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Assessment of miR-150-5 p as diagnostic and prognostic markers of B- chronic Lymphocytic Leukemia in chronic hepatitis C

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Abstract:

Aims: the current study aimed to investigate the role of miR-150-5 p expression as a diagnostic and prognostic marker of B- CLL among Egyptian patients with chronic hepatitis C and its impact on risk stratification and response to therapy.

Patients & Methods: This study was conducted on 25 patients with chronic hepatitis C recently diagnosed as CLL and 25 healthy control All studied patients were subjected to entire history, taking the complete clinical examination, and laboratory investigations including serum LDH, serum β2 microglobulin (β2M), CD38 and ZAP70 expression by flow cytometry. We measured miR-150-5 p expression by qRT-PCR.

Results: The results of this analysis indicated that CLL patients had a significantly lower level of miR-150-5 p expression (0.464±0.16) compared to the control group (0.8±0.76), P <0.001* Additionally, the expression levels of miR-150-5 p were significantly inversely correlated with laboratory and clinicopathological features of CLL as well as the severity of CLL according to Modified Rai staging system and Binet staging system. Interestingly, concerning the treatment of CLL, there were significantly lower levels of miR-150-5 p in no treatment subgroup (n=18, 0.49±0.13) compared to successfully treated subgroups (n=7, 0.77±0.07), P <0.001*

Conclusions: miR-150-5 p expression level was downregulated in CLL patients, particularly patients with HCV infection, and significantly inversely correlated with the severity of CLL. Thus, it could be helpful for diagnostic and prognostic purposes in CLL.

Keywords: HCV; CLL; MicroRNAs; MiR-150-5 p, Expression; CD38; ZAP70; qRT-PCR; β2 microglobulin; Flow cytometry.

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Introduction

Hepatitis C virus (HCV) is a hepatotropic and lymphotropic virus, and its infection affects the B lymphocyte compartments. HCV has been implicated in the pathogenesis of chronic lymphocytic leukemia (CLL) [1]. A vast and growing literature firmly supports the hypothesis that CLL is the most frequent hematological cancer noted in the aging group of patients [2].

There is gathering proof that CLL is deemed as the dysregulated proliferation of lymphocytes in the blood and other organs [3,4]. But interestingly, It is becoming more widely accepted that there is a wide range of clinical and pathological features of CLL due to its complexity [5,6].

MicroRNAs (miRNAs) are a class of endogenous non-coding RNA molecules that regulate vital biological processes, including cell development and malignant transformation. For example, in cancer, miRNAs can act as oncogenes or tumor suppressor genes by regulating the expression of genes that control cell proliferation, differentiation, apoptosis, invasion, and metastasis [7].

Accumulated experimental and clinical evidence indicates that miR-150 is an immuno-miR regulating immune functions, such as proliferation, apoptosis, and differentiation of NK, T, and B cells [8] and lymphocyte activation [9].

It is well established that miRNAs could be used not only as diagnostic and prognostic biomarkers but also as predictive biomarkers. Elegant studies have evaluated the role of early cancer diagnosis in successful and effective treatment. However, inopportunely, many cancer patients are diagnosed late due to insufficient early screening [10]. Thus, this study aimed to gain further insights into the role of miR-150-5 p expression as a diagnostic and prognostic marker of B- CLL among Egyptian patients with chronic hepatitis C and its impact on risk stratification and response to therapy.

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Subjects

This case-control study was conducted on 25 adult patients of both sexes with CLL, and in addition to the 25 healthy control individuals,' the age and sex matched the cases. The diagnosis of CLL was based on current WHO guidelines [11]. Among CLL, we selected 18 Egyptian patients (72%) who were either newly diagnosed with CLL or in the "watch and wait" phase. Those patients were assigned to wait and see strategy and were monitored periodically every three months for disease progression. Interestingly, seven Egyptian patients (28%) were indicated for therapy. The treatment modalities included FCR. (Fludarabine, cyclophosphamide, and rituximab) And ibrutinib and the response to treatment were assessed according to IWCLL [11]. The risk stratification of the patients was done according to modified Rai and Binet staging systems. The Ethics Committee of the Faculty of Medicine, Zagazig University, approved the study protocol with (IRB no. 10213). All participants were assigned informed agreement before their inclusion. The flow chart of the study is demonstrated in figure 1.

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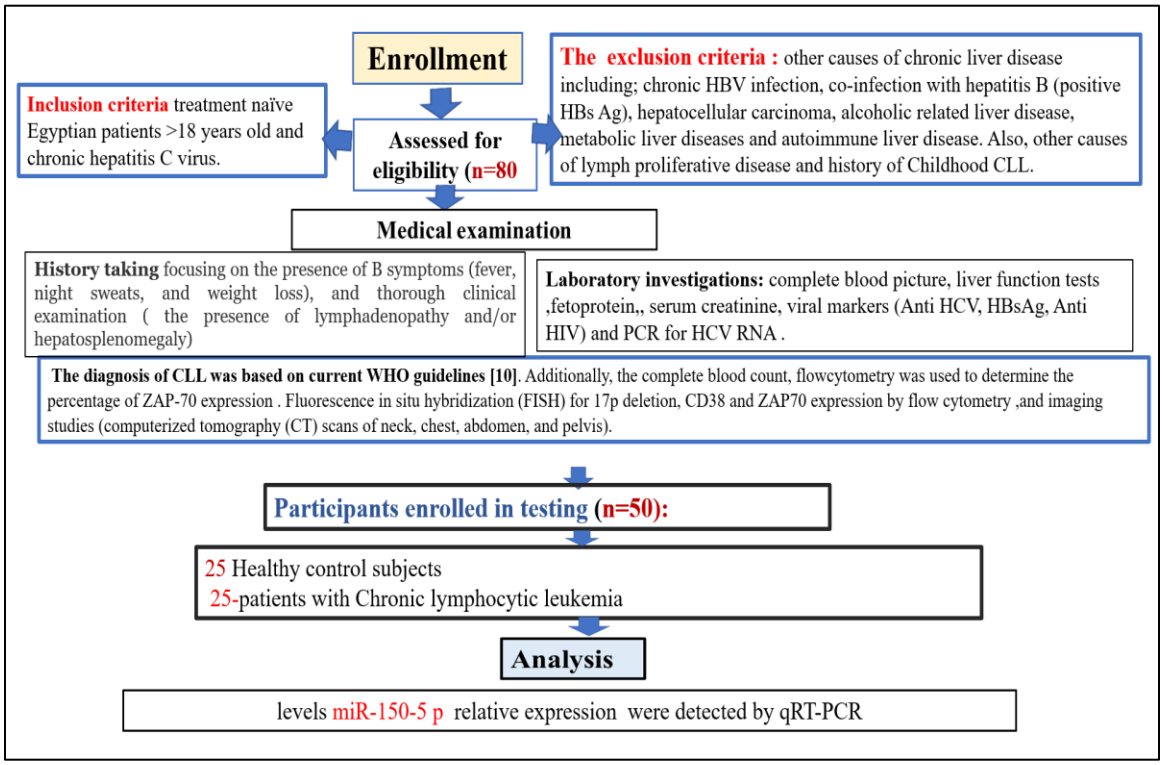

Fig 1. ***Flowchart of the study.***

Laboratory tests

Blood samples were taken from all registered participants from the Departments of Internal medicine, Clinical Oncology, Nuclear Medicine, and Tropical Medicine. Testing was done according to operating techniques in Zagazig university hospital and Medical Biochemistry laboratories, as shown in figure1.

miR-150 expression levels by real-time PCR

According to the company's instructions, the RNA was extracted from EDTA peripheral blood samples. The mRNA expression of the sequences of primers: miR-150 forward: 5′-GCTCTCCCAACCCTTGTACC -3′, reverse: 5′-GTGCAGGGTCCGAGGT-3 and U6 was used as a housekeeping gene; the relative expression level was determined using the 2-ΔΔCT method.

Statistical analysis

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Statistical analyses were performed by SPSS-26 (SPSS Inc, United States). Mean ± SD, or median, was used to express all the data in this study. A chi-square test was used for categorical variables to compare different groups. Student's t-test was used for quantitative variables to compare between two studied groups. Analysis for receiver operating characteristic (ROC) with the area under the curve (AUC) was used to evaluate the diagnostic value of miR-150-5 p. We considered P significant at <0.05 with a 95% confidence interval (CI).

Results

As expected, there were significant differences between the studied groups regarding CLL patients' clinical characteristics, as demonstrated in Table 1.

Table 1. clinical characteristics of studied patients.

Parameters	Control group, (n=25)	CLL group, (n=25)	P value
Age (years)	66.86±5.93	63.57±6.32	0.065
Sex [n (%)]:			
Male	13(52%)	11(44%)	0.389
Female	12(48%)	14(56.7%)	
B symptoms			<0.001*
No	25 (100%)	10(40%)	
Yes	0 (0%)	15(60%)	
Fever:			<0.001*
No	25 (100%)	10(40%)	
Yes	0 (0%)	15(60%)	
Weight loss:			<0.001*
No	25 (100%)	14 (56.7%)	
Yes	0 (0%)	11(44%)	
Bleeding:			<0.001*
No	25 (100%)	19 (76%)	
Yes	0 (0%)	6 (24%)	
Spleen:			<0.001*
No	25 (100%)	13(52%)	
Yes	0 (0%)	12(48%)	
Lymphadenopathy:			<0.001*
No	25 (100%)	19(76%)	
Yes	0 (0%)	6(24%)	

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Anemia:		7 (28%)	<0.001*
No	25 (100%)	18 (72%)	
Yes	0 (0%)		
Thrombocytopenia:			<0.001*
No	25 (100%)	16 (64%)	
Yes	0 (0%)	9 (36%)	
AIHA			<0.001*
No	25 (100%)	23 (92%)	
Yes	0 (0%)	2 (8%)	
ITP			<0.001*
No	25 (100%)	23 (92%)	
Yes	0 (0%)	2 (8%)	

CLL; chronic Lymphocytic Leukemia, AIHA; autoimmune hemolytic anemia, ITP; immune thrombocytopenia, p-value significant when p ≤ 0.05.

The current research concerning laboratory tests showed significant differences between the studied group's regards. WBC, ALC, hemoglobin, platelets. As expected, there were significantly higher values of WBC and ALC in the CLL group compared to controls. On the other hand, there were significantly lower values of hemoglobin and platelets in the CLL group compared to the control group. Concerning severity and diagnostic markers, LDH, serum β2M, ZAP-70% 17p del/ p53 mutation, and CD38% were significantly higher in the CLL group than in controls. Regards risk stratification, according to the Rai staging system, 10(40%) patients had low risk, 9(36%) patients had intermediate risk, and 6(24%) patients were high-risk. (Table 2, P<0.01*). We also used the Binet staging system to evaluate CLL's severity further. According to Binet staging system, 11(44%) patients in stage A, 8(32%) patients in stage B and 6(24%) patients in stage C. (Table 2, P<0.01*).

Table 2. laboratory characteristics of the studied patient.

Parameters	**Control group, (n=25)**	**CLL group, (n=25)**	**P value**
WBC (× 109/L)	6.13±1.93	78.70±55.11	<0.001*
ALC (× 109/L)	2.73±0.93	58.484±46.32	<0.001*
Hemoglobin (g/dl)	13.5±0.61	10.3±1.96	<0.001*
Platelets (× 109/L)	237.2± 35.3	192.4±96.5	<0.001*
AST (IU/L)	18.59±3.9	19.4±3.2	0.719
ALT(IU/L)	21.93±3.5	22.9±5.3	0.412
Total bilirubin (mg/dl)	0.988±0.133	1.001±0.272	0.7943

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Albumin (g/dl)	4.14±0.16	4.10±0.22	0.465
S. creatinine (mg/dl)	0.72±0.22	0.74±0.25	0.576
Alpha-fetoprotein(ng/ml)	8.89±2.9	8.9 ±5.2	0.592
LDH	200.2± 41.3	422.4±137.5	<0.001*
Serum β2M (ng/dl)	2.52 ± 0.77	5.80 ± 1.26	<0.001*
ZAP-70%			<0.001*
ZAP-70- (<20%)	25(100%)	17(68%)	
ZAP-70+(≥ 20%)	0(0%)	8(32%)	
CD38%			<0.001*
CD38- (<20%)	25(100%)	16 (64%)	
CD38+ (≥20%)	0(0%)	9 (36%)	
17p del/ p53 mutation			<0.001*
Positive	0(0%)	3(12%)	
Negative	25(100%)	22(88%)	
Modified Rai staging system:			<0.001*
Low risk	0(0%)	10(40%)	
Intermediate risk		9(36%)	
High-risk		6(24%)	
Binet staging system.	0(0%)		<0.001*
Stage A		11(44%)	
Stage B		8(32%)	
Stage C		6(24%)	

*Hb; hemoglobin, WBC; white blood cells, ALC; absolute lymphocyte count, AST; aspartate aminotransferase, ALT; alanine transaminase, LDH; lactate Dehydrogenase ZAP-70%; Zeta-chain-associated protein kinase 70. A chi-square test was used for categorical variables to compare different groups. Student's t-test was used for quantitative variables. *P < 0.05 when compared with the control group.*

The Circulatory miR-150-5 p expression levels in studied groups.

CLL patients (0.464±0.16) had a significantly lower level of miR-150-5 p expression than the control group (0.88±0.12), figure 2, p <0.001*.

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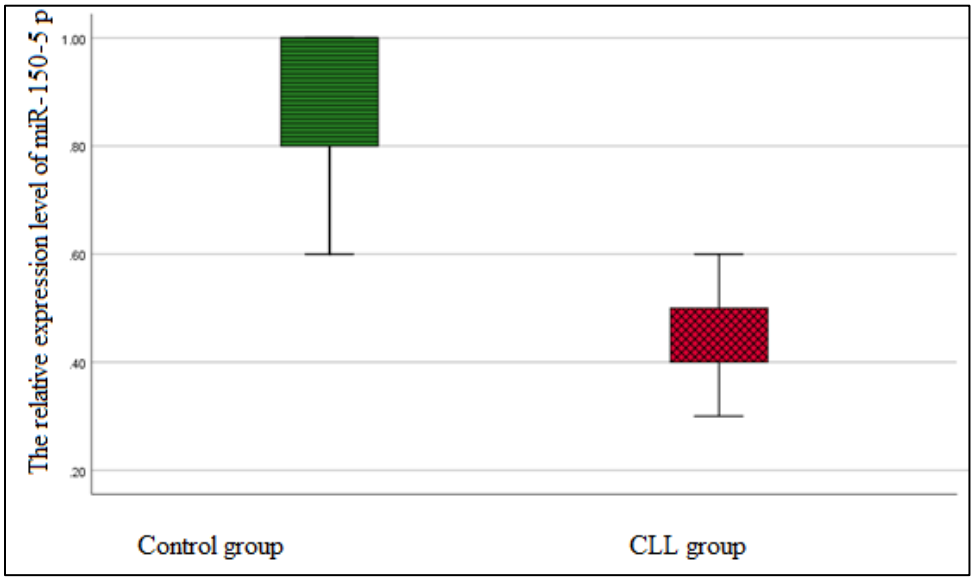

Figure 2. ***Comparison between studied groups regards relative expression level of miR-150-5 p.***

The relationship between the relative expression level of miR-150-5 p and clinicopathological characteristics in CLL patients.

According to the current study, there were statistically significant differences regards HCV infection percentage among CLL patients, with about 19 (76%) of CLL patients not infected with HCV and 6 (24%) of CLL patients infected with HCV P <0.001*. In addition, 11(55%) of patients had B symptoms, 6(24%) of patients had Lymphadenopathy and 12(48%) of patients had HSM. (Table 3).

Table 3. The relationship between the relative expression level of miR-150-5 p and clinicopathological characteristics in CLL patients.

Parameters	CLL patients (n=25)	miR-150-5 p
Non-HCV	19 (76%)	0.466±0.206
HCV	6 (24%)	0.61±0.16
P value	<0.001*	0.051
B symptoms [n (%)]		
Present	10(40%)	0.57±0.15
Absent	15(60%)	0.49±0.07
P value		0.289
Site of involvement [N (%)]		
Lymphadenopathy	6 (24%)	0.47±0.80
HSM	12(48%)	0.51±0.01
P value		
		0.604

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ZAP-70%		
ZAP-70- (<20%)	17(68%)	0.77±0.15
ZAP-70+(≥ 20%)	8(32%)	0.52±0.19
P value		
		0.003
CD38%		
CD38- (<20%)	16 (64%)	0.75±0.14
CD38+ (≥20%)	9 (36%)	0.53±0.19
P value		
		0.006
17p del/ p53 mutation		
Positive	3(12%)	0.533±0.05
Negative	22(88%)	0.71±0.20
		0.138
Modified Rai staging system:		
Low risk	10(40%)	0/75±0. 2
Intermediate risk	9(36%)	0.59±0.01
High-risk	6(24%)	0.46±0.2
P value		
		0.008
Binet staging system.		
Stage A	11(44%)	0.76±0.3
Stage B	8(32%)	0.57±0.2 0.46±0.1
Stage C	6(24%)	0.004
P value		
Treatment of CLL		
No treatment	18(72%)	0.49±0.13
Chemotherapy	7(28%)	0.77±0.07
P value		<0.001*

The relationship between the relative expression level of miR-150-5 p and treatment in CLL patients.

In the current study, 18(72%) patients were newly diagnosed with CLL or in the "watch and wait" phase. Those patients were assigned to wait and see strategy and were monitored periodically every three months for disease progression. As a result, seven Egyptian patients (28%) were indicated for therapy and successfully treated with a good response.

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The treatment modalities included FCR. (Fludarabine, cyclophosphamide, and rituximab) and ibrutinib. Interestingly, there were significantly lower miR-150-5 p in the no-treatment subgroup (n=18, 0.49±0.13) compared to successfully treated subgroups with good response (n=7, 0.77±0.07), table 3.

Pearson correlations between the relative expression level of miR-150-5 p with clinical and laboratory parameters among the CLL group

The relative expression level of miR-150-5 p was negatively correlated with WBC ALC, serum β2M, ZAP-70%, CD38%, and LDH. In contrast, miR-150-5 expression level was positively associated with hemoglobin, and platelet, P <0.001* (Table 4).

Table 4. ***Correlation of serum levels of miR-150-5 p with clinical and laboratory characteristics in CLL patients.***

parameters	miR-150-5 p	
	r	p
WBC	-0.842	<0.001*
ALC	-0.820	<0.001*
Hemoglobin	0.723	<0.001*
Platelets	0.446	<0.001*
AST	-0.138	0.341
ALT	-0.162	0.260
Total bilirubin	-0.179	0.213
Albumin	0.158	0.274
S. creatinine	0.076	0.599
Alpha-fetoprotein	-0.098	0.374
LDH	-0.528	<0.001*
Serum β2M	-0.478	<0.001*
ZAP-70%	-0.384	<0.001*
CD38%	-0.503	<0.001*
17p del/ p53 mutation	-0.276	0.182

linear regression analysis in the CLL group

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According to the current findings, the miR-150-5 p levels had significant correlations with different laboratory parameters. To address this point, we applied linear regression analysis. As a result, we revealed that ALC and platelets were the main predictors of the relative expression levels of miR-150-5 p among other laboratory tests, P <0.001* (Table 5).

Table 5. linear regression analyses to examine the main independent variables against the relative expression level of miR-150-5 p within CLL patients.

	Unstandardized Coefficients		Standardized Coefficients			95% C.I.	
Model	B	S.E.	Beta	t	P value	Lower Bound	Upper Bound
Constant	-0.704	0.187		-3.764	0.000	-1.080	-0.328
WBC	0.001	0.001	0.109	0.952	0.346	-0.001	.002
ALC	0.102	0.018	0.670	5.841	<0.001*	0.067	00.138
Hemoglobin	0.001	0.001	0.147	1.222	0.228	0.000	0.002
Platelets	0.077	0.030	0.505	2.574	<0.001*	0.017	0.137
LDH	0.000	0.000	-0.181	-1.040	0.303	-0.001	0.000

The accuracy of miR-150-5 p expression levels for discriminating CLL from control by ROC analysis

The exciting finding of the current research is that the power of miR-150-5 p expression levels to differentiate CLL from control was evaluated using ROC analysis. The AUC was 0.965 (95% CI = 0.901-1.000) with sensitivity = 96.2%, specificity = 98 %, and the cutoff values (0.553), figure 3, P-value <0.001*.

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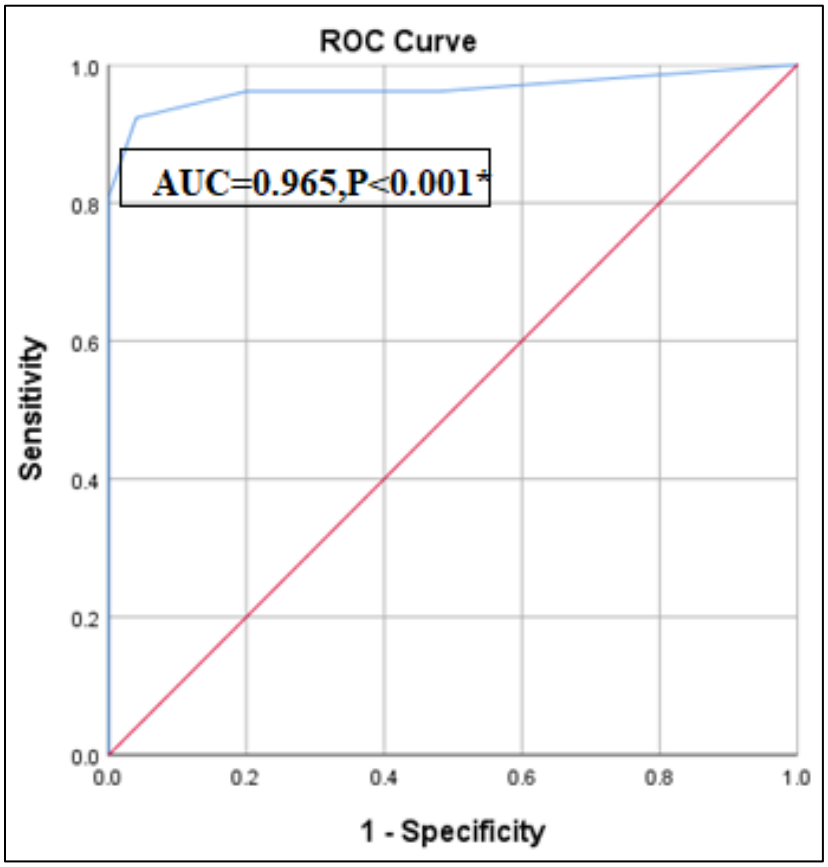

Fig 3. ***Receiver operating characteristic curve of the relative expression levels of miR-150-5 p for diagnosis CLL.***

Discussion

Several studies reported that HCV infects not only hepatocytes but also extrahepatic cells, for example, B lymphocytes [12]. The oligoclonal proliferation of the infected B-cells and the presence of HCV in lymphocytes could initiate growth dysregulation and predispose the lymphocyte to develop molecular changes and malignant B-cell lymphoproliferative disease, for example, CLL [13]. Indeed, the prevalence of monoclonal B-cell lymphocytosis (MBL) in HCV-infected patients was higher than in the general population [14].

CLL is more prevalent in the elderly, and to overcome any drawbacks in the results, we selected a control group matched to the CLL group regarding age and sex. Therefore, the present study aimed to explore the prevalence of HCV infection among studied CLL

patients, and we detected that there were statistically significant differences regards HCV infection percentage among CLL patients, with about 19 (76%) of CLL patients not infected with HCV and 6 (24%) of CLL patients were infected with HCV.

Similarly, Vladareanu et al. observed a higher frequency of chronic lymphoproliferative disorders related to HCV infections in particular older women [15]. They contributed their findings to persistent activation by HCV leading to B–clone proliferation in the bone marrow and liver and fatal alteration. HCV antigens such as E2 protein have the binding site CD 81, representing the specific hepatocyte and B–lymphocyte receptor for HCV[16].

On the contrary, other studies detected non-statistically significant differences regards the prevalence of HCV infection among CLL [patients [17,18]. This difference could be due to the small sample size and the low prevalence of HCV in the countries where these studies were conducted [19].

This is consistent with a previous finding that miRNAs abundantly expressed in CLL may regulate the expression of genes encoding proteins participating in the critical molecular pathways, such as those involved in BCR signaling [20]. Interestingly, the most important results of this analysis indicated that CLL patients had a significantly lower level of miR-150-5 p expression than other studied groups. Additionally, the expression values of miR-150-5 p were significantly inversely correlated with laboratory and clinicopathological features of CLL as well as the severity of CLL according to the Modified Rai staging system and Binet staging system.

In line with this, Ling et al. study found miR-150 as a tumor suppressor in osteosarcoma. Concerning the influence of treatment with doxorubicin-induced apoptosis by targeting RUNX2, they revealed that its level improved after treatment. Remarkably, these findings propose that miR-150 could be a potential therapy in future osteosarcoma treatment [21]. Similar results detected by Mraz et al. postulated that low-level expression of miR-150 was associated with unfavorable clinical and prognostic markers of CLL [22].

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Similar effects were observed in Szurián et al. research, which investigated miR-150 in progenitor B- and T-cells and observed under-expression in them. Nevertheless, miR-150 overexpressed in mature cells. Intriguingly they detected that the epigenetic biomarker was correlated with poor prognosis in CLL [23].

However, compared to our data, Chiaretti et al. observed a significant upregulation of mir-150 in CLL patients compared to the healthy group and recommended that mir-150 could initiate or progress proliferation in CLL. However, they recommended further investigations to elucidate these findings [23]

Interestingly, concerning the treatment of CLL, In the current study, 18(72%) patients did not receive treatment as we mentioned before, and seven Egyptian patients (28%) received treatment modalities, including FCR. (Fludarabine, cyclophosphamide, and rituximab) and ibrutinib. Interestingly, there were significantly lower miR-150-5 p in the non-treatment subgroup than in successfully treated subgroups. But on the other hand, treating patients with CLL increases miR-150-5 p levels. So, we could use this epigenetic marker as a diagnostic and prognostic marker.

Despite these pieces of evidence, there is a substantial gap in our knowledge about the diagnostic and prognostic role of miR-150-5 p in hematological cancers. To address these issues, we further evaluated our findings with Pearson correlations. We detected that the relative expression level of miR-150-5 p was negatively correlated with WBC ALC, serum β2M, and ZAP-70%, respectively CD38%, and LDH. In contrast, miR-150-5 expression level was positively correlated with hemoglobin and platelet. In addition, linear regression analysis revealed that ALC and platelets were the main predictors of the relative expression levels of miR-150-5 p among other laboratory tests.

To further understand the observations, we performed a ROC analysis. The power of miR-150-5 p expression levels to differentiate CLL from control had sensitivity (96.2%) and specificity (98 %).

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Conclusions: The current research findings revealed that miR-150-5p values were under-expressed in CLL patients, particularly patients with HCV infection, and significantly inversely correlated with clinical and laboratory characteristics as well as the severity of CLL. Thus, it could be helpful for diagnostic and prognostic purposes in CLL.

The limitation and recommendations of the study

The fundamental limitations of the current study were the small sample size of the analysis and the case-control study design, as we needed to assess the prevalence of HCV in CLL. Therefore, we recommend further extensive cross-sectional studies and multicenter to confirm the study results so we can use mir-150-5p diagnostic and prognostic epigenetic markers in CLL.

List of abbreviations

CLL: Chronic lymphocytic leukemia.

HCV: hepatitis C virus.

MiRNAs: MicroRNAs.

β2M: β2 microglobulin .

CD38: Cluster of differentiation 38

ZAP70: Zeta-chain-associated protein kinase 70

RT-PCR: A real-time polymerase chain reaction

IWCLL: International Workshop on Chronic Lymphocytic Leukemia

ALC: absolute lymphocyte count

Footnotes.

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Data availability

All the data obtained and analyzed are included in this manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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Authors' contributions: all authors participated in collecting data, writing the manuscript, and revising it.

References

1. Bianco E, Marcucci F, Mele A, et al. Prevalence of hepatitis C virus infection in lymphoproliferative diseases other than B- cell non-Hodgkinís lymphoma, and myeloproliferative diseases: An Italian multi-center case-control study. Haematologica 2004; 89: 70-76.

2. Hallek M, Shanafelt TD, Eichhorst B. Chronic lymphocytic leukemia. Lancet 2018 Apr;391(10129):1524-1537.

3. Karan-Djurasevic T, Pavlovic S. Somatic hypermutation status and gene repertoire of immunoglobulin rearrangements in chronic lymphocytic leukemia. Lymphocyte updates: cancer. Autoimmunity and Infection 2017 Jul;49.

4. Agathangelidis A, Ljungström V, Scarfò L, et al. Highly similar genomic landscapes in monoclonal B-cell lymphocytosis and ultra-stable chronic lymphocytic leukemia with low frequency of driver mutations. Haematologica 2018 May;103(5):865-873.

5. Guièze R, Wu CJ. Genomic and epigenomic heterogeneity in chronic lymphocytic leukemia. Blood 2015 Jul;126(4):445-453.

6 Parikh SA, Shanafelt TD. Prognostic factors and risk stratification in chronic lymphocytic leukemia. Semin Oncol 2016 Apr;43(2):233-240.

7. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol. 2007;12:1–12. doi: 10.1016/j.ydbio.2006.08.028

8. Xiao C, Calado DP, Galler G, et al. MiR-150 Controls B cell differentiation by targeting the transcription factor c-Myb. Cell. (2007) 131:146–59. doi: 10.1016/j.cell.2007.07.021

9. Zhou L, Park JJ, Zheng Q, Dong Z, Mi Q. MicroRNAs control iNKT and regulate T-cell development and function. Cell Mol Immunol. (2011) 8:380–7. doi: 10.1038/cmi.2011.27.

10. Abdul-Maksoud R S, Elsayed R S, Elsayed W S, et al . Combined serum miR-29c and miR-149 expression analysis as diagnostic genetic markers for colorectal cancer. Biotechnol Appl Biochem. 2021;68(4):732–43.

11. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018;131(25):2745–60.

12- Stanislas P., Vallet-Pichard A, Olivier H. Extrahepatic cancers and chronic HCV

infection. Nature Reviews Gastroenterology & Hepatology 2018; 15: 283-290.

13- Ghia P. Another piece in the puzzle: Is there a -cell lymphocytosis? Haematologica 2011; 96 (8): 1089-1091.

14- Rawstron A, Green M, Kuzmicki A, et al. Monoclonal B lymphocytes with the characteristics of lymphocytic leukemia are present in 3.5% of

adults with normal blood counts. Blood 2003; 100: 635-639.

15.Vladareanu AM, Ciufu C, Neagu AM, et al. The impact of hepatitis viruses on chronic lymphoproliferative disorders - Preliminary results. J Med Life. 2010;3:320–9.

16. Quinn ER, Chan CH. The B–cell receptor of a hepatitis C virus (HCV) associated non–Hodgkin's lymphoma binds the viral E2 envelope protein, implicating HCV in lymphomagenesis. Blood. 2001;98(13):3745–3749.

17. Bianco E, Marcucci F, Mele A, et al. Prevalence of hepatitis C virus infection in lymphoproliferative diseases other than B-cell non-Hodgkin's lymphoma, and myeloproliferative disorders: An Italian Multi-Center case-control study. Haematologica. 2004;89:70–6.

18. Murashige N, Kami M, Iwata H, Kishi Y, Matsuo K. No relationship between hepatitis C infection and risk of myeloid malignancy. Haematologica. 2005;90:572–4.

19. Gentile G, Mele A, Monarco B, et al. Hepatitis B and C viruses, human T-cell lymphotropic virus types I and II, and leukemias: A case-control study. The Italian Leukemia Study Group. Cancer Epidemiol Biomarkers Prev. 1996;5:227–30.

20. Mraz M, Chen L, Rassent L Z, et al. miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1Blood. 2014 Jul 3; 124(1): 84–95.

21. Ling Z, Gentao Fan, Danhua Yao, et al. MicroRNA-150 functions as a tumor suppressor and sensitizes osteosarcoma to doxorubicin-induced apoptosis by targeting RUNX2Exp Ther Med. 2020 Jan; 19(1): 481–488.

22. Szurián K, Csala I, Piurkó V, Deák L, Matolcsy A, Reiniger L. Quantitative miR analysis in chronic lymphocytic leukaemia/small lymphocytic lymphoma-proliferation centres are characterized by high miR-92a and miR-155 and low miR-150 expression. Leuk Res. 2017;58:39–42. doi: 10.1016/j.leukres.2017.04.002.

23.Chiaretti S, Valerio Fulci V, Marina Goldoni BS, e al. The MicroRNA (miR) Profile in B-Cell Chronic Lymphocytic Leukemia (CLL) Reveals a Differential Expression of miR-21, miR-155, and miR-150 between Leukemic and Normal B Lymphocytes and of miR-

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150, miR-29bc, and miR-223 between IgVH Mutated and Unmutated Patients. Blood, 2006Nov 16; 108(11):298.