OPEN ACCESS

GAVIN PUBLISHERS

Hematological Diseases and Therapies

Research Article

Hosny M, et al. Hem Disease Therapies 4: 129.

DOI: 10.29011/2577-1418.0000129

Role of miR_155a and miR_181a in Chronic Lymphocytic Leukemia

Nashwa EL-Khazragy[1*], Mohamed Hosny[2], Mostafa M Elhady[2], Ahmed El-Agmawy[3], Nahla S Hassan[2]

[1]Clinical Pathology and Hematology Department, Faculty of Medicine, Ain Shams University Biomedical Research Department, Cairo, Egypt*

[2]Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo. Egypt

[3]Clinical Oncology & Nuclear Medicine Department Faculty of Medicine-Al-Azhar University, Cairo, Egypt

***Corresponding author:** Nashwa El-Khazragy, Clinical Pathology and Hematology department, Faculty of Medicine, Ain Shams University Research Institute (MASRI), Biomedical Research department, Abbassia, Cairo, Egypt, P.O. box 11381. Tel: 01015037888; Managing Director of Global Research Labs, Cairo, Egypt Email: nashwaelkhazragy@med.asu.edu.eg , nashwa@globalresearchlabs.com*

***Citation:** EL-Khazragy N, Hosny M , Elhady MM, El-Agmawy A, Hassan NS (2019) Role of miR_155a and miR_181a in Chronic Lymphocytic Leukemia. Hem Disease Therapies 4: 129. DOI: 10.29011/2577-1418.0000129*

Received Date: 23 September, 2019; **Accepted Date:** 10 October, 2019; **Published Date:** 15 October, 2019

Abstract

Objective: We aim to investigate potential of miRNA155a and miRNA 181a in diagnosis of Chronic Lymphocytic Leukemia (CLL).

Methods: The study was done on 80 CLL patients, we have investigated that the expression levels of two miR-RNAs "miR-181a & miR-155a" using quantitative PCR.

Results: We found high miRNA155a expression and low miRNA181a expression in CLL patients. Cut-off expression levels of miR155 at 19.5 showed 85% sensitivity and 100% specificity. While cut-off expression levels of miR181 at 10.8 showed 75% sensitivity and 80% specificity. High expression of miRNA155a was significantly correlated to low hemoglobin levels and higher total leukocyte count. Low expression of miRNA181a was significantly correlated to high hemoglobin levels and lower total leukocyte count. Conclusion: miRNA 155a could have a role in CLL pathogenesis and progression while miRNA 181a may have a role in suppression of malignant cells. Both have the potential to be used as a diagnostic biomarker.

Keywords: Chronic Lymphocytic Leukemia; miRNA155a; miRNA181a

Introduction

Chronic Lymphoblastic Leukemia (CLL) is the most common form of adult leukemia. It accounts for 25 to 30 percent of all leukemia's in the United States [1]. According to Surveillance, Epidemiology and End Results program (SEER) in 2018, CLL new cases in United States were 20,940 accounting for 1.2% of all new cancer cases, while deaths from CLL were 4,510 accounting for 0.7% of all cancer deaths [2]. CLL is a B Cell Neoplasm. The accumulating malignant cells in CLL are the same in Small Lymphoblastic Lymphoma (SLL). If the disease is primarily in the blood it's termed CLL, if primarily nodal it's termed SLL.

CLL is considered a disease of elderly. The median age at diagnosis is 72 and only 10% of patients are 40 years old or younger [3]. Several staging systems were proposed to classify the disease prognosis. Rai and binet staging systems are the ones preferred by most physicians [4,5].

The Rai staging system considers the concept of gradual increase in the body burden of leukemic lymphocytes as an indicator of the disease prognosis. Accumulation of leukemic lymphocytes starts in the blood causing lymphocytosis (stage 0), then lymphadenopathy (stage 2) and hepatosplenomegaly (stage 3). Eventually leukemic cells infiltrate the bone marrow leading to anemia (stage 4) and thrombocytopenia (stage 5) [3]. Whereas The binet staging system takes the five main involvement sites into consideration, which are cervical, Axillary and inguinal lymph nodes, spleen and liver. Patients are classified according to the number of affected sites and the presence of anemia or thrombocytopenia. [5].

The pathogenesis of CLL is still not clearly understood but its thought that nearly all cases of CLL are preceded by a prema-

lignant condition called Monoclonal B cell lymphocytosis (MBL). MBL with CLL phenotype is present in 5 -15 % of population above the age of 60 and have a rate of nearly 1% progression to CLL / SLL [6,7]. the pathogenesis of MBL appears to be multifactorial and related to abnormal response to antigenic stimulation. Progression of MBL to CLL/SLL in a minority of patients seems to be related to chromosomal abnormalities like deletions (13q14), (11q), (17q), and Trisomy 12 [8-10].

Molecular Genetic abnormalities were also identified in patients with or without chromosomal abnormalities including cell cycle control genes like TP53, Notch signaling genes and inflammatory pathway genes [11-13]. MicroRNAs (miRNAs) are small non-coding RNAs of nearly 22 Nucleotides. They have a role in post-transcriptional repression by silencing mRNAs of Protein coding genes. miRNAs are involved in cellular metabolism, apoptosis and cancer formation [14-28].

miRNAs are generally integrated in the function of every living cell, they show important roles in normal hematopoiesis [14]. miRNA 155a and 181a are two miRNAs of special concern and relation to the development of leukemia's and lymphomas [15-26] but their role in CLL is yet to be established. miRNA 155 normally controls B and T Cell Differentiation and controls germinal center reaction, whereas miRNA 181 normally blocks differentiation of human progenitor cells [16]. We aim to evaluate the diagnostic and prognostic value of miR-155a and miR-181a in chronic lymphocytic leukemia.

Results

Demographics and Clincopathological Characteristics

The mean age for all participants was 50±12.4 years. Mean age of CLL group and Control group was 58.1±12.4 and 56.3±17.1 respectively. 65% of our patients were males, 35% were females. Patients with hemoglobin level ≤ 10 g/dl were 38 (47.5%). Patients with TLC > 50.000 were 52 (65%). Patients with platelet count ≤ 100.000 were 32 (40%). 62.5% of patients had a cytogenetic abnormality; 20 patients had del13q, 16 had trisomy12, 8 had del11q and 6 had del17p (Table 1).

Table 1: Demographic data of the studied group

Parameter	Subgroup	N	Statistics	
Age (years)	ALL	100	mean±SD	50±13.2
			Range	24 - 83
	CLL	80	mean±SD	58.1±12.4
			Range	34 – 83
	Control	20	mean±SD	56.3±17.1
			Range	28 – 80
Age subgroups	≤50	22	N (%)	22 (28%)
	>50	58	N (%)	58 (72%)
Gender	Male	52	N (%)	52 (65%)
	Female	28	N (%)	28 (35%)
Hemoglobin (grm/dl)	CLL	80	mean±SD	9.8±2.5
			Range	2.3 – 14.0
Hemoglobin subgroups	≤10	38	N (%)	38 (47.5%)
	>10	42	N (%)	42 (52.5%)
TLC (x10^3 /L)	CLL	80	Median (Q_1-Q_2)	65.8 (38.5 – 159.8)
			Range	14.6 – 364.0
TLC subgroups	≤50,000	28	N (%)	28 (35%)
	>50,000	52	N (%)	52(65%)

Platelet count (x10^6/L)	CLL	80	Median (Q_1-Q_2)	155.5(91.7 – 213.5)
			Range	29.0 - 421
Platelet subgroups	≤100,000	32	N (%)	32 (40%)
	>100,000	48	N (%)	48 (60%)
Cytogenetic abnormality	No	30	N (%)	15 (37.5%)
	Yes	50	N (%)	25 (62.5%)
Cytogenetic abnormality type	del13q	20	N (%)	20 (40%)
	Trisomy12	16	N (%)	16 (32%)
	del11q	8		8 (16%)
	del17p	6		6 (12%)

(Q_1-Q_2): 25th percentile-75th percentile

miRNA155a and miR-181a Expressions in CLL patients

miR155a median expression levels in the CLL group was significantly higher (67.6) than the control group (3.5) (p =0.001). Patients with hemoglobin level ≤ 10 g/dl had higher miR155a expression (169) than patients with hemoglobin level > 10 g/dl (27) (p=0.001) (Table 2, Figure 1). Regarding the expression of miR181a; it was found that the median expression levels in the CLL group was significantly lower (7.4) than the control group (298.2) (p =0.001). Patients with hemoglobin level ≤ 10 g/dl had lower miR181a expression (3.7) than patients with hemoglobin level > 10 g/dl (10.7). (p =0.001). Patients with TLC > 50.000 had lower miR-181a expression [5] than patients with TLC ≤ 50,000 (10.6) (p =0.003). Patients with platelet count ≤100,000 had lower miR181a expression (3.5) than patients with platelet count >100,000 (p =0.02) (Table 3, Figure 1).

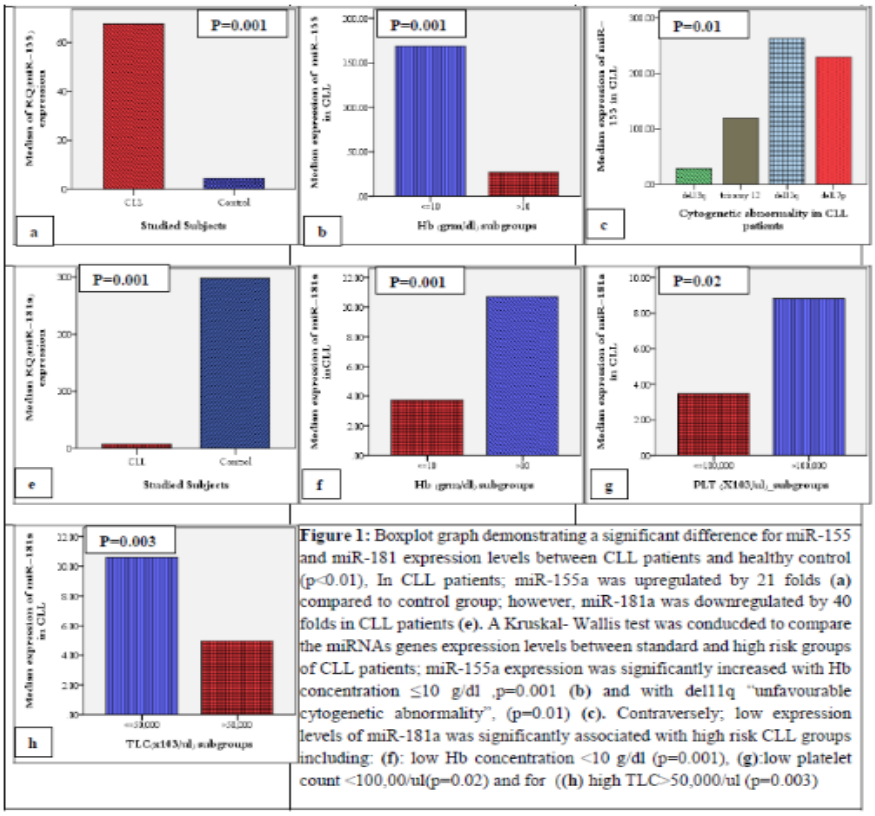

Figure 1: Boxplot graph demonstrating a significant difference for miR-155 and miR-181 expression levels between CLL patients and healthy control (p<0.01). In CLL patients; miR-155a was upregulated by 21 folds **(a)** compared to control group; however, miR-181a was downregulated by 40 folds in CLL patients **(e)**. A Kruskal- Wallis test was conducded to compare the miRNAs genes expression levels between standard and high risk groups of CLL patients; miR-155a expression was significantly increased with Hb concentration ≤10 g/dl ,p=0.001 **(b)** and with del11q "unfavourable cytogenetic abnormality", (p=0.01) **(c)**. Contraversely; low expression levels of miR-181a was significantly associated with high risk CLL groups including: **(f)**: low Hb concentration <10 g/dl (p=0.001), **(g)**:low platelet count <100,00/ul(p=0.02) and for **((h)** high TLC>50,000/ul (p=0.003)

Table 2: Comparative analysis for expression of miR-155a among different CLL-risk subgroups

Group	Subgroup	Median (Q_1-Q_2)	Range	X^2	P value
Subjects	CLL	67.6 (25.7-162.2)	14.1 – 714	23.5	0.001
	Control	3.5 (2.3 – 4.7)	0.8 – 3.5		
Age (years)	≤50	147 (34 – 222.2)	16.3 – 621.7	2.2	0.1
	>50	55.7 (25.6-122.8)	14.1 – 714.1		
Gender	Male	77.4 (20 – 150)	14.0 – 461.2	0.13	0.7
	Female	56.5 (26 – 185)	19.0 – 714.0		
Hemoglobin subgroups	≤10	169 (118 – 223)	19.6 - 714	23.1	0.001
	>10	27 (19 – 41)	14.0 - 110		
TLC subgroups	≤50,000	31.3 (19.6 – 92)	14.0 – 229	2.7	0.1
	>50,000	111 (27 – 203)	15 – 714		
Platelet subgroups	≤100,000	177 (63 – 215)	16.5 -461	3.2	0.07
	>100,000	46.3 (26 – 113)	14.0 - 714		
Cytogenetic abnormality	No	41.0 (26 – 92)	14.0 – 622	1.9	0.2
	Yes	94.4 (28 – 184)	15.0 - 714		
Cytogenetic abnormality type	del13q	28 (19 – 94)	15 - 185	4.5*	0.01
	Trisomy12	119 (9.8 - 162	19.6 – 215		
	del11q	263 (169 -385)	123 – 461		

*: F value (ANOVA), X^2: Chi-Square (Kruskal-Wallis).

Table 3: Comparative analysis for expression of miR-181a among different CLL-risk subgroups

Group	Subgroup	Median (Q_1-Q_2)	Range	X^2	P value
Subjects	CLL	7.4 (4.1 – 10.8)	0.4 – 29.8	25.6	0.001
	Control	298.2 (254 – 336)	241 – 343		
Age (years)	≤50	4.8 (4.1–10.5)	1.1 – 12.5	0.7	0.4
	>50	8.1 (4.4-11.9)	0.4 – 29.9		
Gender	Male	7.4 (4.4-10.9	0.4 – 30.0	0.1	0.7
	Female	7.3 (1.6 – 10.7)	0.95 – 20.4		
Hemoglobin subgroups	≤10	3.7 (1.3 – 6.6)	0.4 – 10.5	21.3	0.001
	>10	10.7 (8.2 – 14.6)	4.8 – 30.0		
TLC subgroups	≤50,000	10.6 (8.1 – 13.2)	3.7 – 30.0	8.8	0.003
	>50,000	5.0 (1.7 – 9.4)	0.4 – 23.0		
Platelet subgroups	≤100,000	3.5 (1.5 – 6.9)	0.6 – 14.6	5.6	0.02
	>100,000	8.8 (5.1 -12.0)	0.4 – 30.0		

Cytogenetic abnormality	No	9.4 (6.9 – 10.8)	0.7 – 29.8	1.8	0.18
	Yes	6.2 (2.7 -10.5)	0.4 -22.6		
Cytogenetic abnormality type	del13q	8.5 (5.1 -20.3)	1.5 – 22.6	2.2*	0.12
	Trisomy12	7.2 (3.2 -9.6)	0.6 – 12.0		
	del11q	2.6 (1.4 -3.7)	0.4 – 4.7		
	del17p	3.7 (2.4 -9.2)	0.9 -14.6		

*: F value (ANOVA), X^2: Chi-Square (Kruskal-Wallis).

Diagnostic Performance of miR-155a and miR-181a in CLL

Cut-off expression levels of miR155 at 19.5 showed 85% sensitivity and 100% specificity. While cut-off expression levels of miR181 at 10.8 showed 75% sensitivity and 80% specificity (Table 4, Figure 2). Correlation between miR-155a, miR-181a and hematological parameters in CLL patients.

Table 4: Diagnostic performance of miR-155 and miR-181a in CLL patients

Parameter	Cut-off	AUC	Sensitivity (%)	Specificity (%)
miR-155 (log^{10})	19.5	0.97	85	100
miR-181 (log^{10})	10.8	0.93	75	80

AUC: area under the curve

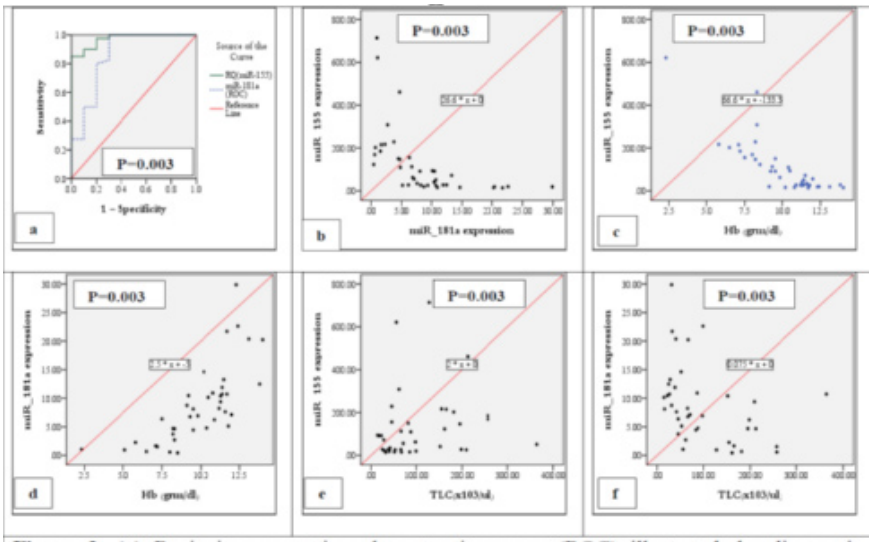

Figure 2. (a) Recievieng operating charactrestics curve (ROC) illustrated the diagnostic performance of miR-155a and miR-181a in, miR-155a gene expression shows 85% senstivity and 100% specificity; however, the sentivity and specificity of miR-181a are 75% and 80% specificity; respectively. A Spearmans correlation analysis was conducted; a strong negative correlation was observed between miR-155a and miR-181a in CLL patients **(fig b)** (r=-0.8,p=0.003), a strong negative correlation was found between miR-155a, miR-181a and Hb concentration g/dl (r:-0.8, p=0.001) **(Fig c,d)**.The TLC ($x10^3$ /ul) showed a strong significant correlation **(e)** with miR-181a (r:-0.5, p=0.001) , while a moderated significant correlation was found with miR-155a gene expression in CLL patients **(f)**.

Expression of miR-155 had a significant negative correlation with hemoglobin levels (P =0.001) and a significant positive correlation with TLC (P=0.03). Expression of miR-181a had a significant positive correlation with hemoglobin levels (P =0.001) and a significant negative correlation with TLC (P =0.001) (Table 5 Figure 2).

Table 5: Correlation between expression levels of measured miRNA and hematological parameters in CLL patients

Parameter	miR-155 (log^{10})			miR-181a (log^{10})		
	r	P value	Sig	r	P value	sig
Hb (grm/dl)	-0.8	0.001	HS	-0.8	0.001	HS
TLC ($x10^3$/ul)	0.3	0.03	S	-0.5	0.001	HS
Plat ($x10^6$/ul)	-0.1	0.36	NS	0.2	0.13	NS
miR-181a (log^{10})	-0.8	0.001	HS	-----	-----	-----

r: correlation coefficient

Methods

The present study was conducted on 100 subjects; they represent two groups; 80 patients were diagnosed as Chronic Lymphocytic Leukemia (CLL) and twenty healthy individuals as a control group. The CLL patients attended at hematology department; Ain Shams University Hospitals; Cairo, Egypt from January 2015 till May 2018. The diagnosis of CLL was based on Complete Blood Counts (CBC), PB and bone marrow films morphological examination, immunophenotyping, cytogenetic and molecular analysis. The CLL patients were categorized according to the risk factors into standard and high risk groups; the considered parameters for grouping includes patient's age, gender, Total Leucocyte Counts (TLC); hemoglobin concentration; platelet counts and detection of minimal residual disease after induction therapy. Assessment of Minimal Residual Disease (MRD) was performed using a lineage-specific monoclonal panel: for B-and T- cell lineage. MRD was considered positive when immature cells exceeded 0.01% of all marrow nucleated cells after induction chemotherapy. A Peripheral Blood (PB) samples were collected in K2 EDTA vacutainers from all subjects after taking the patients approval; a written consent was signed from each subjects in accordance with the declaration of Heliniski. The Clinic pathological features are presented in Table 1.

miRNA Extraction and Purification

miRNA was extracted from PB Mononuclear Cells (MNCs) that it is isolated by ficoll hypaque density gradient centrifugation using a miRNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA concentration and integrity was assessed spectrophotometrically at 260 and 280 nm. The extracted and purified miRNAs was reverse transcribed into cDNA using miScript II RT Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

miRNA-155 and miR-181 Expression Analysis

The expression levels of miR-155a and miR-181a was measured in PB samples using the SYBR-green fluorescent-based primer assay (Hs-miR-155; cat no: MS00031486 and Hs-miR-181a; Cat no: MS0008827); the small nucleolar RNA, C/D box 48 (SNORD48), (NCBI RefSeq: NR_002745.1) as a reference gene. The PCR amplification was performed on the 5-plex Rotor-Gene PCR System using miScript SYBR Green PCR Kit and the (Qiagen, Hilden, Germany). The thermal protocol was adjusted according to manufacture instructions as follows: 15 min for DNA Taq Polymerase activation at 95^0c followed by three cycling steps by 40 cycles; each cycle consist of: denaturation at 94^0c for 15 minutes, primer annealing at 55^0c and extension at 70^0c for 30 sec), the. At the extension step; the fluorescence data was collected. The gene expression was calculated using the $2\Delta\Delta Ct$ method; the housekeeper gene "SNORD48" was used as an endogenous reference control for normalization purposes. Validation of gene amplification efficiencies of the miRNAs targets was assessed by a validation experiment; a serial dilutions of a control cDNA was used as a template. Accordingly; the linear increases of miRNAs and the reference gene calibration curves highlight the 106.3% and 104.2% amplification efficiencies, respectively, as well as the absence of PCR inhibition by the template.

Statistical Analysis

Statistical analysis was performed using SPSS v.23 (Chicago, IL, USA). The expression levels of miRNAs are compared between CLL patients and healthy controls as well as between standard and high risk CLL subgroups using the non-parametric Mann–Whitney U test. In addition, the Receiver Operating Curve (ROC) was conducted to assess the diagnostic and prognostic potential of miR-155 and miR-181a in CLL. Spearman's correlation analysis was used to find out the relation between miRNAs expression and different hematological parameters. Significance was set at $\leqslant 0.05$.

Discussion

MicroRNAs are valuable indicators for predicting the clinical behavior of Chronic Lymphocytic Leukemia (CLL). Structur-

ally, miRNAs are short (19- to 25-nucleotide) RNAs that target messenger mRNA and regulate the expression of protein-coding genes. We found that miRNA155a expression were significantly upregulated and miRNA 181a significantly downregulated in CLL patients. Both miRNA 155a and 181a demonstrated high sensitivity (85% and 75% respectively) and specificity (100% and 80% respectively) at certain cut-off values for diagnosis of CLL. miRNA 155a was significantly correlated to unfavorable disease outcome such as lower hemoglobin levels and higher total Leukocyte Count (TLC), while miRNA 181a was correlated to favorable outcomes such as higher hemoglobin levels and lower total leucocyte count.

In a recent study, found that miRNA155a was highly expressed in peripheral blood mononuclear cells of 88 patients diagnosed with CLL. In these patients, miRNA155a was correlated to lower overall survival rate [17]. Similarly, in a meta-analysis of 11 studies found poor overall survival outcome in patients with high miRNA155a expression and concluded that miRNA 155a was a promising prognostic biomarker in this patient population [18]. In our study, measurement of levels of miRNA155a in the CLL group was significantly higher than the control group.

In addition, miRNA155a expression coincided with high TLC and low hemoglobin. Correlation analysis between the marker and hematological parameters was significant. This was consistent with other studies which reported worse prognosis in patients with high levels of miRNA155a expression [19,20]. A study by [20] showed that miRNA155a was able to increase granulocyte/monocyte expansion in bone marrow of mice with pathological features resembling myeloid metaplasia [20]. Some studies suggested that the JAK/STAT3 pathway-implicated in CLL pathogenesis- were linked to upregulation of miR155a expression in CLL cells [21,22]. It was also found that the miR-155a expression was upregulated in peripheral blood mononuclear cells in CLL patients. They also demonstrated a link between miRNA155a and IL-9 which induces the JAK-STAT3 pathway creating a positive feedback loop that can be an important mechanism of CLL progression and aggressiveness [23].

Conversely, we found miRNA181 expression levels to be lower in the CLL group compared to our Control group. This has been confirmed by some previous studies; [21] showed miRNA181a to be downregulated in CLL [24,27], and lower expression levels of this marker were linked to high disease aggressiveness. This could be due to the fact that miRNA181a affects the P53 system-the most important prognostic indicator of CLL- and improving sensitivity of malignant cells to chemotherapy and subsequently increasing the rate of apoptosis [25]. This is probably the reason miRNA18a were correlated significantly to higher hemoglobin levels and lower TLC in the CLL group.

Whether use of these miRNAs in diagnosing or predicting outcomes of CLL is still a matter of investigation. Recent reports were in agreement with our results, miRNA155a and miRNA 181a could play a crucial role in diagnosing malignancy and in predicting its prognosis [26]. We used a cut off level at which miRNA 155a offered 85% sensitivity in diagnosing CLL. miRNA 181a showed lower sensitivity at 75%. Specificity of both biomarkers were high (100% and 80% respectively).

In Conclusion, levels of both miRNA155a and 181a were significantly correlated to hematological parameters of malignancy. MiRNA155a appeared to be involved in progression and unfavorable outcome while miRNA18a appeared to be protective against progression. Both markers were sensitive and specific for diagnosis of the diseases.

Conclusion

miRNA 155a could have a role in CLL pathogenesis and progression while miRNA 181a may have a role in suppression of malignant cells. Both have the potential to be used as a diagnostic biomarker.

Acknowledgement

We thank our colleagues from (Clinical Hematology Department, Ain Shams University Hospital) who supplied notion and knowledge that substantially studies despite the fact that they may not contain with all the interoperations/conclusions of this paper. We'd additionally like to reveal our gratitude to the patients who are the backbone of this study, also we would like to show our great thanks to the three "anonymous" reviewers for there so-referred to as insight. We also are immensely thankful for his or her comments on an in advice model of the manuscript, in spite of the truth that any errors are our personal and should now not tarnish the reputations of those esteemed folks.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A (2018) "Cancer statistics". Chronic Lymphocytic Leukemia - Cancer Stat Facts; CA Cancer J Clin 68: 7-30.
2. Hallek M, Pflug N (2010) "Chronic lymphocytic leukemia". Ann Oncol vii154-vii164.
3. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, et al, (1975) "Clinical staging of chronic lymphocytic leukemia". Blood 219-234.
4. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, et al (1981) "A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis". Cancer 48: 198-206.
5. Rossi D, Sozzi E, Puma A, De Paoli L, Rasi S, Spina V, et al. (2009): "The prognosis of clinical monoclonal B cell lymphocytosis differs from prognosis of Rai 0 chronic lymphocytic leukaemia and is recapitulated by biological risk factors". Br J Haematol 146: 64-75.
6. Shanafelt TD, Kay NE, Rabe KG, Call TG, Zent CS, Maddocks K, et al. (2009): "Brief report: natural history of individuals with clinically recognized monoclonal B-cell lymphocytosis compared with patients with Rai 0 chronic lymphocytic leukemia". J Clin Oncol 27: 3959-3963.

7. Garcìa-Marco JA, Price CM, Ellis J, Morey M, Matutes E, Lens D, et al. (1996 Nov): "Correlation of trisomy 12 with proliferating cells by combined immunocytochemistry and fluorescence in situ hybridization in chronic lymphocytic leukemia". Leukemia 10: 1705-1711.

8. Kalachikov S, Migliazza A, Cayanis E, Fracchiolla NS, Bonaldo MF, Lawton L, et al. (1997 Jun): "Cloning and gene mapping of the chromosome 13q14 region deleted in chronic lymphocytic leukemia". Genomics 42: 369-377.

9. Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, et al. (2000): "Genomic aberrations and survival in chronic lymphocytic leukemia". N Engl J Med. ;343: 1910-1906.

10. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, et al. (2011) "Other novel cancer genes in chronic lymphocytic leukemia". N Engl J Med 365: 2497-506.

11. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. (2015): "Mutations driving CLL and their evolution in progression and relapse". Nature 526: 525-530.

12. Puente XS, Beà S, Valdés-Mas R, Villamor N, Gutiérrez-Abril J, et al. (2015): "Non-coding recurrent mutations in chronic lymphocytic leukaemia". Nature 526: 519-524.

13. Bartel DP (2009) "MicroRNAs: target recognition and regulatory functions". Cell 136: 215-233.

14. El-Khazragy N, Noshi MA, Abdel-Malak C, Zahran RF, Swellam M. (2018 Oct): "miRNA-155 and miRNA-181a as prognostic biomarkers for pediatric acute lymphoblastic leukemia". J Cell Biochem 120: 6315-6321.

15. Vasilatou D, Papageorgiou S, Pappa V, Papageorgiou E, Dervenoulas J (2010) "The role of microRNAs in normal and malignant hematopoiesis". Eur J Haematol 84: 1-16.

16. Papageorgiou SG, Kontos CK, Diamantopoulos MA, Bouchla A, Glezou E, Bazani E, et al. (2017) "MicroRNA-155-5p Overexpression in Peripheral Blood Mononuclear Cells of Chronic Lymphocytic Leukemia Patients Is a Novel, Independent Molecular Biomarker of Poor Prognosis". Dis Markers [Internet] 1-10.

17. Zhang X, Wang Y, Guo Q, Diao Y, Liu H, Song G, et al. (2018) "Prognostic role of microRNA-155 in patients with leukemia". A meta-analysis. Clin Chim Acta [Internet] 483: 6-13.

18. Ferrajoli A, Shanafelt TD, Ivan C, Shimizu M, Rabe KG, Nouraee N, et al. (2013) "Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia". Blood [Internet] 122: 1891-1899.

19. O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, et al. (2018) "Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder". J Exp Med [Internet] 205: 585-594.

20. Hartman ML, Kilianska ZM (2018) "Lipoprotein lipase: a new prognostic factor in chronic lymphocytic leukaemia". Contemp Oncol (Poznan, Poland) 16: 474-479.

21. ENCODE Project Consortium (2018) "A User's Guide to the Encyclopedia of DNA Elements (ENCODE)". Becker PB, editor. PLoS Biol [Internet] 9: e1001046.

22. Chen N, Feng L, Qu H, Lu K, Li P, Lv X, et al. (2018) "Overexpression of IL-9 induced by STAT3 phosphorylation is mediated by miR-155 and miR-21 in chronic lymphocytic leukemia". Oncol Rep [Internet] 39: 3064-3072.

23. Zhu D-X, Zhu W, Fang C, Fan L, Zou Z-J, et al. (2018) "miR-181a/b significantly enhances drug sensitivity in chronic lymphocytic leukemia cells via targeting multiple anti-apoptosis genes. Carcinogenesis". [Internet] 33: 1294-1301.

24. Kater AP, Dicker F, Mangiola M, Welsh K, Houghten R, Ostresh J, et al. (2018 Nov 9): "Inhibitors of XIAP sensitize CD40-activated chronic lymphocytic leukemia cells to CD95-mediated apoptosis". Blood [Internet] 106: 1742-18.

25. Hou Y, Wang J, Wang X, Shi S, Wang W, et al. (2018 Nov 12): "Appraising MicroRNA-155 as a Noninvasive Diagnostic Biomarker for Cancer Detection: A Meta-Analysis". Medicine (Baltimore) [Internet] 95: e2450.

26. El-Khazragy N, Elshimy A, Hassan S, Matbouly S, Safwat S, et al. (2018) "Dysregulation of miR-125b predicts poor response to therapy in pediatric acute lymphoblastic leukemia". J Cell Biochem.

27. El-Khazragy N, Mohamed Noshi A, Abdel-Malak C, Zahran R, Swellam M (2018) "miRNA-155 and miRNA-181a as prognostic biomarkers for pediatric acute lymphoblastic leukemia". J Cell Biochem 1-7.

28. Swellam M, El-Khazragy,N, (2016) "Clinical impact of circulating microRNAs as blood-based marker in childhood acute lymphoblastic leukemia". J Cell Biochem 37: 10571-10576.