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ORIGINAL ARTICLE

Evaluation of Micro RNAs in Aplastic Anemia Patients Presenting at Tertiary Care Hospital LUMHS, Hyderabad

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ABSTRACT

Background: Bone marrow failure that results in pancytopenia causes aplastic anemia clinically.
Aim: To determine the frequency of positive microRNAs in plasma of patients with aplastic anemia.
Study Design: Descriptive cross-sectional study.
Methodology: Aplastic patients were assessed for microRNAs level and patients were considered positive if the level was ≥2
Results: Majority 20(66.7%) of the patients was aged ≤30 years with only 10(33.3%) patients aged above 30 years. There were 22(73.3%) male and 8(26.7%) female patients with a male to female ratio of 2.8:1. Severe, very severe and non-severe aplastic anemia was recorded in 18(60%), 4(13.3%) and 8(26.7%) patients respectively. Taking a cut-off value of ≥27 IU/mL, 15(50%) patients were positive for microRNAs.
Conclusion: It was concluded that a substantial proportion of patients with aplastic anemia were positive for microRNAs and the observed frequency increased with increasing severity of disease which suggested potential role of microRNAs in the diagnostic workup and management of aplastic patients.
Keywords: Aplastic Anemia and Micro-RNAs.

INTRODUCTION

Aplastic anemia is a bone marrow failure that results in pancytopenia clinically due to immune attack on bone marrow. The incidence of aplastic anemia in the West of 2 per million per year but incidence increases in Asia[1]. It is caused by auto-immune destruction of hematopoietic progenitor cells[2]. Thus micro RNA helpful in disease severity and response to therapy[3].

Its challenging to monitor its progress as we need to develop immune markers for disease.[4] Expression of microRNA changes is unknown but released into circulation as a result of apoptotic and necrotic cell death[5,6]. MiR-150-5p expressed in megakaryocyte lineage cells so it promotes MiR-150-p5 promotes megakaryocytes in aplastic anemia[7,8].

MicroRNAs, regulate normal cell homeostasis and are involved in many physiologic processes, including Hematopoiesis[9-11]. For high valid outcome, the pilot study was done for positive microRNAs in aplastic anemia and for sample size calculation.
This research helped in determining positive microRNAs in aplastic anemia patients as prognostic biomarker. As there is lack of local data regarding the role of positive microRNAs in aplastic anemic patients hence present project was carried out.

The objective of the study was to determine the frequency of positive micro-RNAs in plasma of patients with aplastic anemia.

METHODOLOGY

This study enrolled (n= 30) aplastic patients. These patients were assessed for microRNAs level and patients were considered positive if the level was ≥27 IU/mL. The frequency of positive microRNAs was noted. Almost 5 ml Blood sample in EDTA tubes was collected from subjects diagnosed as aplastic anemia on bone biopsy. CBC was performed. EDTA tube was centrifuged and plasma was separated and stored at -90 0C till the analysis. Real time PCR was performed on Rotor Gene Q 5-PLEX 9001570. Plasma microRNAs testing include RNA Extraction and Amplification. RNA extraction was done for the purification of cell free total RNA, which primarily included small RNAs from small volumes (up to 200µl) of serum and plasma using the mRNeasy Serum /plasma Kit. Prepared master mix, added template CDNA into individual plate and mixed and then performed real time cycler and interpreted the results after completion of cycles.

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Statistical analysis: Data analyzed by SPSS v.26. Chi-square test was applied with p-value ≤ 0.05 as significant. Descriptive data presented as frequency and percentages.

RESULTS

Majority 20(66.7%) of the patients was aged ≤30 years with only 10(33.3%) patients aged above 30 years. There were 22(73.3%) male and 8(26.7%) female patient. Other descriptive parameters of enrolled subjects were shown in table-1.

Table-1: Baseline Parameters (n=30)

Parameters	Categories	Frequency
Age	≤30 years	20 (66.7%)
	>30 years	10 (33.3%)
Mean ± SD	29.7±18.7	
Gender	Male	22 (73.3%)
	Female	8 (26.7%)
Residence	Rural	17 (56.7%)
	Urban	13 (43.3%)
Occupation	Field Worker	11 (36.7%)
	Laborer	10 (33.3%)
	Other	9 (30.0%)
Severity of Aplastic Anemia	Severe	18 (60.0%)
	Very Severe	4 (13.3%)
	Non Severe	8 (26.7%)
Addiction	Yes	12 (40.0%)
	No	18 (60.0%)
Hemoglobin (g/dl)	4.7±1.6	
Micro-RNAs Level (IU/mL)	25.6±10.7	

Taking a cut-off value of ≥27 IU/mL, 15 (50.0%) patients were positive for micro-RNAs as shown in Table-2.

Table-2: Frequency of Positive Micro-RNAs among Patients with Aplastic Anemia

MicroRNAs	Frequency (n)	Percentage (%)
++++	15	50.0
------	15	50.0

Statistically insignificant difference in the frequency of positive micro-RNAs across various subgroups of patients based on age, gender, residence, occupation, history of addiction. (Table-3).

Table 3: Comparison of positive Micro-RNAs among subgroups

Parameters	Subgroups	Positive Micro-RNAs	P-value
Age (years)	≤30 years	10 (50.0%)	1.000
	>30 years	5 (50.0%)	
Gender	Male	11 (50.0%)	1.000
	Female	4 (50.0%)	
Residence	Rural	8 (47.1%)	0.713
	Urban	7 (53.8%)	
Occupation	Field Worker	5 (45.5%)	0.904
	Laborer	(50.0%)	
	Other	5 (55.6%)	
Addiction	Yes	6 (50.0%)	1.000
	No	9 (50.0%)	

The frequency of positive microRNAs increased with increasing severity of aplastic anemia; non-severe versus severe versus very severe aplastic anemia (37.5% vs. 50% vs. 75%; p-value=0.472). The difference was however statistically insignificant as shown in Table-4.

Table-4: Positive Micro-RNAs across Various subgroups based on severity of anemia

Severity of Aplastic Anemia	microRNAs		
	Positive (n=15)	Negative (n=15)	Total
Severe Aplastic Anemia (n=18)	9 (50%)	9(50%)	18(100%)
Very Severe Aplastic Anemia (n=4)	3 (75%)	1(25%)	4(100%)
Non Severe Aplastic Anemia (n=8)	3 (37.5%)	5(62.5%)	8(100%)

P value 0.472

DISCUSSION

Mean age of the patients with aplastic anemia was 29.7±18.7 years in current project. Our observation is in line with that of Ehsan et al[12] (2011) who reported similar mean age of 27.9±18.7 years among patients presenting with aplastic anemia at Shaikh Zayed Hospital, Lahore. In another study conducted at Shaikh Zayed Hospital, Lahore, Ghazanfar et al[13] (2014) reported similar mean age of 32.0±15.7 years among patients of aplastic anemia.

We observed that there was a male predominance among patients with aplastic anemia with a male to female ratio of 2.8:1. Our observation is in line with that of Ehsan et al[12] (2011) who reported similar male predominance with male to female ratio of 2.8:1 among patients presenting with aplastic anemia at Shaikh Zayed Hospital, Lahore. In another study conducted at Shaikh Zayed Hospital, Lahore, Ghazanfar et al[13] (2014) observed similar male predominance among patients of aplastic anemia and reported a male to female ratio of 2.5:1. Wali et al[14] in 2011 (2.8:1). Our observation is also in line with that of Shah et al[15] (2011) who reported a similar male predominance (M:F, 2.1:1) among Indian such patients while Islam et al[16] (2010) reported it to be 1.9:1 in Bangladesh.

In the present study, severe, very severe and non-severe aplastic anemia was recorded in 18(60%), 4 (13.3%) and 8(26.7%) patients respectively. Our observation is in line with that of Hanif et al[17] (2007) who reported comparable frequency of severe (63.6%), very severe (9.1%) and non-severe (27.3%) aplastic anemia at National Institute of Child Health, Karachi. Ehsan et al[12] (2011) reported similar frequency of 68%, 12% and 20% for severe, very severe and non-severe aplastic anemia at Shaikh Zayed Hospital, Lahore. In a similar Indian study, Shah et al[15] (2018) observed comparable frequency of severe, very severe and non-severe aplastic anemia and reported it to be 62.6%, 4.4% and 33% respectively. Islam et al[16] (2010) observed similar frequency of severe (55.9%), very severe (8.8%) and non-severe (35.3%) aplastic anemia in Bangladesh.

In the present study, 50% of patients with aplastic anemia were positive for microRNAs and the observed frequency increased with increasing severity of disease; non-severe versus severe versus very severe aplastic anemia (37.5% vs. 50% vs. 75%; p-value=0.472). The present study is first of its kind and creates new paradigm for scientific research. In the present study, patients with aplastic anemia were positive for microRNAs thus microRNAs have a role in the diagnostic evaluation of patients suspected of aplastic anemia in future hematological practice. We also observed that the frequency of positive microRNAs increased with increasing severity of disease which suggests probable role of microRNAs in the risk stratification and management planning of patients with aplastic anemia in future clinical practice.

Limitations: Single centre study with small sample size and financial constrains.

CONCLUSION

It was concluded that a substantial proportion of patients with aplastic anemia were positive for microRNAs and the observed frequency increased with increasing severity of disease thus microRNAs have a role in the diagnostic workup and management of patients with aplastic anemia.

Author's contribution: MS,SK&NUAA: Conceptualized the study, analyzed the data, and formulated the initial draft., **YAS&RJ:** Contributed to the proof reading, **IDU:** Collected data.

Conflict of interest: None

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