wjpmr, 2025, 11(2), 1-10

SJIF Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

IMPACT OF LNC RNA-NEAT1 ON MULTIPLE DRUG RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Article Received on 04/12/2024 **Article Revised on 25/12/2024** **Article Accepted on 15/01/2025**

ABSTRACT

The biological impact and therapeutic potential of long non-coding RNAs (lncRNAs) in chronic lymphocytic leukemia (CLL) remain an area of concern and research. Many studies have found that lncRNAs play a role in cancer progression as well as chemotherapy resistance. Herein, we investigated the impact of lncRNA NEAT1 on multiple drug resistance in CLL. We examined NEAT1 expression in B-CLL patients and in normal B-cells. This study aimed to determine the NEAT1 expression and its correlation with multiple-drug resistance (ATP-binding cassette transporter A3 ABCA3) gene expression in CLL patients using quantitative real-time polymerase chain reaction (qRT-PCR). Our results showed that NEAT1 expression was significantly lower in CLL patients compared to healthy controls (P-value = 0.016). Our results also showed a significant correlation between the expression levels of NEAT1 and ABCA3 genes in CLL patients (P-value < 0.05).

KEYWORDS: We examined NEAT1 expression in B-CLL patients and in normal B-cells.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in the Western world, representing more than 30% of all leukemia cases **(Siegel et al., 2020)**. It can be asymptomatic disease in 40- 60% of patients are accidentally diagnosed medical routine check-up, while the rest of patients may come with symptoms like lymphadenopathy, splenomegaly, recurrent infections and/or autoimmune diseases (such as hemolytic anemia or thrombocytopenia), in addition to other symptoms like weakness, fever, night sweats and fatigue **(Rodrigues et al., 2016)**. CLL is a malignancy characterized by clonal expansion of CD5+ B-cells that show morphologically mature appearance and accumulate in the blood, bone marrow and secondary lymphoid tissues, resulting in lymphocytosis, bone marrow infiltration, lymphadenopathy and splenomegaly **(Kipps et al., 2017)**. The CLL has a highly heterogeneous clinical course, ranging from an indolent behavior to an aggressive disease that needs prompt treatment in almost 30% of cases. These differences have been associated with a number of markers of the leukemic cells, including chromosomal aberrations, mutational status of the Immunoglobulin heavy chain variable region genes (IGHV), TP53 inactivation, CD38 and ZAP-70 expression **(Hallek et al., 2018)**. However, despite the availability of these markers, the disease course remains somewhat unpredictable.

The ATP-binding cassette (ABC) transporter superfamily is the biggest transporter gene family across extracellular and intracellular membranes. ABC genes are required for numerous cellular functions and mutations in these genes contribute to a variety of human genetic illnesses such as neurological disease, cystic fibrosis, retinal degeneration, cholesterol and bile transport abnormalities, anemia, and medication responsiveness **(Dean et al., 2001)**. The human genome has 49 ABC genes, which are organized into seven subfamilies labeled A to G **(Vasiliou et al., 2009)**. The association of ABC protein expression with cancer aggressiveness has mostly been connected to their drug-efflux capacity, which leads to MDR, especially in recurring cancers. However, accumulating data suggests that their dysregulated expression may play a critical role in the early events of tumor development, as well as promote tumor progression, resulting in poor patient outcomes, irrespective of chemotherapeutic-drug efflux resistance **(Muriithi et al., 2020)**.

Multidrug resistance (MDR) severely affects the efficacy of chemotherapy in cancer and leukemia patients. The specific role of ABCA3 in drug resistance was widely

described in hematologic diseases, and as a contributing mechanism of drug resistance **(Hupfeld et al., 2013)**. Greater ABCA3 expression in acute myeloid leukemia (AML) samples is related with a poor treatment outcome. In vitro, increased expression leads to resistance to a wide range of cytostatic drugs. ABCA3 is confined within the limiting membranes of lysosomes and multivesicular bodies, where it efficiently sequesters cytostatic. Aside from AML, ABCA3 was found in a variety of lymphohematopoietic tissues and altered cell lines. Subcellular drug sequestration driven by really intracellular ABCA3 was found as a clinically significant mechanism of intrinsic MDR **(Chapuy et al., 2008)**. ABCA3 was found down-regulated in the pancreatic ductal adenocarcinoma **(Mohelnikova-Duchonova et al., 2013)** and colorectal cancer **(Hlavata et al., 2012)**, and up-regulated in breast cancer **(Park et al., 2006)** and acute lymphoblastic leukemia **(Efferth et al., 2006)**.

Long non-coding RNAs (LncRNA) are defined as RNA transcripts of larger than 200 nucleotides that are not translated into proteins **(Rinn & Chang, 2012)**. Several studies shed light on long non-coding RNAs and their biological and pathophysiological roles **(Bonetti & Carninci, 2017)**. LncRNAs play important roles in transcriptional and posttranscriptional gene regulation **(Dykes & Emanueli, 2017)**. Recently, emerging data have reported that lncRNAs are frequently deregulated in multiple cancers, such as gastric cancer **(Liu et al., 2017)**, bladder cancer **(Xie et al., 2017)**, and chronic myeloid leukemia **(Lu et al., 2017)**, and play important roles in biological processes, including proliferation, differentiation, and apoptosis **(Rinn & Chang, 2012)**. The lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1), a nuclear restricted lncRNA, includes two subunits: NEAT1_1 and NEAT1_2 **(Gao et al., 2016)**. NEAT1 plays a crucial role in numerous biological processes, including cellular differentiation **(Sun et al., 2016)** and stress response **(Adriaens et al., 2016)**. **Adriaens et al**. **(2016)** proved that NEAT1 enabled the tumorigenesis of lung adenocarcinoma in vivo by promoting the survival of oncogene targeted cells. NEAT1 expression is also increased in many human cancers, including lung **(Pan et al., 2015)**, esophageal **(Chen et al., 2015)**, Laryngeal cancer **(Wang et al., 2016)**, Colorectal cancer **(Wu et al., 2015)**, Hepatocellular cancer **(Guo et al., 2015)**, Prostate cancer **(Chakravarty et al., 2014)**, Breast cancer **(Choudhry et al., 2015)**, Ovarian cancer **(Pils et al., 2013)**, Glioma **(He et al., 2016)**, and gastric cancers **(Ma et al., 2016)**, although it is decreased in acute promyelocytic leukemia **(Zeng et al., 2014)**.

Multiple drug resistance (MDR) and adverse reactions are the leading causes of cancer treatment failure, with drug resistance attributable mostly to aberrant expression of ATP-binding cassette (ABC) **(Aberuyi et al., 2017)** and RNA-binding proteins (RBPs) **(Hong, 2017)**. It has been demonstrated that lncRNAs and miRNAs play a crucial role in oncogene transcription and tumor cell metastasis **(García-Sancha et al., 2019)**. NEAT1 not only increases cancer cell sensitivity to chemotherapy, but it also activates the P53 signaling pathway and increases apoptosis **(Adriaens et al., 2016)**. NEAT1 expression was found to be up-regulated in tumor tissue following analysis of samples from acute lymphoblastic leukemia (ALL) and multiple myeloma (MM) patients. Nonetheless, a recent study found that patients with acute myeloid leukemia (AML) and chronic myelogenous leukemia (CML) had lower levels of NEAT1 expression. AML and CML are promoted by downregulating NEAT1, which is mediated by the miR-194-5p/DNA methyl-transferase 3A (DNMT3A) **(Duan et al., 2020)** and the miR-766-5p/cyclin dependent kinase inhibitor 1A (CDKN1A) axis **(Yao et al., 2021)**. Therefore, in AML, CML, and MM patients, NEAT1 expression plays a critical role in decreasing drug resistance and delaying the rate of proliferation and apoptosis **(Zhang et al., 2021)**.

These findings lead first to our hypothesis, that NEAT1 may also play a crucial role in drug resistance of Chronic Lymphatic Leukemia (CLL) and therefore, we hypothesized that NEAT1 might be associated with CLL progression in MDR patients.

MATERIALS AND METHODS

The current study was conducted on 50 individuals in a period from Dec 2019 to Sep. 2021 during the pandemic COVID19 time. They were divided into two groups: Group I included 40 patients diagnosed with chronic lymphocytic leukemia (26 males and 14 females). The patient's age ranged from 24 to 83 years old. They were diagnosed based on a Complete Blood Picture, morphological bone marrow (BM) examination, Immunophenotyping, and Cytogenetic analysis. Group II included 10 adults (8 males and 2 females) selected from the Hematology- department- Demerdash Hospital - Ain Shams University. Their ages ranged from 45 to 72 years old.

After obtaining the approval of the research ethics committee of the Faculty of Medicine, Ain Shams University (FMASU 1559/2013), written informed consent was obtained from each patient after informing him or her about the steps of the procedure and the expected effects. Patients were diagnosed based on a Complete Blood Picture, morphological bone marrow examination, Immunophenotyping, and Cytogenetic analysis. The demonstration that known prognostic factors (such as Rai staging) are also prognostically relevant in this group of patients suggests that this group of patients is representative of patients with B-CLL and is not biased. The patients were selected according to the following inclusion and exclusion criteria: Inclusion criteria; B-CLL with a characteristic immunophenotype, including small lymphatic lymphoma, Participants must have evidence of anemia and /or thrombocytopenia. Exclusion Criteria; Prior therapy for CLL, Major surgery within 4 weeks before selection, B-CLL patients with

other malignancies whether de novo or as a complication to B-CLL.

The diagnosis was confirmed using peripheral blood samples examined in morphologic, cytochemical, and immunologic studies (CD38), and molecular studies (immunoglobulin heavy chain gene rearrangement and T-cell receptor rearrangement). MDR is defined as an isolate that is not susceptible to at least one agent in at least three antimicrobial classes.

Sampling and RNA extraction

Isolation of mononuclear cells was performed using a protocol based on the Lymphoprep™ Kit. The total RNA was extracted using the RNX-Plus kit and 2 µg of total RNA was used for cDNA synthesis by a cDNA synthesis kit, by the specific temperature protocol (sequentially, 5 minutes at 25°C, 60 minutes at 42°C, and 5 minutes at 70°C). The cDNA was then stored at -20°C for further studies. Bone marrow samples were evaluated for monoclonality (IGHV rearrangement) at diagnosis and again at the end of the first year of treatment using PCR analysis. Persistent monoclonality of T-cell gamma receptors or IGHV gene rearrangement after 1 year of treatment, or observation of any sign of leukemia relapse before this time, was considered as MDR.

Quantitative reverse transcription PCR (qRTPCR)

RNA was extracted by phenol-chloroform isolation using TRIzol reagent (Invitrogen) and then purified using the MirVANA kit total RNA isolation procedure (Ambion). Following isolation, RNA was treated with Turbo DNase (Ambion). cDNA was prepared with the SuperScript First-Strand Synthesis System (Invitrogen). qRT-PCR was performed using SYBER Green master mix (Applied Biosystems). Real-time PCR was performed using the real-time PCR kit (Applied Biosystems Step One™ Real-Time PCR system). Briefly, 1µL of 0.01µg/mL of the synthesized cDNA, 0.4µL of 100nM forward and reverse primers, 5µL of SYBR green Master Mix, and 3.2µL of ddH_2O were added to the real-time tubes. that included a preincubation at 95°C (5 minutes), denaturation at 95°C (20 seconds), annealing at 60°C (30 seconds), and product expansion at 72°C (25 seconds). Delta CT was determined by subtracting the CT value of the housekeeping gene from the CT value of the lncRNA. Relative expression levels were calculated using the 2-ΔΔCt method. Detection was performed using an ABI Prism 7700 detection system (Applied Biosystems). The housekeeping gene GAPDH was used as an endogenous control to study the relative expression of LncRNA NEAT1 and ABCA3 mRNAs. Custom-designed primers were used for all SYBER Green reactions. Custom-designed primer sequences are provided in supplemental information.

Statistical analysis

All experiments were repeated at least three times, and all data were presented as the means ±SD. GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA) was used to analyze the data and draw the graphs. Student's t test was used for comparisons between the two groups, and one way ANOVA was used for comparisons among more than two groups. $p<0.05$ was considered statistically significant.

RESULTS

The present study was performed in Global Medical Research Lab. Our study evaluated lncRNA NEAT1 and mRNA ABCA3 gene expression as molecular diagnostic and prognostic factors for chronic lymphocytic leukemia and correlate their expressions with different clinic-pathological parameters including phenotypes, hematological data and disease outcome. CLL group: including 40 patients were diagnosed as CLL with different clinical phenotypes; their ages range from (24 – 83) years and a mean value of 58.0 ± 12.0, and Control group: including 10 normal persons; their ages ranged from (45 – 72) years and a mean value of 58.0 ± 8.4; these data were illustrated in **Table 1**.

Table 1: Demographic data of the studied subjects.

Characteristics	CLL patients	Control	P value
Age means (yr.) ± SD. median (range)	58.0 ± 12.0 60 (24.0 – 83.0)	58.0 ± 8.4 55 (45-72)	0.234 NS
Sex N (%) Male Female	 26 (65) 14 (35)	 8 (80) 2 (20)	

2- Patients with B-CLL phenotype Description

The CLL patients (n=40) were sub classified according to Rai staging into Low, Intermediate and High classes. Intermediate class represents the commonest group 20 (50 %), followed by High class which represents 11 (27.5%) and Low class which represents 9 (22.5%). According to size of enlarged nodal area involvement into 2 classes, 16 patients (40%) diagnosed with presence of three or less of enlarged nodule area while 24 patients (60%) diagnosed with presence more than three of nodule area. However, concerning tumor differentiation status patients were divided into poor, Intermediate and well classes, well class represents the commonest group 25 (62.5%), followed by Intermediate class which represents 9 (22.5%) and poor class which represents 6 (15%). Regarding histopathological classification of B-CLL lymphocytes, 29 patients (72.5%) with typical B-CLL lymphocytes and 11 patients (27.5%) with atypical B-CLL lymphocytes.

The prognostic factor IGHV mutation which represents the higher percentage 29 (72.5 %) shows good prognosis

of CLL patients, whereas the IGHV un mutation represents a low percentage 11 (27.5 %) and show poor prognosis of CLL patients. ZAP-70 deficiency is an immunodeficiency disorder involving impaired T-cell activation caused by a signaling defect. ZAP-70 expression divided into two subgroups (<=20% / >20%) in B-CLL group, in B-CLL group the prognostic factor ZAP-70 expression (<=20%) represent the higher percentage 30 (75%), than the ZAP-70 expression (>20%) which represent 10 (25%). Concerning the B-CLL group (n=40), the prognostic factor CD38 expression (>30%) represents a higher percentage 26 (65%), than the CD38 expression (<30%) which represents 14 (35%). The CLL patients were sub classified according to response of treatment into resistance and sensitive responses, the resistance response includes 21 patients (52.5%) while sensitive response includes 19 patients (47.5%). The distribution of patients among different subgroups is illustrated in **Table 2**.

Table 2: Clinical characteristics of the B-CLL group.

Characteristics	Patients N (%)
Rai stage	
I (low)	9 (22.5)
II (intermediate)	20 (50.0)
III (high)	11 (27.5)
No. of nodal area	
Good (<=3)	16 (40.0)
Poor (>3)	24 (60.0)
Tumor Differentiation Status	
Well	25 (62.5)
Intermediate	9 (22.5)
Poor	6 (15.0)
Histopathology of B-CLL lymphocytes	
Typical B-CLL lymphocytes	29 (72.5)
Atypical B-CLL lymphocytes	11 (27.5)
IGVH mutational status	
Mutated	29 (72.5)
Unmutated	11 (27.5)
ZAP-70 level	
Positive >20%	10 (25.0)
Negative <20%	30 (75.0)
CD38 level	
Low level <30%	14 (35.0)
High level >30%	26 (65.0)
Response of treatment	
Resistance	21 (52.5)
sensitive	19 (47.5)

Expression levels of Lnc-NEAT1 and ABCA3 in studied subjects

The median value of expression of LncRNA NEAT1 in B-CLL patients was 0.28 in contrast to the median value of the control group which was 1.13 which revealed that the lncRNA was down-expressed in B-CLL patients by almost 4-folds. On the other hand, the median expression of mRNA ABCA3 in B-CLL patients was upregulated from 1.79 in the control group to 41.2 in the studied patients with about 23-fold higher compared to the control groups **Table 3**. According to the expression level of the NEAT1, it showed to be down-regulated by 4 folds, which might work as a tumor suppressor gene; on the other side, the ABCA3 gene is up-regulated in CLL patients compared to health control which might act as an oncogenic gene.

Table 3: Expression levels of LNC NEAT1 and mRNA ABCA3 in the studied subjects.

Variable	Gene expression (log^{10})		
	CLL (N=40)	Control (N=10)	Statistics
LncRNA NEAT1 (log^{10}) Median (Min-Max)	0.28 (0.01 - 3.73)	1.13 (0.58-1.56)	U=39 p=0.016
mRNA ABCA3 (log^{10}) Median (Min-Max)	41.2 (16.68-93.05)	1.79 (0.23-2.22)	U=48 p=0.0001

Expression of NEAT1 and ABCA3 in different diagnostic parameters among B-CLL patients

Clinical features are categorized into subgroups to evaluate the median values of expression levels of lncRNA NEAT1 and mRNA ABCA3 among the B-CLL prognostic criteria.

In respect to clinicopathological characterization for Rai-staging, the B-CLL patients are classified into three classes: low class with a median value (0.13) in NEAT1 and (71.51) in ABCA3, intermediate class with a median value (0.29) in NEAT1 and (37.93) in ABCA3 and high class with a median value (0.32) in NEAT1 and (32.67) in ABCA3. These data show a significant difference in the expression of both genes in B-CLL group (P<0.01). In respect to tumor differentiation status, B-CLL patients were classified into three groups: well group with median value (0.23) in NEAT1 and (56.89) in ABCA3, intermediate group with median value (0.32) in NEAT1 and (35.26) in ABCA3 and poor group with median value (0.52) and (37.02) in ABCA3. These data show a significant difference (P<0.01) in mRNA ABCA3 and a highly significant difference (P<0.000) in lncRNA NEAT1 expression levels in B-CLL group. Concerning histopathology of lymphocytes, the B-CLL patients are classified into two groups: typical B-CLL lymphocytes group with median value (0.23) in lncRNA NEAT1, (54.95) in mRNA ABCA3 and atypical B-CLL lymphocytes group with median value (0.32) in lncRNA NEAT1, (32.67) in mRNA ABCA3. These data show a significantly difference in the expression levels of both genes in B-CLL group. However, molecular studies for immunoglobulin variable heavy chain (IGVH) in the B-CLL patients are classified into two groups: unmutated (IGVH) group with a median value (0.61) in lncRNA NEAT1, (33.13) in mRNA ABCA3 and mutated (IGVH) group with a median value (0.23) in lncRNA NEAT1, (44.02) in mRNA ABCA3. These data show a highly significant difference in mRNA ABCA3 expression (P<0.05) and a non-significant difference in lncRNA NEAT1 expression (P=0.40) in B-CLL group. Zeta-associated protein-70 (ZAP-70) of the B-CLL patients are classified into two groups: negative group with a median value (0.27) in lncRNA NEAT1, (42.24) in mRNA ABCA3 and positive group with a median value (0.38) in lncRNA NEAT1, (38.44) in mRNA ABCA3. These data show no significant difference in expression levels of both genes in B-CLL group according to ZAP-70. But Cluster of differentiation 38 (CD38), the B-CLL patients are classified into two groups: low level of CD38 group with a median value (0.19) in lncRNA NEAT1, (48.74) in mRNA ABCA3 and high level of CD38 group with a median value (0.37) in lncRNA NEAT1, (36.40) in mRNA ABCA3. These data show a highly significant difference in expression levels of lncRNA NEAT1 (P=0.006) and a significant difference in expression levels of mRNA ABCA3 (P=0.04) in B-CLL group. Treatment response, the B-CLL patients were divided into two groups: sensitive group with a median value (0.37) in lncRNA NEAT1, (0.37) in mRNA ABCA3 and resistance group with a median value (0.13) in lncRNA NEAT1, (69.35) in mRNA ABCA3. These data show a highly significant difference in both genes expression levels in B-CLL. These data are presented in **Table 4** and **Fig. 1**.

Table (4): Distribution of NEAT1 and ABCA3 expression in the B-CLL group according to Rai-staging, tumor differentiation status, histopathology of lymphocytes, IGVH mutations, ZAP-70, CD38 clusters and Response to treatment.

Parameters	NEAT1	ABCA3
Rai Staging		
Low (n=9) Median (Min-Max)	**0.13 (0.03-1.13)**	**71.51 (29.86-89.88)**
Intermediate (n=20) Median (Min-Max)	**0.29 (0.01-3.73)**	**37.93 (16.68-93.05)**
High (n=11) Median (Min-Max)	**0.32 (0.04-1.20)**	**32.67 (22.63-58.89)**
Statistics	**F=7.46**	**F= 5.75**
P value	**P<0.01 {S}**	**P<0.01 {S}**
tumor differentiation status		
Well (n=25) Median (Min-Max)	**0.23 (0.03-2.57)**	**56.89 (16.68-93.05)**
Intermediate (n=9) Median (Min-Max)	**0.32 (0.01-3.73)**	**35.26 (22.63-65.80)**
Poor (n=6) Median (Min-Max)	**0.52 (0.04-1.20)**	**37.02 (24.59-58.89)**
Statistics	**F=27.9**	**F= 5.47**
P value	**P<0.000 {HS}**	**P<0.01 {S}**
histopathology of lymphocytes		
Typical (n=29) Median (Min-Max)	**0.23 (0.01-3.73)**	**54.95 (16.68- 93.05)**
Atypical (n=11) Median (Min-Max)	**0.32 (0.04-1.2)**	**32.67 (22.63- 58.89)**
Statistics	**U=70**	**U=81**
P value	**P<0.05 {S}**	**P<0.01 {S}**
IGVH mutations		
-Ve (n=11) Median (Min-Max)	**0.61 (0.01-1.2)**	**33.13 (22.63- 58.89)**
+Ve (n=29) Median (Min-Max)	**0.23 (0.02-3.37)**	**44.02 (16.68- 76.37)**
Statistics	**U=75.5**	**U=96**
P value	**P<0.40 {NS}**	**P<0.05 {S}**
ZAP-70		
-Ve (n=30) Median (Min-Max)	**0.27 (0.02-0.01)**	**42.24 (16.68- 24.59)**

+Ve (n=10) Median (Min-Max) **Statistics** **P value**	**0.38 (3.73-1.2)** **U=135.5** **P<0.66 {NS}**	**38.44 (93.05- 58.89)** **U=114** **P<0.27 {NS}**
CD38 clusters **High (n=26) Median (Min-Max)** **Low (n=14) Median (Min-Max)** **Statistics** **P value**	 **0.37 (0.02-3.73)** **0.19 (0.01-1.13)** **U=48** **P<0006 {HS}**	 **36.40 (16.68- 76.37)** **48.74 (25.46- 66.95)** **U=33** **P<0.04 {S}**
Response to treatment **Sensitive (n=19) Median (Min-Max)** **Resistance(n=21) Median (Min-Max)** **Statistics** **P value**	 **0.37 (0.04-3.73)** **0.13 (0.01-2.57)** **U=54** **P<0.0001 {HS}**	 **31.27 (16.68- 86.22)** **69.35 (27.85- 93.05)** **U=54** **P<0.0001 {HS}**

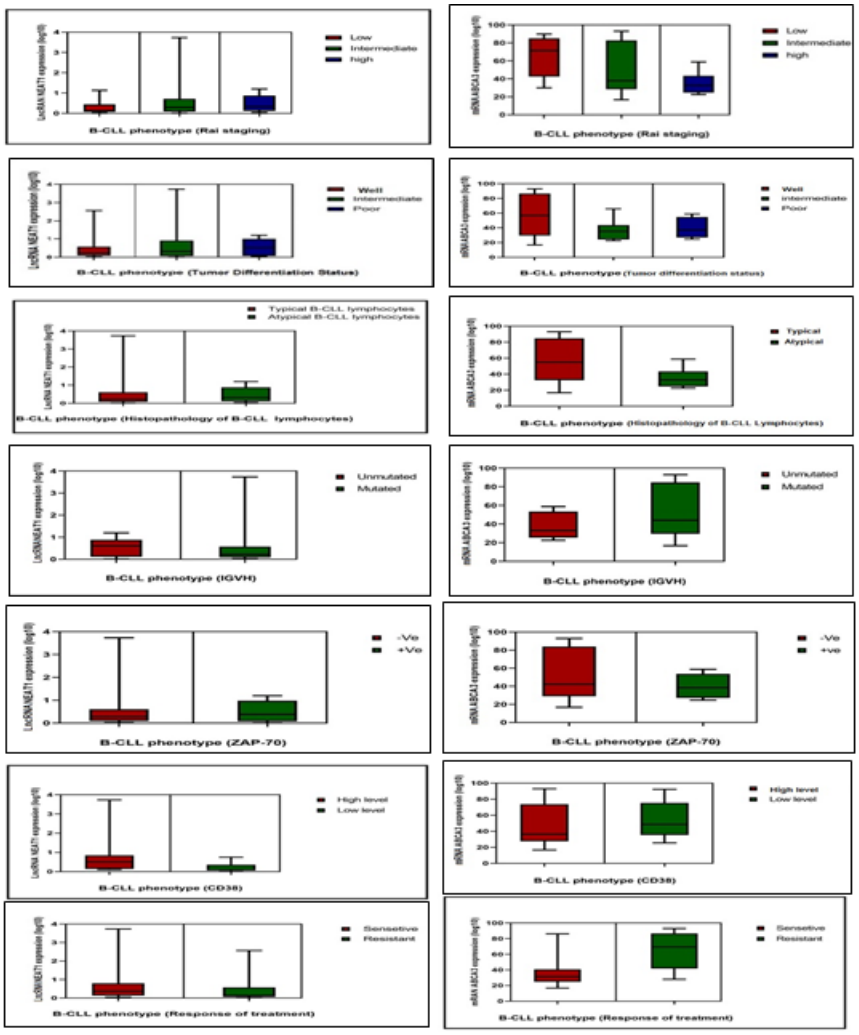

Figure (1): Boxplot graph demonstrating lncRNA NEAT1 and mRNA ABCA3 expression level in B-CLLL patients with Rai staging, tumor differentiation status, histopathology of lymphocytes, Immunoglobulin variable heavy chain (IGVH), ZAP-70, clusters of differentiation 38 (CD38), response to treatment.

DISCUSSION

Long non-coding RNA (lncRNA) has received a lot of interest in recent years because it plays a role in numerous biological processes, including transcriptional gene regulation, cell development, and differentiation. LncRNAs deregulation has been linked to tumor development, progression, and metastasis in a variety of cancers, including hematological malignancies, while studies' investigating a potential pathogenic function in CLL is limited **(Dahl et al., 2018)**.

The current study was performed on 40 CLL patients and 10 healthy individuals as a control group were selected from the Hematology-department- Demerdash Hospital -

Ain Shams University. The expression level of lncRNA NEAT1 and mRNA ABCA3 were examined using a real time PCR assay. We aimed to investigate the lncRNA NEAT1 expression in CLL patients and in healthy ones, find out the correlation between lncRNA NEAT1 and multi-drug resistance gene ABCA3 in CLL patients. Also, correlate lncRNA NEAT1 expression patterns with prognosis to outline its possible role in CLL outcome.

In our study, we focused on nuclear paraspeckle assembly transcript 1 (NEAT1), a well-known novel nuclear lncRNA located on chromosome 11q13 **(Clemson et al., 2009)**. NEAT1 has been recognized as a critical architectural component of a paraspeckle structure, and it has been demonstrated that it regulates several biological processes including cellular differentiation and stress response via the paraspeckles pathway **(Souquere et al., 2010)**. NEAT1 was discovered to be up-regulated in a variety of cancer tissues and cell lines, and it was a crucial mediator in cancer progression by regulating cell death, cell proliferation, and cell cycle **(Adriaens & Marine, 2017)**. In this light, NEAT1 has been proposed as a potential diagnostic marker and a novel target for cancer treatment. Aside from that, NEAT1 was discovered to contribute to chemoresistance and/or radio-resistance in lung cancer, nasopharyngeal carcinoma and ovarian cancer, implying that NEAT1 may be a possible biomarker for chemo-sensitivity **(Yang et al., 2017)**.

Many efforts have been made to investigate NEAT1's functional role in cancer advancement, however the underlying molecular processes by which NEAT1 contributes to cancer progression remain unknown. An significant study found that p53 can produce NEAT1-containing paraspeckles, which then influence replication stress and chemo-sensitivity in cancer cells **(Adriaens et al., 2016)**.

Further analysis showed that increased NEAT1 in cancer patients may be a reliable prognostic factor for hepato-gastroenterol cancers. Apart from the function role NEAT1 in cancer, NEAT1 also had other functional roles, which was found to contribute to the pathogenesis of lupus **(Zhang et al., 2016)**; NEAT1 is also crucial for the development of mammary gland and lactation **(Standaert et al., 2014)**; deregulated NEAT1 expression was found in the Huntington's disease as well **(Sunwoo et al., 2017)**, implying that NEAT1 has a wide range of functions. Increased NEAT1 expression is associated with ovarian cancer **(Adriaens et al., 2016)** and death-free survival in colorectal cancer **(Li et al., 2015)**.

Aside from the studies on solid tumors, NEAT1 was revealed to play a regulatory role in leukemia **(Blume et al., 2015)**. In the present study, the QPCR-analysis results implied that low NEAT1 expression was significantly associated with poor prognosis in patients with B-CLL cancer. Chronic lymphocytic leukemia has a highly variable clinical course that ranges from indolent to aggressive disease that requires immediate treatment in nearly 30% of cases. These changes have been linked to a variety of leukemic cell markers, including chromosomal aberrations, Immunoglobulin heavy chain variable region (IGHV) genes mutational status, TP53 inactivation, and ZAP-70 and CD38 expression **(Hallek et al., 2018)**. Despite the availability of these markers, the illness progression remains somewhat unexpected.

In addition, some researchers evaluated NEAT1 expression in other types of hematological tumors, including B-cell acute lymphoid leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), Multiple myeloma (MM), B cell-lymphoma cell lines, and different types of normal B-cell populations (such as normal peripheral blood B-cells and naïve and memory B cells purified from tonsils or spleen). Statistical tests were carried out using R functions, with a significant cutoff of p-value < 0.01. The expression of NEAT1 in MM was significantly higher than in normal controls; however this deregulation didn't correlate with prognoses. However, NEAT1's possible participation in many cellular stress response systems, such as TP53 pathways and the unfolded protein response makes it a promising option for a targeted therapy in MM. Furthermore, the elevated NEAT1_1 levels found in MM suggest that NEAT1_1 may be involved in unrelated functions of Primary Sjogren's Syndrome. **Ronchetti et al. (2020)** estimated the two isoforms of NEAT1 (NEAT1-1 and NEAT1-2) in 310 newly diagnosed Binet A B-CLL patients and in normal B-cell populations. For NEAT1 determination a quantitative real-time PCR (qRT-PCR) approach was used that was capable of discriminating the NEAT1 global expression. Although CLL samples exhibited a median NEAT1 expression equivalent to that of normal B cells, a subset of samples had high global NEAT1 expression levels. They evaluated whether changes in NEAT1 expression could correlate with other factors that typically differentiate CLL prognostic groups. Global NEAT1 expression was similar in CLL and MBL cases, with no significant difference between IGHV-mutated or unmutated cases or those with distinct cytogenetic abnormalities. Globally, NEAT1 expression did not differ significantly across most subgroups **(Ronchetti et al., 2020)**.

NEAT1 dysregulation has been observed in a variety of solid tumours, where it is frequently linked with a poor prognosis, as well as in hematological malignancies, where it appears to influence several biological processes. Abnormal promyelocytic leukemia/retinoic acid receptor alpha (PML-RARα) activity correlates with downregulated NEAT1 in acute promyelocytic leukemia, potentially affecting myeloid differentiation **(Zeng et al., 2014)**.

There is still a lack of information about NEAT1 expression and probable deregulation in CLL. **Blume et al., (2015)** revealed that NEAT1 expression can be

increased during DNA damage responses in CLL patients with unaltered TP53 function **(Blume et al., 2015)**.

NEAT1 functional mechanism in cancer is still unknown. NEAT1, a critical component of the paraspeckle structure that has been shown to be involved in transcriptional regulation of gene expression, may be able to directly regulate its target gene by controlling the expression of adenosine-to-inosine hyperedited mRNAs via nuclear retention of target transcripts. Furthermore, NEAT1 was shown to respond to cellular stimuli and ligand signaling in a manner similar to the coding transcriptome, implying that NEAT1 has a function other than its association with paraspeckles **(Chakravarty et al., 2014)**. Certainly, more research will be required to determine the oncogenic mechanism of NEAT1 in cancer.

In conclusion, the current study suggests that plasma lncRNA NEAT1 and mRNA ABCA3 can be served as diagnostic biomarkers for CLL according to their high significance. However, ABCA3 is predicted to be used as a good diagnostic marker for CLL than NEAT1.

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