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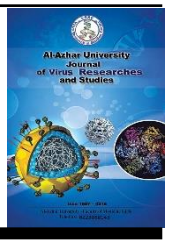

Identification of Circulating Micro RNA-155 as a Potential Biomarker for Detecting Acute Myeloid Leukemia

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Abstract

MicroRNA-155(MiR-155) is one of the important miRNAs that contributes greatly to the pathogenesis of diverse hematological malignancies with complex oncogenic as well as tumor repressor roles depending on the disease context and tissue type. It likely plays a very important role in the pathogenesis of acute myeloid leukemia (AML) through regulating cell signal transduction pathways of cell proliferation, differentiation and apoptosis. Several studies reported that the expression of miR-155 is low in normal hematopoietic cells and often upregulated in AML and it is an important indicator to assess the diagnosis, treatment and prognosis of patients with AML and further associated with tumor progression. 25 adult patients newly diagnosed with AML, their ages ranged from (18 to 60) years old, and 25 cases apparently healthy individuals, their ages were matched with patients, were included into the study. Complete blood count (CBC), Peripheral blood smear examination, bone marrow aspiration (BMA), immunophenotyping, cytogenetic analysis, molecular analysis, serum lactate dehydrogenase level (LDH) and MiR-155 expression level using Real-Time PCR were done. Significant upregulation in miR-155 gene expression in AML group when compared to control group. Hemoglobin concentration, and platelets count were lower in AML group than control group, however total leucocytic count (TLC) and LDH, were increased in AML group than control group. MiR-155 gene expression showed significant positive correlation with TLC, LDH, peripheral blasts and bone marrow blasts. Otherwise, no significant correlations were found between miR-155 gene expression with age, hemoglobin concentration and platelets count in AML patients. Upregulated miR-155 gene expression was significantly associated with failure of CR, relapse and death. AML cases with high miR-155 gene expression showed significantly shorter OS and DFS when compared to low miR-155 gene expression.

Keywords: AML, MicroRNA, miR-155.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by the clonal expansion and loss of differentiation of myeloid blasts in the bone marrow and peripheral blood. It starts in bone marrow and most often it quickly moves into the blood, usually begins in cells that turn into white blood

cells, but it may start in other blood-forming cells, as well. It sometimes spread to other body parts as the lymph nodes, spleen, liver, central nervous system and testicles [1]. Acute myeloid leukemia is one of the most common types of acute leukemia in adults. It is fairly rare overall, accounting for only about 1% of all cancers. AML is slightly more common among men than women AML is generally a disease of older people and is uncommon before the age of 45. The average age of people when they are first diagnosed with AML is about 68, but AML can occur in children as well [2]. MicroRNAs are a class of small, non-coding single-stranded RNAs comprising approximately 19–22 nucleotides. MiRNAs are involved in a variety of biological processes, such as proliferation, apoptosis and differentiation of cells and hematopoiesis. MiRNAs may act as oncogenes or tumor suppressors in malignant diseases. As a result, the expression profile of cancer miRNAs can define tumor type, susceptibility, prognosis and response to treatment [3]. Aberrant expression of miR-155 has been found to be associated with various types of hematological malignancies. It can have either an oncogenic or a tumor-repressor effect, depending on the nature of the tissue and the type of malignancy. The expression of miR-155 is often upregulated in AML (~10- to 50-fold above normal), where it likely mediates aberrant cell differentiation and proliferation [4]. MicroRNA-155 expression is an independent prognostic marker for patients with cytogenetically normal AML. Patients with higher miRNA-155 expression experience significantly lower rates of complete remission and shorter disease-free and overall survival [5]. The relevance of miR-155 in AML open a new path to the possibility that the depletion or inhibition of miR-155 may provide a therapeutic angle [6].

2. Materials and Methods

This case control study was carried out in the Clinical Pathology Department, International medical center, started from December 2019 and up to 2 years. 50 subjects were included in this study aged from (18 – 60) years old, of both sexes (22 males and 23 females), they were classified into 25 adult patients newly diagnosed with AML and 25 apparently healthy adult subjects, age and sex matched normal healthy donors for bone marrow transplantation. The selection of patients was based on inclusion and exclusion criteria.

2.1. Patient Inclusion Criteria

a) Age ≥ 18 years.

b) Newly diagnosed AML patients.

2.2. Patient Exclusion Criteria

a) Patients who were diagnosed with other malignancies at any time period **.**

b) Previous exposure to chemotherapy or radiotherapy**.**

All studied groups were subjected to general medical evaluation, full clinical examination and laboratory investigation for appropriate diagnosis:

I) Complete blood picture: 2 ml blood samples were withdrawn under complete aseptic condition on EDTA tubes for CBC analysis using Sysmex XN-1000 SA-01 .

II) Peripheral blood smear examination: examination of Leishman-stained peripheral blood smears for differential leucocytic count and assessment of blast cell number and morphology .

III) Bone marrow aspiration (BMA): for morphologic examination by Leishman-

stained smear, blasts count detection and cytochemistry.

IV) Immunophenotyping: 3ml BMA sample were collected on heparin tubes for acute leukemia panel that includes CD13, CD117, myeloperoxidase (MPO), CD33, CD34, CD64, CD11c, CD19, human leucocytic antigen-DR (HLA DR) and major histocompatibility complex class II (MHC II), using Beckman Coulter Navios Flow cytometer. v) Cytogenetic analysis: 3ml of BMA sample were collected on heparin tubes and analyzed for t (8:21), inv (16), t (8,16).

VI) Molecular analysis: 3ml EDTA BMA sample for Fms-related tyrosine kinase3-Internal tandem duplications (FLT3-ITD) and Nucleophosmin1 (NPM1) detection .

VII) Serum LDH level: were performed by fully automated chemistry analyzer cobas E411(Germany).

2.3. Specific Laboratory Investigation

2 ml EDTA BMA were transferred to 10 ml centrifuge tube to which added an equal volume of balanced salt solution (final volume 4 ml), which was layered onto the Ficoll-Paque media solution, so mixing the Ficoll-Paque media solution and separating the layer of mononuclear cells,2 volumes of balanced salt solution were added to the mononuclear cells in the centrifuge tube. Centrifuge at 1200 rpm for 15 min twice. The supernatant was removed. The cell pellets were collected.They were preserved using Qiazole reagent (100 µ sample+500 µ Qiazole) then stored in -80 freezer till the time of analysis of miRNA 155 by Real Time Polymerase chain Reaction (RT-PCR) in a single assay to avoid repeated freeze - thaw cycles.

3. Results

Table .1 shows no statistically significant difference between studied groups as regard age and sex. Mean age of patients was 44.6 years, they comprised of 14 males (56%) and 11 females (44%). The reference control group was matched in age and sex. A significant decrease in hemoglobin concentration, and platelets count in AML group compared to control group ($p < 0.001$ for each). Table .2 shows a significant increase in TLC and LDH, was reported in AML group compared to control group ($p = 0.001$, $p < 0.001$ respectively). Median peripheral and marrow blasts at diagnosis in AML group were (70 and 47 respectively). As regard clinical data in AML group, pallor was the commonest presentation (92%), followed by lymphadenopathy (32%), hepatomegaly (28%), fever/infection (24%), bleeding tendency (16%), Splenomegaly (16%), and skin lesions (12%). As regard immunophenotypes of all studied cases, 100% had positive CD13, 96% had positive CD33, 96% had positive MPO, 80% had positive CD117, 48% had positive HLA-DR, 48% had positive CD34, 32% had positive CD64, 24% had positive CD11c, 16% had positive CD14, 8% had positive CD19 and 32% had positive MHC II. All studied samples were subjected to t (8,21) and inv16; 24% had positive t (8,21) and 4% had positive inv16. Only one case was subjected to t (8,16) and revealed a positive result. Eleven samples were tested for FLT3 ITD mutations, 18.2% had FLT3 ITD mutations, while 10 cases were tested for NPM, and none had NPM mutations. Complete remission (CR) was achieved in 18 cases (72%), while 7 cases failed to achieve CR (28%). Out of those achieved CR, 5 cases relapsed (27.8%). Five cases died during the whole period of the study (20%). Mean OS was 10.9 months, Cumulative OS was 80%. Mean DFS was 9.7 months, Cumulative DFS was 71.8%. There was significant up regulation in median miR-155 gene expression in AML group when compared to control group (median =10.2 versus 1.9, $p<0.001$). There was 5.4-fold increase in miR-155 gene expression in AML group in relation to that in control group.

Table (1): Comparison of age and gender distribution in different studied groups.

		Control (n=25)		AML (n=25)		*p*
Age (years)	**Mean ± SD**	39.9	12.7	44.6	14	0.219
Males	**N, %**	13	52%	14	56%	0.777
Females	**N, %**	12	48%	11	44%	

Age was compared by t test. Gender was compared by Chi square.

Table (2): Comparison of laboratory results between different studied groups.

	Control (n=25)		AML (n=25)		*P*
	Median	**Range**	**Median**	**Range**	
Total leucocytic count ($X10^9$/L)	7.4	4.1-11	47	1.2-452	0.001
Hemoglobin concentration (g/dL)	13.1	11.8-16	8.3	5.9-11	<0.001
Platelet count ($X10^9$/L)	218	145-430	35	4-117	<0.001
LDH	105	79-164	765	202-3134	<0.001
Peripheral blasts (%)	-	-	70	10-95	-
Marrow blasts (%)	-	-	47	20-98	-

Man, Whitney test was used for comparison

Table (3): Clinical characteristics of studied cases:

Clinical data	AML (n=25)	
	No.	**%**
Pallor	23	92%
Fever/infection	6	24%
Bleeding tendency	4	16%
Skin lesion	3	12%
Splenomegaly	4	16%
Hepatomegaly	7	28%
Lymphadenopathy	8	32%

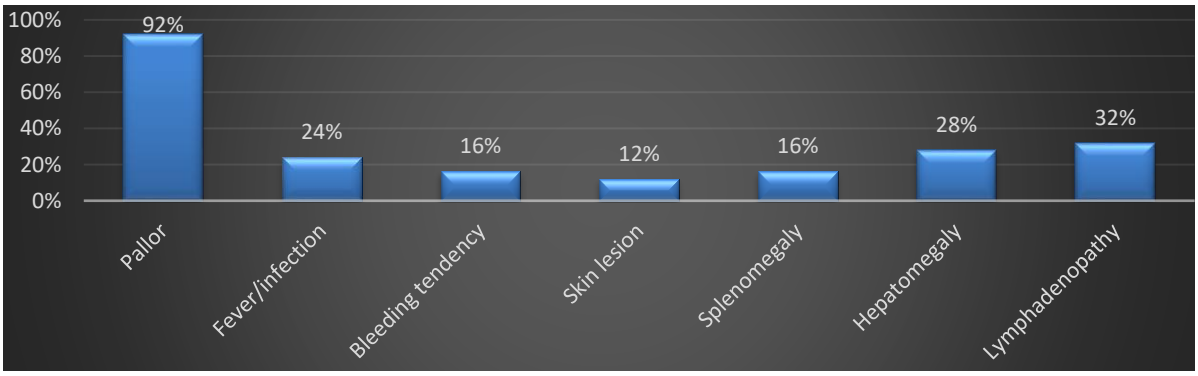

Figure (1): Column chart of clinical data among studied AML cases.

Table (4): FAB classification of studied cases:

FAB classification	AML (n=25)	
	Frequency	**%**
M1	**7**	**28%**
M2	**10**	**40%**
M4	**7**	**28%**
M5	**1**	**4%**

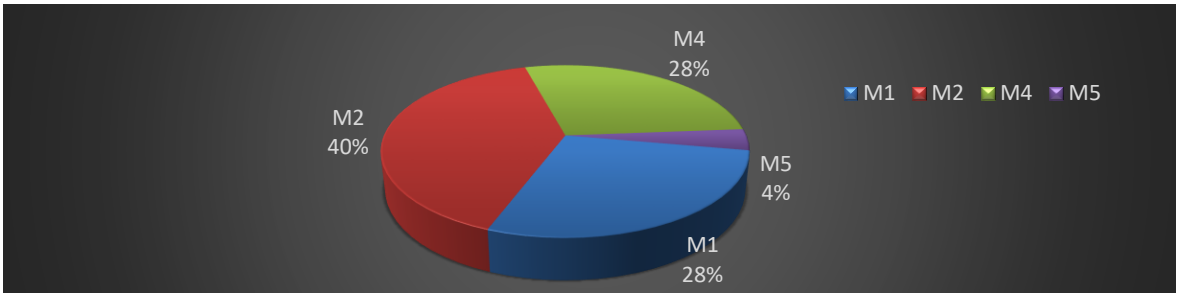

Figure (2): FAB classification of studied cases.

Table (5): Immunophenotypes of all studied cases

	AML (n=25)	
	No.	%
MPO	24	96%
CD13	25	100%
CD33	24	96%
CD117	20	80%
CD34	12	48%
HLA-DR	12	48%
CD64	8	32%
CD11c	6	24%
CD14	4	16%
CD19	2	8%
MHC II	8	32%

Table (6): Studied cytogenetic and molecular among AML cases

		AML (n=25)	
	Total done	No.	%
t (8,21)	25	6	24%
inv16	25	1	4%
t (8,16)	1	1	100%
FLT3 ITD mutation	11	2	18.2%
NPM mutation	10	0	0%

Table (7): Clinical outcome of studied cases

Clinical outcome	AML (n=25)	
	No.	%
CR	18	72%
Failure of CR	7	28%
No relapse	13/18	72.2%
Relapse	5/18	27.8%
Alive	20	80%
Died	5	20%

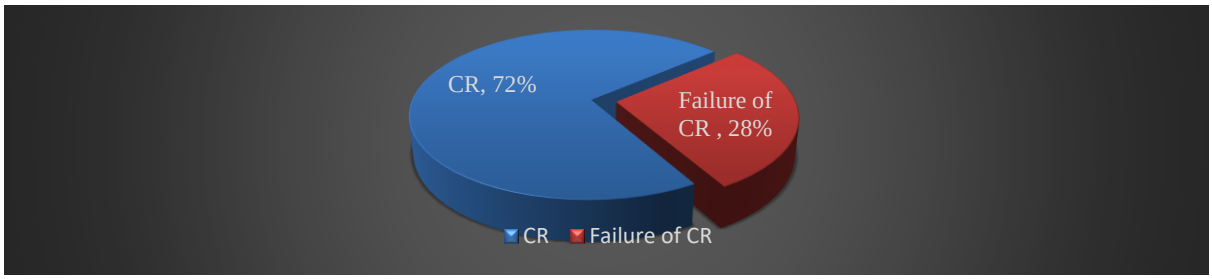

Figure (3): CR among studied cases.

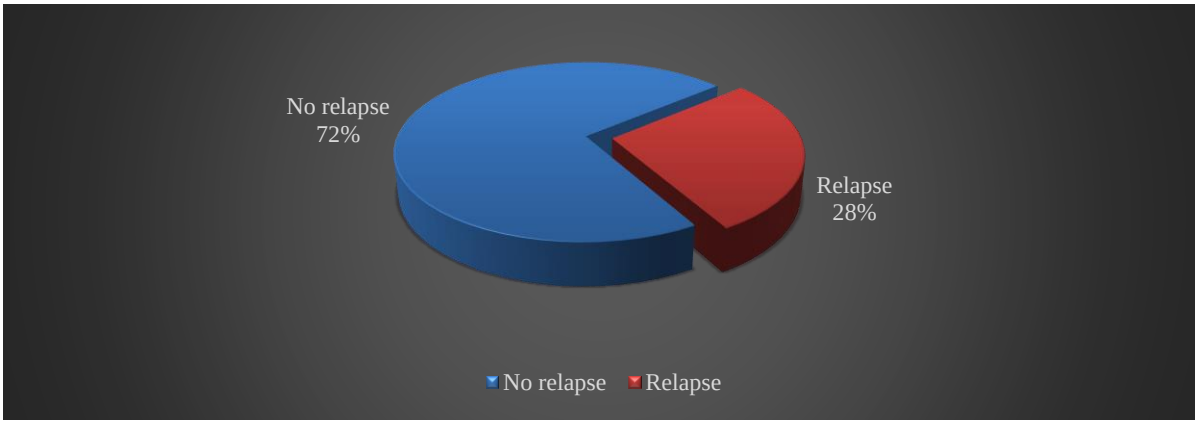

Figure (4): Relapse among studied cases

Figure (5): Relapse among studied cases

Table (8): Clinical outcome of studied cases

	AML (n=30)		
	1-year cumulative survival (%)	**Mean *(months)***	**CI 95%**
1 year-OS	80	10.9	9.2-12.6
1 year-DFS	71.8	9.7	7.8-11.5

Table (9): Comparison of miR-155 gene expression concentration between different studied groups

	Control (n=25)		**AML (n=25)**		***P***
	Median	**Range**	**Median**	**Range**	
MiR-155 gene expression	**1.9**	**0.4-3.6**	**10.2**	**3.8-18.1**	**<0.001**

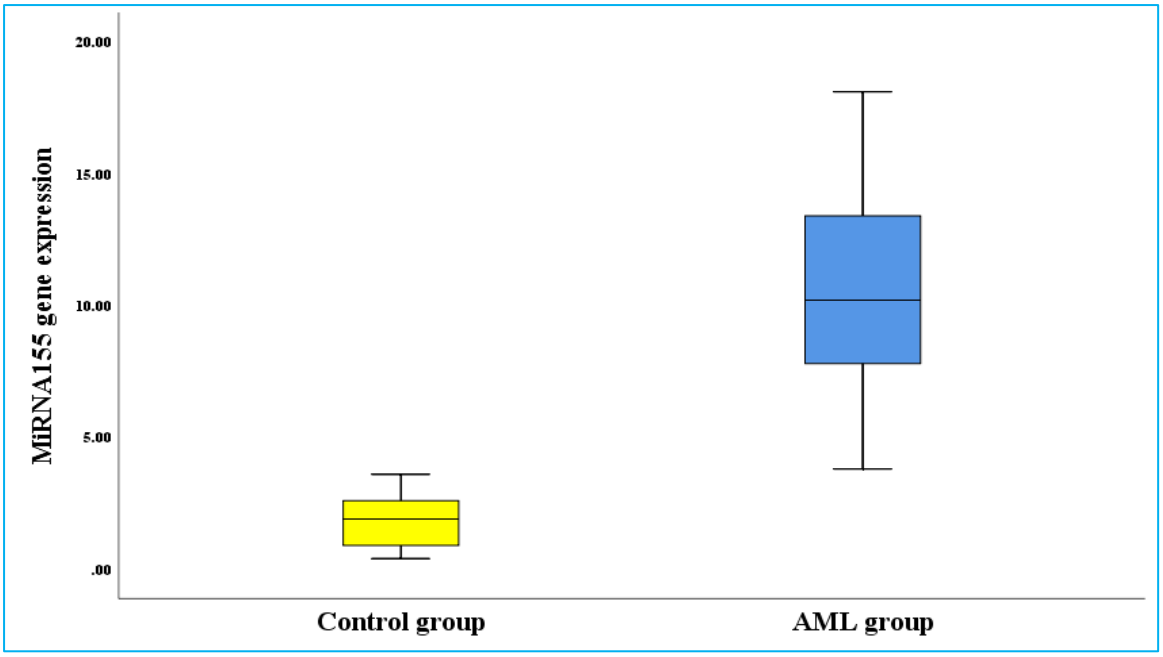

Figure (6): MiR-155 gene expression in AML and control groups

Table (10): Validity of miR-155 gene expression for prediction of non-remission among AML cases.

	MiR-155 gene expression
AUC (95% CI)	0.853 (0.703-1)
Cut off value	13.1
Sensitivity (%)	71.4
Specificity (%)	88.9
PPV (%)	71.4
NPV (%)	88.9
Accuracy (%)	84

A receiver operating characteristic (ROC) curve of miR-155 gene expression was conducted for discrimination between remission and non-remission in all studied AML cases. MiR-155 gene expression showed an AUC of 0.853. At cut off value of 13.1, sensitivity was 71.4%, specificity was 88.9%, PPV was 71.4%, NPV was 88.9%, and accuracy was 84%. That means, if an AML case had miR-155 gene expression at diagnosis above of 13.1, susceptibility of non-remission will be high, with sensitivity of 71.4% and specificity of 88.9%. MiR-155 gene expression showed significant positive correlation with TLC, LDH, peripheral blasts and bone marrow blasts. Otherwise, no significant correlations were found between miR-155 gene expression with age, hemoglobin concentration and platelets count in AML group. As shown in Figure .12 upregulated miR-155 gene expression was significantly associated with failure of CR, relapse and death. No significant associations were found regarding gender and FAB. All studied AML cases were stratified according to median level of miR-155 gene expression (median=10.2) into two subgroups: Low miR-155 gene expression (AML cases with miR-155 gene expression<10.2) and high miR-155 gene expression (AML cases with miR-155 gene expression≥10.2). AML cases with high miR-155 gene expression showed significantly shorter OS and DFS when compared to low miR-155 gene expression (OS=91.7% versus 69.2%; mean OS=12.3 versus 9.6 months, p=0.028; DFS=91.7% versus 25%; mean DFS=11.3 versus 5.9, p=0.005).

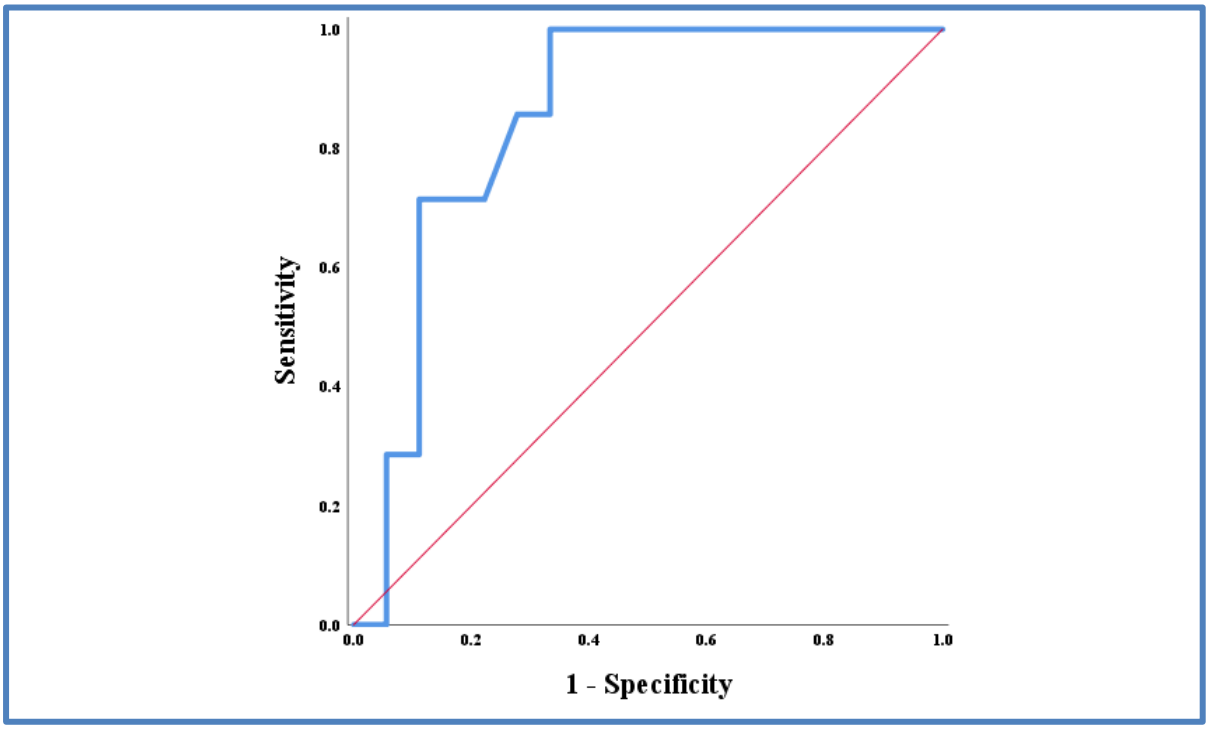

Figure (7): ROC curve of miR-155 gene expression for prediction of non-remission in all studied cases.

Table (11): Correlations between miR-155 gene expression and studied parameters in AML cases

	AML (n=25)	
	r	*p*
Age	**0.289**	**0.154**
Total leucocytic count	**0.564**	**0.003**
Hemoglobin concentration	**0.032**	**0.881**
Platelet count	**0.059**	**0.778**
LDH	**0.399**	**0.048**
peripheral blast	**0.448**	**0.025**
Marrow blasts	**0.499**	**0.011**

r, Spearman's correlation coefficient. Spearman's correlation test was used.

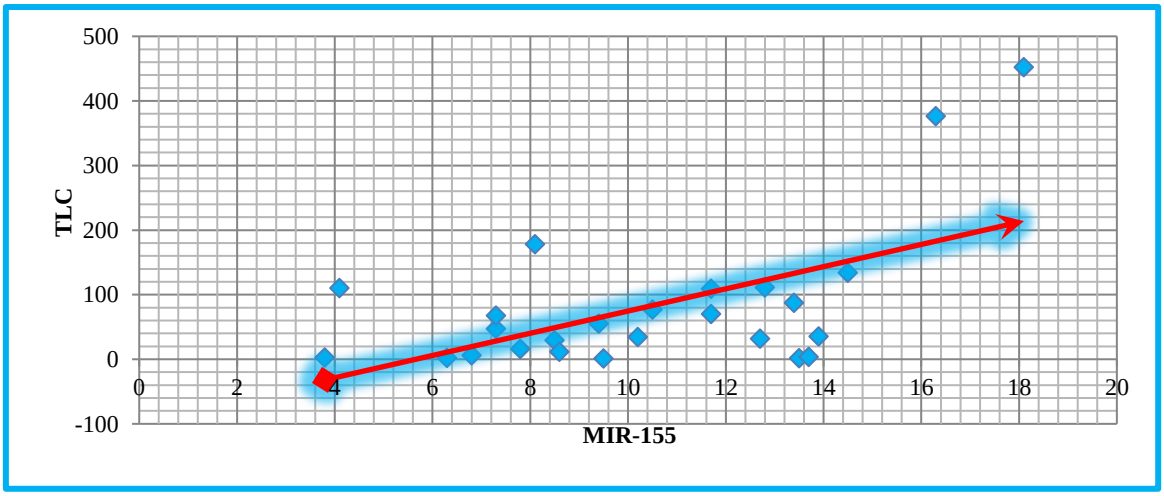

Figure (8): Positive correlation between miR-155 and TLC in AML group.

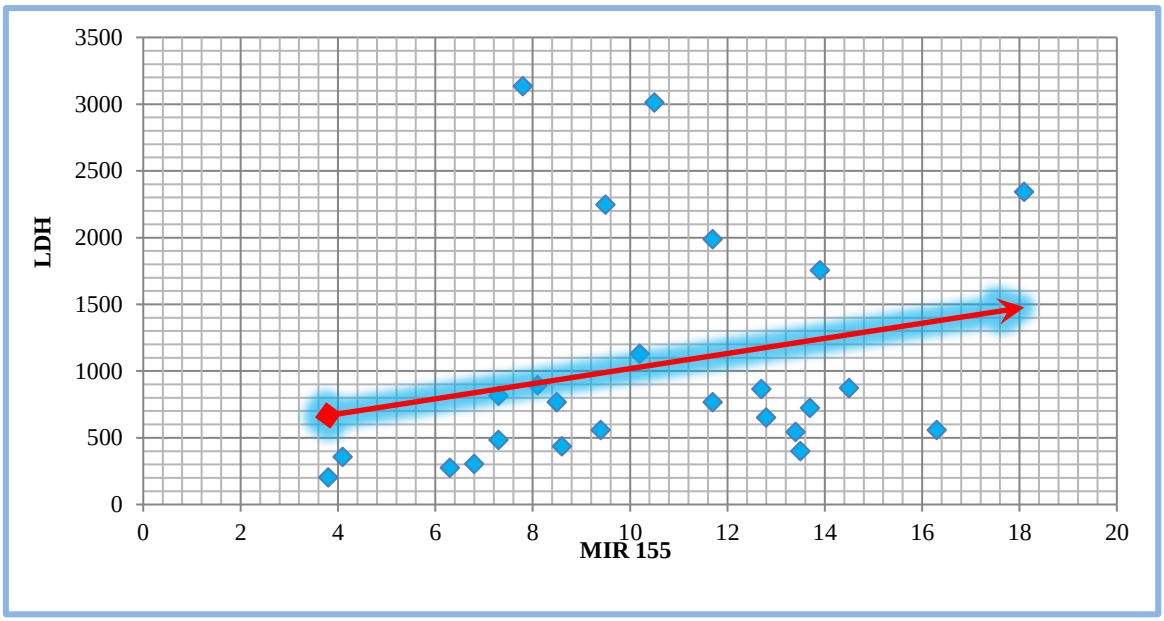

Figure (9): Positive correlation between miR-155 and LDH in AML group.

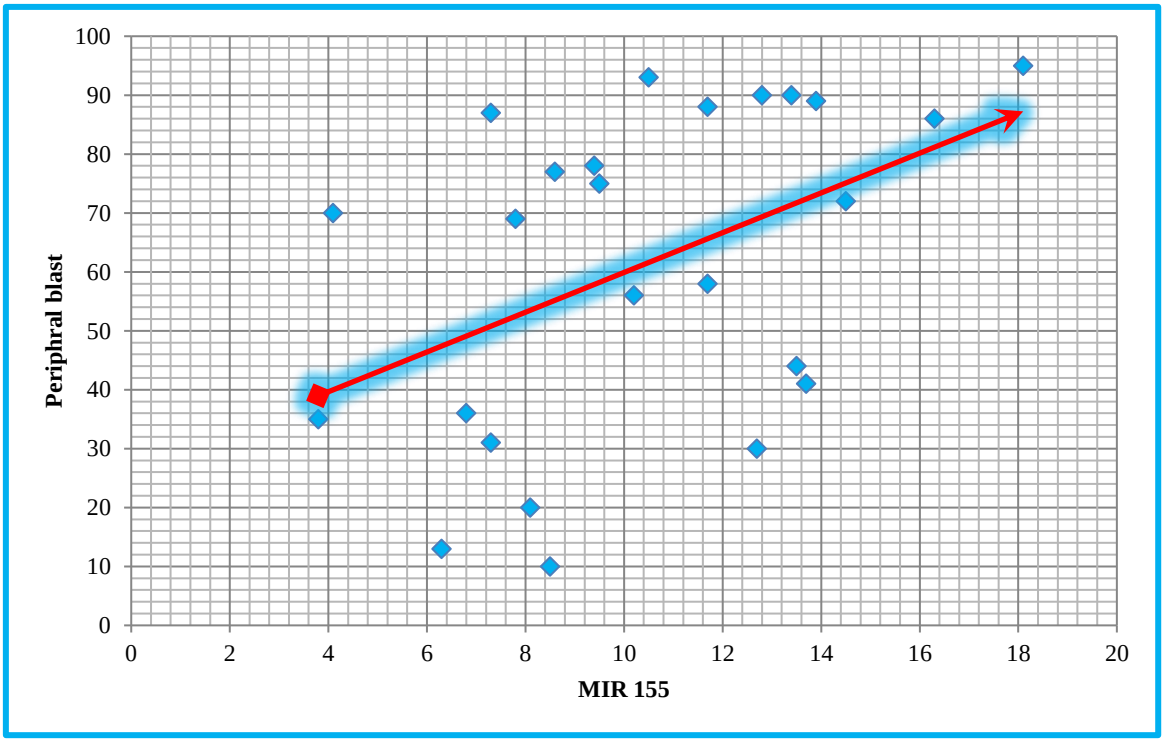

Figure (10): Positive correlation between miR-155 and peripheral blasts in AML group.

Cox regression analysis was conducted for prediction of OS in AML cases, using age, gender, marrow blasts, LDH, FLT3 ITD mutation, miR-155 gene expression as covariates. Higher marrow blasts, LDH and miR-155 gene expression were significantly associated with shorter OS in univariable analysis. Using significant covariates (with $p<0.05$) in univariable analysis into multivariable analysis revealed that only up regulated miR-155 gene expression was suggested to be independent risk predictor for shorter OS in AML cases.

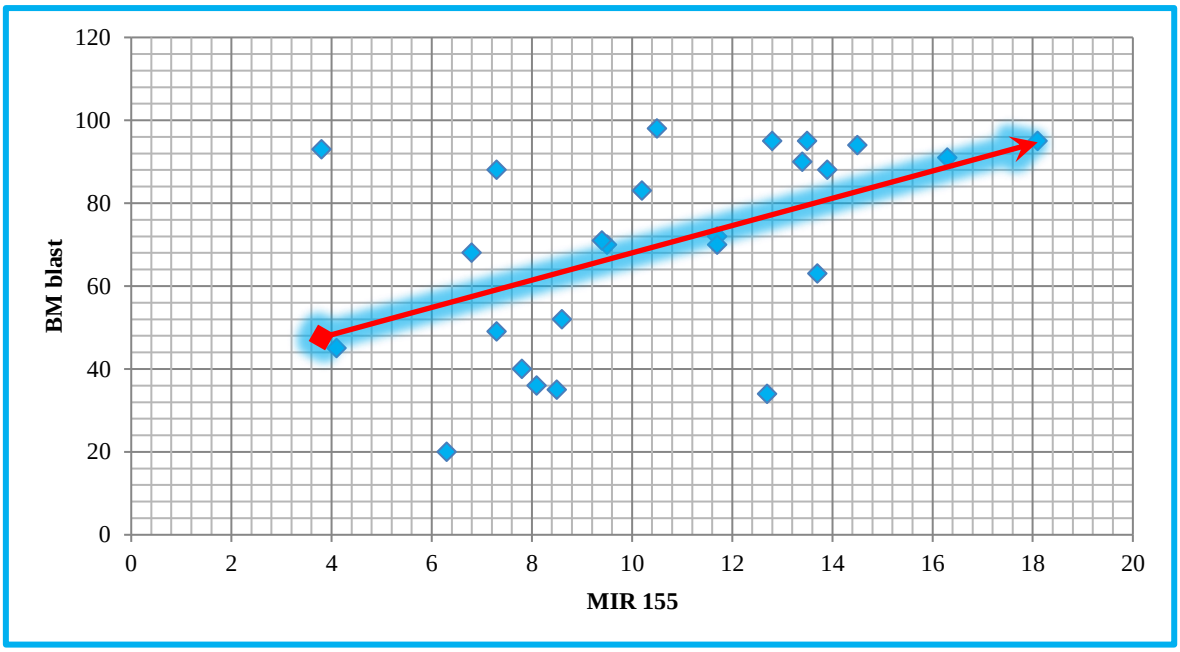

Figure (11): Positive correlation between miR-155 and BM blasts in AML group

Table (12): Association of miR-155 gene expression according to studied parameters in AML group

		MiR-155 gene expression		*p*
		Median	**Range**	
Gender	**Males**	10	3.8-16.3	0.603
	Females	10.2	4.1-18.1	
FAB	**M1**	13.4	3.8-18.1	0.487
	M2	9.55	4.1-14.5	
	M4	8.5	6.3-12.8	
	M5	11.7	11.7-11.7	
CR		8.55	3.8-18.1	**0.007**
Failure of CR		13.5	10.2-16.3	
No relapse		7.8	3.8-11.7	**0.003**
Relapse		12.8	9.5-18.1	
Alive		9	3.8-13.7	**0.005**
Died		14.5	9.5-18.1	

Mann Whitney test was used for comparison between parameters except for FAB, at which Kruskal Wallis test was used for comparison

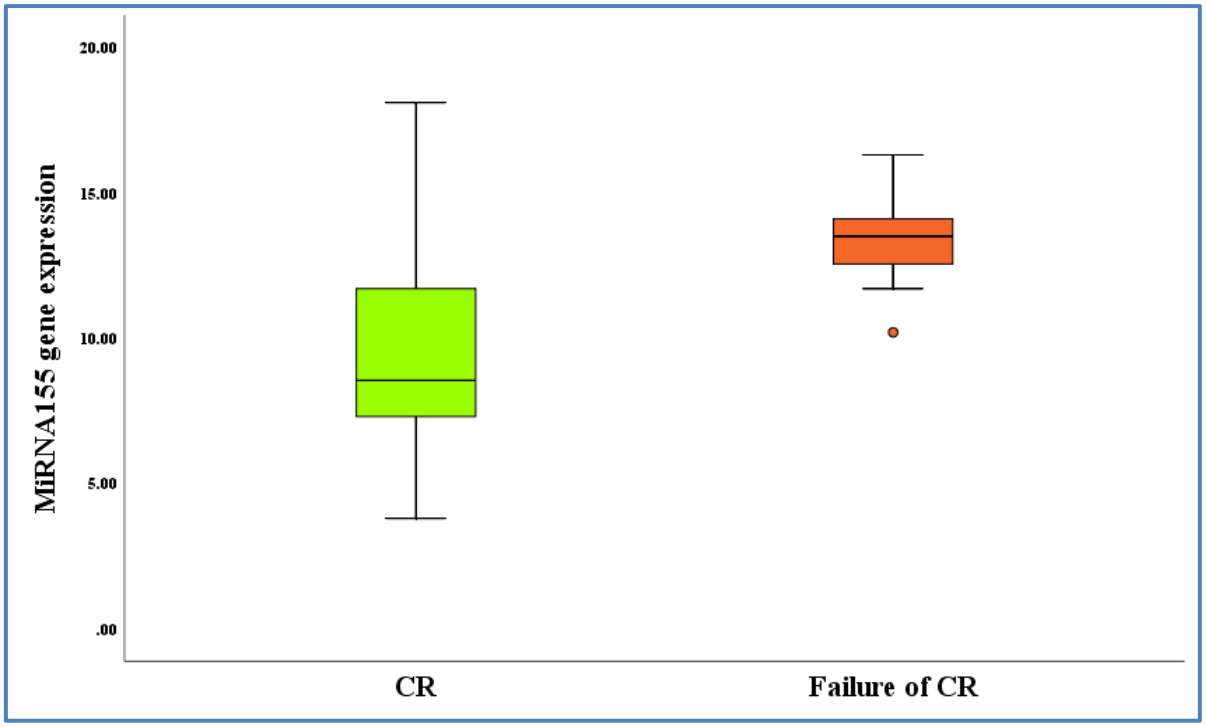

Figure (12): MiR-155 gene expression according to CR in AML group

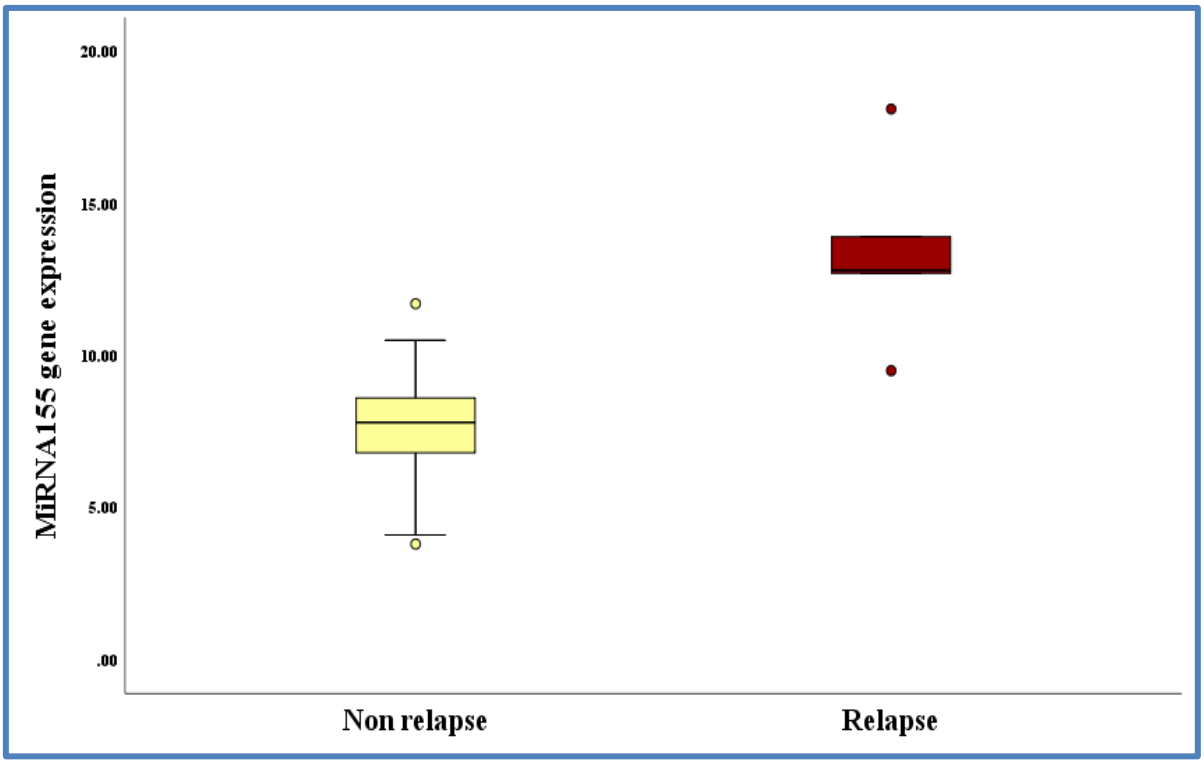

Figure (13): MiR-155 gene expression according to relapse in AML after CR.

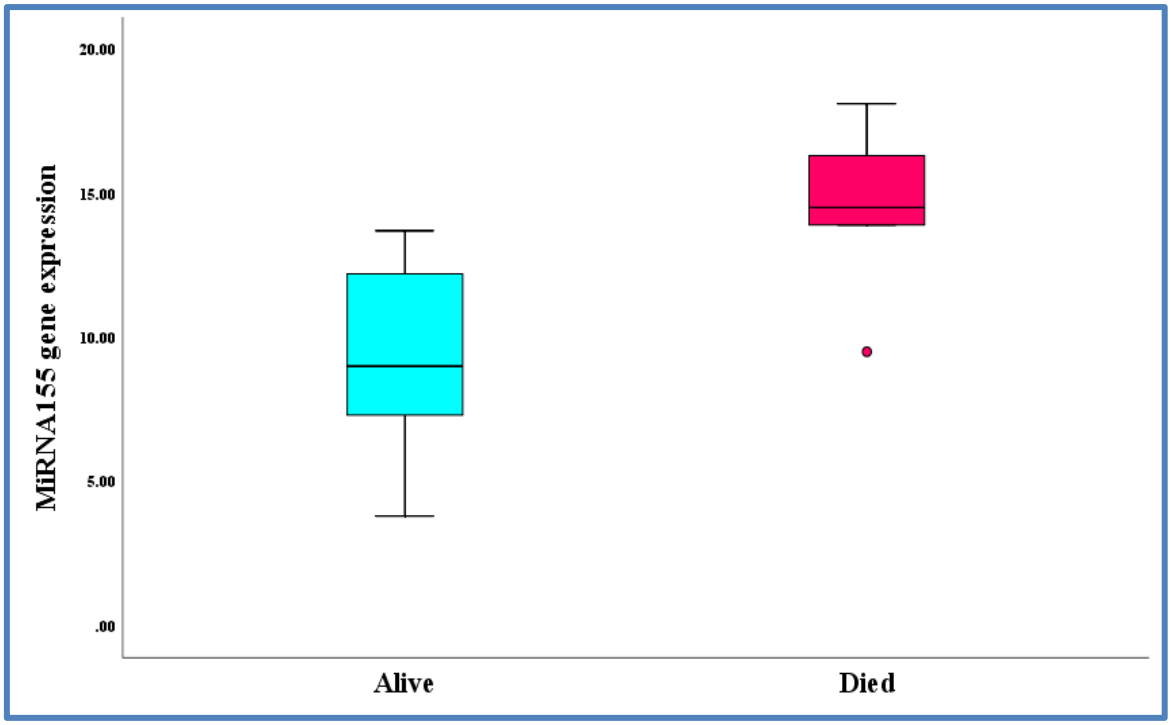

Figure (14): MiR-155 gene expression according to mortality rate among AML group.

Table (13): Comparison of survival times according to median miR-155 gene expression in AML group

		Low miR-155 gene expression N=12	High miR-155 gene expression N=13	*p*
OS	**1-year cumulative OS (%)**	91.7	69.2	**0.028**
	Mean OS (months)	12.3	9.6	
	95% CI	10.8-13.7	6.8-12.4	
DFS	**1-year cumulative DFS (%)**	91.7	25	**0.005**
	Mean DFS (months)	11.3	5.9	
	95% CI	9.8-12.7	2.8-9	

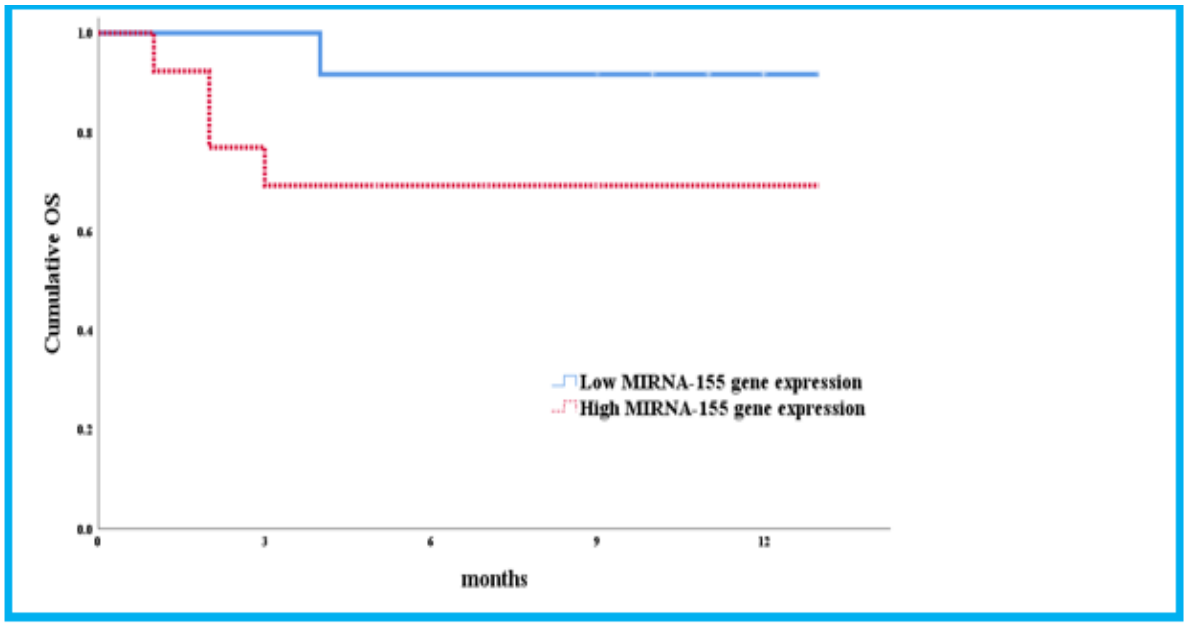

Figure (15): OS according to MiR-155 gene expression in AML group.

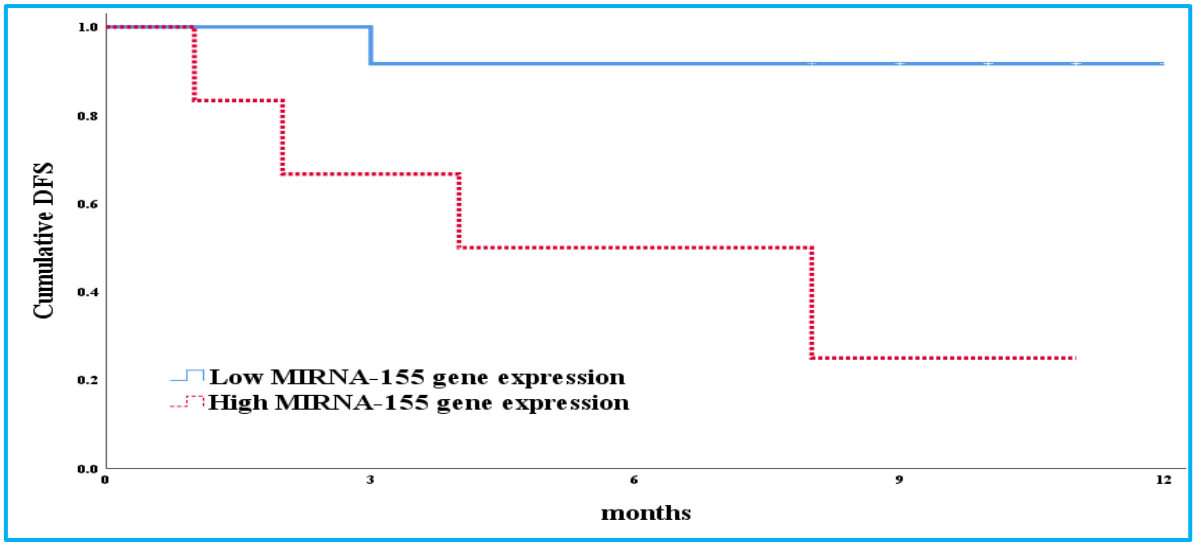

Figure (16): DFS according to MiR-155 gene expression in AML group.

Table (14): Cox regression analysis for prediction of OS in AML group

	Univariable				Multivariable			
	p	**HR**	**95% CI**		***p***	**HR**	**95% CI**	
Age	0.133	1.104	0.970	1.256				
Gender	0.277	0.296	0.033	2.652				
Marrow blasts	**0.024**	1.507	1.183	1.946	0.170	1.106	0.958	1.277
LDH	**0.002**	1.011	1.004	1.019	0.784	1.001	0.998	1.005
FLT3 ITD mutation	0.644	1.683	0.186	15.248				
MIRNA-155 gene expression	**0.007**	1.673	1.153	2.428	**0.049**	1.441	1.011	2.076

Table (15): Cox regression analysis for prediction of DFS in AML group

	Univariable				Multivariable			
	p	**HR**	**95% CI**		***p***	**HR**	**95% CI**	
Age	0.683	1.014	0.950	1.082				
Gender	0.788	0.782	0.130	4.711				
Marrow blasts	0.171	1.031	0.987	1.078				
LDH	**0.035**	1.012	1.003	1.041	0.171	1.001	1.000	1.003
FLT3 ITD mutation	0.298	1.298	0.587	2.198				
MIRNA-155 gene expression	**0.016**	2.290	1.166	4.495	**0.043**	2.910	1.034	8.189

Cox regression analysis; HR, hazard ratio; CI, confidence interval.

Cox regression analysis was conducted for prediction of DFS in AML cases, using age, gender, marrow blasts, LDH, FLT3 ITD mutation, miR-155 gene expression as covariates. Higher LDH and miR-155 gene expression were significantly associated with shorter DFS in univariable analysis. Using significant covariates (with $p<0.05$) in univariable analysis into multivariable analysis revealed that only up regulated miR-155 gene expression was suggested to be independent risk predictor for shorter DFS in AML case.

4. Discussion

MicroRNAs play a pivotal role in leukemogenesis, and its expression signatures have been performed to classify cancers since 2005. This study is aimed at exploring the significances of miR-155 in AML patients. Regarding demographic data, there was no significant difference between cases and controls regarding age and sex which is a good indicator of the strong similarity between patients and controls regarding these criteria. In our study, AML was more common in males than females, there were 14 male (56%), and 11 female (44%), this came in agreement with [7] who reported that out of 107 patients of AML, selected randomly, male percentage was 54.2% while female patients' percentage was 45.8%. Asif [8] also reported that out of 56 adult cases of AML 33 (59%) were males and 23 (41%) were females. Greater incidence of all leukemia in males appears to rise as males are relatively more exposed to work-related and environmental hazards [9]. As regard ages of our AML patients, mean age was 44.6 ±14 years this came in agreement with [10] study on 55 patients newly diagnosed with AML denoted that, the mean age of their AML patients was 45.8 ± 15.1 years. Chang [7] also observed that majority of patients (85.9%) were between 25-60 years. As regard clinical data in AML group, our study represented that pallor was the commonest presentation (92%), followed by lymphadenopathy (32%), hepatomegaly (28%), fever/infection (24%), bleeding tendency& Splenomegaly (16% for each), and skin lesions (12%). In the study done by [7] majority of the AML patients (81%) presented with fever, pallor, and weakness, (49%) had weight loss (13%) presented with bleeding and swollen gums. Hepatomegaly and splenomegaly were observed in (48%) and (45%) patients respectively and lymph node enlargement was noted in (33%) patients. In the present study, the comparison of hematological data at diagnosis between two studied groups showed a significant decrease in hemoglobin concentration, and platelets count ($p < 0.001$ for each) and a significant increase in TLC ($p = 0.001$) between cases and controls. These results are consistent with the anticipated effect from infiltration of bone marrow with immaturely blast cells, these results were agreed with [11] who studied clinical presentation of acute myeloid leukaemia on 626 AML subjects during a period of 11 years ($p < 0.0001$ for each). In our study the median of BM blasts in AML cases was (47%) also [10] study reported a blasts cells count with a median of (42%). Zidan [12] who studied clinical outcomes of adult patients with AML reported a median of (60%). A significant increase in LDH in our study was reported in AML group compared to control group ($p<0.001$) this came in agreement with [13]. The distribution of AML patients by FAB classification in our study showed that the most frequent subtype was M2 (40%) followed by M1 and M4 (28% each) and M5 (4%). According to a study done by [9] on adult patients of AML, the most frequent subtype was M2 followed by M3 and M4. Asif [8] study showed that the most common subtype was AML-M1 (32%) followed by M3, M4 (19.6% each), M2 (14%) and AML-M5 (9%), respectively. AML-M6, M7 and M0 were observed in one patient (1.8%) each. In this study, regarding the clinical outcome of studied cases CR was achieved in 18 cases (72%), while 7 cases failed to achieve CR (28%), out of those achieved CR, 5 cases relapsed (27.8%) and 5 cases died during the whole period of the study (20%). Saber [10] study found that CR was achieved in 25 cases (45.5%), while 4 cases showed non-CR (7.3%), 6 cases relapsed (10.9%) and 20 cases died by the end of the study (36.4%). Our study found that the range of the expression level of miR-155 in patients (3.8-18.1) with a median expression level (10.2) and in healthy control (0.4-3.6) with a median (1.9). Elgohary [14] who studied 101 subjects, that were classified into 61 adult patients with newly diagnosed AML

and 40 apparently healthy adult subjects, found that the range of the expression level of miR-155 in patients (3.1 -17.9) with a median expression level (5.35) and in healthy control (0.3 - 3.3) with a median (1.3). Ramamurthy [15] also showed the expression levels of miR-155 in the patients group range from (0.043 - 25.630) with a median expression level of (0.825) this difference may be due to the number of patients as they evaluated miR-155 expression in 198 AML patients. In the present study when we compared miR-155 expression level in the two studied groups, the fold change was (5.4) fold increase in miR-155 gene expression in AML group in relation to that in control group ($p< 0.001$). The expression level of miR-155 was significantly higher in AML patients than in controls. Zhi [16] measured miR-155 expression level in 140 adult AML patients and 135 healthy control individuals, they found that there was (4.79) fold change and ($p < 0.05$). O'Connell [17] also found that there is (4.5) fold higher when they investigated bone marrow samples from 24 AML patients compared with 6 healthy donors. We found that high miR-155 expression level exhibited a statistically significant positive association with TLC with a range of (1.2 - 452) and median (47) ($p = 0.003$). Also [5] who studied the impact of miR-155 on the outcome of adults AML found that high expression level of miR-155 was significantly associated with high TLC ranged from (1.0 – 450) and median (37.9) ($p < 0.001$) when they investigated 363 adult AML patients, and this came also in accordance with [15] with a WBCs range (0.2 - 827.2) ($P = 0.002$). In this study miR-155 gene expression showed significant positive correlation with LDH ($p= 0.048$), this came in agreement with [18] demonstrated that miR-155 level was positively correlated with serum LDH with a range (111-3910.8) and a median (529.05) and ($P = 0.02$) and this also in agreement with [19]. In the opposite side of our result, [14] reported that there wasn't significant correlation with miR-155 expression level and LDH without giving any explanation. In our study miR-155 gene expression showed significant positive correlation with peripheral blasts and marrow blasts with ($p=0.025$ and 0.011) respectively. Marcucci [5] reported that at diagnosis of AML cases, high miR-155 expressers had higher percentages of peripheral blood and BM blasts than low expressers with a ($P= 0.004$ and < 0.001 respectively). Elgohary [14] also reported that there was high significant differences between high and low miR-155 expression levels as regards the percentage of both BM and peripheral blood blast cells ($p = 0.006$ each). In our study we found that there were no significant correlations between miR-155 gene expression level and age, sex, hemoglobin concentration and platelets count in AML group ($P>0.05$), this came in concordance with [18] and [14]. Our studied patients were subdivided according to FAB classification into, M1 (7patients), M2 (10 patients), M4 (7patients) and M5 (1patients), we did not find any significant correlation between miR-155 expression levels and FAB classification ($p > 0.05$). Elgohary [14] study also subdivided patients according to FAB classification into, M2 (13 patients), M4(16 patients) and M5 (23 patients) and did not find any significant difference between miR-155 expression levels and FAB classification ($p > 0.05$). This goes hand in hand with [15] ($p > 0.05$). In contrast to our results [17] identified that AML patients classified as (M4) & (M5) over expressed miR-155, but they did not give any explanation for this association. In the present study we found that, after induction therapy upregulated miR-155 gene expression (>13.1) was significantly associated with failure of CR with sensitivity of 71.4% and specificity of 88.9% comparing with low miR155 level (<13.1) ($p=0.007$). Elgohary [14] also found that after induction therapy, CR rate was significantly lower in the patient group with miR-155 level ≥ 9.8 (38.5%) than those with miR-155 level < 9.8 (97.4%), (p

< 0.001) indicating that high level of miR-155 level confers a poor outcome. Hu [20] who studied expression of miR-155 in AML and its clinical significance on 80 patients with AML and 11 cases of negative control, reported that the remission rate in miR-155 high expression group and low expression group were being 59.09% versus 87.5% ($p < 0.05$). In contrast to our study [18] found no significant difference for miR-155 expression between remission group and non-remission group after the first induction therapy ($p > 0.05$) without any explanation. In our study by following up the patients, we found that cases with upregulated miR-155 gene expression was significantly associated with higher rate of relapse and death (p=0.003 and 0.005) respectively comparing with low expressors. These results came in agreement with [5] who reported that the risk of relapse or death of high miR-155 expressers was approximately twice that of the low expressers. Elgohary [14] also reported that there was a highly significant increase in the percentage of patients, who had relapsed during follow up together with increased the percentage of dead patients in high miRNA-155 expression level group compared to those with low miRNA-155 expression level ($p < 0.01$), also this in agreement with [21]. In our study AML cases with high miR-155 gene expression group showed significantly shorter OS and DFS when compared to low miR-155 gene expression group (OS=91.7% vs 69.2%; mean OS=12.3 vs 9.6 months, (p=0.028); DFS=91.7% vs 25%; mean DFS=11.3 vs 5.9, ($p = 0.005$). Similar to our findings, [15] demonstrated that high miR-155 expression as compared with low miR-155 expression was associated with shorter OS ($P < 0.01$) and DFS ($P < 0.01$). These came in agreement with results done by [21] who studied miR-155 dysregulation in AML including 153 younger (< 60 years) and 210 older (≥ 60 years) patients, the results of their careful analysis show that miR-155 expression levels constitute an independent prognostic factor in patients with AML. While higher miR-155 expression levels had a negative impact on the outcome of younger and older patients, higher miR-155 expression was associated with lower CR rate, and shorter DFS and OS. Overall, the discrepancies in results between different studies might be due to differences in sample size, age group, clinical characteristics or ethnic origin of the studied patients.

References

1. Lazarevic V, Orsmark-Pietras C, and Lilljebjorn H (2018): Isolated myelosarcoma is characterized by recurrent NFE2 mutations and concurrent preleukemic clones in the bone marrow. Blood; 131:577-581.

2. Siegel R, Miller K, and Jemal A (2019): Cancer statistics, CA Cancer J Clin.; 68, 7–30 .

3. Papayannidis C, Sartor C, Marconi G, Fontana MC, Nanni J, Cristiano G, Parisi S, Paolini S, and Curti A (2019): Acute myeloid leukemia mutations: therapeutic implications. Int J Mol Sci.; 20:11 .

4. Higgs G and Slack F (2013): The multiple roles of microRNA-155 in oncogenesis. J Clin Bioinforma; 3(1):17 .

5. Marcucci G, Maharry K, and Metzeler K (2013): Clinical Role of micro-RNAs in Cytogenetically Normal Acute Myeloid Leukemia: miR-155 Upregulation Independently Identifies High-Risk Patients. Journal of Clinical Oncology; 31: 2086-2093 .

6. Wallace JA, Kagele DA, and Eiring AM (2017): miR-155 promotes FLT3-ITD-induced myeloproliferative disease through inhibition of the interferon response. Blood; 129: 3074-86 .

7. Chang F, Shamsi TS, and Waryah AM (2016): Clinical and Hematological Profile of Acute Myeloid Leukemia (AML) Patients of Sindh. J Hematol Thrombo Dis.; 4: 239 .

8. Asif N and Hassan K (2013): Acute Myeloid Leukemia amongst Adults. JIMDC; 2: 58-63 .

9. Chaudhari S, Desai J, Adam A, and Mishra P (2014): JAK/STAT as a novel target for treatment of leukemia. Int J Pharm Sci.; 6: 1-7 .

10. Saber S, Samir A, and Salah A (2019): CD47 Expression in Egyptian Patients with Acute Myeloid Leukemia Journal of Bioscience and Applied Research; 5(4):456 -465 .

11. Shamsi T (2016): Clinical and Hematological Profile of Acute Myeloid Leukemia (AML) Patients of Sindh. Journal of Hematology & Thromboembolic Diseases; 4, 239-249 .

12. Zidan A, Omran A, Ghonaim R, Abu-Taleb F, and Elgohary T (2019): The Prognostic Value of Vitamin D Insufficiency & Vitamin D Receptor Gene Polymorphism in Adult Acute Myeloid Leukemia Patients. Journal of Cancer Therapy; 10: 361-370 .

13. Soliman A, Abdel A, Afify R, and Ibrahim N (2016): BAALC and ERG Expression in Egyptian Patients with Acute Myeloid Leukemia, Relation to Survival and Response to Treatment. Maced J Med Sci.; 4(2):264-270 .

14. Elgohary T, Abu-Taleb F, and Ghonaim R (2019): The Impact of miRNA-155 Expression on Treatment Outcome in Adult Acute Myeloid Leukemia Patients. Journal of Cancer Therapy; 10, 203-214 .

15. Ramamurthy R, Hughes M, Morris V, and Bolouri H (2016): miR-155 Expression and Correlation with Clinical Outcome in Pediatric AML. Pediatric Blood & Cancer; 63: 2096-2103 .

16. Zhi F, Cao X, Xie X, Wang B, Dong W, and Gu W (2013): Identification of Circulating MicroRNAs as Potential Biomarkers for Detecting Acute Myeloid Leukemia. PloS One, Journal Pone; 8 (2): 56718 .

17. O'Connell RM, Rao DS, and Chaudhuri AA (2008): Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med.; 205:585-94.

18. Xu L, Guo Y, Cen J, Yan W, and He H (2015): Overexpressed miR-155 Is Associated with Initial Presentation and Poor Outcome in Chinese Pediatric Acute Myeloid Leukemia. European Review for Medical and Pharmacological Sciences;19: 4841-4850 .

19. Narayan N, Morenos L, Phipson B, Willis SN, Brumatti G, and Eggers S (2017): Functionally distinct roles for different miR-155 expression levels through contrasting effects on gene expression, in acute myeloid leukaemia. Leukemia; 31: 808-20.

20. Hu XL and Tang AP (2016) Expression of miR-155 in Acute Myeloid Leukemia and Its Clinical Significance. Zhongguo.; 24(4): 980-984.

21. Croce CM (2013): MicroRNA Dysregulation in Acute Myeloid Leukemia. Journal of Clinical Oncology; 31: 2065-2066.