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Study of CD38 Gene Polymorphism in Sudanese Patients with CLL

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Abstract: Chronic lymphocytic leukemia is the most common form of adult leukemia worldwide, insidious and fatal. Recent findings showed the importance of CD38 signaling in the pathogenesis of CLL, CD38 gene single nucleotide polymorphism may affect CD38 expression and contribute to the increased risk of CLL carcinogenesis, now it is considered not only as a risk factor but also as a prognostic marker and targeted therapy to CLL. In this study we are interested to shed light on CD38 polymorphism in Sudanese patients with CLL (frequency, Its correlation with age, gender of patients and its impact on some hematological parameters). This work aimed to Study CD38 gene polymorphism in Sudanese patients with chronic lymphocytic leukemia. A total of 39 blood samples was collected from Sudanese patients who were diagnosed with CLL, CBC was counted for each patient and genomic DNA was extracted by the salting out method, then the extracted DNA was amplified by allele specific PCR., The Amplified products were run on agarose gel electrophoresis and then stained DNA fragments were visualized under ultraviolet gel documentation system. Result*s:*showed that the frequency of CD38 gene polymorphism (CT) allele, was 32(82%) of cases, against 7(18%) who have a wild type allele (CC). We reported that there is a significant association between CD38 genotype and age, but not gender of patients, P-values = 0.001, % CI= -0.588(0.395-0.876), P-value=0.451, OR=1.575 (0.295-8.414) respectively, also (CT) allele genotype appeared to have an influence on the elevation of some hematological parameters (but not Hb Conc(g/dl), regarding patents with (CT) allele, the mean of Hb conc(g/dl), TWBCS count(C/L), Lymphocytes and PLTS count(C/L)) were (9±2.68, 45.96±28.6, 37.95±23.09 and130.1±46.23) while in wild type allele (CC) were (10.91±1.88, 18.33±11.87, 13.29±10.58 and 88.27±39.90) respectively, P-values=(0.304, 0.017, 0.009and 0.033), Our results indicate that Frequency of (CT) genotypic allele was higher than wild type allele (CT) and it may cause significant elevation to some hematological parameters. Conclusion: In summary, CD38 genotype (CT) allele appeared also to have an influence in the development of CLL, thus genotyping is useful for those patients, Considered as predisposing factor or risk factor for CLL and may affect severity of disease, even helpful as prognostic factor for CLL. We recommended to do further study with larger sample size, study of CD38 as flow marker and its impact on prognosis. The effect of the CD38 genotype on the other hematological findings has to be studied further.

Key words: Chronic lymphocytic leukemia · Single nucleotide polymorphism and CD38 antigen

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults [1]. It is defined as a malignancy of mature clonal B cells derived from proliferation in pseudofollicles in the bone marrow and secondary lymphoid tissue, where they receive support from stromal cells as well as immune cells, such as follicular dendritic cells and mature T helper cells [2]. CLL is highly heterogeneous in terms of progression, response to treatment and survival. Thus, design of appropriate therapeutic strategy requires a good prediction of the course of the disease. Since Rai and Binet staging systems are not well suited to predict the clinical course at an early stage, molecular and cellular features are analyzed to determine whether a patient has a favorable or unfavorable prognosis. One of the most widely used marker in diagnosis and prognosis of chronic lymphocytic leukemia is CD38 [3].

CD38 (cluster of differentiation 38), also known as cyclic ADP ribose hydrolase [4], it is a glycoprotein encoded by the CD38 gene which is located on

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chromosome 4, found on the surface of many immune cells (white blood cells), including CD4+, CD8+, B lymphocytes and natural killer cells. CD38 also functions in cell adhesion, signal transduction and calcium signaling [5].

CD38 Polymorphism: The 4p15 locus, where the CD38 gene is situated, is one of the polymorphic gene loci suspected of involvement in the pathogenesis of CLL and the heterogeneous course of the disease. CD38 antigen seems to be not only a molecular marker for poor prognosis, but also a factor contributing to CLL development. Some investigators have identified the CD38 locus as playing a causative role of CLL among Polish and Ukrainian Caucasians [6]. There is an update on genomic aberrations detected by SNP array in CLL samples mostly related to disturbances in apoptosis, as well as immune system function and multidrug resistance, which have impact on disease development and progression [7]. in the current study we aimed to study CD38 gene polymorphism, frequency, Its correlation with age, gender of patients and its impact on some hematological parameters in Sudanese patients with chronic lymphocytic leukemia.

MATERIALS AND METHODS

This is a cross-sectional study with descriptive design of qualitative & quantitative Variables (Age, gender, CBC and CD38 gene polymorphism), it was performed at Khartoum state at Khartoum oncology (RICK) Center, during the period, from Jun to December 2018. The study was approved by research ethical committee of Al-Neelain University, our study included a total of 39 Sudanese patients known with CLL, diagnosed by bone marrow examination and flowcytometry, old and new cases and aged from 45 to 77 years, males and females. Other causes of lymphocytosis were excluded, Samples were collected by random selection method, informed consent and structured questionnaire were obtained from each participant before sample collection, Five ml of venous blood samples were collected from each patient by standard venipuncture procedure and transferred into two EDTA containers for PCR and complete blood count. (CBC) was performed immediately after sample collection by using automated cell counter (Sysmex KN 21x), then genomic DNA was extracted by salting out method, then analyzed by allele specific PCR. at graduate studies laboratory, faculty of medical laboratory sciences, Al-Neelain University, Khartoum Sudan. Regarding exon 3 position 418, CD38 418-F1 5_-TCAGTT CACACAGGTCCAGC-3_ and CD38 418-F2 5_-TCAGT TCACACAGGTCCAGT-3_ were used as forward primers and CD38 418-R 5_-TTCAATCTTCACAGGGCCCAG-3_ as reverse[8]. For each sample, two separate PCR reactions were carried out, to detect missense rs1800561 (418C>T, Arg140Trp), one with the primers CD38 418-F1 and CD38 418-R and the other with CD38 418-F2 and CD38 418-R, with mixes consisting of:

Volume Reagents
4µg genomic DNA
3µl Master Mix (5x FIREPol® Master Mix)
1µl of each primer
to a final volume of 20µl ddH2O (deionized, nuclease free)

PCR reactions were performed using Techne (TC-412t) Thermal Cycler as the following steps:

Initial denaturalization at 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 66°C for 30 seconds, and 72°C for 30 seconds, was carried out. After a final extension at 72°C for 10 minutes.

5 µl of each PCR product (ready to load) was electrophoresed on 2% agarose gel, stained with 0.05 µg/ml ethidium bromide, that floated on 1X TBE buffer which used as a running buffer. Then it was applied on 100 volt/60current for 30 min.1µl DNA ladder of 100 bp was used as molecular weight marker with each patch of samples. After resolving, samples demonstrating the expected-size fragment in only one tube was genotyped as homozygous and samples demonstrating amplification in both tubes were genotyped as heterozygous [9]. Using gel documentation system (SYNGENE), The data was analyzed by (statistical package for social Science) SPSS (version 25) computerized program, mean, frequency and Correlation were determined.

RESULTS

Our study revealed that the frequency of CD38 gene polymorphism (CT) allele, was 32(82%) of patients, against 7(18%) who had a wild type allele (CC) Table (1). Among them 26(67%) were males and 13 (33%)were females as shown in Fig. 1.

Regarding their Age the mean was 60 years old, the Frequency of (CT) allele was found in 17(44%), among >60 years old patients and 22(56%) among those who ≤ 60 years old, with a (P-values = 0.001), % CI= -0.588 (0.395-0.876), {Pearson's R= -0.532, Spearman Correlation= -.532}, Fig. 2.

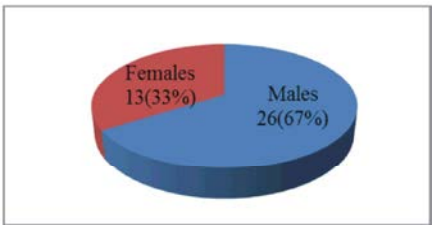

Fig. 1: Distribution of CLL Patients according to gender

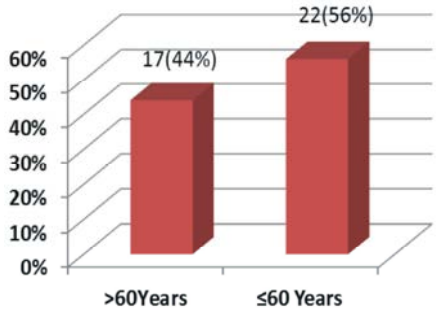

Fig. 2: Distribution of CD38 gene polymorphism among Sudanese patients with CLL according to age

Table 1: Differentiation of CD38 gene alleles among Sudanese patients with CLL

CD38 genotype (rs1800561)	Frequency	Percentage (%)
CT (SNP)	32	82.0
CC (wild type allele)	7	18.0
Total	39	100.0

Table 2: The frequency of CD38 gene polymorphism among Sudanese male and female patients with CLL

	CD38 genotype (rs1800561)				
Gender	----------			P-value	OR (CI Lower-CI Upper)
-	*CT*	*CC*	Total	-	-
Male	22 (68.8%)	4 (57.1%)	26 (67%)	-	-
Female	10 (31.3%)	3 (42.9%)	13 (33%)	0.451	1.575 (0.295-8.414)
Total	32 (100.0%)	7 (100.0%)	39 (100.0%)	-	-

Table 3: The mean of some hematological parameters {Hb Conc(g/dl), TWBCS count(C/L), Lymphocytes and PLTS Count(C/L)} among CLL patients

Parameters	Minimum	Maximum	Mean ± SD
Hb Conc(g/dl)	4.80	14.00	9.99 ±2.57
TWBCS Count(C/L)	8.67	115.	41.0 ± 28.37
Abs. Lymphocyte Count (C/L)	7.08	92.40	33.5 ±23.33
Platelets Count (C/L)	47.0	219.0	122.6 ±47.54

Table 4: Correlation between CD38 SNP and some hematological parameters {Hb conc(g/dl), TWBCS count(C/L), Lymphocytes and PLTS count(C/L)}

CD38 genotype (rs1800561)			
Parameters	CT (Mean±SD)	CC (Mean±SD)	P-value
Hb Conc(g/dl)	9±2.68	10.91±1.88	0.304
TWBCS Count(C/L)	45.96±28.6	18.33±11.87	0.017
Abs. Lymphocyte Count (C/L)	37.95±23.09	13.29±10.58	0.009
Platelets Count (C/L)	130.1±46.23	88.27±39.90	0.033

According to Gender of patients, Frequency of (CT) was 22(68.8%) in males, while 10(31.3%) in females, considering 7, (CC) alleles, 4 (57.1%) in males, 3 (42.9%) in females, with (P-value=0.451), OR=1.575 (0.295-8.414), {Pearson's R.094 Spearman Correlation .094}, Table(2).

The impact of CD38 gene polymorphism on the hematological parameters was studied, the mean of {Hb Conc(g/dl), TWBCS count(C/L), Lymphocytes and PLTS count (C/L)} were {9.99, 41.0, 33.5 and 122.6} respectively. as shown in Table (3).

The CD38 SNP(CT) alleles, the mean of Hb conc(g/dl), TWBCS count(C/L), Lymphocytes and PLTS count(C/L) were (9±2.68, 45.96±28.6, 37.95±23.09 and 130.1±46.23) while in wild type allele (CC) was 10.91±1.88, 18.33±11.87, 13.29±10.58 and 88.27±39.90), while p-value for each parameter was (0.304, 0.017, 0.009, 0.033) respectively as shown in Table (4).

DISCUSSION

CD38 gene polymorphism had been reported as it has high frequency with CLL, also with other disease in many published studies, several studies reported the association between CD38 gene polymorphisms and risk of CLL.

The present study revealed that CD38 gene polymorphism(CT) allele, has higher genotypic frequency than wild type allele (CC), among CLL patients, there is a significant correlation between SNP of CD38 gene and age but not gender of patients, The correlation coefficient is positive weakly strong correlation according to {Pearson's and Spearman Correlation} and also higher frequency among patients whose their ages (≤ 60) years, Regarding, gender of patients, {SNP were higher in males than in females} even though, The correlation coefficient is strongly negative correlation according to, {Pearson's Spearman Correlation and it may cause significant elevation to some hematological parameters.

We found that CD38 gene polymorphism caused significant increase in some hematological parameters {TWBCS count(C/L), Absolute Lymphocytes and PLTS count (C/L)} whereas, No significant correlation between the SNP and Hb conc (g/dl).

TWBCS count(C/L) and Absolute Lymphocytes count(C/L) were strongly correlated with CD38 gene SNP, although it seems to be weakly correlated with PLTS count(C/L).

Our findings agree with study done by Jamroziak *et al* in Poland [10]. They found that frequency of (C C) allele was 428 (98.1%) while (CT) was 8 (1.9%) with P-value 0.97 in CLL patients, in this study the frequency of CD38 gene polymorphism (CT) allele, was 32(82%) and 7(18%) with wild type allele (CC), which demonstrated that CD38 gene SNP may be considered as risk factor for CLL. This study indicated polymorphisms were promising candidate biomarkers for evaluating risk of disease. Their results, considered that there is low (rs1800561 T allele) frequency, they did not detect any individuals homozygous for this allele, which is consistent with our findings. And it did not associate with age of patients, which is disagreed with our findings variation of genetic background might justify gap of results between our study and other studies.

There are several previous studies that investigated the association between polymorphisms and risk of diseases, but the results were inconsistent. A case-control study conducted in the Spain by Marı´a Francisca *et al*. [11] and the results do not support the hypothesis that the CD38 gene polymorphism is related to the risk of SLE, that suggest no individual bearing T in this position was detected among patients with SLE, nor did our healthy controls. These results, support the absence (or the extremely low frequency) of this polymorphism in white population.

Other study which performed by Yagui *et al*. [12] detected an association between CD38 gene polymorphism and type 2 diabetes mellitus in Japan. has been suggested the (CT) allele frequencies were significantly different between the Type II diabetes mellitus patients, as (4, 0.065% and P-value = 0.004) respectively, CD38 gene SNP may contribute to the development of Type II diabetes mellitus in the presence of other genetic defects in beta cell function and insulin action., this is support our Hypothesis, that CD38 gene polymorphisms is associated with some diseases.

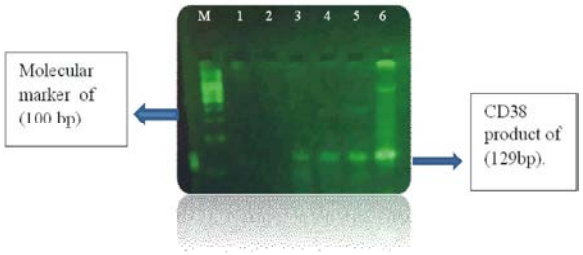

Photo 1: Agarose Gel Demonstrating allele specific PCR Genotyping of CD38 Gene, SNP (CT), The presence of a 129 bp band on both indicates (CT) genotype, (The absence of a 129bp band (e F2) indicates (CC) genotype, Lane 1&2 represent Control –ve, {it was co amplified in all the samples}. Lanes 3 to 6 represent (CT) positive genotype. M marker, 100-bp DNA ladder.

CONCLUSIONS

In conclusion our results suggested that, Single nucleotide polymorphism of CD38 gene is more common than wild type allele among Sudanese patients with CLL and it has a significant correlation with age but not gender of CLL patients especially in males more than females and also among elders (≤ 60 years) and it may cause significant elevation to some hematological parameter {TWBCS count (C/L), Lymphocytes and PLTS count (C/L)}, whereas there is no significant correlation with Hb conc(g/dl). because that genotyping is seems to be very useful for those patients, as predisposing factor or risk factor for CLL and may affect severity of disease, even though its helpful as prognostic factor for CLL.

Recommendations: We recommended to do further study with larger sample size, study of CD38 as flow marker and role of CD38 gene polymorphisms on the patient prognosis., although its impact on prognosis. The effect of the CD38 genotype on the other hematological findings has to be studied further.

Limitations: The study was limited by the small sample size even though it provides a preliminary data of CD38 gene polymorphisms on the patient prognosis. And doesn't correlate CD38 as Flow marker.

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Conflict of Interest: All authors declare that they have no conflict of interest.

Informed Consent: Informed consent was obtained from all individual participants included in the study before sample collection.

REFERENCES

1. Dighiero, G. and T.J. Hamblin, 2008. Chronic lymphocytic leukaemia. Lancet, 371(9617): 1017-1029.
2. OsA, S. Burgler, A. Ribes, A. Funderud, D. Wang, K.M. Thompson, G.E. Tjønnfjord, B. Bogen and L.A. Munthe, 2013. Chronic lymphocytic leukemia cells are activated and proliferate in response to specific T helper cells. Cell Rep., 4: 566-577.
3. Bürgler, S., 2016. Journal of Blood Disorders and Medicine CD38 in Chronic Lymphocytic Leukemia (CLL) - From a Diagnostic Tool to a Therapeutic Target? Editorial 2016 Volume: 1.2 http://dx.doi.org/10.16966/2471-5026.e103.
4. Malavasi, F., A. Funaro, S. Roggero, A. Horenstein, L. Calosso and K. Mehta, 1994. Human CD38: a glycoprotein in search of a function. Immunol. Today, 15: 95.
5. Deaglio, S., K. Mehta and F. Malavasi, 2001. Human CD38: a revolutionary story of enzymes and receptors. Leuk Res., 25: 1.
6. Aydin, S., D. Rossi, L. Bergui, G. D'Arena, E. Ferrero, L. Bonello, P. Omedé, D. Novero, F. Morabito, A. Carbone, G. Gaidano, F. Malavasi and S. Deaglio, 2008. CD38 gene polymorphism and chronic lymphocytic leukemia: a role in transformation to Richter syndrome? Blood. 111: 5646-5653.
7. Ferrero, E. and F. Malavasi, 1999. The metamorphosis of a molecule: from soluble enzyme to the leukocyte receptor CD38. J. Leukoc. Biol., 65: 151.
8. Ferrero, E., F. Saccucci and F. Malavasi, 1999. The human CD38 gene: polymorphism, CpG island and linkage to the CD157(BST-1) gene. Immunogenetics, 49: 597.
9. Kawasaki, E., 1990. Sample preparation from blood, cells and others fluids. In Innis M, D Gelfand, J. Snisky and T. White, 1990. PCR Protocols: A Guide to Methods and Applications. San Diego: Academic Press.
10. Jamroziak, K., Z. Szemraj, O.G. Izydorczyk, J. Szemraj, M. Bieniasz and B. Cebula, 2009. Krzyszt of Giannopoulos, Ewa Balcerczak, Dorota Jesionek-Kupnicka, Malgorzata Kowal, Aleksandra Kostyra, Malgorzata Calbecka, Ewa Wawrzyniak, Marek Mirowski, Radzislaw Kordek and Tadeusz Robak, 2009. Cancer Epidemiology, Biomarkers & Prevention, CD38 Gene Polymorphisms Contribute to Genetic Susceptibility to B-Cell Chronic Lymphocytic Leukemia: Evidence from Two Case-Control Studies in Polish Caucasians/March, 18(3).

11. María Francisca González-Escribano, F. Aguilar, B. Torres, J. Sánchez-Román and A. Núñez-Roldán, 2004. CD38 polymorphisms in Spanish patients with systemic lupus erythematosus Author links open overlay pane, 2004.Servicio de Inmunología, Sevilla, Spain Unidad de Colagenosis, HU Virgen del Rocío, Servicio Andaluz de Salud, Sevilla and Spain, 2004.

12. Yagui, K., F. Shimada, M. Mimura, N. Hashimoto, Y. Suzuki, Y. Tokuyama, K. Nata, A. Tohgo, F. Ikehata, S. Takasawa, H. Okamoto, H. Makino, Y. Saito and A. Kanatsuka, 1998. A missense mutation in the CD38 gene, a novel factor for insulin secretion: association with Type II diabetes mellitus in Japanese subjects and evidence of abnormal function when expressed *in vitro*, Diabetologia, 41: 1024±1028.