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# Estimation of SLC25A3 Gene Expression in Chronic Myelogenous Leukemia Iraqi Patients

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## ABSTRACT

**Background:** Chronic myelogenous leukemia is a malignant hematological disease of hematopoietic stem cells. It is difficult to adapt treatment to each patient's risk level because there are currently few clinical tests and no molecular diagnostics that may predict a patient's clock for the advancement of CML at the time of chronic phase diagnosis. Biomarkers that can differentiate people based on the outcome at diagnosis are needed for blast crisis prevention and response improvement.

**Objective:** This study is an effort to exploit the SLC25A3 gene as a potential biomarker for CML.

**Methods:** RT-qPCR was applied to assess the expression levels of the SLC25A3 gene.

**Results:** In comparison to the mean ΔCt of the control group, which was found to be 0.409±1.69, the mean ΔCt of CML patients was found to be 0.256±2.7 ($p$ = 0.701). Furthermore, when grouped by gender, there were no significant differences between CML patients and controls, whereas a significant difference was only detected when grouped by age. Additionally, the mean of gene expression folding of the SLC25A3 gene in CML patients was found to be 4.589±8.552 which is >1. Finally, no statistically significant link was discovered when the fold of expression was correlated with the age and gender of CML patients.

**Conclusions:** Gene expression folding ($2^{-\Delta\Delta Ct}$) was greater than 1 in CML patients which indicates that the gene could be used as a potential marker for the diagnosis, targeted therapy, and monitoring of prognosis in CML.

**Keywords:** CML (chronic myeloid leukemia), SLC25A3 (solute carrier family 25 member 3).

## INTRODUCTION

15-20% of all adult leukemias are chronic myelogenous leukemia (CML), a malignant hematological disease of hematopoietic stem cells. In CML, cells are only partially developed, and these immature cells divide excessively and inefficiently. They build up in lymphoid tissues and peripheral blood, where they can cause anemia, thrombocytopenia, and leukopenia [(1)]. With a median age of onset of 55 years, CML is more prevalent in older individuals. The majority of the time, there is no clear cause of CML, and there is a lack of information regarding its etiology, but exposure to high doses of radiation, whether accidental or therapeutic, as well as prolonged exposure to industrial chemicals like benzene, seem to be possible risk factors [(2)].

Therefore, CML is a sporadic disease with no evidence of family history among monozygotic (identical) twins or close relatives of patients [(3)]. In CML, the bone marrow overproduces immature granulocytes, which then leak into the bloodstream. Additionally, the myeloblast percentage is less than 10%, and the proportions of neutrophils, monocytes, myelocytes, and eosinophils are higher than usual. The reciprocal chromosomal translocation t (9;22) (q34;q11), which results in the Philadelphia chromosome, a tiny 22q- containing a BCR-ABL fusion gene expressing a BCR-ABL chimeric protein with dysregulated tyrosine kinase activity, is a significant cytogenetic feature of CML[(4)].

The chronic phase, the acceleration phase, and the blast crisis phase are the three phases of CML. The acceleration and explosion crisis phases are only reached by a small percentage of patients in the chronic period. This may happen as a result of additional genetic changes in leukemic stem cells that cytogenetic analysis can identify. The stage of the disease can be determined, and the best monitoring and therapy can be started by comparing laboratory data, including CBC, cytogenic, and molecular tests, which are used in the diagnosis of CML [(5)]. The solute carrier family 25member 3 gene (SLC25A3) has a molecular weight of 12,789 bases and is localized at 12q23. SLC25A3 is a gene that codes for a protein called Pic, which has a molecular weight of 40095 Da and a molecular size of 362 amino acids. By facilitating the movement of inorganic phosphate (Pi) into the mitochondrial matrix, this protein performs the dual roles of phosphate and copper transport, By way of exchanges with OH or symports with H+ in electroneutral transport. The availability of transport of copper can be regulated by phosphate levels, and SLC25A3 depletion or deletion lowers the overall mitochondrial copper levels necessary for cytochrome c oxidase production [(6)].

Cancer cells have altered sensitivity to the mitochondrial and apoptotic pathways. Therefore, it is not surprising that a large number of mitochondrial carrier family members are engaged in the development of cancer. (PiC) controls the mitochondrial permeability transition pore's ability to release cytochrome c, and its suppression has anti-apoptotic effects [(7)].

Cancer cells have considerably higher transmembrane potential than healthy cells do, and several of their transporters are changed. Drugs that target mitochondria have been developed as a result of these variations [(8)].

Overexpression of this carrier activates the intrinsic apoptosis pathway, which has been shown in studies to have a potential function in mitochondria-



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dependent cell death [9]. Furthermore, disruption of ATP production has been connected to phosphate carrier SLC25A3 deficiency [10]. Our study aims to the estimation of SLC25A3 gene expression in CML patients and its possible implication as a biomarker for the disease.

## MATERIALS AND METHODS

### Blood Collection

The sample included 140 people who were split into two groups, healthy controls and people with chronic myelogenous leukemia. Eighty patient samples (49 male and 31 female) were gathered from the international hematology center in Baghdad, Iraq. The patient's ages ranged between 30 and 65. The 60 healthy samples, whose ages ranged from 30 to 50, were taken from volunteers at the international blood bank in Baghdad, Iraq (38 male, 22 female). A venipuncture was used to draw 2 milliliters of blood from each participant. For RT-qPCR analysis, the collected blood was immediately placed in the tubes containing TRIzol TM Reagent.

### Ready to Use PCR Master Mix for cDNA Synthesis from Total RNA

TRIzolTM Reagent was used to isolate RNA from samples, and the synthesis of cDNA from RNA templates was performed using the Biorad thermocycler system/USA. The *AccuPower* RT PreMix is a master mix for cDNA synthesis consisting of an easy-to-resuspend, lyophilized mixture of *M-MLV* Reverse Transcriptase, RNase inhibitors, dNTPs, reaction buffer, tracking dye, and patented stabilizer. First-strand cDNA synthesis from RNA is accomplished using the master mix kit. Reverse transcription PCR is used for downstream applications (cDNA amplification). Only the template RNA, primers, and water were needed to initiate the reaction because all the necessary ingredients had been pre-mixed at the ideal concentrations, and the work was done following the manufacturing specifications.

### The Protocol of cDNA Synthesis

- Template RNA and oligo-dT were mixed in a sterile tube as indicated in **Table 1.**

**Table (1):** Reaction volume and components of the used reverse transcription reaction used to prepare cDNA from total RNA.

| Components | | 20 µl reaction |
|---|---|---|
| Template RNA | Total RNA | 16 µl |
| Primer | Oligo dT18 | 2 µl |
| DEPC-DW | | 2 µl |

- Synthesized cDNA was stored at -20°C or utilized immediately as a PCR template.

### Primer's Design and Preparation

Using the NCBI gene bank database, the SLC25A3 gene's cDNA was sequenced along with a housekeeping gene termed GAPDH. RT-qPCR primers with melting temperatures of 58 °C, lengths of 20–24 nucleotides, and PCR amplicon lengths of 155–185 base pairs were created using Prime software (Table 2). The working solution for GAPDH and SLC25A3 was created from lyophilized primers by dissolving them in nuclease-free water as directed by the manufacturer to create a stock solution containing 100 pmoles/µL of each primer, which was then kept at -23°C. A working solution with a concentration of 10 pmole/µL was obtained by diluting 10 µl of the primer stock solution with 90 µl of nuclease-free water, then stored at -23 °C until use.

**Table 2:** Primers sequences
**RT-qPCR**

| No. | primers | Sequences 5′→3′ | | Annealing Temp. |
|---|---|---|---|---|
| 1 | SLC25A3 | F | GGTTGCTCCTCTCTGGATGA | 58 °C |
| 2 | SLC25A3 | R | AACCTGCTACAAATGTTACAACCA | 58 °C |
| 3 | GAPDH | F | CCCACTCCTCCACCTTTGAC | 58 °C |
| 4 | GAPDH | R | TTCCTCTTGTGCTCTTGCTG | 58 °C |

The Bioneer exicycler quantitative thermal block/Korea was used to identify and measure gene expression. AccuPower® GreenStar™ RT-qPCR master mix accurately detects target genes from a variety of samples. This real-time PCR reagent employs a dsDNA binding dye-based product that simultaneously evaluates fluorescence at each cycle to determine gene amplification. The kit has excellent detection and quantification capabilities for gene expression. The thermostable DNA polymerase, dNTPs, cofactors, and fluorescent dye are all included in this kit as one tube of lyophilized format (freeze-and-dried format). To proceed with real-time RT-PCR, only the template, primers, and nuclease-free distilled water are needed. The master mix was placed in a reaction vessel together with the template cDNA and the specific primers to initiate the reaction as shown in Table 3 and the work was done following the manufacturing specifications.

**Table (3):** Reagents of quantitative real-time PCR used in gene expression experiment

| RT-PCR Reagents | |
|---|---|
| **Components** | **Amount** |
| 2X Master Mix | 1X |
| RT-PCR Forward-Primer (10 picomole/microliter) | 1 µl |
| RT-PCR Reverse-Primer (10 picomole/microliter) | 1 µl |
| Template cDNA | 2 µl |
| Nuclease free water | 16 µl |
| Total | 20 µl |

The reaction was conducted under **Table 4** listed conditions.

**Table (4):** RT-qPCR conditions.

| Step | Condition | Duration | Cycle |
|---|---|---|---|
| Pre-Denaturation | 95°C | 3-5 min. | 1 |
| Denaturation | 95°C | 5-30 sec. | 1 |
| Annealing/Extension 40-45 /Detection | 55-60°C | 30-35 sec. | 40-45 |

The threshold cycle (CT) for each sample was calculated using a real-time cycler program. The mean values were estimated after duplicate samples were tested. The ΔΔCt method was used to normalize the expression data for SLC25A3 (the chosen gene) versus GAPDH (the housekeeping gene), and the findings were reported as fold changes in gene expression.

**Statistical Analysis**

Version 22 of SPSS for Windows was used to analyze the data (SPSS Inc. Chicago, Illinois, United States). Categorical variables were expressed using numbers and percentages, and Pearson Chi-square tests were used to see whether there were any significant differences. Parametric variables were given as mean ± standard deviation (SD), and the least significant difference (LSD) test was used to determine whether there were any significant differences between the parametric variables. Interquartile range and median were used to express nonparametric variables (IQR). The Spearman rank-order correlation analysis was used to evaluate correlations between variables.

**Ethical Approval**

**The Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research in Iraq both certified this research as being ethical.**

## RESULTS

**Gender, Age and CML**

338 cases of chronic myelogenous cancer were reported overall, according to the 2019 Iraq Annual Cancer Registry. Incidence and percentage were 0.94% and 0.86%, respectively. The National Center of Hematology in Baghdad, Iraq, provided the National egistry with all of its data.

**Tables 5 and 6** show the findings for the CML patient group and the healthy control group according to gender and age. There was no statistically significant difference in the gender distribution of the CT and CML patient groups (males and females: 35% and 22.1% vs. 27.1% and 15.8%, respectively). The age range for CML patients was 30 to 65 years, whereas it was 30 to 50 years for healthy individuals. Patients with CML on average were older than those with CT (41.9 1.1 vs. 35.8 4.5 years old), and the difference was significant ($P <$ 0.001).

**Table (5):** Frequencies of CML patients and controls depending on gender.

| Groups | Gender | N (%) | p-value |
|---|---|---|---|
| Patients (N. =80) | Male | 49 (35%) | 0.8 NS |
| Controls (N. =60) | Male | 38 (27.1%) | |
| Patients (N. =80) | Female | 31 (22.1%) | |
| Controls (N. =60) | Female | 22 (15.8%) | |

NS: Non-Significant at the 0.01 level.

**Table (6):** Mean age parameters among CML patients and controls

| Groups | Patients (N. =80) (mean± SD) | Controls (N. =60) (mean± SD) | p-value |
|---|---|---|---|
| Age (years) | 41.9 ± 1.1 | 35.8 ± 4.5 | <0.001** |

**Significant at the 0.01 level.

**Expression Level (ΔCt) of SLC25A3 Gene**

The mean ΔCt of the SLC25A3 gene in CML patients was 0.256±2.7 compared to the mean ΔCt of the control group which was determined to be 0.409±1.69, no significant difference was discovered ($p$ = 0.701) between the two groups **(Table 7).** There were no significant differences between CML patients and controls when expression level (**Δ**CT) was categorized by gender and age categories.

**Table (7):** Expression level (ΔCT) of SLC25A3 gene in CML patients and controls. groups.

| Gene expression Level (ΔCT) | Patients (N. =80) (mean± SD) | Controls (N. =60) (mean± SD) | p-value |
|---|---|---|---|
| **SLC25A3** | 0.256±2.7 | 0.409±1.69 | 0.701 NS |

NS: Non-Significant.

**Gene Expression Folding ($2^{-\Delta\Delta Ct}$) of SLC25A3**

In this investigation, we discovered that the mean expression folding $2^{-\Delta\Delta Ct}$ of the SLC25A3 gene in CML patients was 4.589 ± 8.552 **(Table 8)** which indicates that SLC25A3 could serve as a candidate biomarker for CML.

**Table (8):** The SLC25A3 gene expression folding $2^{-\Delta\Delta Ct}$.

| Categories | No. of cases | Gene expression ($2^{-\Delta\Delta Ct}$) SLC25A3 (mean± SD) |
|---|---|---|
| CML | 80 | 4.589 ± 8.552 |

**Correlation of Gene Expression Folding $2^{-\Delta\Delta Ct}$ of SLC2 5A3 Gene with Age in CML Patients**

According to age, CML patients were split up into three groups in the current study.

- < 40 years (37 patients).
- 40 – 50 (27 patients).
- 50 years (16 patients).

In this investigation, we discovered that the mean expression folding $2^{-\Delta\Delta Ct}$ of the SLC25A3 gene in

CML patients was 5.135 ± 14.27 for ages under 40, 8.085 ± 17.97 for ages between 40 and 50, and 3.506 ± 8.08 for age over 50. Age group and SLC25A3 gene expression did not significantly correlate. According to **Table 9**, the correlation coefficients for the mean gene expression values of the SLC25A3 gene were 0.015, -0.231, and -0.043 for <40 years, 40-50 years, and >50 years, respectively. There was no discernible difference in terms of statistics.

**Table (9):** The SLC25A3 gene expression folding $2^{-\Delta\Delta Ct}$ correlation with age in the CML patients' group.

| Age categories | Number of cases (N=80) | Gene expression ($2^{-\Delta\Delta Ct}$) SLC25A3 (mean± SD) | r | p-value |
|---|---|---|---|---|
| <40 years | 37 | 5.135±14.27 | 0.015 | $0.932^{NS}$ |
| 40-50 years | 27 | 8.085±17.97 | -0.231 | $0.245^{NS}$ |
| >50 years | 16 | 3.506±8.08 | -0.043 | $0.874^{NS}$ |

NS: Non-Significant.

**Correlation of Gene Expression Folding $2^{-\Delta\Delta Ct}$ of SLC25A3 Gene with Gender in CML Patients**

As demonstrated in **Table 10,** there was no statistically significant difference in the mean gene expression folding $2^{-\Delta\Delta Ct}$ for the SLC25A3 gene in the female patient group and the male patient group (4.29±8.31 vs. 4.72±8.7; *p*=0.828, respectively).

**Table (10):** Correlation of The SLC25A3 gene expression folding $2^{-\Delta\Delta Ct}$ with gender in CML patients.

| $2^{-\Delta\Delta Ct}$ | Female group (N. =31) (mean± SD) | Male group (N. =49) (mean± SD) | p-value |
|---|---|---|---|
| SLC25A3 | 4.29 ± 8.31 | 4.72 ± 8.7 | 0.828 NS |

NS: Non-Significant.

## DISCUSSION

The high frequency of CML or the short life expectancy of elderly patients after diagnosis may be attributed to the low mean age at onset. Our findings support the study by **Ahmed *et al.*** which found that the mean age of CML patients in Iraq at diagnosis was 40.39 ± 14.30 which is younger than the globally reported age at initial diagnosis [(11)].

In Asia, as opposed to Western populations, a lower age of onset between 38 and 40 years has been noted. According to **Gorre *et al.*** CML is extremely common among people between the ages of 20 and 40 (58.8%, with a mean age of 30.74 ± 6.10) and >40 (33.8%, with a mean age of 50.73 ± 7.50) [(12)]. Altogether demonstrate that the majority of middle-aged individuals are more susceptible to getting CML. Additionally, a study by **Lin *et al***. demonstrated that the incidence and death of CML in men were higher globally than those in women [(13)], however, the findings from this study revealed that gender did not significantly affect incidence. The gender ratio (male to female ratio) in general observation differs throughout different geographic regions, as well as for various Asian countries, according to a study by **Gorre *et al.*** published in 2021 [(12)].

The predominance of men may be explained by their genetic makeup, innate immunological variations, hormone levels, potential occupational exposure to different types of radiation and toxins, nutrition, smoking, and drinking habits, as well as their genetics.

Inorganic phosphates are transported from the cytosol into the mitochondrial matrix via the mitochondrial phosphate carrier (PiC), which is encoded by the SLC25A3 gene. Copper availability and transportation can be regulated by phosphate levels. The overall amounts of mitochondrial copper are decreased by SLC25A3 deletion or depletion [(6)].

Cytochrome c is released from mitochondria via phosphate carriers, and its suppression had anti-apoptotic effects [(14,15)]. In mouse hearts, SLC25A3 gene knockout somewhat desensitized mPTP to calcium-induced apoptosis but did not entirely prevent mPTP opening [(16)], these findings imply that phosphate carriers, like AAC and ATP-Mg/phosphate carriers, may play a role in mPTP opening and, consequently, carcinogenesis. Phosphate carrier activity was found to be increased in rat liver carcinoma [(17)], and SLC25A3 associated with several other genes was found to be differentially expressed in the early and late chronic phase of chronic myeloid leukemia [(18)]. Because there are currently few clinical markers and no molecular tests that can predict an individual patient's clock for the progression of CML at the time of chronic phase diagnosis, it is challenging to tailor treatment to each patient's risk level [(19)].

The usual treatment for chronic phase chronic myeloid leukemia is a tyrosine kinase inhibitor, however, existing clinical scoring systems are unable to reliably predict the wide range of patient outcomes. To enhance care and make it easier for patients to join in clinical studies aimed at molecular level depth for blast crisis prevention and response improvement, biomarkers that can separate individuals based on the outcome at diagnosis are required. For clinical, biological, and technical reasons, it has been difficult to identify a small and manageable gene set that accurately predicts prognosis in the majority of CML patients [(20)]. The goal of this CML research will be the correlation of gene expression-based biomarkers with CML patients. The variability seen in our results considering expression levels of SLC25A3 (ΔCT) could be explained by what is typical of most carriers, which is that they exhibit both elevated and decreased transcriptional expression in a range of malignancies [(21)]. We found that the fold changes of gene expression ($2^{-\Delta\Delta Ct}$) were greater than 1 which implicate that the SLC25A3 gene could be employed as a marker for CML. The simultaneous targeting of numerous carriers

offers a promising strategy that could considerably improve the efficacy and specificity of future cancer therapeutics, however, there is currently no research looking into the synergistic effects of different mitochondrial transporters.

Age is a significant risk factor for the emergence of numerous diseases, identification of genes whose expression changes with age has been important in discovering pathways whose behavior is altered by age, as well as in identifying aging biomarkers and therapeutic targets [22]. These alterations may affect the degree to which genes are expressed, the splicing of the mRNA that is produced, or the genetic regulation of gene expression, several human genes have shown age-related changes in the heterogeneity of gene expression between people [23].

In a recent study, immunological T-cells from young and elderly people were compared, unstimulated cells showed no difference in cell-to-cell variability, but when the immune system was activated, the older cells showed increased heterogeneity [24]. Our findings support the idea that greater variability in gene expression patterns is not always related to age. According to Medawar's theory, the power of purifying selection is stronger on genes expressed early in life compared to later in life, yet several highly proliferative tissues show the opposite tendency. High levels of malignancy and somatic mutations linked to the age of expression are present in these non-Medawarian tissues. Genes that are under genetic control, however, are subject to more lenient restrictions [25].

In **Whitney *et al.*** analysis of multiple sequential samples from the same individuals to identify donor-specific patterns of gene expression, a surprising and intriguing finding was the obvious negative association between Ig gene expression and donor age [26], which is consistent with the idea that age and genetic expression aren't always related. Furthermore, a study by **Yamamoto *et al.*** divided participants into six age bins ranging from 20 to 80 years old and compared the mean difference in gene expression distances between elderly and young people as well as the slope of the inter-individual JSD, they identified tissues with higher variability in both young and old populations with remarkably similar results, Pearson's R= 0.8 [25]. Thus, aging does not always lead to increasing variability in gene expression patterns, contrary to the initial hypothesis.

Human physiology, anatomy, and illness susceptibility vary significantly depending on gender. Disease characteristics including prevalence, progression, age of onset, and treatment response frequently vary by sex, and many immunological and inflammatory illnesses exhibit a startling gender bias in incidence and severity [27,28]. Different factors, including hormones, sex chromosomes, genotype-sex effects, behavioral differences, and environmental exposures, have been suggested as the causes of these sex disparities [29]. Although the vast majority of prevalent diseases show obvious sex differences in symptoms or prevalence, sex is typically neglected or improperly taken into account in studies [30].

Although differences in cancer incidence, susceptibility, and prognosis have been extensively characterized, it is unclear why these differences take place at the genetic level. Numerous malignancies exhibit differences in incidence and mortality rates linked to sex-specific disparities, some of these differences may be caused by various levels of hormones, particularly estrogen, or sexual chromosomal dosage [31]. With a few exceptions, such as thyroid cancer and lung cancer in nonsmokers, malignancies of the larynx, esophagus, and bladder, which have a male preponderance of 2:1 and 4:1 respectively, and typically have a bad prognosis, have been seen to occur more frequently in men than in women [32,33]. Mechanistic mathematical models of signaling activity were employed by **Çubuk *et al.*** to ascertain how gender-specific gene expression activities affect cell functionality and fate. In addition, the mechanistic modeling framework was used to mimic medication interventions and investigate how gender-specific variable gene expression affects drug action mechanisms, they found that some cancers, such as glioblastoma or rectum cancer, are almost insensitive to these gender-specific traits [34], as we discovered in our investigation into CML.

## CONCLUSION

We concluded that the mean ΔCT of the SLC25A3 gene was variable when compared between patients and control (0.256±2.7, 0.409±1.69; $p = 0.701^{NS}$), but the mean for gene expression folding $2^{-\Delta\Delta Ct}$ was greater than 1 in CML patients (4.589± 8.552) which indicates that the gene could be implicated as a marker for diagnosis or targeted for therapy in CML. Furthermore, we found that the gene expression wasn't statistically correlated with differences in age groups or gender of patients. We suggest further research into a subset of mitochondrial solute carrier genes in synergisty and studying its relationship with CML. Finally, patients with CML were seen to have an average age and median age at onset of 36.34 years and 35 years, respectively. These values were lower than the ones internationally reported.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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