

Expression Analysis of MicroRNA-155-5p in Patients with Polycythemia Vera and Essential Thrombocythemia

Sarah I. Khaleel^{1*}, Jaffar N. Alalsaidissa¹

¹ Department of Pathology and Forensic Medicine, College of Medicine University of Baghdad, Baghdad, Iraq

ABSTRACT

Background: Polycythemia Vera (PV) and Essential Thrombocythemia (ET) are classical myeloproliferative neoplasms (MPNs) characterized by somatic mutations in hematopoietic stem and progenitor cells (HSPCs), leading to abnormal proliferation of myeloid cell lineages. In addition to genetic mutations, dysregulation of microRNAs (miRNAs), a class of small non-coding RNAs (20–30 nucleotides in length), has been implicated in disease pathogenesis. **Objective:** This study aimed to evaluate the expression levels of miRNA-155-5p in patients with PV and ET compared to healthy controls and to investigate its correlation with common prognostic factors. **Methods:** A total of 52 patients were enrolled in the study, including 28 with PV and 24 with ET, along with 20 healthy individuals as controls. Plasma samples were extracted from peripheral blood, and miRNA-155-5p expression was analyzed using quantitative real-time polymerase chain reaction (RT-qPCR). **Results:** miRNA-155-5p was significantly upregulated in both PV ($p = 0.0001$) and ET ($p = 0.0001$) patients compared to the control group. Among PV patients, those with a history of thrombosis exhibited significantly higher miRNA-155-5p levels than those in the ET group ($p = 0.01$). However, no significant correlations were found between miRNA-155-5p expression and age, sex, JAK2 mutation status, hemoglobin (Hb) levels, white blood cell (WBC) count, or platelet (PLT) count. **Conclusion:** The findings suggest that miRNA-155-5p dysregulation may play a role in the pathogenesis of PV and ET, particularly in patients with thrombotic complications. Further studies are needed to elucidate the molecular mechanisms underlying miRNA-155-5p involvement in MPNs and its potential as a biomarker for disease progression and prognosis.

Keywords: Myeloproliferative Disorder, Polycythemia Vera, Essential Thrombocythemia, MicroRNA

INTRODUCTION

The classical myeloproliferative neoplasms (MPNs), also referred to as Philadelphia-negative MPNs, including condition such as Polycythemia Vera (PV), Essential Thrombocythemia (ET), and primary myelofibrosis are syndromes characterized by a stereotypical, but often overlapping, set of pathological and clinical features [1] these disorders defined by uncontrolled proliferation of one or more terminal myeloid cell lines in the peripheral blood due to somatic mutations that occur in (HSPCs) [2]. the most common mutations occur in Ph-ve MPN are *JAK2* mutation which is the most common molecular abnormality found in myeloproliferative neoplasms (MPN) [3], *JAK2* mutation is found in about 95% of polycythemia vera (PV) and 50–60% of essential thrombocythaemia (ET) or primary myelofibrosis (PMF) [4] other mutations include *MPL* and *CLAR* mutations that are responsible for activation the physiologic signal-transduction pathways responsible for hematopoiesis constitutively. The real mechanism by which these mutations impact on human HSC development and proliferation in the bone marrow microenvironment to produce clonal haematopoiesis and MPN remains unknown [5].

Every kind of myeloproliferative neoplasm has the capacity to change into a different type in addition to sharing phenotypic mimicry, which presents difficulties for diagnosis, risk assessment, and treatment selection. Furthermore, despite years of research, the cause and progression of myeloproliferative neoplasms remain unknown, and therapy is primarily supportive. However, a revolution in the field occurred with the identification of driver mutations, which were found in over 90% of patients and provided important light on their underlying mechanisms and significant insight into their pathogenesis [6]. MicroRNAs, also known as miRNAs, are a class of unpaired RNA molecules that do not encode proteins and typically

consist of 22 nucleotides on average. Their main job is to attach to target messenger RNA (mRNA) and operate as gene repressors, controlling the expression of genes and proteins. By preventing translation or destabilising the mRNA target at the post-transcriptional stage, this regulation is accomplished. Furthermore, miRNAs are essential for controlling apoptosis, proliferation, and differentiation of cells [7]. It is possible that dysregulated microRNAs contribute to the pathophysiology of myeloproliferative neoplasms. Differentiating between distinct forms of myeloproliferative neoplasms may be possible by analysing the expression patterns of related microRNAs in the pathogenesis of these tumours [8]. One of the most well-studied miRNAs, miRNA-155-5p, is essential for many physiological and pathological processes, including Down syndrome, cancer, inflammatory responses, viral infections, immunity, and hematopoietic lineage differentiation [9].

MATERIAL AND METHODS

This research utilized a cross-sectional design. and included patients diagnosed with PV and ET based on the 2016 World Health Organisation criteria for MPNs [10]. Additionally, a control group consisting of healthy people was also included in the study. With the agreement of the local Ethics Committee of the College of Medicine, University of Baghdad, Samples of peripheral blood from a total of (52 patients and 20 healthy volunteers) were recruited for the study. The research was done Following the principles that are specified in the Declaration of Helsinki. Informed consent was obtained from every participant. The clinical data obtained from patients' records at presentation included: age, sex, time and history of presentation, mutational status, blood indices at presentation (HB, total WBC count and PLT count). All patients who were included must

be tested for JAK2V617F mutation and should be negative for BCR-ABL1 fusion gene and none of the cases exhibited blastic transformation or fibrotic phase at any stage.

An EDTA tube was utilised to collect a 2mL sample of peripheral blood from each patient and control participant in the study. After plasma was collected and centrifuged within three hours, it was transferred to a 1.5 mL Eppendorf tube containing 300 µl of DNA/RNA Shield. The tube was then stored at a temperature below -20°C . RNA was extracted and stored at a temperature below -20°C for a duration of two weeks prior to the two-step RT-PCR analysis of MiRNA-155-5p expression.

RNA Extraction and Reverse Transcriptase PCR Reactions (RT-PCR)

Direct-zolTM RNA MiniPrep method (ZYMO research) was used to extract RNA from peripheral whole blood. In reverse transcriptase reactions, 3µl of total RNA extract and 0.5µl of stem-loop RT primer were utilised, 10 µl RNase Free water, 2 µl Prime ScriptTM Reverse Transcriptase (Takara Bio, USA). for quantitative PCR (qPCR), a reagent system was used, this system composed of (a fluorescent DNA-binding dye, GoTaq[®] Hot Start Polymerase, MgCl₂, dNTPs and a proprietary reaction buffer).

The reaction was performed on an automated Thermal Cycler (Sacace, Italy). The PCR conditions were as follows: denaturation at 95°C for 20 s, followed by 40 cycles of 20 s at 95°C , 20 s at 60°C , and the final extension step of 20s at 72°C . followed by Analysis of relative gene expression data using real-time quantitative PCR and the $2(-\Delta\Delta\text{Ct})$ method [11].

Statistical Analysis

Input of data was performed with Microsoft Excel 2019. A variety of qualitative and quantitative variables were utilised to record the data in preparation for analysis. the statistical application for social sciences (SPSS version 26) was utilised to conduct the analysis. Tables and graphs were employed to summarise the data along with measures of dispersion (standard deviation) and frequency (mean). Utilising the chi-square test, the relationship between categorical variables has been determined. In contrast to the utilisation of the ANOVA test to compare categorical variables with continuous variables, the independent sample (t-test) is employed to compare categorical variables. The area under the receiver operating characteristic curve (ROC curve) is utilised to determine the cut-off value. The liner regression test is a method utilised to assess the relationship between two continuous variables. The criterion for establishing statistical significance was a two-tailed p value that was equal to or less than 0.05.

Results

Out of 52 patients, 28 (53.8%) were PV, and the remainder 24(46.2%) were ET. The average age in the patient group was (53.6 ± 12.3) years, while in the control group was (52.1 ± 15 years). The mean miRNA 155-5p was notably elevated in the PV and ET group compared to the control group, and this difference was statistically significant ($p=0.0001$) (Table 1and Figure 1) while the average miRNA 155-5p levels in PV and ET showed no statistically significant difference between both PV and ET ($p=0.71$) (Table2).

Table 1: miRNA 155-5p Across Studied Groups

	Control	PV	ET	P Value
Mean $\pm$ SD	0.32 ± 0.28	1.1 ± 0.9	1.03 ± 0.9	0.0001

Table 2: miRNA 155-5p Across MPN Groups

	PV	ET	P Value
Mean $\pm$ SD	1.1 ± 0.9	1.03 ± 0.9	0.71

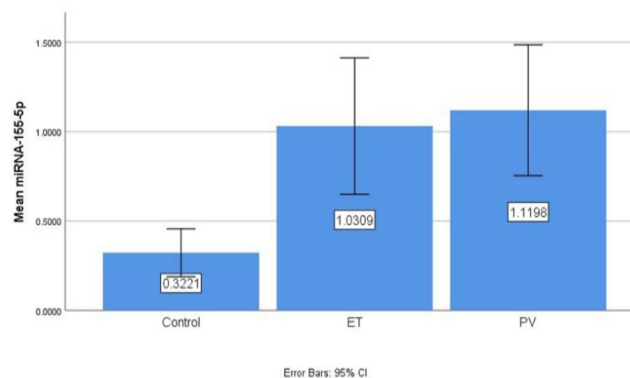
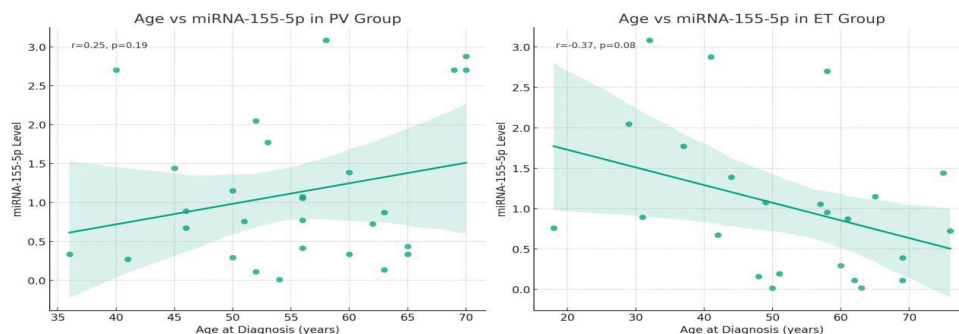


Figure 1: The Mean miRNA 155-5p Across Studied Groups



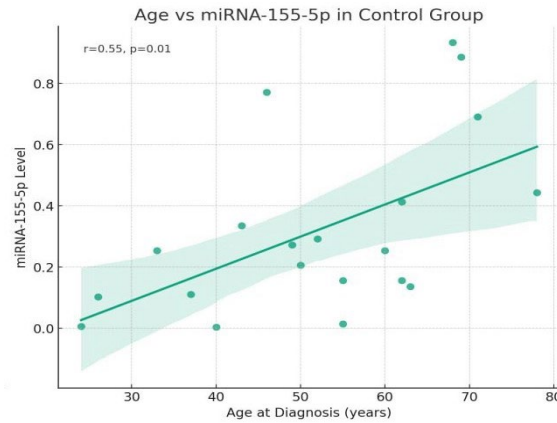


FIGURE 2: The Association between miRNA 155-5p and Age Across Studied Groups

The mean miRNA 155-5p levels among females and males in the MPN group and in the control, group showed, no significant difference between males and females within the MPN group and within the control group ($p>0.05$), while a significant difference was observed between males and females of the MPN and control groups ($p<0.05$). For age, there was no significant correlation between age and miRNA 155-5p level across PV and ET group ($p=0.19$) and ($p=0.08$) respectively, while in control group there was statistically significant correlation between age and miRNA 155-5p level ($p>0.05$) (Figure2).

The evaluation of miRNA 155-5p levels across MPN groups based on thrombosis and splenomegaly revealed a higher miRNA 155-5p level in patients with a history of thrombosis ($p=0.01$). Among MPN groups, patients with a history of thrombosis exhibited a higher miRNA 155-5p level in the PV group compared to the ET group ($p=0.01$),

while no significant difference was observed among patients with no history of thrombosis ($p=0.7$). Regarding splenomegaly and JAK-2 mutation, there was no significant difference observed between patients with and without splenomegaly or JAK-2 mutation ($p>0.05$) (Table 3).

The Association of Hematological Parameters with miRNA 155-5p Level

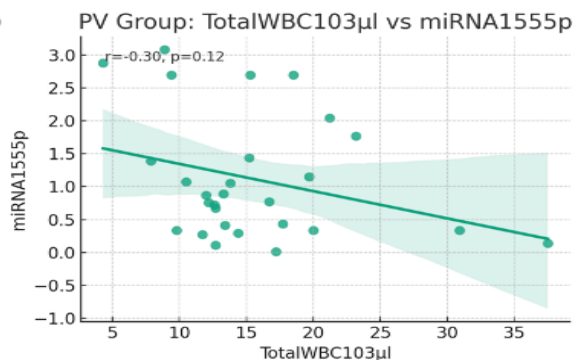
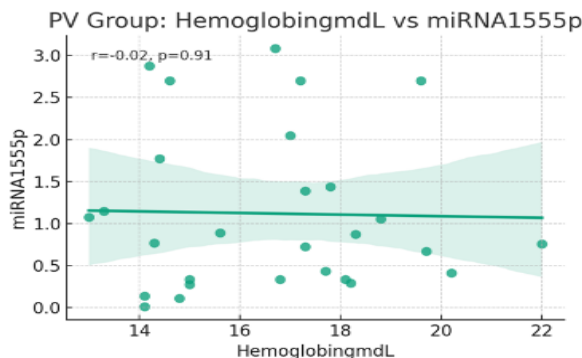
There was no significant statistical association between hematological parameters and miRNA 155-5p level in MPN patients ($p>0.05$) (Table 4 and Figures 3). Furthermore, the assessment of association between each disease of MNP and hematological parameters, again did not show any significant statistical association between miRNA 155-5p level and any of hematological parameters ($p>0.05$)

Table 3: Thrombosis and Splenomegaly Association with miRNA 155-5p Level

	Mean	Total	P Value	PV	ET	P Value
Hx of Thrombosis	No	0.98±0.8	0.01	0.9±0.7	1±0.9	0.71
	Yes	1.8±0.9		2.8±0.2	1.1±0.3	0.01
Splenomegaly	No	0.87±0.7	0.19	1±0.8	0.8±0.7	0.26
	Yes	1.15±0.9		1.1±0.9	1.4±1	0.17
JAK-2 Mutation	Mutated	1.1±0.9	0.18	1.1±0.9	1.1±0.9	0.83
	Non	0.8±0.7		0.29	0.9±0.9	0.34

Table 4: Hematological Parameters with miRNA 155-5p Level

		miRNA 155-5p level		
		MNP Total	PV	ET
Hemoglobin (gm/dL)	r value	0.055	-0.023	0.106
	P value	0.630	0.906	0.621
	N	80	28	24
Total WBC (10 ³ /μl)	r value	-0.001	-0.300	0.290
	P value	0.994	0.120	0.169
	N	80	28	24
Platelets (10 ³ /μl)	r value	-0.059	0.011	-0.038
	P value	0.603	0.957	0.861
	N	80	28	24



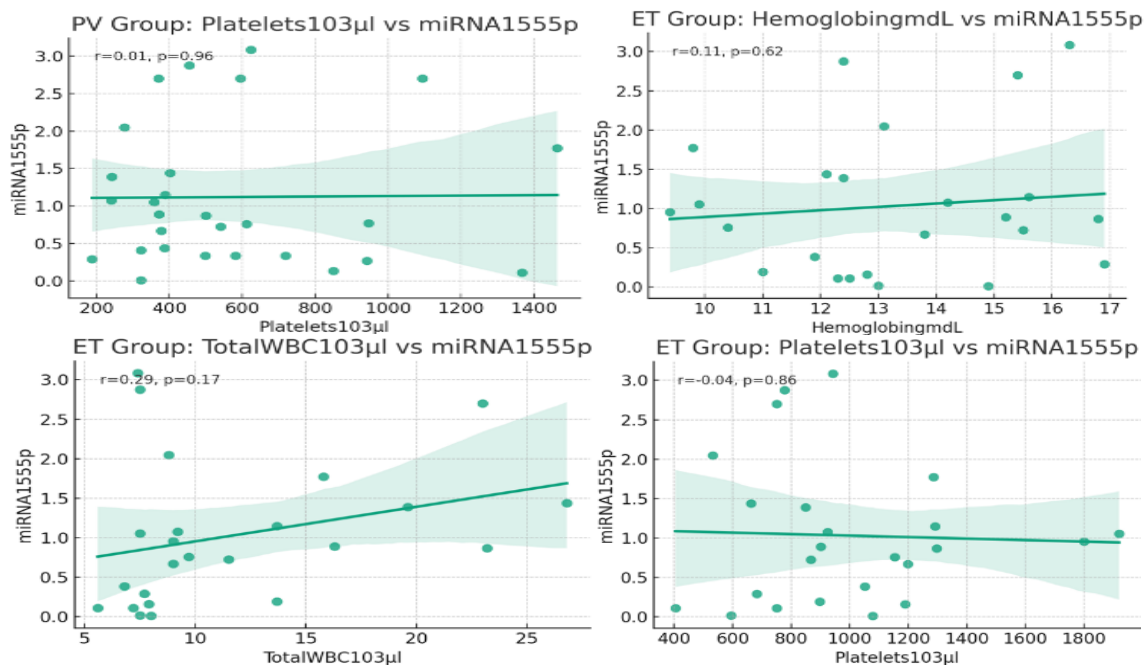


FIGURE 3: Correlation of Hematological Parameters with miRNA 155-5p Level

Discussion

The classical myeloproliferative neoplasms, Polycythemia Vera (PV) and Essential Thrombocythemia (ET), are distinguished by the proliferation of terminally differentiated myeloid cells. These disorders are similar in biology but differ in clinical presentation and histopathological features. The pathophysiology of MPNs is complex and poorly understood, involving the activation of the JAK-STAT pathway, clonal haematopoiesis, and systemic inflammation [12]. MiR-155, a miRNA, regulates the differentiation of B and T lymphocytes and T-helper cells, influencing Th1/Th2 cytokine secretion profiles and inhibiting the establishment of myeloid and erythroid colonies [13]. It is reasonable that miR-155 expression changes in malignant haematological tumors, making it a promising biological marker for malignancies [14]. The purpose of this study is to examine the expression profiles of miR155-5p in the pathogenesis of PV and ET, and its relation to the most significant prognostic factors and investigate their potential as therapeutic targets of the future. In this study and depending on qRT-PCR results, Plasma mir155-5p expression was substantially higher in patients with ET and PV compared to the control group, but was comparable between disease groups (Table 1). Increased mir155 expression carried on by circulating inflammatory cytokines which has a significant part of the pathophysiology of MPNs. These results are compatible with the study of Tombak A et al [15] in India in 2015 and study of Stolyar MA et al [16] in Russia in 2020, while another study of Tran et al [17] in Denmark in 2020 show different results with downregulation of mir155-5p in ET patients and study of Hussein K et al [18] show non-significant level of mir155-5p in ET. In this study the male to female ratio is (0.6:1) which is in contrast to another study of Antonioli E et al [19] report 104 MPN patients revealed (1.04:1), and another study Carpenter L et al [20] report 1049 MPN patients revealed (1.07:1) and another studies as study of Wu J et al [21] revealed (0.7:1). These differences in ratios may be due to variable sample size in these studies. The level of miRNA 155-5p is not significantly associated with sex of patients with MPNs (no significant difference between males and

females within the MPN groups ($p > 0.05$) in this study which is in accordance with Stolyar MA et al [16] study. The age of the patients included in this study, ranged between (20-78 years) which is approximate to an Iraqi study of Mohammed SK et al [22] which was (30-72 years) and another study of Liu D et al [23] in China who revealed (32-82 years) and a study of Singh S et al [24] in India revealed (28-82) and quite different from Salim M et al [25] study in Erbil revealed (46-73 years). These differences in age may be due to variable demographic data in these studies. There is no significant correlation between the age of the patients with MPN and level of miRNA 155-5p which is compatible with Tombak A et al [15] study and Stolyar MA et al [16] study. There was no significant difference between JAK-2 mutational status and level of miRNA 155-5p of patients with MPN groups included in this study which is compatible with Stolyar MA et al [16] study, probably deregulated miRNA 155-5p acts independently from JAK2 signaling in pathogenesis of MPN. Thrombosis is another prognostic factor in MPN that shows a higher miRNA 155-5p level in patients with a history of thrombosis ($p = 0.01$) with the highest level being in patients with PV. This result is not in agreement with study of Tran et al [17] that shows downregulation of miRNA 155 in ET patients. The assessment of correlation between each disease of MPN and hematological parameters (HB level, WBC and PLT counts) did not show any significant correlation between miRNA 155-5p level and any of hematological parameters which is compatible with Stolyar MA et al [16] study.

Conclusion

1-The aberrant expression of miRNA155-5p may contribute to MPN pathogenesis. 2-Expression levels of haematopoiesis-specific miRNA, miRNA 155-5p are not affected by age, sex, JAK2V617F status and haematological parameters

Recommendation

1-Additional investigation is currently required to comprehend the pathogenesis of these disorders. 2.More investigation into the role miRNAs play in JAK2V617F clonal expansion, distinct haematopoiesis

in various MPNs, disease progression, and leukaemia transformation will deepen our knowledge of MPN pathogenesis and offer new

opportunities for disease-modifying or disease-eradicating therapeutic approaches.

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