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The Role of microRNA in The Genetic Disturbance of Patients with Chronic Myeloid Leukemia

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Abstract :
Background: Cancer development is one of the many effects of dysregulation of miRNA expression patterns; miRNA has been found to express abnormally in hematological neoplasia such as chronic myeloid leukemia and solid malignancies. Resistance and response degrees following TKI treatment correlate with miRNA expression. Hence in this study, we tried study the relationship of miRNA- 181c and miRNA-150 between different p210 BCR-ABL transcript levels and the role of miRNA-181c and miRNA-150 between different levels of imatinib optimal response in CML patients. Method: Our study included 60 CML patients divided into two groups based on response to imatinib therapy,30 samples of the optimal molecular response of CML patients, and ٣٠ samples of failed molecular response CML patients30, samples of apparently healthy volunteers were included and evaluated as control. Results: According the BCR-ABL P210 % results there was a significant difference (P = < 0.0001), between the responder and the failure response CML patients, the result of miRNA-181c showed significant difference between both CML patients (P = 0.0012) while miRNA-150 results there was high significant difference (P=0.0001 >).Assessed miRNA-181c and miRNA-150 expression levels in different responses and failure responses of CML patients, there was a highly significant difference (P = 0.0044) (P =0.0002) respectively, a cutoff value of response vs. failure response of miRNA-181c and miRNA-150 (7.24) (1.784) respectively, with high sensitivity, can be of diagnostic value to differentiate between response and failure response. Conclusion: Changing gene expression with different amounts of miRNAs impacts drug-gene interactions, with consequences for cell growth and death. Gene expression of miRNA- miRNA- 181c and miRNA-150 among CML patients of imatinib therapy were higher in response patients than in failure response patients. The gene expression level of miRNA- 181c and miRNA-150 differs through different responses in CML patients.

Keywords: CML, Imatinib mesylate, miRNA181-c, miRNA.150-

دور الحامض النووي الرايبي المجهري في الاضطرابات الوراثية لمرض سرطان الدم النخاعي المزمن

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مستخلص:
الخلفية: تطور السرطان هو أحد الآثار العديدة لخلل تنظيم أنماط التعبير الحمض النووي الريبي. تم العثور على الحمض النووي الريبي للتعبير بشكل غير طبيعي في الأورام الدموية مثل سرطان الدم النخاعي المزمن والأورام الخبيثة الصلبة. ترتبط درجات المقاومة والاستجابة بعد علاج مثبط التايروسين كاينز (TKI) بتعبير الحمض النووي الريبي ومن ثم، في هذه الدراسة، حاولنا دراسة العلاقة بين miRNA- 181c و miRNA-150 بين مستويات نسخ مختلفة من نقاط توقف البروتين الورمي - P210 (BCR- ABL) ودور miRNA-181c وmiRNA-150 بين مستويات مختلفة من الاستجابة المثلى لإيماتينيب في مرضى سرطان الدم النخاعي المزمن. الطريقة: تضمنت دراستنا ستين مريضًا بسرطان الدم النخاعي المزمن تم تقسيمهم إلى مجموعتين بناءً على الاستجابة للعلاج بإيماتينيب، ثلاثين عينة من الاستجابة الجزيئية المثلى لمرضى سرطان الدم النخاعي المزمن وثلاثين عينة من مرضى سرطان الدم النخاعي المزمن. تم تضمين ثلاثين عينة من المتطوعين الأصحاء على ما يبدو وتقييمها كمجموعة تحكم. النتائج: وفقًا لنتائج P210 منطقة مجموعة نقاط التوقف (BCR-ABL)، كان هناك فرق كبير (P = <0.0001) بين المستجيبين وفشل الاستجابة لمرضى سرطان الدم النخاعي المزمن. اظهرت نتيجة miRNA-181c فرقًا معنويا كبيرًا بين كل من مرضى سرطان الدم النخاعي المزمن (P = 0.0012) بينما كانت نتائج miRNA-150 فرقًا معنويا كبيرًا (P = <0.0001). قيمت مستويات تعبير miRNA-181c وmiRNA-150 في استجابات مختلفة وفشل الاستجابة لمرضى سرطان الدم النخاعي المزمن، كان هناك فرق ذو دلالة إحصائية (P = 0.0002) (P = 0.0044) على التوالي، نقطة القطع للاستجابة مقابل فشل الاستجابة 1.784 =miRNA-150 7.24 =miRNA-181c، مع ارتفاع الحساسية، يمكن أن تكون ذات قيمة تشخيصية للتمييز بين الاستجابة وفشل الاستجابة. الخلاصة: يؤثر تغيير التعبير الجيني بكميات مختلفة من الجزيئات الدقيقة على تفاعلات الجينات الدوائية، مع عواقب على نمو الخلايا وموتها. كان التعبير الجيني لـ miRNA- 181c وmiRNA-150 بين مرضى سرطان الدم النخاعي المزمن لعلاج إيماتينيب أعلى في المرضى المستجيبين مقارنة بمرضى فشل الاستجابة. يختلف مستوى التعبير الجيني لـ miRNA-181c وmiRNA-150 من خلال الاستجابات المختلفة في مرضى سرطان الدم النخاعي المزمن.

الكلمات المفتاحية : سرطان الدم النقوي المزمن – ايماتنيب ميسيلات miRNA-181c, miRNA-150 .

Introduction:

Hematopoiesis is described as the ability of self-renewing cells to form mature blood cells[1]. The increase of immature cells in the bone marrow and abnormal hematopoiesis defined Leukemia is a highly diverse hematological malignancy[2]. Agranulocytosis, marrow hypercellularity, and splenomegaly are all symptoms of CML, which is caused by a mutation in a pluripotent stem cell[3]. This myeloproliferative neoplasm is a clonal hematopoietic stem cell (HSC) neoplasm marked by an increase in myeloid linage cells at all stages of development[4]. The increase of Philadelphia chromosome-positive (Ph+) myeloid cells is a defining feature of CML patients. A reciprocal translocation between the Abelson (ABL) protooncogene on chromosome 9 and the breakpoint cluster area (BCR) on chromosome 22 results in the Ph chromosome, t (9;22) (q34; q11). This results in the production of an abnormal mRNA product, p210 BCR-ABL, a fusion protein with constitutive ABL tyrosine kinase (TK) activity[5]. This kinase regulates several downstream substrates, including A serine threonine kinase also known as protein kinase B (Akt), Myelocytomatosis MYC and c-Jun N-terminal kinase (JNK), which are all required for normal cell proliferation and survival. The hyperactivity of the BCR-ABL kinase, on the other hand, breaks this delicate balance and drives cells to uncontrolled proliferation and survival, both of which provide a growth advantage to malignant cells with this mutation, ultimately leading to CML pathogenesis[6]. For the efficient treatment of CML, tyrosine kinase inhibitors (TKIs) are required to suppress the kinase activity of the BCR-ABL protein[7]. Imatinib mesylate is a tyrosine kinase inhibitor that inhibits downstream BCR-ABL signaling by blocking the ATP binding site of the protein[6]. Although imatinib, works for the great majority of CML patients, resistance can develop either spontaneously or during treatment[4]. MicroRNAs (miRNAs) are short noncoding RNAs that affect cell survival and development after transcription. Overexpression of oncogenic miRNAs (oncomiRs) or reduced expression of tumor suppressor miRNAs have been found in malignancies[8]. Over 30% of basic genes, which are involved in key biological processes such as proliferation, differentiation, survival, invasion, and programmed cell death, some research has suggested that miRNA expression profiles could be used as biomarkers for leukemia diagnosis, prognosis, and treatment response[9].

Materials and Method

Subjects: This study was carried out between September 2021- July2022 at Baghdad Teaching Hospital/ Medical City. The current study included 60 CML samples who were over the age of 18 and had been on imatinib therapy for more than a year. Patients were divided into groups based on treatment response and BCR-ABL transcript levels. The European Leukemia-Net (ELN)

guidelines were used to define treatment response criteria[10].30 samples optimal responders (p210 BCR-ABL transcript levels less than 0.1%) and 30 samples as failure molecular responders (p210 BCR-ABL transcript levels greater than 1%)30 , samples of apparently healthy volunteers were used as controls. At the time of sampling, an automated blood count analyzer was used to obtain and calculate blood count indices. This study was approved by the scientific ethics committee/ All the study experiments were performed at the University of Mustansiriyah University - College of Science- Department of biology.

Statistical analysis: GraphPad Prism 7.0 was used for statistical analysis, to detect the effect of different parameters in study, discrete variables presented using their number and percentage, chi square test was used to analyze. For non-parametric data such variables analyzed by Kruskal-Wallis test for comparison between different groups (response, and failure response and control). The probability was calculated for Variables that followed normal distribution One way ANOVA used for their analysis. For post Hoc analysis, Tukey U test used for those analyzed by One way ANOVA, while Dunn's multiple comparison test for those analyzed by Kruskal-Wallis test. The receiver operator curve (ROC) was used to investigate the expression of the level miRNA in distinguishing failure response cases from optimal response cases, and (P 0.05) were deemed statistically significant.

Results

Patients and control group general characteristics: Out of 60 CML patients on imatinib therapy, patients were divided into two groups based on response to therapy, thirty samples were with optimal response with (mean age 45.97 ± 2.23 years, M:F ratio 12: 18) and thirty samples were with failure response with (mean age 49.87 ± 2.25years , M:F ratio 18: 12)and thirty samples of apparently healthy volunteers were included and evaluated as a control with (mean age 30.93 ± I,Voyears , M:F ratio .

The following patient characteristics were included based on a complete blood count: The median white blood cells count (cell/ cm3) for the response, failure response CML patients and control group was (6.6x10³) (3.9-15.3), 7.05)x10³), (3.1-12.6)6.15) ,x10³), (0.8-18.3) respectively ,they showed no significant difference between all studied groups. While the median hemoglobin level g/dl to µL for the response, failure response CML patients and control group was (12.15 g/dl) (6.8-15.3)13.1) , g/dl) (9.6-15.9)15.8) , g/dl) (10.2-17.3) respectively, showed no significant difference (P=0.5386) for both patients CML groups ,but showed a significant difference among patients groups and control group according to the hemoglobin level (P = <0.0001) showed reduced hemoglobin levels than the control. The median platelets count xI·^{3}/µL for the response, failure response CML patients and control group was

(235×10^3) (17-391)48-) (10^3×210.5) , 159-450) (١٠^3×237) ,(1237) respectively showed no significant difference between all student groups. There was a significant difference in all CML cases based on imatinib therapy response based on QPCR results for p210 BCR-ABL transcripts indices for each patients (P=,(0.0001 > between the response and the failure response CML patients with a highest transcript level in failure response CML group with mean (7.463 ± 3.345%) and optimal response group with mean (0.0145 ± 0.03%) in relation to BCR-ABL transcript levels.Distribution of mean BCR-ABL p-210 level among different responses of CML patients groups. The mean BCR-ABL1 (< 0.0032), (0.01-0.0032)0.1-0.01) ,) and (>1), the result showed in this study, the mean **±** SE BCR-ABL p-210 level different response groups and failure response group = (0.0019 ± 0.0005, 0.0056 ± 0.0007, 0.0271 ± 0.0036, 3.345±7.463) respectively, shown high significant difference between all studied patients (p= < 0.0001).

Assessed of mean miRNA expression level among response group and failure response group of CML patients, the mean folding miRNA-181c expression for response and failure response = (2821± ١٥٠٩ and 12.61 ± ٧,٦٣٧) respectively, the result showed high significant difference between both patients groups (P= 0.0012).The mean folding miRNA-150 expression (391.8± ٣٤٩,٣ and 1.919 ± ٠,٤٠٨١) for both response and failure response, respectively. The result showed high significant difference between both patients groups (P= < 0.0001).

Assessed of mean miRNA-181c expression level among different responses groups and failure response group of CML patients showed significant difference result in the mean BCR-ABL1 (< 0.0032), (0.01-0.0032)0.1-0.01) ,) and (>1), were expression (4614±7.397, 24.13 ± 2953 ,2.172 ± 251.1 and 12.61 ± 1.482) respectively, with high level among deeper responders CML patients respectively, as shown in figure 1(A). As for of miRNA- 150 showed significant differences result in the mean BCR-ABL1 (< 0.0032), (0.01-0.0032), 0.1-0.01)) and (>1), were expression (1203±116424.35 ± 59.16 ,8.502 ± 14.17 , and 1.919 ± 0.4081) respectively, with higher level among deeper responders CML patients, as shown in figure1 (B).

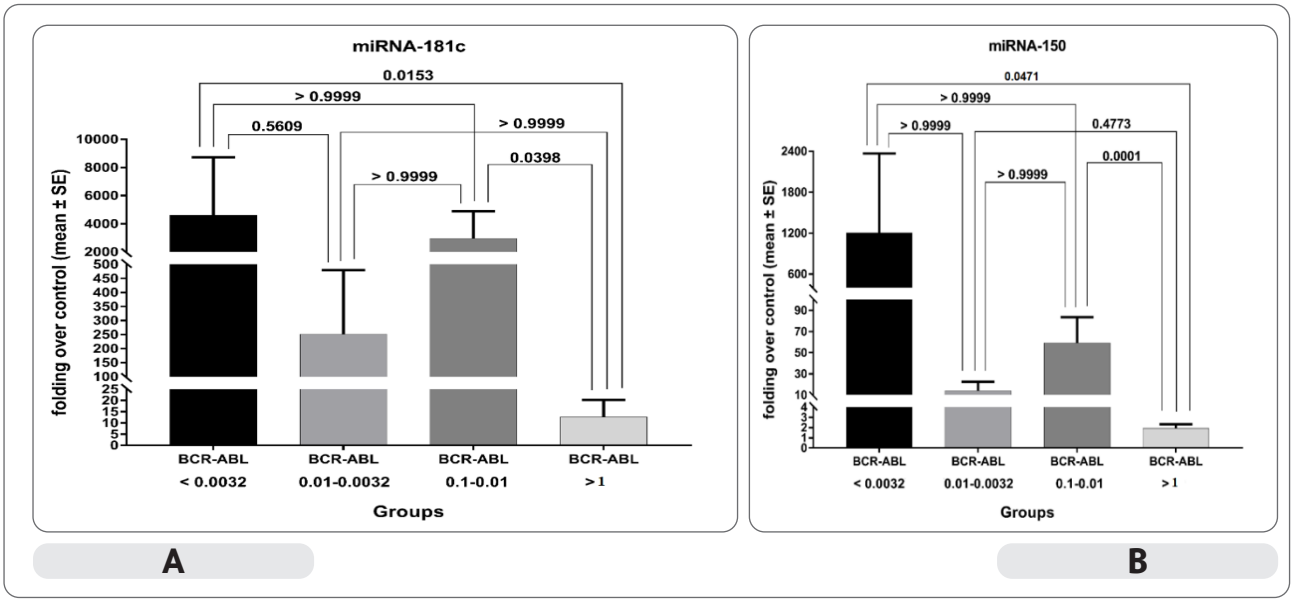

Figure 1: (A) MiRNA -181c expression level through different responses groups and failure response of CML patients (B) MiRNA -150 expression level through different responses groups and failure response of CML patients

Based on table 1 and table 2 to determine a cutoff value, a receiver operating characteristic (ROC) curve analysis was used of miRNA-181c and miRNA-1501.784) (7.24)) respectively, with high sensitivity can be of diagnostic value to differentiate between response and failure response. as shown in figure 2 and figure 3.

Table 1: The ROC analysis for miRNA-181c expression

Comparison groups	AUC	CI 95% of AUC	P-value	Optimum Cut off value	SN (%)	(%) SP
Control vs. Response	0.733	0.575-0.892	0.0019	1.486	73.3	100
Response vs. Failure response	0.74	0.610-0.870	0.0014	7.24	76.7	66.7

Table 2: The ROC analysis for miRNA-150 expression

Comparison groups	AUC	CI 95% of AUC	P-value	Optimum Cut off value	SN (%)	SP (%)
Control vs. Response	0.833	0.7-0.967	<0.0001	1.197	83.3	100
Response vs. Failure response	0.819	0.715-0.922	<0.0001	1.784	70	73.3

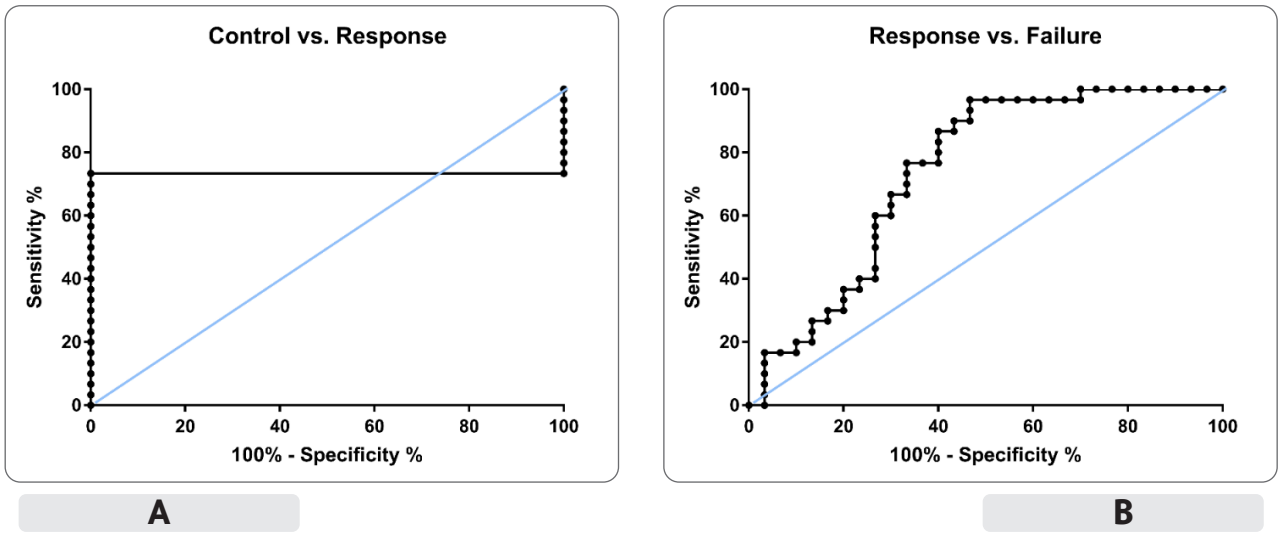

Figure 2: ROC analysis for miRNA-181c expression
(A) for control vs. response (B) for response vs. failure response

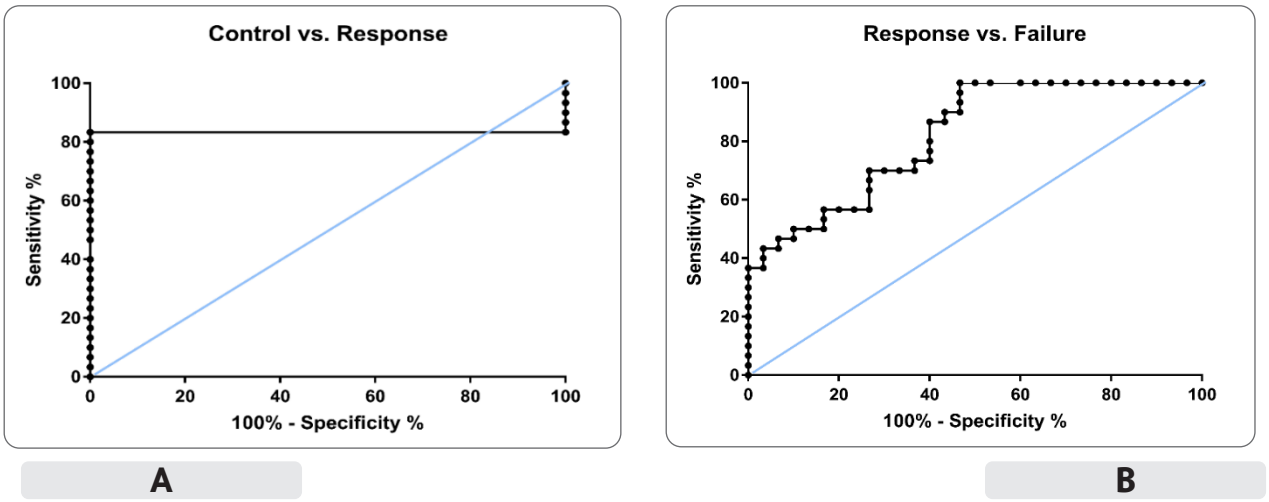

Figure 3: ROC analysis for miRNA-150 expression
(A) for control vs. response (B) for response vs. failure response

Discussion: When comparison differential expression of microRNAs in CML patients who responded to TKI therapy ("responders") versus those who did not ("non responders"). The goal is to identify microRNAs as predictive biomarkers of TKI sensitivity as well as to aid in the investigation of potential microRNA-mediated TKI resistance mechanisms for therapeutic use[11]. MiRNA levels in the blood were shown to alter considerably in newly diagnosed CML patients before and throughout the first two weeks of Imatinib treatment, suggesting the pos-

sibility of identifying easily detectable biomarkers to track TKI response[12]. In our study, include 60 CML patients with mean age of (45.97 ± 2.23, 49.87 ± 2.25, ١,٧٥ ± 30.93) years for both response, failure response CML patients and control group, respectively, without significant statistical differences between both patients groups. This is comparable to the ELN 2020 review, Asians' younger age distribution[10]. Our studied groups showed significant difference of age between the control from one side and different groups of CML patients from the other side (P = <0.0001). Chronic myeloid leukemia (CML) affects people of all ages, and its prevalence rises with age, prior to the introduction of imatinib, older age was a risk factor. Since then, CML patients' outcomes have improved, and older age appears to have lost its negative impact[13]. white blood cells count they showed no significant difference between both patients group, also between patients different response groups and control, our studied failure response CML patients were with failure molecular response. While the median hemoglobin level g/dl to µL showed no significant difference for both patients CML groups ,but showed a significant difference among patients groups and control group (P = <0.0001), showed reduced hemoglobin levels than the control, which might be related to the long-term use of imatinib treatment[14]. Anemia increase gradually with the prolongation of medication[15]. The median platelet count among all studied groups was within the normal range, but this does not rule out the presence of various stages of CML in this study. According to ELN recommendations, achieving CHR within 3 months of starting therapy is an optimal response. With TKI therapy, nearly all patients with chronic CML achieve a CHR[16]**.** In our study, we compared the expression level of miRNA with the degree of response achieved after treatment for CML patients with failure response, through assessment miRNA-181c expression levels in different responses and failure response of CML patients, showed significant difference result in the mean BCR-ABL1(< 0.0032), (0.01-0.0032)0.1-0.01) ,) and (>1), were expression (4614±7.397,2.172 ± 251.1 , 24.13 ± 2953 and 12.61 ± 1.482) respectively, with high level among deeper responders CML patients . There was a significant difference between (< 0.0032 vs. >1) and (0.1-0.01 vs. > 1), (P =0.0153) (P =0.0398) respectively, without significant difference among other studied patients groups. MiRNA -181c is involved in a tumor-suppression pathway gene silencing inhibits the translation process of the mRNA into protein by intervening in gene expression in imatinib-resistant vs. imatinib-responder patients. MiRNA and target genes play roles in the molecular mechanisms underlying CML resistance. Because increasing BCR-ABL levels leads to abnormal miRNA expression, there will be a decrease in apoptosis capability, which may cause more

disease progression in imatinib resistant patients vs. increasing apoptosis of leukemic cells in imatinib-responder patients[17]. MiRNA-181c may function to regulate therapy response, activating miRNA-181c or inactivating its target gene pathway could be a potential strategy for reversing drug resistance in human CML. In CML, downregulation of miRNA-181c may cause the PI3K/AKT pathway to become overactive and ST8SIA4 to express itself excessively. Inhibiting the PI3K/AKT signaling system may reduce cancer cell proliferation and induce apoptosis in a variety of malignancies[18].

As for miRNA-150 expression levels in different responses and failure response of CML patients, showed significant difference result in the mean BCR-ABL1(< 0.0032), (0.01-0.0032), 0.1-0.01)) and (>1), were expression (1203±116424.35 ± 59.16 ,8.502 ± 14.17 , and 1.919 ± 0.4081) respectively, with higher level among deeper responders CML patients. The result significant differences appeared between (< 0.0032 vs. >1) and (0.01-٠,١ vs. >1), (P = 0.0471) (P =0.0001) respectively, without significant difference among other studied patients groups. When neoplasia develops, progresses, or responds to treatment, miRNA-RNA expression, which is a dynamic process, reflects changes at the cellular level[19]. BCR-ABL and miRNA-150 have a strong association in this study's molecular remission data. This suggests that miRNA -150 may be helpful in predicting outcomes once patients using imatinib for chronic myeloid leukemia have achieved molecular remission, high expression of miRNAs targeting BCR-ABL sensitized the CML cells to imatinib treatment, suppressed proliferation and induced apoptosis. Relapse or full molecular remission could be the results of this association[20].

Conclusion

Changing gene expression with different amounts of miRNAs has an impact on drug-gene interactions, with consequences for cell growth and death. Gene expression different level miRNA-181c and miRNA-150 among of CML patients of imatinib therapy were high expression in response patients than failure response patients. The gene expression level of miRNA-181c and miRNA-150 difference among different level BCR-ABL-210 transcript in optimal response CML patients.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

Ethical Clearance: This study was approved by the scientific ethics committee/ All the study experiments were performed at the University of Mustansiriyah University - College of Science - Department of biology.

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