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Research Article

# Analysis of MicroRNA -155-5p Expression in Patients with Primary Myelofibrosis

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**Abstract**

**Background:** Primary myelofibrosis is a chronic myeloproliferative neoplasm characterized by abnormal megakaryocyte proliferation and fibrosis that destroys healthy bone marrow. This results in extramedullary hematopoiesis, variable blood cell deficiencies, hepatosplenomegaly, general symptoms, progression to leukemia, and a reduced lifespan. Myelofibrosis can occur as a de novo myeloproliferative neoplastic disorder or evolve from other myeloproliferative neoplasms, including Polycythemia Vera or Essential Thrombocytosis. MicroRNAs (miRNAs) are short, non-protein-coding RNA molecules, typically 18–24 nucleotides in length. Dysregulation of miRNAs may contribute to the disease phenotype.
**Objective:** To investigate the expression level of MicroRNA-155-5p in patients with Primary Myelofibrosis compared to healthy controls and its correlation with common clinic-pathological factors.
**Methods**: twenty-eight patients with Primary Myelofibrosis and twenty healthy subjects were examined as controls. Expression analysis of MicroRNA-155-5p was performed using reverse transcription-quantitative polymerase chain reaction (qRT-PCR) on plasma isolated from peripheral blood.
**Results:** MicroRNA-155-5p expression was significantly upregulated in patients with Primary Myelofibrosis (P = 0.0001). However, no significant correlations were found between MicroRNA-155-5p and age, sex, Janus kinase 2 mutation status, or hematological parameters, including hemoglobin, white blood cell count, and platelet count.
**Conclusion:** MicroRNA-155-5p expression is not influenced by age, sex, Janus kinase 2 mutation status, or hematological parameters. Aberrant expression of MicroRNA-155-5p may contribute to the pathogenesis of Primary Myelofibrosis, warranting further research to understand the disease mechanisms better.
**Keywords:** Essential thrombocytosis; MicroRNA; Myeloproliferative neoplasms; Polycythemia Vera; Primary Myelofibrosis.

## Introduction:

The classical myeloproliferative neoplasms (MPNs) are characterized by the proliferation of terminally differentiated myeloid cells. Primary myelofibrosis (PMF) is the most aggressive of the classic MPNs, marked by extensive heterogeneity in clinical manifestations and molecular markers (1). A significant proportion of patients harbor activated mutations, including the Janus kinase 2 (JAK2) mutation which drives cytokine-independent proliferation of hematopoietic progenitor cells by constitutively activating both canonical and non-canonical downstream pathways. Other driver mutations, such as calreticulin (CALR) and myeloproliferative leukemia virus oncogene (MPL), mediate persistent JAK-STAT signaling, a key process underlying the disease's pathophysiology (2, 3). Numerous Pathological mechanisms, including defective myeloid cell proliferation, aberrant stem cell trafficking, and increased production of inflammatory cytokines, contribute to the development of PMF (4). These processes lead to progressive changes in marrow histology, where all hematopoietic elements are initially preserved, followed by the accumulation of coarse reticulin fibers arranged in parallel bundles within the increased fibrous tissue. Ultimately, this progression culminates in the osteo-myelosclerotic stage (5,6). These mechanisms disrupt the normal medullary erythropoietic environment, resulting in anemia, bone marrow failure, splenomegaly, infections, bleeding, and constitutional symptoms (7,8). Patients with PMF have a median survival of 5.7 years, with a range of 4 to 7 years post-diagnosis (9). Currently, autologous hematopoietic stem cell transplantation remains the only treatment option capable of potentially prolonging survival or cure PMF (10). MicroRNAs (miRNAs) are a class of single-stranded, non-protein-coding RNA molecules, typically averaging 22 nucleotides in length (11). They bind to target messenger RNA (mRNA) and function as gene repressors, regulating gene and protein expression (12). MiRNAs play a critical role in controlling cellular processes such as apoptosis, proliferation, and differentiation (13).

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Extracellular miRNAs have been extensively studied as potential biomarkers for various conditions and serve as signaling molecules facilitating intercellular communication (14). Among these, miRNA-155-5p is one of the most well-researched miRNAs. It plays a significant role in regulating immune cell differentiation and cytokine secretion, leading to cytokine hypersensitivity in bone marrow progenitors. Altered expression of miRNA-155-5p has been linked to hematological malignancies, making it a promising biological marker for these diseases (15).

The study aimed to analyze the expression level of miRNA-155-5p in patients with Primary Myelofibrosis (PMF) compared to healthy controls and evaluate its correlation with common clinicopathological factors.

**Patients, Materials, and Methods:**

This study employed a cross-sectional design and included patients diagnosed with Primary Myelofibrosis (PMF) according to the 2016 World Health Organization (WHO) criteria for myeloproliferative neoplasms (MPNs)(17). A control group of healthy individuals was also included.

With approval from the local Ethics Committee of the College of Medicine, University of Baghdad, 48 participants (28 patients with PMF and 20 healthy volunteers) were recruited. The study adhered to the Declaration of Helsinki ethical standards, and all participants provided written informed consent. Clinical data obtained from patients' records included:

**Demographics:** Age and sex

**Disease Characteristics:** Time and history of presentation, JAK2 mutation status

**Hematological Parameters:** Hemoglobin (Hb), total white blood cell count (WBC), and platelet count (PLT).

**Inclusion Criteria**

**JAK2V617F mutation** and confirmed negative for the **BCR-ABL1 fusion gene**. Patients were tested for the

None of the patients exhibited blastic transformation**.**

**Sample Collection and Processing**

For each participant, 2 mL of peripheral blood was collected in an EDTA tube. Plasma was separated by centrifugation within 3 hours of collection and then transferred into a 1.5 mL Eppendorf tube containing 300 µl of DNA/RNA Shield for preservation and stored at a temperature below -20 °C. The RNA was extracted within a period of two weeks and stored at a temperature below -20°C till the time of assessing the expression of MiRNA-155-5p using a qRT-PCR method.

**RNA isolation and Reverse Transcriptase PCR procedures (RT-PCR)**

Direct-zol™ RNA MiniPrep method (Cat. # R2051, ZYMO research, USA) was used to extract RNA from peripheral whole blood. Reverse transcriptase reactions contained 3µl isolated total RNA, 0.5µl stem-loop RT primer, 10 µl RNase Free water, and 2 µl Prime Script TM Reverse Transcriptase (Cat. # RR037A, Takara Bio, USA). For quantitative PCR (qPCR), a reagent system was used, this system was composed of (a fluorescent DNA-binding dye, GoTaq® Hot Start Polymerase, MgCl2, dNTPs, and a proprietary reaction buffer). The process was carried out using an automated Thermal Cycler (Sacace, Italy). The PCR conditions were as follows: denaturation at 95°C for 20 seconds, followed by 40 cycles of 20 seconds at 95°C, 20 seconds at 60°C, and the final extension step of 20 seconds at 72°C followed by the analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\text{delta deltaCT}}$ method (18).

**Statistical analysis:** The method of inputting data was performed with Microsoft Excel 2019. The analysis was conducted using the statistical package for social sciences (SPSS version 26). A randomly selected sample (*t*-test) was employed to compare continuous parameters with categorical parameters. Chi-squared tests have been used to measure the association between categorical parameters while (ANOVA) tests were used to compare between categorical variables. ROC curve (receiver operating characteristic curve) was used to measure the area under the curve to measure the cutoff value. The linear regression test was used to measure the association between two continuous variables.

**Results:**

Out of 28 patients with PMF, 19 of them were males comprised 67.8% while 9 were females and comprised 30% (9). The mean age±SD of patients was (53.6 ± 12.2) years, whereas in the control group was (52.1 ± 15 years). The mean±SD MiRNA 155-5p was notably elevated in the patient group (1.04 ± 0.82) with PMF compared to the control group (0.32 ± 0.28), and this difference was statistically significant (*P*=0.0001; Table 1, and Figure 1)

**Table1: MiRNA 155-5p level across studied groups**

| | Control | PMF | *P* value |
|---|---|---|---|
| Mean ± SD | 0.32 ± 0.28 | 1.04 ± 0.82 | 0.0001 |

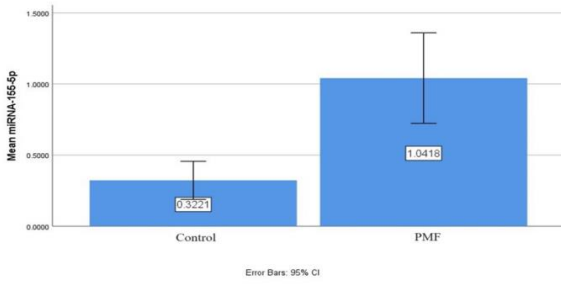


**Figure. 1: The mean MiRNA 155-5p levels across the studied groups.**

**Age and Sex association with MiRNA 155-5p level**
The mean MiRNA 155-5p levels among males and females within the PMF group showed no significant difference (*P*=0.81). A significant difference was observed among males and females of the PMF and control groups (*P*=0.001).
Regarding the age of participants, there was no significant correlation between age and MiRNA 155-5p across both patients and control groups (*P*>0.05) (Figures 2A and B).

**JAK-2 mutation status, splenomegaly, and hematological parameters association with MiRNA 155-5p level**
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 2).

**Table 2: Association of splenomegaly and JAK2 mutation with MiRNA 155-5p mean level in PMF patients**

| Variable | | Mean± SD | *P* value |
|---|---|---|---|
| Splenomegaly* | No | 0.7±0.2 | 0.21 |
| | Yes | 1±0.8 | |
| JAK-2 mutation** | Mutated | 1.1±0.8 | 0.36 |
| | Unmutated | 0.8±0.5 | |

* The mean level of MiRNA-155-5p ± standard deviation in Primary Myelofibrosis patients with splenomegaly compared to those without splenomegaly.
** The mean level of MiRNA-155-5p ± standard deviation in Primary Myelofibrosis patients with JAK-2 mutation compared to those Unmutated

Regarding hematological parameters, there was no significant correlation between hematological parameters and MiRNA 155-5p level in PMF patients (*P*>0.05; Table 3, and Figures 2C, D, and E).

**Table 3: Hematological parameters correlation with MiRNA 155-5p level**

| | | **PME** |
|---|---|---|
| Hemoglobin (gm/dL) | No. | 28 |
| | r value | 0.048 |
| | *P* value | 0.810 |
| Total WBC (10^3/µl) | N | 28 |
| | r value | 0.056 |
| | *P* value | 0.777 |
| Platelets (10^3/µl) | N | 28 |
| | r value | -0.294 |
| | *P* value | 0.129 |

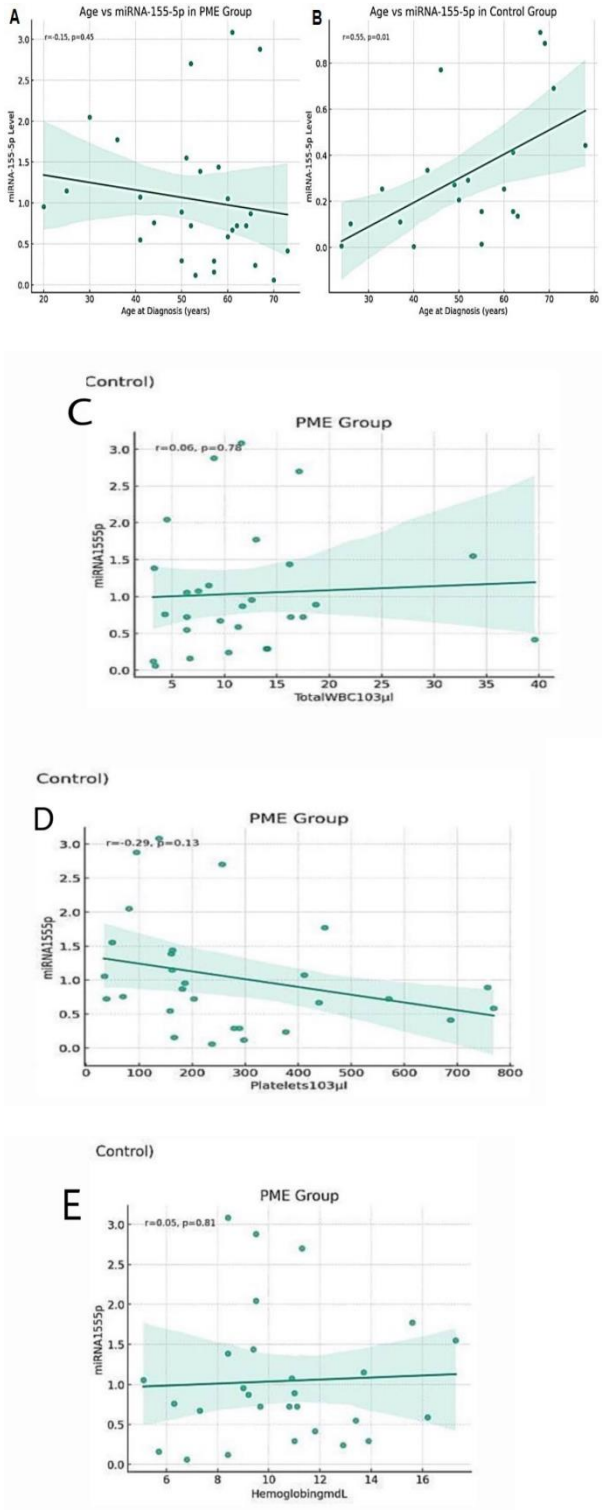


**Figure 2** The association of miRNA 155-5p level with age of studied groups (A and B), total WBC count (C), platelets count (D), and hemoglobin level (E),

**Discussion:**
In this study and depending on qRT-PCR results, plasma mir155-5p expression was substantially elevated in patients with PMF in contrast with the control group. These results agreed with the study of Tombak *et al.* (22), and the study of Norfo *et al.* (23) where both demonstrated upregulation in mir155 expression leading to increased proinflammatory

cytokine production which has a significant role in the pathophysiology of MF. The male-to-female ratio was (2:1) while the study of Alwan AF (24) showed (1.1:1), different from the study of Tombak *et al.* (22) which showed (0.6:1). 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 the sex of patients with PMF (no significant difference between males and females within PMF groups). The average age in the patient category was (53.6 ± 12.3) years while in the research of Tombak et al (22), was (54.8 ± 16,5) years, there is no significant correlation between the age of patients within PMF and the amount of miRNA 155-5p which was in agreement with the study of Tombak, *et al.* (22).

There were no significant differences between JAK-2 mutational status and level of miRNA 155-5p of patients with PMF groups included in this study was in agreement with Stolyar *et al.* (25) study and Tombak *et al.* (22), probably dysregulated miRNA 155-5p operates autonomously in the development of MPN, separately from JAK2 signaling.

The assessment of the correlation between the expression of miRNA155-5p and hematological parameters (HB level, WBC, and PLT counts) did not show any significant correlation between miRNA 155-5p level and any of the hematological parameters which were in agreement with Stolyar *et al.* (25) study.

It should be taken into account in future miRNA research that cells within the bone marrow environment, in addition to the mutated cell clone, contribute to the pathogenesis of MPNs and the expression of miRNAs.

**Limitations:** one of the limitations of the current study is the sample size**.**

**Conclusion:**

The aberrant expression of miRNA155-5p may contribute to PMF pathogenesis. Expression levels of miRNA 155-5p are not affected by age, sex, JAK2V617F status, and hematological parameters.

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**Authors' Declaration:**

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current research, have been given permission for re-publication attached to the manuscript. The project was approved by the Research Ethics Committee in the College of Medicine, University of Baghdad (issue number 26B, 25 Jan 2023)

**Conflict of interest**: None.

**Funding:** None

**Authors' contributions**: Both authors (Dr. Jaffar Nori Sarah I. Khaleel ) worked together to conduct a literature search, Data analysis & interpretation, Manuscript preparation, editing, and review.

***References***

***1.*** *Bose P, Masarova L, Amin HM, Verstovsek S. Philadelphia chromosome-negative myeloproliferative neoplasms (Chapter 6). In: Kantarjian HM, Wolff RA, Rieber AG, eds. The MD Anderson Manual of Medical Oncology. 4th ed. McGraw-Hill, LLC: China; 2022. pp. 119-162. https://accessmedicine.mhmedical.com/content.aspx?bookid=3151&sectionid=264035510*

*2.Gangat N, Tefferi A. Myelofibrosis biology and contemporary management. Br J Haematol. 2020 Oct;191(2):152-170. .*
*https://doi.org/10.1111/bjh.16576*

*3. Tefferi A. Primary myelofibrosis: 2021 update on diagnosis, risk-stratification and management. Am J Hematol. 2021 Jan;96(1):145-162.*
*https://doi.org/10.1002/ajh.26050*

*4. Tremblay D, Mascarenhas J. Next Generation Therapeutics for the Treatment of Myelofibrosis. Cells. 2021 Apr 27;10(5):1034.*
*https://doi.org/10.3390/cells10051034*

*5. Curto-Garcia N, Harrison C, McLornan DP. Bone marrow niche dysregulation in myeloproliferative neoplasms. Haematologica. 2020 May;105(5):1189-1200.*
*https://doi.org/10.3324/haematol.2019.243121*

*6. Ng ZY, Fuller KA, Mazza-Parton A, Erber WN. Morphology of myeloproliferative neoplasms. Int J Lab Hematol. 2023 Jun;45 Suppl 2:59-70.*
*https://doi.org/10.1111/ijlh.14086*

*7. Chifotides HT, Bose P, Verstovsek S. Momelotinib: an emerging treatment for myelofibrosis patients with anemia. J Hematol Oncol. 2022 Jan 19;15(1):7.*
*https://doi.org/10.1186/s13045-021-01157-4*

*8. Passamonti F, Harrison CN, Mesa RA, Kiladjian JJ, Vannucchi AM, Verstovsek S. Anemia in myelofibrosis: Current and emerging treatment options. Crit Rev Oncol Hematol. 2022 Dec;180:103862.*
*https://doi.org/10.1016/j.critrevonc.2022.103862*

*9. Fuentes-Mattei E, Bayraktar R, Manshouri T, Silva AM, Ivan C, Gulei D,et al. miR-543 regulates the epigenetic landscape of myelofibrosis by targeting TET1 and TET2. JCI Insight. 2020 Jan 16;5(1):e121781*
*https://doi.org/10.1172/jci.insight.121781*

*10. Sastow D, Tremblay D. Emerging Treatment Options for Myelofibrosis: Focus on Anemia. Ther Clin Risk Manag. 2023 Jun 28;19:535-547.*
*https://doi.org/10.2147/TCRM.S386802*

*11. Tefferi A. Primary myelofibrosis: 2023 update on diagnosis, risk-stratification, and management. Am J Hematol. 2023 May;98(5):801-821.*
*https://doi.org/10.1002/ajh.26857*

*12. Abbas DD, Al-Rubaie HA. Association of Microrna-153-3p Expression in Response to*

*Treatment with Imatinib in Patients with Chronic Myeloid Leukemia. The Egyptian Journal of Hospital Medicine. 2023 Jan 1;90(1):1049-52.* https://doi.org/10.21608/ejhm.2023.280208

*13. Mehjabin A, Kabir M, Micolucci L, Akhtar MM, Mollah AKMM, Islam MS. MicroRNA in Fibrotic Disorders: A Potential Target for Future Therapeutics. Front Biosci (Landmark Ed). 2023 Nov 29;28(11):317.* https://doi.org/10.31083/j.fbl2811317

*14. Mohd Yacob A, Muhamad NA, Chang KM, Akmal Hisham H, Mat Yusoff Y, Ibrahim L. Hsa-miR-181a-5p, hsa-miR-182-5p, and hsa-miR-26a-5p as potential biomarkers for BCR-ABL1 among adult chronic myeloid leukemia treated with tyrosine kinase inhibitors at the molecular response. BMC Cancer. 2022 Mar 26;22(1):332.* https://doi.org/10.1186/s12885-022-09396-5

*15. Szelenberger R, Kacprzak M, Saluk-Bijak J, Zielinska M, Bijak M. Plasma MicroRNA as a novel diagnostic. Clin Chim Acta. 2019 Dec;499:98-107.* https://doi.org/10.1016/j.cca.2019.09.005

*16. Elton TS, Selemon H, Elton SM, Parinandi NL. Regulation of the MIR155 host gene in physiological and pathological processes. Gene. 2013 Dec 10;532(1):1-12.* https://doi.org/10.1016/j.gene.2012.12.009

*17. Garmezy B, Schaefer JK, Mercer J, Talpaz M. A provider's guide to primary myelofibrosis: pathophysiology, diagnosis, and management. Blood Rev. 2021 Jan;45:100691.* https://doi.org/10.1016/j.blre.2020.100691

*18. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM,et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022 Sep 15;140(11):1200-1228.* https://doi.org/10.1182/blood.2022015850

*19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8.* https://doi.org/10.1006/meth.2001.1262

*20. Tremblay D, Yacoub A, Hoffman R. Overview of Myeloproliferative Neoplasms: History, Pathogenesis, Diagnostic Criteria, and Complications. Hematol Oncol Clin North Am. 2021 Apr;35(2):159-176.* https://doi.org/10.1016/j.hoc.2020.12.001

*21. Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X. miR-155: an important role in inflammation response. Journal of immunology research. 2022 Apr 6;2022* https://doi.org/10.1155/2022/7437281

*22. Tang L, Peng Y, Li C, Jiang HW, Mei H, Hu Y. Prognostic and Clinicopathological Significance of MiR-155 in Hematologic Malignancies: A Systematic Review and Meta-analysis. J Cancer. 2019;10(3):654-64.* https://doi.org/10.7150/jca.28537

*23. Tombak A, Ay OI, Erdal ME, Sungur MA, Ucar MA, Akdeniz A,et al. MicroRNA expression analysis in patients with primary myelofibrosis, polycythemia vera, and essential thrombocythemia. Indian J Hematol Blood Transfus. 2015 Dec;31(4):416-25.* https://doi.org/10.1007/s12288-014-0492-z

*24. Norfo R, Zini R, Pennucci V, Bianchi E, Salati S, Guglielmelli P, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. miRNA-mRNA integrative analysis in primary myelofibrosis CD34+ cells: role of miR-155/JARID2 axis in abnormal megakaryopoiesis. Blood. 2014 Sep 25;124(13):e21-32.* https://doi.org/10.1182/blood-2013-12-544197

*25. Alwan AF. Clinical features and therapeutic outcome of 30 patients diagnosed with primary myelofibrosis at the National Center of Hematology. J Fac Med Baghdad. 2015 Jan. 4;56(4):362-6.* https://doi.org/10.32007/jfacmedbagdad.564545

*26. Stolyar MA, Gorbenko AS, Bakhtina VI, Martynova EV, Moskov VI, Mikhalev MA, et al. Investigation of miR-155 level in the blood of patients with chronic lymphocytic leukemia and Ph-negative myeloproliferative neoplasms. Klin Lab Diagn. 2020;65(4):258-264. Russian.* https://doi.org/10.18821/0869-2084-2020-65-4-258-264

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# تحليل تعبير MicroRNA -155-5p في المرضى المصابين بالتليف النقوي الأولي

**ساره اسماعيل خليل، فرع علم الامراض والطب العدلي، كلية الطب ،جامعة بغداد، بغداد ، العراق.**
**جعفر نوري جعفر، فرع علم الامراض والطب العدلي، كلية الطب ،جامعة بغداد، بغداد ، العراق.**

**الخلفية:** تليف النقوي للعظم هو ورم نقوي تكاثري مزمن يتميز بتكاثر غير نمطي للخلايا العملاقة الليفية، مما يؤدي إلى تدمير نخاع العظام الصحي وبالتالي حدوث تكون الدم خارج نخاع العظام، وتقلبات في مستويات نقص خلايا الدم، وتضخم الكبد والطحال، وأعراض عامة، وتطور نحو اللوكيميا، وانخفاض متوسط العمر المتوقع. يمكن أن يكون التليف النقوي إما اضطراب تكاثري نقوي بدئي يسمى التليف النقوي الأولي (PMF) أو يمكن أن يتطور من الأورام النقوية التكاثرية الأخرى، بما في ذلك كثرة الحمر الحقيقية (PV) أو كثرة الصفيحات الأساسية .(ET) الميكروRNAs ، أو miRNAsاختصارا، هي نوع من RNAs أحادية الشريط وغير مشفرة للبروتينات بطول متوسط يبلغ 22 نيوكليوتيد. قد يعتبر اضطراب تنظيم الميكرو RNA (miRNA) كعوامل إضافية تؤثر على نمط المرض
**أهداف هذه الدراسة:** التحقيق في مستوى تعبير miRNA-155-5P في مرضى التليف النقوي الأولي (PMF) مقارنة بالأشخاص الأصحاء ومقارنة ارتباطه بالعوامل السريرية المرضية الشائعة.
**المرضى والمواد وطرائق العمل:** تم فحص ثمانية وعشرين مريضا بالتليف النقوي الأولي .(PMF) وتم استخدام عشرين شخصا صحيا كمجموعة ضابطة. تم إجراء تحليل التعبير عن miRNA-155-5p بواسطة تفاعل البلمرة المتسلسل الكمي في الوقت الحقيقي (RT-RCR) باستخدام البلازما المعزولة من الدم المحيطي للمرضى .
**النتائج:** كان تعبير miRNA-155-5p مرتفعا في مرضى التليف النقوي الأولي .(p=0.0001) الارتباطات بين miRNA-155-5p والعمر، الجنس، حالة JACK2 ومعايير الدم( الهيموغلوبين عدد الخلايا البيضاءWBC ، عدد الصفيحات الدموية PLT) لم تكن ذات دلالة إحصائية .
**الاستنتاجات:** تشير نتائجنا إلى أن المتغيرات الإضافية، بما في ذلك التعبير غير الطبيعي عنmiRNA-155-5p ، قد تساهم في مرض التليف النقوي، لذا هناك حاجة لمزيد من الأبحاث لفهم التسبب في هذه الاضطرابات في العصر الحالي.
**الكلمات المفتاحية**: كثرة الصفيحات الأساسية، الميكروRNA ، الأورام النقوية التكاثرية، كثرة الحمر الحقيقية، التليف النقوي الأولي.