A STUDY OF ASSOCIATION BETWEEN RECEPTOR OF TYROSINE KINASE ORPHAN LIKE RECEPTOR (ROR1) AND CHRONIC LYMPHOCYTIC LEUKIMIA (CLL) IN EGYPTIAN PATIENTS

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Introduction

Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia worldwide as in the Egyptian population. In Egypt, it accounts for 3.08 % of lymphoid malignancies and 0.5 % of all cancers.CLL diagnosis requires the existence of at least 5×10^9 /L circulating mature B-lymphocytes in the peripheral blood for 3 months. The immunophenotype of typical lymphocytes in case of CLL includes the co-expression of weak monotypic surface immunoglobulin, CD5, CD19, CD23 and weak or absent CD79B, CD22 and FMC7. Identification and targeting antigens that are exclusively overexpressed in malignant but not normal cells are crucial for the success of cancer management. In the previous years the application of tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) as therapeutic, diagnostic and screening targets for cancers become more promising. Theses antigens have a vital role in the growth and survival of malignant cells. ROR-1 is one of the promising TAA in CLL.

Aim of the Work

The aim of our study

To assess the role of ROR 1in diagnosis of CLL and in minimal residual disease (MRD) of CLL.

Subjects and Methods

Our study was carried out on 30 newly diagnosed CLL patients with immunophenotypic chronic panel with score 4/5 or 5/5 from Alexandria Main University Hospital hematology department and 30 healthy age and sex matched normal subjects. Cases were objected to history taking, full clinical examination, routine laboratory investigations including CBC, liver and kidney functions tests. At the onset of diagnosis Chronic panel for CLL diagnosis was done with flowcytometry technique using: CD5, CD19, CD2, CD20, CD22, CD23, CD37, CD79b, FMC7, kappa and lambda chains. ROR 1expression was measured at the onset of diagnosis and six months from therapy start by flowcytometry technique using PE Mouse Anti-Human ROR1from BD Pharmingen™ brand. The study was carried on fresh whole EDTA blood prepared and fixed for immunostainig with Anti-human ROR1 and conjugated with flurochrome R-PE (R phycoereythrin) on the instrument BD FASCanto™II.

Results

There was statistically significant increase ROR1 in patients group at onset of diagnosis and significant decrease after 6 months. There was a positive significant correlation between ROR1 expression at onset of CLL diagnosis.

ROR1 expression at onset of diagnosis and after 6 months of therapy

ROR1 at onset of diagnosis	**Patients group**	**Control group**
Mean ± SD	78.14± 22.59	1.70±0.77
U-test P value	98.0 0.001	
ROR1 after 6 months of therapy	At onset of diagnosis	After 6 months
Mean ± SD	78.79±19.84	64.61±24.72
U-test P value	3.98 0.041	

U- Mann Whitney test P was significant if ≤ 0.05

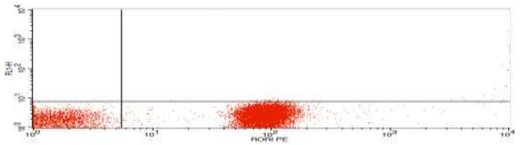
The table shows that ROR1 expression firstly comparing the marker expression patients group from control group then comparing ROR1 expression in survived patients within patients group at onset of diagnosis then its expression after 6 months of starting treatment. Measuring ROR1 expression at onset of diagnosis in patients group ranged from 10-98.3 % with mean value 78.14±22.59 and in control group ranged from 0.5-3.1 with mean value 1.70±0.77. There was statistical significant increase in patients group than in control group regarding ROR1 at onset of diagnosis. Within the patients group ROR1 expression at onset of diagnosis ranged from 10-98.3% with mean value 78.79±19.84and after 6 months ranged from 10.2-94% with mean value 64.61±24.72. There was statistical significant decrease after 6 month than at onset of diagnosis in patients' group in survived patients

Diagnostic performance of ROR1 expression

the table illustrates the role of measuring ROR1 expression by flowcytomerty in CLL as a reliable diagnostic test as ROR1 expression was 100% sensitive and specific for CLL diagnosis with a cut off point more than 70 %

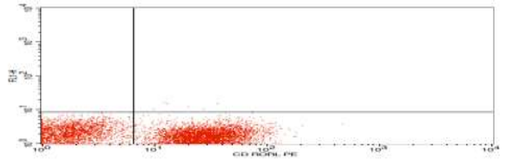
Area	**Cut off value**	**Asymptotic Sig.[b]**	**Asymptotic 95% Confidence Interval**	
			Lower Bound	**Upper Bound**
1.000	> 70%	0.0001*	1.000	1.000
Sensitivity	100.0			
Specificity	100.0			
Accuracy	100.0			

Asymptotic Sig.[b]means Asymptotic significance beta



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	0	0.00	0.00	**	**	**	**
UR	30	0.25	0.19	3979.71	603.25	110.69	21.36
LL	3304	21.31	11.01	1.61	1.47	2.03	1.85
LR	10162	78.44	40.54	96.65	88.50	2.81	2.52

Diagram of ROR1 shows 78.9% expression in CLL newly diagnosed patient



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.01	0.00	4.97	4.97	8.60	8.60
UR	7	0.08	0.02	27.22	21.54	12.71	12.43
LL	3234	36.16	10.78	1.89	1.87	2.36	2.11
LR	5701	63.75	19.00	31.69	27.50	1.75	1.61

Diagram of ROR1 shows 63.7% expression in the previous patient six months after starting therapy

Conclusion

From our results it was concluded that ROR1 is considered very reliable marker to be added to the diagnostic immunophenotyping panel for CLL.

In addition, the level of ROR1 expression showed significant decrease after 6 months in our patients confirming that ROR1 could be used as a marker for minimal residual disease detection in CLL.