Original Article

Dysregulation of *miR-30a-5p* and lncRNA *NORAD* Expression and Their Association with Autophagy Genes *ATG5* and *Beclin1* in Chronic Lymphocytic Leukemia: A Case-Control Study

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Received: 30 December 2025
Revised: 15 May 2026
Accepted: 06 June 2026

What's Known

- Chronic lymphocytic leukemia (CLL) is a common adult lymphoid malignancy with impaired apoptosis and dysregulated autophagy.
- Non-coding RNAs, including long non-coding RNAs (lncRNAs) and miRNAs, regulate cancer cell survival and drug resistance.
- Non-coding RNA Activated by DNA Damage *(NORAD)* is involved in autophagy regulation and tumor progression in several cancers.
- Autophagy-related 5 *(ATG5)* and *Beclin*1 are key autophagy-related genes linked to cancer development.

What's New

- This study is the first to demonstrate the *miR-30a-5p*/*NORAD* axis and its downstream regulation of *ATG5*/*Beclin1*-mediated autophagy in CLL.
- miR-30a-5p suppresses *NORAD* expression, thereby inhibiting *ATG5*/*Beclin1*-mediated autophagy and reducing CLL cell survival.
- These findings identified a novel therapeutic axis and suggested that targeting the *miR-30a-5p/NORAD* pathway might offer new anti-leukemic strategies.
- The *miR-30a-5p/NORAD* axis may serve as a potential biomarker and therapeutic target in CLL.

Abstract

Background: Chronic lymphocytic leukemia (CLL), a prevalent hematologic malignancy in adults, is marked by the pathological accumulation of impaired mature B lymphocytes. Non-coding RNAs, especially microRNAs and long non-coding RNAs (lncRNAs), serve as master regulators of fundamental cellular processes in CLL, from cell proliferation and death to the autophagy pathway, directly influencing disease development. This study aimed to investigate the association between microRNA-30a-5p (*miR-30a-5p*) and lncRNA noncoding RNA activated by DNA damage (*NORAD*) expression and the autophagy-related genes, including autophagy-related 5 (*ATG5*) and *Beclin1,* in patients with CLL.

Methods: This case-control study was conducted in Kermanshah, Iran, between March 2023 and February 2024. Peripheral blood samples were collected from 59 CLL patients and 59 healthy controls recruited through consecutive sampling. Following isolation of peripheral blood mononuclear cells (PBMCs), total RNA was extracted, and cDNA was synthesized. Gene expression was measured using real-time PCR, and statistical tests including the independent-samples *t* test, Mann-Whitney U-test (for non-normally distributed data), and Spearman's correlation coefficient (for non-parametric correlations) were applied to identify expression differences and relationship patterns.

Results: The study identified a significant decrease in *miR-30a-5p* expression (CLL=0.65±0.25 vs. Control=1.02±0.21, P=0.004) and a significant increase in lncRNA *NORAD* (CLL=3.5±1.1 vs. Control=1.01±0.15, P=0.03) and *Beclin1* (CLL=2.31±0.6 vs. Control=1.01±0.27, P=0.01) levels in patients relative to healthy controls. Notably, *miR-30a-5p* levels showed an inverse pattern relative to *ATG5* and *Beclin1*, while *NORAD* demonstrated a coordinated increase with these autophagy genes.

Conclusion: This study demonstrated a significant correlation between dysregulation of *miR-30a-5p* and lncRNA *NORAD* and the altered expression of autophagy-related genes *ATG5* and *Beclin1* in CLL patients. These findings suggested that *miR-30a-5p* and lncRNA *NORAD* might serve as potential biomarkers in CLL, although functional studies are required to establish causal relationships.

Please cite this article as: Ghiassi S, Kiani AA, Ahmadpour F, Maleki A. Dysregulation of *miR-30a-5p* and lncRNA *NORAD* Expression and Their Association with Autophagy Genes *ATG5* and *Beclin1* in Chronic Lymphocytic Leukemia: A Case-Control Study. Iran J Med Sci. doi: 10.30476/ijms.2026.110337.4591.

Keywords ● Leukemia, Lymphocytic, Chronic, B-Cell ● MicroRNAs ● RNA, Long noncoding ● Autophagy ● Beclin-1 ● Autophagy-related protein 5

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia, with an estimated age-adjusted annual incidence rate of 4.6 cases per 100,000 individuals.[1] Diagnosis typically occurs at a median age of approximately 70 years, reflecting the disease's prevalence among older adults.[2] Fewer than 10% of individuals diagnosed with CLL are under the age of 45. The disease exhibits a male predominance, with a male-to-female ratio of approximately 1.9:1, a pattern that appears consistent across diverse ethnic groups. It is projected that around 0.6% of both males and females will be diagnosed with CLL at some stage in their lives.[1] CLL primarily impacts adults and accounts for about 25% of all newly identified cases of leukemia. CLL typically exhibits a more indolent progression than other forms of leukemia, often resulting in delayed symptom recognition and diagnosis.[3] In CLL, mature cluster of differentiation ($CD5^+$) B lymphocytes progressively accumulate in the blood, bone marrow, lymph nodes, and spleen, forming a malignant clone that defines the disease.[4] A substantial subset of patients with CLL remains asymptomatic and does not require therapeutic intervention until specific clinical indications emerge, whereas a distinct group experiences rapid disease progression. This clinical diversity underscores the variability of CLL outcomes and the complexity of prognosis.[5] The pathogenesis of CLL frequently begins with large-scale chromosomal aberrations, such as deletion of 13q, deletion of 11q, or trisomy 12, followed by the acquisition of additional genetic mutations that contribute to disease progression and increased aggressiveness.[6]

Autophagy is a vesicle-based cellular mechanism in which cellular materials are packaged into vesicles called autophagosomes, which fuse with lysosomes for degradation.[7] It is triggered by various stressors, including nutrient deprivation, oxidative stress, and microbial exposure.[8] When autophagy functions properly, it maintains cellular health and normal physiological function. However, when this process is impaired, it can contribute to various diseases, from cancer to neurodegenerative disorders.[9, 10] Beyond stress responses, autophagy maintains cellular homeostasis under normal conditions and actively supports processes such as cell growth and differentiation.[11] In cancer, autophagy plays a dual role, sometimes suppressing tumors, other times promoting them, depending on the cancer type and stage. Under normal physiological conditions, it functions as a protective mechanism that enhances longevity and inhibits tumorigenesis by alleviating genotoxic stress.[7] Recent studies have revealed that once cells become cancerous, autophagy can promote tumor growth and dissemination by sustaining cancer cell survival under stress.[9, 12, 13] *Beclin1* and autophagy-related 5 (*ATG5*) are key genes involved in the regulation of autophagy. Numerous studies assessed autophagic activity by measuring the expression levels of these genes or their corresponding protein products, revealing divergent, sometimes even opposing, expression patterns across different cancer types.[5, 14] In the context of CLL, Mahoney and colleagues reported that the expression levels of *Beclin1* and *ATG5* were comparable to those observed in normal B-cells.[14] Another team found that *Beclin1* mRNA levels were notably lower in CLL cells.[15] Indeed, the alterations in autophagy levels associated with CLL remain insufficiently characterized.[5]

microRNAs (miRNAs), a class of short non-coding RNAs, are key post-transcriptional regulators of gene expression and have been implicated in essential cellular processes including growth, proliferation, differentiation, apoptosis, and stress responses.[16] Dysregulation of miRNA expression is widely recognized as a contributing factor in cancer development. Recent studies have increasingly linked aberrant miRNA profiles to both the initiation and progression of CLL.[17] The important role of *microRNA-30a* (*miR-30a-5p*) in regulating cancer cell growth across various cancers has been demonstrated.[18] However, its role in CLL has rarely been addressed.

Long noncoding RNAs (lncRNAs), a subclass of non-coding RNAs exceeding 200 nucleotides in length and lacking protein-coding capacity, are integral to diverse cellular processes including epigenetic regulation, differentiation, and apoptosis. Accumulating evidence highlights their significant involvement in tumorigenesis and progression across multiple human malignancies, notably leukemia. Noncoding RNA activated by DNA damage (*NORAD*) is a recently identified and evolutionarily conserved lncRNA implicated in DNA damage repair and replication processes. Aberrant *NORAD* expression has been associated with multiple cancer types. However, its regulatory mechanisms and expression profile in CLL remain poorly understood.[19] In this study, we investigated whether the expression levels of *miR-30a-5p* and lncRNA *NORAD* were associated with the expression of autophagy genes *ATG5* and *Beclin1* in peripheral blood mononuclear cells (PBMCs) of CLL patients.

Materials and Methods

Study Design and Population

This case-control study involved 59 patients diagnosed with CLL and 59 healthy individuals as controls. Patients were recruited through consecutive sampling from those referred to the Hematology-Oncology Clinic of Imam Reza Hospital, Kermanshah, Iran, between March 2023 and February 2024. All CLL diagnoses were confirmed by a pathologist based on standard diagnostic criteria, including lymphocytosis (>5×10^9/L persistent for at least 3 months) and immunophenotyping demonstrating a CD5$^+$, CD19$^+$, CD23$^+$ clonal B-cell population.

The control group was selected through consecutive sampling from individuals attending the hospital for routine health check-ups or cancer screening programs during the same time period. Controls were frequency-matched to cases based on age (±5 years) and sex to ensure comparability between groups. The collected data included disease stage (Rai), organ enlargement, blood counts (white blood cell count [WBC], hemoglobin [Hb], platelets [PLT]), lifestyle factors (smoking, alcohol), and demographics (age, height, weight, body mass index [BMI]).[20]

Sample Size Calculation

The sample size was calculated based on a previous study investigating gene expression differences in CLL patients.[5] Considering a two-sided test with a 95% confidence level (α=0.05) and 90% statistical power (β=0.10), and anticipating a minimum detectable effect size of 0.6 in gene expression levels between groups, the required sample size was determined to be 50 participants per group. To account for potential dropouts or inadequate samples, 59 participants were recruited in each group. The sample size calculation was performed using G*Power software (version 3.1.9.7; Heinrich Heine University Düsseldorf, Germany).

Inclusion and Exclusion Criteria

The inclusion criteria for the CLL group were: confirmed diagnosis of CLL based on standard diagnostic criteria (persistent lymphocytosis >5×10^9/L and immunophenotyping confirmation), age ≥18 years, willingness to provide informed consent, and no prior chemotherapy or immunotherapy treatment at the time of blood sampling. The exclusion criteria for the CLL group included: presence of other concurrent malignancies, autoimmune disorders, active infections (human immunodeficiency virus [HIV], hepatitis B virus [HBV], hepatitis C virus [HCV]), previous treatment for CLL, and Richter's transformation.

The inclusion criteria for the control group were: age ≥18 years, no clinical or laboratory evidence of hematological disorders, normal complete blood count (CBC), and no history of malignancy. The Exclusion criteria were: history of any malignancy, autoimmune diseases, chronic inflammatory conditions, active infections, and family history of hematological malignancies in first-degree relatives.

Ethical Approval

The study received ethical approval from the Ethics Committee of Kermanshah University of Medical Sciences (code: IR.KUMS.REC.1403.248). Written informed consent was obtained from all participants, including both patients and healthy controls, before enrollment and blood sample collection, following a comprehensive overview of the study and a clear explanation of their voluntary participation.

Blood Samples Collection

Blood samples were obtained from all participants using ethylenediaminetetraacetic acid (EDTA)-coated tubes to preserve cellular integrity for molecular assays. Peripheral blood mononuclear cells (PBMCs) were subsequently isolated via density gradient centrifugation using Ficoll solution (Biosera Company, France). Briefly, whole blood was diluted with phosphate-buffered saline (PBS), layered onto Ficoll, and centrifuged at 2,800×g for 20 min. The resulting buffy coat layer, comprising lymphocytes, monocytes, and thrombocytes, was carefully aspirated, washed twice with PBS, resuspended, and stored at -70 °C for downstream molecular analyses.[21]

RNA Isolation and Quantitative Real-time RT-PCR

The expression levels of *miR-30a-5p*, lncRNA *NORAD*, and the autophagy-related genes *ATG5* and *Beclin1* were quantified via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Total RNA was isolated from PBMCs using the Total RNA Purification Kit (Akoteb Company, Iran), in accordance with the manufacturer's instructions. RNA purity and integrity were evaluated using spectrophotometric analysis and 2% agarose gel electrophoresis, respectively. Following quantification, complementary DNA (cDNA) synthesis was performed using cDNA synthesis kits (Akoteb Company, Iran), with separate protocols applied for the autophagy-associated genes (*ATG5* and *Beclin1*) and non-coding RNAs (*miR-30a-5p* and lncRNA *NORAD*), according to the manufacturer's guidelines.

For *miR-30a-5p* quantification, a stem-loop reverse transcription primer was used for the specific reverse transcription of mature miRNA, followed by SYBR Green qPCR with a miRNA-specific forward primer and a universal reverse primer.

Gene expression analysis for *ATG5*, *Beclin1*, *miR-30a-5p*, lncRNA *NORAD*, and the reference genes *beta-2-microglobulin* (*β2M*) and *miRNA housekeeping* (*miR HK*) was conducted using qRT-PCR with SYBR Green qPCR Master Mix (Akoteb Company, Iran). All reactions were carried out in triplicate on the Rotor-Gene 6000 platform (Corbett Life Science, Australia). Relative quantification of gene expression was determined using the $2^{-}\Delta\Delta CT$ method.[22]

Amplification efficiencies for all primers were calculated using the standard curve method with serial dilutions of cDNA. Efficiencies ranged from 92% to 105% for all target and reference genes, confirming appropriate PCR conditions.

Primer sequences for each target gene are provided in table 1.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 22; SPSS Inc., Chicago, IL, USA). Results were presented as mean±SEM. Group comparisons were assessed using the independent *t* test for normally distributed variables. The Mann-Whitney U test was used for gene expression as the data did not follow a normal distribution (as assessed by the Shapiro-Wilk test). Spearman's rank correlation coefficient was used instead of Pearson's correlation, since several variables were not normally distributed, making Spearman's nonparametric approach more appropriate. A P value less than 0.05 was considered statistically significant.

Results

Participant Characteristics

Table 2 summarizes the demographic and laboratory parameters of the study participants. Each group consisted of 59 participants, as determined by our sample size calculation and frequency-matching approach. Comparative analysis of age and sex revealed no statistically significant differences between patients with CLL and healthy controls (P=0.572 and P=0.546, respectively), confirming successful frequency-matching. In contrast, CLL patients demonstrated significantly elevated WBC counts (P<0.0001) and reduced Hb levels (P<0.0001) compared with healthy individuals.

Table 1: Nucleotide sequences of primers employed in quantitative real-time reverse transcription PCR (qRT-PCR)

Gene	Primer Position	Primers Sequences	Product Size
β2M	Forward	TGCCGCATTTGGATTGGATG	**195**
	Reverse	GAGCTACCTGTGGAGCAACC	
Beclin1	Forward	GGAGCTGCCGTTATACTGTTCTG	186
	Reverse	CTGCCTCCTGTGTCTTCAATCT	
ATG5	Forward	AAGATGTGCTTCGAGATGTGTG	144
	Reverse	TCACTTTGTCAGTTACCAACGTC	
miR-30a-5p	Forward	GTCGTATCCAGTGCGTGTCGTGGAGT	48
	Reverse	CGGCAATTGCACTGGATACGAC	
lncRNA NORAD	Forward	GCAGACTATTCATTGGGTGTTTGG	94
	Reverse	AACTAATCTCAGGAGCACAGAACC	
miR HK	Forward	CGATATCATGAAGCAGCCGATCATG	55
	Reverse	CGTCGACTTACATGCCATCTTCCATATTGT	

β2M: *Beta-2-microglobulin*; *Beclin1: Coiled-coil myosin-like BCL2-interacting protein*; *ATG5*: *Autophagy related 5*; *miR-30a-5p*: *MicroRNA-30a-5p*; *NORAD*: *Noncoding RNA activated by DNA damage*; *miR HK*: *MicroRNA housekeeping*

Table 2: Baseline demographic information and laboratory measurements of the study population

Variables		CLL group (n=59)	Control group (n=59)	P value
Age (years, mean±SEM)		69.83±4.68	63.76±3.8	0.570
BMI (Kg/m^2, mean±SEM)		23.23±0.38	26.37±0.58	<0.0001
Hb (g/dL, mean±SEM)		8.82±0.09	13.96±0.15	<0.0001
WBC (10^3/mm^3, mean±SEM)		85.03±8.93	6.59±0.32	<0.0001
PLT (10^3/mm^3, mean±SEM)		155.6±10.23	260.06±18.21	0.016
Sex, n (%)	Male	39 (66.1)	41 (69.5)	0.546
	Female	20 (33.9)	18 (30.5)	

Data are presented as mean±standard error of the mean (SEM) or frequency (%). The independent *t* test was used for continuous variables (age, BMI, Hb, WBC, PLT) after confirming normality using the Shapiro-Wilk test. The Chi square test was used for sex comparison. P<0.05 was considered statistically significant. CLL: Chornic lymphocytic leukemia; BMI: Body mass index; Hb: Hemoglobin; WBC: White blood cell count; PLT: Platelets

Furthermore, BMI was significantly lower in the patient group (P<0.0001) than in the control group. In addition, PLT counts were notably reduced in patients with CLL compared to healthy individuals (P=0.016).

Clinical Manifestations in the CLL Patient

As shown in figure 1, 70% of the patients exhibited no signs of inflammation, while 10% presented with splenomegaly and 20% with lymphadenopathy. Additional analyses revealed that the healthy control group had no prior medical conditions and no family history of cancer. Among the patients, only 6 out of 59 individuals were identified as smokers. Furthermore, 16.7% of the patients reported a family history of cancer, and approximately 13.3% were current smokers. All 59 CLL patients were staged according to the Rai classification system. Based on the presence of lymphadenopathy, splenomegaly, Hb levels, and platelet counts, the distribution of Rai stages was as follows: 15 patients (25.4%) were classified as stage 0 (low risk), 5 (8.5%) as stage I (intermediate risk), 3 (5.1%) as stage II (intermediate risk), 28 (47.5%) as stage III (high risk), and 8 (13.6%) as stage IV (high risk). The distribution of Rai stages is summarized in table 3.

Expression Levels of ATG5, Beclin1, lncRNA NORAD, and miR-30a-5p in PBMC of CLL and Control Groups

As illustrated in table 4 and figure 2, the expression levels of *Beclin1* and lncRNA *NORAD* genes in PBMCs of the CLL group were significantly elevated compared to the control group (P=0.03 and P=0.028, respectively).

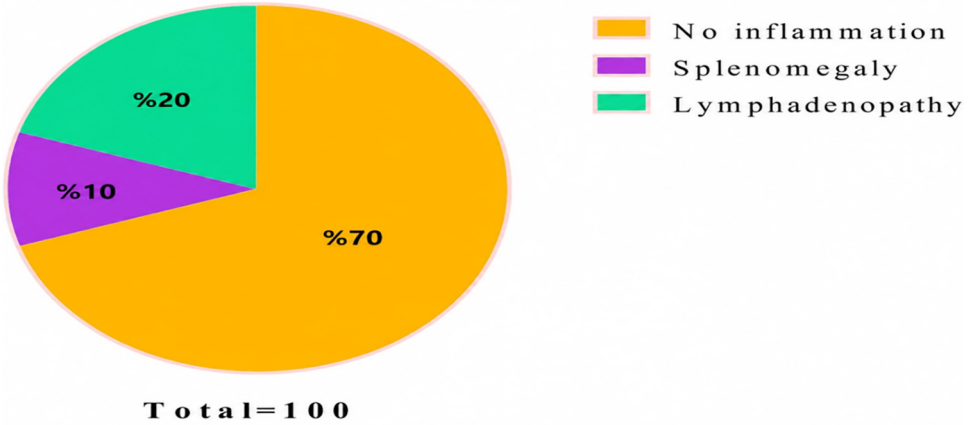

Figure 1: The figure shows clinical manifestations in chronic lymphocytic leukemia (CLL) patients. Seventy percent of the patients exhibited no inflammatory symptoms, 20% had lymphadenopathy, and 10% had splenomegaly. CLL: Chronic lymphocytic leukemia

Table 3: Rai staging of chronic lymphocytic leukemia (CLL) patients

Rai stage	Risk category	Number of patients, n (%)
0	Low	15 (25.4%)
I	Intermediate	5 (8.5%)
II	Intermediate	3 (5.1%)
III	High	28 (47.5%)
IV	High	8 (13.6%)
Total		59 (100%)

Data are presented as numbers (n) and percentages (%). Rai stages: 0 (low risk), I (intermediate risk), II (intermediate risk), III (high risk), IV (high risk). Lymphocytosis (≥5,000/μL) is present in all stages. CLL: Chronic lymphocytic leukemia

Table 4: Expression levels of *ATG5*, *Beclin1*, lncRNA *NORAD*, and *miR-30a-5p* genes in peripheral blood mononuclear cells of chronic lymphocytic leukemia and control groups

Group / Gene expression	Control group	CLL group	P value
ATG5 (mean±SEM)	1.017±0.21	1.24±0.27	0.7
Beclin1 (mean±SEM)	1.01±0.27	2.31±0.6	0.03
lncRNA NORAD (mean±SEM)	1.01±0.15	3.5±1.1	0.028
miR-30a-5p (mean±SEM)	1.02±0.21	0.65±0.25	0.004

Data are presented as mean±SEM. The Mann-Whitney U test was used for group comparisons as the data did not follow a normal distribution (it was assessed by the Shapiro-Wilk test). P<0.05 was considered statistically significant. PBMC: Peripheral blood mononuclear cell; CLL: Chronic lymphocytic leukemia; SEM: Standard error of the mean; *ATG5: Autophagy related 5; miR-30a-5p: MicroRNA-30a-5p; Beclin1: Coiled-coil myosin-like BCL2-interacting protein; NORAD: Noncoding RNA activated by DNA damage*

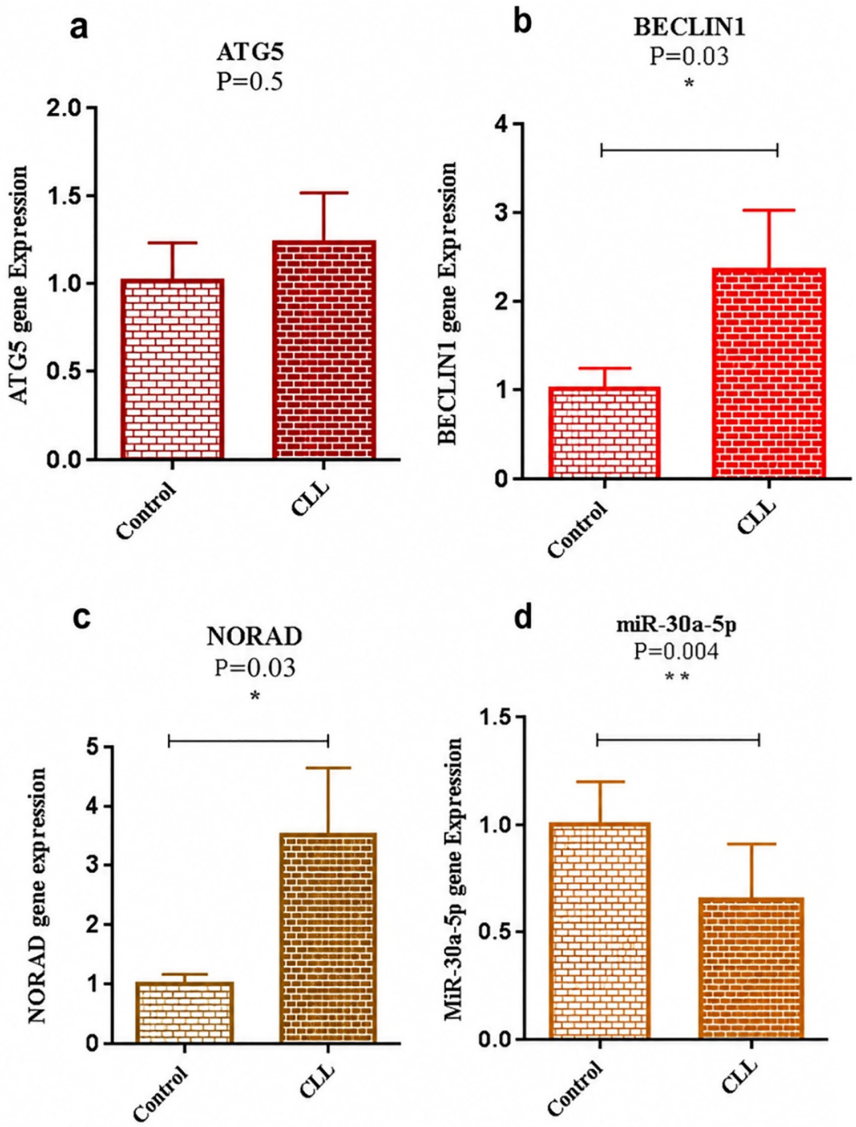

Figure 2: The figure shows the expression levels of autophagy-related and non-coding RNA genes in peripheral blood mononuclear cells (PBMCs) of chronic lymphocytic leukemia (CLL) patients and healthy controls. The panels display the relative expression of (a) *ATG5*, (b) *Beclin1*, (c) lncRNA *NORAD*, and (d) miR-30a-5p in PBMCs from 59 CLL patients and 59 healthy controls. Data are presented as mean±SEM. The Mann-Whitney U test was used for group comparisons as the data did not follow a normal distribution (it was assessed by the Shapiro-Wilk test). The asterisk (*) indicates a statistically significant difference compared to the control group at P<0.05. Exact P values are as follows: *Beclin1*, P=0.03; lncRNA *NORAD*, P=0.028; *miR-30a-5p*, P=0.004. SEM: Standard error of the mean; *ATG5*: Autophagy related 5; *miR-30a-5p*: MicroRNA-30a-5p; *Beclin1*: Coiled-coil myosin-like BCL2-interacting protein; *NORAD*: Noncoding RNA activated by DNA damage

In contrast, the expression of *miR-30a-5p* was markedly reduced in the PBMCs of CLL patients relative to controls (P=0.004). No statistically significant difference was observed in the expression of the *ATG5* gene between the patient and control groups (P=0.712).

Correlation between the Gene Expression Levels of ATG5, Beclin1, lncRNA NORAD, and miR-30a-5p Genes with Baseline Demographic Information and Laboratory Measurements in CLL and Control Groups

Spearman's rank correlation analysis revealed a moderate positive correlation between *Beclin1* and *ATG5* expression (ρ=0.45, P<0.0001). A weak positive correlation was observed between lncRNA *NORAD* expression and WBC count (ρ=0.22, P=0.04). Conversely, *miR-30a-5p* expression demonstrated a weak negative correlation with *Beclin1* expression (ρ=-0.31, P=0.004) and a moderate negative correlation with lymphocyte count (ρ=-0.49, P=0.0004). Based on biostatistical consultation, the simplified regression equations for these correlations were as follows: *Beclin1*=0.45×*ATG5*; *Beclin1*=-0.31×*miR-30a-5p*; Lymphocyte count=-0.49×*miR-30a-5p*; and WBC count=0.22×lncRNA *NORAD*. The results obtained from the Spearman correlation analysis are presented in figure 3.

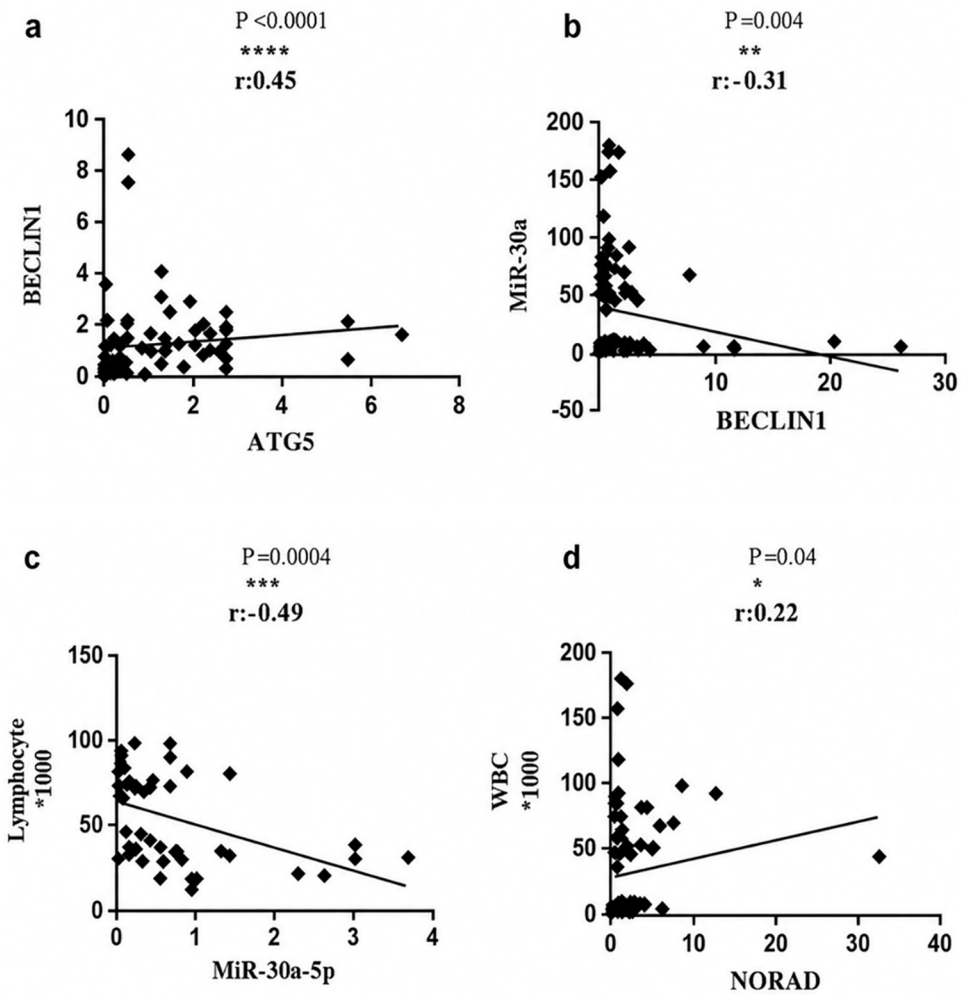

Figure 3: The figure shows the Spearman correlation analysis between gene expression levels and laboratory parameters in chronic lymphocytic leukemia (CLL) patients. The panels display: (a) a significant positive correlation between *Beclin1* and ATG5 expression (ρ=0.45, P<0.0001), (b) a significant negative correlation between *Beclin1* and *miR-30a-5p* expression (ρ=-0.31, P=0.004), (c) a significant negative correlation between miR-30a-5p expression and lymphocyte count (ρ=-0.49, P=0.004), and (d) a significant positive correlation between lncRNA *NORAD* expression and WBC count (ρ=0.22, P=0.04). Spearman's rank correlation coefficient (ρ) and P values are shown for each analysis. WBC: White blood cell count; *ATG5*: Autophagy-related 5; *miR-30a-5p*: *MicroRNA-30a-5p*; *NORAD*: Noncoding RNA activated by DNA damage; The asterisks indicate the levels of statistical significance as follows: *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001. Accordingly, panel (a) is denoted by **** (P<0.0001), panel (b) by ** (P=0.004), panel (c) by *** (P=0.0004), and panel (d) by * (P=0.04). WBC: White blood cell count; *ATG5*: Autophagy related 5; *Beclin1*: Coiled-coil myosin-like BCL2-interacting protein*; miR-30a-5p:* MicroRNA-30a-5p; *NORAD*: Noncoding RNA activated by DNA damage

Correlation between Rai Stage and Gene Expression Levels

Spearman's rank correlation analysis was performed to evaluate the association between Rai stage (0, I, II, III, IV) and expression levels of *miR-30a-5p*, lncRNA *NORAD*, *ATG5*, and *Beclin1*. The results showed no significant correlation between Rai stage and any of the studied molecular markers (all P>0.05). These findings suggested that the expression of these molecular markers was independent of clinical stage in our patients.

Discussion

This study is the first to identify a potential association between *miR-30a-5p* and lncRNA *NORAD* expression and the autophagy-related genes *ATG5* and *Beclin1* in PBMCs of patients with CLL. The results indicated no significant differences in age or sex between CLL patients and healthy controls. However, CLL patients exhibited markedly higher WBC counts and lower Hb levels, BMI, and PLT counts, all statistically significant. The present investigation revealed a novel association of *miR-30a-5p* suppression and lncRNA *NORAD* upregulation with autophagy pathway gene expression changes in PBMCs of CLL patients. While the patient and control groups were well-matched demographically, CLL patients displayed the characteristic hematologic profile of elevated

WBCs coupled with reduced Hb, PLT, and BMI. These findings were consistent with established CLL pathophysiology.[23]

Notably, CLL patients exhibited significantly elevated *Beclin1* and lncRNA *NORAD* expression alongside marked downregulation of *miR-30a-5p*, while *ATG5* levels remained unchanged. This pattern partially aligned with the findings of Kong and colleagues, who reported increased *Beclin1* in CLL.[5] However, we did not replicate their finding of altered *ATG5* expression described in their study. This discrepancy might reflect the context-dependent nature of autophagy regulation in leukemia, where molecular pathways exhibit considerable plasticity across different cellular environments. Recent literature highlighted autophagy as a dual-function mechanism in acute leukemia, capable of both promoting and inhibiting disease progression.[24] *Beclin1*, a component of the PIK3C3 class of phosphatidylinositol-3-kinases, is essential for autophagosome formation and has been implicated in tumorigenesis, neurogenesis, and the regulation of cell death.[25] Extensive research has identified *Beclin1* as a key initiator and regulator of autophagy. In leukemia cells, its expression is typically reduced. However, upregulation of *Beclin1* has been shown to elicit antitumor effects through the activation of autophagic pathways.[26] *Beclin1* and *ATG5* are key upstream regulators of autophagy, yet their expression profiles and prognostic significance vary across cancer types. Reduced *Beclin1* expression correlated with poor survival in non-small cell lung cancer, while elevated *ATG5* levels were linked to improved disease-free survival in breast cancer. Conversely, high *Beclin1* expression was observed in malignant melanoma and cutaneous squamous cell carcinoma, and *ATG5* overexpression was noted in prostate cancer. Interestingly, diminished *Beclin1* expression was associated with favorable outcomes in gastric cancer and CLL.[27] However, the functional roles of *Beclin1* and *ATG5* in CLL remained inadequately characterized.

miRNAs play dual roles in cancer biology, with some promoting tumorigenesis through enhanced proliferation, angiogenesis, invasion, and immune evasion, while others function as tumor suppressors. Beyond their well-established roles in oncogenesis, they critically regulate hematopoiesis and contribute significantly to leukemogenesis through post-transcriptional mechanisms. Consequently, dysregulation of miRNA expression disrupts hematopoietic homeostasis, potentially leading to the onset of leukemia.[28] Our findings supported prior evidence indicating the involvement of *miR-30a-5p* in the modulation of autophagy in cancer. Similarly, the tumor-suppressive role of *miR-30a-5p* was consistent with Chen and colleagues' report of its *Beclin1*-inhibitory function in gastrointestinal stromal tumors.[29] Spearman correlation analysis demonstrated a strong positive association between *Beclin1* and *ATG5* expression in PBMCs ($P<0.0001$). The lncRNA *NORAD* expression was also positively correlated with WBC count ($P=0.032$). In contrast, *miR-30a-5p* expression exhibited significant inverse correlations with both *Beclin1* expression ($P=0.008$) and lymphocyte count ($P<0.001$). Emerging evidence highlighted a significant association between genomic mutations and dysregulated lncRNA expression in tumorigenesis, including leukemia. lncRNAs were identified as key modulators of autophagy-related gene expression, influencing key leukemic processes, such as apoptosis, proliferation, epithelial-mesenchymal transition, migration, and angiogenesis, through autophagy-linked signaling pathways.[30] Our findings were further supported by emerging evidence of lncRNA *NORAD* overexpression in leukemia, particularly Eslami and colleagues' demonstration of its upregulation in acute myeloid leukemia (AML).[19]

Several limitations should be acknowledged. First, given the correlational nature of our study, no causal relationships can be inferred; functional experiments are required to establish mechanistic links. Second, as gene expression was measured in total PBMCs rather than purified $CD19^+$ B cells, the observed differences might partially reflect variations in cellular composition rather than true molecular dysregulation within malignant cells. Third, our analysis was confined to mRNA expression, whereas autophagy is a dynamic process that necessitates protein-level and functional flux analyses. Fourth, regarding clinical staging, we used the Rai classification based on available data. However, complete Binet staging could not be performed due to insufficient documentation of individual lymph node regions and hepatomegaly. Fifth, we lacked access to cytogenetic data (e.g., del(13q), del(11q), del(17p), trisomy 12), IGHV mutation status, and TP53 disruption status, all well-established prognostic markers in CLL. Their absence limited our ability to characterize the molecular heterogeneity of the cohort and reflects the resource-limited context of this study. Sixth, the modest sample size and single-center design underscored the need for validation in larger, multi-center cohorts. Future studies incorporating purified B cells, protein-level and functional assays, and comprehensive

cytogenetic and molecular profiling will be essential to confirm our findings.

Conclusion

This study demonstrated a significant correlation between the expression levels of *miR-30a-5p* and lncRNA *NORAD* and key autophagy-related genes (*ATG5* and *Beclin1*) in patients with CLL. Downregulation of *miR-30a-5p* might be associated with altered expression of *ATG5* and *Beclin1*, while upregulation of lncRNA *NORAD* might correlate with leukemic cell characteristics. These findings suggest that *miR-30a-5p* and lncRNA *NORAD* could serve as potential biomarkers in CLL, offering a foundation for future research into autophagy-based interventions in hematologic malignancies. Nonetheless, further functional studies are required to establish causal relationships and elucidate the underlying mechanisms.

Acknowledgment

This research project was funded by Kermanshah University of Medical Sciences (Grant No: 4040530). This study was extracted from the MSc thesis of Sahar Ghiassi at the Department of Laboratory Sciences, School of Allied Medical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran. The authors would like to thank all the patients and healthy volunteers who participated in this study.

Authors' Contribution

All authors contributed to the conception and design of the study. S.G. performed the experiments, contributed to data acquisition, and wrote the first draft of the manuscript. A.A.K. contributed to the conception of the study and provided scientific ideas. F. A. performed statistical analyses and contributed to data interpretation. A.M. contributed to the conception of the study, provided scientific ideas, critically revised the manuscript for important intellectual content, and supervised the overall research process. All authors reviewed and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work and meet the four ICMJE criteria for authorship.

Declaration of AI

No artificial intelligence-based technologies were used in the production of the submitted work.

Conflict of Interest: None declared.

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