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Association of Microrna-153-3p Expression in Response to Treatment with Imatinib in Patients with Chronic Myeloid Leukemia

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ABSTRACT

Background: It is thought that the susceptibility of chronic myeloid leukemic (CML) cells to imatinib (IM) is increased by high miR-153-3p expression.

Aim: To establish the association of miR-153-3p expression with treatment response to IM in CML patients.

Methods: Sixty CML patients were included and divided into two groups consistent with their response to treatment whether sensitive or resistant to IM. Ten healthy normal participants were enrolled as control group. RNA was extracted from serum to work out miR-153-3p expression utilizing real-time quantitative reverse transcription polymerase chain reaction. The primers were supplied by Macrogen Inc.

Results: 27 patients were sensitive to imatinib and 33 were resistant to imatinib. The ratio of male to female was 1.14:1. The bulk (58%) of patients were within the age range of 41-60 years. Weight and gender did not significantly differ between the two patient groups. The mean patients' CT of miR-153-3p was significantly above the control group and the sensitive group. The mean DCT value in resistant group was significantly above that of the sensitive group while insignificantly above that of the control group. The mean DDCT in resistant group was significantly higher than that of the sensitive group. The miR-153-3p expression showed significantly lower fold change than the sensitive group.

Conclusion: There is miR-153-3p expression downregulation in resistant CML patients indicating unresponsiveness to treatment with imatinib.

Keywords: CML, Imatinib, MiRNA-153-3p, Response to treatment.

INTRODUCTION

Thirteen percent of all leukemia cases are caused by a malignant proliferative disorder called chronic myeloid leukemia (CML). Condition that developed from hematopoietic stem cells and is identified by the Philadelphia (Ph) chromosome and the presence of the fusion gene BCR-ABL [1, 2]. Tyrosine kinase inhibitors (TKIs), such as imatinib (IM), are utilized as first-line therapy since chemotherapy is considered the most effective treatment for CML [3]. But the main obstacle to successful treatment of the illness is chemotherapy resistance [4].

Since blood cancers cannot be surgically treated, unlike solid tumors, as a result, it is vital to research the mechanisms underlying treatment resistance in blood malignancies and figure out how to combat it. Approximately 22 nucleotides in length, microRNAs (miRs) are a category of a short single-stranded non-coding RNAs that regulate the epigenetic state of certain targets by modifying the translation of target genes or by cleaving mRNA [5]. BCR-ABL1 expression is the distinguishing molecular feature of CML and is hence the target for TKI treatment [6]. Since some of the IM-resistant patients had no mutations on the BCR-ABL1 oncogene, resistance to IM and other TKIs has been acknowledged as the main problem for CML treatment and monitoring [7].

Since miRs are powerful regulators, they may contribute to the emergence of drug resistance because they regulate other genes involved in drug transport or the activation of essential signalling pathways in addition to the gene's expression *BCR-ABL1* [8, 9]. It has been discovered that IM-resistant CML cells exhibit downregulation of the miR-153-3p, which has been linked to many different sorts of malignancies. By blocking the autophagy mediated by B cell lymphoma 2 that is caused by up-regulation of miR-153-3p. IM sensitivity was considerably boosted. Whereas down-regulating miR-153-3p mitigated these effects in IM-resistant CML cells, and lowered the survival rate of IM-resistant CML cells [8].

PATIENTS AND METHODS

This cross-sectional analysis examined 60 CML patients were enrolled. Two groups of patients were selected sequentially according to their response to treatment with IM according to the following criteria:

- CML patients who are sensitive to IM after a minimum of 3 months without interruption of treatment, with their *BCR-ABL1* transcript levels ≤ 10%.
- CML patients who are resistant to IM and failed to achieve complete hematologic response and *BCR-ABL1* transcript levels > 10% (IS) after three to six months of therapy or partial cytogenetic response after 3 to 6 months into a therapeutic regimen.

Exclusion criteria:

Patients who stopped or interrupted imatinib for any reason, or taking drugs other than IM.

Ten normal healthy people were recruited as a control group. From each patient and control, a sample of three mL peripheral blood was withdrawn and collected in gel tube. Serum was separated by

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centrifugation within 2 hours after collection, and then 0.5 mL was added to 1.5 mL of TRIzol reagent in an Eppendorf tube with a minimum storage temperature of - 20°C. RNA was extracted within 2 weeks and stored below – 20°C until the time of testing miR-153-3p expression using two-step polymerase chain reaction (PCR) for real-time quantitative reverse transcription (RQ-PCR).

The GenBank database of the National Center for Biotechnology Information was used to obtain the miRNA gene's cDNA sequences. RQ-PCR primers were used. Premier 3 software with melting temperature between 58 to 62°C, PCR amplicon length should be between 75 and 150 base pairs, primer length should be between 18 and 23 nucleotides.

These primers were provided in lyophilized form by Macrogen, Inc. The extracted cDNA concentration was evaluated using a Quantus Fluorometer and ranged between 3-5 ng/L. Data on miRNA expression was normalized using the RNU43 housekeeping gene as a reference gene. Quantification of relative gene expression is calculated according to Pfaffl MW method: Fold change $2^{-\Delta\Delta CT}$ [10, 11].

Ethical approval: The research was completed in accordance with the Helsinki Declaration. An approval was received from the College of Medicine's Research Ethics Committee, University of Baghdad. All participants gave their informed consents.

Statistical analysis

Data analysis was done utilizing SPSS version 26 (IBM, United States). The data were presented using straightforward values for frequency, percentage, mean, standard deviation, and the range from minimum to maximum. ANOVA used to check the difference between more than two independent means followed by Post Hoc tests. Using the Pearson Chi-square test, the disparities between various percentages were evaluated. Student *t* test, with application of Fisher Exact test whenever applicable. Significance was explained as a P value of equal to or less than 0.05.

RESULTS

Out of 60 patients, 32 (53.3%) were male, and the remainder were female. The age range of the majority (35/60, 58%) was 41-60 years old.

In this study, 27 patients were sensitive to imatinib and 33 were resistant to IM. For the 60 CML patients, the mean weight was 79.9 ± 14.3 kg between the two patient groups, there was no obvious difference. (p= 0.622) as shown in table (1).

Table (1): The mean values of body weight in the two patients groups

Parameter	Patients' groups	N	Mean ± SD	P*
Weight (Kg)	Sensitive	27	78.19 ± 11.049	0.622
	Resistant	33	80.09 ± 17.26	

* Student t test

There was no significant association of gender with the groups of the patients whether sensitive or resistant to imatinib (p= 0.320) as illustrated in table (2).

Table (2): The association between patients' groups with the gender

Parameters		**Patients' groups**		**P***
		Resistant to IM	Sensitive to IM	
Gender	Female: n (%)	14 (42.4%)	14 (51.9%)	0.320
	Male: n (%)	19 (57.6%)	13 (48.1%)	

* Chi square.

Analyzing the means of age, the hemoglobin level, WBC count, and platelet level of patients' groups, there were no statistically insignificant differences between resistant and sensitive to the treatment with IM groups (p= 0.149, 0.758, 0.989 and 0.579, respectively) as shown in table (3).

Table (3): The relationship between patients' groups with the means of age and hematological parameters

Parameters	**Patients' groups**		**P***
	Resistant to IM	**Sensitive to IM**	
	(Mean ± SD)		
Age (years)	45.79±15.091	50.67±9.401	0.149
Hemoglobin (g/dL)	12.71±1.82	12.57±1.65	0.758
WBC ($\times 10^9$/L)	6.94±1.75	6.95±1.75	0.989
Platelet ($\times 10^9$/L)	260.18±68.6	270.96±51.16	0.579

* Student *t* test

The CT level of *RNU43* and *miR-153-3p* genes, DCT and DDCT levels were higher in resistant group compared to control and sensitive groups respectively. There were statistically significant differences between the control, sensitive and resistant cases in the CT of RNU43, CT of miR-153-3p, DCT, DDCT and gene expression level with p values of 0.0001, 0.0001, 0.011, 0.001, and 0.003 respectively (Table 4).

Table 4 Comparison of mean values of CT, Delta CT, Delta Delta CT and Fold change expression of miR-153-3p between patients' groups (Sensitive, n=27; and Resistant, n= 33) and control group (n= 10).

Parameters		**Mean**	**SD**	**F**	**P***
CT RNU43	Control	28.4362	2.1506	10.701	**0.0001**
	Sensitive	28.0728	0.9968		
	Resistant	30.3929	2.5319		
CT miR-153-3p	Control	31.0136	1.9611	24.006	**0.0001**
	Sensitive	30.2405	2.2608		
	Resistant	34.3843	2.5948		
DCT	Control	2.5774	2.4492	4.863	**0.011**
	Sensitive	2.1676	2.1203		
	Resistant	3.9913	2.4376		
DDCT	Control	0	2.4492	7.755	**0.001**
	Sensitive	-0.4098	2.1203		
	Resistant	1.8541	2.3962		
Folding	Control	2.3752	2.2581	6.518	**0.003**
	Sensitive	2.8710	3.5368		
	Resistant	0.6735	0.7877		

* ANOVA test

Pairwise comparisons of the CT mean of RNU43 using Scheffé (Post Hoc) test revealed significantly higher values than the control and the sensitive groups (p= 0.032 and 0.0001 respectively). Also, the CT mean of miR-153-3p in resistant group was significantly higher than in the control and the sensitive groups (p= 0.001 and 0.0001 respectively). The mean value of DCT in resistant group was significantly higher than that of the sensitive group (p= 0.014), while insignificantly higher than that of the control group (p= 0.248). The mean value of DDCT in the resistant group was substantially higher than in the sensitive group. (p= 0.001). Finally, the fold change of the resistant group was significantly lower than the sensitive group with p-value of 0.004 (Table 5).

Table 5 Multiple comparisons of the resistant group with each of control and sensitive groups according to PCR parameters

Parameters			**Mean± SD**	**Interval of 95% Confidence**		**P***
				Lower limit	Upper limit	
CT RNU43	Resistant	Control	28.4362±2.1506	0.1339	3.7796	**0.032**
		Sensitive	28.0728±0.9968	1.0097	3.6305	**0.0001**
CT miR-153-3p	Resistant	Control	31.0136±1.9611	1.2100	5.5312	**0.001**
		Sensitive	30.2405±2.2608	2.5906	5.6970	**0.0001**
DCT	Resistant	Control	2.5774±2.4492	-0.6838	3.5116	0.248
		Sensitive	2.1676±2.1203	0.3156	3.3317	**0.014**
DDCT	Resistant	Control	0±2.4492	-0.2249	3.9332	0.090
		Sensitive	-0.4098±2.1203	0.7693	3.7585	**0.001**
Folding	Resistant	Control	2.3752±2.2581	-3.8848	0.4813	0.157
		Sensitive	2.8710±3.5368	-3.7669	-0.6281	**0.004**

* Post Hoc test.

DISCUSSION

In this study, the ratio of male to female was 1.14:1 which is the same as that of **Alves *et al.*** [12] study (1.14:1), but it is less than that reported by **Larson *et al.*** [13] study (1.7:1), and close to other Iraqi studies (1.38:1 and 1.3:1) reported by **Khazaal *et al.*** [14], while in contrast to another local study conducted by **AL-Jader *et al.*** [15] where the ratio was reversed (1:1.27).

The age range in our study (41-60 years) was comparable with **Larson *et al.*** [13] who included patients in the range of 18-70 years, in **Alves *et al.*** [12] study was 18-78 years and in **Peng *et al.*** [16] study was 14-70 years. But it was different from **AL-Jader *et al.*** [15] who reported a range of 23-63 years, while **Abdullah *et al.*** [17] found 44/130 (33.85%) of patients belonged to the age category 31-40 years.

According to the body weight, the mean body weight of CML patients (79.9 ± 14.3 kg) was less than that reported by **Larson *et al.*** [13] study (85.9 ± 16.8 kg). Patient groups, whether sensitive or resistant, did not significantly correlate with body weight, which is compatible with **Peng *et al.* study** [16] who stated that body weight does not appreciably impact the pharmacokinetics of imatinib.

There was no significant association of gender with groups of patients whether sensitive or resistant. The same result was reported by other studies [17-19].

Hematological parameters showed statistically insignificant difference between resistance and sensitive groups according to the treatment with IM which is incompatible with **Peng** study [16].

The PCR parameters were higher in resistant group compared to sensitive and control group. There was significant variance between the control, sensitive and resistant cases according to CT-RNU43, CT-miR-153-3p, DCT, DDCT and gene expression level. The relative gene expression of miR-153-3p was lowest in resistant group which is compatible with the study of **Li *et al.*** [4] where the miR-153-3p gene expression was down regulated in resistant group.

CONCLUSION

There is significant reduction of miR-153-3p gene expression among resistant group in comparison with control and sensitive groups. While, the fold change was slightly upregulated in the sensitive group. This suggest that patients with down regulated miR-153-3p are unlikely to benefit from imatinib treatment and better to start therapy with different choice of TKI.

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