Expression of Ten-Eleven Translocation 1 Gene in Acute Leukemia

Hamed HB[1], Naguib EM[2], Metry MBM[1], Elnaggar MG[1]

[1] Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
[2] Clinical Pathology Department, Faculty of Medicine, Assiut University, Egypt.

Abstract:

Background: The Ten-Eleven Translocation 1 gene (TET1) is a member of the TET methyl cytosine dioxygenase family of enzymes (TET1, TET2, and TET3). TET1 has contrasting roles in myeloid and lymphoid transformation being either an oncogene or a tumor suppressor. This work aimed to study the expression level of the TET1 gene in acute leukemia patients and its correlation with the clinical and pathological criteria of these patients.

Methods: This study was conducted on 73 acute leukemia patients. Bone marrow samples were analyzed using Real-Time PCR 7500s.

Results: There was a significant correlation between the expression levels of TET 1 gene in acute leukemia patients and their clinical and pathological criteria. It has been found that expression levels of TET1 gene in patients' samples were higher in AML, not otherwise specified (NOS), and T lymphoblastic leukemia/lymphoma patients and lower in B lymphoblastic leukemia/lymphoma, NOS patients. Besides, this study showed a significant relation between TET1 gene and the percentage of blast cells in peripheral blood (P.B), bone marrow (B.M) and generalized lymphadenopathy. In AML patients, the higher percent of blast cells was associated with the upregulated TET1 gene, while TET1expression was decreased in patients with enlarged lymph nodes. However, in B-ALL patients, the TET1 expression level was significantly lower in patients with a high percent of blast cells in P.B and B.M, generalized lymphadenopathy, and fever.

Conclusion: The TET1 gene has dual roles in myeloid and lymphoid leukemia even within lymphoid leukemia. TET1 exerts an oncogenic role in AML and T-ALL in contrast to its tumor suppressor role in B-ALL, thus rendering TET1 a potential target for treating this form of hematopoietic malignancy.

Keywords: AML, ALL, TET1, DNA demethylation, real-time PCR.

Received: 9 August 2022
Accepted: 29 August 2022

Authors Information:

Hosny Badrawy Hamed
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: badrawyh@yahoo.com

Eman Mohamed Naguib
Clinical Pathology Department, Faculty of Medicine, Assiut University, Egypt
email: Eman_nagiub@yahoo.com

Monica Botros Mosaad Metry
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: monecabotros@yahoo.com

Mohamed Galal El-Naggar
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: mohamed1979@aun.edu.eg

Corresponding Author:
Mohamed Galal El-Naggar
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: mohamed1979@aun.edu.eg

Introduction:

The gene known as TET1 is responsible for producing a 235.3 kDa nuclear protein with a total of 2,113 amino acids.

Carboxyl-terminal catalytic core section includes Cysteine-rich domain essential for activity and double-stranded helix (DSBH) domain containing Iron (Fe II) and alpha-ketoglutarate (-KG) binding sites. Within the DSBH domain, a low complexity insert is also present in the catalytic core. In addition to its catalytic domain, TET1 also has an N-terminal Cysteine-X-X-Cysteine (CXXC) domain that facilitates direct DNA binding [1].

TET1 has a role in various physiological and pathological processes. It is an epigenetic regulator of the DNA and RNA demethylation processes. Therefore, it regulates gene expression at both the transcriptional and post-transcriptional levels. Besides, through global and site-specific demethylation, it can also regulate cell fate decisions [2], cellular DNA damage response [3], embryonic stem cell development and pluripotency [4], reproduction [5,6] neuronal development and activity [7], and cancer [8].

TET1 is required for normal hematopoiesis. According to Mohr, TET1 is essential for maintaining the reconstitution capacity of hematopoietic stem cells (HSCs) and increasing long-term HSC populations and quiescent side populations [9].

Acute leukemia is a malignant clonal disorder defined by the World Health Organization standards (WHO) as greater than 20% of the cells in the bone marrow (B.M) being blasts [10]. These blast cells replace the normal B.M cells and infiltrate various

organs with a rapidly progressive fatal course if untreated. It has two major forms; acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) [11]. TET1 was initially identified as a fusion partner of the myeloid/lymphoid (or mixed-lineage) leukemia (MLL) gene in some cases of AML and was named LCX (leukemia -associated protein with a CXXC domain) gene [12]. Dysregulation of the TET1 gene mediates the pathogenesis of leukemia through different mechanisms. These proposed pathogenic mechanisms are that high TET1 expression either correlates with the upregulation of RNA transport and ribosome biogenesis pathways driving tumorigenesis [3,13] or Cell transformation and leukemogenesis are triggered by TET1 and MLL fusion proteins, which together activate the HoxA9/myeloid ecotropic viral integration Site 1 (Meis1)/PBX 3 signaling cascade [14]. Whereas TET1 downregulation correlates with genetic and epigenetic changes in HSCs, which accumulate and predispose to developing leukemia [15] or TET1 acts as a tumor suppressor where loss of TET1 impacts the Wnt pathway and genes involved in the suppression of differentiation in AML [9].

Aim of the work:

We studied the expression level of the TET1 gene in acute leukemia patients and its correlation with the clinical and pathological criteria of these patients.

Methods:

This study was done on 73 patients of newly diagnosed acute leukemia based on the WHO (2016) classification (37 AML patients + 36 ALL patients; 28 B-ALL, and 8 T-ALL). Those patients were presented to South Egypt Cancer Institute Assiut University hospital in the period between February 2019 and March 2020. Patients' ages ranged from 2 to 76 years old, according to sex, 44 patients were males and 29 patients were females. According to age, 44 patients were children (2-18 years) and 29 patients were adults (19-76 years).

Twenty subjects, apparently healthy (10 males and 10 females) aged 3 to 80 years were included in the study as a control group. The research program was approved by the ethical committee, Faculty of Medicine, Assiut University.

All subjects were included after having informed consent. A History was taken from all patients who were exposed to complete clinical examination. Some laboratory investigations were also carried out, including complete blood count (CBC) with differential count, bone marrow aspiration (BMA), flowcytometry, cytogenetic and molecular testing, and TET1 by real-time PCR.

Bone marrow and peripheral blood samples were collected in Ethylene Diamine Tetra Acetic acid (EDTA) vacutainer tubes.

- Two ml of B.M samples were collected from acute leukemia patients via bone marrow aspiration in a complete aseptic precaution.

- Two ml of P.B samples were collected from healthy individuals (control group) via direct venous puncture under complete aseptic conditions.

Steps for measuring TET1 expression level:

A- Purification of total cellular RNA from human whole blood using QIAamp RNA Blood Mini Kit (Cat. No. 52304).

Erythrocytes were selectively lysed, and leukocytes were recovered by centrifugation. Then leukocytes were lysed through highly denaturing conditions that inactivate RNases, allowing the isolation of intact RNA. After the homogenization of the lysate by brief centrifugation through a QIAshredder spin column, ethanol was added to adjust binding conditions, and the sample was applied to the QIAamp spin column. The RNA was bound to the silica membrane during a brief centrifugation step. Contaminants were washed away, and total RNA was eluted in 35 µl of RNase-free water.

B- Reverse Transcription and Quantification Using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit K1622 and thermal cycler:

The isolated total cellular RNA was first incubated with the primer at 65o C to denature the RNA secondary structure and then was chilled quickly on the ice, so the primer was annealed to RNA. Then reverse transcriptase, deoxynucleotide triphosphate (dNTPs), and buffer were added. The reverse transcription reaction was extended at 60 o C for 42 minutes to allow transcription to occur. This reaction was then heated to 70 o C for 5 minutes to inactivate the enzyme.

C- Detection of TET1 gene expression level using 7500 fast real-time PCR (Applied Biosystems) USA.

Reaction set-up is at room temperature as the master mix includes Maxima Hot Start Taq DNA polymerase. The template DNA was added to the prepared reaction master mix. The PCR cycling starts with an initial denaturation step of 10 min at 95°C to activate maxima hot start Taq DNA polymerase. Denaturation at 95 °C for 15 seconds was followed by annealing/extension at 60 °C for 1 minute, for a total of 40 cycles.

D- Analysis of the data and results interpretation:

1- The cycle threshold (Ct) was determined, and Δct was calculated for each patient and the control.

2- We calculated the ΔΔct for each patient, then, we applied the equation 2-ΔΔct for calculation of the relative quantification (RQ); if more than 1, it would be upregulated while, if less than 1, it would be downregulated. The data was presented as a fold change. Different groups of acute leukemia patients were compared with the healthy control group and tested for statistical significance using mean ± standard deviation (SD).

Statistical analysis:

The collected data was analyzed by computer program SPSS" ver. 21" Chicago. USA. Data was expressed as mean, standard deviation and number, percentage. The T-test or Mann-Whitney test was used to determine significance for numeric variables. The Chi-Square or Fisher exact test was used to determine

significance for categorical variables. P < 0.05 is significant.

Results:

This cross-sectional study has been conducted to assess the expression of TET 1 gene in patients with newly diagnosed acute leukemia based on 2016 WHO classification (n=73); 37(50.7%) patients were AML and 36 were ALL (49.3%) with B-ALL and T-ALL being 28 (38.3%) and 8 (11.0%) respectively.

There was no significant difference in age or sex between patients and controls.

Among the AML patients studied, 56.8% had monocytic differentiation, 16.2% had AML without maturation, and 16.2% had AML with maturation. As shown in "table 1" and "figure 1" the remaining AML patients (10.8%) had cytogenetic abnormalities; 5.4% had t (15;17) and the other 5.4% had t (8;21).

Table 1. Classification of studied acute myeloid leukemia patients

AML (n=37)	Frequency	%
AML, NOS	**33**	**89.2**
AML without maturation	6	16.2
AML with maturation	6	16.2
AML with monocytic differentiation	21	56.8
AML with recurrent cytogenetic abnormalities	**4**	**10.8**
t (15;17) +ve	2	5.4
t (8;21) +ve	2	5.4

AML, NOS: acute myeloid leukemia, not otherwise specified

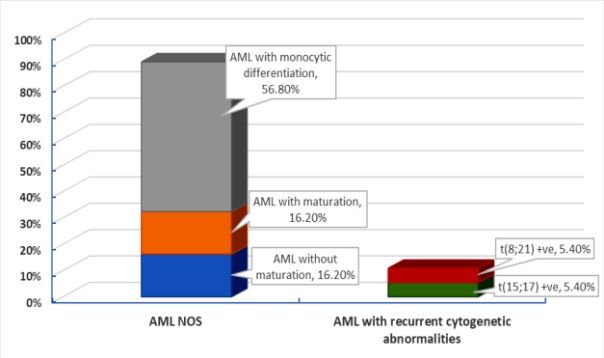

Figure 1. Classification of studied acute myeloid leukemia patients

The most common presenting symptoms of the studied AML patients were fever and bleeding tendency found in 81.1% and 64.9% of patients, respectively, as shown in “table 2” and “figure 2”.

The CBC with a differential count of the studied AML patients showed that the white blood cell (WBC) count ranged from (1.1 – 278) x103/mL with a mean count of 69.2 x103/mL ± 68.7.

The blast cell percentage ranged from (7 % – 98%) with the mean value of 70.9% ± 22.6.

Table 2. Clinical characteristics of acute myeloid leukemia patients (n=37)

Symptoms	Frequency	%
Fever	30	81.1
Bleeding tendency	24	64.9
HSM	20	54.1
Enlarged lymph nodes	12	32.4
Others*	10	18.9

Others (bone pain, scalp abscess, anemic manifestations only or patients discovered accidentally)

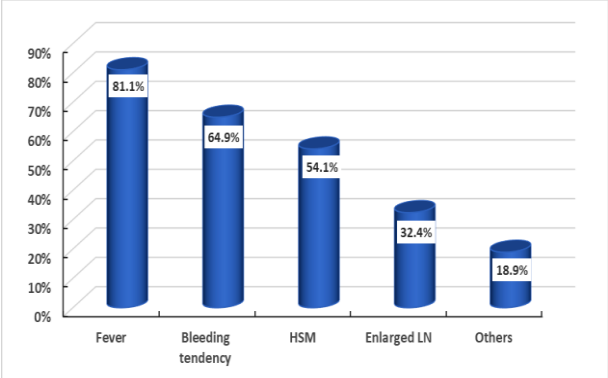

Figure 2. Presenting symptoms of studied acute myeloid leukemia patients

The hemoglobin concentration ranged from (5 – 13) g/dL with a mean value of 8.7 g/dL ± 2.

The platelet count ranged from (5 – 161) x109/L with a mean value of 57.1 x109/L ± 47.2. However, the blast cell percentage of the BMA samples ranged from (25% – 98%) with a mean value of 78.5% ± 20.5, all reported in “table 3”.

Table 3. Hematological parameters of acute myeloid leukemia patients (n= 37)

Variable	(Min-max)	Mean ± SD
WBCs (x103/mL)	(1.1 – 278)	69.2 ± 68.7
WBCs blasts (%)	(7 – 98)	70.9 ± 22.6
Hemoglobin (g/dL)	(5 – 13)	8.7± 2
Platelets (x109/L)	(5 – 161)	57 .1± 47.2
BMA blasts (%)	(25 – 98)	78.5 ± 20.5

WBCs: white blood cells, BMA: bone marrow aspirate, SD: Standard Deviation

As for ALL, most of the studied B-ALL patients (63.9%) were diagnosed as B lymphoblastic leukemia/lymphoma, NOS. The remaining 13.9% carried cytogenetic abnormalities; 5.6% with a Breakpoint cluster- Abelson tyrosine kinase gene (BCR-ABL) fusion gene and 8.3% with MLL gene

rearrangement, where T lymphoblastic leukemia/lymphoma patients were 22.2 %, as shown in "table 4" and "figure 3".

Table 4. Classification of studied acute lymphoblastic leukemia patients

ALL (n=36)	Frequency	%
B lymphoblastic leukemia/ lymphoma, NOS	**23**	**63.9**
B lymphoblastic leukemia/lymphoma with cytogenetic abnormalities	**5**	**13.9**
• BCR-ABL +ve	2	5.6
• MLL gene rearrangement +ve	3	8.3
T lymphoblastic leukemia/lymphoma	**8**	**22.2**

ALL: acute lymphoblastic leukemia, NOS: not otherwise specified, BCR-ABL: Break point cluster- Abelson tyrosine kinase gene, MLL: Myeloid/lymphoid or mixed-lineage leukemia

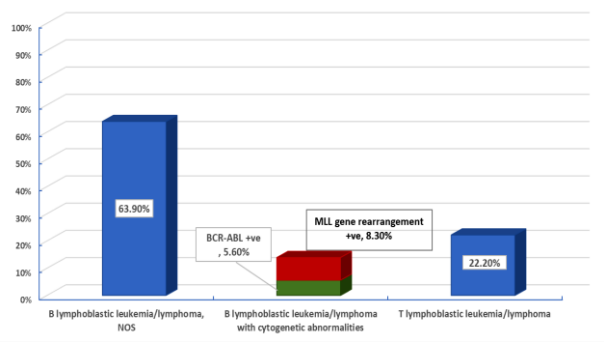

Figure 3. Classification of studied acute lymphoblastic leukemia patients.

"Table 5" and "figure 4" showed that the most common presenting symptoms of the studied ALL patients were fever and enlarged lymph nodes which were found in 83.3% of patients.

Table 5. Clinical and laboratory characteristics of acute lymphoblastic leukemia patients (n=36)

Symptoms	Frequency	%
Fever	30	83.3
Bleeding tendency	13	36.1
HSM	28	77.8
Enlarged lymph nodes	30	83.3
Others*	18	50.0

*Others (bone pain, mediastinal mass, CNS manifestations). HSM: hepatosplenomegaly

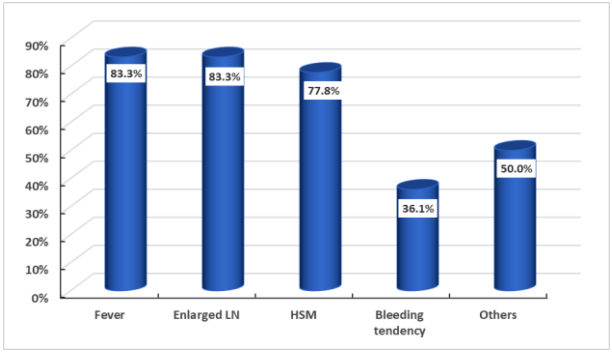

Figure 4. Presenting symptoms of studied acute lymphoblastic leukemia patients.

The WBC count ranged from (2.2 – 156) x103/mL with a mean of 61.3 x103/mL ± 48.5 on the CBC with a differential count of the patients studied. The blast cell count ranged from (18% – 96%) with the mean value 68.9% ± 26.2. The hemoglobin concentration ranged from (6 – 14) g/dL with a mean value of 9.5 g/dL ± 1.9. The Platelets count ranged from (5 – 241) x109/L with a mean value of 60.9 x109/L ± 53.4. However, the blast count of the BMA samples ranged from (40% – 98%) with a mean value of 86.9% ± 12.8; "table 6".

Table 6. Hematological parameters of acute lymphoblastic leukemia patients (n= 36)

Variable	(Min-Max)	Mean ± SD
WBCs ($x10^3$/mL)	(2.2 – 156)	61.3 ± 48.5
WBCs blasts (%)	(18 – 96)	68.9 ± 26.2
Hemoglobin (g/dL)	(6 – 14)	9.5 ± 1.9
Platelets ($x10^9$/L)	(5 – 241)	60.9 ± 53.4
BMA blasts (%)	(40 – 98)	86.9 ± 12.8

WBCs: white blood cells, BMA: bone marrow aspirate, SD: Standard Deviation

The RQ was obtained and interpreted as up-or down-regulated in the patients (n=73) compared with expression in healthy controls (n=20). No significant difference was found in TET1 gene expression concerning age and sex, as shown in "table 7".

Table 7. TET 1 gene expression (n=73) in relation to age and sex of studied patients

Variable	Upregulation (n=41)	Downregulation (n=32)	P-value*
Age (Mean ± SD)	21.4 ± 17.6	17.9 ± 18.5	0.467
Sex			
• Male	24 (54.5%)	20(45.5%)	0.731
• Female	17 (58.6%)	12 (41.4%)	

*Mann-Whitney test was used. ^Significant p-value (< 0.05).

Concerning the diagnosis, “table 8” showed no significant difference in TET1 expression between AML and ALL patients. However, in ALL, TET1 was significantly downregulated in 64.3% of B-ALL patients, and significantly upregulated in 100% of patients.

Table 8. TET1 expression levels in acute leukaemia patients

Diagnosis		Upregulation n=41 (56.2%)	Downregulation n=32 (43.8%)	P-value*
AML		23 (62.2%)	14 (37.8%)	0.295
ALL		18 (50%)	18 (50%)	
ALL	**B-ALL**	10 (35.7%)	18 (64.3%)	0.001^
	T- ALL	8 (100%)	0	

*Mann-Whitney test was used. ^Significant p-value (< 0.05).
AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia

A significant increase in the TET1 gene expression was found in 66.7% of patients with AML, NOS “table 9”. Also, the TET1 gene was downregulated in AML patients with enlarged lymph nodes as revealed in “Table 10” and “figure 5”.

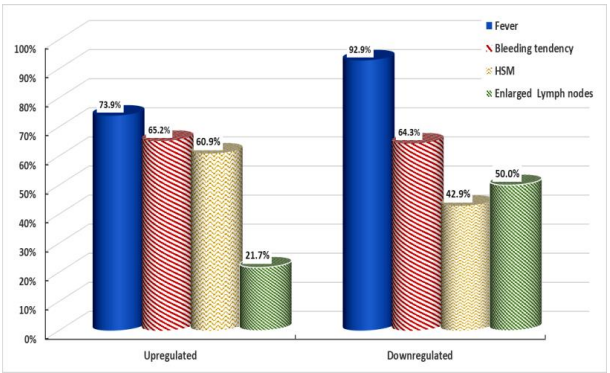

Figure 5. Clinical characteristics of studied acute myeloid leukemia patients in relation to TET 1 gene expression.

About the hematological parameters, the upregulated TET1 gene in AML patients was associated with a higher percent of peripheral blast compared to cases of the downregulated gene (75.3% ± 23.2% vs. 63.6% ± 20.3%). This difference was statistically significant, as shown in “table 11” and “figure 6”.

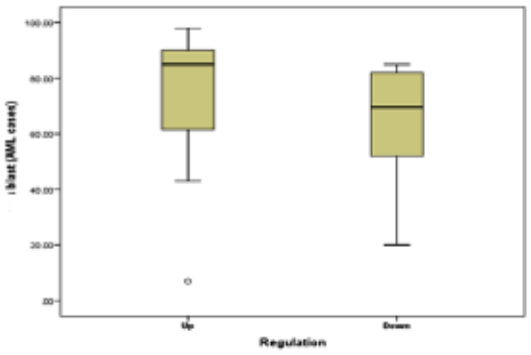

Figure 6. Percentage of peripheral blast of studied acute myeloid leukemia patients according to TET 1 gene expression.

Concerning ALL, "table 12" showed that the TET1 gene expression level was significantly lower in 76.9% of patients with B lymphoblastic leukemia/lymphoma, not otherwise specified, but significantly higher in all 8 (100%) T lymphoblastic leukemia/lymphoma patients.

Also, ALL patients with fever and enlarged lymph nodes showed significant TET1 gene downregulation “table 13” and “figure 7”.

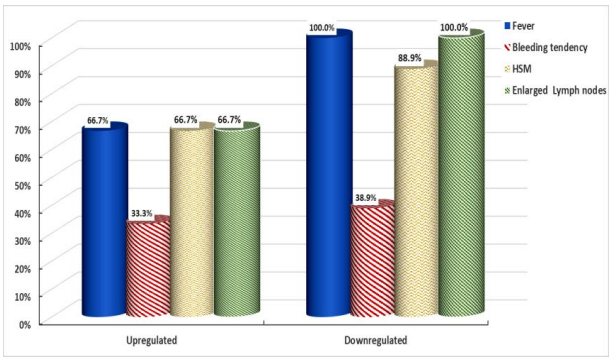

Figure 7. Clinical characteristics of studied acute lymphoblastic leukemia patients in relation to TET 1 gene expression

Moreover, “table 14” and “figure 8”. showed that the downregulated TET1 gene was linked to a higher percentage of peripheral and bone marrow blasts than the upregulated gene.

Table 9. TET1 expression levels in acute myeloid leukemia patients

AML subtype	Upregulation	Downregulation	P-value*
AML, NOS	**22 (66.7%)**	**11 (33.3%)**	**0.007^**
AML without maturation	4 (66.7%)	2 (33.3%)	
AML with maturation	5 (83.3%)	1 (16.7%)	0.618
AML with monocytic differentiation	13 (61.9%)	8 (38.1%)	
AML with recurrent cytogenetic abnormalities	1 (25%)	3 (75%)	0.157
t(15;17) +ve	1 (50%)	1 (50%)	0.248
t(8;21) +ve	0	2 (100)	

*Chi-square test was used. ^ Significant p-value (< 0.05)
AML, NOS: acute myeloid leukemia, not otherwise specified

Table 10. Clinical characteristics of studied acute myeloid leukemia patients (n=37) concerning TET 1 gene expression

Symptoms	Upregulation n=23	Downregulation n=14	P-value*
Fever	17 (73.9%)	13 (92.9%)	0.154
Bleeding tendency	15 (65.2%)	9 (64.3%)	0.954
HSM	14 (60.9%)	6 (42.9%)	0.286
Enlarged lymph nodes	5(21.7%)	7 (50%)	< 0.001^

*Chi-square test was used. *Significant p-value (< 0.05)
HSM: hepatosplenomegaly

Table 11. Hematological parameters of studied acute myeloid leukemia patients according to TET 1 gene expression

Variable	Upregulation n=23 (Mean ± SD)	Downregulation n=14 (Mean ± SD)	P-value*
WBCs ($x10^3$/mL)	85.8 ± 79.1	42 ± 34.6	0.056
WBCs blasts (%)	75.3 ± 23.2	63.6 ± 20.3	0.030^
Hemoglobin (g/dL)	8.5 ± 2.2	8.9 ± 1.7	0.470
Platelets ($x10^9$/L)	52.9 ± 43.9	64 ± 53.2	0.583
BMA blasts (%)	79.9 ± 18.5	76.1 ± 24.1	0.627

*Mann-Whitney test was used. ^Significant p-value (< 0.05)
WBCs: white blood cells, BMA: bone marrow aspirate

Table 12. TET1 expression levels in acute lymphoblastic leukemia patients

ALL subtype	Upregulation	Downregulation	P-value*
B lymphoblastic leukemia/lymphoma, NOS	**6 (23.1%)**	**17 (76.9%)**	**0.001^**
B lymphoblastic leukemia/lymphoma with cytogenetic abnormalities	**4 (53.8%)**	**1 (46.2%)**	**0.058**
• BCR-ABL +ve	1 (50%)	1 (50%)	0.171
• MLL gene rearrangement +ve	3 (100%)	0	
T lymphoblastic leukemia/lymphoma	**8 (100%)**	**0**	**< 0.001^**

*Chi-square test was used. *Significant p-value (< 0.05).
ALL: acute lymphoblastic leukemia, NOS: not otherwise specified, BCR-ABL: Break point cluster- Abelson tyrosine kinase gene, MLL: Myeloid/lymphoid or mixed-lineage leukemia

Table 13. Clinical characteristics of studied acute lymphoblastic leukemia patients (n=36) in relation to TET 1 gene expression

Symptoms	Upregulation n=18	Downregulation n=18	P-value*
Fever	12 (66.7%)	18 (100%)	0.007 ^
Bleeding tendency	6 (33.3%)	7 (38.9%)	0.729
HSM	12 (66.7%)	16 (88.9%)	0.109
Enlarged lymph nodes	12 (66.7%)	18 (100%)	0.007 ^

Chi-square test was used. *Significant p-value (< 0.05)
HSM : hepatosplenomegaly

Table 14. Hematological parameters of studied acute lymphoblastic leukemia patients according to TET 1 gene expression

Variable	Upregulation n=18 (Mean ± SD)	Downregulation n=18 (Mean ± SD)	P-value*
WBCs (x10^3/mL)	73.1 ± 51.1	48.8 ± 43.7	0.165
WBCs blasts (%)	58.8 ± 25	78.4 ± 24.3	0.012^
Hemoglobin (g/dL)	9.5 ± 2	9.4 ± 1.8	0.791
Platelets (x10^9/L)	72.7 ± 64.3	48.5 ± 36.9	0.248
BMA blasts (%)	84.4 ± 13.2	89.2 ± 12.4	0.049^

Mann-Whitney test was used. ^Significant p-value (< 0.05)
WBCs: white blood cells, BMA: bone marrow aspirate, SD: Standard Deviation

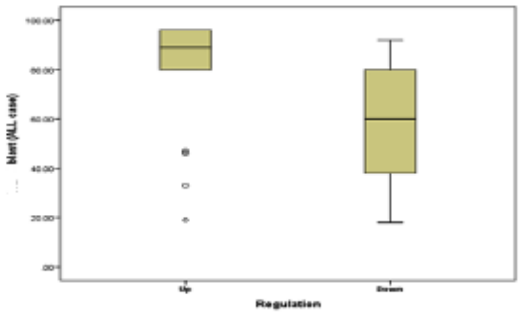

Figure 8. Percentage of peripheral blast of studied acute lymphoblastic leukemia patients according to TET 1 gene expression

Discussion:

Acute leukemia is a malignant clonal disorder originating in HSCs characterized by the proliferation of blast cells in the B.M, replacing the normal B.M cells. It has two major forms: ALL and AML [11]. The TET1 gene is an epigenetic regulator of DNA demethylation regulating gene expression by establishing transcriptional activation or suppression in a tissue- and gene-specific manner. Besides, TET1 regulates cell fate decisions; therefore, dysregulation contributes to tumorigenesis. TET1 functions as both an oncogene and a tumor suppressor gene in tumorigenesis, depending on the cellular context, other epigenetic regulators, and cell type-specific interacting partners [8].

In our study, we reported a significant TET1 gene upregulation in 62.2% of the studied AML patients. A significant high TET1 level was found in 66.7% of patients with AML, NOS; 66.7%, 83.3%, and 61.9 % of AML without maturation, AML with maturation, and AML with monocytic differentiation groups of patients, respectively. These results were in contrast with Jiang et al. concerning TET1 expression in AML with a monocytic differentiation group of patients, and this might be because they included only AML patients aged more than 14 at diagnosis [16].

Regarding AML with recurrent cytogenetic abnormalities in our study, 100% of patients with AML carrying t(8;21) showed TET1 downregulation with no difference in TET1 expression level among AML patients with t(15;17), conflicting with previous studies [9,16,17]. The discrepancy between studies could be the result of the small number of patients in our study.

Clinically, we found that TET1 gene expression was significantly decreased in patients with enlarged lymph nodes, which was in agreement with Cimmino et al. study [18].

Our study demonstrated that a higher percent of blast cells in P.B was significantly associated with patients who expressed higher levels of the TET1 gene compared to cases of the downregulated gene. No significant correlation between TET1 level and the blast percentage in B.M, age, sex, WBCs count, hemoglobin level, or platelet counts in concordance with Wang et al. study [19]; however, Kim et al. declared that WBCs count, hemoglobin level, and platelet count were all decreased with TET1 upregulation [20]. This disagreement between studies might be caused by the inclusion of animal P.B and B.M samples by Kim et al. study [20].

These findings supported the role of TET1 in leukemogenesis. High TET1 expression in AML patients was attributed to activation of cancer-specific oncogenic mechanisms and was correlated with the upregulation of RNA transport and ribosome biogenesis pathways initiating leukemia [3,13,21].

As for ALL, our study found that in B lymphoblastic leukemia/lymphoma patients, the TET1 gene was significantly downregulated in concordance with a number of studies who reported that TET1 gene downregulation in HSCs and progenitor cells provided a basis for DNA damage [22-24]. Low TET1 gene expression caused widespread genetic and epigenetic changes in HSCs, which accumulated with the continuous proliferation and blocked B cell maturation, predisposing them to develop leukemia of B cell origin and revealing its tumor suppressor role in B lymphoblastic leukemia/lymphoma [15].

On the other hand, in our study, all B lymphoblastic leukemia/lymphoma patients carrying MLL gene rearrangements showed TET1 upregulation. Similarly, several studies reported that MLL fusion proteins directly increased TET1 expression, which promoted cell proliferation and inhibited cell differentiation and apoptosis, leading to cell transformation and leukemogenesis [14,25]. Furthermore, other studies identified the TET1 gene as a fusion partner of the MLL gene in cases of acute leukemia with t (10;11) (q22;q23) producing the MLL-TET1 (MT1) fusion protein, which resulted in an imbalance in TET1-mediated DNA demethylation and inhibited cellular differentiation, thus participating in leukemogenesis. [18,20]. However, the upregulation of the TET1 gene in MLL rearranged leukemia patients in our study was not significant may be due to the small number of B-ALL patients carrying MLL gene rearrangement. TET1 expression levels in B lymphoblastic leukemia/lymphoma patients with the BCR-ABL fusion gene did not differ significantly. In previous studies, no correlation with this cytogenetic marker could be found.

As for T lymphoblastic leukemia/lymphoma patients, this study reported a significantly high expression of the TET1 gene in all patients, in agreement with Jiang et al. who found that high TET1 expression in T lymphoblastic leukemia/lymphoma patients correlated with the upregulation of RNA transport driving tumor genesis [16].

Previous research found that poly (ADP-ribose) polymerase enzyme (PARP), an enzyme essential for single-strand DNA damage repair, positively influenced TET1 expression, causing hypomethylation and, as a result, increased TET1 expression in T lymphoblastic leukemia/lymphoma [26,27]. High expression of TET1 promoted the growth of T-ALL cells by regulating the expression of DNA repair genes and by protecting the

genome from damage by maintaining the expression of factors required for the growth and genomic integrity of T-ALL cells, indicating that high TET1 enzymatic activity was advantageous for the leukemic growth and chemoresistance of T-ALL cells [26,27].

Altogether, these findings support the opposing roles of the TET1 gene in ALL, being a tumor suppressor in B lymphoblastic leukemia/lymphoma [22] and an oncogene in T lymphoblastic leukemia/lymphoma [28]. Besides, we demonstrated that a higher percent of blast cells in P.B and B.M was significantly associated with patients who expressed lower levels of the TET1 gene compared to cases of the upregulated gene. However, Huang et al. reported that decreased TET1 expression reduced the proportion of blast cells in both B.M and P.B and was associated with minimal leukemia cell infiltration [14]. Whereas Zhao et al. found no significant correlation between TET1 expression and the percentage of B.M blasts [22]. The discrepancy between the studies may be due to the fact that they included animal P.B and B.M samples.

There was no significant correlation between TET1 expression and age, sex, WBCs count, hemoglobin level, or platelet counts, which is concordant with what was stated in Wang et al. study [19].

The TET1 expression level was significantly lower in patients with generalized lymphadenopathy and fever, agreeing with Cimmino et al. indicating the role of TET1 downregulation in the pathogenesis of ALL [18].

Other than these previous correlations, no correlation was found between TET1 expression levels and other laboratory and clinical data.

These previous findings may indicate that the TET1 gene has dual roles in myeloid and lymphoid hematological malignancies, even within lymphoid leukemias. The TET1 gene is significantly upregulated in AML, and T lymphoblastic leukemia/lymphoma, and significantly downregulated in B lymphoblastic leukemia/lymphoma.

Conclusion:

A statistically significant relation between TET1 expression level and blast percentage in P.B and enlarged lymph nodes in AML patients was found, the higher percent of blast cells was associated with the upregulated TET1 gene, while TET1 expression was decreased in patients with enlarged lymph nodes.

Besides, we also found a statistically significant relation between TET1 expression level and blast percentage in both P.B and B.M, and generalized lymphadenopathy and fever in B-ALL patients. The TET1 expression level was significantly lower in patients with a high percent of blast cells in P.B and B.M, generalized lymphadenopathy, and fever.

The TET1 gene plays dichotomous roles as we demonstrated that TET1 exerts an oncogenic role in AML and T-ALL in contrast to B-ALL in which TET1 acts as a tumor suppressor, thus rendering TET1 as a potential target for treating this form of hematopoietic malignancy.

List of abbreviations:

ALL Acute lymphoblastic leukemia
AML Acute Myeloid leukemia
B.M. Bone marrow
BCR-ABL Break point cluster- Abelson (ABL) tyrosine kinase gene
CBC Complete blood count
CD Cluster of differentiation
CXXC Cysteine -X-X-Cysteine domain
Ct The cycle threshold
dNTPs Deoxynucleotide triphosphate
DNA Deoxyribonucleic acid
DSBH double-stranded β helix
EDTA Ethylene Diamine Tetra Acetic acid
HSC Hematopoietic stem cells
LCX Leukemic associated protein with CXXC domain
MLL myeloid/lymphoid or mixed-lineage leukemia
MT1 MLL – TET1
NOS not otherwise specified
PARP poly (ADP-ribose) polymerase
PB Peripheral blood
PCR Polymerase chain reaction
PML/RARA Promyelocytic leukemia/Retinoic Acid Receptor Alpha
RNA Ribonucleic acid
RQ-PCR Real-time quantitative polymerase chain reaction
RQ Relative quantification method
SD Standard Deviation
TALL T cell acute lymphocytic leukemia
TET1 Ten-Eleven Translocation 1
WBCs white blood cells
WHO World Health Organization

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

H.B.H.: Conceptualization, Supervision, Investigation, Writing-review & editing. E.M.N.: Supervision, Investigation, Writing -review & editing. M.B.M.: Methodology, Investigation, Data curation, Writing- original draft. M.G.E.: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing- original draft, Supervision. All authors have read and approved the manuscript.

Acknowledgements: (Not applicable)

Ethics approval and consent to participate:

The protocol was approved by the Institutional Review Board of Faculty of Medicine, Assiut University (protocol number 17100336 and 12 September 2017 of approval). Our study conformed to all requirements as governed by the declaration of Helsinki.

References:

1. Tsagaratou A, Lio CJ, Yue X, et al. TET Methylcytosine Oxidases in T Cell and B Cell Development and Function. Front Immunol.

2017;8:220.
2. Melamed P, Yosefzon Y, David C, et al. Tet Enzymes, Variants, and Differential Effects on Function. Front Cell Dev Biol. 2018;6:22.
3. Coulter JB, Lopez-Bertoni H, Kuhns KJ, et al. TET1 deficiency attenuates the DNA damage response and promotes resistance to DNA damaging agents. Epigenetics. 2017;12(10):854-64.
4. Fidalgo M, Huang X, Guallar D, et al. Zfp281 Coordinates Opposing Functions of Tet1 and Tet2 in Pluripotent States. Cell Stem Cell. 2016;19(3):355-69.
5. Gan H, Wen L, Liao S, et al. Dynamics of 5-hydroxymethylcytosine during mouse spermatogenesis. Nat Commun. 2013;4:1995.
6. Yosefzon Y, David C, Tsukerman A, et al. An epigenetic switch repressing Tet1 in gonadotropes activates the reproductive axis. Proc Natl Acad Sci U S A. 2017;114(38):10131-6.
7. Tao H, Xie P, Cao Y, et al. The Dynamic DNA Demethylation during Postnatal Neuronal Development and Neural Stem Cell Differentiation. Stem Cells Int. 2018;2018:2186301.
8. Good CR, Panjarian S, Kelly AD, et al. TET1-Mediated Hypomethylation Activates Oncogenic Signaling in Triple-Negative Breast Cancer. Cancer Res. 2018;78(15):4126-37.
9. Mohr F. A balanced expression of TET1 is important for normal hematopoiesis: Universität Ulm; 2015.
10. Rose-Inman H, Kuehl D. Acute leukemia. Emerg Med Clin North Am. 2014;32(3):579-96.
11. Hiddemann W. Handbook of acute leukemia: Springer; 2016.
12. Ono R, Taki T, Taketani T, et al. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11)(q22;q23). Cancer Res. 2002;62(14):4075-80.
13. Good CR, Madzo J, Patel B, et al. A novel isoform of TET1 that lacks a CXXC domain is overexpressed in cancer. Nucleic Acids Res. 2017;45(14):8269-81.
14. Huang H, Jiang X, Li Z, et al. TET1 plays an essential oncogenic role in MLL-rearranged leukemia. Proc Natl Acad Sci U S A. 2013;110(29):11994-9.
15. Li X, Zeng X, Xu Y, et al. Mechanisms and rejuvenation strategies for aged hematopoietic stem cells. J Hematol Oncol. 2020;13(1):31.
16. Jiang X, Hu C, Ferchen K, et al. Targeted inhibition of STAT/TET1 axis as a therapeutic strategy for acute myeloid leukemia. Nat Commun. 2017;8(1):2099.
17. Weng H, Huang H, Huang H, et al. TET1 Modulates DNA Replication in Leukemia Cells Via a Catalytic-Independent Mechanism through Cooperating with KAT8. Blood. 2019;134:1249-.
18. Cimmino L, Dawlaty MM, Ndiaye-Lobry D, et al. TET1 is a tumor suppressor of hematopoietic malignancy. Nature Immunology. 2015;16(6):653-62.
19. Wang J, Li F, Ma Z, et al. High Expression of TET1 Predicts Poor Survival in Cytogenetically Normal Acute Myeloid Leukemia From Two Cohorts. EBioMedicine. 2018;28:90-6.
20. Kim HS, Oh SH, Kim JH, et al. MLL-TET1 fusion protein promotes immortalization of myeloid progenitor cells and leukemia development. Haematologica. 2017;102(11):e434-e7.
21. Ma C, Ji P, Xie N, et al. Cytosine Modifications and Distinct Functions of TET1 on Tumorigenesis. 2019.
22. Zhao Z, Chen L, Dawlaty, et al. Combined Loss of Tet1 and Tet2 Promotes B Cell, but Not Myeloid Malignancies, in Mice. Cell Rep. 2015;13(8):1692-704.
23. An J, Rao A, Ko M. TET family dioxygenases and DNA demethylation in stem cells and cancers. Exp Mol Med. 2017;49(4):e323.
24. Li W, Xu L. Epigenetic Function of TET Family, 5-Methylcytosine, and 5-Hydroxymethylcytosine in Hematologic Malignancies. Oncol Res Treat. 2019;42(6):309-18.
25. Ko M, An J, Pastor WA, et al. TET proteins and 5-methylcytosine oxidation in hematological cancers. Immunol Rev. 2015;263(1):6-21.
26. Sahin D, Bamezai S, Ciccarone F, et al. TET1 Promotes Leukemic Growth in T-ALL Via Maintenance of 5-Hydroxymethylation Marks and Can be Antagonized By the PARP Inhibitor Olaparib. Blood. 2016;128(22):737.
27. Bamezai S, Demir D, Pulikkottil AJ, et al. TET1 promotes growth of T-cell acute lymphoblastic leukemia and can be antagonized via PARP inhibition. Leukemia. 2021;35(2):389-403.
28. Poole CJ, Lodh A, Choi JH, et al. MYC deregulates TET1 and TET2 expression to control global DNA (hydroxy)methylation and gene expression to maintain a neoplastic phenotype in T-ALL. Epigenetics Chromatin. 2019;12(1):41.