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Association of *SCARB1* Gene Expression with Chronic Myeloid Leukemia Progression in a Sample of Iraqi Patients

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ABSTRACT

The present research design examines the relationship between SCARB1 gene expression and the progression of chronic myeloid leukemia (CML) in Iraqi patients. The variations in gene expression between patients with CML and healthy controls were investigated. The gender and age correlations with CML patients were included, as was the association of gene expression folding of the SCARB1 gene with clinical data (WBC, RBC, hemoglobin, platelets, and BCR-ABL gene). The results displayed a significant difference in the mean gene expression level (ΔCt) of the CML group when compared to the matching ΔCt values in the healthy control group. The gene expression folding of the SCARB1 gene indicates considerable changes in expression, which reached 0.134. There was no significant correlation for CML patients in regards to their age or gender, and with all studied clinical parameters except WBC and the BCR-ABL gene, there was no significant correlation. Our findings suggest that the degree of SCARB1 gene expression may serve as a determinant biomarker for CML prognosis.

Keywords: age, gender, chronic myeloid leukemia.

العلاقه بين SCARB1 وتطور مرض ابيضاض الدم النيقياني المزمن في عينه من المرضى العراقيين التعبير الجيني لجين ال

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الخلاصه

صمم البحث الحالي العلاقة بين التعبير الجيني لـ SCARB1 ونمو سرطان ابيضاض الدم النيقاني المزمن (CML)في المرضى العراقيين. تم الكشف عن الاختلافات في التعبير الجيني بين المرضى ومجموعة الاصحاء. تم تضمين العلاقه بين الجنس والعمر مع مرضى سرطان الدم النخاعي المزمن ، بالإضافة إلى ارتباط التعبير الجيني مع البيانات السريرية (WBC ، RBC، الهيموغلوبين ، الصفائح الدموية ، وجين .(BCR–ABL أظهرت النتائج اختلافًا معنويًا في متوسط مستوى التعبير الجيني للمجموعات المريضة عند مقارنتها بقيم المطابقة في مجموعة الاصحاء ، يشير التعبير الجيني لجين SCARB1 إلى تغييرات كبيرة في التعبير ، والتي اظهرت زيادة في مستوى التعبير في مرضى سرطان ابيضاض الدم النيقاني المزمن(1.056) ، لم تكن هناك علاقه ذات دلالة إحصائية في مرضى سرطان ابيضاض الدم النيقاني المزمن فيما يتعلق بعمرهم أو جنسهم او

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البيانات السريريه باستثناء BCR–ABL gene و . WBC تشير النتائج التي توصلنا إليها إلى أن درجة التعبير الجيني لـ SCARB1 قد تكون عاملاً محددًا لتشخيص تقدم سرطان ابيضاض الدم النيقاني المزمن.

1. INTRODUCTION

CML is a chronic myeloproliferative disease with a 3-5-year chronic course [1]. Obesity and adult weight gain are important risk factors for CML [2]. Prior to CML diagnosis, there was an elevated prevalence of several malignancies and autoimmune illnesses, showing that CML patients had a hereditary propensity to cancer and autoimmunity [3] [4]. CML patients often have minimal symptoms, such as stomach fullness caused by anemia-related tiredness, splenomegaly, bone pain caused by packed bone marrow, hemorrhage, malaise, weight loss, or night sweats. During a physical examination, an enlarged spleen is frequently noticeable, although an enlarged liver is less common [5]. CML's etiology is yet unknown. Response to high levels of ionizing radiation is the most well-established risk factor among Japanese atomic bomb survivors in Hiroshima and Nagasaki [6]. This proliferation of mutant stem cells will result in some developed cells, increasing total myeloid bulk [1]. CML is a clonal hematopoietic stem cell illness characterized by cytogenetic abnormalities caused by cell proliferation and division with the Philadelphia chromosome [7]. A reciprocal translocation between the long arms of chromosomes 9 and 22, known as the Philadelphia chromosome, takes place in a hematopoietic cell. The *BCR-ABL1* gene complex, also known as t (9; 22), was formed by combining the breakpoint cluster area (BCR) gene on chromosome 22 (22q11) with the proto-oncogene Abelson (ABL1) on chromosome 9 (9q34) (q34; q11) [8]. CML affects men more than women and can affect people of all ages; however, it is most commonly associated with the elderly [9].

The human scavenger receptor class B type I (SCARB1), which is the primary receptor for HDL-cholesteryl ester absorption, is encoded by the SCARB1 gene. By affecting the expression of the SR-BI protein and blood lipid levels, polymorphisms in this gene may influence the susceptibility to coronary heart disease (CHD) and cerebral infarction (CI) [10]. The SCARB1 gene is found on 12q24.31 and spans approximately 75 kb with 13 exons. Scavenger receptor class B type 1 (*SCARB1*, also known as SR-BI) is a multiligand cell surface receptor that is expressed in macrophages and the liver, indicating that it plays an important role in the removal of excess cholesterol from the body [11]. The SCARB1 rs5888 gene polymorphism has been linked to human disorders such as CHD, cancer, and leukemia, according to several studies [12]. The fusion oncoprotein Bcr-Abl, which has been found to be abnormally expressed in chronic myeloid leukemia (CML) cells and may therefore be essential for the survival and maintenance of LSCs, is produced by a reciprocal translocation that causes CML (9:22). [13] employed gene microarrays to investigate the variations in gene expression that correspond with the development of AP/BC. The transcription factors JUN-B and FOS, the marker PRAME, the WNT/β-catenin pathway, alternative kinase pathways, and these factors have all been linked to advanced CML. *SCARB1*, which distinguished between individuals with CP and those with BC, was discovered via an additional study [14].

This study was aimed at studying the correlation between SCARB1 gene expression and the incidence of CML in the Iraqi population and the possibility of using it as either a protection or risk factor.

2.MATERIALS AND METHODS

Subjects

The national center for research and treatment of hematology in Baghdad, Iraq, provided the study's participants: 50 CML patients with ages ranging from 8 to 75, gender split into 21 males and 29 females. On the basis of a complete blood count (CBC), a bone marrow examination, and a BCR-ABL gene test, patients were given a CML diagnosis.

Whole blood was obtained from each patient and then placed in a tube containing TRIzol™ Reagent for mRNA collection for RT-qPCR analysis.

Total RNA Extraction

RNA was extracted from samples using the TRIzol™ Reagent Kit (Sigma, USA) by adopting the manufacturer's protocol.

The concentration and purity of extracted RNA were detected using a nanodrop spectrophotometer (Q5000 UV-VIS, Origin) by measuring the absorbance at 260/280 nm, while the concentration is measured in ng/l (absorption wavelength of protein and DNA). The purity of RNA that is accepted is between 1.7 and 2.0.

Primer design

The NCBI gene bank database was used to obtain the cDNA sequences of the SCARB1 gene as well as β-Globin as a housekeeping gene. Premier 3 software was used to design RT-qPCR primers with a melting temperature of 65 degrees Celsius, primer lengths of 18–23 nucleotides, and amplicon lengths of PCR of 75–150 base pairs, as shown in Table 1.

Table 1: Primers' sequences

Primer Name	Sequence 5`- 3`	Annealing Temp. (ºC)
β-Globin-F	ACACAACTGTGTTCACTAGC	65
β-Globin-R	CAACTTCATCCACGTTCACC	
SCARB1_exp-F	GGTCCAGAACATCAGCAGGATC	
SCARB1_exp-R	GCCACATTTGCCCAGAAGTTCC	

*SCARB1***Gene Expression**

The GoTaq®1-Step RT-qPCR System Kit (Promega, USA) was used to assess the expression of *SCARB1* genes. It's a one-step RT-qPCR reagent method for quantitative RNA analysis.

Reverse transcription processes need to be put together in an environment free of RNase. All materials were thawed, including the RNA templates, and each solution was carefully blended. The PCR tube rack was loaded with RT-FDmix tubes. According to Table 2, the reaction component was introduced to the RT FDmix tube.

Table 2: Reverse transcription reaction components

Component	Volume
RT FDmix	1 tube
Total RNA	4µl
Nuclease-freeH20	up to 20µl

Then a thermal cycler program was performed as in Table 3.

Table 3: Thermal cycler steps

	Step1	Step2	Step3	Step4

Temperature	25 ºC	42 ºC	85 ºC	4 ºC
Time	10 min	30 min	10 min	∞

The SCARB1 gene expression levels were determined using the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method, which is a sensitive methodology for measuring steady-state RNA levels. A quantitative real-time qRT-PCR SYBR Green test was utilized to confirm the target gene's expression. The current work designed primer sequences for the SCARB1 gene, which were manufactured by Alpha DNA Ltd. (Canada) and stored lyophilized at -23 °C. Housekeeping gene β-globin RNA levels were amplified and utilized to normalize the mRNA levels of the examined genes (Table 1).

The Macrogen Company provided these primers in lyophilized form. Lyophilized primers were diluted in nuclease-free water to make a stock solution with a final concentration of 100 pmol/µl. To create a functional primer solution of 10 pmol/µl, 10 µl of primer stock solution (stored at -20 C) was mixed with 90 µl of nuclease-free water.

The MIC-4 Real-Time PCR System was employed for qRT-PCR. The gene expression fold change and levels were assessed using the 2xqPCR Master Mix Kit ingredients by determining the threshold cycle (Ct). Every reaction was performed twice. Table 4 was used to obtain the needed volume of each ingredient.

Table 4: Quantitative real-time PCR ingredients used within gene expression research

components	Stock	Unit	Final	Unit	Volume 1 Sample
qPCR Master Mix	2	X	1	X	5
RT mix	50	X	1	X	0.25
MgCl2					0.25
Forward primer	10	µM	0.5	µM	0.5
Reverse primer	10	µM	0.5	µM	0.5
Nuclease Free Water					2.5
RNA		Ng/µM		Ng/µM	1
Total volume					10
Aliquot per single rxn	9µl of Master mix per tube and add 1µl of Template				

The cycling routine was done in accordance with the thermal profile as shown in Table 5.

Table 5: Thermal profile underlying gene expression

STEP	°C	m:s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40
Annealing	65	00:20	
Extension	72	00:20	

The threshold cycle (CT) for each sample was determined using the real-time magnetic induction cycler (mic) software. The average values of all samples were computed after they

were run in repetition. The housekeeping gene was used to standardize the expression data of the chosen genes. The ΔΔCt technique was utilized for data analysis, as described by Kenneth and Thomas [15], and the findings were reported as folding changes in gene expression. The gene expression fold-change was computed as follows: Fold change=2-ΔΔCt

3.Statistical analysis

GraphPad Prism version 9.0 was used for the statistical analysis (GraphPad Software Inc., La Jolla, CA). A Student's *t test* and one-way ANOVA were utilized to establish whether or not the group variance was significant. To examine count variances, the Chi-square was used. The Pearson coefficient (r) was used to assess correlation. Data were expressed as mean ± SD and statistical differences were defined as * $p \leq 0.05$ or ** $p \leq 0.0001$ [16].

4.RESULT AND DISCUSSION

cDNA reverse transcription:

The complementary DNA reverse transcription was performed on the second day following RNA extraction. Because cDNA for the SCARB1 gene and the housekeeping gene were required, the same primer reaction was used. The efficiency of cDNA concentration was tested later on by the effectiveness of qPCR. All processes had a 100% yield, showing effective reverse transcription. The annealing temperature of the optimum primers was derived using the following equation based on the Tm of each primer provided in the manufacturer's instructions:

- Melting Temperature (Tm) =2 (A+T) + 4 (G+C).
- Annealing Temperature (Ta) = Tm - (2-5) ºC. [5].

Melting temperatures for the reverse and forward primers were also estimated using the previous equation. The lowest melting temperatures of both forward and reverse primers were determined after comparing the annealing temperatures of the forward and reverse primers [5].

SCARB1 gene expression was evaluated using real-time polymerase chain reaction (RT-qPCR), and the amount of gene expression was equalized to that of a housekeeping gene and quantified using the folding ($2^{-\Delta\Delta Ct}$) and ΔCt values presented in Figures (1), (2).

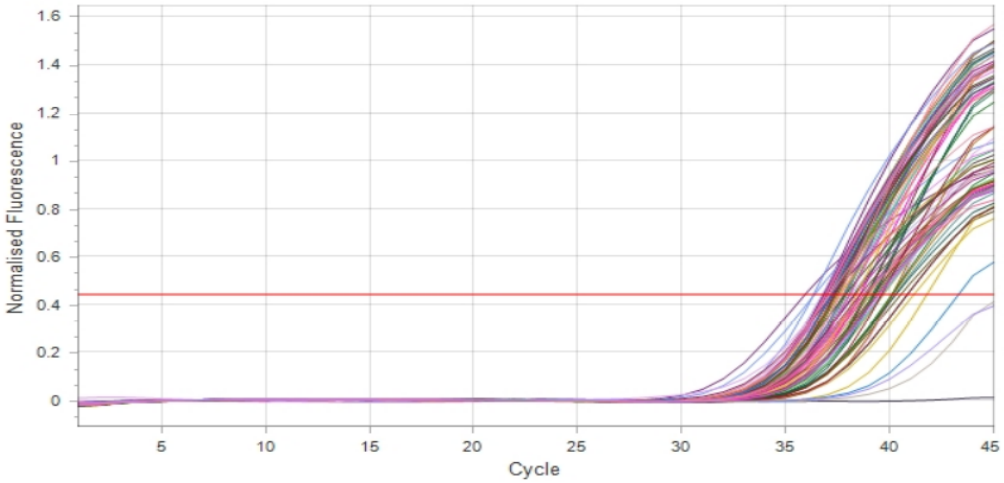

Figure 1: Amplification plots of the SCARB1 gene by RT-qPCR

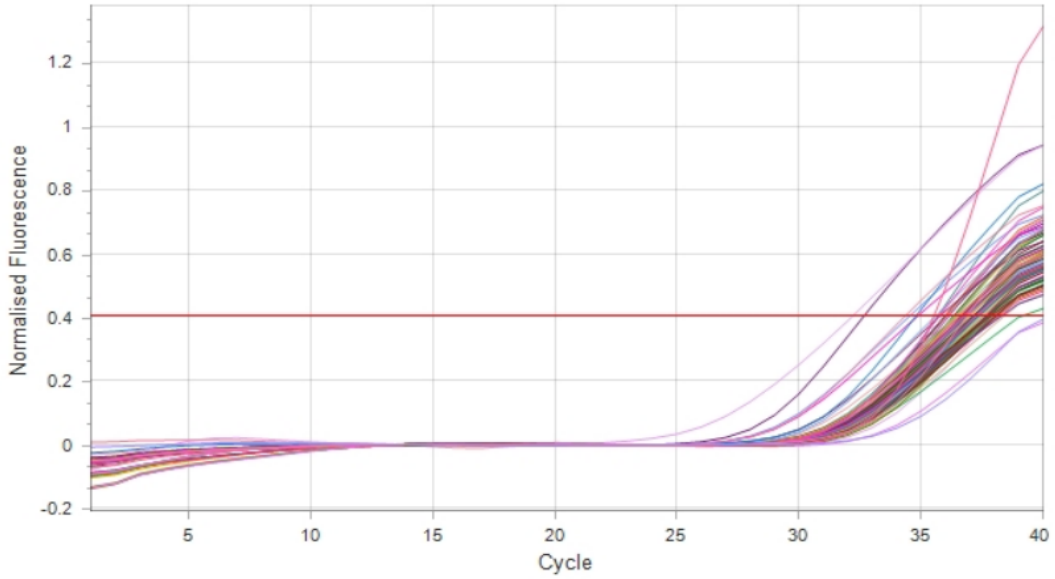

Figure 2: Amplification plots of *β-globin* gene by RT-qPCR

According to figures (3) and (4), a single peak for the amplicon was noticed by a representative melt curve of both the *SCARB1* gene and the *β-GLOBIN* gene after analyzing the samples by RT-qPCR. This observation revealed that the melt curve represented a pure single amplicon of samples, and the amplification specificity was high with the intercalating dye test.

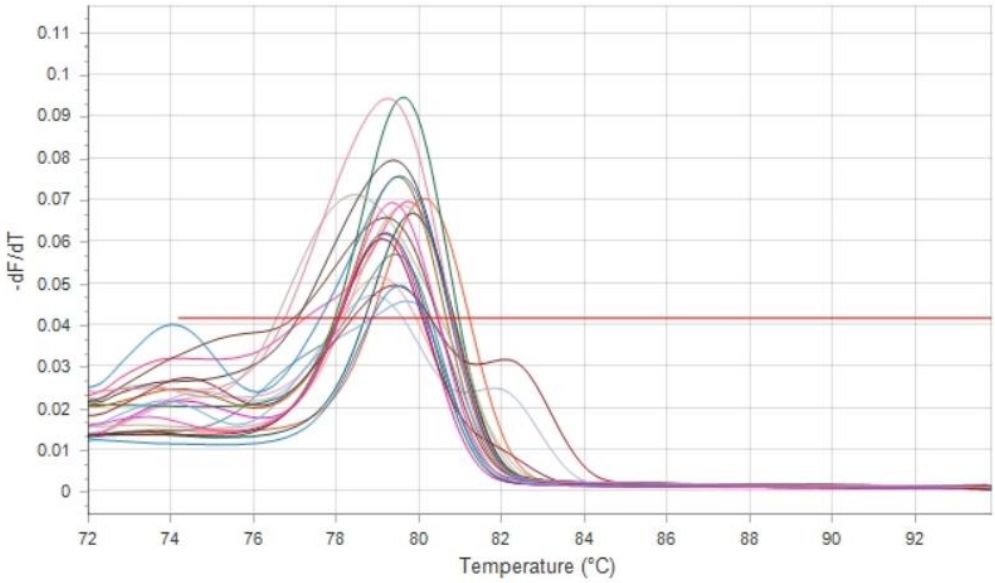

Figure 3: Melt curve of SCARB1 gene amplicons after RT-qPCR analysis showing a single peak threshold of 0.042 starting at 74.18 °C

Figure 4: Melt curve of *β-GLOBIN* gene amplicons after RT-qPCR analysis showing single peaks Threshold 0.023 starting at 72.79 °C

The Expression level of *SCARB1* gene in the studied groups:

SCARB1 gene expression levels in CML patients were greater than levels in healthy controls, and the ΔCT average of the SCARB1 gene in CML patients' blood samples was 0.134 compared to the ΔCT average of the healthy controls (2.424). There was a statistically significant reduction in ΔCt mean (p = 0.0002) for the SCARB1 gene in the diseased group compared to the ΔCt mean in the healthy controls. The current study's expression-folding mean (2-ΔΔct) of SCARB1 reveals a value of 1.056 and was significant at p = 0.0002, as shown in tables 6 and 7.

Table 6: Expression level (ΔCT) of *SCARB1* in CML patients and control groups

Gene expression Level ΔCT	Control (n=50)	Patient (n=50)	*P*
SCARB1	2.424 ± 1.22	0.134 ± 0.89	0.002

Table 7: Mean of gene expression ($2^{-\Delta\Delta Ct}$) of *SCARB1* mRNA in the patient group

Genes	Gene expression ($2^{-\Delta\Delta ct}$) (mean± SD)	*P*
SCARB1	3.00 ± 5.52	0.002

The significant differences in gene expression levels of the *SCARB1* gene between Iraqi CML patients and healthy controls, according to the findings of tables (6), and the increased expression fold for this gene in CML patients indicate a connection between this gene and chronic myeloid leukemia and also provide a qualified applicant to use these genes as an indicator tool for detecting CML evolution. Sweet et al. confirm these findings, stating that molecular alterations serve as prognostic markers in CML patients in the United States [17]. In the United States, additional studies discovered a *SCARB1* gene signature that differentiated between patients in the chronic and blast phases [18]. A prior study conducted in the United

Kingdom by Lucas et al. found that SCARB1 was considerably raised after a period of treatment in CML patients who had a poor response to imatinib therapy, particularly at the final stage, suggesting a greater risk of disease progression [19]. This might be due to *SCARB1* overexpression as a barrier to leukotriene-mediated signaling. In Sweden, leukotriene-mediated signaling pathways have been recognized as critical controllers of acute and chronic inflammation, as well as their participation in the etiology of various malignancies, including breast, lung, prostate, and colon cancers [20].

The mean of gene expression folding in the female patient group for the SCARB1 gene was 3.963± 2.571, compared to 3.726± 2.726 in the male patient group, and the differences were statistically insignificant $p>0.05$, as shown in table (8). Surprisingly, our results agree with Libyan research that found a higher frequency of CML in females (58%) than males (42%), [23]. Our results, however, contradicted Melos and Barnes' findings that the frequency was greater in males than in females [24]. In conclusion, CML can affect people of all ages and both sexes at equal frequencies.

Table 8: Shows the correlation of the mean SCARB1 gene expression fold with gender in CML patients.

Gene expression $2^{-\Delta\Delta ct}$	Patients groups		*p*
	Female group (n=29)	**Male group (n=21)**	
SCARB1	3.963± 2.571	3.726± 2.726	0.7560

As a result, we may deduce that the relationship between gene expression and patient gender may vary depending on the gene type. These findings indicate that CML can harm both men and women at any age. The Philadelphia chromosome results in the formation of a hybrid BCR-ABL gene, which is then translated into chimeric BCR-ABL messenger RNA [25]. As a result, a diagnosis may be determined based on the BCR-ABL gene by identifying the Philadelphia chromosome as well as the features of blood parameters represented by blood count and hemoglobin [26].

As shown in table (9), in which the mean and standard deviation for each measurement in CML patients were reported. The data showed that the mean value for WBC (149.99 x 10^9), RBC (3.93 x 10^9), platelets (274.4 x 10^9), hemoglobin (11.88), and the BCR-ABL gene was 90.7%.

Table 9: Clinical parameter means in CML patient groups

parameters	Mean (n=50)	SD
WBC x 10^9/L	149.99	89.83
RBC x 10^9/L	3.93	0.65
Platelets x10^9/L	274.4	155.7
Haemoglobin g/L	11.28	1.502
BCR-ABL gene	90.7%	36.688

This result showed a highly significant correlation with the SCARB1 gene that was seen only with the WBC and BCR-ABL genes. Furthermore, the differences were not statistically significant. with other clinical parameters including RBC, platelets, and hemoglobin (Table 10).

Table 10: Shows the results of a correlation study of SCARB1 gene expression and clinical indicators in CML patient groups

parameters	SCARB1	
	r	***p***
WBC x 109/L	0.141	0.000
RBC x 10^9/L	0.031	0.892
Platelets x10^9/L	-0.1189	0.4107
Hemoglobin g/L	-0.02098	0.8850
BCR-ABL gene	0.1885	0.000

SCARB1 gene expression was evaluated in CML patients throughout the chronic phase to investigate if there was a link between gene expression levels and clinical outcome. *SCARB1* levels were elevated, showing that the arachidonic acid system is functioning normally up to the degree of *SCARB1* synthesis [19]. Differences in *SCARB1* seen in CML patients may be linked to leukemia rather than a physical reaction to medication [27]. Our finding is the first study in Iraq to link *SCARB1* gene expression with CML, and thus it might be suggested as a biomarker in CML progression. Oehler *et al*. validated these findings by suggesting six genes (NOB1, DDX47, IGSF2, LTB4R, SCARB1, and SLC25A3) as helpful indicators in patients at an initial point in CML development. Gene expression analysis at the diagnosis stage may be effective for identifying individuals with a poor prognosis and clarifying the molecular basis of CML disease progression [28].

5. Conclusion:

According to our findings, there is a significant association between SCARB1 gene expression and CML patients; a significant correlation was also seen with some clinical parameters, especially WBC and the BCR-ABL gene; and no correlation was seen with both age and gender. Thus, the SCARB1 gene expression fold might function as an indicator for CML prognosis.

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