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Comparison between LDH with Zap-70 and CD38 as prognostic Markers in Chronic Lymphocytic Leukemia Sudanese patients - A Pilot Study in Khartoum State.

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Abstract

Background: Several prognostic markers are used in clinical stages of Chronic Lymphocytic Leukemia (CLL); including lactate dehydrogenase enzyme (LDH), CD38 and Zeta associated protein kinase (ZAP70). This study aimed to compare LDH with ZAP70 and CD38 as prognostic markers in Sudanese patients with CLL.

Materials and Methods: Fifty newly diagnosed patients with CLL were enrolled in this cross-sectional study, the age of patients ranged between 36 - 85 years, with mean of 62 years. Of total patients; there were 34 males (68%) and 16 females (32%). Blood samples were obtained and LDH measurement was done by using Dirui CS-T240 automated analyzer while CD38 and ZAP-70 were measured by flowcytometry. Data were analyzed by statistical package for social science (SPSS) computer software program version 21.

Results: The present study revealed a significant association between LDH and Rai staging (p. value= 0.002), when LDH compared with ZA-70 and CD38 as reference prognostic markers; poor correlation between CD38 and LDH was found (R2= 0.086, P value= 0.034) and no correlation between LDH level and ZAP-70 expression (R2= 000, P value= 0.960).

In conclusion: According to the outcomes of this study; ZAP-70 and CD38 cannot be substituted by LDH as a prognostic marker.

Key Words: CLL, LDH, ZAP-70, CD38, prognostic markers, Khartoum state.

Introduction

Rai and Binet staging methods are common clinical stages for Chronic lymphocytic leukemia (CLL) patients prognosis, prognostic markers are used to classify patients who will respond from the beginning of therapy regardless of clinical level, including conventional prognostic factors involving the clinical stage, pattern of bone marrow infiltration, lymphocytes doubling time, B2 microglobulin, thymidine kinase, and LDH (Rai et al,1975) (Gogia et al, 2013). Recently several novel prognostic markers are used based on Immuno-phenotypic or cytogenetic (CD38, Zap-70, and p53 expression), cytogenetic markers (17p deletion, 11q deletion, IGVH mutation) (Hendy et al,2016) (Sellmann et al, 2011).

Some studies compared LDH with Zap-70 and CD38 in patients with CLL in different populations; a study by Shen *et al* on 141 patients with CLL in China showed that serum LDH was significantly higher in patients in the Binet C stage than in Binet A. The study concluded that serum level of LDH and stage Binet are important prognostic markers for chronic lymphocytic leukemia (Shen et al, 2007).

To the best of our knowledge, limited data regarding this issue in Sudanese population was found. According to the fact that; determination of ZAP-70 and CD38 expression are relatively expensive techniques requiring specialized equipment and skills, while LDH is inexpensive test that do not require sophisticated equipment and can be undertaken in routine laboratory; this study aims to compare LDH with ZAP-70 and CD38 as prognostic markers in Sudanese patients with chronic lymphocytic leukemia.

Material and methods

This cross-sectional study was approved by scientific committee of Clinical Chemistry Department, Faculty of Medical Laboratory Science, Al-Neelain University. The study was conducted at Flowcytometery Center in Khartoum- Sudan during the period from June 2018 to December 2018. After obtaining an informed consent; fifty newly diagnosed patients with CLL with age range ranged from 36 to85 years (34 of them were males and 16 were females) who previously untreated were enrolled in this study, patients with documented evidence of renal or hepatic failure were excluded. Clinical data was collected from the clinical records.

Sampling: A venous blood sample was collected from all patients; 3 mL were dispensed in a plain container for LDH measurement using Dirui CS-T 240 analyzer and 3 mL were dispensed in EDTA container for CBC investigation using Sysmex XP–300 and Zap-70, CD38 were investigated by flowcytometer Epiccs XL- MCL four colors Beckman Coulter.

Quality control: The precision and accuracy of all biochemical methods used in this study were checked each time by including commercially prepared control sera while the flowcytometry flow check beads were used to verify instrument alignment.

Statistical analysis: Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 21. Descriptive statistics were used; Means (Standard Deviations) for normally distributed continuous variables, while Medians (Interquartile Range) for non-normally distributed continuous variables. Independent sample T test and one way ANOVA were used for comparison between parameters.

Cruskal Wallis H test was done to determine the association between study variables. *P- value* < 0.05 were considered statistically significant in all tests.

Results

In this study; males were 34 (68%) and females were 16 (32%) with 2.1:1 male to female ratio, the patients mean age was 62 years. Most of patients were in stage I (26%) and stage II (24 %) according to Ria clinical staging system (Figure.1), 26% of patients were presented with anemia and 32% of them were presented with thrombocytopenia (Table 1).

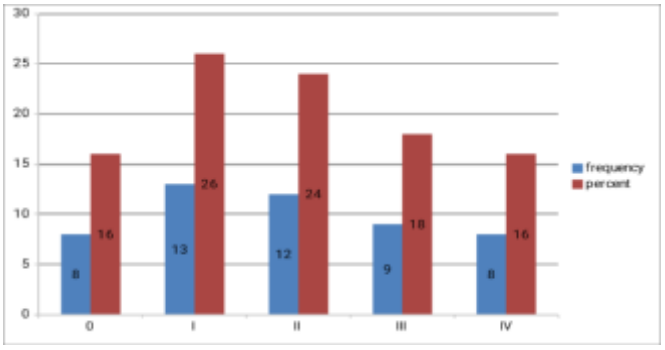

Figure 1: Distribution of Ria stage diagnosis among CLL patients.

Table 1: Hematological characteristics among study group.

Characteristics	**n=50**
Haemoglobin (g/dL), mean ±SD	11.7 ±2.4
Haemoglobin categories	n (%)
Normal	37(74 %)
Anemia	13(26%)
White blood cell count (x109/l) mean ±SD	90.4±75.9
WBC count categories	n (%)
Normal WBC count	0 (0%)
High WBC count	50 (100%)
Platelet count (x109/L), mean ±SD	184.7±83.06
Platelet count categories	n (%)
Normal	34(68%)
Thrombocytopenia	16(32%)

At the level of biochemical profile in this study the findings showed that 32% of patients had increased LDH level, 4% (2) and 2%(1) had abnormal expression of CD38 and Zap-70 respectively (Table 2).

Table 2. : Distribution of biochemical characteristics among CLL patients.

Characteristics n=50	**mean±SD**	**Positive**	**n (%)**
LDH IU/L	464.7±207.3	>460 IU/L	16(32%)
CD38	8.77±13.07	>30%	2(4%)
Zap-70	6.87±12.07	>30%	1(2%)

In the present study a significant association was found between Ria clinical stage and LDH (Table 3). When using CD38 as the reference marker; poor correlation between LDH and CD38 were found but there was no relation between LDH and Zap-70 when using the last as reference marker (Figures 2, 3).

Table 3: Correlation between Ria clinical stage and biochemical profile among study group.

Biochemical results	Clinical stages	*p*-value
LDH U/L	0 I II III IV	0.002
CD38	0 I II III IV	0.643
Zap70	0 I II III IV	0.279

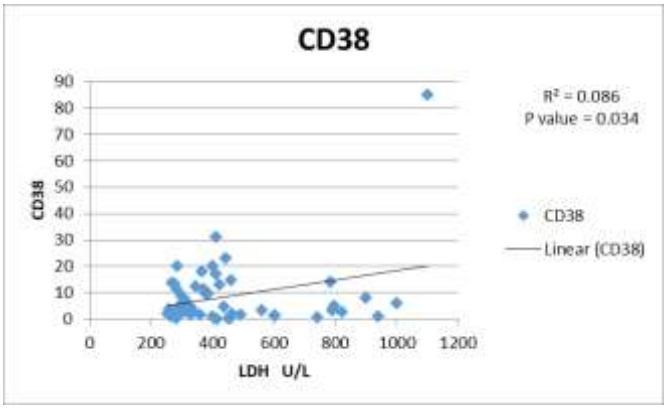

Figure 2: Relationship between LDH with CD38 as reference standard.

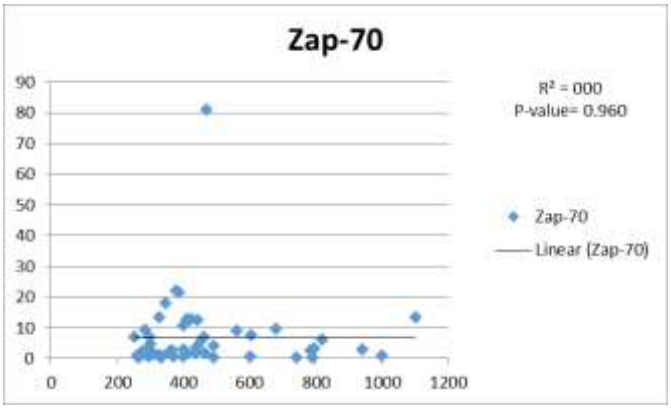

Figure 3: Relationship between LDH with zap-70 as reference standard

Discussion:

It's a fact that LDH, CD38 and ZAP-70 are some of many prognostic markers that used in prognosis of CLL. In the current study which aimed to compare LDH with ZAP-70 and CD38 in Sudanese CLL patients; males were 34 (68%) and females were 16 (32%) with male to female ratio of 2:1, this findings was near to a study results done in Sudan by Ahmed and Osman in 2017, their study found that male to female ratio was 2.4:1(Ahmed & Osman,2017) and near to Egyptian studies (Khorshed et al,2006) (Assem et al, 2009) , the good explanation of the male predominance more than females is that they might be more exposed to occupational and environmental carcinogenic agent as reported by Bhutani *et al* in 2002 (Bhutani et al, 2002) . Regarding to this study; patient's age ranged from 36 to 85 years old with mean age of 62 years; this finding is similar to previous study in Sudanese population which found that the mean age of CLL patients was 60 years (Ahmed & Osman,2017).

The finding of the current study showed that, 13 (26%) of patients were anemic, 13(26%) were thrombocytopenic and all of them had leukocytosis and absolute lymphocytosis as very expected in CLL, this results agreed with reported findings in previous study in Sudanese population in which most of patients presented leukocytosis, anemia and thrombocytopenia (Ahmed & Osman,2017).

The clinical staging of patients of the current study was done using Rai staging which is based on absolute lymphocytosis, enlarged lymph node, spleen, and liver, level of hemoglobin, RBCs and platelets count (Rai et al,1975). In fact majority of patients with newly diagnosed CLL have lower risk disease Rai stage (0-1) (Rai,1987). Regarding the Ria stage classification system, this study revealed that, most of the patients 13 (26%) were classified as stage 1 which considered intermediate risk followed by stage 2 in 12 (24%) of patients, these findings disagreed to findings reported by Enaam *et al* in which the results showed that nearly two-third of the patients (64.7%) were in a high risk group (Abdelgader et al,2018) which also confirmed by other previous studies used Binet stage classification system instead of Ria stage system (Schroers et al,2005) (Basabaeen et al,2019) .

Indeed, the LDH level has been affected by the state of hypoxia in tissues and increased in hematological malignancies like CLL (Mani et al,2006) (Bouafia et al,2004). Sixteen of patients (32%) enrolled in this study had elevated LDH level; this is also agreed with another reports by Mozaheb *et al* showed that LDH was elevated in 20% of the patients (Mozaheb et al, 2012) , also to what reported by a study done in India in 2006 by Mani and Murthy, and a study in Spain by Eva *et al* in 2010; both reported increased LDH levels in 20 -25% of CLL patients at the time of enrollment into the study, results which agreed with a study in Iran by Mozaheb *et al*. , and another study in which LDH was elevated in only 20% of the patients . (Mani et al,2006) (Mozaheb et al, 2012) (Gine et al,2010).

Expressions of ZAP-70 and CD38 by flowcytometry identified by several groups as predictors of disease progression and overall survival in CLL (Schroers et al,2005) (Shanafelt et al,2004) . In Sudan, A study done by Abdelgader *et al* revealed that the CD38 expression at a cutoff value of 7% is significantly associated with low hemoglobin concentration, and therefore, more likely to predict poor outcomes than the higher CD38 cutoff levels (Abdelgader et al,2018). Another study done by Basabaeen and others found that expressions of ZAP-70 and CD38 were not statistically associated with more advanced stages at Rai and Binet of B-CLL patients and CD38 was significantly associated with splenomegaly (Basabaeen et al,2019). To the best of our knowledge, this is the first study conducted in Sudan to investigate and to set LDH if possible as a prognostic marker for CLL patients and to correlate it with patient's outcome and Zap-70, CD38 when used as reference prognostic markers.

In the present study, a significant association between LDH level and Ria stage were found and insignificant association between Zap-70, CD38 expression and Ria stage, this findings disagreed with previous studies done by Del Poeta *et al* and Hus *et al* who found significant correlation between Zap-70 and advance Rai stage (Del Poeta et al,2001) (Hus et al,2006) and agreed with Gogia *et al* study in India which showed no correlation between Zap-70 and CD38 with Rai stage (Gogia et al, 2013). In fact many studies reported that prognostic information given by ZAP-70 and CD38 expression is complementary, and could be used to guide treatment decisions especially in early clinical stages of the disease (Schroers et al,2005) (Hus et al,2006).

In the present study, when LDH compared with ZA-70 and CD38 as reference prognostic markers; there was poor relation between LDH and CD38 and no relation between LDH and ZAP-70.

Conclusion:

Although determination of ZAP-70 and CD38 expression is relatively expensive technique requiring specialized equipment and skills; while LDH is a routine, inexpensive test requires unsophisticated equipment; but ZAP-70 and CD38 cannot be substituted by LDH as prognostic marker as the outcomes of the current study revealed significant correlation between LDH levels and Rai staging, poor correlation between LDH level and CD38 expression and no correlation between LDH level and ZAP-70 expression.

Ethical Consideration:

The study was approved by scientific committee of Faculty of Medical Laboratory Science, Clinical Chemistry Department, Al-Neelain University. An informed consent was obtained from each participant.

Conflict of Interest:

All authors declare no conflict of interest regarding this work.

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