Original Research Article

Study of Soluble CD52 as a Tumor Marker and Indicator of Disease Activity in Chronic Lymphocytic Leukemia

Abstract

Background: Chronic Lymphocytic Leukemia (CLL) is non-curable disease and has variable course among patients. In some patients it progresses fast despite sever treatment and is fatal within 2 to 3 years whereas others require no or minimal treatment and die due to cause other than CLL. The aim of this work was to investigate the circulating soluble CD52 in blood of CLL patients to explore its usefulness as diagnostic and prognostic biomarker.

Methods: This study included 40 cases who were divided into four equal groups; group 1 was patients who were newly diagnosed with CLL, group 2 was cases of chronic lymphocytic leukaemia with complete remission after treatment, group 3 was cases of relapsed chronic lymphocytic leukaemia and group 4 was the control group. All patients were subjected to clinical examinations ,laboratory investigations including: (complete blood counting, liver function tests, kidney function test, LDH Immunophenotyping) and specific laboratory investigation of soluble CD52 using ELISA.

Results: ROC curve for SCD52 to discriminate between newly diagnosed cases and control group with cut of point 201(ng / ml) and AUC 0.890 with p value 0.003 with sensitivity and specificity 80% and to discriminate between relapsed cases from complete remission cases with cutoff point 345 (ng / ml) and AUC 0.840 with (p value 0.010) with sensitivity 80% and specificity 90%. In group 1and group 3, there were positive correlation between SCD52 and staging system, absolute lymphocytic count and LDH, significant negative correlation between SCD52 and platelet count.

Conclusions: The role ofsCD52 as diagnostic and prognostic marker for CLL due to its relation to CLL disease activity.

Keywords: CD52, Tumor marker, Chronic Lymphocytic

Introduction:

Chronic lymphocytic leukemia (CLL) is one of most malignancy in our world with average prevalence of 3.9 new patients per 100,000 population per year, male to female ratio is approximately 2:1 with median age at diagnosis 60 years old, [1]. It is associated with infection due to hypogammaglobulinemia, autoimmune phenomena and secondary malignancies [2].

B-CLL originates in bone marrow and affect B lymphocytes which develop in lymph node producing antibodies fighting infection, B cells CLL grow abnormally and accumulate in bone marrow and blood, where they crowd out healthy blood cells [3].

CLL is non-curable disease and has variable course among patients. In some patients it progress fastly despite sever treatment and is fatal within 2 to 3 years whereas others require no or minimal treatment and die due to cause other than CLL [4].

Therefore assessment of prognosis is crucial step in management of CLL patients. There are many parameters that are used in clinical practice as prognostic indicators. The most viable one is IGVH mutational status, however it is very expensive and not available in all labs [5, 6].

CD52 is glycosyl-phosphatidyl-inositol loosely anchored glycoprotein of 29 KDa [7]. It is present approximately in all normal blood cells and leukemic cells but higher level on lymphocytes and malignant B cells [8], and also it is present on surface of neoplastic lymphocytes in patients with CLL, low grade Lymphoma and most T – cell malignancy [9].It also expressed in some patients with myeloid, monocytic and acute lymphoblastic leukaemia [10].

Soluble CD52 can shed from CLL cells in vitro and in vivo (chemoimmunotherapy) [11]**.** However the correlation between soluble CD52, the disease activity and response to therapy is not thoroughly investigated [12].

The aim of this work was to investigate the circulating soluble CD52 in blood of CLL patients to explore its usefulness as diagnostic and prognostic biomarker.

Patients and Methods:

This study included 30 CLL patients who agreed to participate, newly diagnosed CLL untreated cases and treated CLL patients with complete remission and relapsed patients and 10 healthy controls. Written informed consent was obtained from all patients. The study was done after approval from the Ethical Committee of Tanta Cancer Institute, Tanta, Egypt.

CLL patients who did not agree to participate, blood diseases other than CLL as acute leukemia, CML and others, patients with liver or kidney disease and patients with connective tissue diseases like SLE and RA were excluded.

Patients were divided into three equal groups: group 1 who newly diagnosed cases of CLL, group 2 who cases of chronic lymphocytic leukaemia with complete remission after treatment and group 3 who cases of relapsed chronic lymphocytic leukaemia.

Group 4 included 10 cases of healthy subjects as control group.

All patients were subjected to: Full history taking, complete clinical examination, laboratory investigations including: (complete blood counting, liver function tests, kidney function test, LDH Immunophenotyping) and specific laboratory investigation of soluble CD52 using ELISA.

Blood sample: About 6.0 ml random venous blood sample under sterile conditions was taken from each subject in this study. About 2.0 ml blood was collected on EDTA vacutainer tube for CBC, 3.0 ml was collected and allowed to clot for 10-20 minutes at room temperature and was centrifuged at 2000-3000 rpm for 20 minutes to separate serum, which was stored at -

20°C till use to measure sCD52 in serum by ELIZA and rest of blood sample was collected on plain vacutainer tube used for liver and kidney function tests. Bone marrow samples from CLL patients were collected on EDTA vacutainer tube for immunophenotyping.

Estimation of serum CD52 by ELISA: All reagents, standard solutions and samples were prepared as instructed. The assay was performed at room temperature. The strips were inserted in the frames for use to assay the marker. 50µl standard solution was added to standard well in duplicate. 40µl of each sample was added to sample wells, 10µl anti-CD52 antibody was added to sample wells, and then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well), wells were mixed, the plate was covered with a sealer, incubated 60 minutes at 37°C. The sealer was removed, and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash, overfilled wells with wash buffer. The plate was blotted into paper towels. 50µl substrate solution A was added to each well and then50µl substrate solution B was added to each well. Incubated plate was covered with a new sealer for 10 minutes at 37°C in the dark. 50µl stop solution was added to each well, the blue color was changed into yellow immediately. The optical density (OD value) was determined of each well using a microplate reader set to 450 nm within 30 min after stop solution.

Calculation: A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and best-fit curve through the points on the graph. These calculations performed with computer-based curve-fitting software and the best-fit line determined by regression analysis.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using

range, mean, standard deviation and median. Chi-square test for categorical variables, to compare between different groups, Monte Carlo correction for chi-square when more than 20% of the cells have expected count less than 5, F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups and Post Hoc test for pairwise comparisons and Kruskal Wallis test for abnormally distributed quantitative variables, to compare between more than two studied groups and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. In all tests P value was considered significant if <0.05.

Results:

There was no significant difference between studied groups as regard age and sex. There was significant difference in WBCs , HB and PLT of CLL patients compared with control group ($p < 0.001$, $P=0.023$, $P<0.001$) respectively, There was significant difference in WBCs of group 1 and group 3 compared with control group ($p1= 0.001$, $p1 < 0.001$) respectively and significant difference between group 1 and group 3 compared to group 2 ($p2= 0.003$, $p4 =0.001$) respectively. There was significant difference in HB of group 3separately as compared with control group ($p1 = 0.049$). There was significant difference of PLT count in group1and group 3 compared with control group ($p1 = 0.001$). There was significant difference of PLT count between group 1 and group 3 compared to group 2 ($p2 = 0.040$, $p4 =0.017$). **Error! Not a valid bookmark self-reference.**

Table 1: Comparison between the different studied groups according to demographics data and CBC

	Group 1 (n = 10)		**Group 2 (n = 10)**		**Group 3 (n = 10)**		**Group 4 (n = 10)**		**Test of Sig.**	**p**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Sex										
Male	7	70.0	6	60.0	4	40.0	7	70.0	χ^2= 2.436	^{MC}p= 0.620
Female	3	30.0	4	40.0	6	60.0	3	30.0		
Age (years)										

Min. – Max.	45.0 – 81.0	47.0 – 78.0	54.0 – 77.0	39.0 – 69.0	F= 2.270	0.097
Mean ± SD.	61.0 ± 10.09	62.10 ± 10.52	62.40 ± 6.87	52.80 ± 10.30		
Median	61.50	63.0	63.0	50.0		
CBC						
WBCS(×10^3/µl)					H= 23.513*	<0.001*
Min. – Max.	10.0 – 201.0	2.30 – 16.0	10.0 – 433.0	3.20 – 11.40		
Mean ± SD.	39.61 ± 59.02	7.94 ± 4.32	150.55 ± 193.91	7.21 ± 2.74		
Median	16.15	7.0	14.65	6.80		
p_1	0.001*	0.702	<0.001*			
Sig. bet. grps	p_2=0.003*, p_3=0.674, p_4=0.001*					
HB level(g/dl)					F=3.589*	0.023*
Min. – Max.	7.50 – 13.10	11.0 – 13.70	7.50 – 13.0	11.60 – 13.40		
Mean ± SD.	10.93 ± 1.95	12.14 ± 0.81	10.57 ± 1.92	12.32 ± 0.56		
Median	11.10	12.0	10.25	12.50		
p_1	0.160	0.992	0.049*			
Sig. bet. grps	p_2=0.262, p_3=0.945, p_4=0.992					
PLT (10^3/ul)					H= 17.785*	<0.001*
Min. – Max.	46.0 – 460.0	118.0 – 450.0	18.0 – 211.0	190.0 – 460.0		
Mean ± SD.	145.20 ± 119.47	250.3 ± 129.9	112.0 ± 60.0	316.8 ± 98.15		
Median	106.0	207.5	125.0	350.0		
p_1	0.001*	0.217	<0.001*			
Sig. bet. grps	p_2=0.040*, p_3=0.738, p_4=0.017*					

p: p value for comparing between CLL patients and control group, p1: p value for comparing between group 4 and each other groups, p2: p value for comparing between group 1 and group 2, p3: p value for comparing between group 1 and group 3, p4: p value for comparing between group 2and group 3, *: Statistically significant at $p \leq 0.05$

There was significant difference between absolute lymphocytic counts in CLL patients compared to control group (p value< 0.001). There was significant difference in absolute lymphocytic count and LDH in group 1 and group 3 compared with control group (p1 =

0.018, p1 <0.001, p1<0.001) respectively. There was significant difference in absolute lymphocytic count and LDH between group 1 and group 3 compared to group 2 (p2= 0.018, p4= 0.001, p2 <0.001, p4 <0.001) respectively. Table 2

Table 2: Comparison between the different studied groups according to splenomegaly, LN enlargement, absolute Lymphocytic count and LDH

	Group 1 (n = 10)		**Group 2 (n = 10)**		**Group 3 (n = 10)**		**Group 4 (n = 10)**		**Test of sig.**	**p**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Spleen										
Not enlarge	4	40.0	10	100.0	5	50.0	10	100.0	χ^2= 15.172*	^{MC}p = 0.001*
Enlarge	6	60.0	0	0.0	5	50.0	0	0.0		
LN										
Not enlarge	5	50.0	10	100.0	4	40.0	10	100.0	χ^2= 15.172*	^{MC}p = 0.001*
Enlarge	5	50.0	0	0.0	6	60.0	0	0.0		
Absolute Lymph($\times 10^3/\mu l$)										
Min. – Max.	8.70 – 170.85		0.60 – 4.19		5.50 – 362.79		0.48 – 3.99		H= 20.778*	<0.001*
Mean ± SD.	35.91 ± 53.34		2.26 ± 1.36		109.0 ± 138.52		1.93 ± 1.26			
Median	10.59		1.93		12.42		1.73			
p_1	0.018*		1.000		<0.001*					
Sig. bet. grps	p_2=0.018*, p_3=0.157, p_4<0.001*									
LDH (U/L)	**Group 1 (n = 10)**		**Group 2 (n = 10)**		**Group 3 (n = 10)**		**Group 4 (n = 10)**		**F**	**p**
Min. – Max.	322.0 – 771.0		216.0 – 379.0		450.0 – 876.0		201.0 –372.0		35.271*	<0.001*
Mean ± SD.	556.6 ± 154.6		288.9 ± 48.51		674.1 ± 113.6		288.5 ±61.37			
Median	555.5		299.0		676.5		307.0			
p_1	<0.001*		1.000		<0.001*					
Sig. bet. grps	p_2<0.001*, p_3=0.071, p_4<0.001*									

p: p value for comparing between CLL patients and control group, p1: p value for comparing between group 4 and each other groups, p2: p value for comparing between group 1 and group 2, p3: p value for comparing between group 1 and group 3, p4: p value for comparing between group 2and group 3, *: Statistically significant at $p \leq 0.05$, LDH: lactate dehydrogenase, LN: lymph node

There was no significant difference between two studied groups as regard staging criteria. Table 3

Table 3: Comparison between the two studied groups according to staging criteria

Staging	Group 1 (n = 10)		Group 3 (n = 10)		χ^2	^{MC}p
	No.	%	No.	%		
I	2	20.0	2	20.0	1.288	0.866
II	3	30.0	2	20.0		
III	3	30.0	2	20.0		
IV	2	20.0	4	40.0		

There was significant difference in SCD52 of CLL patients compared to control group($p < 0.001$). There was significant difference in sCD52 of group 1 and group 3 compared with control group ($p1=0.002$, $p1<0.001$) respectively. There was significant difference in sCD52 between group 1 and group 3 compared to group 2 ($p2 =0.046$, $p4 =0.001$) respectively. Table 4

Table 4: Comparison between the different studied groups according to SCD52

SCD52 (ng/ml)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 4 (n = 10)	F	p
Min. – Max.	140.0 – 886.0	177.0 – 356.0	132.0 – 932.0	88.0 – 312.0	12.465*	<0.001*
Mean ± SD.	527.90 ± 296.84	274.0 ± 60.77	669.0 ± 273.1	160.1 ± 82.4		
Median	561.0	282.5	759.5	121.0		
p_1	0.002*	0.616	<0.001*			
Sig. bet. grps	$p_2=0.046^*, p_3=0.439, p_4=0.001^*$					

p: p value for comparing between CLL patients and control group, p1: p value for comparing between group 4 and each other groups, p2: p value for comparing between group 1 and group 2, p3: p value for comparing between group 1 and group 3, p4: p value for comparing between group 2and group 3, *: Statistically significant at $p \leq 0.05$

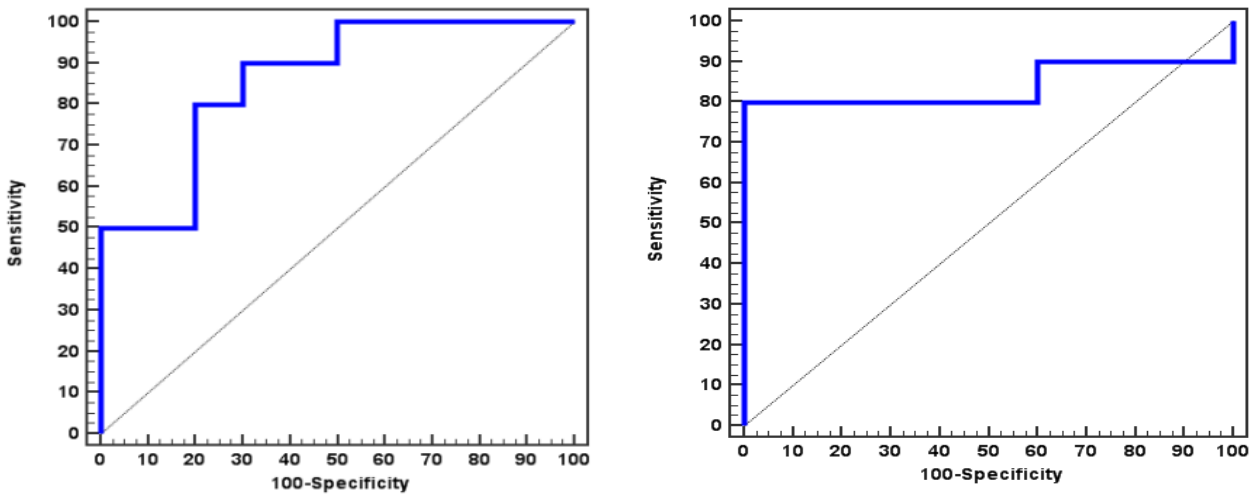
In group 1, there were positive correlation between SCD52 and staging system, absolute lymphocytic count and LDH, significant negative correlation between SCD52 and platelet count, while no correlation was recorded with age, HBand WBCS. In group 2, there was no significant correlation observed. In group 3, significant positive correlation between SCD52 and staging system, WBCS, absolute lymphocytic count and LDH and negative correlation with PLT count. Table 5

Table 5: Correlation between SCD52 and different parameters in each group

	SCD52					
	Group 1		Group 2		Group 3	
	r	p	r	p	r	p
Age	0.094	0.797	0.421	0.226	0.298	0.402
Staging	0.917	<0.001*	-	-	0.946	<0.001*
WBCS(×10³/µl)	0.490	**0.151**	0.212	0.556	0.671	0.034*
Hb level(g/dl)	-0.581	0.078	-0.074	0.672	-0.619	0.056
PLT (10^3/ul)	-0.696	0.025*	-0.084	0.818	-0.851	0.002*
Absolute Lymph(×10³/µl)	0.646	0.044*	0.243	0.499	0.664	0.036*
LDH(U/L)	0.816	0.004*	0.576	0.081	0.840	0.002*

*: Statistically significant at $p \leq 0.05$, LDH: lactate dehydrogenase, Hb: hemoglobin, WBCS: white blood cells PLT: platelets

ROC curve for SCD52 to discriminate between newly diagnosed cases and control group with cut of point 201(ng / ml) and AUC 0.890 with p value 0.003 with sensitivity and specificity 80% and to discriminate between relapsed cases from complete remission cases with cutoff point 345 (ng / ml) and AUC 0.840 with (p value 0.010) with sensitivity 80% and specificity 90%. Figure 1



(A) **(B)**

Figure 1: A) ROC curve for SCD52 to diagnose newly Diagnosed Cases from control B) to diagnose relapsed cases from complete remission cases

Discussion

The CD52 antigen is a glycoprotein with a very small mature protein sequence, comprised of only 12 amino acids, but have a large carbohydrate domain [13].

CD52 is found on the surface of T and B lymphocytes, monocytes/macrophages, eosinophils and on some early hematopoietic cells. It is also present in the male reproductive system [13].The increase in sCD52 levels most likely results from active shedding of the molecule from cell surfaces in a manner similar to that reported in the male reproductive system. Adding phorbol ester as a shedding agent to cultured CLL cells significantly increased the levels of sCD52 in the supernatant fluid. It has been proposed that sCD52 is necessary for preventing the adhesion of sperm to each other and to other cells. Plasma CD52 may have a similar function, i.e. preventing cells from adhering to each other. Regardless of the mechanisms that lead to plasma sCD52, the current data indicate that sCD52 plasma levels reflect the clinical behavior of CLL, namely, that higher levels of sCD52 are associated with more aggressive disease [13].

Antibodies against CD52 are believed to start the killing of cells through antigen cross-linking [14]. Because of this cross-linkage, several cytokines are liberated, among them tumor necrosis factor-α, interferon-γ and interleukin-6 [7].

This study reported significant relation between CLL and decrease HB level especially in relapsed cases (group 3). This is agreed by Littlewood and mandeili [15] who concluded that anemia is the most prognostic factor. On the other hand no significant relation between CLL and anemia in newly diagnosed patients. Also kipps T. *et al.,* [16] who suggested that HB level may remain normal in most of cases but small percent are anemic. Most common cause of anemia may be coincident hematinic deficiency. Additionally, Maura *et al.,* [17] reported that 30% to 37% of patients develop autoimmune hemolytic anemia and this is usually associated with a warm-type antibody against the Rhesus system.

Our study found significant relation between CLL and decrease platelet count with more significance with relapsed cases as a feature of poor prognosis. This is agreed by Ghia *et al.,* [18].

Also this study found significant relation between high total leukocytic count and CLL disease especially high peripheral blood lymphocytosis. This also agreed by Dasgupta *et al.,* [19] as it is a hallmark of diagnosis with present elevation of total leukocytic count and presence of small lymphocyte in peripheral blood.

According to serum LDH level, this study found significant elevation in CLL patients as it is considered a marker of cell damage. Xu *et al.,* [20] recorded that its high level due to cell damage indicates sever disease and worse prognosis.

This study reported significant elevation in sCD52 in group 1 with cut off more than 201 ng /ml with specificity 70% and in group 3 significant elevations in sCD52 with cut off more than 345 ng /ml with specificity 90%. Albitar M. *et al.,* [11] and Giles FJ. *et al.,* [21] recorded that sCD52 is higher in diseased group than healthy and complete remission groups.

According to correlation of sCD52 with different parameter, our study found positive correlation with staging system, absolute lymphocytic count and LDH but negative correlation with PLT count in group 1. In group 2 no correlation found between sCD52 and different parameters. In group 3 found positive correlation with staging system, WBCS, absolute lymphocytic count and LDH but negative correlation with PLT count. Albitar M. et. al., [21] recorded that, with high staging there is high sCD52 that possibly reflect disease aggressiveness, progressive disease or disease burden, and have prognostic impact of sCD52 levels in both early and relapsed patients with CLL that effect on overall survival. There were significant differences in sCD52 level between patients at different Rai stages and Binet stages of disease.

Albitar M. *et al.,* [21] also found weak linear correlation between level of sCD52 and WBCs in mixed population of both early and high-risk group, but in our study positive correlation only with relapsed group and no correlation with early group as regard sCD52 and WBCs.

Conclusions:

The role ofsCD52 as diagnostic and prognostic marker for CLL due to its relation to CLL disease activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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