Research Article

Role of immunophenotyping in diagnosis of CLL patient at South Egypt Cancer Institute.

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Abstract

Objective: To determine the role of immunophenotyping in chronic lymphocytic leukemia (CLL) patients at South Egypt Cancer Institute (SECI). **Methodology:** This study was carried out in Department of Clinical pathology, South Egypt Cancer Institute, from December 2017 to July 2019. We used SECI Flowcytometry lab 4 color for diagnosis. **Results:** 37 patients were included in our study with CLL and 30 apparently healthy control group. For the both groups we performed: CBC, BM aspiration, BM biopsy and immunophenotyping. The mean age in CLL group was (55.92± 9.02) years. while the mean age in control group was (38.37 ± 14.08) years . **Conclusion:** Immunophenotyping has a great role in CLL diagnosis.

Keywords: CLL, BM

Introduction

Chronic lymphocytic leukaemia/ small lymphocytic lymphoma (CLL/SLL) is a monoclonal neoplasm characterized by accumulation of monomorphic small mature B cells that coexpress CD5+ and CD23 in the peripheral blood, bone marrow and secondary lymphoid organs as lymph nodes and spleen. It is usually characterized by an increase in lymphocytes count (there must be monoclonal B-cell) in peripheral blood >5 x 10*9/l. The term SLL is used for cases with a circulating CLL cell count < 5x 10*9/L and documented nodal, splenic, or other extramedullary involvement (Hallek et al., 2015).

B cell receptors of CLL cells demonstrate highly selected immunoglobulin heavy chain variable (IGHV) gene coded by both heavy and light chain genes so called stereotypes, and thus differ from the B cell receptors of much broader diversity found in normal B lymphocytes (Agathangelidis et al., 2012).

Most cases of CLL are diagnosed on the basis of routine blood analysis in asymptomatic patients and discovered accidentally. Less often, lymphadenopathy, splenomegaly, anaemia or thrombocytopenia which lead to (fatigue, malaise and bleeding). In few cases the diagnosis is reached after work-up for other manifestations such as an autoimmune cytopenia (autoimmune haemolytic anaemia, immune thrombocytopenia or erythroblastopenia) or an infection, most frecuently pulmonary. The frecuency of hypogammaglobulinaemia is about 30% at diagnosis and increases over time (Parikh et al., 2013).

Patients whose absolute lymphocyte count (ALC) takes more than 12 months to double have a better prognosis than those whose lymphocyte count takes less than 12 months to double. Experts suggest that a lymphocyte doubling time of less than 6 months may be a measure to define initiation of therapy (Falay and Ozet., 2017).

Circulating leukaemic B cells express CD19 and dim surface IgM,/IgD, CD20, CD22, CD 79 b. They also positive for CD5 and CD43 and strongly positive for CD23 and CD200, CD10 is negative and FMC 7

is usually negative or only weak express (Falay and Özet., 2017).

Methodology:
This study was performed on 37 patients with CLL. Those patients were presented to South Egypt Cancer Institute Assiut University hospital in the period from December 2017 to July 2019. An approval of the medical ethical committee of faculty of medicine AL-Azhar University and South Egypt Cancer Institute Assiut University were obtained.

All patients were presents with:

1- Absolute lymphocytosis in peripheral blood >5000/cmm sustained for at least 3 months.
2- Lmphocytes are small,monotonues, mature –looking with high N/C ratio, round to oval nuclei and clumbed chromatin. Smudge cell is a characteristic feature.
3- Characterastic immunophenotyping: CLL cells coexpress the surface antigen CD5 together with the B-cell antigens CD23, CD19, CD20+(dim), monoclonal kappa or lambda light chain restriction and negative FMC7, CD10.
4- Bone marrow shows increased numbers (>30%) of mature small lymphocytes. Prolymphocytes are <55%.

In our study **Complete blood count (CBC)** was performed by CELL-DYN 3500 (Abbott Diagnostics, Santa Clara, California, USA). At time of diagnosis, bone marrow samples for both **Bone marrow aspirate and biopsy** were collected from posterior superior iliac spine by BM needles (Islam and Jamshidi).

Immunophenotyping by flow cytometry:
The test aimed to detect the presence and level of expression of the cell surface marker. These markers can be demonstrated by the use of labeled cells are acquired using a flow cytometer.

Analysis:
Analysis was done by multicolor flow cytometry (FACS Caliber, BD Biosciences-San Jose, CA, USA).

Forward scatter and side scatter histogram were made to detect the lymphocyte population. Lymphocytes were then gated for further analysis of different monoclonal antibodies
The percentage of cells was calculated on the basis of data obtained from immune-fluoreseence dot-blots. Analysis were carried out using CellQUEST Software (Becton Dickinson) on gated lymphocyte, gate lymphocytes which coexpress CD19 only (to make sure that they were mononuclear cells).

Data Analysis: Data entry and data analysis were done using SPSS version 22 (Statistical Package for Social Science). Data were presented as number, percentage, mean, median and standard deviation. Chi-square and Fisher Exact tests were used to compare between qualitative variables. Kruskal Wallis test and Mann-Whitney test were used in case of non-parametric data. P-value considered statistically significant when $P < 0.05$**.**

Results

This study was performed on 37 patients with CLL (group I). They were compared with sex and age-matched 30 controls. The mean age in CLL group was (55.92± 9.02) years. while the mean age in control group was (38.37±14.08) years. Regarding gender, group I patients (CLL) were 22 males and 15 females. Likewise , controls were 22 males and 8 females. Demographic data of cases and controls in table(1).
In group (I) 2(5.4%) of patient had splenomegaly, 18(48.6%) of patient had lymph node (table 2)

In CLL group the mean concentration of WBCs was (33.43±45.55) which was more than controls (7.23±1.59) with a significant difference between CLL group and control **(P- value 0.000).** The patient group show increase in the number of peripheral lymphocytes with mean (67.51±12.13). The RBCs was show a significant between CLL group and control (P-value = 0.002). On the other hand the mean concentration of hemoglobin was (11.61±2.15) in CLL group, also the mean of control (12.04± 2.15) and there was no significant between

CLL group and control (P-value = 0.640). However ,the mean of PLT in CLL group (164.43±59.54) was lower than controls (233.80±81.90) with a significant difference between CLL group and controls (P- value =0.001). data shown in table (3).

The distribution of expression of Immuno-phenotyping panel for our studied group in table (4).

Table (1): Personal data of the studied groups

Personal data	Group I (n= 37)		Control (n= 30)		P-value[1]
	No.	%	No.	%	
Sex:					0.234
Male	22	59.5	22	73.3	
Female	15	40.5	8	26.7	
Age (years)					<0.001*
Mean ± SD	55.92 ± 9.02		38.37 ± 14.08		

1: Comparison between Group I and Control, SD: standard deviation, n: number

Table (2): Clinical data of the studied group

Clinical data	CLL Group (n= 37)	
	No.	%
Splenomegaly:		
Positive	2	5.4
Negative	35	94.6
Lymph nodes:		
Positive	18	48.6
Negative	19	51.4

Table (3):The hematological data of the studied groups

	Group I (n= 37)	Control (n= 30)	P-value*
WBCs:(10^9/l)			<0.001*
Mean ± SD	33.43 ± 45.55	7.23 ± 1.59	
Median (Range)	29 (13-215)	7.1 (4.9-11.5)	
Lymphocytes %:			<0.001*
Mean ± SD	67.51 ± 12.13	34.43 ± 4.61	
Median (Range)	65 (45-94)	34 (25-43)	
RBCS:			0.002*
Mean ± SD	4.03 ± 0.81	4.61 ± 0.55	
Median (Range)	4.3 (1.9-5.1)	4.7 (3.5-5.5)	
Hb:(g/ dl)			0.640
Mean ± SD	11.61 ± 2.15	12.04 ± 2.15	
Median (Range)	12.0 (5.9-14.2)	12.1 (4.7-15.9)	
Platelets(10^9/l)			<0.001*
Mean ± SD	164.43 ± 59.54	233.80 ± 81.90	
Median (Range)	160 (25-319)	216.5 (117-427)	

WBC : white blood cell , RBCs: red blood cells , HB: haemoglobin,
p-value*: Comparison between Group I and Control

Table (4):The distribution of expression of Immunophenotyping panel for CLL

Lymphoproliferative disorders panel	Group I (n= 37)	
	No.	%
CD5:		
Positive	37	100.0
Negative	0	0.0
CD19:		
Positive	37	100.0
Negative	0	0.0
CD10:		
Positive	0	0.0
Negative	37	100.0
CD20:		
Positive	37	100.0
Negative	0	0.0
CD23:		
Positive	33	89.2
Negative	4	10.8
CD38:		
Positive	6	16.2
Negative	31	83.8
CD200:		
Positive	37	100.0
Negative	0	0.0
SIg:		
Positive	11	29.7
Negative	26	70.3
FMC7:		
Positive	0	0.0
Negative	37	100.0

CD=cluster of differentiation, SIg= surface immunoglobulin

Disscussion

Chronic lymphocytic leukaemia is one of the most common types of leukemia in the Westren world. However, infrequent in the Eastern. It is the most common types of leukaemia diagnosed in adult. Although most patients are asymptomatic at diagnosis, some can have systemic symptoms (hepatosplenomegaly, lymphadenopathy, and cytopenias due to leukaemic infiltration of bone marrow and other organs). As defined in the WHO classification, CLL is a neoplasm composed of monomorphic small mature B cells that coexpress CD5 and CD23 (Hallek et al., 2018).

This study was conducted on 37 patients with CLL, there was a slightly higher prevalence in males (59.5%) over females (40.5%). The mean age of CLL group was (55.92 ± 9.02).

A reduced level of Hgb (10.64 ± 1.9 gm/dl) with a range (7.2-14.2) was observed in our CLL patients in (group I) compared to the control group (12.06 ± 2.12gm/dl) with a range (9.8-15.9). These results are in agreement with earlier studies of Junglee et al. (2012) who reported that in CLL patients Hgb values vary depending upon disease condition (Junglee et al., 2012).

There was lymphocytosis in our studied patients compared to the control group is in agreement with Williams et al., (2008). Patients with CLL had higher number of lymphocytes than the controls; this result was in agreement with the result found by Perry et al., (2012) who reported that the fraction of peripheral blood lymphocytes was significant elevated, when compared to the healthy controls (Perry et al., 2012).

Platelet count was low in CLL patients (164.43 ± 59.54×10^9/l) with a range (25-319) compared to the control group (234.02 ± 80.85 10^9/l) with a range (157-427), it was coincide with the previous study reported that platelets vary from normal platelet count in some patients and low platelet count in others according to stages of disease (Hallek et al., 2015). Also this result was in agreement with Tsang & Parikh, (2017) who reported that CLL patients were frequently associated with autoimmune complications such as auto-immune hemolytic anemia and immune thrombocytopenia which may cause of decrease haemoglobin and platelet count (Tsang & Parikh, 2017).

Role of flow cytometry (FC) in the classification of mature B-cell lymphoid neoplasms can dividing the FC findings in four different groups based on the expression or lack of CD5 and CD10 in combination CD5+CD10- (CLL/SLL, MCL, PLL and DLBCL (rare)), CD5-CD10+ (FL, BL and DLBCL), CD5-CD10- (MZL, HCL) and CD5+CD10+ (CLL/SLL in trans-formation to DLBCL, FL, MCL) (Wang and Youli., 2017).

In this study, we using a multiparameter flow cytometry approach to do immune-phenotype analysis for studied groups as in CLL group 37 Patients was positive CD5/CD19, CD200. Dim positive CD20, Negative CD 10 and FMC7. The immune-phenotype of CLL cells have been integrated into a scoring system that helps in differential diagnosis of CLL from other B-cell leukaemias (Hallek et al., 2018).

Conclusion:

Immunophenotyping has a great role in CLL diagnosis and differentiating it from other Lymphoproliferative disorders.

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