

Expression of Ten-Eleven Translocation 1 Gene in Acute Leukemia

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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). The 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 TET1 gene in acute leukemia patients and its correlation with clinical and pathological criteria of these patients.

Patients & methods:

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

Results:

There was significant correlation between expression levels of TET 1 gene in acute leukemia patients and their clinical and pathological criteria.

Conclusion:

It has been found that expression levels of TET1 gene in patients' samples were higher in 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 the significant relation between TET1 gene and the percentage of blast cells in peripheral blood (P.B) and bone marrow (B.M) and generalized lymphadenopathy. These findings revealed the great role of TET1 gene dysregulation in leukemogenesis; also its possible usage as a potential target for treating this form of hematopoietic malignancy.

1. Background

The Ten-Eleven Translocation 1 gene (TET1) encodes a nuclear protein of 2136 amino acids with a molecular mass of 235.3 kDa. It has a carboxyl-terminal catalytic core region comprising a Cysteine-rich domain required for activity, and a double-stranded β helix (DSBH) domain, which contains the binding sites for Iron (Fe II) and alpha-ketoglutarate (α -KG). The catalytic core also includes a low complexity insert found within the DSBH domain. Besides its catalytic domain, TET1 contains N terminal Cysteine-X-X-Cysteine (CXXC) domain, which mediates direct DNA binding ⁽¹⁾.

The TET1 has a role in various physiological and pathological processes. It is an epigenetic regulator of the DNA and RNA demethylation process. 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 cells development and pluripotency ⁽⁴⁾, reproduction ⁽⁵⁾, ⁽⁶⁾ neuronal development and activity ⁽⁷⁾, and cancer ⁽⁸⁾.

TET1 plays an essential role in hematopoiesis. **Mohr 2015** ⁽⁹⁾ reported that TET1 is critical for maintaining the reconstitution capacity of hematopoietic stem cells (HSCs) and increasing the long-term HSC populations and quiescent side populations.

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) are 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⁾. The TET1 was initially identified as a fusion partner of 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 the high TET1 expression correlates with the upregulation of RNA transport and ribosome biogenesis pathways driving tumorigenesis ⁽³⁾, ⁽¹⁹⁾, whereas TET1 downregulation correlates with genetic and epigenetic changes in HSCs, which accumulate and predispose to develop leukemia ⁽²⁴⁾.

2. Methods

This study was done on 73 patients of newly diagnosed acute leukemia based on 2016 WHO 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. Patient's age 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 an informed consent. History was taken from all patients who 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 (2) ml of B.M samples were collected from acute leukemia patients via bone marrow aspiration in a complete aseptic precaution.

- Two (2) ml of P.B samples were collected from healthy individuals (control group) via direct venous puncture under complete aseptic conditions.

2.1. Steps of 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 65° 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 ° C for 42 minutes to allow transcription to occur. This reaction was then heated to 70 ° 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. Then the cycling steps were denaturation at 95°C for 15 seconds and annealing/extension 60°C for 1 min, along 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 $\Delta\Delta Ct$ for each patient, then, we applied the Eq. $2^{-\Delta\Delta Ct}$ 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 were presented as fold change. Different groups of acute leukemia patients were compared with the healthy control group and tested for statistical significance using mean \pm standard deviation (SD).

3. Results

This cross-sectional study has been conducted to assess the expression of TET 1 gene in patients of 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 were 28 (38.3%) and 8 (11.0%) respectively.

There was no significant difference in age or sex between patients and controls as shown in "Table 1" and "figure 1".

Most of the studied AML patients 89.2% were diagnosed as AML not otherwise specified (AML, NOS), with 56.8% AML with monocytic differentiation, 16.2% AML without maturation, and 16.2% AML with maturation. The remaining AML patients 10.8%, carry cytogenetic abnormalities; 6.5% carry t(15;17) and the other 6.5% carry t(8;21) as shown in "Table 2" and "figure 2".

The most presenting symptoms of the studied AML patients were fever and bleeding tendency found in 81.1% and 64.9% patients, respectively as shown in "Table 3" and "figure 3".

The CBC with a differential count of the studied AML patients showed that white blood cell (WBCs) count ranged from (1.1–278) $\times 10^3/\text{mL}$ with a mean count of $69.2 \times 10^3/\text{mL} \pm 68.7$ SD. The blast cell percentage ranged from (7% – 98%) with the mean value $70.9\% \pm 22.6$ SD. The hemoglobin concentration ranged from (5–13) g/dL with a mean value of $8.7 \text{ g/dL} \pm 2$ SD. The platelets count ranged from (5–161) $\times 10^9/\text{L}$ with a mean value of $57.1 \times 10^9/\text{L} \pm 47.2$ SD. However, the blast cell percentage of the BMA samples ranged from (25% – 98%) with a mean value of $78.5\% \pm 20.5$ SD, all reported in "Table 4".

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 Break point cluster- Abelson tyrosine kinase gene (BCR-ABL) fusion gene and 8.3% with MLL gene rearrangement. T lymphoblastic leukemia/lymphoma patients were 22.2%, as shown in "Table 5" and "figure4".

"Table 6" and "figure 5" show that the most presenting symptoms of the studied ALL patients were fever and enlarged lymph nodes found in 83.3% of patients.

The CBC with a differential count of the studied patients showed that WBCs count ranged from (2.2–156) $\times 10^3/\text{mL}$ with mean $61.3 \times 10^3/\text{mL} \pm 48.5$ SD. The blast cell count ranged from (18% – 96%) with the mean value $68.9\% \pm 26.2$ SD. The hemoglobin concentration ranged from (6–14) g/dL with a mean value of $9.5 \text{ g/dL} \pm 1.9$ SD. The Platelets count ranged from (5–241) $\times 10^9/\text{L}$ with a mean value of $60.9 \times 10^9/\text{L} \pm 53.4$ SD. However, the blast count of the BMA samples ranged from (40% – 98%) with a mean value of $86.9\% \pm 12.8$ SD; "Table 7".

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 8".

Concerning the diagnosis, "Table 9" showed no significant difference in TET1 expression between AML and ALL patients. However, in ALL, TET1 was significantly downregulated in 64.3% B-ALL patients, and significantly upregulated in 100% patients.

A significant increase in the TET1 gene expression was found in 66.7% of patients with AML, NOS "Table 10". Also, the TET1 gene was downregulated in AML patients with enlarged lymph nodes as revealed by "Table 11" and "figure 6".

About the hematological parameters, the upregulated TET1 gene in AML patients was associated with a higher percent of WBCs blast compared to cases of the downregulated gene ($75.3\% \pm 23.2\%$ vs. $63.6\% \pm 20.3\%$). This difference was statistically significant, as shown in "Table 12" and "figure7".

Concerning ALL, "Table 13" revealed that the TET1 gene expression level was significantly decreased in 76.9% of patients with B lymphoblastic leukemia/lymphoma, NOS, whereas it was significantly increased in all 8 (100%) T lymphoblastic leukemia/lymphoma patients.

Also, ALL patients with fever and enlarged lymph nodes showed significant TET1 gene downregulation "Table 14" and "figure 8".

Moreover, the downregulated TET1 gene was associated with a higher percent of peripheral and bone marrow blast compared to cases of upregulated gene "Table 15" and "figure 9".

4. 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. The TET1 plays dual roles in tumorigenesis either, being an oncogene or a tumor suppressor gene depending on the cellular context, other epigenetic regulators, and different interacting partners that are cell type-specific⁽⁸⁾.

In our study, we reported a significant TET1 gene upregulation in 62.2% of the studied AML patients. The 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. 2017**⁽¹³⁾ concerning TET1 expression in AML with monocytic differentiation group of patients, and this might be because they included only AML patients aged more than 14 at diagnosis.

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 Jiang et al. 2017⁽¹³⁾,⁽¹⁴⁾,⁽⁹⁾, respectively. 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. 2015**⁽¹⁶⁾ study.

Our study demonstrated that a higher percent of blast cells in P.B was significantly associated with patients who expressed higher levels of 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, platelet counts in concordance with **Wang et al. 2018**⁽¹⁷⁾ study; however, **Kim et al. 2017**⁽¹⁸⁾ declared that WBCs count, hemoglobin level, and platelets count were all decreased with TET1 upregulation. This disagreement between studies might be caused by the inclusion of animal P.B and B.M samples by **Kim et al. 2017**⁽¹⁸⁾ study.

These findings supported the role of TET1 in leukemogenesis. High TET1 expression in AML patients attributed to activation of cancer-specific oncogenic mechanisms and correlated with the upregulation of RNA transport and ribosome biogenesis pathways initiating leukemia⁽¹⁹⁾,⁽³⁾,⁽²⁰⁾.

As for ALL, our study found that in B lymphoblastic leukemia/lymphoma patients, the TET1 gene was significantly downregulated in concordance with **An, Rao, and Ko 2017**⁽²¹⁾, **W. Li and Xu 2019**⁽²²⁾, and **Zhao et al. 2015**⁽²³⁾ who reported that TET1 gene downregulation in HSCs and progenitor cells provided a basis for DNA damage. Low TET1 gene expression caused widespread genetic and epigenetic changes in HSCs, which accumulated with the continuous proliferation and blocked the B cell maturation, predisposing to develop leukemia of B cell origin and revealing its tumor suppressor role in B lymphoblastic leukemia/lymphoma⁽²⁴⁾.

On the other hand, in our study, all B lymphoblastic leukemia/lymphoma patients carrying MLL gene rearrangements showed TET1 upregulation. Similarly, several studies such as **H. Huang et al. 2013**⁽²⁹⁾ and **Ko et al. 2015**⁽²⁵⁾ 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. Besides, **Cimmino et al. 2015**⁽¹⁶⁾, and **Kim et al. 2017**⁽¹⁸⁾ defined the TET1 gene as a fusion partner of the MLL gene in cases of acute leukemia bearing t(10;11)(q22;q23) producing the MLL – TET1 (MT1) fusion protein, which resulted in imbalance in the TET1-mediated DNA demethylation, as well as inhibited cellular differentiation thus participating in leukemogenesis. 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. We found no significant difference in TET1 expression level among B lymphoblastic leukemia/lymphoma patients carrying the BCR-ABL fusion gene. Correlation with this cytogenetic marker could not be found in previous studies.

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. 2017**⁽¹³⁾ who found that high TET1

expression in T lymphoblastic leukemia/lymphoma patients correlated with the upregulation of RNA transport driving tumor genesis.

Another mechanism was indicated by **Sahin et al. 2016** ⁽²⁶⁾ and **(Bamezai et al. 2021)** ⁽²⁷⁾ that poly (ADP-ribose) polymerase enzyme (PARP), an enzyme essential for single-strand DNA damage repair, positively influenced the TET1 expression causing its hypomethylation and, therefore, increased expression of TET1 in T lymphoblastic leukemia/lymphoma. 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 through maintaining expression of factors required for 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.

All together, these findings support the opposing roles of TET1 gene in ALL, being a tumor suppressor in B lymphoblastic leukemia/lymphoma ⁽²³⁾ 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 TET1 gene compared to cases of the upregulated gene. However, **H. Huang et al. 2013** ⁽²⁹⁾ 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. Whereas **Zhao et al. 2015** ⁽²³⁾ found no significant correlation between TET1 expression and the percentage of B.M blasts. The discrepancy between the studies may be due to 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, and platelet counts, concordant with what stated in **Wang et al. 2018** ⁽¹⁷⁾.

The TET1 expression level was significantly lower in patients with generalized lymphadenopathy and fever, agreeing with **Cimmino et al. 2015** ⁽¹⁶⁾ indicating the role of TET1 downregulation in pathogenesis of ALL.

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

5. Conclusion

According to the results of our study, the TET1 gene was significantly upregulated in AML, NOS, and T lymphoblastic leukemia/lymphoma. The TET1 gene was significantly downregulated in B lymphoblastic leukemia/lymphoma.

As well, 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.

This may reveal that the TET1 gene has dual roles in myeloid and lymphoid hematological malignancies even within lymphoid leukemias, TET1 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 the TET1 as a potential target for treating this form of hematopoietic malignancy.

Abbreviations

Item	Abbreviation
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
7. Tables	

Item	Abbreviation
WBCs	white blood cells
WHO	World Health Organization
7. Tables	

Table 1
Age and sex of studied groups

	Patients (n = 73)	Control (n = 20)	P-value*
Age	19.7 ± 17.8	17.3 ± 16.1	0.585
Mean ± SD	13 (2–76)	14 (3–62)	
Median (min-max)			
Sex			
Male	44 (60.3%)	10 (50%)	0.409
Female	29 (39.7%)	10 (50%)	
* Mann-Whitney and Chi-square tests were used			

Table 2
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

Table 3
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)		

Table 4
Haematological parameters of acute myeloid leukemia patients (n = 37)

Variable	(min-max)	Mean \pm SD
WBCs (x10 ³ /mL)	(1.1–278)	69.2 \pm 68.7
WBCs blasts (%)	(7–98)	70.9 \pm 22.6
Hemoglobin (g/dL)	(5–13)	8.7 \pm 2
Platelets (x10 ⁹ /L)	(5–161)	57.1 \pm 47.2
BMA blasts (%)	(25–98)	78.5 \pm 20.5

Table 5
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

Table 6
Clinical and laboratory characteristics of ALL 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).		

Table 7
Haematological parameters of acute lymphoblastic leukemia patients (n = 36)

Variable	(Min-Max)	Mean \pm SD
WBCs ($\times 10^3/\text{mL}$)	(2.2–156)	61.3 \pm 48.5
WBCs blasts (%)	(18–96)	68.9 \pm 26.2
Hemoglobin (g/dL)	(6–14)	9.5 \pm 1.9
Platelets ($\times 10^9/\text{L}$)	(5–241)	60.9 \pm 53.4
BMA blasts (%)	(40–98)	86.9 \pm 12.8

Table 8
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 \pm SD)	21.4 \pm 17.6	17.9 \pm 18.5	0.467
Sex			
• Male	24 (54.5%)	20(45.5%)	0.731
• Female	17 (58.6%)	12 (41.4%)	

Table 9
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	

Table 10
TET1 expression levels in acute myeloid leukemia patients

AML subtype	Up regulation	Down regulation	P-value*
AML, NOS	22 (66.7%)	11 (33.3%)	0.007[^]
AML without maturation	4 (66.7%)	2 (33.3%)	0.618
AML with maturation	5 (83.3%)	1 (16.7%)	
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)

Table 11
Clinical characteristics of studied acute myeloid leukemia patients (n = 37) in relation to TET 1 gene expression

Symptoms	Upregulation	Downregulation	P-value*
	n = 23	n = 14	
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)			

Table 12
Haematological parameters of studied AML patients according to TET 1 gene expression

Variable	Upregulation	Downregulation	P-value*
	n = 23 (Mean ± SD)	n = 14 (Mean ± SD)	
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)

Table 13
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 [^]

Table 14
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)			

Table 15
Haematological parameters of studied ALL 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)

8.Declarations

Declarations

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 all requirement as governed by declaration of Helsinki.

Consent for publication: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/ relative of the patient. A copy of the consent form is available for review by the Editor of this journal.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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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.

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Tables

Table 1. Age and sex of studied groups

	Patients (n=73)	Control (n=20)	P-value*
Age			
Mean ± SD	19.7 ± 17.8	17.3 ± 16.1	0.585
Median (min-max)	13 (2-76)	14 (3-62)	
Sex			
Male	44 (60.3%)	10 (50%)	0.409
Female	29 (39.7%)	10 (50%)	

* Mann-Whitney and Chi-square tests were used

Table 2. 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

Table 3. 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)

Table 4. Haematological parameters of acute myeloid leukemia patients (n= 37)

Variable	(min-max)	Mean ± SD
WBCs (x10 ³ /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 (x10 ⁹ /L)	(5 – 161)	57 .1± 47.2
BMA blasts (%)	(25 – 98)	78.5 ± 20.5

Table 5. 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

Table6. Clinical and laboratory characteristics of ALL 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).

Table 7. Haematological 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

Table 8. 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%)	

Table 9. 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	

Table 10. TET1 expression levels in acute myeloid leukemia patients

AML subtype	Up regulation	Down regulation	P-value*
AML, NOS	22 (66.7%)	11 (33.3%)	0.007[^]
AML without maturation	4 (66.7%)	2 (33.3%)	0.618
AML with maturation	5 (83.3%)	1 (16.7%)	
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)

Table11. Clinical characteristics of studied acute mueloid leukemia patients (n=37) in relation to 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)

Table12. Haematological parameters of studied AML 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)

Table13. 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^

Table14. 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)

Table15. Haematological parameters of studied ALL 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)

Figures

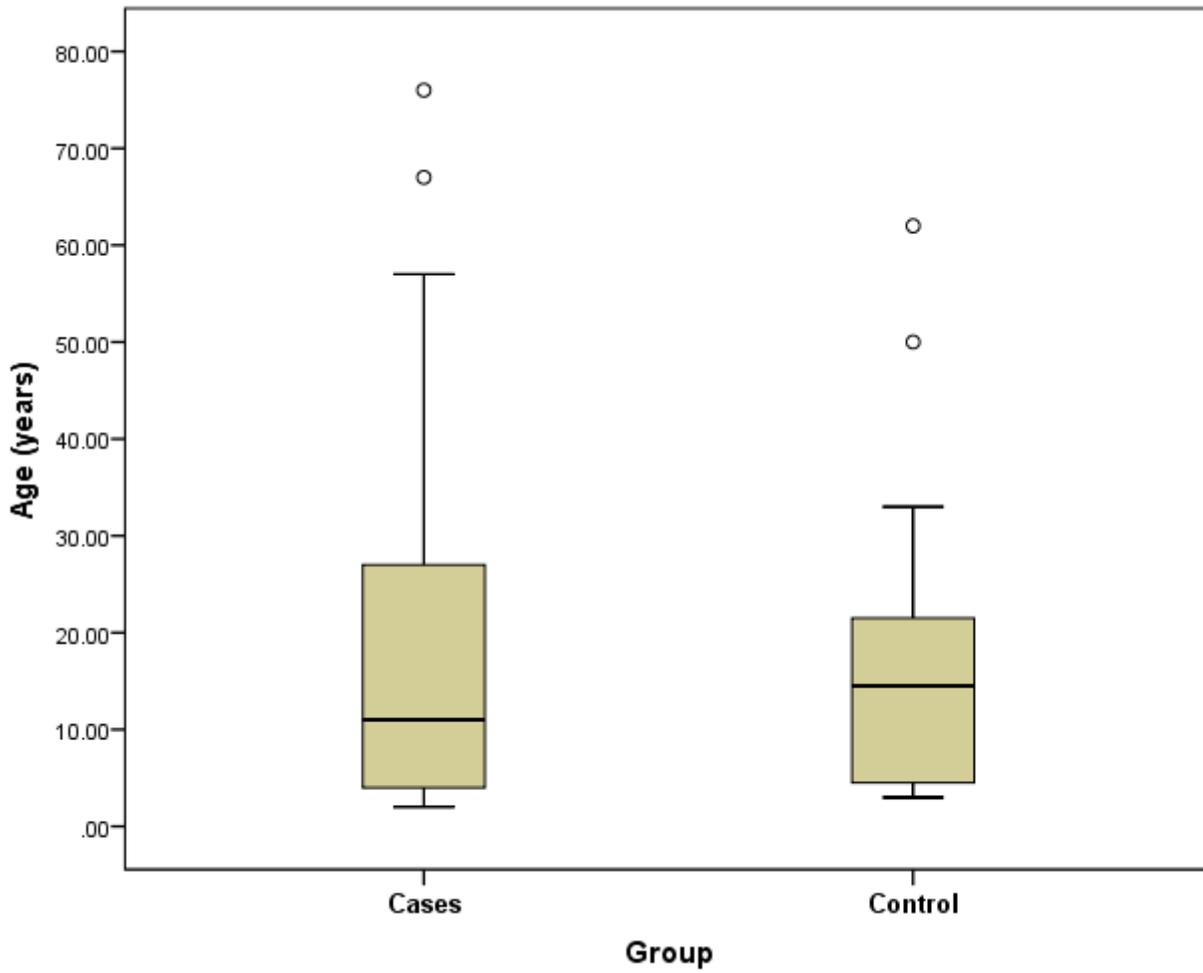


Figure 1

Boxplot of age of study groups

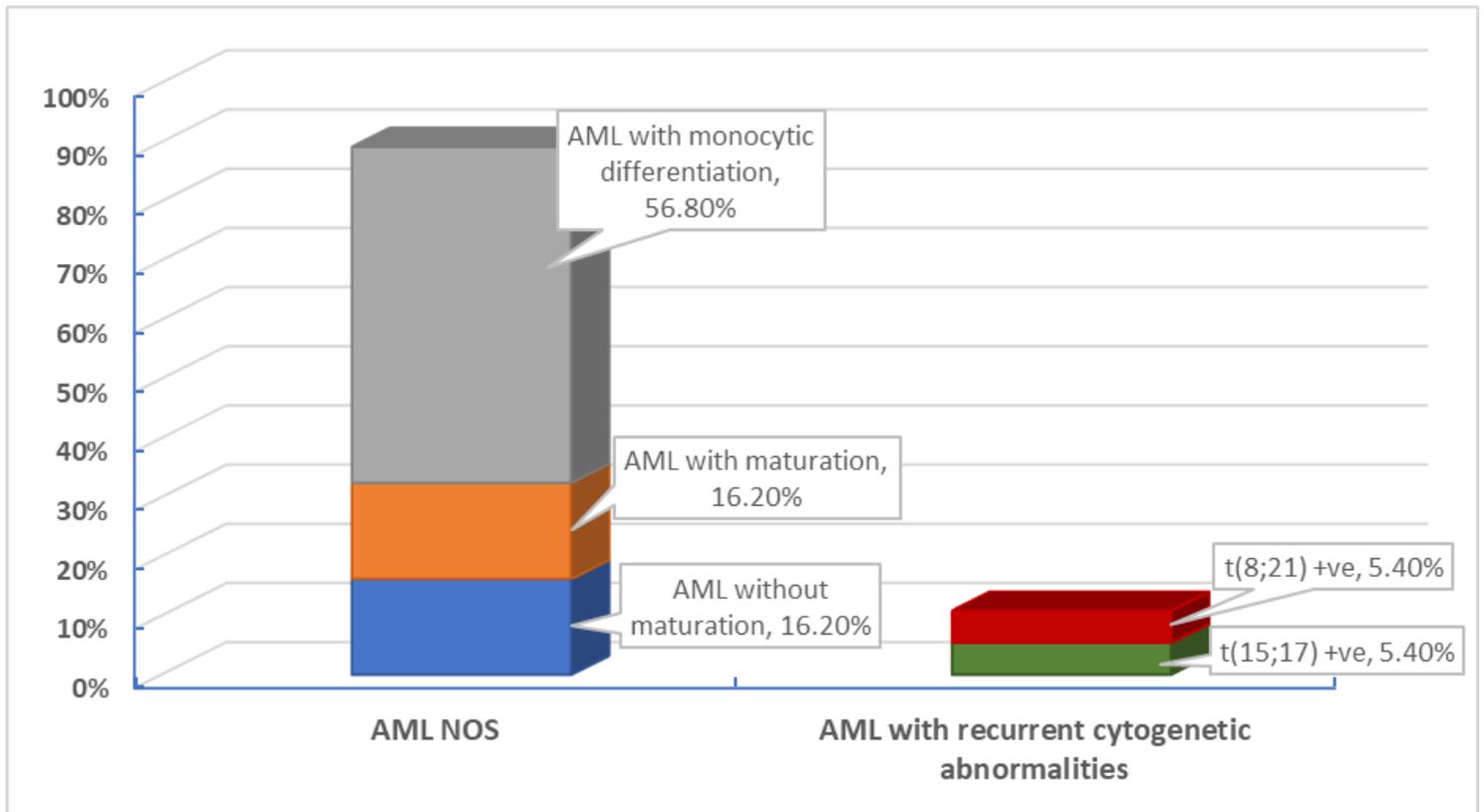


Figure 2

Classification of studied Acute myeloid leukemia patients

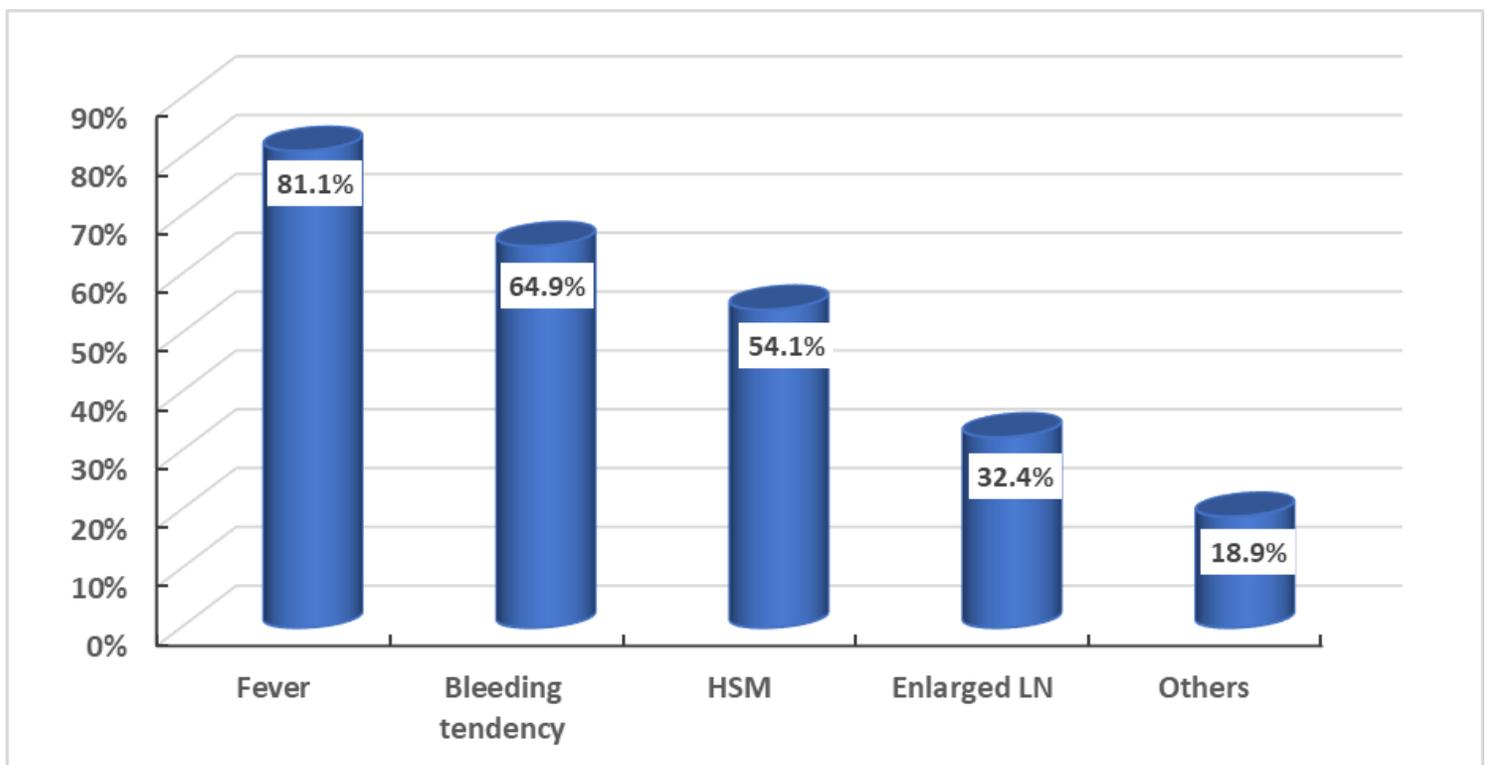


Figure 3

Presenting symptoms of studied acute myeloid leukemia patients

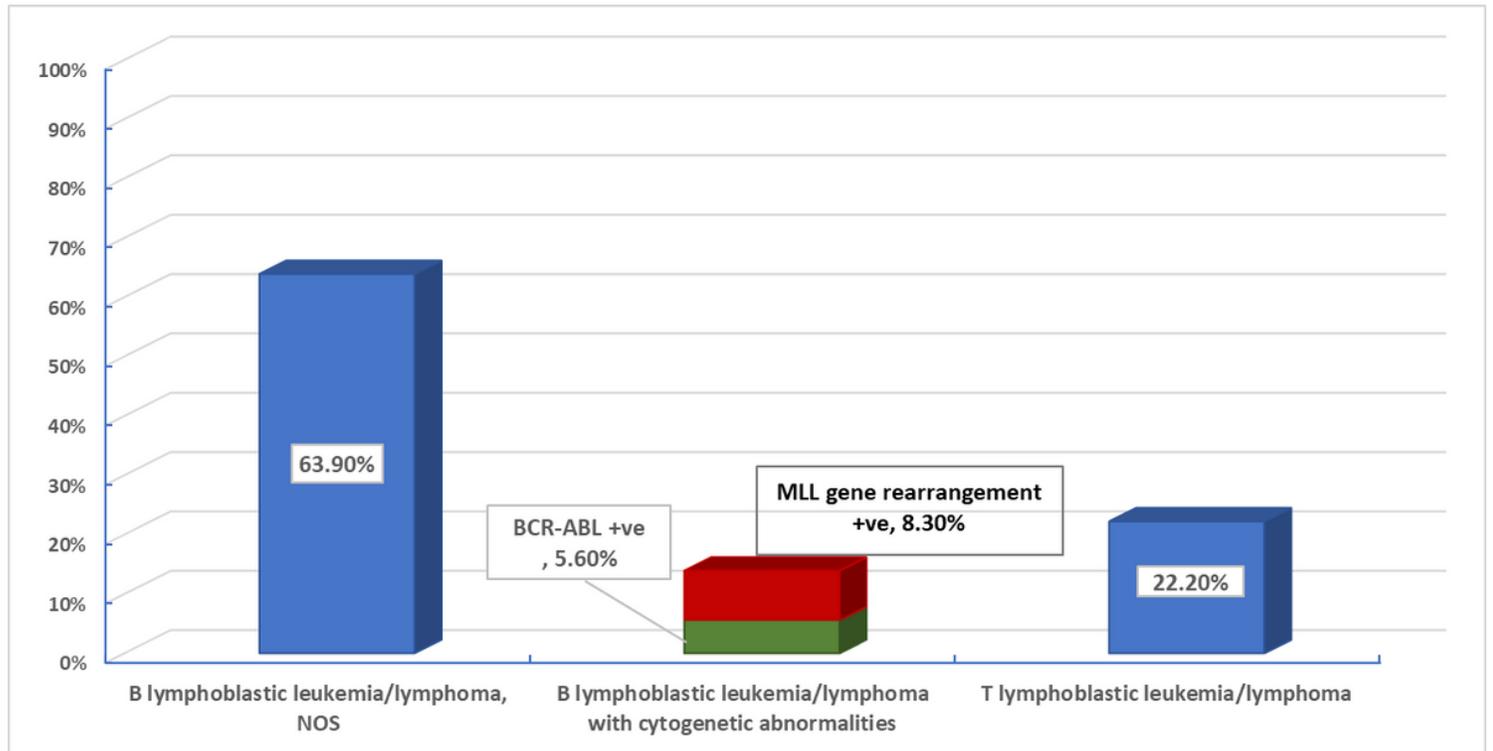


Figure 4

Classification of studied acute lymphoblastic leukemic patients

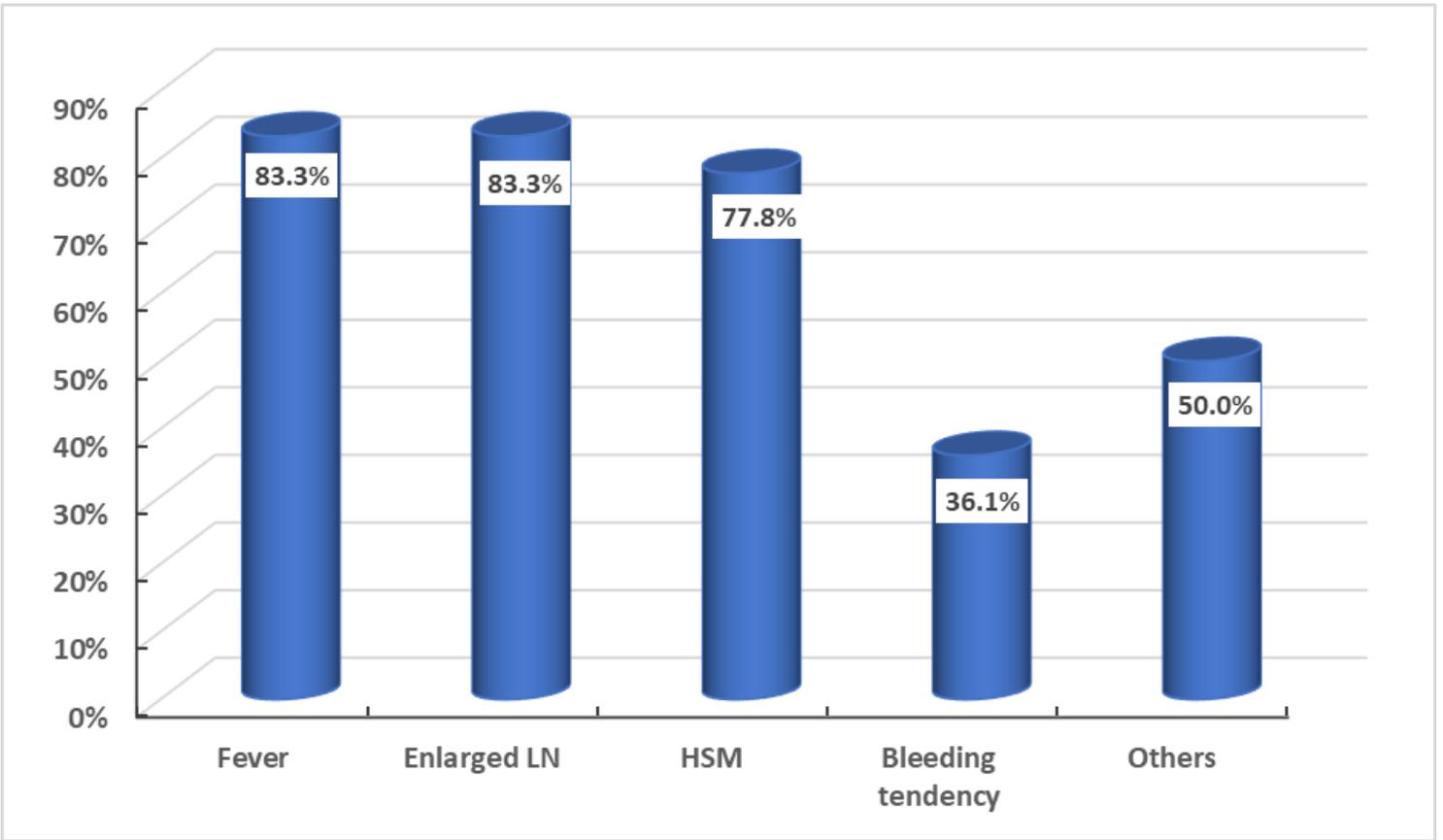


Figure 5

Presenting symptoms of studied acute lymphoblastic leukemia patients.

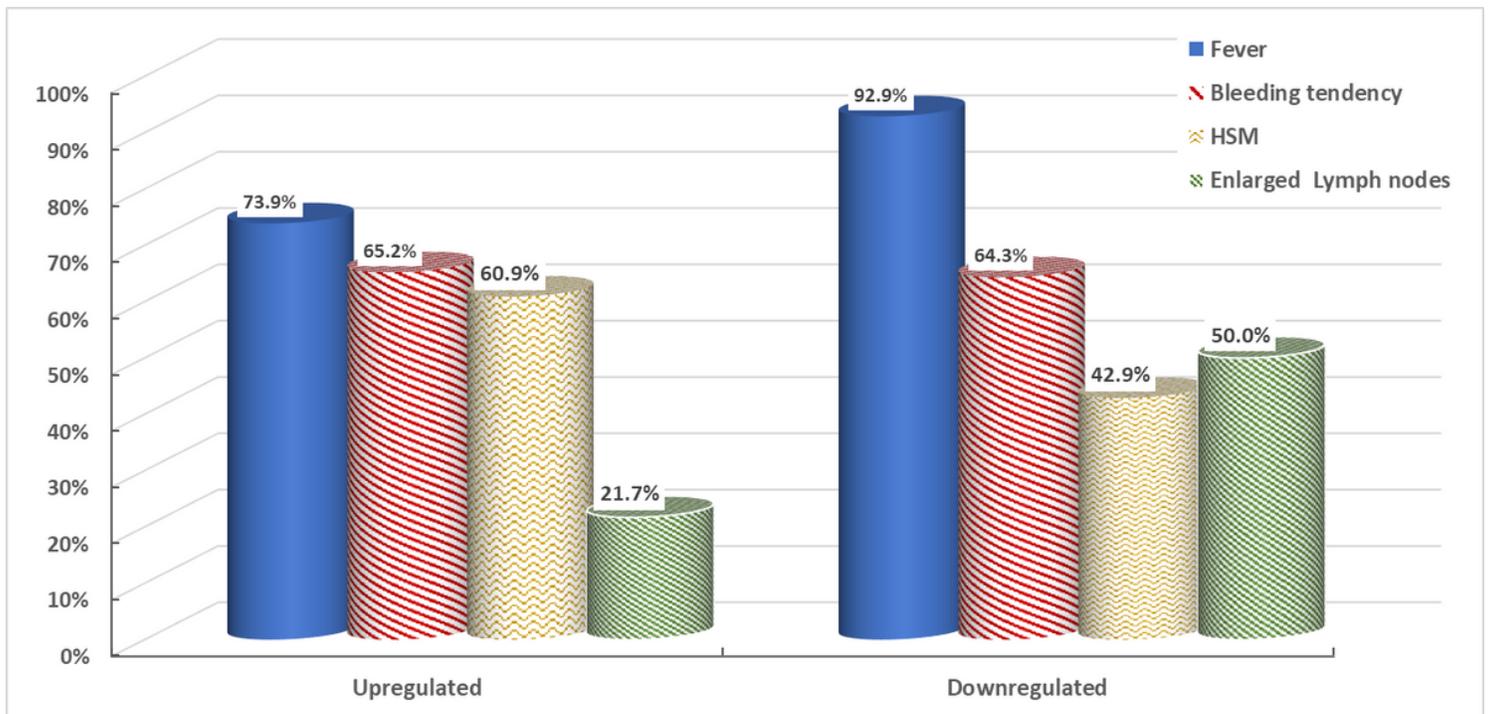


Figure 6

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

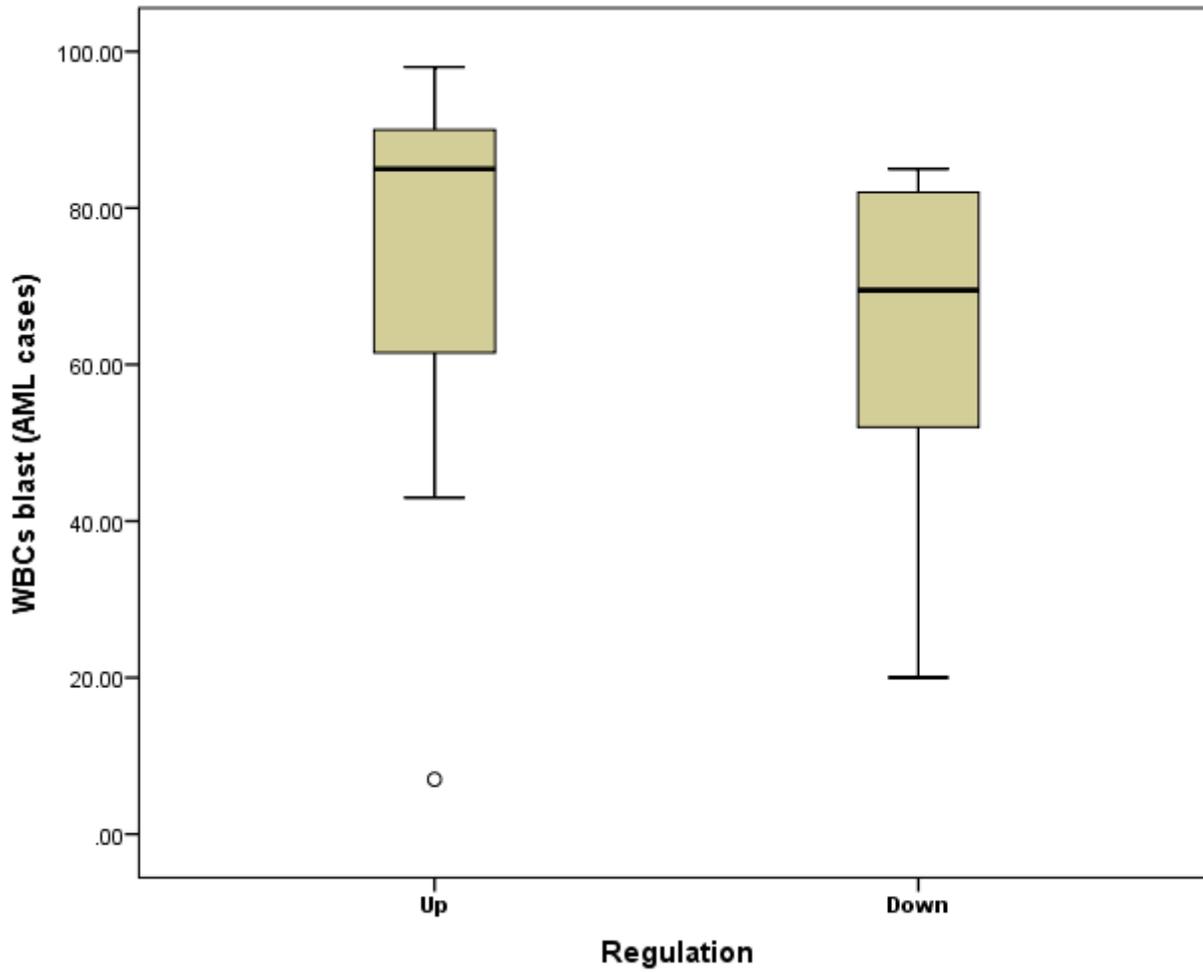


Figure 7

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

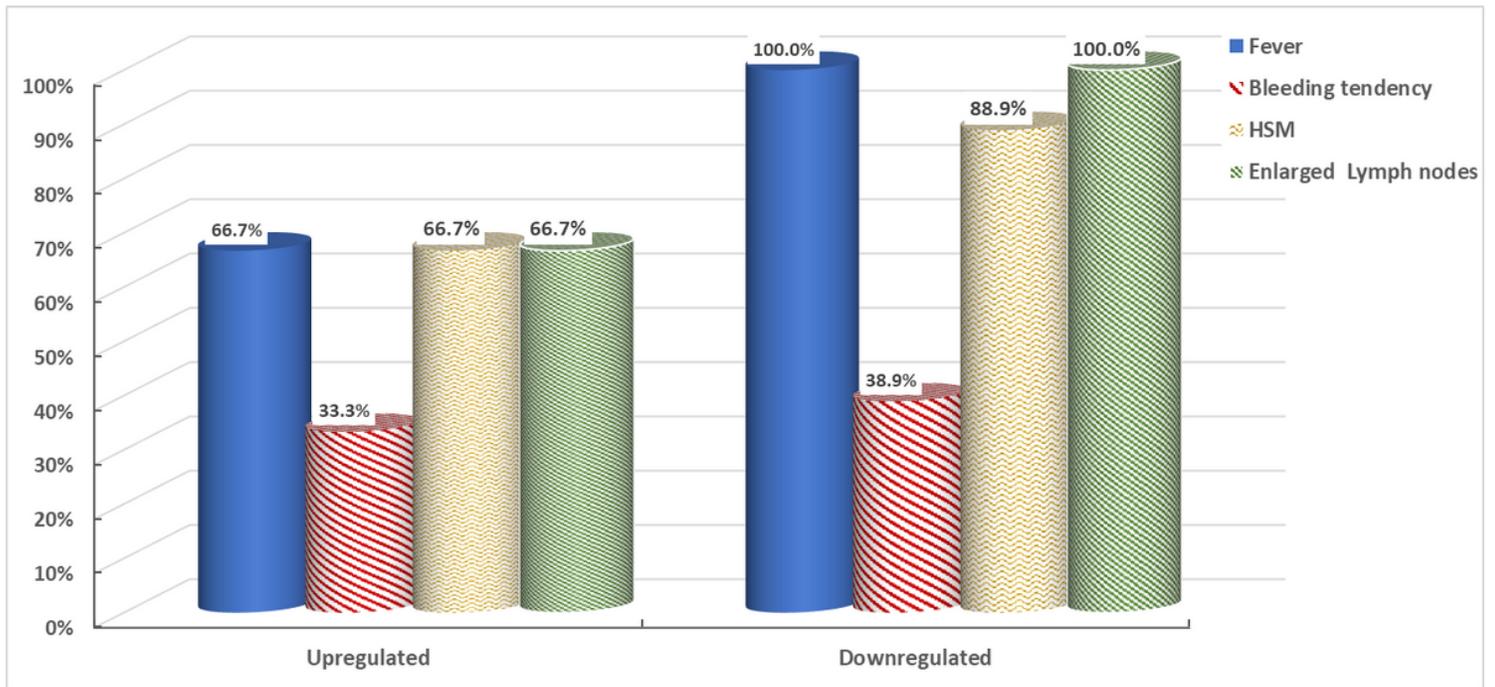


Figure 8

Clinical characteristics of studied acute lymphoblastic leukaemia patients in relation to TET 1 gene expression

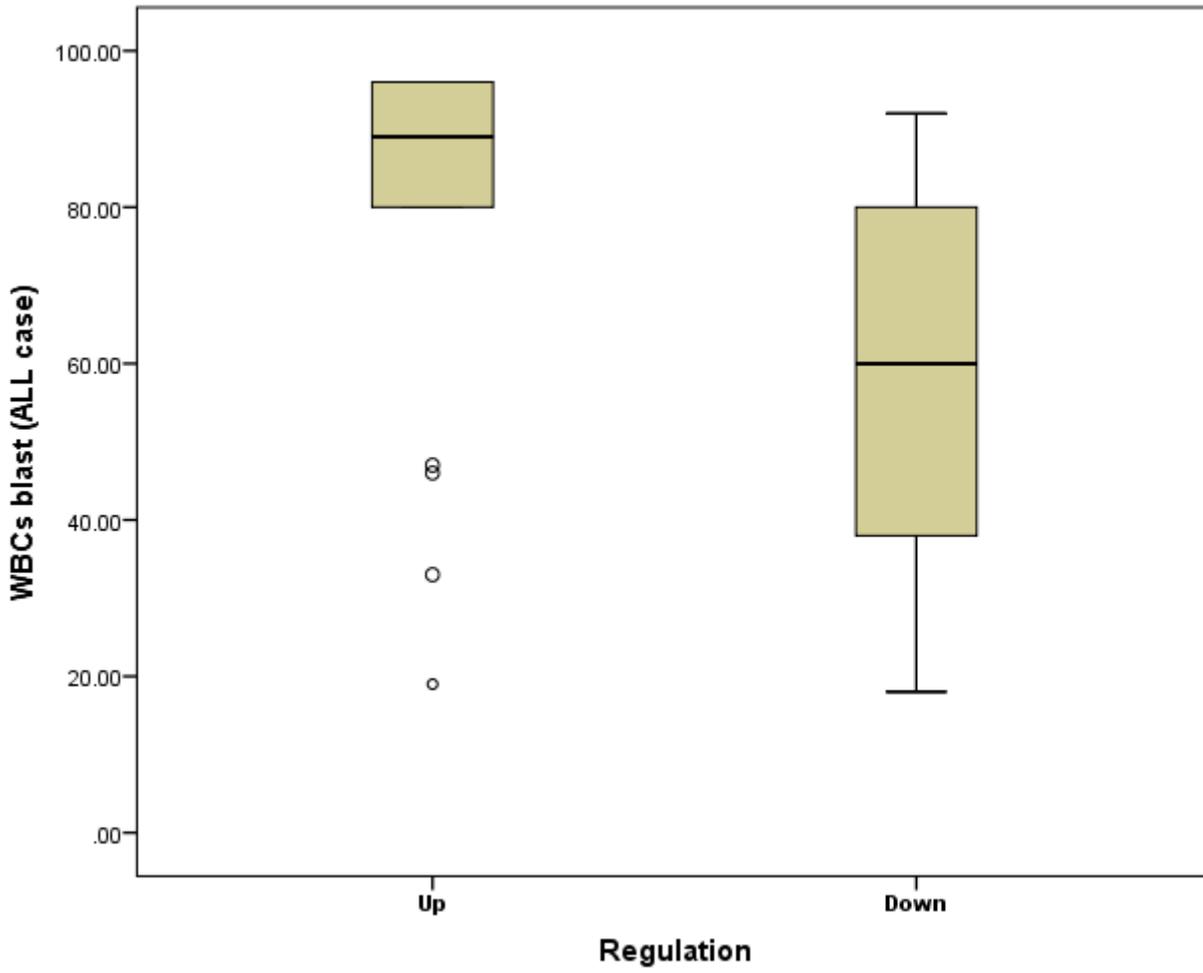


Figure 9

Hematological parameters of studied ALL patients according to TET 1 gene expression