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ORIGINAL ARTICLE

PROGNOSTIC IMPACT OF MICRORNAS (MIR-155, MIR-10A, LET-7A) ON THE OUTCOME OF ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA.

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ABSTRACT

Background: Acute myeloid leukemia is a heterogeneous disease caused by clonal proliferation and disrupted differentiation of malignant myeloid precursors. AML can be classified based on chromosomal abnormalities, acquired somatic mutations, gene expression. Significant changes in gene transcription may lead to relapse after chemotherapy. So, assessment of the prognosis in AML is very crucial.

Aim: To assess prognostic impact of miRNAs (miR-155, miR-10a, let-7a) levels using in AML patients.

Method: 55 subjects were included in this work. They were classified into 2 groups: patient and control group. Patient group is further classified based on cytogenetic and molecular profile into 3 groups: bad, intermediate & good prognosis. miRNAs (mir155, miR-10a, let7a) was assessed using real time PCR [QIAGEN, miScript, Quanti Tect and Rotor-isc (QIAGEN Group) PAXgene (pre Analytix Gmbh)].

Results: There was significant increase in miRNAs (miR-155, miR-10a, let-7a) level among cases compared to control group. There was significant increase in miR-155 level and significant decrease in miR-10 and Let-7a among bad prognosis cases compared to intermediate and good subgroups. There was significant +ve correlation between miR-155 & blast % B.M and significant -ve correlation between (miR-10a, let-7a) and blast % BM among the cases group. There was significant increase in DFS and OS among intermediate & good compared to bad subgroups. ROC curve detected the validity of (miR-155, mir10a & let7a respectively) in diagnosis of AML.

Conclusion: miRNAs (miR-155, miR-10a, let-7a) expression levels were significantly higher in AML patients compared to control group and have prognostic significance in adult patients with AML.

Keywords: miR-155, miR-10a, let-7a, miRNA & AML

INTRODUCTION

Acute myeloid leukemia (AML) is a blood disease in which there is abnormal cell proliferation and cell death with aggregation of immature cells of myeloid lineage in peripheral blood and bone marrow. Also, AML is a clinically and genetically heterogeneous disease [(1)]. It is the commonest acute leukaemia with incidence rising with age [(2)].

MicroRNAs (miRNAs) are noncoding short RNAs (18–25) nucleotides, which are gene expression regulators. More than 60% of mammalian are predicted to be silenced by miRNAs. They are included in many vital

biologic processes as progression of cell cycle, apoptosis, cell differentiation, and immune responses [(3)]. miRNAs are well conserved in both plants and animals. They are found to be a vital and old component of gene regulation. They either present within the cell or circulating miRNA [(4)].

A large number of genes may be targeted by single or multiple miRNAs. In the early 1990s, miRNAs discovery created a new era in understanding gene expression regulation through controlling transcriptional and posttranscriptional processes [(5)]. Now, miRNAs are reported to have roles in cancer biology, involving proliferation, invasion, apoptosis, angiogenesis and metastasis**.** Accumulating evidences reported that miRNAs have important roles in normal hematopoiesis and leukemogenesis [(6)]**.**

miRNAs role in cancers are either oncogenic or tumour-suppressive. Activity patterns of genes of miRNAs could differentiate cancer forms [(7)]**.** In AML, miRNAs are included in deregulation of hematopoietic mechanisms of cell differentiation, proliferation, and survival; sharing in molecular heterogeneity of the disease. So, this will affect response to treatment and predict the clinical outcome of patients [(8)]**.** Numerous studies demonstrated the existence of distinct miRNA profiles in different AML subtypes, indicating that miRNA signature contributed to AML heterogeneity, and suggesting its potential inclusion in clinical setting [(9)]**.** It was found that miR-155, miR-10a and let-7a are involved in different physiological and pathological processes [(10), (11), (12)]**.**

SUBJECTS & METHODS

This study was carried out in the Clinical Pathology and Medical Oncology & Hematology Departments, Faculty of Medicine, Zagazig University Hospitals from 5/2016 to 11/ 2017 on (55) subjects classified into two groups: group I: patient group (40 adult newly diagnosed AML patients) and group II: control group (15 adult apparent healthy individuals).

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All patients were diagnosed using routine methods including CBC, Liver & Kidney functions, LDH level, BM aspiration (morphological examination by Leishman stain & cytochemistry), Immunophenotyping and Cytogenetics. BM aspiration should done for all patients. Follow up of patients was carried out over the study period, which reached 1 year, to evaluate the response to therapy.

- **Patient Inclusion Criteria :**

- Age group ≥17 years old, de novo cases with AML.
- Patient fit for standard induction protocol without chronic illness or organ failure.

- **Patient Exclusion Criteria :**

- Patients refusing participation in the study.
- Previous exposure to chemotherapy and radiation.
- Patients diagnosed with promyelocytic leukemia.
- Patients who were diagnosed with other maliganancies at any time period.

All patients received the standard cytarabine-doxorubicin-based induction chemotherapy regimen 3 & 7 consisting of cytarabine 100 mg/m2/day continuous infusion for 7 consecutive days plus doxorubicin 45 mg/m2/day for 3 days.

Written informed consent was taken from all patients and study protocols were approved by the institutional review board at the faculty of medicine, Zagazig University.

2.1. Specific Laboratory Investigation (Estimation of miR-155, mir-10a & let-7a)

Both groups were subjected to Determination of serum expression of miR-155, miR-10a and let-7a by real-time PCR. miRNAs was extracted from serum using "miRNeasy Mini

kit" (Qiagen, Germany). Reverse transcription was done using "miScript II RT Kit" (Qiagen, Germany). Amplification was done using target specific miScript Primer Assays (forward primers) and the miScript SYBR Green PCR Kit, which contains the miScript Universal Primer (reverse primer) and "QuantiTect SYBR Green PCR Master Mix" (Qiagen, Germany). Real time PCR was done on "Stratagene Mx3005P" platform (Agilent Technologies, USA). All steps were done according to manufacturer recommendations. Threshold cycle (CT) values were registered for each sample well and were normalized against Snord68. Fold changes of miRNA expression were calculated using 2−ΔΔCT method **(13).**

Fold Change = 2−ΔΔCT method,

ΔΔCT = ΔCTpatients − ΔCTcontrol.

ΔCT (relative expression)= CT miRNA of interest – CT of housekeeping RNA.

2.2. Statistical Analysis

The collected data were computerized and statistically analyzed using Statistical Package for Social Sciences (SPSS 20 Inc. Chicago, IL, USA). P value was used to calculate the significance Level for all used statistical tests. The threshold of significance is fixed at 5% level (P-value); P value of >0.05 indicates non-significant results, P value of <0.05 indicates significant results and P value of <0.01 indicates highly significant results.

RESULTS

Both cases and controls are matched as regard age and sex. Patient Prognostic-risk group is further classified based on cytogenetic profile **(2017 ELN risk stratification by genetics) (14)** into 3 groups: bad, intermediate & good prognosis (Table 10). Group Ia (bad prognosis), group Ib (intermediate prognosis) and group Ic (good prognosis) (Figure 1).

There was significant increase in (miR-155, miR-10a, let-7a) level among cases group compared to control group (Table 1).

There was statistical significant increase in miR-155 level and statistical decrease in (miR-10a, let-7a) among bad prognosis cases compared to intermediate and good (Table 2).

There was significant +ve correlation between miR-155 and blast % BM while, there was no correlation between it and (age, Hb, platelets, Blast % PB, LDH & uric acid). On the other hand, there was significant -ve correlation between (miR-10a, let-7a) and blast % BM while, there was no correlation between them and (age, Hb, platelets, Blast % PB, LDH & uric acid) among the cases group (Table 3).

There was statistical significant increase in miR-155 expression level and statistical significant decrease in mir10a & let7a among No CR, relapsed and dead cases compared to other cases in Group I (Table 4), (Table 5), (Table 6).

ROC curve suggested the validity of miRNAs in diagnosis of disease and prediction of bad prognosis among the studied groups which detected cut off (3.0, 3.53 & 2.15) as best fit value of (miR-155, mir10a & let7a respectively) in diagnosis of AML among the studied groups. The sensitivity of miR-155 at cut off 3 was 90%, specificity was 86.7%, the sensitivity of miR-10a at cut off 3.53 was 85%, specificity was 80% and Finally sensitivity of let-7a at cut off 2.15 was 92.5%, specificity was 86.7%. While, it detected cut off (5.15, 6.95 & 4.2) as best fit value of (miR-155, mir10a & let7a respectively) for predicting bad prognosis with sensitivity 80 % of miR-155 at cut off 5.15 and specificity 72%. The sensitivity of miR-10a at cut off 6.95 was 66.7%, specificity was 68% and finally sensitivity of let7a at cut off 4.2 was 73.3%, specificity was 72% (Table 8 & 9), (Figure 2 & 3 &4).

There was statistical significant increase in DFS and OS among intermediate & good prognosis groups compared to bad prognosis group. There was statistical significance increase in the frequency of relapse and death among bad prognosis group compared to intermediate & good prognosis groups (Table 7) (Figure 5 & 6).

Table 1. miR-155, miR-10a & let-7a among the two groups

Variable	Group I (Cases) (n=40)	Group II (Control) (n=15)	M W	P
miR-155 *Mean ± SD* *Range*	7.70 ± 5.06 2.4 – 18.5	1.73 ± 0.95 0.5 – 3.2	**5.36**	**<0.001****
miR10a *Mean ± SD* *Range*	9.46 ± 2.73 3.2 – 21.3	1.98 ± 1.71 0.19 – 4.97	**5.02**	**<0.001****
Let-7a *Mean ± SD* *Range*	5.89 ± 4.02 1.5 – 15.6	0.88 ± 0.70 0.17 – 2.56	**5.42**	**<0.001****

Table 2. Comparison of miRNAs (miR-155, miR-10a, let-7a) among the cases subgroups

Variable	Group Ia (Bad) (n=15)	Group Ib (Intermediate) (n=10)	Group Ic (Good) (n=15)	K	P	LSD
miR-155 *Median* *Range*	13.2 2.5 – 18.5	4.5 2.8 – 10.5	4.1 2.4 – 11.6	**14.02**	**0.001****	**0.005**** [1] **0.001**** [2] 0.44 NS [3]
miR-10a *Median* *Range*	5.4 3.2 – 12.4	10.15 3.2 – 20.5	8.2 3.2 – 21.3	**5.13**	**0.04***	**0..04*** [1] **0.04*** [2] 0.98 NS [3]
Let-7a *Median* *Range*	3.4 1.8 – 8.2	7.15 1.5 – 11.1	6.5 2.1 – 15.6	**7.12**	**0.03***	**0.03*** [1] **0.02*** [2] 0.64 NS [3]

SD: Standard deviation**, K:**Kruskal Wallis test**, NS:** Non significant (P>0.05), ***:**Significant (P<0.05), ****:**Highly significant (p<0.01), **LSD:** Least significant difference**, P1:**Group Ia versus Group Ib**, P2:**Group Ia versus Group Ic**, P3:** Group Ib versus Group Ic.

Table 3. Correlation between miRNAs and age & laboratory parameters among the cases

Variable	microRNAs					
	miRNA155 (n=40)		**mir10a (n=40)**		**let7a (n=40)**	
	R	**P**	**R**	**P**	**R**	**P**
Age (years)	-0.02	0.88 NS	0.15	0.36 NS	0.01	0.93 NS
Hb (gm/dl)	-0.18	0.26 NS	0.13	0.43 NS	0.15	0.37 NS
Platelets ($x10^3/mm^3$)	-0.12	0.47 NS	0.16	0.32 NS	0.23	0.16 NS
Blast cell PB (%)	-0.15	0.35 NS	0.07	0.65 NS	0.01	0.99 NS
Blast cell BM (%)	**0.52**	**0.001 ****	**-0.40**	**0.01***	**-0.39**	**0.01***
LDH (U/L)	0.12	0.47 NS	-0.26	0.10 NS	-0.05	0.74 NS
Uric acid (mg/dl)	0.09	0.57 NS	-0.04	0.82 NS	-0.17	0.29 NS
miR-155	------	------	**-0.39**	**0.01***	**-0.61**	**<0.001 ****
mir-10a	------	------	------	------	**0.53**	**<0.001 ****

r: spearman correlation coefficient, ***:**Significant (P<0.05),
****:**Highly significant (**P**<0.01)

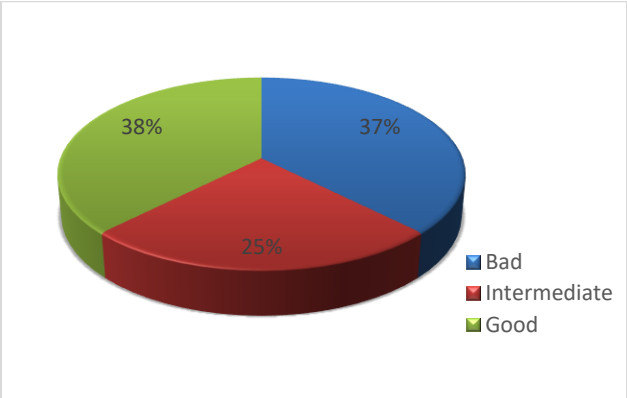

Figure 1. Cytogenetic classification among the cases

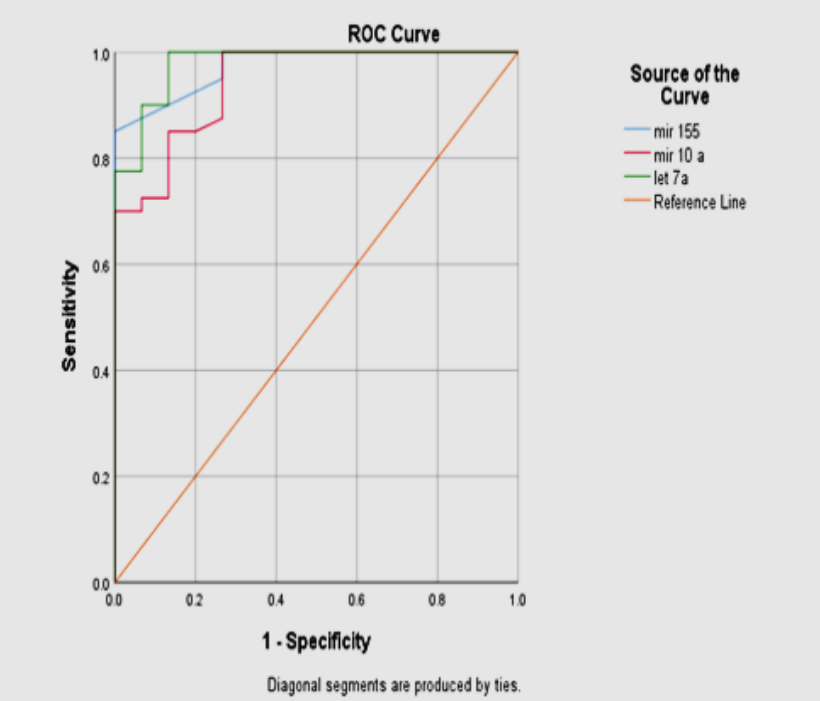

Figure 2. ROC curve for validity of miRNAs in diagnosis of AML among groups

DISCUSSION

It was established that a range of clinical and diagnostic parameters including a variety of laboratory and molecular markers should be used in assessing prognosis for a patient with AML [(15)].

Assessment of prognosis accurately is crucial to the management of AML. Stratifying patients according to their risk of treatment resistance or treatment-related mortality (TRM), prognostic factors help to guide the physician in deciding patient therapy [(16)]. Moreover, miRNAs can act as oncogenes or tumor suppressors, contributing to malignant transformation in solid and hematological tumors; including AML by regulating different mRNA targets [(17)].

Aberrant miRNAs expressions have been variable in different cytogenetic and molecular subtypes in AML cases. A large number of genes in AML patients can be targeted and repressed by these miRs [(18), (19), (20)].

In our study there was significant increase in miRNAs (miR-155, miR-10a, let-7a) level among cases group compared to control group in which; the expression level of miR-155 in patients (2.5 – 18.5), in healthy control (0.5 – 3.2) and P-value (<0.001) & the range of the expression level of miR-10a in patients (3.2–11.1), in healthy control (0.19 – 4.97) and P-value (<0.001) & the range of the expression level of let-7a in patients (1.5 – 8.2), in healthy control (0.17 – 2.56) and P-value (<0.001).

Consistent with our results as regards miR-155, *Ramamurthy et al., (2016) & Schneider et al., (2018)* found that AML patients had overexpression of miR-155 compared to control (P<0.05) [(21), (36)]. It was pointed out that the levels of miR-10a expression were increased in AML cases compared with normal controls (P<0.05) [(22), (23)]. *Zhi et al., (2013)* measured expression levels of different miRNAs including miR-10a & miR-155 which were found to be significantly overexpressed in AML patients compared with control, (p<0.005) [(24)].

As regards let-7a, it was found that the expresson levels of let-7a were significantly increased in CN-AML patients compared to the healthy control, (p<0.001) [(25)]. *Garzon et al.,*

(2008) identified the up-regulation of different miRNAs including miR-10a, let-7 and miR-155 in AML patients compared to normal controls [(26)].

We found that there was significant increase in miR-155 level among bad prognosis cases (median; 13.2) compared to intermediate (median; 4.5) and good subgroups (median; 4.1). While there was statistical decrease in (miR-10a, let-7a) among bad subgroup (median; 5.4, 3.4) compared to intermediate (median; 10.15, 7.15) and good subgroups (median; 8.2, 6.5). Subsequently, we used Fisher LSD test; there was significant difference of the 3 miRNAs between bad and intermediate prognosis groups with LSD: 0.005, 0.04 & 0.03 respectively. Also, there was significant difference of them between bad and good prognosis groups with LSD: 0.001, 0.04 & 0.02 respectively. While, there was no significant difference of them between intermediate and good prognosis groups.

In agree with our results, *Hu and tang, (2016)* divided their patients according to cytogenetic groups into (poor, moderate and favorable). They found that miR-155 expression levels in the moderate and poor prognostic group were significantly higher compared with the favorable prognosis group (P<0.05 in both), but there was no significant difference between poor and moderate prognostic groups (P>0.05) [(27)]. It was reported that miR-155 expression levels were significantly higher in the poor prognostic group than moderate and favorable prognosis group [(28)].

Against to our results, *Palma et al., (2014)* demonstrated contrasting apparent tumor suppressor functions of miR-155 in AML cell lines [(11)]. Recently, these differences between the studies may be explained by a dose-dependent role for miR-155 in AML. The effects of miR-155 in AML are critically dependent on the levels to which miR-155 is expressed [(30), (31)].

As regard miR-10a, it was significantly up regulated in AML patients with good prognostic cytogenetics and molecular genetics compared with normal controls (P<0.05) [(22)]. Also, ***Zhang et al., (2018)*** found that miR-10a has significant differences according to karyotype classifications with miR-10a overexpression in favorable and intermediate prognostic groups as compared to bad prognostic group (p<0.001) (23).

On the contrary, it was found that there was no significant correlation between expression level of miR-10a and cytogenetic classification (p= 0.288) [(32)].

Concerning let-7a, it was postulated that high expression level of let-7a associated with good prognosis [(33)]. High expression of let-7a can be seen as a biomarker for favorable prognosis in CN-AML (P= 0.03) [(34)].

On Correlating the 3 miRNAs with laboratory parameters among the cases group we observed that there was significant +ve correlation between miR-155 and blast % B.M (r: 0.52 and P-value: 0.001) while, there was no significant correlation between it and (age, Hb, platelets, Blast % P.B, LDH & uric acid) (P-value: > 0.05).

It was found that there was no significant correlation between high miR-155 and some of the laboratory parameters including (Hb, Plt and Blast% in B.M & P.B) (p-value: > 0.05) [(35), (36), (37)]. Also, it was found that there was no significant correlation between LDH and miR-155 expression level; p-value: 0.587 [(39)]. On the contrary, others found that there was significant positive correlation between expression level of miR-155 and LDH; p value 0.02 [(28)].

While, others found that high miR-155 had been associated with significant decrease in (hemoglobin concentration and platelet count) and increase in (B.M & P.B blast) [(36), (37), (38)].

On the other hand, there was significant -ve correlation between (miR-10a, let-7a) and blast% B.M (r: -0.4, -0.39 respectively and P-value: 0.01) while, there was no significant correlation between them and (age, Hb, platelets, Blast % P.B, LDH & uric acid) (P-value: > 0.05) among the cases group.

It was reported that miR-10a overexpression was not correlated with sex, age, and some of laboratory parameters (Hb and platelet count and BM blast %) (P-value: > 0.05), but was found to be associated with lower BM blasts% (p=0.049) [23], [32].

Concerning let-7a, it was shown that expression levels of let-7a were not significantly associated with clinical parameters such as age, sex, Hb count [34].

Our study proved the validity of those 3 miRNAs in diagnosis of AML among the studied groups using ROC curve analysis which detected cut off (3.0, 3.53 & 2.15) as best fit value of (miR-155, mir10a & let7a respectively) in diagnosis of AML among the studied groups. The sensitivity of miR-155 at cut off 3 was 90%, specificity was 86.7%, the sensitivity of miR-10a at cut off 3.53 was 85%, specificity was 80% and finally sensitivity of let-7a at cut off 2.15 was 92.5%, specificity was 86.7%.

The results of *Zhi et al., (2013)* suggested the potential of some miRNAs including miR-10a & miR-155 for discriminating patients with AML from healthy subjects with the following AUCs: miR-10a, 0.8129 (95% confidence interval (CI)=0.7610–0.8649); miR-155, 0.9531 (95% CI=0.9259–0.9803) [24]. ROC curve analysis was used to evaluate the diagnostic accuracy of serum miR-10a which could be available as a potential biomarker for discriminating AML from controls [23], [32].

We evaluated prognostic role of the 3 miRNAs expression levels by observing any significant association with response to therapy, disease outcome, and survival. After giving the first induction therapy, we found that there was significance increase in miR-155 expression level among No CR, relapsed and dead cases (P-value: 0.04, 0.03 & 0.04 respectively), statistical significant decrease in mir10a among those cases (P-value: 0.001, 0.04 & 0.04 respectively) & statistical significant decrease in let-7a among those cases (P-value: 0.04, 0.04 & 0.03 respectively) compared to other cases in Group I. So, our results demonstrated that high miR-155 expression level has a bad prognostic impact while, high (miR-10a & let-7a) expression levels have favorable prognosis.

Consistent with our results, it was reported that high miR-155 expression level was associated with unfavorable prognosis with lower CR rate, higher risk for disease relapse or death and decreased OS (p-value: <0.05) [39]. While, *Xu et al., (2015)* did not find significant difference for miR-155 levels between remission and non-remission group after initial induction therapy (*p–value*: 0.511). This may due to the sample type of pediatric patients [28].

As regard miR-10a; it was reported that miR-10a could predict chemotherapy response in AML. Higher miR-10a expression level in CR compared to no-CR patients (*P* = 0.003) [19]. Others postulated that BM miR-10a overexpression is associated with genetic events but was not associated with CR rate and not affects clinical outcome in AML, (P= 0.931) [23].

On the contrary, others suggested that miR-10a expression was significantly higher in cases with relapsed AML when compared to CR patients (p < 0.001) [32].

It was postulated that high let-7a expression is associated with lower risk of death, higher CR rate and lower relapse rate compared to low expression group, (p < 0.05) [34].

After follow up of our patients, we observed that there was statistical significant increase in DFS & OS and significant decrease in the frequency of relapse and death among intermediate & good prognosis groups compared to bad prognosis group (p<0.05). CR% in bad prognostic group was (53.3)% compared to CR% in intermediate and good (70% & 73.3% respectively), (P= 0.48). Also, we found that patients without CR had higher miR-155 and lower (miR-10a & let7a) expression level than those with CR after chemotherapy (p<0.05).

It was demonstrated that, higher *miR-155* expression was associated with lower CR rate, and shorter DFS and OS, (P<0.05) [21], [27], [28].

A meta-analysis done by *Li et al., (2019)* supported our results and revealed that high expression of miR-155 could predict worse OS and DFS regardless of the region, sample types, tumor types, cut off values and independent factors. So, it could be an important predictor of poor prognosis [(39)].

In contrast to our results; higher *miR-155* expression was associated with increased CR rate, and longer DFS and OS [(11)].

It was suggested that the patients with miR-10a overexpression showed no significant differences in DFS and OS as compared with those without miR-10a overexpression (p=0.661 & 0.996 respectively) [(23)]. On the contrary**,** others found that AML patients with high miR-10a expression had both shorter OS than those with low miR-10a expression (p= 0.042). The explanation in difference between this result and ours may be according to the function of the targeted gene by mir-10a whether tumor suppressor or oncogene [(32)]. Let-7a over expression is found to be associated with longer OS (P= 0.0045) and DFS (P= 0.016) [(34)].

Finally, our study proved the validity of those 3 miRNAs (miR-155, miR-10a, let-7a) in predication of bad prognosis among the studied groups. There was significance increase in miR-155 and significant decrease in miR-10 and Let-7a levels among bad prognosis cases compared to intermediate and good subgroups There was significant +ve correlation between miR-10a and let-7a (r: 0.53 and P-value <0.001) and both correlated negatively with miR-155 (r: -0.39, -0.61 and P-value: 0.01, <0.001 respectively).

CONCLUSION

The expression level of miRNAs (miR-155, miR-10a and let-7a) was significantly higher in AML patients than in control group. The 3 miRNAs (miR-155 miR-10a and let-7a) have pivotal roles for early diagnosis and prognosis of adult patients with acute myeloid leukemia.

***Conflicts of Interest:** There is no conflict of interest.

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Supplementary Tables & Figures

Table 4. Relation between miR-155 and disease data among Group I

Variable		No	miRNA-155			MW	p
			Mean	**Sd**	**Range**		
Response:	*CR*	26	6.28	3.99	2.4 – 18.5	**2.06**	**0.04***
	No CR	14	10.33	5.88	2.5 – 17.9		
Relapse:	*No*	16	5.29	2.55	2.9 – 11.6	**2.12**	**0.03***
	Yes	10	8.5	5.75	2.4 – 18.5		
Outcome:	*Alive*	23	5.97	4	2.8 – 17.9	**2.08**	**0.04***
	Dead	17	10.02	5.51	2.4 – 18.5		

SD: Stander deviation, **MW:** Mann Whiteny test, **NS:** Non significant (**P**>0.05) *: Significant (**P**<0.05)

Table 5. Relation between mir10a and disease data among Group I

Variable		No	Mir10a			MW	p
			Mean	**Sd**	**Range**		
Response	*CR*	26	11.39	5.78	3.5 – 21.3	**3.27**	**0.001****
	No CR	14	5.87	3.86	3.2 – 15.3		
Relapse	*No*	16	12.6	5.75	4.6 – 21.3	**2.11**	**0.04***
	Yes	10	8.68	5.2	3.5 – 19.6		
Death	*No*	23	11.1	5.97	3.2 – 21.3	**2.09**	**0.04***
	Yes	17	7.24	4.85	3.2 – 19.6		

SD: Stander deviation, **MW:** Mann Whiteny test, **NS:** Non significant (P>0.05) *: Significant (P<0.05) **: Highly significant (P<0.01)

Table 6. Relation between let7a and disease data among Group I

Variable		No	Let7a			MW	p
			Mean	**Sd**	**Range**		
Response	*CR*	26	6.84	4.5	1.5 – 15.6	**2.06**	**0.04***
	No CR	14	4.12	2.09	1.8 – 8.1		
Relapse	*No*	16	7.76	4.66		**2.07**	**0.04***
	Yes	10	4.79	3.56	2.1 – 15.6 1.5 – 12.3		
Death	*No*	23	7.05	4.40		**2.15**	**0.03***
	Yes	17	4.32	2.87	1.8 – 15.6 1.5 – 12.3		

SD: Stander deviation, **MW:** Mann Whiteny test, **NS:** Non significant (**P**>0.05) *: Significant (P<0.05)

Table 7. Comparison of disease outcome among the cases subgroups

Variable		Group I (Cases) (n=40)		Group Ia (Bad) (n=15)		Group Ib (Intermediate) (n=10)		Group Ic (Good) (n=15)		$\chi 2$	p
		No	**%**	**No**	**%**	**No**	**%**	**No**	**%**		
Response:	*No CR*	14	35	7	46.7	3	30	4	26.7	1.47	0.48
	CR	26	65	8	53.3	7	70	11	73.3		NS
	No	16	40	2	13.3	4	40	10	66.7	8.58	**0.01***
	relapse	10	25	6	40	3	30	1	6.7		
	Relapse										
Outcome:	*Alive*	23	57.5	4	26.7	7	70	12	80	**9.58**	**0.008**
	Dead	17	42.5	11	73.3	3	30	3	20		**

χ^2**:** Chai square test, **NS:** Non significant (P>0.05) ****:** highly significant (P<0.01)

Table 8. Validity of miRNAs (miR-155, miR-10a, let-7a) in diagnosis of disease among the studied groups:

Marker	Cutoff	AUC	CI	Sensitivity	Specificity	+PV	-PV	Accuracy	p-value
miR-155	**≥3**	**0.97**	**0.94 – 1**	**90**	**86.7**	**94.7**	**76.5**	**89.1**	**<0.001****
miR-10a	**≥3.53**	**0.94**	**0.88 – 1**	**85**	**80**	**91.9**	**66.7**	**83.6**	**<0.001****
let-7a	**≥2.15**	**0.98**	**0.94-1**	**92.5**	**86.7**	**94.9**	**81.3**	**90.9**	**<0.001****

AUC: Area under curve CI: Confidence interval **:Highly significant (P<0.01)

Table 9. Validity of miRNAs (miR-155, miR-10a, let-7a) in predication of bad prognosis among the studied cases groups:

Marker	Cutoff	AUC	CI	Sensitivity	Specificity	+PV	-PV	Accuracy	p-value
miR-155	**≥5.15**	**0.85**	**0.70-1**	**80**	**72**	**63.2**	**85.7**	**75**	**<0.001****
miR-10a	**≤6.95**	**0.72**	**0.56-0.87**	**66.7**	**68**	**55.6**	**70.8**	**67.5**	**0.02***
let-7a	**≤4.2**	**0.75**	**0.60-0.90**	**73.3**	**72**	**61.1**	**81.8**	**72.5**	**0.008****

AUC: Area under curve, CI: Confidence interval, *:Significant (P<0.05) **:Highly significant (P<0.01)

Table 10. Prognostic-risk group based on cytogenetic and molecular profile (2017 ELN risk stratification by genetics) (Döhner et al., 2017).

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low†}
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high†}
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low†} (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type *NPM1* and *FLT3*-ITD^{high†}
	Mutated *RUNX1*¶
	Mutated *ASXL1*¶
	Mutated *TP53*

† Low, low allelic ratio (<0.5); high, high allelic ratio (≥0.5);
§ Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.
‖ Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).
¶ These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

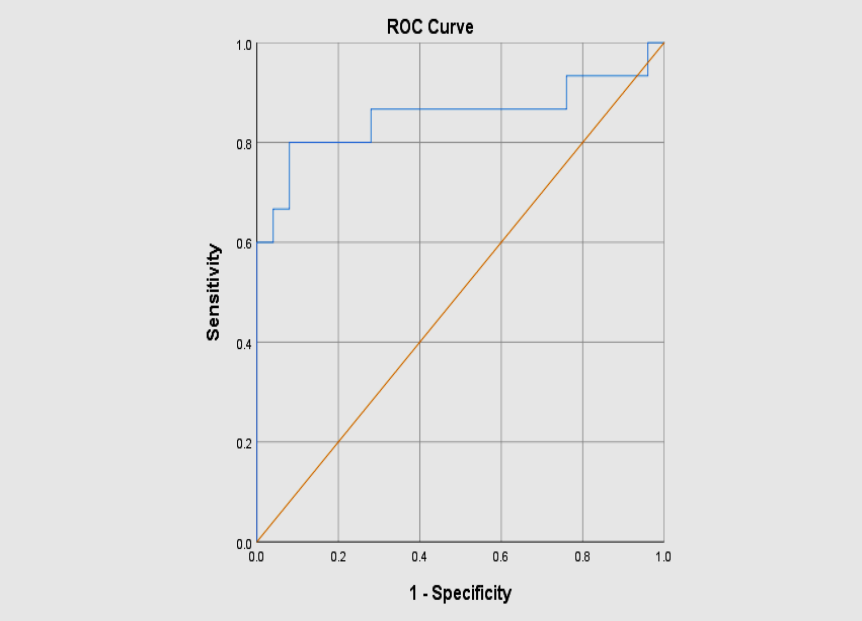

Figure 3. ROC curve for validity of mir155 in prediction of bad prognosis among cases

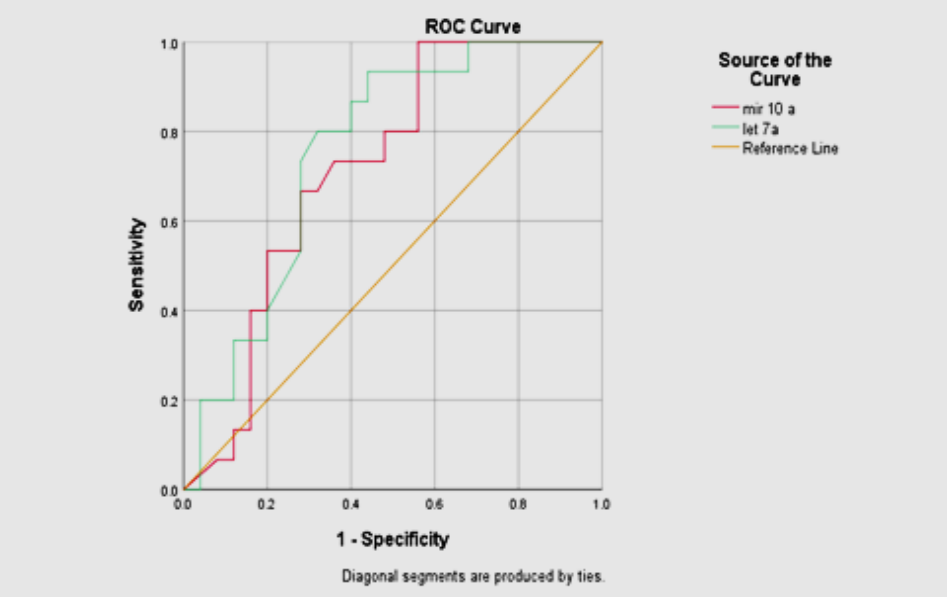

Figure 4. ROC curve for validity of (mir10a, let7a) in prediction of bad prognosis among cases

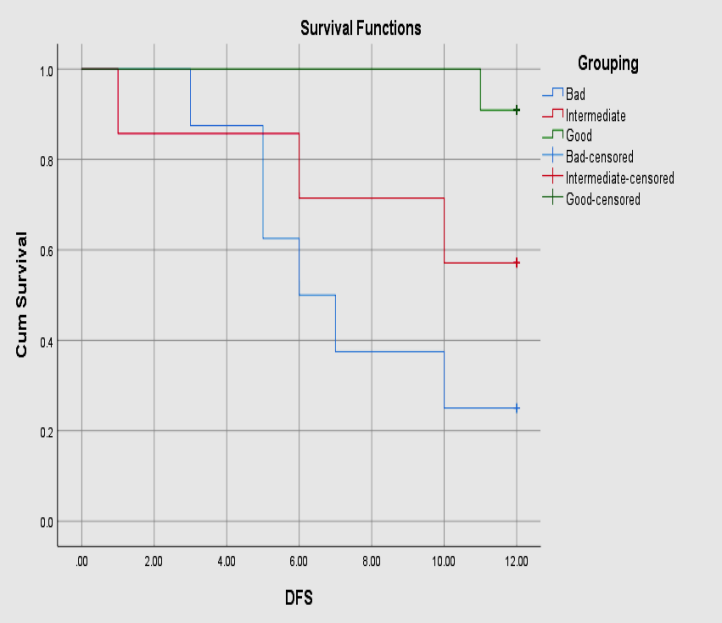

Figure 5. DFS in cases groups

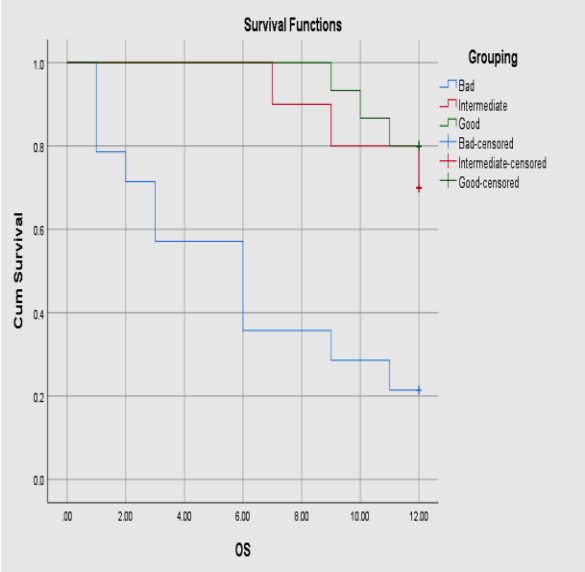

Figure 6. OS in cases groups