Study of CD184 (CXCR4) Expression in Chronic Lymphocytic Leukemia Patients

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ABSTRACT

Background: Chronic lymphocytic leukemia ,most common leukemia in western countries ,is characterized by accumulation of mature CD19,CD5,CD23 positive B cells. Indeed, microenvironments in the bone marrow ,lymph node and other secondary lymphoid organs have been shown to inhibit spontaneous CLL cell apoptosis and enhance chemoresistance. **Aim of the work:** To study the clinical utility of CD184 in CLL patients,so that it can be used as routine investigation to predict the outcome of the disease and to help in choosing treatment regimen, **Patients and methods:** 33 newly diagnosed CLL patients at Nasser institute and Ainshams university hospitals, have undergone CBC , LFt, KFT; Virology, LDH,B2microglobulin, bone marrow aspiration and biobsy, immunophenotyping , cytogenetics, radiological investigation, And CD184 by flowcytometry which has been correlated with these diagnostic and prognostic parameters. **Results:** Positive correlation was found between CD184 and TLC, Lymphocytic count ,LDH,B2microglobulin,RAI stage and revised RAI score, while negative correlation was found between CD184 and both HB and platelet count. **Conclusion**: High CD184 expression associated with poor prognosis of CLL patients,so that it can be used as routine investigation to predict outcome of disease

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INTRODUCTION

With an age-adjusted incidence of 4.3/100 000 inhabitants in the United States, chronic lymphocytic leukemia (CLL) is the most common type of leukemia in Western countries. More than 15 000 newly diagnosed cases and; 4500 deaths are currently estimated ***per year in the United States (National Cancer Institute, 2013).***

The median age at diagnosis lies between 67 and 72 years. More male than female patients are affected by this disease ***(National Cancer Institute, 2013).***

CLL is characterized by the clonal proliferation and accumulation of mature, typically CD5-positive B cells within the blood, BM, lymph nodes, and spleen. The leukemic transformation is in particular deletions on the long arm of chromosome 13 [del(13q14)] .Some of these aberrations cause the deletion of specific micro-RNA genes and increase the resistance of B cells toward apoptosis ***(Calin et al., 2002).*** Additional aberrations of the long arm of chromosome 11 [del(11q)], of the short arm of chromosome 17 [del(17p)], and trisomy 12 seem to occur later in the course of the disease and predict a worse outcome ***(Puente et al., 2011; Quesada et al., 2011; Conde et al., 2012 Rossi et al., 2013)*** survival of CLL cells strictly depends on a permissive

microenvironment composed of cellular components such as macrophages, T cells, or stromal follicular dendritic cells ***(Burger et al., 2009)*** which provide essential proteins (chemokines, cytokines, and angiogenic factors) for activation of crucial survival and proliferative signaling pathways of transformed cells ***(Stevenson et al., 2011; Wiestner et al., 2012).***

Chronic lymphocytic leukemia (CLL), the most common leukemia in Western countries, is characterized by the accumulation of mature CD19, CD5, CD23 positive (CD19+CD5+CD23+) B cells which present a weak proliferation index compared with normal B cells and a defect in apoptosis ***(Dighiero et al., 1991).*** However, these cells rapidly undergo spontaneous apoptosis when they are cultured in vitro, ***(Collins et al., 1989)*** suggesting that in vivo factors contribute to their prolonged survival, and reinforcing the importance of the microenvironment in this context ***(Lagneaux et al., 1999; Stamatopoulos et al, 2003; Caligaris et al., 2009).***

Indeed, microenvironments in the bone marrow, lymph nodes and other secondary lymphoid organs have been shown to inhibit spontaneous CLL cell apoptosis and enhance chemoresistance. These prosurvival effects are largely dependent on microenvironment/CLL cell contact but also on chemokines released in the milieu.

Aim of the Study

To study the clinical utility of CD184 in CLL patients, so that it can be used as routine investigation to predict the outcome of the disease and to help in choosing the treatment regimen. Expression of CD184 will be correlated with other known prognostic parameters as age, LDH, cytogenetics, β2 microglobulin, CBC, Lymphocytic count, Rai stage and, revised Rai score.

PATIENTS AND METHODS

- The study is a prospective study conducted on 33 patient attending at Hematology Unit at Nasser Institute and Ain Shams University Hospitals

Inclusion criteria:

- Patients with newly diagnosed CLL.

Exclusion Criteria

- Patients previously diagnosed as CLL and received treatment before.
- Patients with other solid tumors.

All patients were subjected to:

- Full history taking.
- Thorough physical examination.
- Laboratory investigations including:
  - Complete blood count with differential leucocytic count.
  - Kidney and Liver functions.
  - HBsAg, HCV Ab and HIV Ab.
  - Bone marrow aspiration.
  - Bone marrow biopsy.
  - Immunophenotyping on peripheral blood or bone marrow aspirate.
  - Cytogenetics (Karyotyping and FISH on peripheral blood or bone marrow aspirate for del (17p) and del (11q).
  - Serum LDH.
  - Serum B2 microglobulin.
- Detection of CD184 (CXCR4) expression by flowcytometry
- Radiological investigations
  - CT Neck, Chest, Abdomen and Pelvis.

The level of CD184 expression has been correlated with the known CLL diagnostic and prognostic parameters

and with the course of the disease and the outcome after 6 months

- An approved written consent was taken from all the patients participating in the study.

Methods:

Sampling:

Blood was collected from each patient by withdrawing venous blood by a single puncture technique of the antecubital vein. Samples were dispensed gently into edeta containing tube to perform CD184 by flowcytometry.

Statistical Analysis

Data were collected, coded, revised and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The data were presented as number and percentages for the qualitative data, mean, standard deviations and ranges for the quantitative data with parametric distribution and median with inter quartile range (IQR) for the quantitative data with non parametric distribution

Independent t-test was used in the comparison between two groups with quantitative data and parametric distribution and ***Mann-Whitney test*** was used in the comparison between two groups with quantitative data and non parametric distribution

The comparison between more than two groups with quantitative data and parametric distribution were done by using ***One Way Analysis of Variance (ANOVA) test*** and ***Kruskall-Wallis test*** was used in the comparison between more than two groups with quantitative data and non parametric distribution.

Spearman correlation coefficients were used to assess the significant relation between two quantitative parameters in the same group.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

P > 0.05: Non significant (NS)

P < 0.05: Significant (S)

P < 0.01: Highly significant (HS)

Therapeutic protocols

Twelve patients didn't receive treatment, just for watchful waiting.

Thirteen patients received FCR protocol (fludarapine - cyclophosphamide - rituximab) and the remaining 8 patient received (chlorambucil – rituximab) protocol.

RESULTS

The study is a prospective study conducted on 33 patient attending to the Hematology Unit at Nasser Institute and Ain Shams University Hospitals to study the clinical utility of using CD184 to predict the outcome in CLL patients.

Table (1): Distribution of sex and age in studied population:

		No.	%
Sex	Male	21	63.6%
	Female	12	36.4%
Age	Mean ± SD	53.79 ± 8.05	
	Range	42 - 80	

Sociodemographic characteristics:

Table 1 shows population's sociodemographic characteristics. The mean of age was 53.79 years with a range from 42 to 80. The most of participants were males (63.6%) and females were only 36.4%.

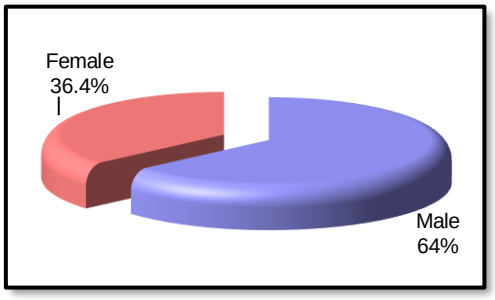

Table (2): Medical history and general examination distribution of included subjects:

		No.	%
HTN	Negative	32	97.0%
	Positive	1	3.0%
DM	Negative	28	84.8%
	Positive	5	15.2%
General examination	NO OG or LN	11	33.3%
	LN	5	15.2%
	HSM	6	18.2%
	HSM LN	11	33.3%
B SYMPTOMS	Negative	14	42.4%
	Positive	19	57.6%

- **Medical history and general examination of included subjects:**

Table 2 showed the medical history and the general examination of the included subjects. According to the medical history of these B-CLL patient. (15%) of patients had a history of diabetes mellitus and (3%) of patients had hypertension.

The general examination of the patients revealed that 33.3% of patients had no organomegaly or lymphadenopathy. On the other hand 33.3% of them had hepatosplenomegaly and lymphadenopathy, 18.2% had only hepatosplenomegaly and 15.2% had only lymphadenopathy. Also, the B symptoms in the studied patients showed that (57.6%) of them had signs of fever, night sweating and weight loss and (42.2%) had no B symptoms.

Table (3): Distribution of CBC

CBC	Min	Max	Mean	SD
Hb	6	15.5	10.65	3.04
Platelet) (*1000/mm^3)	16	329	(177,4)	82.09
TLC) (*1000/mm^3)	16,5	219	(58,132)	49,522
Lymphocyte count(*1000/mm^3)	8.25	154.84	61.18	48.66

Investigations of included subjects:

1. CBC results:

The mean hemoglobin level was 10.65 gm/dL that ranged from severe anemia 6 gm/dL to normal levels of 15.5 gm/dL. The platelets were (16×10^3-329×10^3/mm^3) with a mean of 177×10^3/mm^3. The mean total leuckocytic count was higher than normal levels (58×10^3/mm^3). The mean lymphocytes count was (61×10^3/mm^3).

Table (4): Distribution of LFT, KFt, VIROLGY

		No.	%
LFT	Normal	33	100.0%
	Abnormal	0	0.0%
KFT	Normal	33	100.0%
	Abnormal	0	0.0%
		No.	**%**
VIROLGY	+v HCV Ab	4	12.1%
	-v HCV Ab	29	87.9%
	-v HBsAg	33	100.0%
	-v HIV Ab	33	100.0%

2. Liver, kidney and virology investigations:

The included subjects had normal levels of kidney and liver function tests. Also, all the included subjects were serologically negative for HBV and HIV however 12.1% of them were positive for HCV.

Table (6): Serum LDH, B2 microglobulin and CD184 flow cytometry.

	Min	Max	Mean	SD
B2 microglobulin	1	4.1	2.46	1.15
			No.	**%**
B2Microglobulin	Less than 3.5 mg/l		18	54.5%
	More than 3.5 mg/l		15	45.5%
	Min	**Max**	**Mean**	**SD**
LDH	346	1890	1043.76	547.60
		No.		**%**
LDH	Less than 480 IU/L		7	22%
	More than 480 IU/L		26	78%
	Min	**Max**	**Mean**	**SD**
CD184	10.1%	93%	58.57	25.95

Table 6 indicated the serum levels of B2 microglobulin, LDH as well as the flow cytometer analysis of CD184. The mean of B2 microglobulin was 2.46mg/l with a range from 1 to 4.1 mg/L. (54.5%) of patients had a level of B2 microglobulin less than 3.5mg/l and 45.5% of them more than 3.5mg/dl

The mean LDH level was 1043.76 IU/L with 22% of patients having levels less than 480 IU/L and the rest of patients (78%) had levels more than 480 IU/L.

The flow cytometer analysis of CD184 revealed that the mean levels were 58.57 with a range of 10.1-93%.

CD184 cutoff value:

- +ve patients >20%
- -ve patients < 20%

One patient was negative with CD184 < 20%, while the remaining 32 patients were +ve with CD184 > 20%.

Table (7): Scoring and radiological examination of included patients:

		No.	%
Rai Stage	Stage 0	7	22%
	Stage 1	3	9%
	Stage 2	6	18%
	Stage 3	7	21%
	Stage 4	10	30%
Revised Rai Score	Low	7	22%
	Intermediate	9	27%
	high	17	51%
PAN CT	NO OG or LN	11	33.3%
	LN	5	15.2%
	HSM	6	18.2%
	HSM LN	11	33.3%

- **Scoring and radiological examination:**

Table 7 show that 22% patients at stage 0, 9% patients at stage 1, 18% at stage 2, 21%at stage 3 and 30% patients were at stage 4.

2. Revised rai score indicated that 22% of patients have low score, 27% 0f patients have intermediate score and 51% of patients had high revised rai score.

The computed tomography showed that 33.3% of patients had no organomegaly or lymphadenopathy. On the other hand 33.3% of them had hepatosplenomegaly and lymphadenopathy, 18.2% had only hepatosplenomegaly and 15.2% had only lymphadenopathy.

Table (8): Distribution of Cytogenetic, CD38, ZAP70 and Coombs'.

		No.	%
Cytogenetic	Normal	12	80.0%
	Abnormal	3	20.0%
CD38	Negative	5	62.5%
	Positive	3	37.5%
ZAP70	Negative	5	71.4%
	Positive	2	28.6%
Coombs' test	Negative	12	70.6%
	Positive	5	29.4%

Table (8) showed the distribution of cytogenetic, and other diagnostic parameters. As for the cytogenetic studies, at diagnosis 80% out of (15) patients had normal cytogenetic parameters (CLL pannel by FISH was normal) while only 20% out of (15) patients had abnormal cytogenetic in the form of ($11q^{-}$+12 $13q^{-}$ $17p^{-}$) by FISH technique. The levels of CD38 were positive in (3) out of (8) studied patients 37.5%.

ZAP70 was +ve in 2 out of7 studied patients (28%) and the remaining 5 were negative (71%).

Regarding coombs, test, (5) out of (17) patients were positive (24%), the remaining 12 were negative (71%).

Correlation studies at diagnosis and after treatment:

Table (11): Relation between CD184 among all studied parameters: sex, HTN, DM

		CD184		Independent t-test	
		Mean	SD	t	P-value
Sex	Male	57.82	24.82	0.215	0.831
	Female	59.88	28.91		
HTN	Negative	59.68	25.55	1.414	0.167
	Positive	23.00	0.00		
DM	Negative	60.13	26.74	0.81	0.424
	Positive	49.86	21.16		

Table 11 indicated that there was no correlation between CD184 level with either, sex, hypertension or diabetes mellitus.

Table (12): Relation between CD184: and B Symptoms

		CD184		Independent t-test	
		Mean	SD	t	P-value
B Symptoms	Negative	37.71	21.26	5.467	<0.001
	Positive	73.94	16.82		

Table.12, there was a highly significant correlation between the B symptoms of all patients with the level of CD184 (Fig 1).

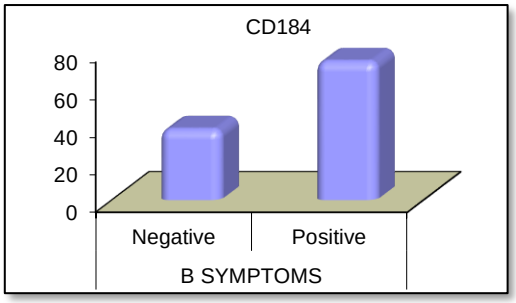

Fig. (1): CD184 in relation to B symptoms among studied patient

Table (13): Correlation between CD184 among all studied parameters

	CD184 initially		CD184 After 6 months	
	r	P-value	r	P-value
Age	0.071	0.695		
Hb	-0.478	0.005	-0.423	0.014
Platelet	-0.415	0.016	-0.645	<0.001
TLC	0.607	<0.001	0.898	<0.001
Lymphocyte count in CBC	0.345	0.049	0.667	<0.001
LDH	0.582	<0.001	0.833	<0.001
B2 mIcroglobulin	0.529	0.009	0.913	<0.001
Lymphocyte (%) in BMA	0.653	<0.001		
Rai stage	0.667	<0.001		
revised Rai score	0.579	<0.001		

P > 0.05: Non significant (NS)
P < 0.05: Significant (S)

As shown in table 13, there was no correlation between the patients age and the CD184 level in the included patients. As for the CBC results, the Hb and platelets level were inversely correlated with the levels of CD184 (Fig 2 & 3).

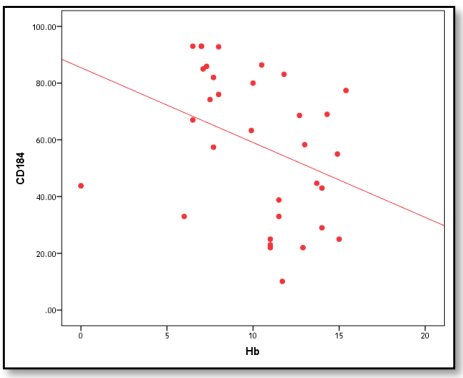

Fig. (2): Reverse relationship between Hb and CD184

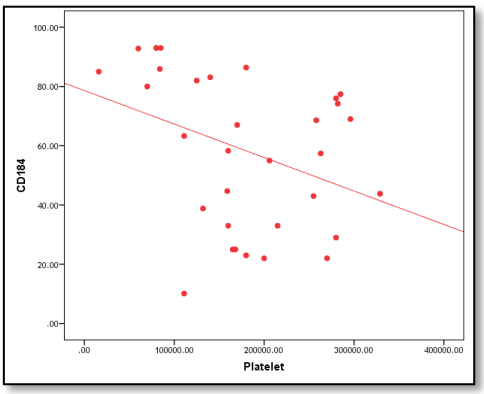

Fig. (3): Reverse relationship between platelet count and CD184

Also, the TLC and lymphocytes showed a positive significant correlation with the levels of CD184 (Fig. 4 & 5).

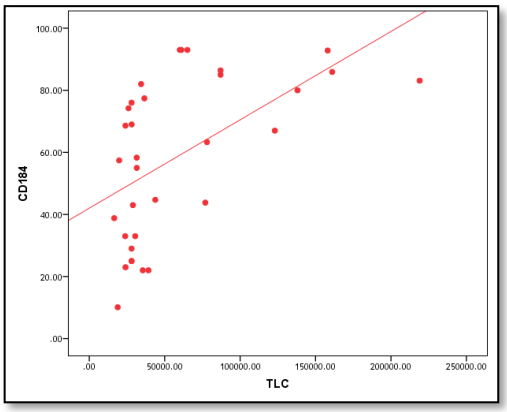

Fig. (4): Positive correlation between TLC and CD184

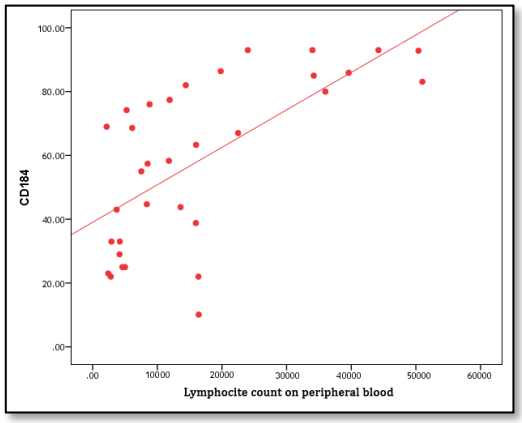

Fig. (5): Positive correlation between lymphocyte count on peripheral blood and CD 184

The serum levels of LDH and B2 microglobulin also showed a positive significant correlation with CD184 level (Fig. 6 & 7).

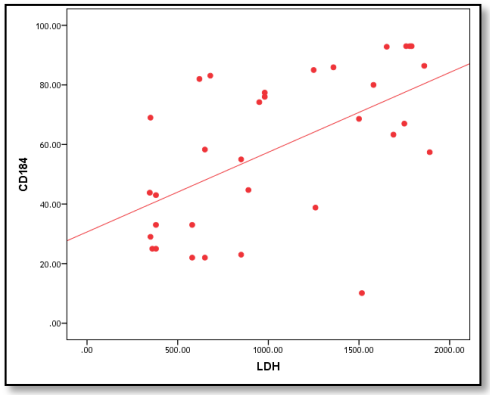

Fig. (6): Positive correlation between LDH and CD184

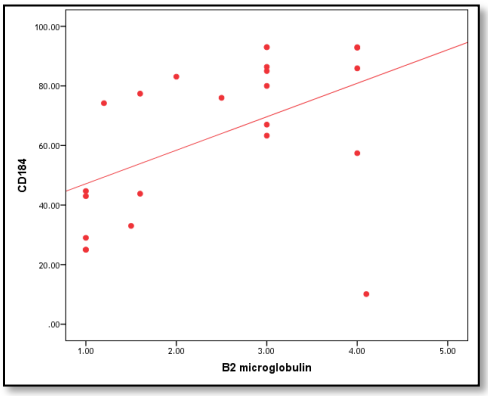

Fig. (7): Positive correlation between B2 microglobulin and CD184

The BMA lymphocytes were significantly correlated with CD184 (Fig. 8). The Rai stage and revised Rai score showed a positive significant correlation with CD184 level (Fig. 9 & 10).

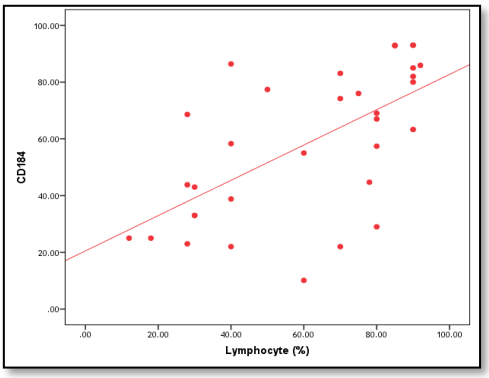

Fig. (8): Positive correlation between Lymphocyte percentage on bone marrow aspiration and CD184

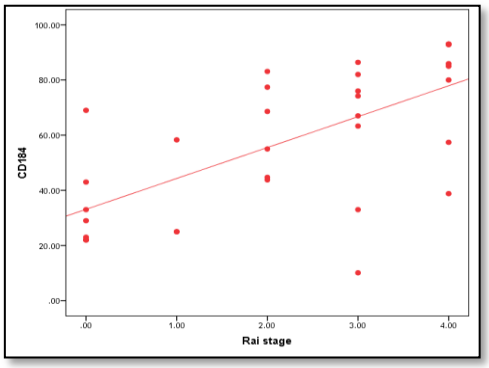

Fig. (9): Positive correlation between Rai stage and CD184

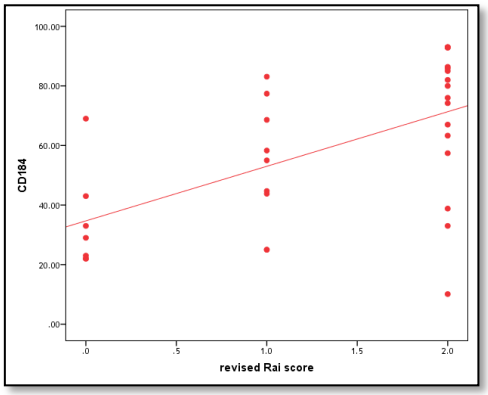

Fig. (10): Positive correlation between revised Rai score and CD184

As regards, the laboratory parameters after 6 months of the follow up period, the serum LDH and B2 microglobulin showed a positive significant correlation with CD184 level (Fig. 11 & 12).

After 6 months

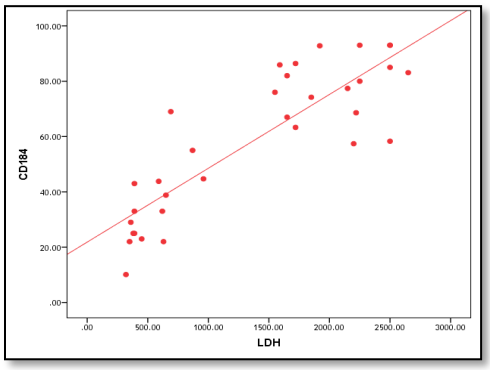

Fig. (11): After 6 month's positive correlation between LDH and CD184

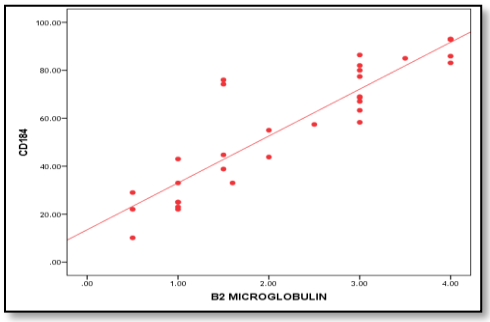

Fig. (12): After 6 months positive correlation between B2microglobulin and CD184

The Hb and platelet count had an inverse significant correlation with CD184 level (Fig. 13 & 14).

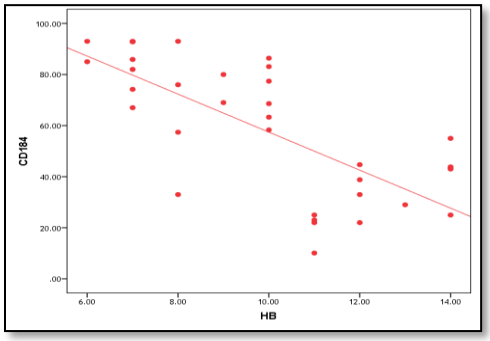

Fig. (13): After 6 months inverse relationship between Hb and CD184

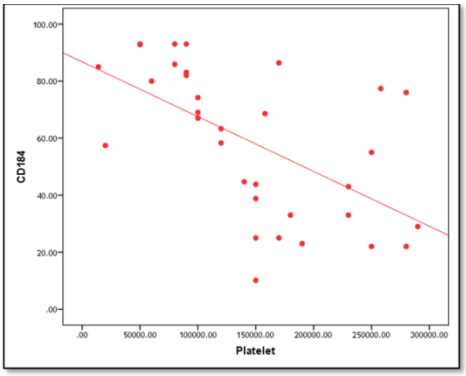

Fig. (14): After 6 months reverse relationship between platelet count and CD184

The total leukocytes count and lymphocytes count in peripheral blood also showed a highly significant correlation with the CD184 level (Fig. 15 & 16).

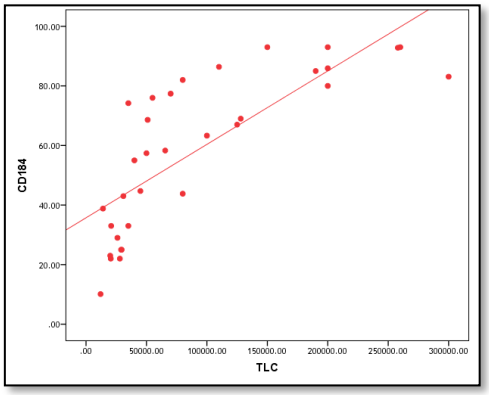

Fig. (15): After 6 months positive correlation between TLC and CD184

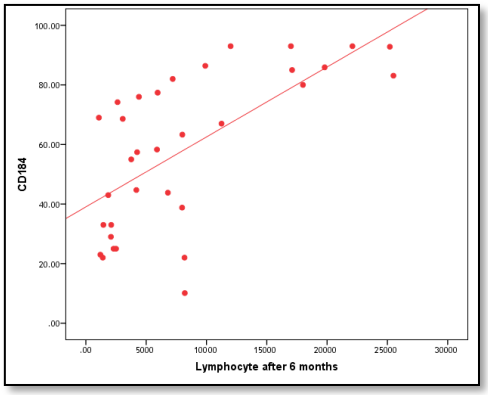

Fig. (16): After 6 months positive correlation between CD184 and lymphocyte count in peripheral blood.

DISCUSSION

Chronic lymphocytic leukemia (CLL) is the leading malignant β tumor in the Western World. The disease is characterized by its heterogenous pathological nature, indolent natural history and variable clinical outcomes ***(Blachly and Blachly, 2016; Parikh and Shanafelt, 2015).***

Mesenchymal stromal cells, nurse like cells (NLC) and lymphoma-associated macrophages (LAM), in concert with T cells, natural killer cells and extracellular matrix components participate in the dialog with the neoplastic B cells. B cell receptor signaling, activation via TNF family members (i.e. BAFF, APRIL), and tissue homing chemokine receptors and adhesion molecules are important in the interaction between malignant B cells and their microenvironment ***(Burger and Gribben, 2014).***

CXCR4 (CD184) is a chemokine and chemokine receptor pair playing critical roles in tumor genesis. Over expression of C-X-C chemokine receptor type 4 (CXCR4) is a hallmark of many hematological malignancies including acute myeloid leukemia, chronic lymphocytic leukemia and non-Hodgkin's lymphoma, and generally correlates with a poor prognosis ***(Li et al., 2016).***

So, the present study aimed to determine clinical utility of CD184 in CLL patients in predicting disease outcome and planning treatment regimen. The study recruited 33 patients with CLL. They were subjected to careful history taking, thorough clinical and laboratory investigations including serological assessment of CD184.

Patients included in the present study comprised 21 males (63.6%) and 12 females (36.4%) with a mean age of 53.79±8.05 years. Similarly, in the study of ***El-Ghammaz et al., (2015)*** males outnumbered females (38 vs 33) and the median age of the studied patients was 57 years and in the study of ***Abousamra et al., (2015)*** males constituted 66.2% of the studied CLL patients and the mean ± SD patients' age was 59.9± 6.78 years.

Documented patients' history in the current study revealed that just 1 patient (3.0%) had hypertension while 5 patients (15.2%) had diabetes mellitus. These data are in accordance with that found by ***(Thurmes et al., (2008)*** who reported that among 1195 individuals with newly-diagnosed CLL, 11.0% had diabetes. However, our data significantly contradicts that reported by ***Mozessohn et al., (2016)*** who noted that in their large series of 2124 CLL patients, 20.2% had diabetes and 35.8% had hypertension. This discrepancy; however, can be explained by the fact that their study populations is markedly aged in comparison to ours with a mean age of 75.6 years.

On examination, 5 patients (15.2%) of our series had lymphadenopathy, 6 patients (18.2%) had hepato-splenomegaly and 19 patients (57.6%) had positive B symptoms. These findings accord with that reported by ***Hassan et al., (2011)*** who diagnosed splenomegaly in 66.7% and hepatomegaly in 43.3% of 30 CLL patients. However, in the study of ***Kamel et al., (2016)***, among 78 patients with newly diagnosed CLL, 49.3% of patients had hepatomegaly, 64.2% had splenomegaly: and 83.6% had lymphadenopathy. This is simply explained by the heterogeneous patients characteristics among various studies according to disease stage and treatment response.

Regarding laboratory data, patients included in the current study had hemoglobin level of 10.65 ± 3.04 gm/dl, platelets count of 177.4±82.09 (*1000/mm^3), TLC of 58,132±49,522 (*1000/mm^3) and lymphocyte count of 61 ± 48,66. These data generally accord with that reported by the study of ***Payandeh et al., (2015)*** in their 109 CLL patients research.

In addition, we identified 4 patients (12.1%) with +ve HCV infection. The low

rate of HCV in CLL patients was also reported by the study of ***Gharagozloo et al., (2001)*** who identified HCV in only 4.0% of the studied CLL patients.

Other laboratory data showed elevated B2 microglobulin in 15 patients (45.5%) and elevated LDH in 26 patients (78%). Both markers are important prognostic factors for CLL as reported by ***(Labib et al., (2014).*** Prognostic Rai classification in the present study involved low risk disease in 7 patients (22.0%), intermediate risk disease in 9 patients (27.0%) and high risk disease in 17 patients (51.0%). This is in accordance with the study of ***Assem et al., (2009)*** who found that 48.0% of their study patients were classified as high risk patients while the remainder are of low and intermediate risk.

Further cytogenetic and immunological analysis of our study patients revealed abnormal cytogenetics in 20.0% out of(15) cases, positive CD38 in 37.5% out of(8) cases, positive ZAP-70 in 28.6% out of (7) cases and positive Coombs test in 29.4% out of(17) cases. In comparison, abnormal cytogenetics was detected in in 52.0% and CD38 was positive in 60.0% of patients included in the study of ***Saad et al., (2008)*** on 25 CLL patients while in the study of ***Abousamra et al., (2009)***, ZAP-70 was positive in 47.8% of patients.

In respect to response, patients included in the present study experienced progressive disease in 13 cases (39.3%), complete response in 2 cases (6.06%), partial response in 10 cases (30.03%) and no response in 8 cases (24.24%). Correspondingly, ***El-Kinawy et al., (2012)*** reported unfavorable outcome in most patients (55.0%) in their series evaluating the prognostic factors in 40 CLL patients.

Correlation analysis of CD184 with other clinical and laboratory values declared a statistically significant inverse correlation between CD184 levels and Hb concentration and platelets count and statistically significant correlation between CD184 and total leukocytic count, lymphocyte count, LDH, B2 microglobulin and Rai stage at the start of the study and after 6 months.

These data are in agreement with the study of ***Barretina et al., (2003)*** who studied CXCR4 expression in B-cell chronic lymphocytic leukemia and noted a significant correlation between CXCR4 and total leukocytic count and lymphocyte count.

While no studies reported the relation between CXCR4 and LDH in CLL patients, the study of ***Guo et al., (2013)*** found a statistically significant correlation between CXCR4 and LDH levels in patients with diffuse large cell lymphoma.

Moreover, the study of ***Ghobrial et al., (2004)*** noted significant increase in CXCR4 with increased Rai stage in their study investigating the clinical relevance of chemokine receptor expression on the progression of B-cell chronic lymphocytic leukemia (B-CLL) in 45 patients with B-CLL.

Perusing relation between CXCR4 expression and the clinical data in the present study including patients' gender, associated comorbidities and general examination data revealed significantly higher CXCR4 values in patients with positive B symptoms. The study of ***Kubeczko et al., (2016)*** noted that CLL patients with positive B symptoms had significantly higher levels of multiple chemokines including IFNγ, CCL3 and CCL4 but not as in our study.

SUMMARY

Chronic lymphocytic leukemia (CLL) is a prevalent malignant tumor characterized by CLL cells homing in the bone marrow microenvironment.

CXCR4 (CD184) is a chemokine and chemokine receptor pair playing critical roles in tumor genesis. It is over expressed in many hematological malignancies including acute myeloid leukemia, chronic

lymphocytic leukemia and non-Hodgkin's lymphoma, and generally correlates with a poor prognosis ***(Li et al., 2016).***

So, the present study aimed to determine clinical utility of CD184 in CLL patients in predicting disease outcome and planning treatment regimen. The study recruited 33 patients with CLL. They were subjected to careful history taking, thorough clinical and laboratory investigations including serological assessment of CD184.

Patients included in the present study comprised 21 males (63.6%) and 12 females (36.4%) with a mean age of 53.79±8.05 years. Documented patients' history in the current study revealed that just 1 patient (3.0%) had hypertension while 5 patients (15.2%) had diabetes mellitus

On examination, 5 patients (15.2%) of our series had lymphadenopathy, 6 patients (18.2%) had hepato-splenomegaly and 19 patients (57.6%) had positive B symptoms.

Regarding laboratory data, patients included in the current study had hemoglobin level of 10.65 ± 3.04 gm/dl, platelets count of 177.4 ± 82.09 (*1000/mm^3), TLC of 58,132 ± 49,522 (*1000/mm^3) and lymphocyte count of 61 ± 48.66.

In addition, we identified 4 patients (12.1%) with +ve HCV infection. Other laboratory data showed elevated B2 microglobulin in 15 patients (45.5%) and elevated LDH in 26 patients (78%).

Prognostic Rai classification in the present study involved low risk disease in 7 patients (22.0%), intermediate risk disease in 9 patients (27.0%) and high risk disease in 17 patients (51.0%).

Further cytogenetic and immunological analysis of our study patients revealed abnormal cytogenetics in 20.0% out of(15) cases, positive CD38 in 37.5% out of(5) cases, positive ZAP-70 in 28.6% out of(8) cases and positive Coombs test in 29.4% out of (17)cases.

In respect to treatment response, patients included in the present study experienced progressive disease in 13 cases (39.3%), complete response in 2 cases (6.06%), partial response in 10 cases (30.03%) and no response in 8 cases (24.24%).

Correlation analysis of CD184 with other clinical and laboratory values declared a statistically significant inverse correlation between CD184 levels and Hb concentration and platelets count and statistically significant correlation between CD184 and total leukocytic count, lymphocyte count, LDH, B2 microglobulin and Rai stage at the start of the study and after 6 months. Also threre was statistically significant inverse relationship between CD184 and response rate,6 months disease free survival.

Perusing relation between CXCR4 expression and the clinical data in the present study including patients' gender, associated comorbidities and general examination data revealed significantly higher CXCR4 values in patients with positive B symptoms.

CONCLUSION

CD184 expression in CLL patients showed statistically significant inverse correlation with Hb concentration and platelets count.

In addition, it showed statistically significant positive correlation with total leukocytic count, lymphocyte count, LDH, B2 microglobulin and Rai stage at the start of the study and after 6 months

RECOMMENDATIONS

- A larger study is recommended to confirm results of the present study.
- CD184 can be used as routine investigation in CLL patient, to predict outcome of the disease and response to treatment.

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