

# Level of microRNA Expression and Blood Picture of Car Repairing Workers Compared with a Sample of Patients with Chronic myeloid leukemia in Anbar Province



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## ABSTRACT

A close relationship was found between exposure to benzene and leukemia. MicroRNAs was used as biomarkers to provide values for cancer diagnosis and prognosis. This study aimed to measure the gene expression levels of MiR-124, MiR-22, and MiR-155 genes and compare their levels between patients, car repair workers, and healthy controls. All samples were collected from Al-Anbar Specialized Center for Oncology, Al-Sinai District. All leukemia patients were of the type of chronic myeloid leukemia (CML) in the chronic phase. Real time-PCR (qRT-PCR) was used to measure the gene expression of the mentioned genes. Gene expression results of MiR-124, MiR-22, and MiR-155 showed statistically significant differences ( $P \leq 0.05$ ) with an increase in the level of the MiR-124 gene by (7.6) in the patient group, increased in the level of MiR-22 gene in the category of mechanics (3.03), and increased in the levels of the MiR-155 gene (3.63) in the group of patients. Blood image analysis showed that there were significant differences ( $P \leq 0.05$ ) with a high level of white blood cells and lymphocytes among workers in car repairing workers. On the other hand, Gran showed a significant increase in the patient group. While RBC and HGB showed a significant decrease in the patient group. Platelets results showed a significant increase in the category of workers in car repair shops. The increase in the level of MiR-124, MiR-22, and MiR-155 genes in patients and car repairing workers coincided with an increase in CBC test values, and this explains the negative effect as a result of exposure to benzene. The results obtained from the study also showed that the gene expression levels of genes MiR-124, MiR-22, and MiR-155 are closely related to the biochemical characteristics of CML patients and car repair workers.

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## Introduction

The International Agency for Research has classified benzene on Cancer (I.A.R.C) as a Group I human carcinogen [1]. Benzene is a highly flammable, volatile, colorless liquid aromatic hydrocarbon used as a solvent or raw material in many industrial processes. At the beginning of the 20th century, benzene was used in many industries, especially printing, shoemaking, rubber, and other industries [2]. Since the 1930s, there has been an association between benzene and leukemia. By 1928, several scientists were reporting unconfirmed cases of leukemia among workers exposed to benzene. In 1963, Italy was one of the first countries in the world to adopt a law banning gasoline use.

Benzene is a solvent in work activities. Vigliani and Alessandra Forni discovered it in the 1960s to cause chromosomal aberrations in the bone marrow, which can cause leukemia [2]. CML is a type of leukemia characterized by a balanced t (22;9) (q34;q11.2) gene transmission [3]. This transmission involves the fusion of the Abelson gene (ABL) from chromosome 9 q34 with the BCR gene on chromosome 22 q11.2. This arrangement is known as the Philadelphia chromosome, and the molecular consequence of this translocation is the production of the fusion oncogene BCR-ABL1, which is then translated into the oncoprotein BCR-ABL [4].

4Biomarkers are used to detect tumors. They are biological molecules (protein or nucleic acid) that are produced when genetic or epigenetic changes occur, as they are present in concentrations higher or lower than normal concentrations in patient tissues, blood serum,

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urine, and other body fluids; these molecules serve as predictive, diagnostic, and prognostic biomarkers [4]. Physiological processes also require precise regulation of gene expression where the mechanism of gene regul [5]. Action is directed after transcription and the control of the translation process from genes to proteins by small molecules of non-coding RNA known as microRNA, which consists of approximately 19–22 nucleotides [6]. MicroRNA is involved in many different pathological conditions, including cancer of all types [7].

MiR-124 is considered an interesting subject for several reasons, including its high degree of conservation in various tissues of living organisms and its abnormal expression associated with cancer arising from different etiologies. These miRNAs are involved in the regulation of cell growth, differentiation, and development. MiR-124 leads to the development of malignant cell transformation and chemotherapy resistance, so miR-124 is considered a biomarker used to develop new therapeutic strategies for treating tumors [8]. As for MiR-22, its roles have begun to emerge at the level of diseases and normal physiology recently, as aberrant expression of MiR-22 has been found in several human diseases, such as cancer, as it contributes to many activities that include tumorigenesis [9]. The level of MiR-155 is very important, as high levels of MiR-155 were found in leukemia patients. Since MiR-155 is considered an oncoming chimera, its target is a tumor suppressor gene. As miR-155 levels in tumors are higher than in normal tissues, different levels have different outcomes [10]. CBC results are used to screen for leukemia, the first step in diagnosing leukemia. In leukemia, there is an increase in WBCs if hemoglobin is low and liver cirrhosis when the platelet count is low [11]. Therefore, the current study aims to measure the gene expression of MiR-124, MiR-22, and MiR-155 genes, compare it with the studied groups, measure the values of CBC indicators, and compare them between CML patients, Car repairing workers, and healthy subjects.

## Materials and Methods

### Sample collection

A comparative study was conducted in the Al-Anbar Specialized Center for Oncology, Al-Sinai District, and

Al-Mawla Laboratory for pathological analysis during the period from September 2022 to January 2022. Their ages ranged from 20 to 55, they were males only, and the leukemia patients were of the type of chronic myeloid leukemia (CML) in the chronic phase. 5 ml of blood was drawn for 60 samples divided into three groups, each group containing 20 samples from a group of leukemia patients. Chronic myelodysplasia, the car repair mechanics group, and the healthy group.

### Sample analysis of a complete picture of the blood

2 ml of blood is placed in a special test tube containing ethylene diamine tetraacetic acid (EDTA), an anticoagulant. The test tube is then left on the mixer for 2-3 minutes to mix the blood with EDTA, after which the test tube is placed in the blood analyzer self from the Swedish company Mythic. The result is given after a few minutes as a complete picture of the blood.

### Extraction RNA and real time-PCR (qRT-PCR)

200 µl of serum and 400 µl of Trizol reagent were added for RNA extraction. According to the company's instructions, RNA was converted to cDNA using a special kit prepared by the English company NEB. Then the real-time quantitative reverse transcription technique (Real Time q RT-PCR) was used to determine the gene expression of the study genes MiR-124, MiR-22, MiR-155, and the calibrator gene U6, as shown in Table (1). Partial data were calculated using the Livak equation and according to the method [12].

**Table 1.** The primers used in the study

Human Gene	Primer sequence 5'–3'	Gene ID
miR-22F	GAGCTGCACTGACCAGTAGG	NR_029494.1
miR-22R	GTGCTGGCAGATGGATCACT	
miR-124F	GCTTTCTAAGGCACGCGGT	406907
miR-124R	CAGTGCTGGGTCCGAGTGA	406947
miR-155F	CTCAGACTCGGTTAATGCTAATCGT GATAGG	
miR-155R	GCTGTGGCAGTGAAGCGTGATTTA TT	
U6-F	GAGAAGATTAGCATGGCCCT	26827
U6-R	ATATGGAACGCTTCACGAATTT GC	

### Statistical Analysis

After collecting and tabulating the data related to the study, the experimental data were presented as (Mean±SD), and the statistical analysis was conducted with (CoStas software) (Monterey, CA, U.S.A.). We

detected differences between the two groups by one-way analysis of variance (ANOVA) and calculated the means test using the least significant difference L.S.D. test at the probability level ( $P \leq 0.05$ ) [13].

## Results

### Comparison of age groups

The statistical results are shown in Table (2). There are no statistically significant differences between the average age of the studied groups, where the average group of patients, car repairing workers, and healthy people were (39.70, 38.65, 37.85), respectively, with standard errors (1.89, 1.99, 2.33) respectively at the probability level (0.850).

**Table 2.** Comparison between different age groups

Class	NO	Mean ± SE of Age (year)
Patients	20	39.70 ± 1.89
Car Repairing Workers	20	38.65 ± 1.99
Healthy	20	37.85 ± 2.33
LSD value	---	5.897 NS
P-value	---	0.850
<b>NS: Non-Significant.</b>		

### Gene expression of MiR-124, MiR-22, and MiR-155 genes

The statistical- analysis results in Table (3) confirmed the existence of significant differences in the gene expression level of MiR-124, MiR-22, and MiR-155 genes among the study groups. The results showed significant differences in the mean of the MiR-124 gene at a probability level of (2.04\*), where the patients gave the highest mean of (7.6). In contrast, the healthy group showed the lowest mean by (1), there were also significant differences between the group of car repairing workers and healthy people, and the value of the MiR-22 gene showed that there were significant differences between the averages of the studied groups at a significant level of (1.134\*), Car repairing workers had the highest average with a value of (3.03), Between patients and healthy people, as the MiR-155 gene indicated that there were statistically significant differences between the studied groups, the patient's group gave the highest average value of (3.63). In contrast, the healthy group had the lowest average (1) at the probability level (1.838\*) in addition to the significant differences between Categories of repairing workers mechanics and healthy people.

**Table 3.** Shows the mean values of gene expression for the study genes

Class Gene	Patients		Car Repairing Workers		Healthy		L.S.D 5%
	AV	SD	AV	SD	AV	SD	
MiR-124	7.6 a	1.99	3.26 b	2.08	1.00 c	0.00	2.04*
MiR-22	2.15 a	0.81	3.03 a	1.37	1.00 b	0.00	1.134*
MiR-155	3.63 a	2.04	3.21 a	1.57	1.00 b	0.00	1.838*

### Blood test results

The results of the statistical analysis in Table (4) confirmed the existence of significant differences between the mean values of the whole blood image at the level of probability ( $P \leq 0.05$ ). And between the tables, there were significant differences in the average values of white blood cells at the probability level (0.034). Car repairing workers had the highest average value of (7.540 mm) with a standard error of ( $\pm 0.341$ ), while the patient group showed the lowest level for this characteristic (6.465 mm<sup>3</sup>) with a standard error of ( $\pm 0.215$ ). Where the car repairing workers gave the highest mean of (37.90%) and a standard error of ( $\pm 9.40$ ), while CML patients gave the lowest mean of (32.16%) and a standard error of ( $\pm 5.93$ ), Granulocytes showed significant differences at a significant level of (0.043). Patients had the highest mean (58.62%) with a standard error ( $\pm 6.52$ ). The lowest mean in healthy subjects amounted to (52.33%) with a standard error of ( $\pm 6.68$ ). RBC showed significant differences with a probability value of (0.000), where the highest mean was found in healthy subjects with a value of (5.077  $\mu$ l) and a standard error of ( $\pm 0.080$ ). In contrast, the lowest arithmetic mean was found in patients with a value of (4.399 $\mu$ l) and a standard error of ( $\pm 0.16$ ), HGB indicated a statistically significant difference at the probability level (0.000), and the highest mean was for healthy people with an amount of (14.630 g/dl) and a standard error of ( $\pm 0.224$ ), while the lowest mean was for patients with a value of (12.780 g / dl) with an error standard of ( $\pm 0.433$ ). As for the PLT value, there were significant differences between the arithmetic means at the level of probability (0.059), where the category of car repairing

workers gave the highest mean of (211.750 mm<sup>3</sup>) and a standard error of ( $\pm$ 13.482). In contrast, the category of patients gave the lowest mean (182.350 mm<sup>3</sup>) and a standard error its amount is ( $\pm$ 4.645).

**Table 4.** Shows the mean values of CBC and standard error of the studied groups

Class	WBC mm <sup>3</sup>	Lymph %	Gran %	RBC $\mu$ l	HGB g/dl	PLT mm <sup>3</sup>
Patients	6.465 $\pm$ 0.215	32.16 $\pm$ 5.93	58.62 $\pm$ 6.52	4.399 $\pm$ 0.168	12.780 $\pm$ 0.433	182.350 $\pm$ 4.645
Car Repairing Workers	7.540 $\pm$ 0.341	37.90 $\pm$ 9.40	56.51 $\pm$ 9.91	4.855 $\pm$ 0.062	14.185 $\pm$ 0.196	211.750 $\pm$ 13.482
Healthy	6.935 $\pm$ 0.283	37.43 $\pm$ 8.21	52.33 $\pm$ 6.68	5.077 $\pm$ 0.080	14.630 $\pm$ 0.224	207.200 $\pm$ 6.968
L.S.D 5%	0.757	5.05	4.98	0.322	0.860	26.94
C.V%	16.6	22.3	14.1	10.6	9.8	21.0
P-Value	0.034	0.049	0.043	0.000	0.000	0.059

## DISCUSSION

The elevated miR-124 gene is due to: miR-124 is the most abundantly expressed miRNA in bone marrow, lymph nodes, the thymus, and peripheral blood mononuclear cells [14]. The function of miR-124 is that it is a potent pro-apoptotic tumor suppressor in many types of cancer; downregulation of miR-124 due to inducible over methylation has been observed in several types of malignancies [15]. The result of this study was in contrast to the findings of [16], who, in his study, including AML patients, indicated that decreased miR-124-1 expression leads to slightly longer survival and relapse-free survival in miR-124-downregulated patients, that downregulation of miR-124-1 predicts positive survival in AML patients The increased expression of miR-22 in methanolysis is due to the specific epigenetic mechanism that benzene exerts on the induction of hematologic malignancies: the alteration of the expression of non-coding genetic material, a set of regulatory RNAs of great importance in many diseases such as allergic, autoimmune, and neoplastic disorders[17]. A study by Su Jung Song[18]. Showed that miR-22 is an oncogene and a key factor in tumorigenesis leading to MDS and, thus, malignancy in vivo. Its expression also increases in hematological malignancies and stimulates blood proliferation as a result of suppressing the expression of (ten-11-translocation 2) TET2; TET2 was identified as the direct

final target of miR-22, as a negative association was found between these two molecules. When miR-22 is released, it affects miR-22 induction of TET2, and CML may additionally promote miR-22, which is regulated by p53 [19]. The results of our study are consistent with the finding that the gene expression of the miR-22 gene was increased. However, Nidal et al. indicated that miR-22 was preferentially decreased in patients with CML [20]. Recent studies have shown that the miR-155 gene promotes cancer cell proliferation and inhibits apoptosis by targeting PTEN, PI3K, and AKT [21]. Stefan et al found that miR-155 is down-regulated in CML[22]. The reason for the rise in white blood cells, lymphocytes, platelets, and granulocytes in auto repair mechanics is due to: The significant increase in total WBC count in motor vehicle workers is due to increased exposure to dust or fumes from toxic chemicals, which leads to an increase in white blood cells in some inflammatory and infectious diseases because the main function of white blood cells is: Provides basic defense [23]. It is known that smoking has a direct negative effect on the blood-forming system, so it enhances the effects of exposure to various chemicals[23]. In addition, car repairing workers are exposed to mineral oils and petroleum derivatives, which lead to skin diseases such as contact dermatitis/eczema / Occupational contact dermatitis[24]. Leukocytosis is a consequence of the chronic inflammatory nature of the disease and its specific immune and inflammatory mechanism[25]. Platelets are also implicated in allergic dermatitis such as eczema as is their association with lymphocytes where thrombocytosis and lymphocytes play a role in allergic diseases[26].

The therapeutic landscape for CML has dramatically improved with the development of small molecule tyrosine kinase inhibitors (TKIs) that effectively interfere through competitive inhibition at the interaction site between the oncoprotein BCR-ABL1 and adenosine triphosphate (ATP), preventing the cellular proliferation of malignant cells. This approach "targeted" the natural history of chronic myeloid leukemia, increasing the 10-year survival rate from about 20% to 80%–90% [27]. Among these TKIs are imatinib, which is considered a front-line treatment for CML in the chronic phase (CML-CP), and three second-generation TKIs (bosutinib, dasatinib, and nilotinib) that

have been associated with similar survival outcomes [28]. This study agreed with the study of Zerihun et al. in the town of Harar, eastern Ethiopia, which compared 30 samples of garage workers and 30 control samples of teachers and students and found statistically significant differences in the parameters WBC, platelets and RBC Hb [23].

In addition to the results of Salah et al. [29], which were designed to find out the negative impact of gasoline on the health of workers at gas stations in Nasiriyah, the study included 90 male samples consisting of (60 samples from gas station workers, and 30 samples from healthy people). His study revealed that there are statistically significant differences in granulocytes (GRAN), hemoglobin (Hb), and red blood cells (RBC). The results of our study are also consistent with the study conducted by Hasan [30], in Kirkuk on 60 workers who are exposed to direct contact with benzene and 60 people who are not exposed to benzene. His study revealed significant differences in the percentage of lymphocytes (LYM%) and a significant difference in white blood cells and platelets.

Our results did not statistically agree with the results of Nommanudien et al. in Kabul, Afghanistan. The study included 53 adult workers, 49 children, and 29 controls to determine the effect of petroleum products on the blood systems of children and adults working in repair shops [31]. There were no significant differences between exposed adults and controls in terms of WBC centrality, LYM lymphocytes, GRA granulocytes, HB hemoglobin, RBC erythrocytes, and PLT platelets, as this study showed that adults were less affected by Occupational exposure to petroleum products.

## CONCLUSION

We conclude from our study that the MiR-124, MiR-22, and MiR-155 genes are elevated in patients with chronic myeloid leukemia and car repair shop workers, and this explains the negative effect as a result of exposure to benzene. In addition to its effect on CBC values, whether its effect is direct, such as inflammation that causes increased WBC, or as a result of Infection with eczema, which caused an increase in PLT and LYM, and smoking leads to an increase in the harmful effect of benzene, such as an increase in GRA values. Imatinib treatment also showed high effectiveness in

regulating RBC and Hb values in patients with chronic myeloid leukemia.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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## Ethics Approval

Informed consent was obtained from each participant and from Anbar University. in the ref: 94 and date:17/7/2023.

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## مستوى التعبير الجيني microRNA و صورة الدم لدى العاملين في ورش تصليح السيارات مقارنة بعينة من مرضى ابيضاض الدم النخاعي المزمن في محافظة الأنبار.

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### الخلاصة:

يعد البنزين مادة كيميائية تسبب الكثير من المشاكل الصحية فقد وجدت علاقة وثيقة بين البنزين و سرطان الدم، كما يمكن استخدام MicroRNA كمؤشرات حيوية التي توفر قيمة لتشخيص السرطان و التنبؤ به. تهدف هذه الدراسة الى قياس مستوى التعبير الجيني لجينات MiR-22 و MiR-124 و MiR-155 ومقارنة مستوياتها بين مجموعة المرضى و عمال ورش اصلاح السيارات و الاصحاء. بالإضافة الى قياس تعداد الدم الكامل و فعالية وظائف الكلى (اليوريا و الكرياتينين) و فعالية وظائف الكبد (البروتين الكلي) و مقارنة قيمها بين مجموعة المرضى و عمال ورش اصلاح السيارات و الاصحاء. طرق العمل: تم جمع العينات من مركز الأنبار التخصصي لعلاج الأورام و من منطقة الصناعة ، تراوحت أعمارهم بين (20-55) سنة. و كانت فئة مرضى ابيضاض الدم من نوع ابيضاض الدم النخاعي المزمن في الطور المزمن. تم استخلاص الحمض النووي الريبي (RNA) من مصل العينات لقياس التعبير الجيني باستخدام PCR في الوقت الحقيقي (qRT-PCR). النتائج: أظهرت نتائج التعبير الجيني بين جينات MiR-124، MiR-22، MiR-155 وجود فروق معنوية ذات دلالة إحصائية عند مستوى احتمال ( $P \leq 0.05$ ) مع زيادة مستوى التعبير الجيني لجين MiR-124 بمقدار (7.6) في فئة المرضى ، و زيادة مستوى التعبير الجيني لجين MiR-22 في فئة عمال ورش اصلاح السيارات بمقدار (3.03) ، يماثلها زيادة مستوى التعبير الجيني لجين MiR-155 بمقدار (3.63) في فئة المرضى. اما نتائج تحليل صورة الدم فقد أظهرت وجود فروق معنوية ذات دلالة إحصائية عند مستوى احتمالية ( $P \leq 0.05$ ) في ارتفاع مستوى خلايا الدم البيضاء و الخلايا اللمفاوية في فئة عمال ورش اصلاح السيارات، من ناحية أخرى أظهرت الخلايا الحبيبية زيادة معنوية في فئة المرضى ، بينما أظهرت خلايا الدم الحمراء و الهيموغلوبين انخفاضاً معنوياً في فئة المرضى، أما بالنسبة للصفائح الدموية فقد أظهرت ارتفاعاً ملحوظاً في فئة عمال ورش اصلاح السيارات . الاستنتاج: تزامنت الزيادة في مستوى التعبير الجيني لجينات MiR-124 و MiR-22 و MiR-155 لدى المرضى و عمال ورش اصلاح السيارات مع زيادة قيم اختبار تعداد الدم الكامل، و هذا ما يفسر التأثير السلبي نتيجة التعرض للبنزين ، كما أظهرت النتائج التي تم الحصول عليها من هذه الدراسة ان مستويات التعبير الجيني لجينات MiR-124 و MiR-22 و MiR-155 ترتبط ارتباطاً وثيقاً بالخصائص البيوكيميائية لمرضى سرطان الدم النخاعي المزمن و عمال ورش اصلاح السيارات.

**الكلمات المفتاحية:** الميكورنا، ابيضاض الدم النخاعي المزمن، صورة الدم، عمال ورش اصلاح السيارات.