Egy. J. Pure & Appl. Sci. 2023; 61 (3):11-33

*Egyptian Journal of Pure and Applied Science*

# Impact of p53-dependent Networks on Prognosis of chronic lymphocytic leukemia

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**ARTICLE INFO**

Received 31 July 2023
Accepted 22 August 2023

***Keywords***
Chronic lymphocytic leukemia (CLL),
P53,
lincRNA-p21,
MiR-34a,
Prognosis.

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**ABSTRACT**

Chronic lymphocytic leukemia (CLL) is a type of blood cancer that is characterized by the accumulation of abnormal lymphocytes in the bone marrow, lymph nodes, and other tissues. The prognosis of CLL varies widely, with some patients experiencing a relatively benign disease course while others progress rapidly to a more aggressive form of the disease. Recent research has identified several molecular pathways that may impact CLL prognosis, including many axes composed of messenger RNA (mRNA) for tumor suppressor pathways, long noncoding RNA (lncRNA), and microRNA (mRNA). Our study on 75 male and female B-CLL patients for p53, lincRNA-p21, and miR-34a biomarkers assessment for CLL prognosis. Our results showed that p53 and lincRNA-p21 increased with no significant difference between males and females. On the other hand, miR-34a expression decreased significantly compared to the control group. Results suggested that lincRNA-p21 and miR-34a may interact in a complex feedback loop that regulates p53 function and cellular processes in CLL. Dysregulation of this feedback loop can contribute to CLL pathogenesis and may serve as a potential therapeutic target. Therefore, p53, lincRNA-p21, and miR-34a proved to be important molecular regulators of CLL pathogenesis and prognosis. Dysregulation of these pathways can contribute to CLL progression and may serve as potential biomarkers or therapeutic targets. Further research is needed to better understand these pathways' complex interactions and develop more effective treatments for CLL patients.

## 1. Introduction

The p53 tumor suppressor pathway's status is one potential component that could affect the prognosis for CLL [1].

It typically develops mutations or deletions in CLL and is a crucial regulator protein of cell cycle progression and apoptosis. what it does Loss has a worse therapeutic response and a more aggressive disease [2, 3].

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western world and affects primarily elderly patients, often older than 70 years of age. The clonal growth of abnormal B cells that co-express T cell-specific antigens and B cell markers (CD19, CD20), as well as CD5, is what leads to CLL [4, 15]. Chromosomal abnormalities, such as deletions of (13q14), (11q23), (17p13), and trisomy, which is the presence of an extra chromosome in a person's genetic makeup and results in a total of three copies of a particular chromosome instead of the usual two, cause the variability in the progression and treatment requirements. These abnormalities are present in more than 75% of CLL patients. Despite the fact that this disorder can affect any chromosome, it is most frequently seen in chromosomes 13, 18, and 21. [5]. Trisomy can have a significant impact on a person's health, and it is often associated with developmental delays, intellectual disabilities, and physical abnormalities.

Trisomy 21, also known as Down syndrome, is the most well-known type of trisomy and affects approximately 1 in 700 births [17, 18]. In the context of hematologic and oncologic conditions, trisomy can also play a role in the development of certain cancers [19, 20, 21]. For example, trisomy 12 is commonly observed in chronic lymphocytic leukemia, a type of blood cancer that affects the white blood cells. Trisomy 8 is also associated with a higher risk of developing myelodysplastic syndromes and acute myeloid leukemia [22, 23, 24]. The most frequent chromosomal anomaly in CLL is trisomy 12, which is more prevalent in morphologically unusual cases, some of which may be undergoing transformation [25].

Trisomy 12 was associated with a statistically significant difference in the occurrence of atypical cases (47%) compared to those without it (7.6%; P .001). In the context of a nationwide therapy study, it is currently being researched to see if this anomaly is linked to a worse prognosis. [26]. Additionally, it has been linked to aberrant lymphocyte shape, illness progression, and poor survival [27]. The deletion of chromosome 13 (13q14), which can be found utilizing cutting-edge diagnostic techniques like southern blot hybridization and fluorescence in situ hybridization, is a common anomaly in B-cell CLL. It happens in up to 70% of cases of mantle-cell lymphoma and 51% of CLL patients [28].

The translocation t(11;14) (q13;q32) on chromosome 14 expresses chromosome 14 abnormalities, which are also frequent and are associated with a high leukocyte count, a poor response to cytostatic treatment, and a higher risk of prolymphocytic proliferation [29]. The oncogene BCL-1 is triggered during this translocation. The BCL-2 oncogene is activated by deletions of the long arm of chromosome 18 (18q21) (q32;q13.1), whereas the BCL-3 oncogene is activated by the translocation t(14;19)(q32;q13.1) [30]. In addition, trisomy can also be used as a diagnostic tool in cancer treatment. Certain trisomy patterns can be used to predict the prognosis of particular cancer or to guide treatment decisions. For example, trisomy 8 is associated with a poorer prognosis in patients with acute myeloid leukemia, while trisomy 13 is associated with a better prognosis in patients with multiple myeloma [23].

Overall, trisomy is a complex genetic condition that can have significant implications for a person's health and well-being. As a hematologist and oncologist, understanding the role of trisomy in the development of certain cancers is crucial for accurate diagnosis and effective treatment planning [24, 5]. LncRNAs are a class of RNA molecules that are longer than 200 nucleotides and do not encode proteins [34, 35, 36]. Instead, they play a crucial role in regulating gene expression by interacting with DNA, RNA, and proteins [37]. LncRNAs can act as transcriptional regulators, post-transcriptional regulators, and epigenetic modifiers, affecting various cellular processes such as proliferation, differentiation, apoptosis, and metastasis [38, 39]. Long non-coding RNAs (lncRNAs) have emerged as important regulators of gene expression, and their dysregulation has been implicated in various human cancers [31, 32, 33].

According to the duration of dissemination Lnc RNAs are further classified into various classes. Large-lncRNA (4,800 nt), medium-lncRNA (4,800–950 nt), and small-lncRNA (200–950 nt) [45, 46]. Small-lncRNAs make up the majority of lncRNAs in humans (58%), whereas medium-lncRNAs make up the majority of lncRNAs in mice (78%) [47, 48]. Additionally, the human genome has less medium-lncRNAs than the mouse genome and more small- and large-lncRNAs. The comparison results are, however, questionable because the amount of carefully annotated lncRNAs by GENCODE released recently is less than half that of NONCODE V3.0,4,50 [49, 50]. While the disparities may just be the result of different annotation levels, if they are shown to accurately reflect the reality, they may indicate that lncRNAs have evolved differently in mice and humans, necessitating more evolutionary investigation [51, 52, 53].

Therefore, lncRNAs are emerging as important players in cancer biology, and understanding their role in cancer development and progression may lead to the development of new diagnostic and therapeutic strategies for cancer patients. Several studies have shown that lncRNAs are dysregulated in various types of cancer, including breast cancer, lung cancer, prostate cancer, and colorectal cancer [33, 40, 44]. For example, the lncRNA HOTAIR has been shown to promote breast cancer metastasis by repressing the expression of tumor suppressor genes. Another lncRNA, MALAT1, has been shown to promote lung cancer metastasis by regulating the alternative splicing of genes involved in cell motility and invasion [42, 43, 31]. Furthermore, lncRNAs have been shown to have diagnostic and prognostic value in cancer.

For instance, the lncRNA PCA3 has been used as a diagnostic marker for prostate cancer, while the lncRNA HULC has been shown to be a prognostic marker for hepatocellular carcinoma [43, 44]. LncRNA p21 is transcribed from the opposite strand of the p21 gene and overlaps with the p21 coding region. It has been shown to regulate p21 expression at both the transcriptional and post-transcriptional levels. At the transcriptional level, lncRNA p21 can interact with the p53 tumor suppressor protein to enhance p21 expression in response to DNA damage. This interaction stabilizes p53 and increases its binding to the p21 promoter, leading to increased p21 transcription [42]. At the post-transcriptional level, lncRNA p21 can regulate p21 expression by acting as a competing endogenous RNA (ceRNA).

It has been shown to bind to microRNAs (miRNAs) that target p21 mRNA, thereby preventing their interaction with p21 mRNA and increasing p21 expression. Additionally, lncRNA p21 can also bind to the RNA-binding protein HuR to stabilize p21 mRNA and enhance its translation. Studies have shown that lncRNA p21 plays a critical role in various biological processes, including cell cycle regulation, apoptosis, DNA damage repair, and cellular senescence [43]. Recent research has also identified a number of p53-dependent networks that play important roles in CLL pathogenesis and progression. For example, the p53 pathway can regulate the expression of key genes involved in DNA repair, metabolism, and cell signaling, and disruption of these networks can promote CLL growth and survival [54, 3, 55].

P53 mutations that affect the function of wild-type p53 in CLL or deletion of the P53 locus on chromosome 17 (17p13.1) can cause P53 aberrations [56, 6]. P53 encodes a tumor-suppressor protein that regulates the cell cycle and apoptosis and promotes DNA repair in response to cellular stress signals such as DNA damage. Chemotherapy acts by causing DNA damage, which activates the P53 pathway and induces death in CLL cells [57, 7]. When P53 is disrupted, chemotherapy fails to elicit apoptosis in CLL cells, allowing the disease to progress and leading to clonal evolution as the cells continue to replicate at a steady rate [69, 8]. Several research have been conducted to investigate the potential prognostic importance of p53 pathway malfunction in chronic lymphocytic leukemia (CLL) [58]. One retrospective analysis of CLL patients who received chemoimmuno therapy found that those with p53 mutations had considerably shorter overall survival and progression-free survival compared to those without mutations.

Other studies have suggested that p53 pathway dysfunction may contribute to resistance to specific therapies, such as B-cell receptor (BCR) pathway inhibitors [59, 9]. Despite these observations, the prognostic worth of p53 pathway status in CLL remains somewhat controversial. Some studies have failed to find a significant connection between p53 mutations and clinical outcomes, and other factors, such as age, disease stage, and genetic abnormalities, may also play important roles in CLL prognosis [60, 10]. However, overall, the available evidence suggests that p53-dependent networks are likely to be significant drivers of CLL pathogenesis and progression, and that p53 pathway dysfunction may serve as a useful prognostic marker in some patients.

Further research is needed to better understand the mechanisms underlying p53 pathway regulation in CLL and to develop more effective therapies targeting this pathway [62]. MiRNAs, on the other hand, serve critical roles in the regulation of gene-expression programs that underpin both normal and pathologic cellular processes, including cancer. When abnormally over-expressed, some miRNAs operate as tumor suppressors, whereas others might promote tumor development, growth, and/or progression to metastasis. Because of their tiny size, miRNAs are rarely found with point mutations; yet, their dysregulation is widespread in many malignancies. [63, 12].

Similar to other RNAs, microRNAs are controlled by different mechanisms such epigenetic repression, transcriptional activation or inhibition, and regulated breakdown rates [64]. Human microRNAs are distributed as follows: 52% are found in intergenic regions, 40% are found in intronic sections of genes, and the remaining 8% are found in exonic regions. Although they are frequently controlled by their host gene and processed from the intron, intronic microRNAs may also have a unique promoter region. Independent promoter elements exist for intergenic microRNAs [65]. By focusing on their own transcription factor(s), upstream signaling might cause feedback loops by starting the transcription of microRNA genes. Zinc finger E-box-binding homeobox 1 (ZEB1) transcriptionally represses MiR-200c, which is implicated in the epithelial-to-mesenchymal transition. [66]

Small non-coding RNA molecules known as microRNAs (miRNAs) serve significant functions in controlling gene expression [67]. They are typically 21-25 nucleotides in length and are transcribed from DNA by RNA polymerase II. After transcription, miRNAs undergo a series of processing steps that involve the actions of several enzymes, including Drosha, Dicer, and Argonaute proteins. The end result of this processing is a mature miRNA molecule that can interact with mRNA targets to regulate gene expression [68]. MiRNAs are involved in a wide range of biological processes, including development, differentiation, apoptosis, and metabolism. They can act as both positive and negative regulators of gene expression, depending on the specific miRNA and mRNA targets involved [69]. In terms of their mechanism of action, miRNAs typically bind to the 3' untranslated region (UTR) of target mRNAs, which can lead to degradation of the mRNA or inhibition of translation.

This can ultimately result in reduced expression of the target gene [71]. MiRNAs have been implicated in a variety of diseases, including cancer, cardiovascular disease, and neurological disorders. They are also being studied as potential targets for therapeutic intervention, as modulating miRNA expression or activity could have a significant impact on disease progression [72]. P53 manage several miRNAs, including the direct p53 target miR-34a.

Inhibiting cell cycle progression by targeting cyclin-dependent kinases and cyclin D1, miR-34a mediates some of p53's pro-apoptotic actions upon DNA damage, promotes apoptosis by targeting anti-apoptotic B-cell lymphoma 2 (BCL2), and suppresses cell growth. MiRNA levels in tumors may be lower than in healthy tissue, which may lead to an increase in the clonal heterogeneity of cancer cells. This would boost the adaptability and survival of cancer cells similarly to genetic noise. enhancing yeast and bacterial survivability in response to shifting environmental circumstances [73, 13]. So, our work aimed to find out the role of lincRNA-p21 as regulator for p53 gene which considered as target in CLL via regulation of miRNA-34a and find out the association between p53 dependent pathway and disease outcome that includes severity, prognosis and response to treatment.

## 2. *Subjects and methods*

The study conducted on 75 individuals whom attended to the Hematology- department- Demerdash Hospital - Ain Shams University and informed consents will be obtained from all of them. The approval of this study will be taken by the Institutional Ethics Committee of Ain Shams University. Individuals in the study will be categorized into two groups, CLL group comprised of 50 patients diagnosed with CLL that were considered as the studied group. Patients diagnosed as CLL patients according to high lymphocyte fractions and fulfilling standard diagnostic criteria for CLL and control group of 25 normal individuals of matched age and sex to the studied group.

**Inclusion Criteria:**

- CLL patients were diagnosed according to the standard diagnostic criteria (WHO-2012).
- Patients' ages were > 15 years old in both CLL and control
- CLL samples were 33 males and 17 females
- Control samples were 14 male and 11 female

**Exclusion Criteria:**

- CLL patients who took therapy were in remission or relapse stage.
- Patients did not take any sort of Chemotherapy
- CLL patients with other malignancies whether De Novo or as a complication to CLL.

The Detailed history of each patient, with special reference to present and past family history was recorded and also Full Patients' Clinical and laboratory data will be collected from patient data sheets.

### 3. Sample collection

3 ml of peripheral blood samples were drawn and put into K2-EDTA-filled vials. Phosphate-buffered saline (PBS) was used to dilute the anticoagulated blood in an amount that was equivalent to it. By carefully pipetting the diluted blood down the side of the tube containing the Ficoll-Hypaque solution, the diluted blood was placed over the Ficoll-Hypaque solution very slowly, and then a centrifuge was used for 40 min at 400 x g, 22 oC. The interface between the plasma (top layer) and the Ficoll-Hypaque (bottom layer) was cleaned of mononuclear cells.

By gently pipetting up and down, the cells were suspended in three volumes of balanced salt solution (PBS), after which 100 g of centrifugal force was applied for 10 minutes at 20 °C. The leukocyte pellet was centrifuged once more after being rinsed with phosphate-buffered saline (PBS) and the supernatant was discarded. When not in use, the leukocyte cells were suspended in PBS and kept at -80°C. Extraction and purification of total RNA, including miRNA, from polymorphorph nuclear leucocytes (PMNL) according to the miRNeasy Serum/Plasma Kit" (Qiagen, Hilden, Germany).

### 4. Reverse transcription to cDNA

(The miScript II RT Kit was used to reversibly transcribe the linc-RNAs p21 and p53. The TaqmanV R MicroRNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany) was used to reverse-transcribe the cDNA templates for miRNA 34a in accordance with the manufacturer's instructions. Lnc-RNAs are poly adenylated by poly (A) polymerase and transformed into cDNA by reverse transcriptase with oligo-dT priming in a reverse transcription reaction with miScriptHiSpec Buffer. Real-time PCR is then performed to quantify the expression of lncRNA using the cDNA.

### 5. PCR in gene expression analysis amplification of P53, lincRNA-p21, miRNA

The Step One Real-Time PCR Analyzer (Applied BioSystems, USA) was used to examine each sample.

P53 QuantiTect Primer Assay (QT00060235) cat no. 249900 (Qiagen, Germany) and QuantiTect SYBR Green PCR Kit cat no. 249900 (Qiagen, Germany) were used to amplify the amount of P53 gene expression from mRNA. QuantiTect primer assay Linc RNA-P21 QuantiTect Primer Assay (LPH09368A) cat no: 330701 (Qiagen, Germany) and QuantiTect SYBR Green PCR Kit cat no: 330701 (Qiagen, Germany) were used to amplify the expression level of the Linc RNA-p21 gene from mRNA. With the use of Hs_miR-43a-5P_1 miScript and the miScript SYBR Green PCR kit from Qiagen (Germany), relative miRNA expression levels for candidate miRNA-43a were examined.

Primer Assay (cat no: 218300) which targets mature miRNA: hsa-miR-43a-5P-1 (cat no: MS00006629) (Qiagen, Germany) and Hs_SNORD68_11 miScript Primer Assay cat no: 218300) as housekeeper gene (HK)which targets SNORD68 small nucleolar RNA, C/D box 68 cat no: (MS000337),and QuantiTect SYBR Green PCR Kit cat no: 249900 (Qiagen, Germany).All samples were analyzed using the StepOneRealTime PCR Analyzer (Applied BioSystems, USA). The resulting amplification and melting curves were analyzed to identify the specific PCR products. The relative gene expression values were calculated using the comparative 2–ΔΔCt method. The relative gene expression levels were calculated by normalization to the GAPDH mRNA level.

Targeting mature miRNA are the primer assay (cat. no. 218300), Hs_SNORD68_11 miScript, and hsa-miR-43a-5P-1 (cat. no. MS00006629) from Qiagen, Germany. Housekeeper gene (HK) primer assay (cat. no. 218300) that targets SNORD68 small nucleolar RNA, C/D box 68 cat. no. (MS000337), and QuantiTect SYBR Green PCR Kit (Qiagen, Germany). The Step One Real Time PCR Analyzer (Applied BioSystems, USA) was used to examine each sample. To determine which particular PCR products were produced, the ensuing amplification and melting curves were examined. The comparative 2-Ct technique was used to calculate the relative gene expression values. By normalizing to the GAPDH mRNA level, the relative gene expression levels were computed.

### 6. Statistical analysis

Our data were analyzed using SPSS (V.21) software using average and slandered deviation. One way ANOVA and student two tailed tests were used.

## 7. Results

The present study was conducted on 75 subjects who were stratified into two groups, the CLL group, and the control group. Our study evaluated Linc- p21, MIRNA- 34a, and the tumor suppressor p53 (P53) gene expression as molecular diagnostic and prognostic factors for B- chronic lymphocytic leukemia, and correlate their expressions with different clinic-pathological parameters including phenotypes, hematological data, and disease outcome. The B-CLL patients were sub-classified according to histopathological stages to I, II, III, and IV stages. Our results showed that 64% (32/50) of the patients were in I and II stages on the other hand only 36% (18/50) were at III and IV (Table 1). On the other hand The B-CLL patients were sub-classified according to cytogenic abnormalities into three groups.

Our results revealed that group de113q represents the majority of B-CLL group 31 (62%), followed by group de11 1q 12 (24%) and finally group de17q which represent 7 (14%) , these data are presented in Table 1. The B-CLL patients were sub classified according to clinical response state to remission group and relapse group. The results showed that 26 (52%) of the patients were in the remission group, on the other hand, 24 (48%) of the patients were in the relapse group (Table 1). In the current study, Linc-p21 expression showed a median (45.6) and interquartile range (IQR) (70) in the B-CLL group and a median (13.6), & IQR (13.3) the in control group. miR34a expression showed a median (0.1) & IQR (0.2) the in B-CLL group and a median (2.2) & IQR (2.3) in the control group.

On the other hand, the data of P53 expression revealed the Median (4.3) in B-CLL & IQR range (5.33) and median (1.06) &IQR range (1.7) in the control group. According to the expression level in our data, the Lincp21 showed to be up-regulated by 4 folds, miR34a works as tumor suppressor gene up-regulated by 0.375 folds, on other hand, the P53is up-regulated by 3 folds in B-CLL patients compared to healthy control respectively (Table 2, fig. 1). Regarding gender, the majority of B- CLL patients were males, they account for 33/50 (66%) in contrast to 17/50 (34%) females. A similar distribution of gender was observed in the control group. Therefore, no significant difference was detected in gender distribution between B- CLL patients and control groups ($\chi^2$ =0.001, p=0.97).

This data reflects homogeneity between B- CLL and controls. According to nodal area distribution among the B-CLL patients, 20 (40%) of the patients had < 3 nodal areas, and 30 (60%) of the B-CLL patients had >3 nodal areas. In respect to gender, the B-CLL patients are classified into two groups: male and female, Linc p21 in the male group showed a Median (53.8), &IQR (84.3), and in the female show a median (36.5), & IQR (32.8). miR 34a in the male group showed Median (0.2), & IQR (0.3) and in the female group showed Median (0.26), & IQR (0.12). P53 in the male group showed a median (4.3) & IQR (3.4) and the female group showed a median (4.6) & IQR (6.5). Our data indicated insignificant differences between gender in B-CLL groups regarding miR34a and P53 expression. On the other hand, there is an insignificant difference between male and female groups regarding the Linc p21. These data are presented in Table 3 and fig. 2.

According to our data analysis of B-CLL patients' blood, the Hemoglobin mean was 10.0 gm/dl, and the median was 9.5 gm/dl. Regarding the total leucocyte count of B-CLL patients, the mean was 71.6510^3/µL and the median was 41.600 10^3/µL. the platelets mean count was 143.310^3/µL and the median 130 10^3/µL, on the other hand, the lymphocytes mean was 60% and the median was 72%. (Table 4). The data analysis and results of ≤ 2 nodal areas showed a median (30.7) and IQR (28.6) regarding Linc-p21, a median (0.3) and IQR (0.2) regarding miR-34a, on the other hand, the median was (2.5) and IQR (2.4) regarding P53 of B.CLL patients.

Regarding > 3 nodal areas, the results showed a median (66.5) and IQR (90.4) regarding Linc-p21, median (0.2), and IQR (0.3) regarding miR-34a.Finally the median was (5.1) and IQR was (5.9) regarding P53 of B.CLL patients (Table 5 and Fig.3). Regarding to histopathological stages, B-CLL patients were classified into four groups (I, II, III, and IV). Stage I-II median (34.7) in Linc-p21 and IQR (23), and stage III-IV median (110.8) in Linc-p21 and IQR (108.4). Regarding miR-34a gene expression, the stage I-II median was (0.35) and the interquartile range was (0.3), in the III-IV stage, the median was (0.1) and the interquartile range was (0.2). On the other hand, in stage I-II median was (2.5) in the P53 gene and IQR was (2.4) and the III-IV stage median was (6.6) and IQR was (3.8). The results are presented in Table 6, Fig. 4.

Cytogenetic analysis divided B-CLL patients into three groups: del13q, del11q and del17p. Linc-p21 expression showed Median (36.3) & IQR (30.8) in del13q group, Median (103), & IQR (115.6) in del11q group and Median (145), & IQR (117) in del17p group. miR-34a expression showed Median (0.35) & IQR (0.2) in del13q group, Median (0.1), & IQR (0.06) in del11q group and Median (0.09), & IQR (0.3) in del17p group. P53 expression showed median (2.6) and IQR (2.5) in del13q group, median (5.6) and IQR (5) in del11q group and median (7.8) and IQR (5.7) in del17q group. These data are presented in Table 7 and Fig. 5.

Also, results showed that the patients of B-CLL were classified into 2 groups (relapsed and remitted). According to Linc-p21, the relapsed median was (80.9) and IQR (110), and remitted median was (35.8) and IQR (28). Regarding miR-34a, the relapsed median was (0.1) and IQR (0.3), and remitted median was (0.35) and IQR (0.2). on the other hand, the median of relapsed group was (5.2) and IQR was (4.6) of the P53 gene, and the median of the remitted group was (2.6) and IQR (2.2). The results showed in Table 9 and Fig. 6.

To evaluate the Linc-p21, miR-34a, and P53 expression as diagnostic predictors in B-CLL; we conducted a receiver operating characteristic (ROC) analysis for each gene. Our result showed that the Linc-p21 expression obtained an area under the curve (AUC) was19 with an 88 % confidence interval and specificity of 80%; in contrast to 0.13 shown in miR-34a expression with a specificity of 92%. On the other hand, P53 expression obtained an area under the curve (AUC) was 2 with sensitivity (76%) and 84% specificity. The AUC, sensitivity, and specificity were significant P< 0.05, so Linc-p21, miR-34a, and P53 in B-CLL patients could be used as diagnostic biomarkers. These data are represented in Table 10 and Fig. 7A.

In addition, to evaluate Linc-p21, miR-34a, and P53 expression as prognostic predictors in B-CLL; we conducted a receiver operating characteristic (ROC) analysis for each gene. Our result revealed that the Linc-p21 expression obtains an area under the curve (AUC) is 49 with 88 % confidence interval, but the miR-34a expression had no (AUC) area under the curve. According to P53 gene expression, the area under the curve was (4) with a sensitivity of (73%) as a biomarker. From the analysis of our results, only Linc-p21 and P53 gene expression could be used as prognostic biomarkers (p <0.05). Results showed in Table 11 and Fig.7B.

## 8. Discussion

A high level of epigenomic heterogeneity was associated with a worse prognosis and a higher rate of relapse in the multi-step and heterogeneous processes that make up the CLL pathogenesis [25, 26]. It is challenging to diagnose at an early stage due to the lack of distinct symptoms and early identification, which results in a poor prognosis. P53, Linc-p21, and miR34a are three molecular prognostic indicators for CLL that are currently known. However, each by itself has a number of drawbacks. Only 15–30% of CLL patients have P53 mutations. In contrast to miR34a, whose statistics were disputed [27], linc-p21 is upregulated in between 40 and 60% of patients and is only associated with worse outcomes in specific subtypes of CLL. This calls for the development of new prognostic markers. [28–30]

Nevertheless, these investigations were conducted on tumor tissue samples and/or cell lines, and as putative mediators of resistance to cancer therapy, The present study tries to investigate the role of an axis composed of long non-coding RNA as Linc-P21 antisense intergenic RNA (Linc-p21) and to investigate its association with microRNA (miR-34a) together with mRNA(P53) as a molecular marker for predicting leukemia development and prognosis in Acute Lymphatic Leukemia (CLL). According to nodal area distribution among our B-CLL patients, 40% of the patients had < 3 nodal areas, and 60% of the B-CLL patients had >3 nodal areas. Linc p21 in the male group showed higher Median &IQR than female median IQR. miR 34a in the male group showed higher Median & IQR in the female group Median & IQR.

P53 in the male group showed less median & IQR than the female group). Our data indicated-significant differences between gender in B-CLL groups regarding miR34a and P53 expression. On the other hand, there is a significant difference between male and female groups regarding the Linc p21. Our data analysis and results of ≤ 2 nodal areas showed up regulation with median (30.7) and IQR (28.6) regarding Linc-p21, a median (0.3) and IQR (0.2) with down regulation regarding miR-34a, on the other hand, the median was (2.5) and IQR (2.4) regarding P53 of B.CLL patients. Regarding > 3 nodal areas, the results showed highest up regulation with median (66.5) and IQR (90.4) regarding Linc-p21, median (0.2), and IQR (0.3) regarding miR-34a. Finally, the median was (5.1) and IQR was (5.9) regarding P53 of B.CLL

Regarding histopathological stages, B-CLL patients were classified into four groups (I, II, III, and IV). Stage I-II lower median (34.7) in Linc-p21 and IQR (23) was recorded than stage III-IV median (110.8) in Linc-p21 and IQR (108.4). In contrast, regarding miR-34a gene expression, the stage I-II median was (0.35) and the interquartile range was (0.3), in the III-IV stage, the median was (0.1) and the interquartile range was (0.2). On the other hand, in stage I-II median was (2.5) in the P53 gene and IQR was (2.4) and the III-IV stage median was (6.6) and IQR was (3.8). In addition, the Cytogenetic analysis divided B-CLL patients into three groups: del13q, del11q and del17p. Linc-p21 expression showed lowest Median & IQRin del13q group, moderate Median & IQR in del11q group and highest Median & IQR in del17p group. miR-34a expression showed the highest Median & IQR in del13q group, Median (0.1), & IQR (0.06) in del11q group and Median (0.09), & IQR (0.3) in del17p group.

P53 expression showed lowest median and IQR in del13q group, moderate median and IQR in del11q group and highest median and IQR in del17q group. Our data analysis indicated that, regarding Linc-p21 gene expression, the difference in del11q and del17q groups was insignificant and regarding miR-34a gene expression, the difference between del13q and del17q groups was insignificant, as well as the difference between del11q and del17q groups also. On the other hand, in P53 gene expression, the difference between the del13q and del11q groups was insignificant. Also, results showed that the patients of B-CLL were classified into 2 groups (relapsed and remitted). According to Linc-p21, the relapsed median was higher and IQR than the remitted median and IQR.

Regarding miR-34a, the relapsed median and IQR was lower than the remitted median and IQR. On the other hand, the median of relapsed group was median and IQR was higher than the median of the remitted group according to P53 gene. Despite the fact that CLL is typically a dormant condition, a sizable proportion of individuals exhibit an aggressive clinical history with resistance to medication or relapse after initial treatment. The fact that less than 50% of patients undergoing the gold standard chemotherapeutic fludarabine, cyclophosphamide, and rituximab (FCR) treatment have a 5-year progression-free survival indicates that resistance to therapy is a serious medical problem in CLL.

Mounting evidence suggests that more than 10,000 newly discovered lncRNAs may have contributed to human solid and hematologic cancers (102). The need for new non-invasive diagnostic and prognostic biomarkers that target human cancer is increasing, which has put the use of lncRNAs in leukemia in the fore front (99). There are around 9,000 distinct human lncRNA genes. Even though some of them can alter gene expression through unique molecular pathways, few of them are involved in myelopoiesis. (103). In the current study, the expression of Linc-p21 in high-risk CLL patients was evaluated and compared to that in the low-risk group; higher expression of Linc-p21 was highly significant associated with CLL patients with total leucocyte counts >50,000, bone marrow blasts percentage >70%, and was significantly associated with CLL patients with hemoglobin concentrations 6 gm/dl, which represented aggressive clinic-pathological features.

Wu et al. also came to the same conclusions, concluding that Linc-p21 overexpression is a distinct biomarker with a bad prognosis in CLL patients (98). The predictive value of Linc-p21 has also been studied in a variety of malignancies, and the outcomes were consistent with our findings. In light of the afore mentioned results, we can draw the conclusion that Linc-p21 expression in CLL has a considerable prognostic significance and that higher expression levels are related to a worse prognosis. Linc-p21 expression was shown to be significantly greater in peripheral blood of de-novo CLL samples as compared to normal healthy control samples in the current investigations using quantitative Real-time PCR (qPCR). High-risk and poor prognosis groups also contributed to the higher expression of Linc-p21.

Our findings are in line with earlier research (Wu et al.), which demonstrated that de-novo CLL significantly upregulated Linc-p21 expression by five folds when compared to healthy controls; additionally, higher expression levels were linked to higher BM blast counts, total leucocyte counts, and lower hemoglobin concentrations and platelet counts. (98). Given that p53 plays a crucial role in controlling the response to chemotherapy, inhibiting the proliferation of aberrant cells, and maintaining genomic integrity, there is considerable interest in developing pharmaceutical strategies that activate p53 (20, 21).

The use of short RNA is one of these strategies; it relies on the non-genotoxic activation of p53 by protecting it from inhibition and MDM2 degradation, stabilizing p53, and activating its transcriptional activity to promote p53-induced death. (20, 21, 24, 25). We make a strong argument for the further investigation of miR34a inhibitors in CLL therapy based on our results that CLL cells are particularly prepared for p53-dependent apoptosis compared to control patients. The major objectives of this work were to assess P53 gene alterations in CLL patients and investigate the prognostic, clinical, and hematological implications of these mutations. More patients than in the Western literature (20%) tested positive for the P53 gene mutation [15].

According to Rossi et al. [15], only 10% of patients had a P53 mutation at the time of diagnosis, which may point to a difference in the disease's biology. About two-thirds of cases of CLL are caused by del 17p coupled with P53 mutations, the most prevalent aberration affecting the P53 gene, according to research by Leroy et al. [16]. This study's main goals were to evaluate P53 gene mutations in CLL patients and look into the prognostic, clinical, and haematological effects of these changes. More patients (20%) tested positive for the P53 gene mutation than in the Western literature [15]. Only 10% of patients had a P53 mutation at the time of diagnosis, according to Rossi et al. [15], indicating a variation in the disease's biology. According to Leroy et al., over two-thirds of CLL cases are caused by del 17p combined with P53 mutations, the most common aberration affecting the P53 gene. [16].

According to the study by Wenlan et al., P53 mutations did not affect core-binding factor (CLL), including inv (16) and t(8;21) CLL, although they did result in a much higher risk of relapse. However, it has been noted (16) AML did not have a significant increase in the P53 mutation-related relapse risk, CLL (8,21) did. and. Their main findings support prior studies' findings [96] that CLL patients with P53 mutations should be assessed based on ethnicity. It only partially clarified how P53 mutations affected the overall survival (OS) of (8,21) CLL. Ethnicity is not a risk factor for this CLL subtype with P53 mutations, according to a comparable analysis for inv (16) AML; additional research is needed to determine any possible risk variables [97]. MiR-34a family members, such as miR-34, were found to be downregulated in CLL blasts, according to Razan et al.

In cytogenetically defined subgroups of CLL, both miRNAs are tumor suppressors, and he demonstrated that low miR-34a expression is an independent predictor of poor prognosis and survival. According to their findings, miR-34a, a STAT5-regulated miRNA, regulates hematopoietic stem and progenitor cell (HSPC) growth by altering cytokine receptor signalling. Restoring miR-34a expression in CLL cells by targeting P53, KRAS, and SOS2 - important elements in the KIT-RAS-RAF-MEK-ERK signalling cascade - would provide high anti-leukemic efficacy while avoiding the rapid development of resistance mechanisms. These findings point to miR-34a playing an opportunistic role in future CLL treatments.

Razan et al discovered that the miR-34a family members miR-34 are down regulated in CLL blasts. Both miRNAs are effective tumor suppressors in several cytogenetically diverse subgroups of CLL, but he established that low miR-34a expression was a separate predictor of poor prognosis and survival. Their findings show that miR-34a, a STAT5-regulated miRNA, regulates hematopoietic stem and progenitor cell (HSPC) growth by modulating cytokine receptor signalling. Targeting P53, KRAS, and SOS2, three critical components of the KIT-RAS-RAF-MEK-ERK signalling cascade, would restore miR-34a expression in CLL cells and give strong anti-leukemic efficacy while delaying the establishment of resistance mechanisms. These data suggest that miR-34a may play an opportunistic role in future CLL therapies. [95].

MiR-34a, a tumor suppressor miRNA, is down regulated in CLL patients with del17p and/or mutant P5322,45, the subgroup with the worst prognosis and responsiveness to treatment. It is also expressed at significantly lower levels in fludarabine-refractory CLL cases compared to non-refractory CLL cases, regardless of 17p/P53 status44. Asslaber and colleagues looked studied the relationship between a single nucleotide polymorphism (SNP309) in the intronic promoter of MDM2, a gene upstream of P53, and the amount of miR34a expressed in P53 wild-type patients. [45]. those with the GG genotype had considerably lower levels of miR-34a expression when compared to those with the TT genotype, and these low levels of miR-34a were connected to a shorter time to therapy. Upregulating or reinstalling miR-34a induces the pro-apoptotic protein Bax and the cell cycle regulator p2144, as well as apoptosis [45]. As a result, MiR-34a is a promising candidate for targeted cancer therapy.

A very effective miRNA that might be utilised to treat CLL is miR-34a. It is a significant tumor suppressor that is downregulated in a variety of human malignancies, including neuroblastoma, glioblastoma, and cancers of the ovary, colon, liver, lung, breast, prostate, pancreas, kidney, bladder, skin, oesophagus, cervix, and urothelium (reviewed by 59,60). MiR-34a expression is downregulated in cases of del17p and/or mutant P5322 in CLL, which makes sense given that miR-34a is a significant downstream target of P5323 [45]. A worse prognosis is also associated with fludarabine-resistant disease and low expression of miR-34a.Re-expression of miR-34a dramatically boosted apoptosis when wild-type P53 was expressed in primary CLL patient cells, but not when P53 was attenuated. Due to its function in cancer, miR-34a is an excellent option for miRNA replacement treatment.

MRX34, the first miRNA mimic to enter clinical trials, is currently being tested in a multicenter Phase I study in patients with liver cancer or those who have liver metastases from other cancers, as well as in patients with hematological malignancies, including CLL. It contains a miR-34amimic. SMARTICLES are used to distribute MRX34, a double-stranded miR-34 mimic, in a safe and efficient manner [60]. Hepatocellular carcinoma anorthotopic mouse models treated with MRX3462,63 showed significant tumor reduction and increased longevity without obvious drug-related side effects. The expression of miR-34 oncogenic targets was discovered to be reduced as a result of the discovery that miR-34 expression was more than 100 times greater in liver cancer cells [64].

Determining the maximum tolerated dose and the recommended Phase II dose in a variety of cancers are the major objectives of the ongoing Phase I clinical trial investigating MRX34. Patients with CLL get MRX34 intravenously, and a treatment schedule consisting of five straight days of treatment followed by two weeks off in 21-day cycles is being investigated. The secondary objectives include examining any biological activity and clinical outcomes, as well as safety, tolerability, and pharmacokinetic profile. According to recently released interim results on safety and preliminary efficacy for 52 individuals, MRX34 has a tolerable safety profile. The primary treatment-emergent adverse events were infusion reactions, which can result in fever, chills, nausea, vomiting, back and flank pain, exhaustion, diarrhea, headache, dehydration, an increase in liver enzymes, a decrease in albumin, hyponatremia, lymphopenia, thrombocytopenia, and neutropenia [64].

The microRNAs 34a and 34b/cThe miR-34b/c cluster is part of the 11q deleted region in CLL [41], the P53 tumor suppressor is part of the 17p deletion [42], and the 13q deletion results in the downregulation of miR15a/16-1 [30]. As a result, we looked into whether the tumor protein p53, the miR-34b/c cluster, and the miR-5a/16-1cluster share a molecular pathway that can shed light on the prognostic effects of 11q, 17p, and 13q deletions in CLL [41]. Several P53 binding sites can be found in the miR-15a/16-1, miR-34b/c, and miR-34a upstream regions. P53 can therefore stimulate the expression of these microRNAs. While miR-34 members target ZAP70, miR-15a/16-1 target P53 and BCL2 [41].

Patients with 13q deletions have increased levels of both Bcl2 and p53 when miR-15a/16-1 is lost [29]. In this case, high levels of Bcl2 reduce the number of apoptotic cells; nevertheless, high levels of p53 maintain a low tumor burden, which explains the sluggish progression of 13q deleted CLL patients, and increase the transactivation of miR-34b/c, which lowers ZAP70 levels [4]. Since miR-15a/16-1 are not eliminated in CLL patients with 11q deletion, P53 is not elevated, providing a reduced level of apoptotic control. Furthermore, because this microRNA is deleted [41], which increases ZAP70 expression [4], P53 transactivation of miR-34b/c is inefficient. Last but not least, deletion of 17p strongly corresponds with unfavorable outcomes, and therapeutic response is frequently subpar.

Another study examined cytogenetic abnormalities, the miR-15a/miR-16-1 cluster, the miR-34 family, the P53 gene, downstream effectors cycle independent kinase inhibitor 1A (p21, Cip1), B-cell CLL/lymphoma 2 binding components 3 (BBC3), and ZAP70 gene expression levels. In CLLs with 13q deletions, the miR-15a/miR-16-1 cluster exclusively targeted P53 (mean luciferase activity for miR-15a vs scrambled control, 0.68 RLU [95% CI, 0.63-0.73]; P=.02; mean for miR-16 vs scrambled control, 0.62 RLU [95% CI, 0.59-0.65]; P=.02).

P53 increased transcription of the miR-15/miR-16-1 and miR-34b/miR-34c clusters in leukemic cell lines, with the miR-34b/miR-34c cluster directly targeting the ZAP70 kinase (mean luciferase activity for miR-34a versus scrambled control, 0.33 RLU [95% CI, 0.30-0.36]; P= 0.02.CLL progression and prognosis are linked to a microRNA/P53 feedback loop. This pathway, which involves microRNAs, P53, and ZAP70, provides a novel pathogenetic explanation for the association of 13q deletions with the indolent form of CLL, as did our findings [19].

Another study discovered that patients with high miR-34a expression experienced much more PNA-induced apoptosis than those with low expression. These variations were discovered in cytogenetic patients. Our data show that miR-34a expression is an apoptotic predictor even in the absence of additional risk factors such as cytogenetic abnormalities. MicroRNA expression analysis appears to be useful in predicting the outcome of RNA-based therapy and acts as an indicator of RNA sensitivity. We discovered that plasma Linc-p21 and P53 were differently expressed in CLL patients, indicating a role in the disease's development. To the best of our knowledge, we are the first to present evidence for the expression of this axis in CLL and its diagnostic and prognostic implications.

Furthermore, we found that plasma levels of Linc-p21, P53, and miR34a have a discriminative ability for CLL, implying that they could be used as surrogate non-invasive biomarkers of CLL diagnosis, with miR34a having greater diagnostic performance. In the current research, Linc-p21, miR-34a, and P53 were found to be associated with clinical samples of CLL. Our findings showed a significant relationship between the three biomarkers, which may reflect their regulatory interlink, which was demonstrated experimentally on leukemic cell lines [96] by contrasting it with earlier research. The three biomarkers were shown to be significantly correlated in the current investigation, which may indicate a regulatory interaction between them that was demonstrated experimentally on leukemic cell lines. The current study indicated that miR-34a functions as a tumor suppressor gene by 11.5 folds and that Lnc-p21 expression is up-regulated by 5 folds in CLL patients compared to healthy controls.

P53 expression is also up-regulated by 3 folds. However, contrary to Sayad et al.'s findings, earlier research on Iranian patients found that Linc-p21 expression levels did not significantly differ between CLL patients and healthy controls, so they could not be used as a conclusive diagnostic or prognostic biomarker for the disease [97]. This peculiar outcome may be explained by a number of restrictions, such as the use of just Iranian patients and a few tiny sample sizes. Gene expression variability supports our study by clearly demonstrating a difference between the two subtypes (1-II and III-IV), with early CLL—the more severe disease in Linc-p21—being associated with higher variability.

The gene expression levels of CLL patients in the early and late phases, however, did not differ significantly (approximately 1.5-fold difference in miR-34a downregulation and P53 upregulation), which is consistent with earlier studies [43, 44]. These findings imply that expression variability between patients may play a crucial role in differentiating between the two disease subtypes, for which the overall level of expression will not serve as a marker and for which only a small number of differentially expressed genes have been identified [24, 43].

Moreover; we demonstrated a significant association between upregulated Linc-p21 with downregulation of miR-43a and higher expression of the P53 gene. Inconsistent with our results; evidence supports the idea that Linc-p21 acts as an oncogene and mediates tumor invasion and metastases. It has been found that Linc-p21 is upregulated in CLL [94]. Additionally, we validated the in vivo experimental data that supported the molecular route of Linc-p21/miR-43a/P53 on blast proliferation in CLL, which ultimately supports the oncogenic function of HOTAIR in the emergence of CLL. Furthermore, we propose that in the near future, the Linc-p21/miR-43a/P53 axis may also offer a novel therapeutic use in CLL.

## 9. Conclusion

Chronic lymphocytic leukemia (CLL) is a disease with a wide range of clinical outcomes. Recent research has revealed a number of molecular pathways, including p53-dependent networks and microRNAs (miRNAs), that may play essential roles in CLL development and progression. In CLL, the p53 tumor suppressor pathway is frequently disturbed, and loss of p53 activity has been linked to more aggressive illness and poorer therapy response. Furthermore, p53-dependent networks can control essential cellular functions in CLL, including DNA repair, metabolism, and cell signalling. MiRNAs are short non-coding RNAs that can influence gene expression and are critical in the development and progression of cancer. Several miRNAs have been proven to be dysregulated in CLL and may act as prognostic biomarkers. MiR-15a and miR-16-1, for example, are frequently deleted or downregulated in CLL and are linked to more advanced disease and poorer outcomes. These findings imply that molecular profiling of p53-dependent networks and miRNA expression may provide important prognostic information for CLL patients.

More studies are needed, however, to better understand the mechanisms underlying these pathways and to develop more effective medicines that target them.

Improved molecular characterization of CLL could eventually lead to personalized therapy methods that enhance results for patients suffering from this difficult disease.

**Table 1.** Descriptive statistics of B-CLL and Healthy control groups

| Groups | Statistics | B-CLL (n=50) | Control (n=25) |
|---|---|---|---|
| **Age (years)** | Mean ±SD<br>Range | 59.0 ±11.8<br>24 – 83 | 56.2 ±8.9<br>44 - 72 |
| **Gender**<br>**Male**<br>**Females** | N (%) | 33 (66)<br>17 (34) | 17 (68)<br>8 (32) |
| **No of nodual areas**<br>**<3**<br>**>3** | N (%) | 20 (40)<br>30 (60) | ---------- |
| **Histopathological stage**<br>**I -II**<br>**III – IV** | n (%) | 32 (64)<br>18 (36) | ---------- |
| **Cytogenetic abnormalities**<br>**Del13q**<br>**Del11q**<br>**Del17q** | n (%) | 31 (62)<br>12 (24)<br>7 (14) | ---------- |
| **Clinical response**<br>**Remission**<br>**Relapse** | n (%) | 26 (52)<br>24 (48) | ---------- |

**Table 2.** The gene expression level of Linc-p21, miR-34a and P53 in B-CLL and Healthy control groups

| Group | **Linc-p21(FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** | **miR-34a (FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** | **P53 (FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** |
|---|---|---|---|
| **B-CLL** | 45.6 (70)<br>13.9 – 189 | 0.1 (0.2)<br>0.01 – 1.23 | 4.3 (5.33)<br>0.01 – 14.7 |
| **Control** | 13.6 (13.3)<br>0.27 – 53.0 | 2.2 (2.36)<br>0.84 – 4.17 | 1.06 (1.7)<br>0.41 – 5.2 |
| **Statistics** | U: 119, p<0.0001 | U: 70, p<0.0001 | U: 201, p<0.0001 |

U: Mann-Whitney U test value, IQR: interquartile ratio, FC: fold change

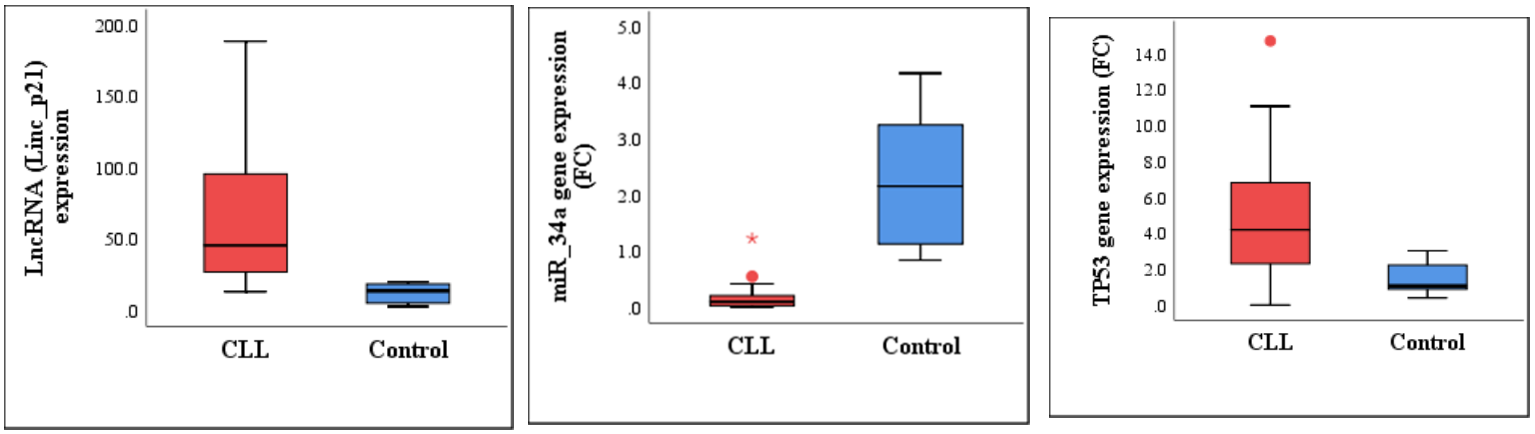


**Fig. 1** Boxplot graph illustrating a significant difference in the expression of Linc-p21 (panel A), miR-34a (panel B), and P53 (panel c) in B-CLL patients compared to the healthy control group.

**Table 3.** The gene expression level of Linc-p21, miR-34a, and P53 in different gender of B-CLL patients

| **Group** | **Linc-p21(FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** | **miR-34a (FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** | **P53 (FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** |
|---|---|---|---|
| **Male** | 53.8 (84.3)<br>13.9 – 188.7 | 0.2 (0.3)<br>0.01 – 1.23 | 4.3 (3.4)<br>0.01 – 147 |
| **Females** | 36.5 (32.8)<br>22.2 – 147.0 | 0.26 (0.12)<br>0.01 – 0.5 | 4.6 (6.5)<br>1.42 – 11.8 |
| **Statistics** | U: 229, p=0.29 | U: 266, p<0.77 | U: 820, p<0.68 |

U: Mann-Whitney U test value, IQR: interquartile ratio, FC: fold change

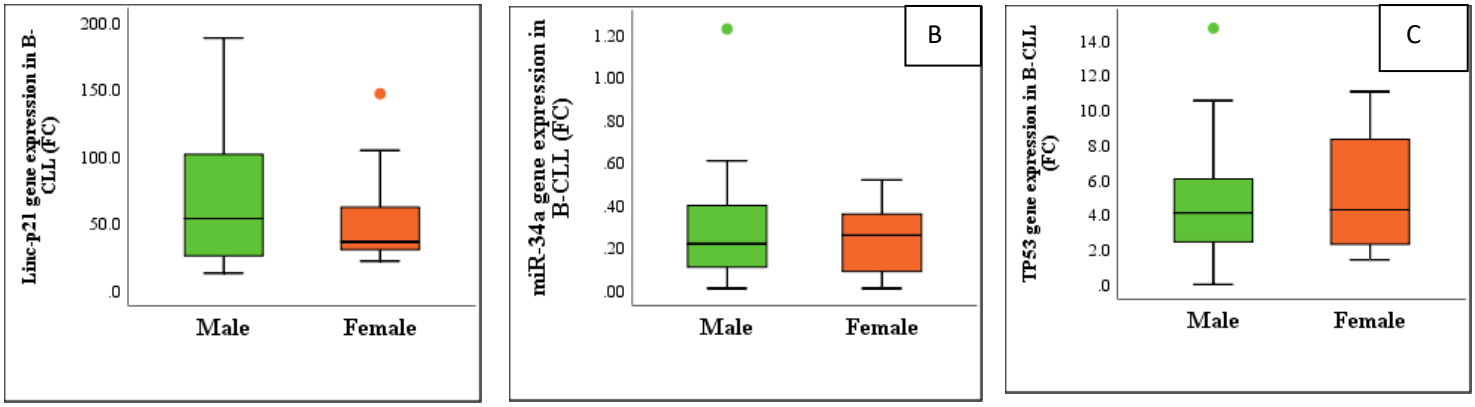


**Fig. 2** Boxplot graph illustrating the non-significant difference in the expression of Linc-p21 (panel A), miR-34a (panel B), and P53 (panel C) in B-CLL patients of different gender groups

**Table 4.** Descriptive statistics of hematological data of B-CLL patients

| Statistics | Hemoglobin (gm/dL) | TLC (10^3/µL) | Platelets (10^3/ µL) | Lymphocytes% |
|---|---|---|---|---|
| **Mean** | 10.0 | 71.65 | 143.3 | 59.9 |
| **Median** | 9.500 | 41.600 | 130.0 | 72.0 |
| **Standard deviation** | 2.10 | 79.2093 | 93.0 | 29.9 |
| **Minimum** | 8 | 39 | 121 | 45 |
| **Maximum** | 11 | 14.074 | 146 | 81 |

TLC: total leukocyte count

**Table 5.** The gene expression level of Linc-p21, miR-34a and P53 in B-CLL patients with infiltration of less and more than three nodal regions

| Group | Linc-p21(FC) *Median (IQR)* *Minimum-maximum* | miR-34a (FC) *Median (IQR)* *Minimum-maximum* | P53 (FC) *Median (IQR)* *Minimum-maximum* |
|---|---|---|---|
| **≤ 2 nodal areas** | 30.7(28.6)<br>13.0 – 116.8 | 0.3 (0.2)<br>0.03 – 0.6 | 2.5 (2.4)<br>0.01 – 8.4 |
| **> 3 nodal areas** | 66.5 (90.4)<br>22.7 – 188.7 | 0.2 (0.3)<br>0.01 – 1.23 | 5.1 (5.9<br>0.01 – 14.7 |
| **Statistics** | U: 113, p=0.001 | U: 242, p<0.25 | U: 159, p<0.005 |

U: Mann-Whitney U test value, IQR: interquartile ratio, FC: fold change

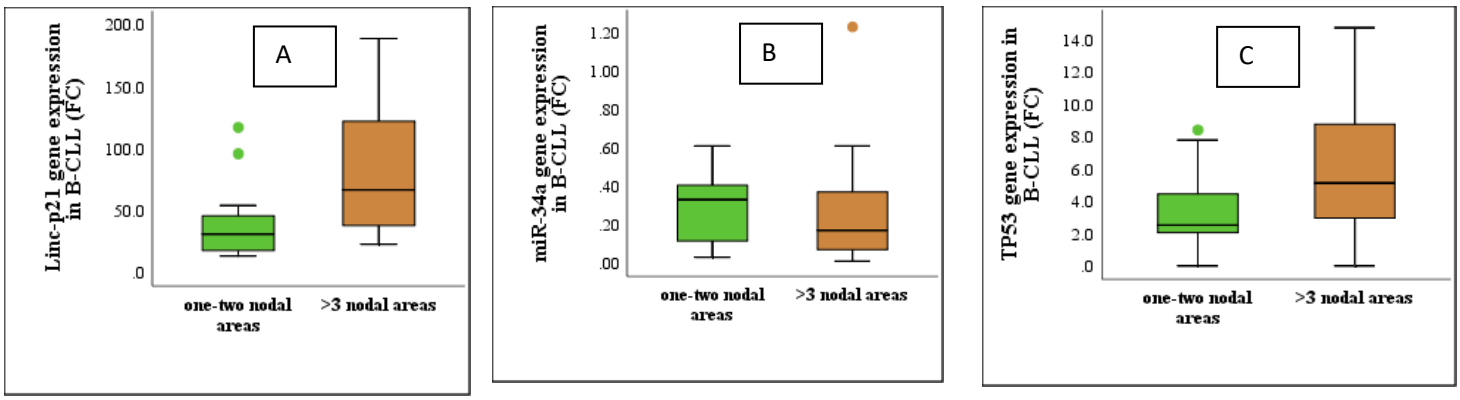


**Fig. 3** Boxplot graph illustrating a significant difference in the expression of Linc-p21 (panel A) miR-34a (panel B). and P53 (panel C) in B-CLL patients' different grades of nodal affection

**Table 6.** The gene expression level of Linc-p21, miR-34a, and P53 in B-CLL patients with different histopathological stages

| Group | Linc-p21(FC)<br>*Median (IQR)*<br>*Minimum-maximum* | miR-34a (FC)<br>*Median (IQR)*<br>*Minimum-maximum* | P53 (FC)<br>*Median (IQR)*<br>*Minimum-maximum* |
|---|---|---|---|
| **I – II** | 34.7 (23.0)<br>13.9 – 99.7 | 0.35 (0.3)<br>0.01 – 1.23 | 2.5 (2.4)<br>0.01 – 10.4 |
| **III – IV** | 110.8 (108.4)<br>30.7 – 188.7 | 0.1 (0.2)<br>0.01 – 0.5 | 6.6 (3.8)<br>4.0 – 14.7 |
| **Statistics** | U: 40.5, p=0.0001 | U: 128, p<0.001 | U: 67, p<0.001 |

U: Mann-Whitney U test value, IQR: interquartile ratio, FC: fold change

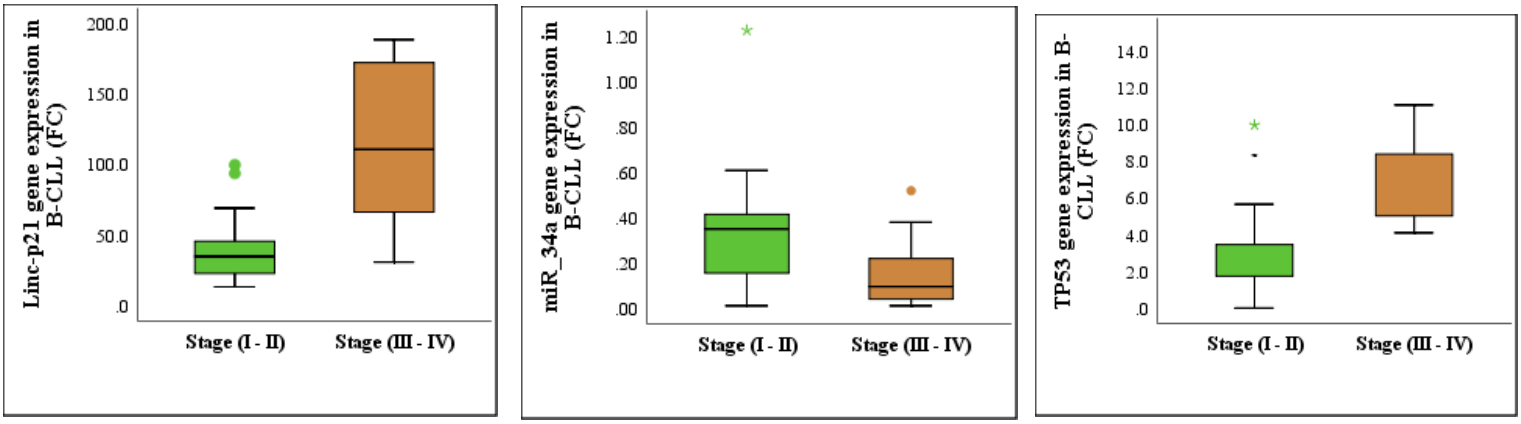


**Fig. 4** Boxplot graph illustrating non-significant difference in the expression of Linc-p21 (panel A) miR-34a (panel B). and P53 (panel C) in B-CLL patients'' different histopathological stages (p<0.01)

**Table 7.** Gene expression level of Linc-p21, miR-34a and P53 in B-CLL patients with different Cytogenetics abnormalities

| Group | Linc-p21(FC)<br>*Median (IQR)*<br>*Minimum-maximum* | miR-34a (FC)<br>*Median (IQR)*<br>*Minimum-maximum* | P53 (FC)<br>*Median (IQR)*<br>*Minimum-maximum* |
|---|---|---|---|
| **del 13q abnormality** | 36.3 (30.8)<br>13.0 – 96.0 | 0.35 (0.2)<br>0.01 – 1.23 | 2.6 (2.5)<br>0.01 – 10.4 |
| **del 11q abnormality** | 103 (115.6)<br>18.3 – 181.0 | 0.1 (0.06)<br>0.04 – 0.6 | 5.6 (5.0)<br>0.01 – 10.6 |
| **del 17q abnormality** | 145 (117)<br>31.0 – 188.7 | 0.09 (0.3)<br>0.01 – 0.6 | 7.8 (5.7)<br>4.9 – 14.7 |
| **Statistics** | F: 178, p=0.001 | F: 3.8, p<0.03 | F: 6.8, p<0.003 |

F: ANOVA test value, IQR: interquartile ratio, FC: fold change

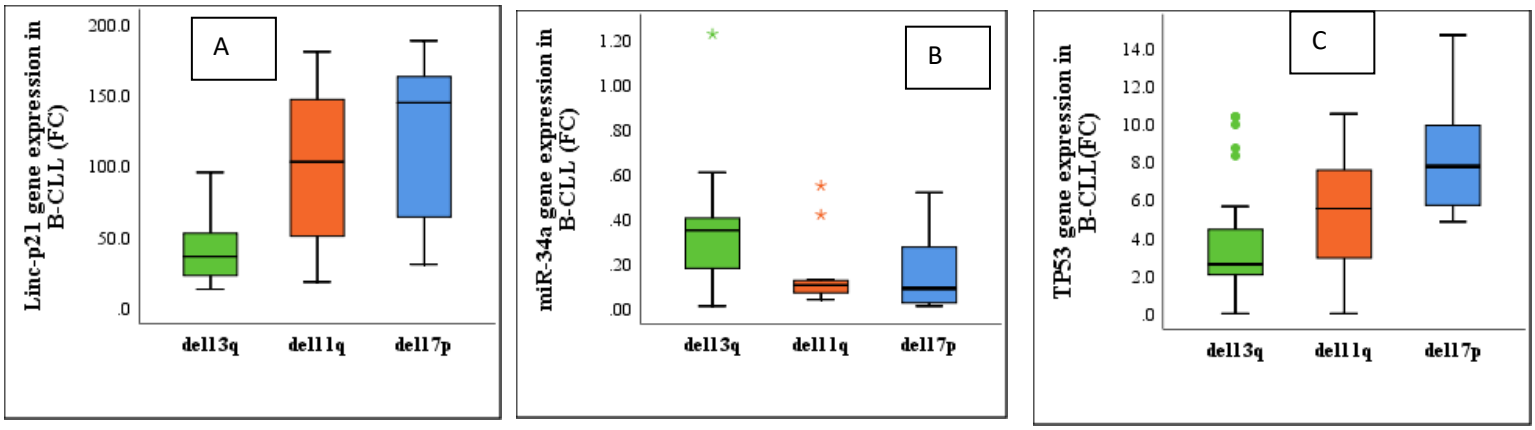


**Fig. 5** Boxplot graph illustrating the significant difference in the expression of Linc-p21 (panel A) miR-34a (panel B). and P53 (panel C) in B-CLL patients'' different cytogenetic abnormalities (p<0.01)

**Table 9.** The gene expression level of Linc-p21, miR-34a, and P53 in B-CLL patients with different Cytogenetics abnormalities

| **Group** | **Linc-p21(FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** | **miR-34a (FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** | **P53 (FC)**<br>***Median (IQR)***<br>***Minimum-maximum*** |
|---|---|---|---|
| **Relapsed** | 80.9 (110)<br>16.0 – 188.7 | 0.1 (0.3)<br>0.01 – 0.5 | 5.2 (4.6)<br>0.01 – 14.7 |
| **Remitted** | 35.8 (28.0)<br>13.8 – 93.7 | 0.35 (0.2)<br>0.04 – 1.2 | 2.6 (2.2)<br>0.01 – 10.4 |
| **Statistics** | U: 148, p=0.001 | U: 165, p<0.004 | U: 190, p<0.018 |

U: Mann-Whitney U test value, IQR: interquartile ratio, FC: fold change

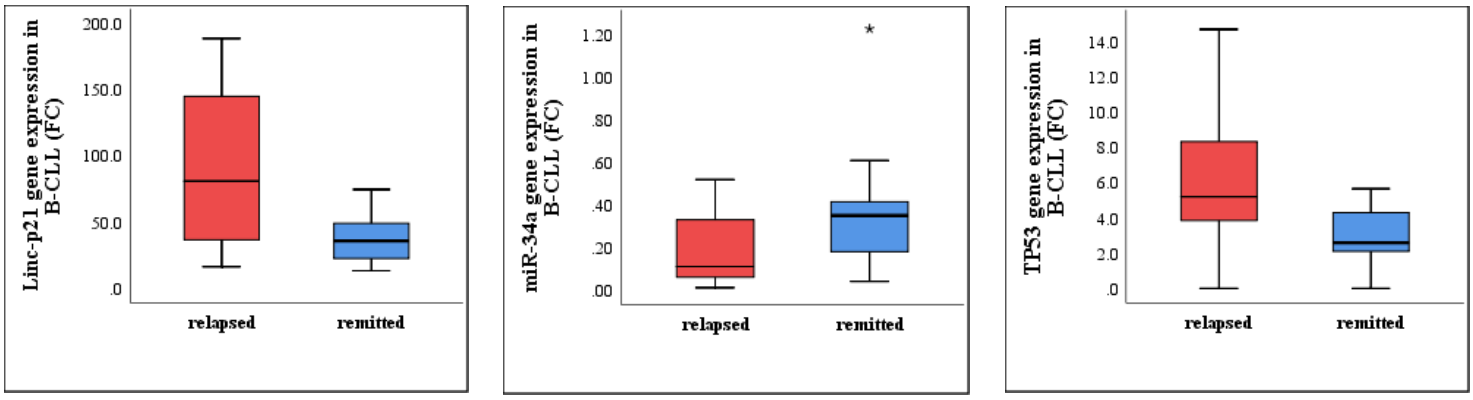


**Fig. 6** Boxplot graph illustrating the significant difference in the expression of Linc-p21 (panel A) miR-34a (panel B). and P53 (panel C) in B-CLL patients different clinical response (p<0.05)

**Table 10.** Diagnostic potential of Linc-p21, miR-34a, and P53 in B-CLL vs healthy controls (ROC curve)

| **Group** | | **Linc-p21(FC)** | **miR-34a (FC)** | **P53 (FC)** |
|---|---|---|---|---|
| **Cut-off value (AUC)** | | 19 (0.9) | 0.13 (0.94) | 2.0 (0.84) |
| **Biomarker Sensitivity (%)** | | 88 | 72 | 76 |
| **Biomarker Specificity (%)** | 80 | 92 | 84 | |
| **p-value** | 0.001 | 0.033 | 0.042 | |

AUC: area under the curve, FC: fold change, ROC: receiving operating characteristics curve

**Table 11.** Prognostic potential of Linc-p21, miR-34a, and P53 for prediction of progression in B-CLL (ROC curve)

| **Group** | | **Linc-p21(FC)** | **miR-34a (FC)** | **P53 (FC)** |
|---|---|---|---|---|
| **Cut-off value (AUC)** | 49.0 (0.8) | NA (0.55) | 4.0(0.7) | |
| **Biomarker Sensitivity (%)** | 88 | NA | 73 | |
| **Biomarker Specificity (%)** | 80 | NA | 62 | |
| **p-value** | 0.001 | 0.52 | 0.02 | |

AUC: area under the curve, FC: fold change, ROC: receiving operating characteristics curve, NA: not applicable.

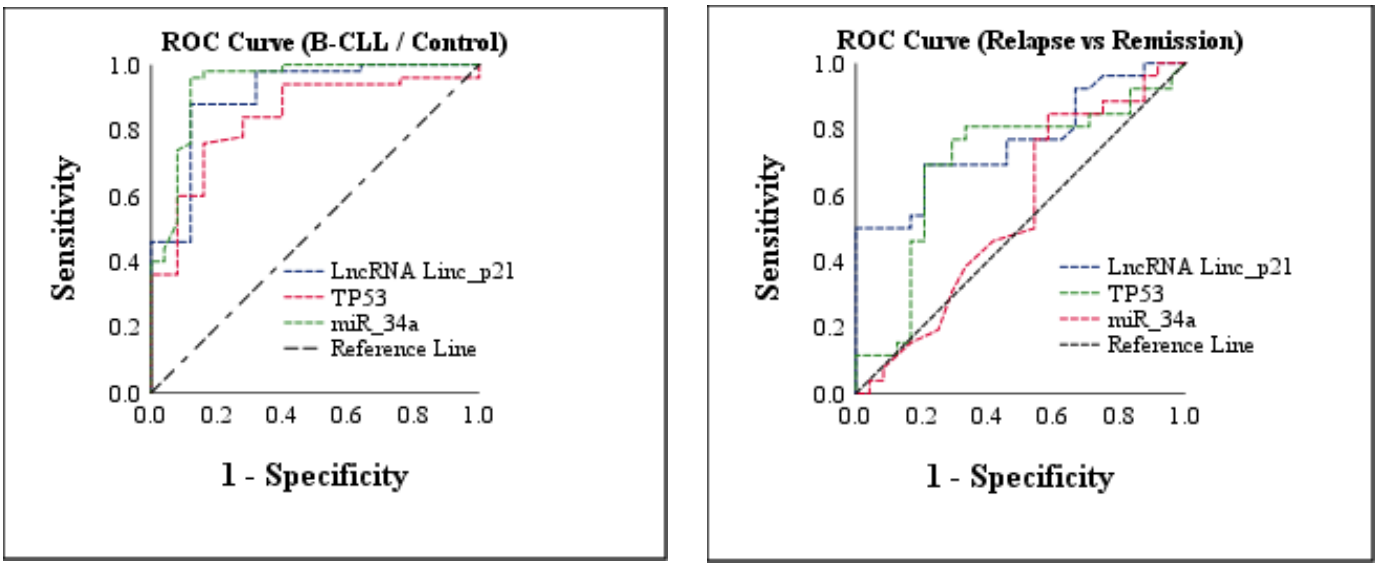


**Fig. 7** ROC curve illustrating the diagnostic potential of Linc-p21, miR-34a, and P53 in differentiating B-CLL patients from healthy controls (Panel A). for prediction to progression in B-CLL patients.

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