BCR-ABL Monitor assay for quantitative assessment of the BCR-ABL transcript level and who are willing to take part in the study.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

All patients have a confirmed Philadelphia positive CML diagnosis and have an informed consent for participation.

5.4.2 Exclusion criteria

Patients with history of liver disease, diabetes, renal failure, stroke, chronic hypertension, gestational diabetes, smoking and alcoholism, patient ingests large amounts of ascorbic acid (chewing chat lower LDH), hepatotoxic drugs and patients already having history of gout excluded from the study.

5.5 Study variables

5.5.1 Dependent variable

Total LDH activity and uric acid concentration

5.5.2 Independent variables

BCR-ABL transcript level, WBC counts, platelet count, Hgb value, HCT value, differential, (hematologic response) treatment status.

5.6 Measurement and Data collection

5.6.1 Sample size and sampling technique

According to Clinical and Laboratory Standard Institute (CLSI) recommendation for method comparison study at least 40 samples meets the criteria. We used 41 more samples improve the confidence in the statistical estimates [43].

Convenient sampling technique was used to include 36 treatments naive and 45 on treatment with a total of 81 CML patients. We used more samples improved the confidence in the statistical estimates.

Thus all consecutive patients on treatment and treatment naive were fulfilling the inclusion criteria and attending Tikur Anbesa specialized hospital hematology clinic coming for BCR-ABL test during the study period were included.

5.6.2 Data collection procedure

Data was collected by seeking hospital record as well as previous medical reports. Also patients are grouped according to their treatment status. Blood sample was collected by following standard operating procedures (SOPs) that is important for the analysis of LDH, BCR-ABL transcript level, uric acid, CBC. With all aseptic and antiseptic precautions 6ml of blood was collected from antecubital vein in a serum separator tube (SST) and EDTA test tube. The blood collected in EDTA test tube was used for BCR-ABL transcript and hematology parameters. The blood collected was allowed to clot for 30 minutes and subjected to centrifugation in centrifuge machine at 3000 rpm for 3 minutes to separate the serum.

5.6.4 Laboratory analysis

During their treatment follow-up appointment at hematology clinic of the Tikur Anbesa Specialized Hospital, blood sample was collected under all aseptic and antiseptic conditions and serum was separated by centrifugation and serum was separated into Nung tubes and stored at -20^{0}_{C}. Different clinical chemistry parameters were analyzed on Mindray 200E fully automated analyzer. And hematological parameters were determined by fully automated 5 part cell counter (SYSMEX XT 2000-I), the BCR-ABL transcript level was estimated by the Cepheid, Gene Xpert RT-PCR molecular analyzers in Tikur Anbessa specialized hospital Laboratory. Serum uric was estimated by PAP- method Enzymatic colorimetric test and serum LDH by Modified IFCC method**.** Routine hematological examinations and liver and renal function tests estimations were done as per standard procedures.

5.7 Quality Assurance

Pre-analytical Consideration: Protocol for sample collection, transportation and processing were strictly followed. Blood was collected from antecubital vein without application of tourniquet in a sterile empty vial.

The quality of the interview questions checked and pre-tested before the detailed work is started. A data collection was done by the principal investigator. Blood sample quality was censured by collecting and processing according to the standard operating procedures.

Analytical Consideration: The manufacturers' instruction was strictly followed while performing the above mentioned tests by complying with the SOPs. Separated serum was used to estimate serum LDH activity, uric acid concentration and organ function tests. The performance of automated clinical chemistry and hematology analyzer was checked by running quality control sample before running the sample used for the study purpose. The following procedure is the quality control procedure that was done for the tests that we have performed.

Quality control for LDH activity and uric acid

Commercially manufactured normal and abnormal quality control sample was analyzed along with patient samples, using Westgard or other quality control rules for acceptance or rejection of the analytical run and used to detect analytical errors**.**

Post-analytical Consideration: The result obtained was collected on data collection sheet. Before dispatching of all the laboratory test results were verified or checked by senior personnel working in Tikur anbessa specialized hospital laboratory.

5.8 Data analysis and interpretation

The data obtained from the tests were ppppinters using Microsoft Excel 2007 and analysis of the data was performed by SPSS version 20. The descriptive statistics such as frequency, median and percentage were calculated. The spearman correlation coefficient (r) was used for measuring the relationship between two variables. We have performed a simple linear regression analysis of the data set, with LDH being the dependant X variable and other biomarkers the independent (Y) variables; the fitted regression lines were plotted. Area under the curve (AUC) was performed which used as an accuracy index for evaluating the diagnostic performance of Lactate dehydrogenase. Statistical significance association between dependent and independent variables were evaluated and P-value < 0.05 is considered to be statistically significance.

5.9 Ethical considerations

Ethical clearance was obtained from Addis Ababa University, Medical Laboratory Science department of ethical review and research committee. After summiting this ethical clearance and support letter written to Tikur Anbessa Specialized Hospital administrators. Permission was also obtained from the clinical director of the hospital allowed the start of the research with Laboratory department. Patients enrolled in the Gleevac International Patient Assistance Program (GIPAP) came for treatment follow-up appointment at hematology clinic of the Tikur Anbesa Specialized Hospital, at the same time coming for testing Xpert BCR-ABL Monitor assay for quantitative assessment of the BCR-ABL in the laboratory. Laboratory results were communicated to Physicians. The study aim, risks, benefits and right for withdrawal anytime from the study was explained for the study participants and informed consent was obtained. Samples were coded and confidentiality of patient data was maintained throughout the study by locking hard copies and password protecting electronic files.

6. Results

6.1 Socio-demographic characteristics of the study population

A total of eighty one (81) CML patients were enrolled of which 52(64.2%) were male with age ranged from 20 to 70 years; with a mean age of 39 years. From where it is evident that highest numbers of cases were occurring in age group of 20-35 years 42(51.9%) followed by 36-50 years 23(28.4%) age group. Among the study population the majority 28.4% were from Oromia region patients followed by Amhara 23.4%, Addis Ababa 19.8%, SNNP 16% and Tigray 8.6%. According to received therapy, we have grouped study subjects in to two main categories: medication treated group 46(56.8%) and treatment naive patients 35 (43.2%). The result of this study were summarized in (Tables 1-8) and (Figures 1 to 4).

Table 1 Socio-demographic Characteristics of study population

Variables	Frequency	Percent (%)
Age		
20-35	**42**	**51.9**
36-50	**23**	**28.4**
51-65	**13**	**16.0**
≥66	**3**	**3.7**
Sex		
Male	**52**	**64.2**
Female	**29**	**35.8**
Treatment status		
On treatment	**46**	**56.8**
Treatment naive	**35**	**43.2**
Region		
Oromia	**23**	**28.4**
Amhara	**16**	**23.4**
Addis Abeba	**19**	**19.8**
SNNP	**13**	**16**
Tigray	**7**	**8.6**
Others	**3**	**3.7**

A majority of the study participant 47 (58%) have elevated value of LDH (450 U/L) above upper normal cut of value. The median serum LDH was 549 U/L, with a minimum serum LDH of 275 U/L, the maximum serum LDH value was 4220 U/L whereas that of BCR-ABL the median transcript level was 41%, and the minimum BCR-ABL transcript level was 0 %, with the maximum level of transcript 91%. The median serum uric acid was 6.3 mg/dL, with the minimum serum uric acid of 2.4mg/dL; the maximum serum uric acid value was 15.5mg/dL (**Table 2**)

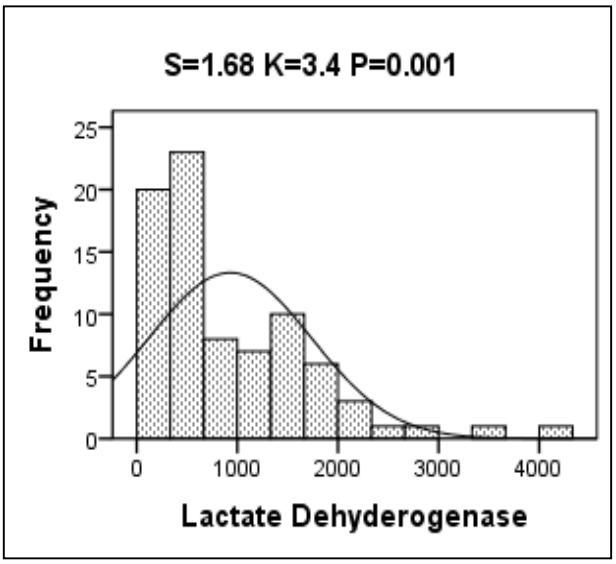
Table 2 Laboratory data Characteristics of the study population

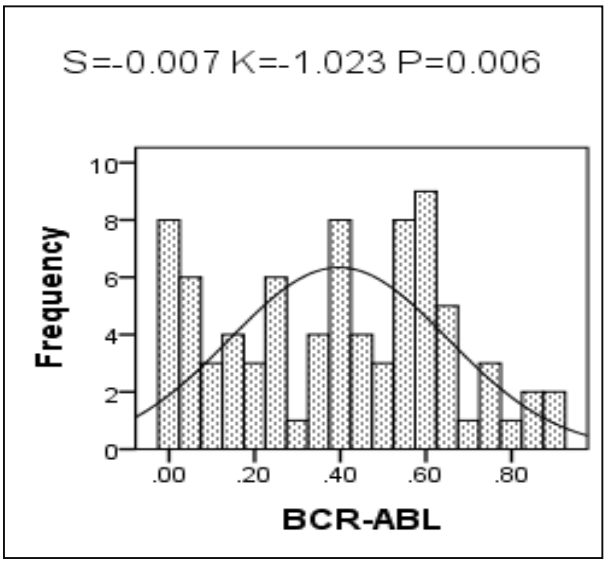
Laboratory data distribution of study population				
	Range	**Minimum**	**Maximum**	**Median**
BCR-ABL (%)	91	0%	91	41
LDH (U/L)	3945	275	4220	549
WBC $x10^9$/l	405.93	1.57	407.50	10.97
RBCs(x 10^6/μL)	3.70	1.56	5.26	3.58
Uric Acid (MG/DL)	13.1	2.4	15.5	6.3
PLT (x 10^3/μL)	793.0	14.0	807.0	170
Hgb (g/dl)	11.2	4.6	15.8	11.5
Hct (%)	32.4	14.5	46.9	34.5
L (%)	79.3	2.8	82.1	27.1
N (%)	73.5	11.6	85.1	55.1
B (%)	31	0	31	3.7

LDH: lactate dehyderogenase**, BCR-ABL:** breakpoint cluster region-Abelson, **WBC**: white blood count, **PLT**: platelet count, **Hb**: hemoglobin, **L:** lymphocyte**, N:** neuterophil**, B:** basophil

6.2 Distribution plots of laboratory Data

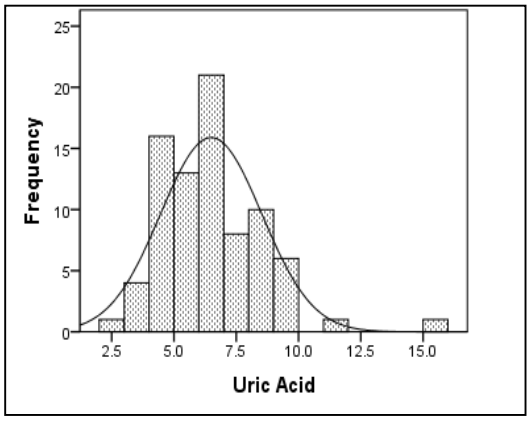
There laboratory distribution showed in (Fig. 1a, 1b, 1c), respectively. Serum LDH, BCR-ABL transcript level and uric acid test for normality. Transform the dependent variable (repeating the normality check on the transformed data by taking the log or square root of the dependent variable indicated that the data represented a continuous parameter that did not meet the assumption of the test for normality, therefore we used non parametric test. So for the skewed data, $p = 0.001$ so there is very strong evidence of non-normality and a non-parametric test should be used.





A. Normal distribution of LDH **B. Normality distribution of BCR-ABL**

S=1.218 P= 0.001



C. Normality distribution of uric acid

Figure 1 (A, B, C) Normal distribution check graph

6.3 Distribution of Lactate dehydrogenase and BCR-ABL according to gender

LDH and BCR-ABL levels were differently distributed depend on the type of gender, where a male shows higher levels of LDH and BCR-ABL as compared to females. (Table 4) was shown the variation in lactate dehydrogenase activity and BCR-ABL in respect to gender, the mean lactate dehydrogenase levels and BCR-ABL in males (999 ± 893U/L), (40±25%) were found to be more than that in females (869±626 U/L), (39±26%).

Table 3 Distribution of lactate dehydrogenase and BCR-ABL according to gender summery

Sex		BCR-ABL	LDH
Female	Mean± SD	39±26%	869±626 u/l
Male	Mean± SD	40±25%	999±893u/l
Total	Mean± SD	39.6±25.4%	953±806u/l

6.4 Association of serum LDH level with BCR-ABL transcript Level

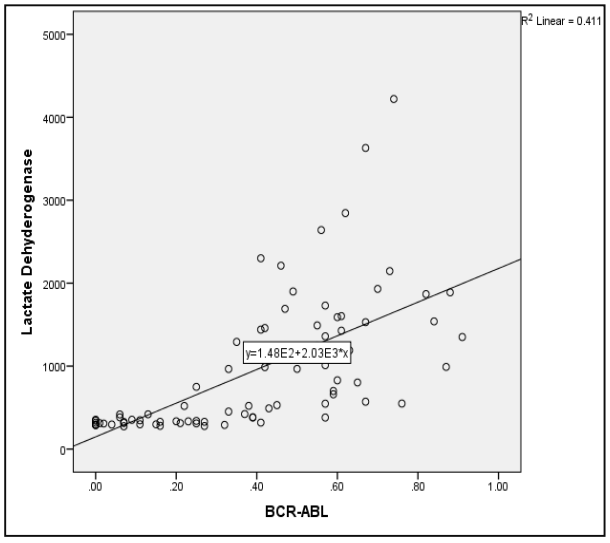
This study shows that strong significant positive correlations between LDH and BCR-ABL (P=0.001). The significant correlation (r=0.79) confirms strong positive correlation between the two variables. LDH also significant correlation with white blood cells, neuterophil, lymphocyte and Basophil (P=0.001). And significant negative correlation with red blood cells, hemoglobin and hematocrite (P=0.001). No significant correlation of LDH and platelet count (P=0.069). At the same time BCR-ABL was shows significant positively correlated with platelets, white blood cell count ,neuteropile, lymphocyte and Basophil in chronic myeloid leukemia (P=0.001).

Table 4 Correlation between lactate dehyderogenase, BCR-ABL, Uric Acid and hematological variables

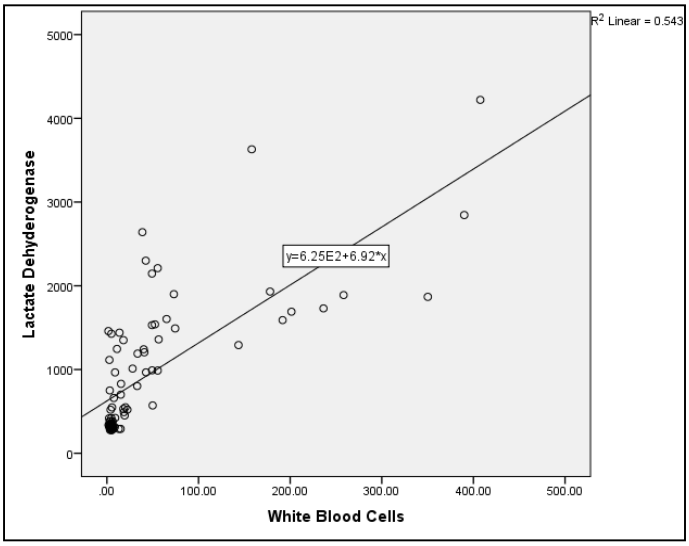
Variables correlated with lactate dehydrogenase			
	Median	Corr. Coefficient	p-value
BCR-ABL (%)	41	0.79	<0.001
WBC x10^9/l	10.97	0.73	<0.001
RBCs(x 10^6/µL)	3.58	-0.38	<0.001
Uric Acid (mg/dl)	6.3	0.39	<0.001
Platelets (x 10^3/µL)	170	0.24	<0.069
Hgb (g/dl)	11.5	-0.50	<0.001
Hct (%)	34.5	-0.42	<0.001
L (%)	27.1	-0.614	<0.001
N (%)	55.1	0.409	<0.001
B (%)	3.7	0.75	<0.001

LDH: lactate dehyderogenase**, BCR-ABL:** breakpoint cluster region-Abelson **WBC**: white blood count, **PLT**: platelet count, **Hb**: hemoglobin, **HCT**: hematocrit **L:** lymphocyte**, N:** neuterophile**, B:** basophile

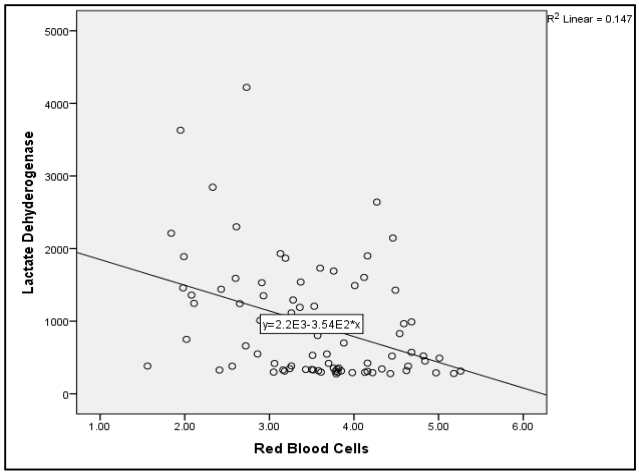
The scatter plot shows there were positive significant correlation between lactate dehydrogenase activity and BCR-ABL and white blood cells. There were negative significant correlation between lactate dehydrogenase activity red blood cells, hemoglobin and hematocrite. Weak correlation between uric acid and BCR-ABL (r=0.295, P=0.008)



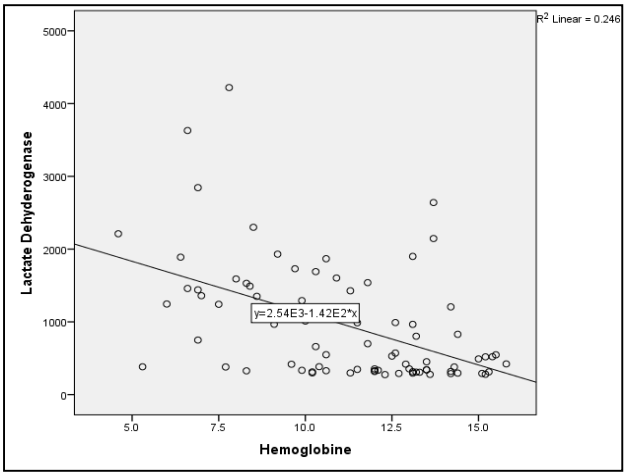
A. LDH and BCR-ABL



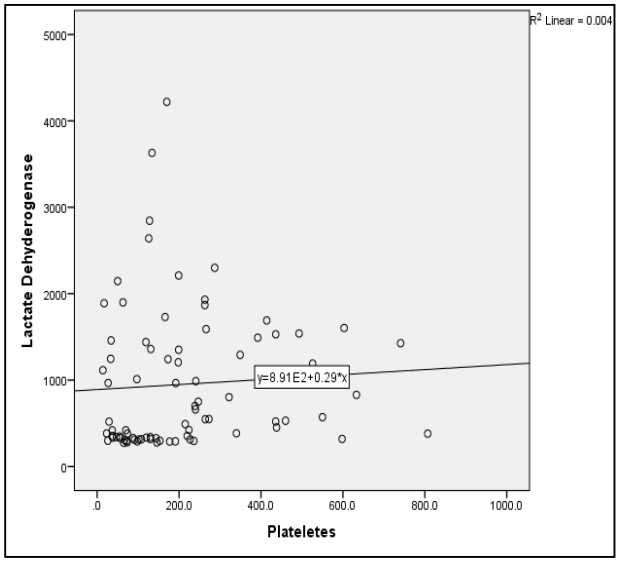
B. LDH and WBC



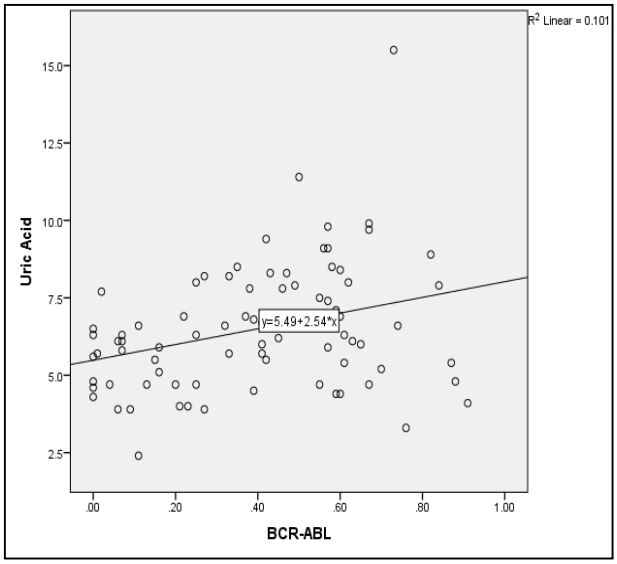
C. LDH and RBC



D. LDH and Hgb



E. LDH and PLT



F. Uric acid and BCR-ABL

Figure 2 scatter plot for LDH, uric acid and BCR-ABL, WBC, RBC, Hgb, and platelets

Regression analysis showed a significant correlation (r = 0.402, *P* = 0.001) between serum LDH and BCR-ABL. There was a significant correlation (r=0.65, *P*=0.001) between LDH and white blood cells. There was weak correlation between LDH and platelets (r= 0.021,) uric acid and BCR-ABL (R = 0.04). Also regression analysis showed week correlation between uric acid and BCR-ABL.

6.5 Association of uric acid with BCR-ABL

We also evaluate the relationship between serum uric acid and BCR-ABL transcript level. This shows that positive correlations were observed between uric acid and BCR-ABL (P=0.008). The significant correlation value of (r=0.295) indicated that very weak correlation between the two variables.

Table 5 Correlation between uric acid and BCR-ABL and hematological variables

Variables correlated with uric acid			
	Median	Correlation Coefficient	p-value
BCR-ABL (%)	41	0.295	<0.008
WBC x10^9/l	10.97	0.389	<0.001
RBCs(x 10^6/µL)	3.58	-0.037	<0.743
Platelets (x 10^3/µL)	170	-0.002	<0.985
Hgb (g/dl)	11.5	-0.016	<0.889
Hct (%)	34.5	0.028	<0.805

BCR-ABL: breakpoint cluster region-Abelson, **WBC**: white blood count, **PLT**: platelet count, **Hgb**: hemoglobin, **HCT**: hematocrite

6.6 Analysis of LDH and BCR-ABL in treatment naive patients and patients on treatment

In order to study whether correlation between LDH and BCR-ABL would be more significant, we split the data into two groups based on their treatment status. The Laboratory parameters in both patient on treatment and treatment naive are LDH, BCR-ABL, and hematological parameters variation between the two studied groups. There was statistically significant increase in LDH, BCR-ABL and white blood cells in treatment naive chronic leukemia than patients on treatment. There were statistically significant decrease in red blood cells and platelets count in treatment naive patients. A Mann-Whitney U test indicated that there was statistically significant difference (U = 301, p = 0.001) in BCR-ABL level between patients on treatment group (median=21%), and treatment naive group (median=57). There was also statistically significant difference (P=0.001) in lactate dehydrogenase activity in treatment naive group cases (median=1246u/l) with compared to patients on treatment group (median=350u/l). Similarly there was also statistically significant

difference in white blood cells, hematocrite, red blood cell, and hemoglobin (**table 9**). For uric acid Mann-Whitney U test indicated that there was no statistically significant (U = 741, p = 0.542) difference in the patient on treatment group (median=6.1) and treatment naive group (Median=6.6).

Table 6 Laboratory parameters based on patient's treatment status

Laboratory data distribution based on treatment status				
Parameters	Patient on treatment Median	Treatment naive Median	**Mann-Whitney U**	**p-value**
BCR-ABL (%)	21	57	301	<0.001
LDH(u/l)	350	1246	315	<0.001
UA (mg/dl)	6.1	6.6	741	<0.542
WBC x10^9/l	5.46	33.46	397	<0.001
RBC(x 10^6/µL)	3.8	3.26	510	<0.005
Hgb(g/dl)	12.95	9.90	430	<0.001
HCT (%)	37.1	30.3	494	<0.003
PLT(x 10^6/µL)	137.0	239.	614	<0.069
Netero(%)	38.8	60.9	413	<0.001
Lympho(%)	39.7	14.5	303	<0.001
Baso(%)	1.0	7.3	269.5	<0.001

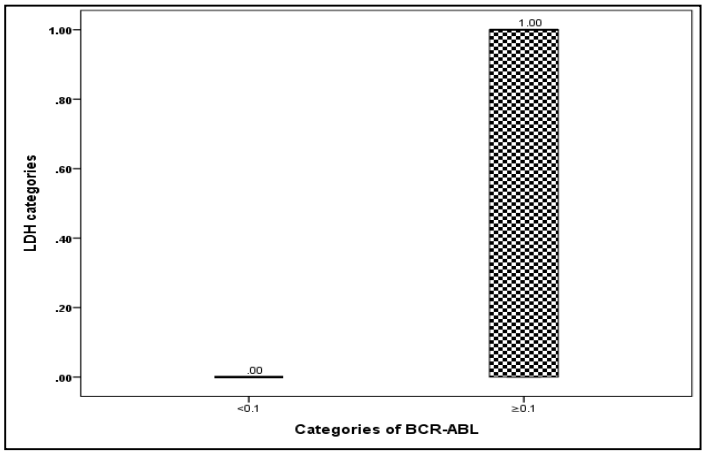
LDH: lactate dehyderogenase**, BCR-ABL:** breakpoint cluster region-Abelson **WBC**: white blood count, **PLT**: platelet count, **Hb**: hemoglobin

6.7 Hematological parameters according to lactate dehydrogenase activity and BCR-ABL

According to National Comprehensive Cancer Network guide lines Complete hematologic response was defined as WBC less than 10x 10^9 /l with normal differential; platelet count less than 450x 109/l. Molecular BCR-ABL undetectable BCR-ABL transcript level less than 10%. According to the activity of lactate dehydrogenase and BCR-transcript level we classified chronic myloid leukemia patients in to two groups, one group in which lactate dehydrogenase activity >450 IU/L (high Lactate dehydrogenase activity group) and BCR-ABL transcript level >10% (high percent transcript level) the other group in which lactate dehydrogenase activity <450 IU/L (Low Lactate dehydrogenase activity group) and their BCR-ABL transcript level <10% (low percent transcript level).

A Mann-Whitney U test indicated that there was statistically significant difference in the high lactate dehydrogenase activity group (Median=967u/l), and low lactate dehydrogenase activity group (median= 315u/l), U = 118, p = 0.001. There was also statistically significant difference in the high BCR-ABL group (median=49%) and low BCR-ABL group (median= 2%), U = 0, p = 0.001. Similarly, there was statistically significant difference between low white cell count group (Median=5.0x10^9/l) and high white blood cell count group (median=18.3x10^9/l), U=249, p=0.003. As indicated in the **table** 7 the two groups did not differ significantly in uric acid, hemoglobin, and hematocrite.

In our study sustained complete hematologic response was noted in 15(18.52%) the median LDH value was 315u/l where as 66(81.48%) had hematologic failure the median LDH value was 967u/l. Those BCR-ABL transcript levels were greater than 10% the median BCR-ABL transcript value was 49% and those BCR-ABL less than 10% the median BCR-ABL transcript level is 2%. Figure shows elevated lactate dehydrogenase activity accompanied with increased BCR-ABL. On the other hand, reduction in lactate dehydrogenase activity accompanied with increased of red blood cells, hemoglobin and hematocrite value **(Table 7)**.



LDH >450 and BCR-ABL >10%

Figure 3 Frequency of categories of BCR-ABL and lactate dehyderogenase

Table 7 Comparison of hematological data according to Lactate dehydrogenase activity and BCR-ABL transcript level in chronic myloid leukemia patients

Comparison of hematological data according to BCR-ABL transcript level					
Variables	**BCR-ABL**	N	Median	Mann-WhitneyU	P value
LDH	<10%	15	**315**	118	
	≥10%	66	**967**		0.001
UA	<10%	15	**5.7**	302	
	≥10%	66	**6.6**		0.019
WBC	<10%	15	**5.0**	249	
	≥10%	66	**18.3**		0.003
Hgb	<10%	15	**13**		
	≥10%	66	**10.7**	370	0.128
HCT	<10%	15	**37.2**		
	≥10%	66	**32.8**	415	0.331
PLT	<10%	15	**98.0**		
	≥10%	66	**195**	291.5	0.013

LDH: lactate dehyderogenase**, BCR-ABL:** breakpoint cluster region-Abelson **WBC**: white blood count, **PLT**: platelet count, **Hb**: hemoglobin, **HCT**: hematocrit

6.8 Roc curve analysis

The area under the curve (AUC) is an effective and combined measure of sensitivity and specificity for assessing inherent validity of a diagnostic test. We have performed ROC curve which is used for clinical decisions and used for determining the validity of biomarkers. The AUC for LDH, WBC, uric acid, RBC, Hgb, HCT, platelets, neuterophil, lymphocyte and basophile was 0.881, 0.889, 0.695, 0.393, 0.374, 0.419, 0.704, 0.769, 0.174, and 0.899 respectively. ROC curve analysis was shown in Figure 4a-4d. and figure 9 Lactate dehydrogenase act as a diagnostic test for chronic myloid leukemia; the sensitivity and specificity, positive predictive value and negative predictive value of LDH were (88% and 67%), (92% and 55%) respectively. We evaluated the prognostic ability of lactate dehydrogenase for discriminating patients have complete hematologic response from failure. In order to establish the differential diagnosis between complete hematologic response

(CHR) and failure, we determined the levels of LDH. By means of the ROC curve, the best cut-off (327U/l) point for this test was calculated.

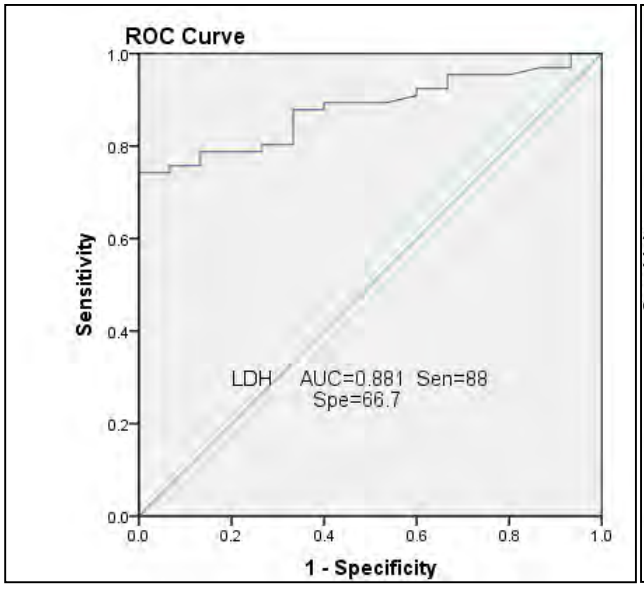
Table 8 Two by two table for BCR-ABL and LDH

2×2 table				
		BCR-ABL		
		≥10%	< 10%	Total
LDH	>327.5U/L	58	5	63
	<327.5U/L	8	10	18
Total		**66**	**15**	**81**

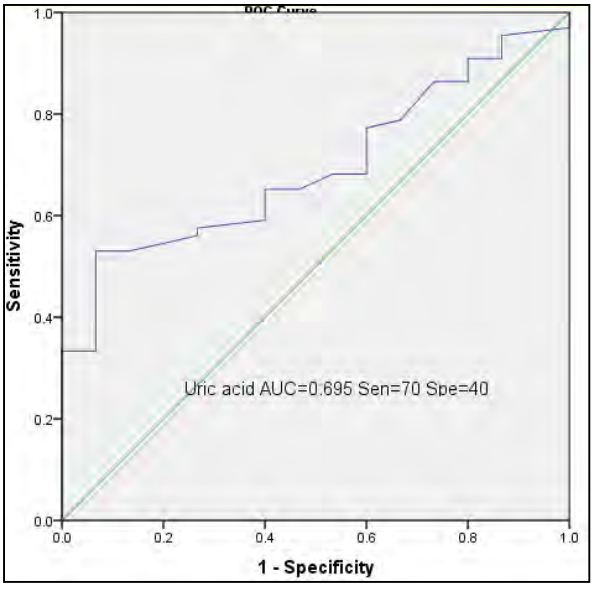
- LDH Sensitivity = 58/58+8= **87.8 %**
- LDH Specificity =10/10+5 = **66.7%**
- Positive predictivity of the test =58/58+5=**92%**
- Negative predictivity of the test= 10/10+8=**55%**
- Inherent validity of the test = 58+10/81=**84%**
- Predictive validity of the test = 58+10/81=**84%**

Regarding the determination of LDH the new cut-off point (327u/l) established for the differentiation between patients has hematologic remission and failure (hematologic remission LDH< 327U/L and failure >327U/L). The area under the ROC curve was 0.881 (p = 0.001).

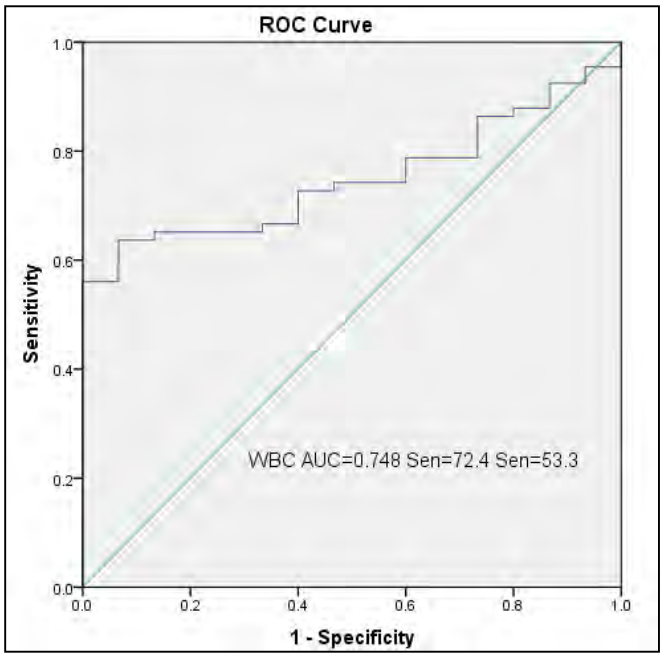
A. Lactate dehyderogenase



B. Uric acid



C. White Blood cells



D. Basophil

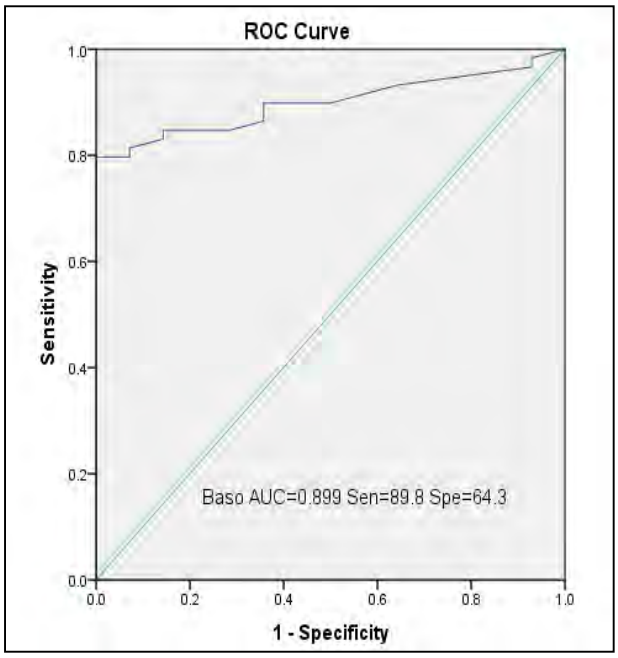


Figure 4 ROC curves

Showing AUC, sensitivity and specificity for (A) Lactate dehydrogenase, (B) Uric acid and (C) White blood cells (D) Basophil . ROC: receiver operating characteristic

7. Discussion

Leukemia is a heterogeneous disease in which there were different in genetic abnormalities. There were different technique for detection and differentiate chronic myloid leukemia. Lactate dehydrogenase acts as glycolytic enzyme increase in malignant cell than in normal cells. Malignant cells have a special kind of metabolism in depend on glycolytic sequence to obtain its own fuel of energy, so the cells move to exhausted glucose from five to ten times more than normal cells, to convert it to lactate [40].

This study was planned to investigate the role of lactate dehydrogenase as prognostic marker compared to BCR-ABL test which is available in developed country but not found in developing countries like Ethiopia for chronic myloid leukemia, also, in differentiation between patients on treatment and without treatment. In this study we tried to choose simple and cheap technique to diagnosis and differentiate chronic leukemia. In the present study, both patients on treatment and treatment naive have statistically significant serum LDH and BCR-ABL levels.

Under rapid proliferation and immaturity of tumor cells, LDH is released due to multiple cytokine activity and cell membrane damage [44]. There were many possible mechanisms which explain the reason of elevated of lactate dehydrogenase in malignancy tumor. First, acidification of the extracellular water space by lactate and the subsequent activation of tumor invasion may be a rational explanation [45]. Second, over expression of LDH, especially LDH5 reflects an up regulated hypoxia-induced factor pathway, which regulates glycolysis, angiogenesis, resistance to apoptosis, and even cancer metastasis [46]. Simonović E *et al.* indicated that the largest number of subjects with elevated values of LDH was in the group with CML (93.14%). It is statistically larger in comparison to all other leukemia groups ($p<0.01$) [47].

Our data demonstrated that increased LDH and BCR-ABL concentration in male more than in female in chronic myloid leukemia cases. That is the mean value of LDH and BCR-ABL is high in male (999U/L, 40%) respectively when compared with the female (869U/L, 39%) this may be due to the sexual hormones. Estrogen regulates and has a negative correlation with LDH activity and so the value of female is less than the male. The other might be the muscle fibers cross sectional area that is high in male than females which reflects the high LDH level in male than females [48].

The present study revealed that strong significant positive correlations between LDH and BCR-ABL (P=0.001). The significant correlation (r=0.79) confirms strong positive correlation between the two variables. Elevation of serum LDH with BCR-ABL transcript level in chronic myloid leukemia patients may be due to the tumor burden activity which reflects the function of leukemic cell number and turnover. In addition to this specifically lactate dehyderogenase increases in chronic myloid leukemia due to oncogenic receptor tyrosine kinase FGFR1 (fibroblast growth factor receptor 1) directly phosphorylates LDH-A. Phosphorylation at Y10 and Y83 enhances LDH-A activity by enhancing the formation of active, tetrameric LDH-A and the binding of LDH-A substrate NADH, respectively. Y10 phosphorylation of LDH-A is common in diverse human cancer cells, which correlates with activation of multiple oncogenic tyrosine kinases. Tyrosine phosphorylation enhances LDH-A enzyme activity to promote the Warburg effect and tumor growth by regulating the NADH/NAD^+ redox homeostasis, representing an acute molecular mechanism underlying the enhanced lactate production in cancer cells [49, 50].

With regard to patient treatment status, our study revealed that lactate dehydrogenase activity was statistically significant in both treatment naive and patients on treatment chronic myloid leukemia. There was statistically significant difference in BCR-ABL level between patients on treatment group and treatment naive group (p = 0.001). This in coincided with Assouline S, and Lipton JH study's they found that an increase in BCR-ABL transcripts in patients may indicate a loss of treatment response [51]. ,

According to European LeukemiaNet (ELN) and U.S. National Comprehensive Cancer Network (NCCN) guidelines**.** A hematologic response indicates improvement in peripheral blood cell counts and may be complete CHR (compete hematologic response) normalized peripheral blood counts, white blood cell count below 10×10^9/L, platelets below 450×10^9/L [51].

In our study sustained complete hematologic response was noted in 15(18.52%) the median LDH value was 315U/L where as 66(81.48%) had hematologic failure the median LDH value was 967U/L [52].

Those BCR-ABL transcript levels were greater than 10 %(failure) the median BCR-ABL transcript value was 49% and those BCR-ABL less than 10% (major molecular response) the median BCR-ABL transcript level was 2%. Elevated lactate dehydrogenase activity accompanied with increased BCR-ABL, white blood cells, and platelets. On the other hand, depression in Lactate

dehydrogenase activity accompanied with increased red blood cells and hemoglobin and hematocrite value [51].

In this study, there were statistically significant (p=0.001) increase in BCR-ABL and LDH, in treatment naive patients compared to patents on treatment. On the other hand, there were statistically significant decrease in red blood cells, hemoglobin and hematocrite value. But there is no difference between uric acid values among the study group. High lactate dehydrogenase activity was highly correlated with increase BCR-ABL transcript level this agree with one study in which patients who presented transcript in the peripheral blood samples and whose LDH concentration was high [33].

The ROC curve revealed that the cut-off LDH value could significantly differentiate patients with different complete hematologic response and failure. And also differentiate patients with major molecular response (BCR-ABL less than 0.1%). By means of the ROC curve, the best cut-off (327U/l) point for this test was calculated. LDH, WBC and Basophil showed better performance (AUC=0.881, 0.889 and 0.899) respectively for the diagnosis of patients with chronic myloid leukemia compared with uric acid, Hgb, HCT, RBC and platelets (0.695, 0.374, 0.419, 0.393, 0.769). In particular, Lactate dehydrogenase and basophile displayed the best prognostic ability. Thus lactate dehydrogenase activity might be a useful in addition to hematologic, routine morphologic, Cytogenetic, and molecular analyses for diagnosis and classification of chronic myloid leukemia. Detection of lactate dehydrogenase activity was comparatively easy to perform due to its availability and price. Lactate dehydrogenase activity could be used as diagnostic tool in follow up the chronic myloid leukemia patients during their treatment.

As far as our knowledge, there is no previous study conducted to evaluate the prognostic value of LDH and uric acid as a clinical biomarker in a cross sectional study in CML patients, directly comparing with BCR-ABL which is the gold standard method of monitoring test. Difficulty in getting very expensive and unavailable tests to count leukemic tumor burden by tumor size or number of tumor cells, Lactate dehydrogenase was viewed as a simple biochemical marker for chronic myloid leukemia tumor burden prediction.

8. Study Strengths and Limitation of the study

8.1 Study Strengths

As far as our knowledge, it is the first study done in Ethiopia, which serves as a benchmark. It also highlights the need for further evaluation. This study did not solely relied on the laboratory result but incorporated major clinical data from medical records.

8.2 Limitation of the study

This study partially relied on medical records which at times were missing. And the population studied was a biased sample since patients came only to afford the price of BCR-ABL test. Unfortunately our study did not include the LDH isoenzyme estimation.

Conclusion

The present study revealed that strong significant positive correlations between LDH and BCR-ABL transcript level in chronic myloid Leukemia. But we found that there was weak correlation between uric acid and BCR-ABL.

With regard to patient treatment status, lactate dehydrogenase activity was statistically significant in both treatment naive and patients on treatment chronic myloid leukemia.

On the basis of our study; we conclude that a high LDH level is an important independent predictor of poor survival for chronic myloid leukemia.

Simple clinical biomarkers that are both inexpensive and widely available would be invaluable to the clinician and patients. Practical monitoring of response, resistance, and intolerance can be used to guide treatment choices over time so that patients have the chance of a significantly better long-term outcome. Total LDH can be used as independent prognostic marker not only in diagnosis of chronic myloid leukemia but also, to monitor patients on treatment and treatment naive.

Recommendation

We confirmed that BCR-ABL and lactate dehyderogenase have strong association. It could become an alternative tool in monitoring treatment outcome of chronic myloid leukemia in combination with hematological parameters. Therefore we recommend lactate dehydrogenase screened periodically through treatment follow-up to monitor any rising trends.

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Annex

Annex I English Versions of Participant Information sheet

English Versions of Participant Information sheet, consent form

Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences

You are invited to participate in a study to be conducted by MSc student Temesgen Sisay at Addis Ababa University, College of Health Sciences and Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction The topic of this study is Importance of lactate dehyderogenase and uric acid as compare to BCR-ABL transcript level among Ethiopian chronic myeloid leukemia patients in Tikur Anbesa Specialized Hospital in Addis Ababa, Ethiopia. Participation in this study is exclusively voluntarily. If you are not interested to participate, there will be no consequences.

What is expected from me as participant of the study?

As a participant of this study, there is additional blood sample taking from you for uric acid and lactate dehyderogenase. And the left over sample will be used for CBC count study.

Potential benefits to participant and/ or to the society

Based on the results obtained from the result you are indirectly benefiting other patients and the society.

Compensation for participation

You will not receive any payment for your participation in this research study.

Confidentiality

On the request paper your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose.

Person to contact

Please direct any questions you may encounter during this study to the principal investigator.

Temesgen Sisay

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Cell phone: +251- 0911560375

Email: tutusatule@gmail.com

Consent form

This page contains an agreement signature to participate in the study entitled "Importance of lactate dehyderogenase and uric acid as compare to BCR-ABL transcript level among Ethiopian chronic myeloid leukemia patients in Tikur Anbesa Specialized Hospital in Addis Ababa, Ethiopia"So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study in "Importance of lactate dehyderogenase and uric acid compare to BCR-ABL transcript level in Tikur Anbesa Specialized Hospital in Addis Ababa, Ethiopia. I know that the left over sample (blood) that I gave is going to be used for this study only.
2. I understand that, all the information and the results are confidential.
3. I understand that I will not get any money for my participation.
4. All the information is explained by phlebotomist and Principal investigator.

Therefore, with full understanding of the situations I agree to give blood for laboratory analysis.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Annex II: Amharic version of Participant Information sheet,

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Annex III Data Collection Sheet

Date: _____ Code No.: _____

Address: Sub-city_____Woreda_____Kebele_____Tele:_____

Section I: Socio Demographic Characteristics

1. Sex: M ☐ ☐ F

2. Age: _____ Years

3. Permanent residence place: Rural ☐ Urban ☐

4. Current visiting status: In patient ☐ Out patient ☐

5. Ethnicity. (a) Amhara ☐ (b) Oromo ☐ (c) Tigray ☐ (d) SNNP ☐ (e) Others (specify)

7. Occupation: _____

8. For all age groups

Have you taken any treatments such as cytotoxic chemotherapy (TKI)? Yes No ☐ ☐

If yes what kind Chemotrapy of do you take? _____

History of previous antibiotic treatment: _____

If yes what kind of antibiotic do you take? _____

Section II. Risk factors associated with high or low Lactate dehyderogenase.

1. HIV status (a) Positive (b) Negative

2. Have you ever encounter chronic hypertension? Yes No

3. Do you have liver disease illness? Yes ☐ No ☐

4. Have history of gout? Yes ☐ No ☐

5. Do you have habit for chewing chat, smoking cigarette or drinking alcohol? Yes ☐ N o ☐

6. Do you have any known underline disease? Yes ☐ No ☐

7. Have you ever been admitted to hospital? Yes ☐ No ☐

12. Have you ever had organ transplant surgical procedure? Yes ☐ No ☐

Annex IV. Standard Operating procedures (Sop)

1. Uric Acid

Purpose To detect hyperuricemia and hypouricemia

Materials

Reagents
Enzyme and standard kits ***N.B*** Enzyme/buffer and standard kit contains sodium azide (0.095%). Do not swallow. Avoid contact with skin and mucous membranes.

Reagents preparation: reagent and standard are ready for use

Reagents stability and storage:

1. Stock reagent, stored at 2-8°C, until expiry date.
2. Working reagent is Stable for 4 weeks at 2 -8°C and for 5days at 15 - 25°C

N.B: The working reagent must be kept light protected.

The specimen Analyze as soon as possible if delayed store at 4-8^{o}c for 3 days

Supplies	
Sample cups	- Reaction well
Micropipette tips(200µL, 1000 µL)	-Reagent bottle
Yellow tips and Blue tips	

Equipment
Fully automated Mindray 200 E analyzer graduated pipette , micropipette(50-200µL, 200-1000 µL) test tube, centrifuge, rack, distilled water

Principle

Determination of uric acid by the reaction with uricase. The formed H_2O_2 reacts under catalysis of peroxidase with 3,5-dichloro-2-hyderoxybenzene-sulfonic acid (DCHBS) and 4-aminophenazone (PAP) to give a red- violet quinoneimine dye as indicator.

Reaction principle

Uric acid + O_2 + $2H_2O$ _____uricase__allantoin+ CO_2+ H_2O_2

$2H_2O_2$+DCHBS+PAP_____peroxidase_____quinoneimine+HCL+$4H_2O$

The reference range is for men 3.4-7.0 mg/dl, and for female 2.4-5.7mg/dl. If the absorbance exceeds linearity range repeats the assay using physiological saline (0.9) % using this dilution multiply by dilution factor.

Sample

Sample type	**Amount required**	**Transport and Storage**	**Stability**
Serum	6µl	•Transport whole blood at RT •Separate serum within 1 Hr. • store serum at 2-8°C or -20°C	• Loss of activity within 7 days •10% at Room Temperature
Heparinised Plasma	6µl	•Transport whole blood at RT •Separate serum within 1hour. store serum at 2-8°C or -20°C	• Loss of activity within 7 days •10% at Room Temperature

Limitations: Gross hemolysis, lipemic and icterus specimen

Special Safety Precautions Use universal safety precaution (wearing gloves lab coat and washing hands) when handling infectious materials

Maintenance

Step	Action
1	Daily Cleaning
2	Weekly cleaning
3	Periodic cleaning
Refer cleaning procedures from chemistry Maintenance SOP.	

Calibration Calibrator preparation: Reconstituted one vial of Autocal with 5ml of distilled water.

Note: Mix gently to avoid formation of bubble for 30 minutes

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
Normal	1	Lyophilized: Until Expiry Date Reconstituted: 24hour at 20-30°C 7days at 2-8°C 1month at -20°C	Once per a testing date	Yes
Pathogenic	1			

Control preparation: Reconstituted one vial of Normal or Pathological with 5ml of distilled water.

Note:

- Controls must be kept at room temperature before use
- Controls must be within range. If out of range, repeat the run. If still out of the range. Investigate for root cause. (Reagent, Calibration and QC Preparation….etc)
- Solve the Problem, Document and rerun the Quality controls and Patient samples.

Procedure Refer to chemistry analyzer manual and test kit insertion.

Result Interpretation	**Step**	**Action**
	1	Manual dilution with 0.9%NaCl and rerun the test if > 14 mg/dl If manual dilution, then multiply the result by the dilution factor

Expected Values		**Reference Range**		**Analytical Range**	**Units**
		Women	2.4-5.7	1-14	Mg/dl
		Men	3- 7		

Limitations Gross haemolysis, lipemic and icterus specimen

1. Interfering factors Drugs like

Loop diuretics, Ethambutol, Vincristine, Pyrazinzmide, Thiazidies, Salicylates, Acetaminophen, Ascorvic acid, Leuodopa , Phenacetin,

Physiologically,

Starvation, High purine diet, Stress

Alchohol abuse can raise the amount of uric acid

Aspirin, Coumarin, Colfibrate, Allopurinal, Corticotrophin, Phenothiazines

Hemolysis will decrease the uric acid amount

Related Procedures and Documents

- Chemistry analyzer operator manual.
- Chemistry analyzer Procedures How to Order Test.
- Preventive Maintenance for chemistry analyzer.
- Refer to Chemistry Analyzer Calibration Procedure.
- chemistry analyzer Start up and Shutdown Procedure

- chemistry analyzer Quality Control Running Procedure
- Specimens Collection and Processing Procedure
- Quality Manual

Reference

- Tietz NW, editor. Text book of Clinical Chemistry.3rd ed. Philadelphia: WB Saunders,2001
- Chernecky & Berger: Laboratory Tests and Diagnostic Procedures, 5th ed. 2008
- Lothar Thomas: Clinical Laboratory Diagnostics, use and assessment of Clinical Laboratory Results 1st ed.1998,
- Mindray 200E fully automated chemistry analyzer

2. Lactate dehyderogenase Standard Operating procedures

Purpose to detect high and low LDH

Materials

Reagents
Enzyme/buffer and substrate kits ***N.B*** Enzyme/buffer and substrate kit contains NADH and sodium azide (0.095%). Do not swallow. Avoid contact with skin and mucous membranes.

Reagents preparation: reagent and standard are ready for use

Reagents stability and storage:

1. Stock reagent, stored at 2-8°C, until expiry date. Buffer must be protected from light. Contamination of reagents must be avoided.

2. Working reagent is Stable for 3 weeks at 2 -8°C and for 3 days at 15 - 25°C

N.B: The working reagent must be kept light protected.

The specimen Analyze as soon as possible if delayed loss of activity within 3 days 8% +4 °C, 2% at 15…25°C.

Supplies	
Sample cups	- Reaction well
Micropipette tips(200µL, 1000 µL)	-Reagent bottle
Yellow tips and Blue tips	
Equipment	
Fully automated mindray 200 E analyzer graduated pipette, micropipette(50-200µL, 200-1000 µL), test tube, centrifuge rack, distilled water	

Principle: Lactate dehydrogenase catalyzes the interconversion of lactic and pyruvic acids using the coenzyme $NAD^{+.}$ Lactate + $NAD^+ \leftrightarrow$ pyruvate +NADH + H^+

Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD^+. The rate of oxidation of NADH to NAD^+ is measured as a decrease in absorbance which is proportional to the LDH activity in the sample.

The reference range is 225-450u/l. If the absorbance exceeds linearity range repeats the assay using physiological saline (0.9) % using this dilution multiply by dilution factor.

Sample

Sample type	**Amount required**	**Transport and Storage**	**Stability**
Serum	6µl	•Transport whole blood at RT •Separate serum within 1 Hr. • store serum at room tem 15-25°C	• Loss of activity within 3 days •10% at Room Temperature
Heparinised Plasma	6µl	•Transport whole blood at RT •Separate serum within 1 Hr. • store serum at room temp.	• Loss of activity within 7 days •10% at Room Temperature

Limitations: Gross hemolysis, lipemic and icterus specimen

Special Safety Precautions Use Universal safety precaution (wearing gloves lab coat and washing hands) when handling infectious materials

Refer to the National Health and Safety Guidelines for standard safety procedures

Maintenance

Step	Action
1	Daily Cleaning
2	Weekly cleaning
3	Periodic cleaning
Refer cleaning procedures from chemistry Maintenance SOP.	

Calibration Calibrator preparation: Reconstituted one vial of Autocal with 5ml of distilled water.

Note: Mix gently to avoid formation of bubble for 30 minutes

Control	Level	Stability	Frequency	Preparation (y/n)
Normal	1	Lyophilized*:* Until Expiry Date Reconstituted*:* 24hr at 20-30°C 7days at 2-8°C 1month at -20°C	Once per a testing date	Yes
Pathogenic	1			

Quality Control	**Control preparation:** Reconstituted one vial of Normal or Pathological with 5ml of distilled water. **Note:** • Controls must be kept at room temperature before use • Controls must be within range. If out of range, repeat the run. If still out of the range. Investigate for root cause. (Reagent, Calibration and QC Preparation….etc) • Solve the Problem, Document and rerun the Quality controls and Patient samples.

Procedure Refer to chemistry analyzer manual and test kit insertion.

Result Interpretation	Step	Action
	1	Manual dilution with 0.9%NaCl and rerun the test if 800u/l If manual dilution, then multiply the result by 10 dilution factor

Expected Values	Reference Range		Analytical Range	Units
	Adult	225-450		u/l

Limitations Gross haemolysis, lipemic and icterus specimen

Source of Error

Erythrocytes contain an LDH concentration approximately 100 to 150 times that found in serum. Therefore, any degree of hemolysis should render a sample unacceptable for analysis. LDH activity is unstable in serum regardless of the temperature at which it is stored. If the sample cannot be analyzed immediately, it should be stored at 25°C and analyzed within 48 hours. LDH-5 is the most labile isoenzyme. Loss of activity occurs more quickly at 4°C than at 25°C. Serum samples for LDH isoenzyme analysis should be stored at 25°C and analyzed within 24 hours of collection

Hyper LDH

liver disease, diabetes, renal failure, hemolytic anemias, stroke, coronary artery disease, chronic lung diseases, connective tissue disorders, disseminated intravascular coagulation and seizures, chronic hypertension, gestational diabetes, multiple pregnancy, smoking and alcoholism.

Low LDH

Vitamin c or high content of ascorbic acid

Related Procedures and Documents

- Chemistry analyzer operator manual .
- Chemistry analyzer Procedures How to Order Test.
- Preventive Maintenance for chemistry analyzer .
- Refer to Chemistry Analyzer Calibration Procedure.

- chemistry analyzer Start up and Shutdown Procedure
- chemistry analyzer Quality Control Running Procedure
- Specimens Collection and Processing Procedures
- Quality Manual

Reference

- Tietz NW, editor. Text book of Clinical Chemistry.3rd ed. Philadelphia: WB Saunders,2001
- Chernecky & Berger: Laboratory Tests and Diagnostic Procedures, 5th ed. 2008
- Lothar Thomas: Clinical Laboratory Diagnostics, use and assessment of Clinical Laboratory Results 1st ed.1998,
- Mindray 200E fully automated chemistry analyzer manual

3. Gene Xpert BCR-ABL Monitor Standard operating procedures

Purpose of the test

The Xpert BCR-ABL Monitor Assay is a real-time RT-PCR (reverse transcription-polymerase chain reaction) assay intended as an aid in the monitoring of the BCR-ABL mRNA transcript in peripheral blood of patients with chronic myelogenous leukemia (CML).

Summary and Explanation

CML is one of the most common hematologic malignancies and accounts for 15–20% of all cases of leukemia. The incidence of CML is approximately 1.6/100,000, meaning 1 out of every 635 men and women will be diagnosed with CML during their lifetime. More than 95% of patients with CML have the distinctive Philadelphia chromosome (Ph1) that results from a reciprocal translocation between the long arms of chromosomes 9 and 22.2 The translocation involves the transfer of the Abelson or ABL1 (ABL) gene on chromosome 9 to the breakpoint cluster region (BCR) of chromosome 22, resulting in a fused BCR-ABL1 (BCR-ABL) gene. The gene fusion produces BCR-ABL, a tyrosine kinase with deregulated activity that plays a keyrole in the development of CML.

Principle of Xpert BCR-ABL Monitor Assay

The Xpert BCR-ABL Monitor Assay is an automated test for quantifying the amount of BCR-ABL transcript as a ratio of BCR-ABL/ABL. The assay is performed on Cepheid GeneXpert Instrument Systems. The Gene Xpert Dx Instrument Systems automate and integrate sample preparation, nucleic acid purification and amplification, and target sequence detection in simple or complex samples using real-time RT-PCR and PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single use, disposable GeneXpert cartridges that hold the RT-PCR and PCR reagents and host the RT-PCR and PCR processes. The Xpert BCR-ABL Monitor Assay includes reagents to detect BCR-ABL gene fusions resulting from two major breakpoints, translocation e13a2 (b2a2) and e14a2 (b3a2) and the ABL transcript as an endogenous control in peripheral blood specimens. The amount of BCR-ABL transcript is quantified as the ratio of BCR-ABL/ABL. Besides an Endogenous Control (ABL), a Probe Check Control (PCC) is also included in the cartridge. The Endogenous Control normalizes the BCR-ABL target and ensures that sufficient sample is used in the assay. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

Reagents and Instruments

Xpert BCR-ABL Monitor Assay Reagents

- Sample Container
- Proteinase K (PK)
- Lysis Reagent (LY)
- Guanidine HCl
- Urea
- Wash Reagent (1)
- Ethanol
- Guanidinium thiocyanate
- Rinse Reagent
- Elution Reagent

Xpert BCR-ABL Monitor Assay Cartridges with integratedreaction tubes

- Bead 1, 2, and 4 (freeze-dried) 1 per cartridge
- Bead 3 (freeze-dried) 2 per cartridge

Storage and Handling

- Store the Xpert BCR-ABL Monitor Assay kit contents at 2–28 °C.
- Do not open the cartridge until you are ready to perform the assay.
- Use the cartridge and reagents within 30 minutes after opening the package.
- Except for the Lysis Reagent, do not use reagents that have become cloudy or discolored.
- Do not use an Xpert BCR-ABL Monitor Assay cartridge after the expiration date.
- Do not open the Xpert BCR-ABL Monitor Assay cartridge lid except when adding sample and reagents.
- Do not use an Xpert BCR-ABL Monitor Assay cartridge if a reagent is added to the wrong opening.
- Do not shake the Xpert BCR-ABL Monitor Assay cartridge

Specimen Collection and Storage

Collect blood specimens in EDTA tubes, Sodium Citrate tubes, or PAXgene Blood RNA tubes. Do not use heparin as the anticoagulant because it can inhibit the PCR reaction. Store blood specimens at 2–8 °C and complete the test within 48 hours after collection.

Procedure

Before You Start

Before you start the procedure, remove the Sample Preparation reagents from storage and briefly microcentrifuge these reagents:

- Proteinase K (PK)
- Lysis Reagent (LY)

• Wash Reagent (1)

• Rinse Reagent (2)

• Elution Reagent (3)

A. Preparing the Sample

1. Add 40 µL of Proteinase K (PK) to the empty 5 mL Sample Container (S).

2. Ensure blood specimen is well-mixed by inverting collection tube 8 times immediately before pipetting. See manufacturer's instructions for each type of blood collection tube.

3. Add 200 µL of blood specimen to the Sample Container (S) already containing Proteinase K.

4. Mix the sample with a Vortex mixer for 5 seconds.

5. Incubate at room temperature for 1 minute.

6. Add 1 mL of Lysis Reagent (LY) to the Sample Container (S).

7. Mix the sample with a vortex mixer for 10 seconds.

8. Incubate at room temperature for 10 minutes.

9. Add 1 mL of reagent grade absolute ethanol (provided by user) to the Sample Container (S).

10. Mix the sample with a vortex mixer for 10 seconds.

11. Set aside.

B. Preparing the Cartridge

1. Remove the cartridge from the sealed pouch.

2. Inspect the cartridge for damage. If damaged, do not use it.

3. Open the cartridge lid and transfer the contents from the following reagents tubes to the cartridge chambers

• Pipette 2.1 mL of the contents from the Wash Reagent vial (1).

• Pipette 1 mL of the contents from the Rinse Reagent vial (2).

• Pipette 1.8 mL from the Elution Reagent vial (3).

4. Pipette the entire contents from the Sample Container (S) into the cartridge chamber S (Sample

5. Close the cartridge lid. Ensure the lid snaps firmly into place.

6. Load the cartridge into the GeneXpert Dx or GeneXpert Infinity instrument

Place the cartridge into the GeneXpert instrument within 15 minutes of adding the sample and reagents into the cartridge

Before starting the test, make sure the Xpert BCR-ABL Monitor Assay definition file is imported into the software.

Quality Control

Each test contains an Endogenous Control (ABL) and a Probe Check Control (PCC). No external controls are required.

Results

Xpert BCR-ABL Monitor Assay results are reported to the International Scale (IS).

BCR-ABL Detected

For a "BCR-ABL has been detected..." result, the GeneXpert software calculates the % BCR-ABL/ABL IS

BCR-ABL Not Detected

For a "BCR-ABL was not detected..." result, the GeneXpert software first calculates a theoretical BCR-ABL ratio by subtracting 32 from the test result's ABL Ct (ABL Ct - 32).

References

1. Jemal A, Siegel R, Ward E, et al. Cancer Statistics 2008; CA Cancer J Clin. 2008;58:71–96.

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fluorescence and Giemsa staining. Nature. 1973;243:290–293.

3. NCCN. Clinical Practice Guidelines in Oncology; Chronic Myelogenous Leukemia. Version 2. 2009.

4. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 2005;105(7):2640–2653.

12.Declaration

I, the under signed, declare that this MSc thesis is my original work and it has not been presented for a degree in any other University. All sources of materials used for this thesis and institutions who gave support have been duly acknowledged.

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