Original Article

Sex-Specific Correlation of microRNA-21 Expression in Chronic Myeloid Leukemia Patients Under Tyrosine Kinase Inhibitor Therapy

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ABSTRACT

Objective: To investigate the relation between expression level of microRNA-21 (miR-21) and patient gender in patients of CML receiving TKI therapy.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the University of Al-Qadisiya, College of Medicine, Iraq from 1st April 2025 31st August 2025.

Methods: This study included 50 chronic myeloid leukemia patients under tyrosine kinase inhibitors treatment. Molecular analysis of miR-21 expression was performed using real-time quantitative PCR. Statistical analysis, including correlation and cross-tabulation, was used to investigate the relation between gender, miR-21 expression, tyrosine kinase inhibitors type, and treatment response.

Results: A statistically significant association was observed between microRNA-21 expression level and patient sex (r= 0.312, p=0.027). The cohort demonstrated a slight predominant of male patients (56%), while no statistically significant differences were detected in molecular response rates between genders.

Conclusion: The association between microRNA-21 expression level and patient sex indicates the presence of a potential sex-specific regulatory mechanism influencing microRNA-21 in chronic myeloid leukemia.

Key Words: Chronic myeloid leukemia, microRNA-21, Gender differences, Sex specific, Tyrosine kinase inhibitors

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INTRODUCTION

Chronic myeloid leukemia (CML) is hematological disease categorized by malignant transformation of hematopoietic stem cells carrying the Philadelphia chromosome, and has an annual incidence of about 2 cases per 100,000, representingapproximately15% of newly diagnoses leukemia cases in the adult population.[1,2] A key event in pathogenesis of CML is the fusion of Ableson murine leukemia (ABL1) gene located on chromosome 9 with the breakpoint Cluster Region (BCR) gene on chromosome 22.[3]

This chromosomal rearrangement leads to formation of the BCR:ABL1 oncoprotein, a constitutively active tyrosine kinase that drives leukemogenesis by promoting uncontrolled proliferation and survival of CML cells.[4,5] Tyrosine kinase inhibitors (TKI) are a class of pharmacological drugs that disrupt protein kinase -mediated signal transduction pathways and have been approved by the US Food and Drug administration (FDA) for the treatment of CML.[6,7] Despite this success , significant variability in patient response and toxicity profiles persists. Recent research has highlighted the function of non-coding RNA, epically microRNAs (miRNAs), in regulating these outcomes.[8] microRNA-21 (miR-21) is a key oncomiR, and its elevated expression has been associated with weak prognosis. And drugs resistance in various cancers, including CML.[9,10]

Growing evidence indicates that gender influence cancer susceptibility, disease progression, and therapeutic response, including in CML.[11] Clinical studies have identified gender-related variation in CML patient characteristic such as differences in platelet count and spleen size. These disparities are commonly attributed to a combination of hormonal influences, genetic differences linked to sex chromosomes, and gender specific variations in drug metabolism.[12] Considering that miR-21 expression has been reported to vary by gender in other cancer types, it is important

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to explore whether gender similarly affect the molecular profile of patients with CML.[13]

The purpose of this study was to evaluate the relationship between microRNA-21 (miR-21) expression levels and patient gender in individuals with CML undergoing TKI therapy, in order to gain insights in to possible sex-specific molecular mechanisms.

METHODS

A cross-sectional study was conducted at Al-Diwaniyah General Hospital and Department of Pharmacology and Therapeutics, faculty of Medicine, University of Al-Qadisiya, Iraq from from 1st April 2025 31st August 2025 vide letter No. 301 date 23-2-2025 and 50 patients diagnosed with Philadelphia chromosome-positive chronic myeloid leukemia (CML) were included. All of whom were on therapy with tyrosine kinase inhibitors (TKIs) such as Imatinib, Nilotinib, or Bosutinib. For diagnosis and what follows, standard procedures included a complete blood count and BCR-ABL1molecular analysis by real-time quantitative PCR. The entire RNA was taken from whole blood samples using TRI pure RNA extraction reagents (ELK, China) following the manufacturer's protocol, which involved lysis with Triazol, chloroform extraction, isopropanol precipitation, and 70% ethanol washing. RNA concentration was measured using the Quantus™ Fluorometer (Promega, USA). The isolated RNA was converted into complementary DNA (cDNA) using the reverse transcription ADDBio kit (Korea). The reaction of the mixture (20 µl total volume) included 4 µl of RNA and was incubated at 50°C for 60 minutes for reverse transcription.

The microRNA-21 (miR-21) expression level was quantified by [RT-qPCR] using the Add Script RT-qPCR Syber master (AddBio, Korea) on a BioRAD (USA) real-time qPCR machine. The housekeeping gene was GAPDH (Table 1). Reaction mix and preparation of RT-qPCR amplification is shown in Table 2. The thermal cycling conditions of the thermal conditions were carried in Table 3.

Total RNA was extracted from whole blood samples. The microRNA-21 (miR-21) expression level was quantified by [RT-qPCR] using the AddScript RT-qPCR Syber master (AddBio, Korea) on a BioRAD (USA) real-time qPCR machine, with GAPDH as the housekeeping gene. The data was analyzed through SPSS-25. The correlation of miR-21 expression with patient gender was assessed using Pearson's correlation coefficient. Cross-tabulation and Chi-square tests were used to compare the distribution of TKI type and treatment response between male and female patients. Statistical significance was assigned to p-values less than 0.05.

RESULTS

There were 28 (56%) males and 22 (44%) females with male: female ratio is 1.27:1 (Fig. 1). The average age of male patients with CML was found to be higher than that of female patients, 51.78 years versus 47.18 years, respectively. However, from a statistical perspective, this apparent age difference between the sexes is not significant because the p-value is 0.275 (Fig. 2)

The disease's duration varied greatly, ranging from a minimum of one year to a maximum of 26 years. The median (inter-quartile range) of disease duration is 8 (15.25) years with average of 9.82±7.93 years. With 42% of cases, the largest percentage of patients had disease duration of less than five years. Since it accounted for 10% of cases, the lowest percentage was recorded during the 6-10 year period. 20%, 16%, and 12% of cases at 11-15 years, 16-20 years, and >20 years disease duration, respectively (Table 4, Fig. 3).

Table No. 1: Primers for House Keeping Gene (GAPDH) and for Gene of Interest (miRNA21)

Gene	Primer	Sequence (5'->3')
House Keeping Gene (GAPDH)	Forward	GAAGGTGAAGGTCGGAGTC
	Reverse	GAAGATGGTGATGGGATTTC
Gene of Interest (miRNA-21)	Forward	TTGTCGGGTAGCTTATC
	Reverse	GTCAGACAGCCCATCGA

Table No. 2: Preparation RT-qPCR amplification

Substance	Amount
H2O	4 µl
AddScript RT-qPCR	10 µl
Forward primer (0.05 pmol/20 µl)	2 µl
Reverse primer (0.05 pmol/20 µl)	2 µl
cDNA	2 µl
Total	20 µl

Table No. 3: The thermal conditions were carried out using BioRAD (USA)

Temperature		Time/Ses	Repeat
Initials denaturations	95°C	300	1
Denaturations	95°C	20	40x
Annealings	According to gradient protocol	30	40 x with machines read
Extensions	72°C	30	40 x
Melting analyses	94°C	15	1
Melting analyses	61°C	60	1
Melting analyses	+ 0.3°C of 95°C	15	Machines reading

96% of the cases in this project were in the chronic phase, and 4% were in the blast phase (Fig. 4).

Table No. 4: Disease duration of patients with chronic myeloid leukemia

Duration (years)	Results
Mean±SD	9.82±7.93
Minimum - Maximum	1 – 26
Median (IQR)	8 (15.25)
< 5 years	21 (42%)
6 – 10 years	5 (10%)
11- 15 years	10 (20%)
16 – 20 years	6 (16%)
> 20 years	6 (12%)

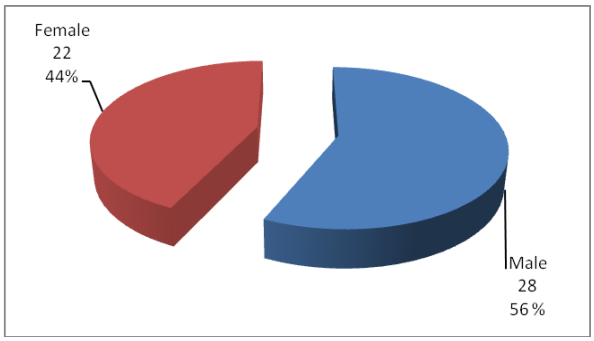

Figure No. 1: Frequency of genders

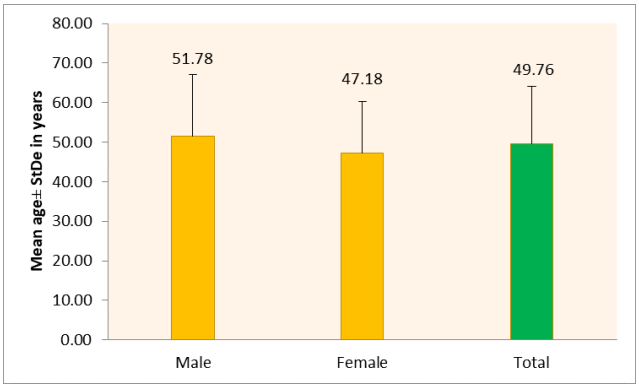

Figure No. 2: Relation between sex and mean of age of patients having CML

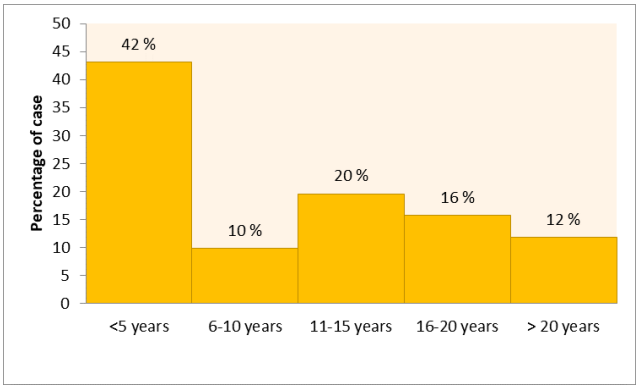

Figure No. 3: The proportions of case of CML classified based on duration of disease

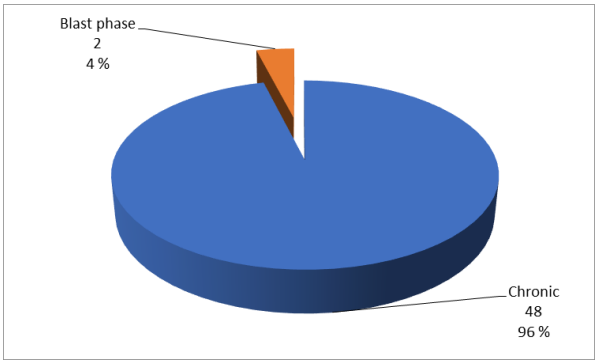

Figure No. 4: The proportion of cases of CML categorized into chronic phase and at blast phase

The primary finding of this study was the significant statistical correlation observed between miR-21 expression and patient sex (r=0.312, p=0.027) [Table 5].

Table No. 5: Correlation of miR-21 expression with patient sex

Characteristic	miRNA-21 (r)	miRNA-21 (p)
Sex	0.312	0.027

DISCUSSION

Interferon-α was the first successful therapy for CML, a blood cancer identified by neoplastic alteration of hematopoietic stem cells. Prior to the advent of new therapeutic approaches like Imatinib., a tyrosine kinase inhibitor, that drug is the sole option available to treating CML. patient. However, transplantation of allogenic stem-cell kind is only curing therapy for CML, and recent tyrosine kinase inhibitors. Numerous projects has proposed micro-RNAs taking part with the pathogenic mechanism of cancerous changes and progression of disease, and miR-21 was reported to show elevation in blood cancers.[14] According to Chan et al[15], miR-21 is a highly significant candidate involved in oncogenic activity. In a cell-line of glioblastoma culture, it was proposed this microRNA-21 knockout activated caspases, increased apoptosis, and caused cell death, indicating the upregulation of miR-21 may be a mechanism combating programmed cell death.

In the present study, the average age of CML patients was 49.76 years (median of 46.5 years) and it ranged between 14 and 77 years and approximately half of our patients were above 40 years and below 60 of age. In our study, there were 28 males CML cases and 22 of female cases, making the male to female ratio as 1.27:1 and we reported no significant differences in average of age between male patients and female patients. In 2023, Laabidi et al[16] reported a little female predominance (sex-ratio 0.78), which contradicts our findings. They also reported that the mean age of CML patients was 50

years, which is fairly similar to the mean age obtained in our study.

In this study, the median disease duration was 8 years and the range of duration was between one and 26 years. Duration of less than 5 years was shared by the major fraction of patients (42%). Chronic phase was the dominant phase as it accounted for 96% of the cases whereas blast phase was seen in only 4% of cases. With respect to use of TKI, in our study we observe significant change from Imat. use as an initial form of therapy to other new generations such as bosutinib and nilotinib. In our study, drug toxicity was seen in 18% of cases.

The finding of a statistically significant association between miR-21 expression and patient gender ($r=0.312$, $p=0.027$) is a novel and important observation that warrants further discussion. This result aligns with a growing body of literature suggesting that sex-related differences exist in the molecular landscape of various cancers.[4,7]

The underlying mechanism for this gender-dependent expression of miR-21 in CML is likely multifactorial. One hypothesis relates to the influence of sex hormones. Studies in other cancers have shown that estradiol can down regulate miR-21 expression.[10] Given that CML is a disease with known gender-related clinical characteristics[5], it is plausible that hormonal differences between male and female patients influence the regulatory pathways of miR-21, which is a known oncomiR.

An alternative explanation may involve gender-specific variations in the metabolism of tyrosine kinase inhibitors. Although our cross-tabulation analysis did not reveal a statistically significant difference in the types of TKIs prescribed between males and females, it is well-documented that cytochrome P450 (CYP) enzyme activity which plays a key role in the metabolism of TKIs such as Imatinib and Nilotinib - differs between genders.[12] These differences could lead to variations in TKI plasma concentrations, potentially influencing the cellular milieu and thereby modulating the expression of stress-responsive microRNAs including miR-21.

This investigation is constrained by its cross-sectional nature and the absence of direct measurements of hormonal levels. Nevertheless, the significant correlation identified underscores the importance of considering gender as a pivotal factor in future research exploring miR-21 as a biomarker in chronic myeloid leukemia. Subsequent studies should aim to quantify sex hormone concentrations and directly assess their relationship with miR-21 expression, in order to clarify the underlying regulatory mechanisms.

CONCLUSION

A significant association was observed between miR-21expression and the gender in CML patients receiving TKI therapy. This result underscores the importance of incorporating gender as a biological variable in molecular profile studies of CML and suggests the involvement of hormonal or metabolic regulatory mechanism in modulating miR-21 expression.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Abbas Ridha Jasim, Hussein. A Sahib
Drafting or Revising Critically:	Abbas Ridha Jasim, Hussein. A Sahib
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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REFERENCES

1. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2022 update on diagnosis, therapy, and monitoring. Am J Hematol 2022;97(9): 1236-56,
2. Shah NP. Chronic myeloid leukemia, JNCCN 2024;22(1):43-69.
3. Jain P. BCR-ABL Fusion transcripts in patients with chronic myeloid leukemia in chronic phase (CML-CP): Experience with ponatinib. Blood 2013;122(21): 2727.
4. Al Hamad M. Contribution of BCR-ABL molecular variants and leukemic stem cells in response and resistance to tyrosine kinase inhibitors: a review. F1000 Res 2022; 10.
5. Zenebe B, Nigussie H, Belay G, Seboka N. A review on characterization of BCR–ABL transcript variants for molecular monitoring of chronic myeloid leukemia phenotypes. Hematolol 2023; 28(1): 12.
6. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2016 update on diagnosis, therapy, and monitoring. Am J Hematol 2016;91(2): 252-65.
7. Delphine R, Hughes TP. Critical Reviews in Oncology / Hematology Development of asciminib. Novel Allosteric Inhibitor of BCR-ABL1 2022; 171.
8. Mohamad SFS, Ramlee Ra, Elias MH. The Role of MicroRNAs in Chronic Myeloid Leukemia (CML) Treatment, Biomarkers, and Resistance to Tyrosine Kinase Inhibitor: A Bioinformatics-Based Analysis. J Trop Life Sci 2024;14(3): 621-42.
9. Mirza MAB, Guru SA, Abdullah SM, Rizvi A, Saxena A. microRNA-21 expression as

prognostic and therapeutic response marker in chronic myeloid leukaemia patients. Asian Pacific J Cancer Prev 2019; 20(8): 2379-83.

10. Feng YH, Tsao CJ. Emerging role of microRNA-21 in cancer (Review)," Biomed Reports 2016;5(4):395-402.
11. Karantanos T, Jain T, Moliterno AR, Jones RJ, Dezern AE. Sex-related differences in chronic myeloid neoplasms. Clin Observation Underlying Biol 2021;16.
12. Caserta S, Gangemi S, Murdaca G. Gender differences and miRNAs expression in cancer: implications on prognosis and susceptibility. Int J Mol Sci 2023; 24(14): 1154.4
13. Hasáková K, Bezakova J, Vician M, Reis R, Zeman R, Herichova I. Gender-dependent expression of leading and passenger strand of miR-21 and miR-16 in human colorectal cancer and adjacent colonic tissues. Physiol Res 2017; 66: 5575-82.
14. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, et al. microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006;103(7):2257-61.
15. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;14: 602933.
16. Laabidi B, Slama N, Ouahchi I, Boufrikha W, Adnene M. Leukemia research reports chronic-phase chronic myeloid leukemia: incidence of bcr / abl transcript and its correlation with presenting features, response to treatment , and survival. Leuk Res Reports 2023; 20: 100373.