RESEARCH ARTICLE

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Telomerase Gene Expression in Relation to Serum Protein and Hematological Parameters in Acute Myeloid Leukemia Patients

Yusur Ridha Alnaqashli*, Hameed Majeed Jasim

Abstract

Background and Objective: Acute myeloid leukemia (AML) is a hematological malignancy marked by the abnormal proliferation of myeloid precursor cells (blasts) in the bone marrow and peripheral blood, leading to disrupted blood cell production. The telomerase reverse transcriptase (*hTERT*), a key component of the telomerase enzyme, is often overexpressed in various cancers, including AML, contributing to cellular immortality. This study aimed to investigate the expression levels of the *hTERT* gene, serum protein concentrations, and hematological parameters in newly diagnosed AML patients, comparing these findings to AML patients in remission and healthy controls. **Methods:** Blood samples were collected from three groups: 10 newly diagnosed AML patients, 35 AML patients in remission, and 40 healthy controls. Hematological parameters, including white blood cell (WBC), red blood cell (RBC), platelet (PLT), and hemoglobin (Hb) levels, were measured. Serum *hTERT* protein concentrations were analyzed using enzyme-linked immunosorbent assay (ELISA), and *hTERT* gene expression was assessed through reverse transcription-quantitative PCR (RT-qPCR). **Result:** The study demonstrated that newly diagnosed AML patients had significantly higher *hTERT* gene expression and serum protein levels compared to both remission patients and healthy individuals. Hematological analyses revealed elevated WBC counts alongside reduced RBC, PLT, and Hb levels in AML patients relative to controls. **Conclusion:** Increased *hTERT* expression and serum protein levels are valuable biomarkers for diagnosing and monitoring AML. These findings highlight the therapeutic potential of targeting *hTERT* and underscore the importance of conducting further studies on larger patient cohorts to validate these results.

Keywords: Acute myeloid leukemia (AML)- *hTERT* gene expression- Hematological parameters- Genetic biomarkers

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Introduction

Acute myeloid leukemia (AML) is a hematological malignancy characterized by the proliferation of malignant myeloid cells in the bone marrow and peripheral blood. It is the most common form of leukemia in adults and the second most common in children. AML is defined by the rapid expansion of myeloid blasts, which disrupt the normal production of blood cells. This disease is associated with a high mortality rate and poses significant treatment challenges, particularly in elderly patients [1]. AML arises from the genetic and epigenetic transformation of undifferentiated myeloid precursor cells, leading to uncontrolled proliferation, impaired differentiation, defective maturation, and disruption of apoptotic and cell cycle regulatory mechanisms. These changes result in ineffective hematopoiesis, failure of the bone marrow, and compromised production of red blood cells. AML is also known for its genetic heterogeneity, variable prognosis, and rapid progression. These factors complicate diagnosis and necessitate a multidisciplinary approach involving specialists in clinical hematology, medical genetics, pathology, and clinical immunology. Precise identification of the genetic events driving AML is essential for accurate diagnosis, prognostic evaluation, and therapeutic decision-making [2, 3].

Telomerase is a ribonucleoprotein enzyme located at the ends of chromosomes. It comprises two main subunits: telomerase RNA (hTR), which serves as a template for telomere synthesis, and the catalytic subunit, human telomerase reverse transcriptase (*hTERT*), which determines telomerase activity levels [4-6]. Telomerase prevents the progressive loss of genetic material caused by the end-replication problem by adding repetitive telomeric sequences (TTAGGG) to the 3' ends of linear chromosomes [7, 8]. Telomerase is reactivated in 85-90% of human malignancies, including AML, where its re-expression enables cellular immortality. Elevated *hTERT* expression has been strongly linked to the development of various cancers, including leukemia, as it restores telomerase activity [9, 10].

The present study aimed to investigate the role of *hTERT* gene expression and serum protein concentration in newly diagnosed AML patients, AML patients in remission, and healthy controls. By analyzing these factors, the study seeks to explore their potential as

*College of Biotechnology, Al-Nahrain University, Baghdad, Iraq.*For Correspondence: yusur.alnaqashli@gmail.com*

diagnostic and prognostic biomarkers for AML.

Materials and Methods

Subjects

This study employed a case-control design involving the collection and analysis of blood samples from three groups: newly diagnosed AML patients, AML patients in remission, and healthy controls. A total of 10 newly diagnosed AML patients, 35 AML patients in remission, and 40 healthy controls participated. Blood samples (10 mL) were collected from all AML patients attending the Hematology Center at Baghdad Medical City between January and June 2022. Clinical data for each patient were obtained from hospital records and case sheets. Blood samples were processed by dividing them into tubes containing TRIzol™ reagent for RNA extraction and tubes designated for serological testing.

Hematological and Serological Parameters

Hematological parameters, including white blood cell (WBC) count, red blood cell (RBC) count, platelet (PLT) count, and hemoglobin (Hb) levels, were measured in all participants. These measurements were performed using a Hemoanalyzer 30T. Telomerase reverse transcriptase (TERT) serum protein levels were assessed in AML and healthy control samples using a TERT ELISA Kit (Shanghai YL Biont, China) according to the manufacturer's protocol.

TERT Gene Expression

Total RNA was extracted from blood samples using TRIzol™ Reagent (Thermo-Fisher, USA). Briefly, 2 mL of TRIzol reagent was added to 5 mL of blood to lyse cells while preserving RNA integrity. After homogenization, phase separation, and RNA precipitation, the RNA concentration was measured using a Quantus Fluorometer. For *hTERT* gene expression analysis, primers were designed using the Origene platform, with β-globin serving as the housekeeping gene for normalization [11]. Reverse transcription-quantitative PCR (RT-qPCR) was performed in a single-step reaction using the GoTaq® 1-Step RT-qPCR System (Promega, USA) in a total volume of 10 µL. The reaction mixture included 5 µl of GoTaq® 1-Step RT-qPCR System (Promega, USA), 0.25 µl of RT mix Mix (Promega, USA), 0.25 µl of MgCl2 (Promega, USA), 0.5 µl of each primer, TERT Forward primer: 5'-GCCGATTGTGAACATGGACTACG-3' and TERT Reverse primer: 5'-GCTCGTAGTTGAGCACGCTGAA-3', β-globin forward primer: 5'-GCTCGCGCTACTCTCTCTTT-3' and β-globin reverse primer: 5'-TCTGAATGCTCCACTTTTTCAA-3', 1 µl RNA template and 2 µl nuclease free water (Promega, USA). Reverse-transcription-quantitative (RT-qPCR) protocol on the Mic qPCR system was as follows: an RT. Enzyme Activation step (37 "C for 15 min) was followed by Initial Denaturation (95 "C for 5 min) then denaturation, annealing and extension steps repeated for 40 cycles (95 "C for 20 s, 63 "C for 20 s, 72 "C for 20 s). Gene expression was analyzed using the 2^−ΔΔCT method, with β-globin as the reference gene. The calculations included:

ΔCT= CT target gene –CT of reference gene (Equation 1)
ΔΔCT= ΔCT of each sample –average control ΔCT (Equation 2)
Folding change = 2-ΔΔCt (Equation 3)

Statistical Analysis

Data analysis was performed using SPSS version 24 and GraphPad Prism version 9. Continuous variables were compared using the Mann-Whitney U test (non-parametric equivalent of the independent samples t-test) and ordinary one-way ANOVA. A p-value of < 0.05 was considered statistically significant for all analyses.

Results

The demographic data of the study participants are summarized in Table 1. The age of AML patients ranged from 15 to 70 years, with the highest incidence observed in the 45–59 years age group. The distribution of cases was approximately equal between males and females.

The hematological analysis of AML patients is presented in Table 2. The total white blood cell (WBC) count in AML patients was significantly elevated compared to healthy controls ($P<0.01$). Conversely, red blood cell (RBC) count, platelet (PLT) levels, and hemoglobin (Hb) concentrations were significantly lower in AML patients than in healthy controls ($P<0.01$).

As shown in Figure 1, the serum levels of TERT protein were significantly higher in both newly diagnosed AML patients (1273.86 ± 301.90 ng/L) and AML patients in remission (1236.97 ± 540.11 ng/L) compared to healthy controls (334.59 ± 86.59 ng/L) ($P<0.01$).

The expression of the *TERT* gene was analyzed using RT-qPCR, and the results are illustrated in Figure 2. A significant increase in *TERT* gene expression was observed

Table 1. Age and Sex Distribution among AML Patients and Healthy Control

Age	AML Patients						Healthy Controls		
Group (year)	Newly Diagnosed			Treated					
	No. (%)	Female	Male	No. (%)	Female	Male	No. (%)	Female	Male
15-29	2 (2.4)	2.0 (2.4)	/	11 (13.0)	5 (5.9)	6 (7.1)	12 (14.2)	6 (7.1)	6 (7.1)
30-44	5 (5.9)	5.0 (5.9)	/	7 (8.3)	1 (1.2)	6 (7.1)	10 (11.8)	4 (4.7)	6 (7.1)
45-59	1 (1.2)	/	1 (1.2)	15 (17.7)	6 (7.1)	9 (10.6)	13 (15.3)	10 (11.8)	3 (3.5)
60-75	2 (2.4)	2.0 (2.4)	/	2 (2.4)	1 (1.2)	1 (1.2)	5 (5.9)	4 (4.7)	1 (1.2)
Total	10	9.0	1	35	13	22	40	24	16

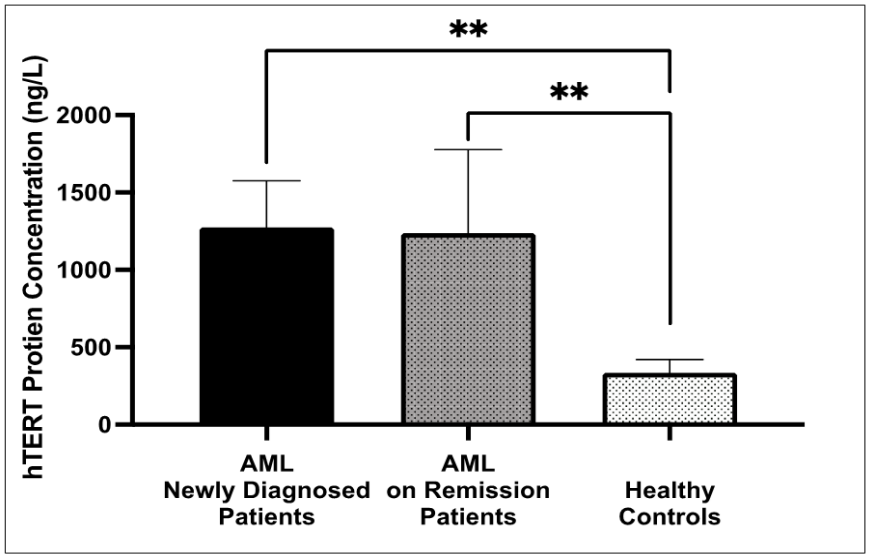

Figure 1. hTERT Serum Protein Concentration in Acute Myeloid Leukemia Newly Diagnosed Patients, Acute Myeloid Leukemia treated patients and healthy control. Error bars represent standard deviation; Error bars represent standard deviation; **: Significant (P<0.01).

Table 2. Hematological Parameters among AML Patients and Healthy Controls

Subjects	Hematological parameter (Value±SD)			
	WBC x 10^3/µl	RBC x 10^6/µl	PLT x 10^3/µl	Hb (g/dL)
Healthy Control	6.74 ± 1.59	4.68 ± 0.53	256.6 ± 69.91	13.31 ± 1.83
AML Newly Diagnosed Patients	18.45± 12.48	2.55 ± 0.67	39.78 ± 27.38	7.41 ± 1.43
AML on Remission Patients	14.65 ±13.28	2.83 ±0.90	70.73 ± 2	8.80 ±1.97
Pa	0.002**	0.0001**	0.0001**	0.0001**
Pb	0.003**	0.0001**	0.0001**	0.0001**
Pc	0.2NS	0.29 NS	0.18 NS	0.04*

SD, Standard deviation; NS, Non- significant; *, significant (P≤0.05); **, significant (P≤0.01); Pa, value between healthy control and newly diagnosed AML patients; Pb, value between healthy control and AML on remission patients; Pc, Value between AML newly diagnosed patients and AML on remission patients.

in both newly diagnosed AML patients and AML patients in remission compared to healthy controls (P≤0.01). Furthermore, the expression levels in both AML groups were significantly higher than those of healthy controls.

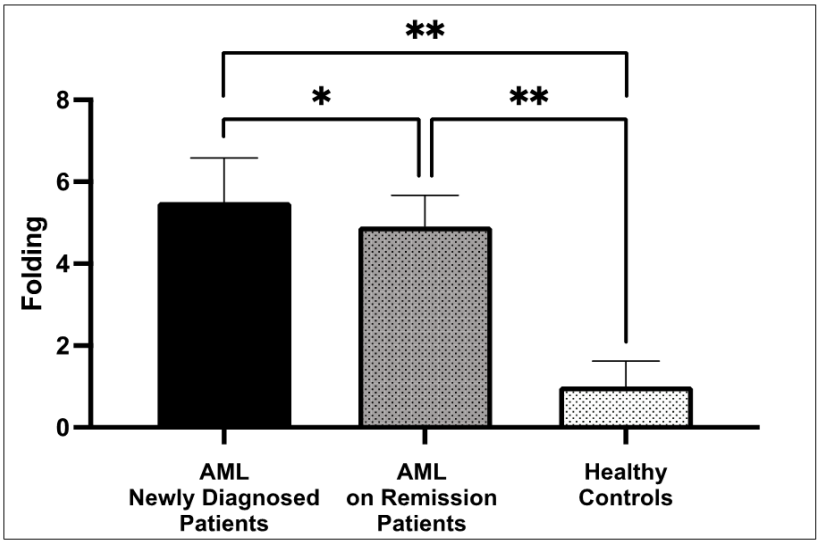

Figure 2. Expression of *hTERT* Gene in Acute Myeloid Leukemia Newly Diagnosed patients, Acute Myeloid Leukemia treated patients and healthy controls. Error bars represent standard deviation; *: Significant (P<0.05); **: Significant (P<0.01).

Discussion

The findings of this study revealed a significant increase (P≤0.01) in WBC counts and a significant decrease in RBC counts, hemoglobin (Hb), and platelet (PLT) levels in AML patients compared with healthy controls. These results align with those of Imad, Ghaloub [12], who highlighted the importance of these hematological parameters as critical indicators of AML patient health, particularly in assessing disease progression or response to therapy. AML arises from a cascade of molecular events within hematopoietic stem cells, which inhibit differentiation and promote unchecked proliferation. Consequently, immature myeloid blast cells accumulate in the bone marrow and circulation. This accumulation typically leads to increased WBC production and reduced RBC and platelet levels, consistent with the pathophysiology of AML [13].

In addition, this study demonstrated elevated *TERT* serum protein levels in both AML groups compared to healthy controls. This finding aligns with Singh [14], who reported increased *TERT* levels in various stages of breast cancer, suggesting its potential role as a biomarker in malignancies. Specifically, Singh's study found elevated *TERT* levels in 88.2% of stage III, 77.5% of stage II, and 51.9% of stage I breast cancer patients.

Our study also found a significant increase (P≤0.01) in *TERT* gene expression in newly diagnosed AML patients and AML patients in remission compared with healthy controls. This elevation in *TERT* mRNA expression may influence AML pathogenesis, as suggested by Shehata and Sallam [15]. However, the prognostic significance of *TERT* expression remains unclear due to the heterogeneity of AML, necessitating larger studies to establish its predictive value. *TERT* encodes the catalytic subunit of telomerase, the enzyme responsible for maintaining telomere length. Telomerase activity, enabled by *TERT*, facilitates the continuous replication of cancer cells. While *TERT* is suppressed in most somatic cells, it is strongly expressed in 85%–95% of malignancies, playing a pivotal role in tumorigenesis. Mechanisms driving elevated *TERT* expression in tumors include *TERT* promoter mutations, alternative splicing, amplification, promoter methylation, and disruption of telomere position effect (TPE) machinery [16, 17]. Targeting telomerase is a promising therapeutic approach, with ongoing clinical trials exploring various telomerase inhibition strategies that induce telomere shortening and cancer cell death.

Interestingly, this study also revealed significantly higher *TERT* gene expression in newly diagnosed AML patients compared to those in remission (P≤0.01). These findings are consistent with Huh et al. [18], who reported elevated *TERT* mRNA levels at diagnosis compared to remission. Similarly, AlJobori and Jaafar [19] noted significantly higher *TERT* levels in patients who failed to achieve full hematological remission compared to those who did. However, conflicting evidence exists; for example, Cogulu et al. [20] found no significant difference in *TERT* mRNA expression between cases of full remission and recurrence. These discrepancies may arise from the heterogeneity of AML, variations in genetic and molecular characteristics of leukemic cells, differences in treatment regimens, and individual therapeutic responses [21].

A primary limitation of this study is the relatively small sample size, which may restrict the generalizability of the findings. Additionally, the study population was drawn from a specific demographic, which may not fully represent the global diversity of AML patients. Future research should aim to include larger, more diverse cohorts to validate these findings and assess their applicability across different populations.

In conclusion, the findings of this study demonstrate that *hTERT* gene expression levels are significantly higher in newly diagnosed AML patients compared to those in remission. This underscores the potential of *hTERT* as a valuable biomarker for the diagnosis, prognosis, and monitoring of AML. The strong correlations observed between *hTERT* levels and hematological markers further support its utility in assessing AML severity and treatment efficacy.

In summary, this study contributes to a deeper understanding of the molecular pathways underlying AML and lays the groundwork for developing innovative diagnostic and therapeutic strategies targeting telomerase activity.

Author Contribution Statement

The work was designed, performed by Yusur Alnaqashli. Supervised and edited by Hameed M Jasim. All authors reviewed and approved the final article.

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General

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Ethical Declaration

The research protocol for this study was reviewed and approved by the college of biotechnology, Al-Nahrain University. This study was applied at the Hematology Center in Baghdad Medical City, Baghdad, Iraq (Approval Number: 9251 on 02/03/2022). A consent from each participant was taken before study started.

Conflict of Interest

There is no conflict of interest among the author

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