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Research article

Phe354Leu polymorphism of the liver kinase B1 gene as a prognostic factor in adult egyptian patients with acute myeloid leukemia

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ABSTRACT

Background: The human liver kinase B1 (*LKB1*) gene is a significant tumor suppressor widely expressed in all fetal and adult tissues. Despite its established role in solid tumors, the biological and clinical implications of *LKB1* gene alterations in hematological malignancies have not been sufficiently recognized.
Aim: This study aimed to determine the frequency of the *LKB1* Phe354Leu polymorphism in adult Egyptian patients with cytogenetically normal AML (*C*N-AML), evaluate its clinical prognostic significance, and investigate its effect on the therapeutic outcome and patient survival.
Methods: Direct sequencing of amplified exon eight of the *LKB1* gene was performed to detect the Phe354Leu polymorphism in 72 adult de novo *C*N-AML patients.
Results: The *LKB1* Phe354Leu polymorphism was detected in 16.7% of patients and associated with younger age and lower hemoglobin levels ($p < 0.001$). Patients in the mutated group had significantly higher total leukocytic count and bone marrow blasts ($p = 0.001$ and $p < 0.001$, respectively). The most common FAB subtypes in mutated patients were M4 and M2. The relapse rate was significantly higher in the mutated group ($p = 0.004$). There was a significant association between the FLT3-ITD polymorphism and *LKB1* F354L ($p < 0.001$). The mutated group had shorter overall survival ($p = 0.003$). In multivariate analysis, the Phe354Leu polymorphism was a significant independent prognostic variable for the overall and disease-free survival of the studied patients ($p = 0.049$).
Conclusion: The *LKB1* Phe354Leu polymorphism was diagnosed at younger ages in Egyptian *C*N-AML patients and represented a poor independent prognostic factor in *C*N-AML. Patients who carried this polymorphism had shorter overall survival and more frequent relapses. Our findings may provide insight into the design of therapeutic targets, and molecular testing of the *LKB1* gene is recommended for proper risk stratification of *C*N-AML patients.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease in adults with varying presentations and responses to therapy, and further research is necessary to gain insights into its pathogenesis [1]. Approximately 50% of AML patients have a normal karyotype

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and are classified as intermediate risk [2,3]. This group is diverse, and additional molecular markers are increasingly crucial for prognostic assessment and treatment decisions [4,5]. Many patients with cytogenetically normal AML (*C*N-AML) lack reliable prognostic markers and thus require further molecular characterization [6].

The liver kinase B1 (*LKB1*) gene, also known as serine/threonine kinase 11 (STK11), is a tumor suppressor gene located on chromosome 19p13.3 spanning 23 kb and composed of nine coding exons and one non-coding exon [7,8]. *LKB1* encodes 2.4 kb mRNA transcribed from telomeres to centrosomes [9]. The *LKB1* protein is approximately 50 kDa serine/threonine kinase with 433 amino acids (aa) in humans [10]. Its catalytic domain is unrelated to any known protein kinase and spans from aa49 to aa309 [11,12].

LKB1 is widely expressed in almost all fetal and adult tissues. However, it is expressed to a greater extent in fetal tissues, with its leading expression site being the testicular epithelial and seminiferous tubules [13,14]. There are two widely expressed splice variants: *LKB1*L (long) and *LKB1*S (short) [15,16]. *LKB1* is located in the nucleus, with a small portion in the cytoplasm. It forms a heterotrimeric complex with MO25 and the STE20-related adaptor (STRAD) that regulates the activity and stability of the kinase [17,18].

LKB1 is the primary kinase of 13 AMPK-associated protein kinases. It phosphorylates AMPK and AMPK-like kinases to regulate polarity, adhesion, metabolism, growth, and cell survival [19]. Moreover, *LKB1* regulates other cellular processes, such as DNA damage checkpoints and various signal transduction pathways [20]. *LKB1* is mutated or deleted in Peutz-Jeghers syndrome (PJS) and various cancers [10]. It is inactivated in non-small-cell lung cancer (NSCLC), malignant melanoma, and cervical cancer [21,22].

Clinical implications of *LKB1* gene alterations in hematological malignancies, particularly AML, have not been well established. This study aimed to determine the frequency of the *LKB1* Phe354Leu polymorphism in Egyptian adult patients with *C*N-AML, evaluate its clinical prognostic significance, and investigate its effect on therapeutic outcomes and patient survival. These findings may provide insights into the role of the *LKB1*/AMPK signaling pathway in the development of new anti-cancer drugs.

2. Materials and methods

This prospective cohort study was conducted at the Clinical Pathology Department of Zagazig University Hospital between December 2018 and July 2021. The study included 72 de novo AML patients admitted to the Medical Oncology Department. The study was approved by the Institutional Review Board of Zagazig University (approval number: ZU-IRB# 5015) and conducted as per the Helsinki Declaration. Written informed consent was obtained from all patients after thoroughly clarifying the nature and purpose of the study.

The inclusion criteria included newly diagnosed, *C*N-AML patients without any other evident malignancy before starting induction chemotherapy. Exclusion criteria included patients under 16, those who had relapsed, AML related with therapy, and individuals with acute promyelocytic leukemia (M3).

Patients underwent a comprehensive medical evaluation, including thorough history taking, clinical examination, and basic laboratory investigations. CBC using Sysmex Xn (Sysmex, Japan), ESR using VISION–B (YHLO Biotech, China), liver and kidney functions, and serum lactate dehydrogenase (LDH) using Cobas 6000 (Roche Diagnostics, Germany), PT and APTT using CS-1500 (Sysmex, Japan), aspiration of the bone marrow and assessment using Leishman and peroxidase stains, immunophenotyping utilizing a FACSCanto II flow cytometer with Diva software (Becton Dickinson, USA), cytogenetic analysis (karyotyping), and PCR for FLT3-ITD mutant identification were performed. Radiological examinations such as Pevli-abdominal sonography, echocardiography, and chest X-rays were conducted.

2.1. LKB1 gene amplification

The genomic DNA extraction was performed using the QIAamp DNA Blood Kit (cat. No. 51183, Qiagen GmbH, Hilden, Germany) and spin column technique for diagnosing bone marrow mononuclear cells according to the manufacturer's guidelines. The *LKB1* phe354Leu mutation was detected by amplifying *LKB1* exon 8 using the forward primer 5′-GAG CTG GGT CGG AAA ACT G-3′ and the reverse primer 5′-AGA AGCTGT CCT TGT TGC AGA-3' [23]. The PCR reaction was carried out in a final volume of 25 μl containing 12.5 μl of Taq PCR Master Mix (cat. No. 201443, Qiagen, Germany) containing dNTPs, MgCL 2, hot start Taq DNA polymerase, and Taq buffer, 1 μl of each primer (10 pmol), 200 ng of genomic DNA, and nuclease-free water up to 25 μl. The reaction was subjected to denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. The PCR products were checked on 2.0% agarose gel electrophoresis and visualized as bands at 354 bp after staining with ethidium bromide on an ultraviolet transilluminator. The amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's guidelines.

Cycle sequencing was performed using the Big Dye Terminator Ready Reaction Cycle Sequencing Kit V3.1 (cat. No. 4337454, Thermo Fisher Scientific Inc., Applied Biosystem, USA), followed by purification of the products with the Big Dye X Terminator Purification Kit (Thermo Fisher Scientific Inc., Applied Biosystem, USA), according to the manufacturer's guidelines. The purified products were sequenced using the Applied Biosystems ABI PRISM 3500 Genetic Analyzer (Applied Biosystem, USA), and Sequencing Analysis v5.4 software was employed to analyze the results (Applied Biosystems). The data obtained were analyzed and interpreted using the web tool Nucleotide Blast (https://blast.ncbi.nlm.nih.gov).

Follow-up of the patients was performed on day 21 after induction chemotherapy by conducting a complete blood cell count and bone marrow aspirate to assess their remission status. Complete remission (CR) was defined as normocellular bone marrow containing less than 5% blast cells and peripheral blood with at least 1×10^9/L neutrophils and $\geq 100 \times 10^9$/L platelets. Patients who achieved remission received three to four courses of high-dose cytarabine (1–2 g/m^2 every 12 h on days 1, 3, and 5; total, 12 g/m^2) as consolidation [24]. The patients were followed once every three months with physical examinations and complete blood cell counts. A

marrow examination was performed for any doubt of relapse on clinical examination or blood smear. The patients were followed up for 18 months to evaluate overall survival (OS) and disease-free survival (DFS).

2.2. Treatment plan and follow-up

The patients were administered an induction 3 + 7 regimen, consisting of a continuous infusion of cytarabine (100 mg/m^2) every day for seven days, combined with three days of doxorubicin (30 mg/m^2). Patients over 60 years of age or with poor performance status were treated with the 2 + 5 regimen (cytarabine 100 mg/m^2 daily for five days with three days of doxorubicin 25 mg/m^2) or low-dose cytarabine 10 mg/m2/12 h for 14 days. For the consolidation, three to four courses of high-dose cytosine arabinoside (3 g/m^2 every 12 h on days 1, 3, and 5; a total of 18 g/m^2) were utilized [24].

On day 21, following induction chemotherapy, CBC and bone marrow aspirates were conducted to evaluate the patient's progress toward remission. Complete remission was defined as normocellular bone marrow with fewer than 5% blast cells and peripheral blood with at least 1 × 10^9/L neutrophils and ≥100 × 10^9/L platelets [24]. Patients underwent complete blood cell counts and physical examinations once every three months. If there were indications of relapse based on a clinical examination or blood test, a bone marrow investigation was carried out. The OS and DFS of the patients were evaluated after 18 months of follow-up.

2.3. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 28 (IBM, Armonk, New York, USA) was employed for data analysis.

Table 1
Demographic and clinical data in patients' groups.

Variable	All patients (72)		Mutated (12)		Wild (60)		Test of Sig	p-value
Sex (M/F)	40/32		8/4		32/28		$\chi2 = 0.72$	0.396
Age, (years) mean ± SD (Range)	47.79 ± 15.30 (23–86)		35.42 ± 4.78 (25–45)		50.27 ± 15.50 (23–86)		t = 6.112*	<0.001*
Clinical data (n, %)							$\chi2$	
Anemic symptom	50	69.4	12	100	38	63.3	6.336*	0.013*
Bleeding	26	36.1	6	50.0	20	33.3	1.204	0.330
Gum swelling	14	19.4	0	0.0	14	23.3	3.476	0.106
L.N	14	19.4	2	16.7	12	20.0	0.071	1.000
Fever	64	88.9	12	100	52	86.7	1.800	0.337
Bony pain	50	69.4	12	100	38	63.3	6.336*	0.013*
Ultrasound (n, %)							$\chi2$	
Spleen	48	66.7	6	50.0	42	70.0	1.800	0.197
Liver	14	19.4	4	33.3	10	16.7	1.773	0.231
LN	14	19.4	2	16.7	12	20.0	0.071	1.000
ESR mean ± SD (Range)	85.82 ± 21.31 (28–126)		84.83 ± 22.89 (38–126)		86.02 ± 21.18 (28–116)		t = 0.174	0.862
LDH Median (Range)	340 (137–1230)		750 (440–1230)		320 (137–1190)		U = 115.0*	<0.001*
WBCs (103/μl) Median (Range)	73.5 (1.3–192)		118.5 (80–150)		64.25 (1.3–192)		U = 130.50*	0.001*
Hb (g/dl) mean ± SD (Range)	8.14 ± 1.18 (5.50–11 =)		7.03 ± 1.03 (5.5–8.5)		8.36 ± 1.09 (6–11)		t = 3.878	<0.001*
Platelets(103/μl) Median (Range)	30.5 (10–106)		24.50 (14–87)		31 (10–106)		U = 312.0	0.468
BM Blast (%) Median (Range)	58 (26–93)		87.5 (77–92)		55 (26–93)		U = 68.00*	<0.001*
FAB (n, %)							$\chi2$	
M0	2	2.8	0	0.0	2	3.3		
M1	6	8.3	2	16.7	4	6.7		
M2	20	27.8	4	33.3	16	26.7		
M4	34	47.2	6	50.0	28	46.7	4.122	0.683
M5	8	11.1	0	0.0	8	13.3		
M6	1	1.4	0	0.0	1	1.7		
M7	1	1.4	0	.0	1	1.7		
CR (Not in CR/CR) n (%)	30/42 (41.7/58.3)		6/6 (50/50)		24/36 (40/60)		$\chi2 = 0.411$	0.521
Death (dead/Alive) n (%)	32/40 (44.4/55.6)		8/4 (66.7/33.3)		24/36 (40/60)		$\chi2 = 2.88$	0.090

$\chi2$: chi-square test; Student's t-test; U: Mann–Whitney test; *: statistically significant at $p \leq 0.05$; WBCs: white blood cells count; HB: hemoglobin level; BM: bone marrow; ESR: erythrocyte sedimentation rate; LDH: lactate dehydrogenase; FAB: French-American-British classification; CR: complete remission.

Quantitative variables were expressed using their means, standard deviations, medians, and ranges, depending on the type of data. Categorical variables were compared using the chi-square test and expressed using their absolute frequencies. The chi-square trend test was used to compare ordinal data between the two groups. The Shapiro-Wilk test was applied to validate the assumptions employed in parametric tests. The Mann–Whitney test (for data that are not normally distributed) and the independent sample *t*-test (for data that are normally distributed) were used to compare quantitative data between the two groups.

The log-rank test (Mantel-Cox test) was used to compare estimates of the hazard functions of the two groups at each recorded event time. The Cox model, a regression technique, was used to estimate the adjusted hazard ratio (HR) of a given endpoint linked with a certain risk factor. Statistical significance was set at $p < 0.05$; a highly significant difference was evident at $p \leq 0.001$. All factors significant in univariate analysis were used for multivariate Cox regression.

3. Results

To determine the frequency of the Phe to Leu polymorphism (Phe354Leu) of the *LKB1* gene in our study, we screened 72 adult patients with de novo *C*N-AML by PCR of *LKB1* exon 8, followed by direct sequencing. Their ages ranged from 23 to 86 years, with a median of 45 years, and comprised 55.6% males and 44.4% females. Wild *LKB1* was detected in 60/72 patients (83.3%), with Phe 354 normally encoded by the TTC codon (wild group), while the remaining 12/72 patients (16.7%) carried mutated *LKB1* encoded by the TTG (mutated group). This reveals the c.1062C > G polymorphism, resulting in a Phe354Leu change in the protein. All the polymorphisms were heterozygous missense point mutations. Additionally, we identified two novel, previously unreported mutations in *LKB1*: c.1087 A > G, p. T363A detected in three patients, and c.1023 T > G, p. L341W in two other patients. These were heterozygous missense point mutations.

There was no statistically significant difference in sex between the two groups of patients. However, the age of the mutated group was significantly lower than that of the wild group ($p < 0.001$). There was no statistically significant difference in clinical characteristics and ultrasound findings between the two groups of patients, apart from anemic symptoms and bone aches that were significantly more evident in the mutated group than in the wild group ($p = 0.013$). The hemoglobin level was statistically significantly lower in the mutated group than in the wild group ($p < 0.001$). The total leukocytic count, percentage of BM blasts, and serum LDH levels were higher in the mutated group than in the wild group ($p = 0.001$). The demographic, clinical, and laboratory features of both patient groups are summarized in Table 1.

There was no statistically significant difference between the two groups of patients in the morphological pattern of AML, according to the Franco-American-British (FAB) classification (Table 1). The most common FAB subtypes noticed in the mutated group were M4 (6/12, 50%), followed by M2 (4/12, 33.3%); the M1 subtype was found only in two cases, and none of them had M0, M5, or M6 ($p = 0.9$) (Table 1). The FLT3-ITD was found in 23.6%. There was a significant association between the FLT3-ITD mutation and *LKB1* Phe354Leu. (66.7%, 8/12) of the mutated group had concurrent mutations compared to (15%, 9/60) of the wild group ($p < 0.001$) (Table 4).

Table 2
Univariate and multivariate Cox analysis of overall survival factors.

	Mean ± SD	Univariate			Multivariate		
		P1 value	Crude hazard ratio	(95% CI)	P2 value	Adjusted hazard ratio	(95% CI)
Sex:							
Male	12.33 ± 0.88	0.037*	2.14	(1–4.52) *			
Female	14.91 ± 0.89				0.09	1.9	(0.9–4.11)
Age:							
≤ 45	13.06 ± 0.98	0.753	1.12	(0.56–2.23)			
> 45	13.89 ± 0.83						
WBCs (10^3/μl):							
≤ 67.5	13.33 ± 0.87	0.505	1.3	(0.63–2.52)			
> 67.5	13.59 ± 0.94						
HB (g/dl):							
≤ 9	13.55 ± 0.69	0.991	1.01	(0.4–2.61)			
> 9	13.08 ± 1.79						
Platelet (10^3/μl):							
≤ 31	13.28 ± 0.8	0.411	1.3	(0.66–2.74)			
> 31	13.7 ± 1.05						
FLT3-ITD:							
Absent	9.94 ± 1.26	<0.001**	3.23	(1.58–6.58)	0.027*	2.47	(1.11–5.49)*
Present	14.56 ± 0.69						
BM blast%:							
≤ 58%	13.56 ± 0.85	0.808	1.1	(0.54–2.18)			
> 58%	13.39 ± 0.98						
Phe354Leu:							
Mutated	8.83 ± 1.94	0.003**	3.1	(1.4–7.1) *	0.049*	1.98	(1–4.7)
Wild	14.4 ± 0.6						

CI: Confidence interval; OS: overall survival; WBCs: white blood cells count; HB: hemoglobin level; BM: Bone marrow; p1: log rank test; p2: Cox regression; *: statistically significant at $p \leq 0.05$; **: statistically highly significant at $p \leq 0.001$.

Among *C*N-AML patients, 42 out of 72 (58.3%) achieved complete remission (CR). The CR rate was higher in patients with wild *LKB1* (36/60, 60%) than in patients with mutated *LKB1* (6/12, 50%), although this difference was not statistically significant (Table 1).

Concerning treatment outcomes during the follow-up period, 66.6% (24/36) of the wild group remained in CR, while 33.3% (12/36) of those who achieved CR relapsed. In contrast, all those who achieved CR in the mutated group (6/6) experienced a relapse. There was a statistically significant increase in the relapse rate in the mutated group compared to the wild group ($p = 0.004$).

After an 18-month follow-up, 32 patients were deceased (44.4%), and 40 were alive (55.6%). No statistically significant difference was found in mortality rates between patient groups. The mean OS was 13.47 months (95% CI 12.2–14.74 months). The mean OS was significantly longer in wild *LKB1* cases (14.4 months; 95% CI 13.22–15.58 months) than in mutated cases (8.83 months; 95% CI 5.02–12.64 months) ($p = 0.003$) (Fig. 1). The mean DFS was 12.54 months (95% CI 11.19–13.9 months). The mean DFS was significantly shorter in mutated cases (4.66 months; 95% CI 3.57–5.76 months) than in wild *LKB1* cases (13.86 months; 95% CI 12.77–14.95 months) ($p < 0.001$).

Univariate analysis of different prognostic variables was performed to assess the prognostic significance of *LKB1* Phe354Leu for OS. It was found that *LKB1* Phe354Leu could be a negative prognostic marker for OS, male sex, and the FLT3-ITD mutation ($p = 0.003$, 0.037, and <0.001, respectively). In multivariate analysis, only the Phe354Leu and FLT3-ITD mutations were found to have a significant independent prognostic impact on the OS of the studied patients ($p = 0.049$ and 0.027, respectively) (Table 2).

Logistic regression was used to determine the effects of various parameters on the probability of DFS. There was a significant association between DFS and both Phe354Leu and FLT3, with both demonstrating significantly increased risk in univariate and multivariate analyses. There was no significant association between DFS and gender, age group, white blood cell count, platelet count, or BM blasts (Table 3).

4. Discussion

According to the WHO classification of hemato-lymphoid malignancies, cytogenetically normal acute myeloid leukemia (*C*N-AML) is distinct. In the past 15 years, several mutations in genes such as FLT3, NPM1, and CEBPA have been identified and demonstrated prognostic significance in *C*N-AML. Combining these molecular markers may help predict an unfavorable prognosis instead of relying on a single marker [25].

DNA methylation signature plays a crucial role in leukemogenesis. This signature involves serine glycine pathway enzymes as methylation substrate enhancers and *LKB1* as the primary regulator [26]. The molecular identification of the *LKB1*/AMPK signaling pathway as a cancer suppressor axis further supports the link between cancer and metabolism [27]. Furthermore, research on hematopoietic stem cells (HSC) and leukemia cells has highlighted the potential benefits of *LKB1*/AMPK modulation in hematological cancers [28,29]. However, genetic changes contributing to leukemogenesis and defining molecular risk in approximately 15–20% of *C*N-AML cases remain unknown [30].

Table 3
Univariate and multivariate Cox analysis of disease-free survival factors.

	Mean ± SD	Univariate			Multivariate		
		P1 value	Crude hazard ratio	(95% CI)	P2 value	Adjusted hazard ratio	(95% CI)
Sex:							
Male	12.35 ± 1.08	0.868	1.1	(0.4–2.7)			
Female	12.73 ± 0.88						
Age:							
≤ 45	11.55 ± 1.04	0.106	2.1	(0.8–5.8)			
> 45	13.65 ± 0.83						
WBCs (10^3/μl):							
≤ 67.5	13.48 ± 0.82	0.188	1.9	(0.7–4.8)			
> 67.5	11.62 ± 1.08						
HB (g/dl):							
≤ 9	11.94 ± 1.8	0.075	5.1	(0.7–38.2)			
> 9	15.13 ± 0.82						
FLT3-ITD:							
Present	7.75 ± 1.77	<0.001**	4.54	(1.68–12.3) *	0.032*	3.6	(1.11–11.63) *
Absent	13.68 ± 0.6						
Platelet (10^3/μl):							
≤ 31	11.47 ± 1.11	0.385	1.5	(0.6–3.8)			
> 31	13.44 ± 0.83						
BM blast%:							
≤ 58%	13.06 ± 0.87	0.908	1.1	(0.4–2.68)			
> 58%	12.17 ± 1.01						
Phe354Leu:							
Mutated	4.67 ± 0.56	<0.001**	44.6	(6.9–288) *	<0.001**	66.4	(7.25–607.5) *
Wild	13.86 ± 0.56						

CI: confidence interval; DFS: disease-free survival; WBCs: white blood cells count; HB: hemoglobin level; BM: Bone marrow; p1: log rank test; p2: Cox regression; **: statistically highly significant at $p \leq 0.001$.

Table 4
Association between Phe354Leu polymorphism and FLT3-ITD.

FLT3-ITD	Phe354Leu		Test	
	Mutated (12)	Wild (60)	Phi	P
Positive	8 (66.7%)	9 (15%)	0.453	<0.001[a]
Negative	4 (33.3%)	51 (85%)		

[a] Statistically highly significant at $p \leq 0.001$.

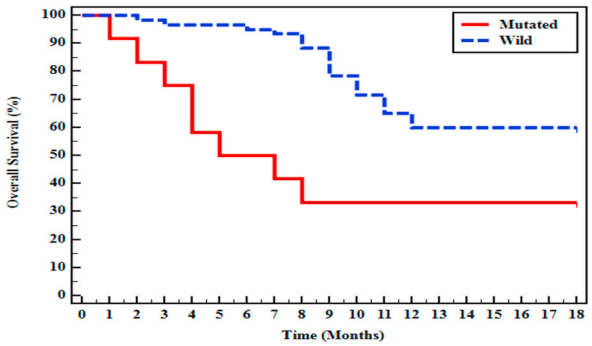

Fig. 1. Kaplan–Meier survival curve for overall survival of patients with Phe354Leu (n = 72).

This study aimed to assess the frequency of *LKB1* Phe354Leu in adult Egyptian patients with *C*N-AML, given the limited information available on this polymorphism in Egyptian patients, and to investigate its association with various clinical and laboratory findings. Additionally, the study aimed to determine the role of this polymorphism as a prognostic factor in adult de novo AML patients.

The study found that *LKB1* Phe354Leu was present in 16.7% of *C*N-AML patients, which is higher than the frequency reported by Yang et al. (7%) [23]. This polymorphism is located in the *C*-terminal region of the *LKB1* gene rather than in the kinase domain. This alteration leads to reduced *LKB1*-mediated AMPK activation, inhibits downstream signaling, and diminishes the ability of *LKB1* to maintain cell polarity. Therefore, this change in Phe354Leu is hypothesized to be associated with cancer predisposition [31].

In addition, the Phe354Leu mutation has been found in 6.3% of Koreans with left-sided colorectal tumors [32]. Other studies have reported that *LKB1* mutations are less common in Asian patients with NSCLC (3%) than in Caucasian patients (30%) [33,34]. However, our results are consistent with a study that found *LKB1* mutated in 13.6% of NSCLC cases and reported its association with the KRAS mutation in approximately 6.5% of cases [35].

Suzuki et al. also found that the Phe354Leu mutation was present in 14.4% of lung adenocarcinoma cases [36]. In intrahepatic cholangiocarcinoma, sequencing identified nine missense mutations in the *LKB1* gene, including Phe354Leu, in two cases [37].

A previous study reported a significant decrease in *LKB1* mRNA expression in 69% of AML cases [38]. Downregulation of *LKB1* and induced aberrant methylation can lead to genome instability, inactivation of cancer suppressor genes, or higher expression of oncogenes [38]. *LKB1*'s tumor suppressor activity in AML can be mediated by the repression of mTOR mRNA translation [39].

Using next-generation sequencing, researchers studying acute lymphoblastic leukemia found that genetic alterations in *LKB1* occurred in approximately 18% of cases. The most significant number of new mutations were detected in the *LKB1* gene [40].

The discrepancy in mutation frequency between studies may be due to various factors, such as differences in research design, racial background, or the type of malignancy. Additionally, the detection techniques used in various studies may have varying sensitivity levels, which can impact mutation frequency results.

Most studies have investigated *LKB1* gene alterations and their prognosis in solid tumors. However, this study focused on the clinical characteristics and laboratory results of AML patients. Our findings revealed that the age of the mutated group was significantly lower than that of the wild group. This indicates that younger AML patients with a polymorphism may benefit from allogeneic stem cell transplantation, consistent with previous publications showing that patients with *LKB1* Phe354Leu were younger [23,41]. However, other studies have shown no significant age difference [42]. In our study, there were no statistical differences in the polymorphism distribution concerning sex, which is consistent with that of Yang et al. [23].

Our patients in the mutated group showed a significant increase in WBC count, bone marrow blast percentage, and LDH level. In contrast, their hemoglobin levels were significantly lower than in the wild group. This finding can be explained by encroachment on normal BM erythropoiesis or its inhibition. Furthermore, this finding is consistent with a previous study that found Phe354Leu positive cases had higher WBC counts, lower hemoglobin values, and higher bone marrow blast cells [23]. This might be attributed to the impairment of *LKB1* function as a tumor suppressor gene, which normally suppresses cancer cell growth by inhibiting the cell cycle via p53 through AMPK signaling. Additionally, it reduces HIF-1, a transcription factor enhanced in hypoxic environments, activating the genes necessary for cancer cells to survive [43].

This study found a significant association between Phe354Leu and the FLT3-ITD mutation. AML patients with FLT3 have a high leukemic burden and poor prognosis [44]. This finding is consistent with another study in which Phe354Leu occurred concurrently

with FLT3, NPM, and CEBPA, significantly impacting AML patients' outcomes [23].

The most common FAB subtype found in the mutated group was M4, followed by M2, and only two patients had M1. However, this finding did not reach significance. This concurs with other studies that revealed that M4 was 50% in Phe354Leu-positive cases, followed by M2 [23].

There was no significant difference in the complete remission (CR) achievement rate between the mutated and wild groups. However, all those who achieved CR in the mutated group relapsed, indicating a significantly higher relapse rate than in the wild group. The current study is the first to examine the impact of the *LKB1* mutation on AML response to treatment. Therefore, we cannot compare our findings to those of previous studies. Nonetheless, studies have highlighted the negative prognostic value of *LKB1* mutations in patients with metastatic lung adenocarcinoma treated with either immunotherapy or chemotherapy [35]. Furthermore, the loss of *LKB1* was associated with an increased risk of recurrence and death in patients with resected colon cancer [42]. Patients with low *LKB1* expression had significantly worse tumor differentiation, larger tumors, early lymph node metastasis, advanced TNM stage, and poor treatment response [45].

In the present study, patients in the mutated group had significantly shorter OS than those in the wild group. The hazard ratio revealed that at any given time, the mutated group was 3.1 times more likely to have a shorter OS than the wild group, with statistical significance. This finding is consistent with a previous study that reported the unfavorable effect of *LKB1* mutation on AML [23].

Additionally, the current study found that the mean time of DFS was significantly shorter in the mutated group than in the wild group. *LKB1* Phe354Leu and FLT3-ITD were identified as independent prognostic factors in AML using multivariate Cox analysis. This finding is consistent with a study that concluded *LKB1* Phe354Leu might be crucial to leukemogenesis and could be considered a poor prognostic factor with a shorter DFS [23]. A recent study has identified *LKB1* mutations as a driver of leukemic transformation, showing a more than 50% decrease in *LKB1* mRNA expression at the blast phase in myeloproliferative neoplasms [46].

A multivariate colon cancer study revealed that loss of *LKB1* expression was an independent prognostic factor for reduced DFS [42].

The study showed that *LKB1* Phe354Leu is present in many Egyptian patients with *C*N-AML (16.7%). This mutation was diagnosed at younger ages in Egyptian *C*N-AML patients and was associated with worse overall and DFS, representing a poor independent prognostic factor in *C*N-AML. Furthermore, it occurs concurrently with FLT3 mutations, which have a worse impact on survival. Some patients with this mutation may benefit from early hematopoietic stem cell transplantation treatment.

However, our study had limitations, such as the small sample size and being conducted in a single center. Further research using a larger *C*N-AML patient cohort with extensive molecular profiling data was required to determine the frequency and prognostic significance of the *LKB1* Phe354Leu polymorphism in the presence of other gene mutations. Additionally, studies are needed to illustrate the clinical implications of *LKB1* mutations in AML to aid in categorizing patients for more intensive therapy. Further research is also necessary to highlight the precise pathogenic role of the *LKB1*/AMPK signaling pathway, which can be used to develop targeted therapy for patients with this mutation. Molecular testing for the *LKB1* gene is recommended for proper AML risk stratification before starting treatment, providing insight into the design of new therapies.

Author contribution statement

Ola A. Hussein: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Hany A. Labib: Conceived and designed the experiments; Analyzed and interpreted the data.
Rasha Haggag: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Maha Mahmoud Hamed Sakr: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Ethical approval

All procedures involving human subjects complied with the Institutional Review Board's (IRB) ethical standards (ZU-IRB# 5015). The study was performed following the Helsinki Declaration and approved by the IRB.

Informed consent

All participants gave their informed consent.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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