

Evaluation of PTEN Gene Expression and its Association with Treatment Response and Hematological Parameters in Adult Patients with Acute Myeloid Leukemia

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ABSTRACT

Background: Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by the uncontrolled proliferation of immature myeloid cells in the bone marrow (BM) and peripheral blood. The phosphatase and tensin homolog (PTEN) gene plays a crucial role in tumor suppression by regulating apoptosis and cell survival through inhibition of the PI3K/AKT signaling pathway. Dysregulation of this pathway has been implicated in leukemogenesis and treatment resistance. **Objective:** This study aimed to evaluate PTEN gene expression in newly diagnosed AML patients and assess its association with hematological parameters, treatment response, and short-term survival. **Methods:** A cross-sectional study was conducted on 63 de novo AML patients before initiation of chemotherapy and 15 healthy individuals as a control group. RNA was extracted from peripheral blood samples, and PTEN gene expression was quantified using reverse transcriptase polymerase chain reaction (RT-PCR). Hematological parameters were analyzed, and correlations with treatment outcomes, including complete remission (CR) and non-remission (NR), as well as six-month survival status, were assessed. **Results:** There was no significant difference in PTEN gene expression between AML patients and healthy controls ($p = 0.625$). Additionally, PTEN expression levels were not significantly associated with remission status ($p = 0.328$) or survival outcomes after six months ($p = 0.994$). A negative correlation was observed between PTEN expression and patient age; however, no significant associations were found between PTEN expression and sex or hematological parameters. **Conclusion:** The findings indicate that PTEN gene expression does not show significant alterations in AML patients compared to healthy controls and is not a predictive marker for remission or short-term survival. Further studies with larger cohorts and longer follow-up periods are required to determine the potential role of PTEN expression in AML pathogenesis and treatment resistance

Keywords: AML, PTEN, RT-PCR, Gene expression.

INTRODUCTION

Acute myeloid leukemia is a highly aggressive malignancy characterized by the fast multiplication of immature cells in the myeloid line in the bone marrow. The disease may be exhibited in peripheral blood and as an extramedullary disease [1]. Diagnosis of AML includes peripheral blood and bone marrow smear reviews, flow cytometric assessment, BMA, and BMB, and extensive cytogenetic and molecular genetic study when called for [2, 3].

This type of signaling regulates apoptosis by activating the PI3K/AKT. Normally, the signaling pathway is triggered by the receptor of the epidermal growth factors. The mechanism is the pathway's signaling and regulation. The protein p53 is the role model of this signaling pathway. The pathway regulates the cell's response to apoptosis. The pathway's regulation is critical, with p53 playing a pivotal role in triggering cellular apoptosis in response to DNA damage [4].

Although PTEN anomalies are implicated in various cancers, their function in AML is to regulate a myriad of cellular processes including genomic stability, cell survival, migration, proliferation, and metabolism through its enzyme-dependent and independent (scaffold) mechanisms[5]. Compared to solid tumors, alterations in PTEN are less frequently observed in hematologic malignancies [6]. This study aimed to investigate the PTEN gene expression in newly diagnosed de novo AML patients to demonstrate its prognostic value and its relation to laboratory findings at diagnosis.

PATIENTS, MATERIALS, AND METHODS

This cross-sectional study was performed on 63 newly diagnosed de novo AML patients who were sequentially selected. Fifteen age- and sex-matched healthy individuals with no history of chronic inflammatory diseases or hematologic disorders were enrolled to serve as a control group. Patients under 18, those with secondary AML, AML-M3, other malignancies, pregnant or lactating women, and those with comorbid diseases were all excluded. Patients were assessed for response to remission induction therapy after 1 month of starting chemotherapy by assessment of complete blood count (CBC), PB, and BMA blast percentage.

Patients were divided into two groups: the complete remission (CR) group, which included those who achieved CR (BM blast count $<5\%$, no circulating blast cells, absence of extramedullary disease, absolute neutrophil count (ANC) $\geq 1.0 \times 10^9/L$, platelet count (PLT) $\geq 100 \times 10^9/L$) or complete remission with incomplete hematological recovery (CRi) (meeting all CR criteria except ANC $< 1.0 \times 10^9/L$ or PLT count $< 100 \times 10^9/L$), and the no response (NR) group [7]. Patients were followed up for six months to document the survival status. All subjects involved in this study were informed, and their consent was obtained verbally before sample collection. The research was approved by the Research Ethics Committee in the College of Medicine, University of Baghdad (issue no. 144, dated 4 Oct 2022). The patient history was reported and clinical examination was performed.

Assessment of the patient's medical records was conducted, focusing on the diagnosis, therapy. The results of the patient's CBC, blood films, BMA and flowcytometry analysis were collected from the patients' records. PB mononuclear cells (PBMcs) were isolated from EDTA-anticoagulated blood by Lymphocyte Separation Media, Catalog no. LSM-B (Capricorn, Germany). The separation solution contains Ficoll™ density gradient media. After carefully layering two milliliters of blood and two milliliters of phosphate-buffered saline (PBS) over the 3 milliliters of media, the mixture was centrifuged at 400 g for 30 minutes. PBS was used twice to wash the separated PBMcs. The isolated PBMcs were added to a sufficient amount of Trizol (TRI) reagent (ratio 1:3) and mixed thoroughly for 5 minutes to ensure complete lysis and homogenization of a sample to be used in the next RNA isolation. The RNA was extracted using the Direct-zol™ RNA MiniPrep kit (Catalog no. R2051, Zymo Research, USA) according to the directions provided by the manufacturer. A Nanodrop (Nabi, South Korea) was used to measure and confirm the concentration and purity of the extracted RNA by assessing the ultraviolet absorbance at wavelengths of 260 and 280 nm. Then the extracted RNA was converted to complementary DNA (cDNA) using Prime Script™ RT reagent Kit (Perfect Real Time, Catalog No. RR037A, TaKaRa, Japan) according to the manufacturer's guidelines.

The reaction mixture (2 µL of PrimeScript mix and 8 µL of RNA) was incubated at 37°C for 15 minutes (reverse transcription), then at 85°C for 5 seconds (Inactivation of reverse transcriptase with heat treatment), then the cDNA was cooled to 4°C until it was stored at -80°C. Using the GoTaq® qPCR Master Mix Kit (Catalog no. A6001, Promega, USA), the generated cDNA was submitted to quantitative real-time polymerase chain reaction (qRT-PCR) for gene expression analysis. A reaction mixture was prepared with a final volume of 20 µL that contained 1 µL of each forward and reverse primer, 5 µL of nuclease-free water, 3 µL of cDNA, and 10 µL of the master mix. The reaction mixture was then added to each PCR tube or well of an optical-grade PCR plate with thermal cycling protocol as follows: One cycle at 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, 40 cycles at 60°C for 1 minute, and 100 cycles at 90°C for 15 seconds. Then, using a computer connected to the RT-PCR device, the required software was started. The primer sequences of the PTEN and the GAPDH (the reference internal control) genes were: Forward (5'-CACACGACGGGAAGACAAGTTC -3'), Reverse (5'-TCCTCTGGTCCTGGTATGAAGAATG-3') and Forward (5'-AATGGGCAGCCGTTAGGAAA -3'), and Reverse (5'-GCGCCCAATACGACCAAATC -3'), respectively. Using the comparative CT method and the GAPDH gene as a housekeeping gene, Real Time PCR was performed on the patients prior to treatment as well as the

control group to ascertain the expression of the PTEN gene. The relative amount of PTEN normalized to GAPDH was calculated using the following equation;

$$\text{Fold change} = 2^{-\Delta\Delta CT} [8]$$

Where

$$\Delta\Delta CT = (CT_{\text{target gene}} - CT_{\text{GAPDH}})_{\text{patient}} - (CT_{\text{target gene}} - CT_{\text{GAPDH}})_{\text{control}}$$

Statistical Analysis

The detailed description for each variable was made on the Statistical Package for Social Sciences (SPSS) version 26. The data was represented using the mean, standard deviation, and frequency (percent) according to the type of variable. Mann-Whitney U test was used to evaluate the difference between two independent continuous non-normally distributed variables. The Spearman correlation was used to evaluate the relationship between continuous variables that do not follow a normal distribution. A P-value equal to 0.05 or below, with a confidence level of 95%, was deemed significant.

Results

The mean age (±SD) of AML patients was 50 ± 19 years (range 18 – 80 years). The male to female ratio was 1: 2.15. The means of white blood cells (WBC), hemoglobin (Hb), PLT, ANC, PB blast and BM blast were 37.7±50.2 ×10⁹/L, 8.1±2.1 g/dL, 73.4±66.7 ×10⁹/L, 4.5±7 ×10⁹/L, 42.1±30%, and 53.1±29.2%, respectively. The mean (±SD) levels of PTEN gene expression in AML patients and control groups were 3.84558 ± 6.61759, and 1.63019 ± 1.64494, respectively. As a result, the PTEN expression level in the control group is 1.6; any value above the control level indicates overexpression, whereas values below the control level indicates downregulation.

PTEN was down-regulated in 36 (57.1%) and up-regulated in 27 (42.9%) patients. "An insignificant increase in PTEN gene expression in AML patients was observed in comparison with the control group (p= 0.625), Table 1. PTEN gene expression showed no significant association with sex (p= 0.126)". After 28 days of chemotherapy, the therapeutic response was CR in 20 patients (31.7%), NR in 32 patients (50.8%), death during treatment in 8 patients (12.7%), and death prior to treatment in 3 patients (4.8%). No statistically significant difference was found in means of PTEN gene expression between AML patients with CR and NR (p= 0.328). After six months of follow-up, 36 patients (57.1%) were still alive, while 27 (42.9%) had died. there was no statistically significant difference between survival status in AML patients and PTEN gene expression in both alive and deceased patients (p= 0.994).

Table 1: Association of PTEN Gene Expression in AML Patients with Sex, Remission and Survival Status.

Characteristics	PTEN Gene expression (Mean ± SD)	P value*
AML patients (N= 63)	3.84558 ± 6.61759	0.625
Control (N= 15)	1.63019 ± 1.64494	
Males (N= 20)	5.52677 ± 9.09950	0.126
Females (N= 43)	3.06363 ± 5.02162	
Complete remission (N= 20) **	3.61653 ± 8.41028	0.328
No response (N= 32) **	3.65844 ± 5.53585	
Alive (N= 36)	4.01776 ± 7.28747	0.994
Deceased (N= 27)	3.61602 ± 5.73079	

*Mann-Whitney U test. **11 patients who passed away before or during treatment are not included in the assessment of remission status because they did not receive or finish the induction chemotherapy.

There was negative statistically insignificant correlation of PTEN expression with age, Hb and PLT in 63 AML patients (Table 2), and

positive statistically insignificant correlation of PTEN expression with WBC, ANC, PB blast percentage and BM blast percentage ($P > 0.05$).

Table 2: Spearman Correlation between PTEN Gene Expression with Age and the Hematological Parameters in AML Patients.

Variable	PTEN Gene expression	
Age	r	-0.167
	p	0.190
WBC	r	0.135
	p	-0.292
Hb	r	-0.088
	p	0.494
PLT	r	-0.169
	p	0.185
ANC	r	0.140
	p	0.275
PB blast %	r	0.221
	p	0.082
BM blast %	r	0.124
	p	0.333

Discussion

The mean age of AML patients was comparable to other Iraqi studies [9, 10], and with British and Egyptian studies [11, 12], respectively. At presentation the mean of WBC count in AML patients was parallel to other Iraqi studies [9, 13, 14], in which the means of WBC count were $35.9 \pm 23.2 \times 10^9/L$, $35.7 \pm 43.1 \times 10^9/L$, $36.4 \pm 58.7 \times 10^9/L$, respectively, in addition, the results of other Egyptian study [15] was also close to our result with a mean of $37.19 \pm 45.6 \times 10^9/L$. The mean Hb level was analogous with other Iraqi studies [13, 16, 17], in which the means of Hb were 8.06 ± 1.9 g/dL, 7.9 ± 2.09 g/dL, and 7.8 ± 1.65 g/dL, respectively, in addition to another non-local study [18], in which the mean of Hb was 8.01 ± 2.04 g/dL. The mean of PLT in this study was concurrent with other studies in [13, 14], in which the means of PLT were $72.36 \pm 52.46 \times 10^9/L$, $76.1 \pm 92.1 \times 10^9/L$, respectively. The mean of PB blast is close to that of Al-Khawaja, et al. and Ghosh, et al. in which they found PB blast to be 45.5%, and 41.4%, respectively [19, 20]. While the mean of BM blast was comparable to other studies [16, 20], that reported the means of BM blast 55.9% and 57.6%, respectively. This study evaluated the expression of the PTEN gene in patients who newly diagnosed with de novo AML employing a real-time qRT-PCR as a quantitative technique, compared to a control group. Several studies have employed immunohistochemical assays utilizing antibodies to identify protein molecules in both solid and hematological malignancies [21, 22]. This approach offers a distinct benefit compared to the semi-quantitative IHC test. Therefore, it can serve as an alternate and complementary method for assessing the biomolecular status [23, 24]. PTEN gene expression was down-regulated in 57.1% and up-regulated in 42.9% of AML patients. However, these findings indicate that there was no significant difference in PTEN gene expression in AML patients as compared to the control group. In contrast to this finding, other studies in China (in which both IHC and qPCR methods were used to detect PTEN gene expression in BM samples of 59 children with AML) [22], Egypt [4] and Iran [25], which used PCR technique, reported a significantly decreased of PTEN gene expression in AML patients when compared to

the control group. A local study used the qRT-PCR to detect the expression of PTEN gene from blood samples in patients with breast and thyroid cancer reported high expression of PTEN in both types of cancer [26]. "In addition, another Iraqi study in which they found significant increase in PTEN gene expression among patients diagnosed with endometrial carcinoma who were residing in regions with elevated levels of depleted uranium exposure, which are considered high-risk places" [27]. Also, several non-local studies have revealed an up-regulation of PTEN gene expression in breast cancer in Denmark [28], Iran [23, 29], and Egypt [30]. No other Iraqi studies have been found investigating the expression of PTEN in AML patients.

Our findings contradict the mechanical function of the PTEN gene by indicating its overexpression in AML. Further studies are necessary to comprehend this disparity. However, there was no significant correlation between the level of PTEN gene expression and age or hematological parameters and no significant association with sex, these findings agreed with Zayed et al. study [31]. Also, Esmaeili et al. [25] did not find any significant correlation between PTEN gene expression and blast percentage. Furthermore, no significant correlation was observed between the degree of PTEN gene expression and both the clinical response to the initial cycle of induction chemotherapy and survival status. These findings are consistent with Zayed et al., 2017 [31], and Yang et al., 2007 [32] studies. In contrast, Sadek et al. [4] found a significant relationship between PTEN gene expression and one-year OS. It is necessary to conduct long-term follow-up studies to examine the potential association between the level of PTEN expression in AML and the likelihood of relapse as well as the total duration of patient survival.

Conclusions

There was no significant association between PTEN gene expression in AML patients and remission status or with alive or deceased status after 6 months of follow-up. Contrary to the expectation, there was no observed increase in the expression levels of the PTEN gene.

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