A STUDY OF EUCHROMATI C HISTONE-LYSINE N-METHYLTRANSFERASE 1 EXPRESSION IN EGYPTIAN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS.

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INTRODUCTION

Chronic lymphocytic leukemia is a indolent hematological disorder characterized by monoclonal B lymphocyte proliferation. It is the most common adult leukemia in Western populations and comprises up to 30 percent of leukemias developed world. Identification of the cases that need early and prompt treatment still leaves many patients with untailored treatment options.

Study of epigenetics in leukemia may provide early identification and effective treatment option. There has been intensive work on the role of various methyltransferases in leukemias, however EHMT1 expression levels in Egyptian patients with CLL, and their prognostic value are still inconclusive.

A previous study have suggested that EHMT1 is over expressed is CLL cases with worse prognosis cytogenetic abnormalities (p = 0.05), however expression level of EHMT1 has not been studied against CD38 and Zap70.

AIM OF THE WORK

To study the expression of EHMT1 in newly diagnosed CLL patients and evaluate its role as a prognostic marker for CLL.

PATIENTS AND METHODS

This study was conducted on 25 newly diagnosed chronic lymphocytic leukemia patients and 25 healthy controls recruited from Alexandria University Hospital haematology clinic. After approval of the Ethics Committee of Alexandria University, Faculty of Medicine, a written informed consent was obtained from the patients. Patients with history of malignancy, chemotherapy, radiotherapy, pregnancy and organ failure were excluded.

The 50 subjects (patients and controls) were subjected to comprehensive history taking, clinical examination, CBC. The 25 CLL cases samples were analysed for CD38 and Zap70 by Becton Dickinson, FACS caliber flow cytometer equipped with Cell Quest software [USA]. Real Time PCR for EHMT1 expression for all the 50 subjects was analysed using the. Rotor-Gene Q thermocycler (QIAGEN, US.A) by Roto gene q series software version 2.3.4 (U.S.A). Follow up for the 25 cases of CLL with CBC and clinical findings, screening (hepatosplenomegaly and lymphadenopathy) was done.

RESULTS

EHMT1 expression levels was compared in 24 cases against 25 controls, after removal of outlier. The CLL group had a mean EHMT1 expression of 6.68 ± 4.67 while the control group had a mean expression of 0.98± 1.46. The difference was statistically significant (P = <0.001).

Table 1: Table for ROC curve analysis to discriminate CLL cases (n = 24) from healthy controls (n = 25).

|	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Fold change	0.904	<0.001*	0.797 – 1.000	>3.182	83.33	90.0	90.9	81.8
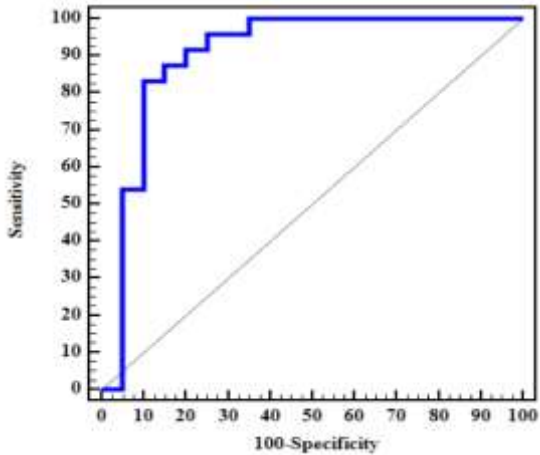
Figure 1:
ROC curve to discriminate CLL cases(n = 24) from healthy controls (n =25).
The cutoff of 3.182 correctly discriminated CLL cases from healthy controls.

Ten CLL cases were started on treatment while fifteen were not eligible for treatment. Eight of the ten cases responded to therapy while 2 cases had no improvement of their hematological parameters.

The CLL group that responded to treatment had a mean EHMT1 expression of 4.288±0.126while the CLL group that did not respond to treatment had a mean EHMT1 expression of 9.374 ± 6.170. The difference was statistically significant (p = 0.044)

Table 1: Prognostic performance for Fold change to discriminate treatment responders (n = 8) from non-responders (n = 2) in the cases group.

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
Fold change	1.000	0.037*	1.000 – 1.000	>4.377	100.0	100.0	100.0	100.0
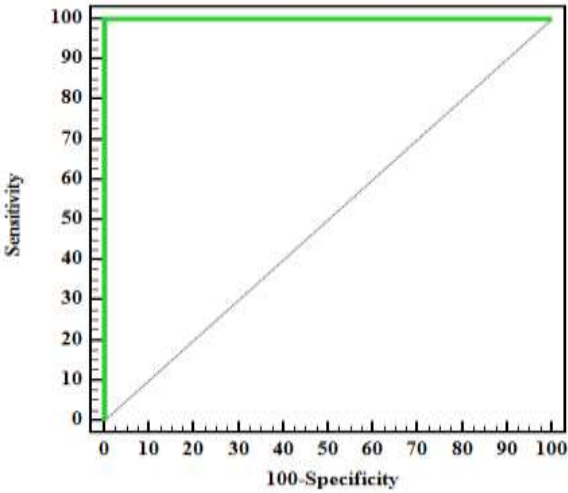
Figure 2:
ROC curve for Fold change to discriminate treatment responders (n = 8) from non-responders (n = 2) in cases group.

The cutoff of 4.377 correctly discriminated treatment responders from non responders. Eighty eight percent of the CLL cases were Zap70 positive while thirty two percent were CD38 positive .Double positive cases for CD38 and Zap70 were 24% of total CLL cases, double negatives cases(for CD38 and Zap70) were 4%. Single positive for Zap70 (negative for CD38) were 64%, while 8% were single positive for CD38(negative for Zap70). The CD38 subgroups analysis revealed that allclinical and CBC parameters with the exception of platelet count (p = 0.020) were statistically insignificant.EHMT1 expression between the subgroups was also not statistically significant (p= 0.190). Analysis of CLL cases based on Zap70 positive and negative was insignificant on all hematological and clinical parameters, except for Hb (p= 0.042). EHMT1 expression level between the Zap70 subgroups was insignificant (p=0.441).

CONCLUSION

EHMT1 is over expressed in Egyptian CLL patients and the EHMT1 expression levels were higher in patients resistant to CLL treatment. Significant correlation between EHMT1 and absolute lymphocyte count in CLL patients were demonstrated. Expression levels was statistically significant between treatment responsive and non responsive CLL cases.

Overall, the use of the EHMT1 in clinical practice represents a promising approach, considering the accumulating evidence on its functions and expression levels.