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Evaluation of Gene Expression of *AQP-1* Gene in Chronic Myeloid Leukemia of Iraqi Patients

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Abstract: Chronic myeloid leukemia is a type of cancer characterized by myeloproliferative disorder in the hemopoietic stem cell (HSC) compartment. It is characterized by the overproduction of myeloid cells which are a type of white blood cell. This study aimed to evaluate the expression levels of the aquaporin-1 (*AQP1*) gene in Iraqi patients diagnosed with chronic myeloid leukemia. The study was conducted on 40 subjects, aged 8 to 63 years. Blood samples were collected from July/2024 to October/2024 in Baghdad, divided into 20 patients with CML (12 females, 8 males) and 20 healthy controls (12 females, 8 males) attending the Baghdad Private Lab. *AQP1* genes were assessed using qRT-PCR. *AQP-1* gene expression revealed that the outcome of the fold value in patients was 28.2 compared to the control 24.1, and the relative expression (2-ΔΔ Ct) for the same gene was decreased (downregulation), which was 0.80. These results suggest that reduced expression of the *AQP1* gene in chronic myeloid leukemia patients could have implications for fluid regulation, kidney function, and disease management. Further studies are needed to clarify the mechanisms underlying decreased *AQP1* expression in leukemia and its clinical significance

Keywords: Membrane protein, leukemia, *AQP1*, *aqp1* gene, chronic myeloid leukemia.
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Introduction

Chronic myeloid leukemia is a myeloproliferative disorder in the hemopoietic stem cell (HSC) compartment (1). This disease is characterized by a reciprocal t (9;22) chromosomal translocation, resulting in the formation of the Philadelphia (Ph) chromosome containing the BCR-ABL1 gene (2, 3). AQPs are a family of 12 currently described proteins that act as channels and have been classified into three groups, the first is classical, which acts to allow the passive transport of water; the second is the aquaglyceroporins, which in addition to water also facilitate the transport of small uncharged solutes, such as glycerol, ammonia, and urea; and the third was unorthodox, which have been more recently discovered and whose function is not fully understood (4).

Aquaporin-1 (*AQP1*) is a channel-forming integral membrane protein found in all living organisms from bacteria to humans and some viruses (5). AQPs mainly facilitate the transmembrane diffusion of water and various small solutes are involved in cellular trafficking and many physiological processes. Their primary role is to allow water to flow rapidly into and out of cells, surpassing the relatively slow process of simple diffusion through the lipid bilayer (6).

This protein was the first to be measured, and a high-resolution structure was determined. Studies have identified a clear gating mechanism of action of *AQP1* and that alteration of osmotic conditions could induce a reversible protein kinase C (PKC) dependent change in the membrane localization of *AQP1*, which suggests a regulatory mechanism by trafficking (7) the protein is found in many different tissues in the body, including red blood cells, kidneys, and lungs. Mice and humans lacking *AQP1* have been shown to have urinary concentration deficiency during water deprivation (8, 5).

Aquaporins are alike in basic structure, with monomers containing six transmembranes and two short helical segments that enclose cytoplasmic and extracellular vestibules linked by aqueous pores. They have several conserved motifs in their short helical segments as well as NPA sequences (9).

Growing data suggest their possible involvement in cell volume regulating events associated with various non-infectious diseases. Consequently, AQPs have become a potential drug target in clinical medicine (9). In addition to the above expression studies, recent molecular and biochemical studies have alluded to the role of AQPs in human carcinogenesis. *AQP1* is shown to play a role both in angiogenesis and cell cycle control, assisting cancer development (10).

There are many studies in Iraq to predictive biomarker for CML progression and development (11,12)respectively.

Little is known about the role of the AQP gene in CML. This study aimed to determine the gene expression of *AQP-1* in a sample of Iraqi patients with CML analyzed according to the clinical baseline and laboratory data to find their role in disease severity. This investigation may clarify the functions of the AQP gene in the CML pathophysiology.

Materials and Methods

This case-control study was conducted between July 2024 and October 2024, to investigate the potential association between *AQP-1* gene expressions. Participants were recruited from a private hospital in Baghdad. A total of 40 participants were included in the study, consisting of 20 subject cases with CML, along with 20 healthy controls denoted as Control (CO) for brevity. Also, they were matched as possible. The patient and health were evaluated under the supervision of a hematologist. Consent was obtained, and participants provided information about their family medical history, outlining risks and general information.

Sample Collection

Venous blood samples (5mL) were collected from (20) diagnosed CML patients and (20) control subjects after obtaining a comprehensive medical history, their ages ranged from 8-63 years. About 250µl of EDTA-blood was transferred in 500µl of TRIzol mix well and stored at -20Ċ. The blood was used for RNA extraction to detect gene expression of the AQP-1 gene.

Outcome Measurement

Genomic DNA was eliminated using DNase (Promega were total RNA was isolated from sorted cells using TRIzol reagent (Invitrogen) per the manufacturer's instructions. The purity and concentration of RNA were quantified using a Quibt 4 (ThermoFisher Scientific/USA). The reverse transcription of RNA (1 µg/sample) was performed using an iScript cDNA Synthesis kit (ThermoFisher/USA) per the manufacturer's instructions. Quantitative reverse transcription

polymerase chain reaction (qRT-PCR) was conducted using SYBR Green Real-Time PCR Master Mixes (NEB/UK) and a StepOnePlus Real-Time PCR System (Applied Biosystems, USA). The results were normalized with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and fold changes were studied with the formula $2^{-\Delta\Delta CT}$. The primer sequences used for qRT-PCR are recorded as follows (from the 5′ end to 3′ end): Depending on NCBI, specific oligonucleotide primers were designed by Geneious prime software, Table 1.

Table (1): Primers involved in Real-Time-Quantitative PCR

Primers	Primer Sequence 5`-3`
AQP-1 gene	Forward- CCTGGCTGATGGTGTGAACTC
	Reverse- TGTCCAAGGGCTACAGAGAGG
HAGPD (Housekeeping genes)	Forward- TCAAGGCTGAGAACGGGAAGCT
	Reverse- CTGCAAATGAGCCCCAGCCTT

Statistical Analysis

The data were analyzed using Microsoft Excel and IBM SPSS V26. The results reported in this study were expressed as mean± SD. Independent t-tests were used to test between sexes, and one-way ANOVA was used to test between study groups. Probability values less than 0.05 and 0.01 were considered significant and highly significant differences, respectively.

Results and discussions

Chronic myeloid leukemia disease affects all ages; here, the youngest patient was eight years old, while the oldest was 63. de la Fuente et al., (2014) (13) found that chronic myeloid leukemia in children and young people is a relatively rare form of leukemia that shows increased incidence with age. Some evidence suggests that the molecular basis differs from that in adults.

The randomly collected leukemia group included 20 patients, of whom 27 % were males and 73% were females. The mean age for the patient group was 24.26 years. The control group comprised 20 participants, of whom 73 % were females and 27 % were males, its mean age was 25.71 years.

Based on the findings, the total occurrence of CML was significantly greater in females than males. The results of this study were in disagreement with Radivoyevitch et al., (2014) (14) as they proposed that males have an elevated risk of developing CML or a shorter latency from initiation to diagnosis of CML. Also, in a series of diagnosed CML patients reported by Rohrbacher and Hasford, (2009)) (15), they found that the proportion of Philadelphia (Ph)/BCR-ABL-positive chronic myeloid leukemia (CML), was more frequent in males than in females.

Determination of *AQP-1* gene in the blood of groups' specimens

Quantitative RT-PCR was used to determine the expression of *AQP-1* gene in different experimental category. SYBER green dye was used in this experiment as an indicator of gene expression that emits green light as an indication of its binding to cDNA. The emission was measured after each qPCR cycle and the amplification results of each cycle were called CT (cycling threshold). The expression of genes was normalized to the scale of housekeeping gene GAPDH and quantified by the (Δ Ct) value and folding ($2^{(-\Delta\Delta Ct)}$).

Table 2 below shows the CT of *AQP1* gene descriptive statistics like

median and Percentiles, the p-value for compassion between studied groups shows a statistically significant difference < 0.01.

Table (2): Illustrates the descriptive statistics value of *AQP-1* gene CT in patient and control.

	Leukemia	Control	P Value
Mean	28.2	24.1	<0.01
SD	6.52	5.45	
Median	26.7	22.4	
75 % Percentiles	34.5	27.7	
25 % Percentiles	22.2	19.4	

The result in Table 3 shows the lowest level of CT of GAPDH expressed by the control while the Leukemia patients group showed the highest and were significantly different (P< 0.008).

Table (3): The significant difference between CT of GAPDH of Leukemia patients and control

	Leukemia	Control	P value
Mean	23.3	20.8	< 0.008
SD	5.08	4.62	
Median	20.9	19.2	
75 % Percentiles	27.2	23.7	
25 % Percentiles	18.6	16.7	

Table 4 below Illustrates that there are statistically high significant differences between the studied groups < 0.001 and the statistically significant difference was presented between the levels of the median of Leukemia as well as Leukemia vs. Control.

Table (4): The statistically significant difference between levels of the median of Leukemia and *vs.* control

	Leukemia	Control	P Value
mean	4.7	3.3	< 0.001
SD	2.5	1.4	
Median	4.2	3.2	
75 % Percentiles	6.8	4.0	
25 % Percentiles	3.0	2.3	

As shown below in table 5. The descriptive statistics of ΔΔCt of Leukemia patients group

Table (5): Illustrates comparison of ΔΔCt between control and Leukemia patient

	Leukemia	Control
mean	0.80	N/A
SD	1.15	
Median	0.90	
75 % Percentiles	1.66	
25 % Percentiles	0.07	

The comparison of folding between control and Leukemia subjects showed a statistically significant difference, Table 6.

Table (6): Illustrates a comparison of folding between control and Leukemia

	Leukemia	Control	P value
Mean	0.85	1	< 0.001
SD	1.33	0.4	
Median	0.92	1	
75 % Percentiles	3	0.7	
25 % Percentiles	0.47	0.2	

Based on the equation of Livak and Schmittgen (2001) (16) the results of the sample were analyzed and the expression fold of the aquaporin gene was determined for each sample. The table above shows that the Δ Ct value was 2.4 after comparing with the corresponding ΔCt value. Simultaneously an increase in the relative expression ($2^{-\Delta\Delta Ct}$) of the same gene amounted to 4. The amplification plot and the melting curve for the aqp-1 gene for patients and control subjects using RT-q PCR were positive as shown in figure (1).
And figure (2).

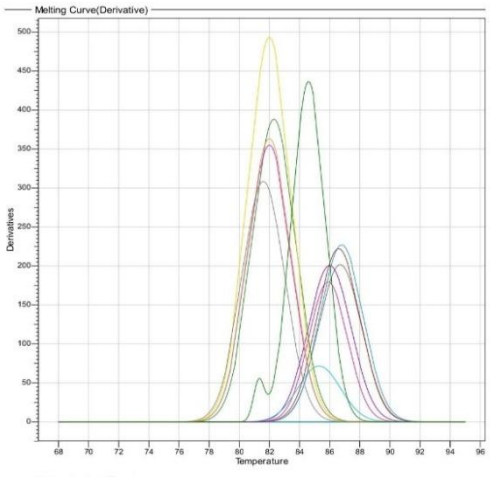

Figure (1): Amplification curves of aqp-1 gene of different subjects of patient groups which was determined by RT-q PCR. Each curve represents separate specimens (patients and control) that were positive and (GAPDH) housekeeping genes.

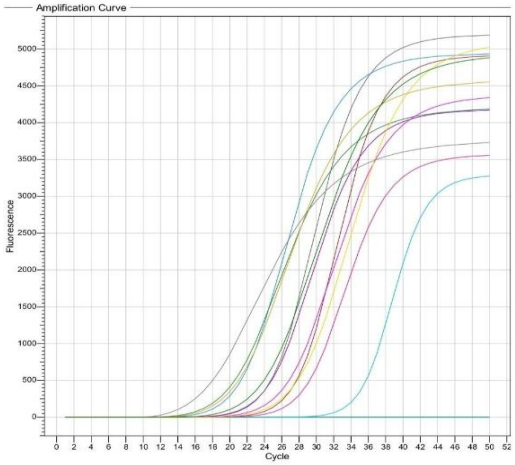

Figure (2): Melt Curves of aqp-1 gene of different subjects of patient groups which was determined by RT-q PCR.

In leukemia, particularly acute myeloid leukemia (AML), altered AQP1 expression may influence the proliferation and survival of leukemic cells. The down-regulation of AQP1 could affect cell migration, invasion, and apoptosis, potentially leading to more aggressive disease phenotypes as Yin et al., (2020) (17) mentioned.

Accumulating evidence elucidate that the induction of epithelial-mesenchymal transition (EMT) and aberrant expression of microRNAs (miRNAs) are associated with

tumorigenesis, tumor progression, metastasis, and relapse in cancers, including chronic myeloid leukemia (CML) (Xishan et al., 2015) (18). They institute that miR-320a expression was reduced in K562 and CML cancer stem cells. The expression of mesenchymal markers in miR-320a-expressing cells returned to normal levels by the renovation of BCR/ABL expression. Thus, miR-320a acts as a novel tumor suppressor gene in CML and miR-320a can decrease migratory, invasive, proliferative, and apoptotic behaviors, as well as CML EMT, by attenuating the expression of BCR/ABL oncogene

Allegra et al., (2022) (19) reported that in several conditions such as cancer tumor progression might be develop by aquaporins in modifying tumor angiogenesis, cell volume adaptation, proteases activity, cell-matrix adhesions, actin cytoskeleton, epithelial-mesenchymal transitions, and acting on several signaling pathways aperient cancer progression. Close connections have also been identified between the aquaporins and hematological malignancies. However, it is difficult to recognize a unique action exerted by aquaporins in different hemopathies, and each aquaporin has specific action that vary according to the class of aquaporin examined and the different neoplastic cells. However, the expression of aquaporins is altered in cell cultures and patients with acute and chronic myeloid leukemia, lymphoproliferative diseases, and multiple myeloma, and seems to be able to impact the efficacy of treatment and could have a prognostic significance, as greater expression of aquaporins is correlated to progress overall survival in leukemia patients.

On the other hand, Aquaporins (AQPs) have previously been associated with increased expression in solid tumors. However, their expression in hematologic malignancies, including CML, has not been described yet (20).

Meanwhile, Wei et al., (2015) (21) found the over-expression of aquaporin-1 modify erythroid gene expression in human erythroleukemia K562 cells. AQP1 over-expression effectively inhibited cell proliferation and induced cell growth arrest in the G1 phase of K562 cells. Significant enrichment of genes involved in “oxygen transporter activity” including hemoglobins (HBD, HBG, HBB, HBE1, and HBQ1), HEMGN, and, the silencing of HEMGN by RNA interference in K562-AQP1 cells resulted in the down-regulation of these genes (22). hypothesized that the gene expression is due to hypoxia conditions that can lead to changes in gene expression patterns, including down-regulation of AQP1. Hypoxia-inducible factors that are stabilized under low oxygen conditions may not favor the expression of AQP1 leading to reduced levels of this protein (23).

Additionally, Wang and Owler, (2011) (24) were researched to determine the expression of AQP1, AQP4 in pediatric brain tumors. Twenty tumor bank specimens were used, the expression of AQP1 and 4, and they found that some brain tumors expressed high levels of AQP1 and 4 but had a more variable pattern of staining. AQP1 and 4 have relevance to pediatric brain tumors and are worthy of further investigation in developing potential therapeutic strategies.

CML is characterized by the Philadelphia chromosome (Ph chromosome) (25) suggested that chromosomal abnormalities can affect gene expression. These alterations may directly impact the regulatory elements controlling AQP1 transcription, resulting in decreased expression. For instance, mutations in transcription

factors or epigenetic modifications could silence the AQP1 gene (26) suggested that the suppressing pathways of the BCR-ABL fusion gene would normally maintain AQP1 expression, while the mechanisms behind AQP1 down-regulation in CML are not fully understood.

Conclusion

The current study suggests that the downregulation of the *AQP1* gene in chronic myeloid leukemia (CML) patients may play a significant role in the pathophysiology of the disease. Reduced expression of *AQP1* could impact cellular water transport and contribute to the altered microenvironment observed in CML. This downregulation may also influence tumor cell proliferation, survival, and response to therapy, highlighting the potential importance of *AQP1* as a biomarker or therapeutic target.

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Approval

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