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INVESTIGATION OF *DICER1* AND *BAFF* GENE MUTATIONS IN B-CELL NON-HODGKIN LYMPHOMA

B HÜCRELİ NON-HODGKİN LENFOMADA *DICER1* VE *BAFF* GEN MUTASYONLARININ ARAŞTIRILMASI

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ABSTRACT

Objective: *DICER1* and *BAFF* gene mutations are effective in T-cell lymphoma progression. Therefore, *DICER1* and *BAFF* genes may have a role in the progression of B-cell lymphomas. For this reason, it was aimed to determine the role of *DICER1* and *BAFF* genes in the development of B-NHL.

Materials and Methods: The study included DNA samples from 60 patients diagnosed with B-NHL who had applied to the Istanbul University, Institute of Oncology, Department of Clinical Oncology between 1991 and 1997. DNA materials obtained from lymphocytes of 30 healthy individuals matched with the patients in terms of age, gender, and race were used as a control group. The c.+3473A>G (rs3742330) polymorphism in the DICER1 gene and the c.-871C>T (rs9514828) single nucleotide polymorphism in the BAFF gene were examined using the PCR-RFLP method. In addition, the presence of mutations in the 11th and 25th exons of the DICER1 gene was evaluated by SANGER sequencing. The results of the control and patient groups were analyzed for polymorphism and mutation presence using Chi-square and Fisher tests.

Results: The polymorphic regions of c.+3473A>G(rs3742330) in the *DICER1* gene and c.-871C>T (rs 9514828) in the *BAFF* gene were examined in the patient and control groups, but no statistically significant relationship was found. When the exon 11 and exon 25 of the *DICER1* gene were investigated, no statistically significant relationship was found between the patient and control groups (p>0.05).

Conclusion: The absence of a difference between the patient and control groups suggests that different genetic mechanisms may be involved in the formation of B-NHL. The small population in the study is one of the reasons why no significant difference was found between the results. Additional studies are needed in larger patient and control groups.

Keywords: B-cell non-Hodgkin lymphoma, *DICER1*, *BAFF*, mutation, SNP

ÖZ

Amaç: *DICER1* ve *BAFF* geni mutasyonlarının T hücreli lenfoma progresyonunda etkili olduğu bilinmektedir. Bu durum B hücreli lenfomaların progresyonunda da *DICER1* ve *BAFF* genlerinin rolünün olabileceğini düşündürmüştür. *DICER1* ve *BAFF* geninin ekspresyon ve mutasyonlarını araştıran birçok çalışma bulunmasına rağmen, B hücreli non-Hodgkin lenfoma progresyonunda tümör baskılayıcı etkisinin nasıl oluştuğu ile ilgili olarak yeterli bilgi bulunmamaktadır. Bu sebeple çalışmada, *DICER1* ve *BAFF* genlerinin B-NHL gelişimindeki rolünün ne olduğunun belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 1991-1997 yılları arasında İstanbul Üniversitesi, Onkoloji Enstitüsü, Klinik Onkoloji Anabilim Dalı'na başvurmuş ve B-NHL tanısı almış 60 hastaya ait DNA örnekleri dâhil edilmiştir. Kontrol grubu olarak hastalarla yaş, cinsiyet ve ırk olarak eşleştirilmiş 30 sağlıklı kişinin lenfositlerinden elde edilen DNA materyalleri kullanılmıştır. *DICER1* geninde yer alan c.+3473A>G(rs3742330) polimorfizmi ile *BAFF* genindeki c.-871C>T(rs9514828) tek nükleotid polimorfizmi PCR-RFLP yöntemiyle incelenmiştir. Ayrıca *DICER1* geninin 11. ve 25. ekzonları mutasyon varlığı açısından SANGER dizi analizi yöntemiyle değerlendirilmiştir. Kontrol ve hasta grubunun sonuçları polimorfizm ve mutasyon varlığı açısından Ki-kare ve Fisher testleriyle analiz edilmiştir.

Bulgular: *DICER1* genindeki c.+3473A>G(rs3742330) ile *BAFF* genindeki c.-871C>T(rs 9514828) polimorfik bölgeleri hasta ve kontrol grubunda incelenmiş ancak istatistiksel olarak anlamlı bir ilişki bulunamamıştır. *DICER1* geninin 11. ve 25. Ekzonları araştırıldığında ise yine hasta ve kontrol grubu arasında istatiksel olarak anlamlı bir ilişki bulunamamıştır (p>0,05).

Sonuç: Hasta ve kontrol grupları arasında bir farkın bulunmaması, B-NHL oluşumunda farklı genetik mekanizmaların etkisinin olabileceğini düşündürmektedir. Çalışmadaki popülasyon sayısının az olması, sonuçlar arasında anlamlı bir fark bulunamamasının nedenlerinden biridir. Daha geniş hasta ve kontrol grubunda ek çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: B hücreli non-Hodgkin lenfoma, *DICER1*, *BAFF*, mutasyon, SNP

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INTRODUCTION

Cancer covers more than a hundred different types of diseases that originate from various organs and many cell types in the human body and are characterized by the uncontrolled proliferation of cells and metastasis to different organs (1).

Non-Hodgkin lymphoma (NHL) is the most common hematological malignancy worldwide and accounts for approximately 3% of cancer diagnoses and deaths (2). According to GLOBACAN 2020 data, 544,352 people in the world are newly diagnosed with NHL and 259,793 of these patients die from NHL. Although NHL is more common in men than in women worldwide, mortality rates are also higher in men. According to the incidence rates for both sexes, 44.3% of the cases are seen in the Asian population. The 5-year prevalence in the adult population was 2.6% in both sexes, while the incidence of NHL was slightly higher in males than females (3). The incidence in Turkey is 6.5 per 100,000 in men and 4.4 in women (4).

NHL is a neoplasm of lymphoid tissues arising from B cell precursors, mature B cells, T cell precursors, and mature T cells. NHL consists of several subtypes, each with different epidemiologies, etiologies, immunophenotypic, genetic, clinical features, and response to therapy. The most common mature B-cell neoplasms are Follicular lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, Mantle cell lymphoma, marginal zone lymphoma, and primary CNS lymphoma (5). B-cell lymphomas are clonal tumors of mature and immature B cells that make up the majority (80-85%) of NHLs. NHLs are a heterogeneous group of lymphoproliferative malignancies with different behavioral patterns and responses to therapy. NHL usually originates in lymphoid tissues and can spread to other organs (6).

In the literature, it has been shown that *DICER1* and *BAFF* genes have important roles in cancer development. Although there are many studies investigating the expression and mutations of *DICER1* and *BAFF* genes, there is not enough information about how these genes act as tumor suppressors in B- NHL progression. For this reason, it was aimed to determine the role of *DICER1* and *BAFF* genes in the development of B- NHL and to contribute to the literature.

MATERIAL and METHOD

Patient population

DNAs isolated from blood samples collected at that time belonging to 60 Caucasian patients with a diagnosis of B- NHL who applied to Istanbul University, Institute of Oncology, Department of Clinical Oncology between 1991-1997 and never received any treatment were used.

The control group consisted of 30 white people who were selected by matching the patient group in terms of age, gender, and ethnicity between 2014 and 2015, and who did not have a family history of cancer and systemic disease. Peripheral blood samples were taken after the subjects who volunteered for the control group were informed about the study before they were included in the study, and after the informed consent form was signed.

The study was approved ethically at the Ethics Committee meeting dated 18.04.2014 and numbered 08 and was carried out at Istanbul University, Institute of Oncology, Department of Basic Oncology, Cancer Genetics Research Laboratory (Date: 18.04.2014, No: 08).

DNA isolation

Since the DNAs of the patient group were used for other research and thesis studies before, they were ready. Peripheral blood samples of the control group were collected within the scope of this study. After 10 mL of peripheral blood sample taken from healthy individuals in EDTA tubes was transferred to 50 mL DNA tubes, the DNA isolation process was started. DNA was isolated from lymphocytes obtained from blood samples using the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. Measurement of the concentrations of the isolated DNAs was carried out using the Nanodrop 2000 Spectrophotometer [Thermo Scientific].

Genetic changes analyzed in patient and control group

Within the scope of the study, the c.+3473A>G (rs3742330) polymorphism in the *DICER1* gene and the c.-871 C>T (rs9514828) single nucleotide polymorphism in the *BAFF* gene were examined by the PCR-RFLP method, while the exon 11 and exon 25 mutations of the *DICER1* gene were evaluated by Sanger Sequencing. The genetic changes analyzed in the patient and control groups are shown in Table 1.

Table 1: Genetic changes analyzed in patient and control group

Study group	Analyzed genetic change
Patient Group (n=60) ve Control Group (n=15)	*DICER1* gene exon 11 and exon 25 mutations
Patient Group (n=60) ve Control Group (n=30)	*DICER1* gene c.+3473A>G (rs3742330) polymorphism and *BAFF* gene c.-871C >T (rs9514828) polymorphism

DICER1: Dicer 1, Ribonuclease III, *BAFF:* B cell activation factor

Primer selection

For the study, forward and reverse primer sequences of the *DICER1* and *BAFF* genes were designed using the IDT Oligo Analyzer computer program (www.itdna.com). The primer sequences of the gene regions and the properties of these primer sequences are given below.

BAFF c.-871C >T (rs 9514828) SNP İçin Kullanılan Primerler [Forward (5'- GGC ACA GTC AAC ATG GGA GT- 3'); Reverse (5'- CCT TGA AGG AAG TGT GGA AGT A- 3')]

Primers Used for *DICER1* c.+ 3473A>G (rs 3742330) SNP [Forward (5-' TGG CGT CTC CAA CAA CTT TA - 3'); Reverse (5-' CCT GCC TTG ACA ACA TGA AA- 3')]

Primers Used for *DICER1* gene Exon 11 [Forward (5'- GTA CAG AGG CAG ACA GCA TAC - 3'); Reverse (5'- GAC TTA AAC TGT GCA ACA TTC CC- 3')]

Primers Used for *DICER1* gene Exon 25 [Forward (5'- AGA AAC TAC ATC TGT GGA CTG C - 3'); Reverse (5'-GGC AGT TTC TGG TTC CAT TTC - 3')].

Restriction enzymes and enzyme recognition sites
Restriction endonucleases to be used in the study were selected by planning to reveal the nucleotide change in the relevant SNP region of the *DICER1* and *BAFF* genes, and the following enzymes were used. In the literature, it was determined that the same enzymes were used for the examination of these sites (17, 25).

BanI restriction endonuclease:

BanI enzyme recognition site 5'... G↓ G Y R C C.... 3' R: A or G

3'... C C R Y G↑ G.... 5' Y: C or T

BsrBI restriction endonuclease:

BsrBI enzyme recognition site5'...C C G ↓ C T C...... 3'

3'...G G C ↑G A G...... 5'

Sequencing of gene regions by The Sanger Method
Firstly, Polymerase Chain Reaction (PCR) was performed from the obtained DNAs. Then, purification, DTCS (Dye Terminator Cycle Sequencing Reaction), and Ethanol precipitation processes were performed, respectively. The obtained samples were thawed and loaded into a sequence analysis device (Beckman Coulter, Ceq 8000 Genetic Analyzer) to perform sequence analysis.

Polymerase chain reaction - Restriction fragment length polymorphism (PCR-RFLP)
In order to examine the presence of c.+3473A>G (rs3742330) in the *DICER1* gene and c.-871C>T (rs9514828) SNPs in the *BAFF* gene in DNA samples isolated from the peripheral blood of the patients and control groups, first the relevant gene regions were amplified by the PCR method. After PCR, amplified DNA products were cut with BanI restriction enzyme to detect c.+3473A>G polymorphism in the *DICER1* gene and with BsrBI restriction enzyme to detect c.-871C>T polymorphism in the *BAFF* gene. In order to determine the size of the DNA fragments fragmented from the recognition sites by restriction endonucleases, 1 µL of 6 X Orange-Dye loading buffer and 5 µL of DNA sample were mixed and 5 µL of this mixture was loaded into the wells on a 2.5% agarose gel and accompanied by a Molecular Weight Marker. After the agarose gel electrophoresis, the bands of the DNA fragments were visualized with the UV imaging system. Samples cut and uncut with restriction enzymes were determined.

Statistics
SPSS v.18 computer program was used in the statistical evaluation of the results of the patient and control groups. Statistical differences between groups were evaluated with the Chi-square test, Yates Chi-square test, and Fisher test.

RESULTS

Distribution of patients with B- NHL according to subgroups, 49 patients with Diffuse Large B-cell Lymphoma (DLBCL) (81.6%), 2 patients with Small-cell Lymphocytic Lymphoma (SLL) (3.33%), 5 patients with Diffuse Mixed Small and Large Cell Lymphoma (8.33%), 1 patient with Marginal Zone Lymphoma (MZL) (1.6%), 2 patients with Malt Lymphoma (3.33%), 1 patient with Burkitt Lymphoma (BL) (1.6%). While the mean age of the patient groups by gender was 51.55 (±15.42) for male patients, the mean age of female patients was 48.3(±18.45). The mean age of the healthy control group was 51.25(±14.79) in men and 45.3(±14.27) in women for the group consisting of 30 people. In the control group consisting of 15 individuals selected from the same healthy control group, the mean age was 48.6(±17.09) in males and 46.6 (±14.94) in females. There is no statistical difference between the patient group and the healthy control groups in terms of age. The characteristics of the patient and control groups are shown in Table 2.

Table 2: Distribution of patient groups by NHL subtypes

Patient groups	Female n (%)	Male n (%)	Total n (%)
DLBCL	18 (36.7%)	31 (63.3%)	49 (81.6%)
SLL	0 (0%)	2 (100%)	2 (3.33%)
Diffuse Mixed Small and Large Cell Lymphoma	2 (40%)	3 (60%)	5 (8.33%)
MZL	0 (0%)	1 (100%)	1 (1.6%)
Malt Lymphoma	0 (0%)	2 (100%)	2 (3.33%)
Burkitt Lymphoma	0 (0%)	1 (100%)	1 (1.6%)
Total	20 (33.3%)	40 (66.7%)	60 (100%)

DICER1: Dicer 1, Ribonuclease III, BAFF:,B cell activation factor, NHL: Non-Hodgkin Lymphoma, DLBCL: Diffuse Large B-cell Lymphoma, SLL: Small-cell Lymphocytic Lymphoma, MZL: Marginal Zone Lymphoma

In the evaluation of the *DICER1* gene exon 11 sequence analysis of 60 patients, a heterozygous change, indicated as Heterozygote 1827C>T(rs150087634), was detected in one patient sample. No mutation or genetic change was observed in the exon 11 sequence analysis results of the other patients. The results of the sequence analysis evaluations for the exon 11 of the *DICER1* gene of the individuals in the patient group are shown in Figure 1.

The DICER1 gene of the healthy control group was not detected in any of the individuals in the healthy group in the evaluation of exon 11 sequence analysis. The results of the sequence analysis evaluations for the exon 11 of the *DICER1* gene of the individuals in the control group are shown in Figure 2.

No mutations or genetic changes were detected in the sequence analysis performed for exon 25 of the *DICER1* gene in the DNA samples of 60 individuals in the patient group. The results of the sequence analysis evaluations for the exon 25 of the *DICER1* gene of the individuals in the patient group are shown in Figure 3.

When DNA samples of 15 individuals in the healthy control group were evaluated by sequence analysis for the presence of a mutation in the exon 25 of the *DICER1* gene, no mutation

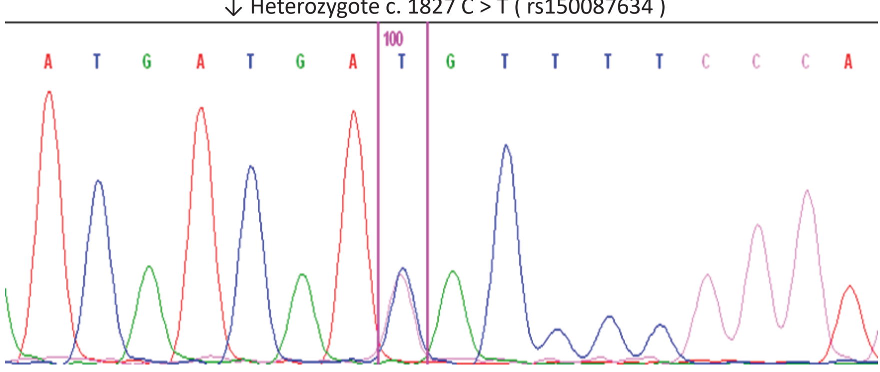

Figure 1: Sequence analysis result of *DICER1* gene exon 11 in patient group

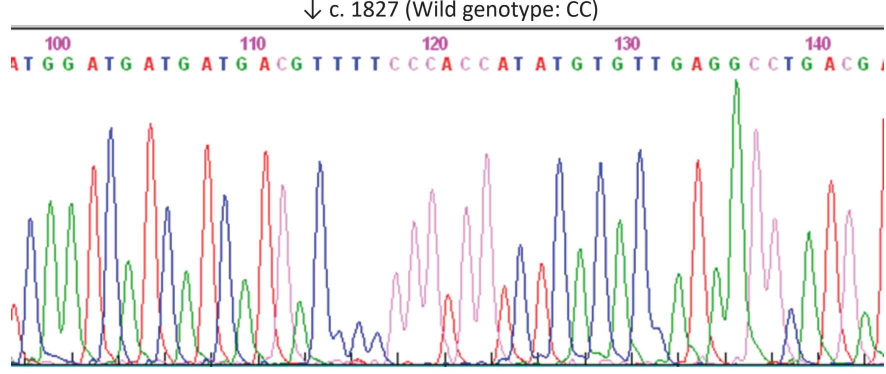

Figure 2: Sequence analysis result of *DICER1* gene exon 11 in control group

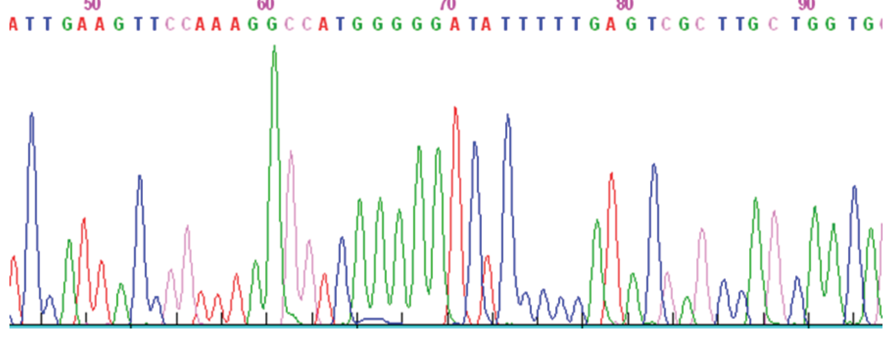

Figure 3: Sequence analysis result of *DICER1* gene exon 25 in patient group

or genetic change was detected. The results of the sequence analysis evaluations for the exon 25 of the *DICER1* gene of the individuals in the control group are shown in Figure 4.

When DNA samples of the patient group were evaluated by PCR-RFLP method, it was determined that 44 patients were wild genotype (AA) and 16 patients were heterozygote genotype (AG) for the c.+3473 region of the *DICER1* gene. *DICER1* gene c.+3473 PCR-RFLP imaging of the patient group is shown in Figure 5.

When the DNA samples of 30 individuals in the healthy control group were evaluated by PCR-RFLP method, it was determined that 24 individuals were in the wild genotype (AA) and 6 individuals were heterozygote genotype (AG) for the c.+3473 region of the *DICER1* gene. c.+3473 PCR-RFLP imaging of the *DICER1* gene of the control group is shown in Figure 6.

When DNA samples of 60 individuals in the patient group were analyzed by PCR-RFLP method, 20 patients were found to be wild genotype (CC), 29 patients were heterozygote genotype (CT), and 11 patients were homozygote genotype (TT) for the c.-871 region in the *BAFF* gene. PCR-RFLP imaging of the c.-871 region in the *BAFF* gene of the patient group is shown in Figure 7.

When DNA samples of 30 individuals in the control group were evaluated by PCR-RFLP method for the c.-871 region of the *BAFF* gene, 10 individuals were found to be wild genotype (CC),

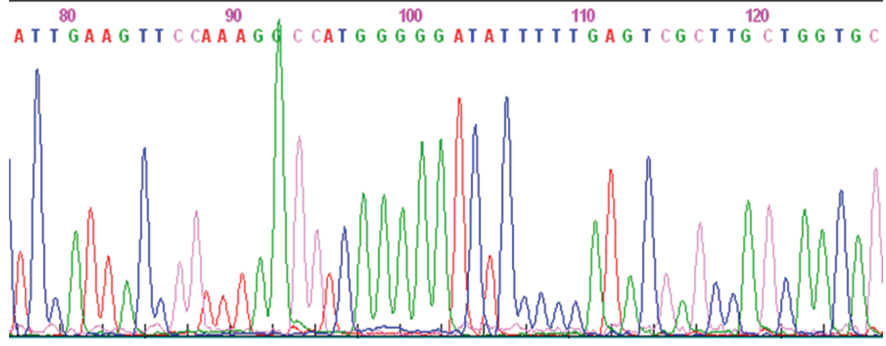

Figure 4: Sequence analysis result of *DICER1* gene exon 25 in control group

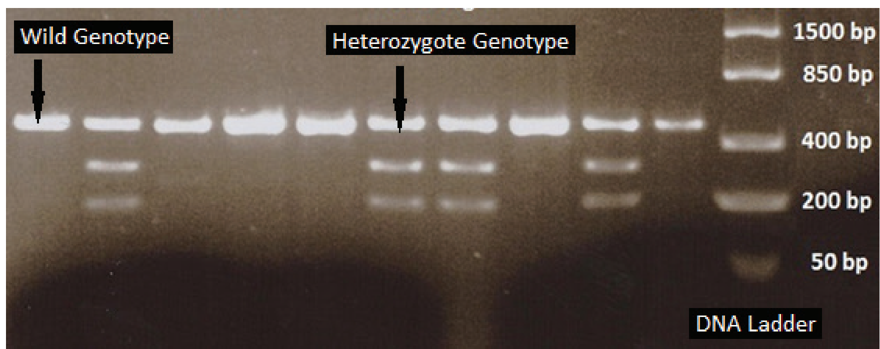

Figure 5: PCR-RFLP imaging of *DICER1* gene c.+3473 region for the patient group

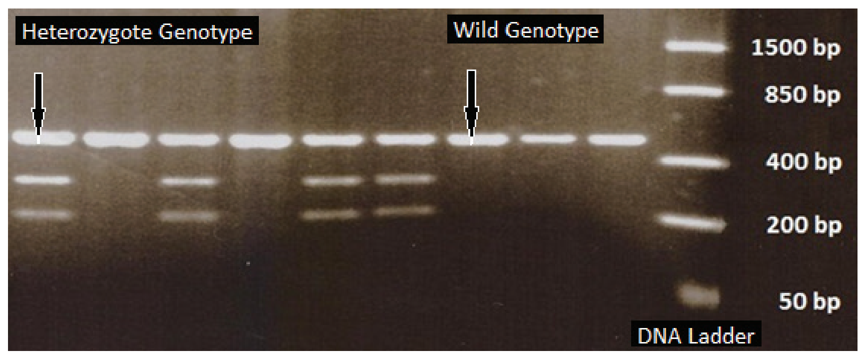

Figure 6: PCR-RFLP imaging of *DICER1* gene c.+3473 region for the control group

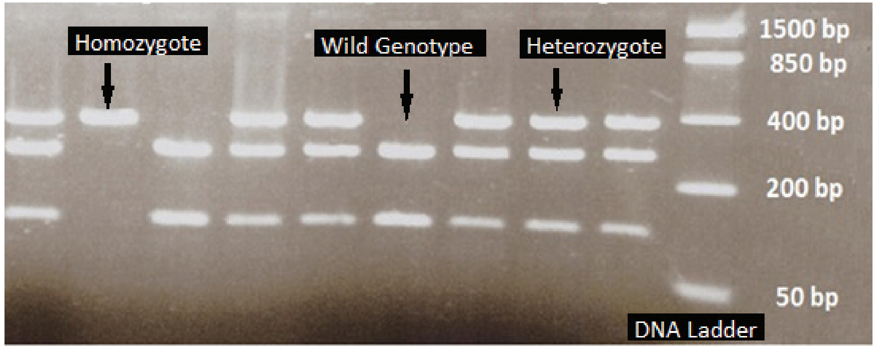

Figure 7: PCR-RFLP imaging of the c.-871 region in the *BAFF* gene of the patient group

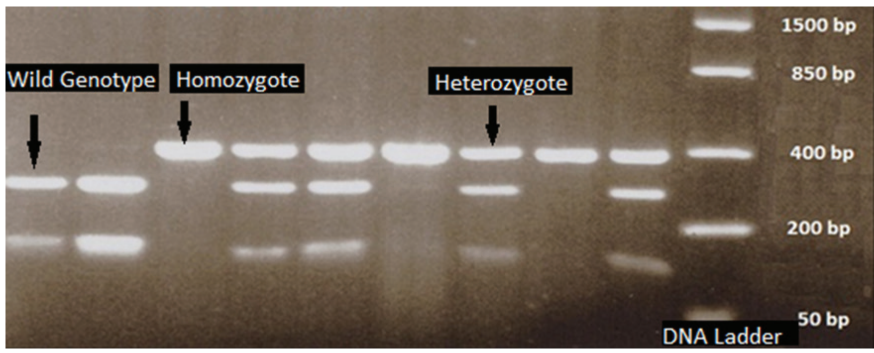

Figure 8: PCR-RFLP imaging of the c.-871 region in the *BAFF* gene of the control group

12 individuals were heterozygote genotype (CT), and 8 individuals were homozygote genotype (TT). PCR-RFLP imaging of the c.-871 region in the *BAFF* gene of the control group is shown in Figure 8.

DNA samples of the patient group (n=60) and control group (n=30) were evaluated for the *DICER1* c.+3473A>G polymorphism with the Yates Chi-Square test. χ2yates=0.1880, degrees of freedom=1 and p= 0.66. Since p>0.05, no statistically significant relationship was established between the patient group and the healthy group in terms of the c.+3473A>G polymorphism of the *DICER1* gene (Table 3).

When the *BAFF* gene c.-871C >T polymorphism in 60 patients with B- NHL and 30 healthy control groups was evaluated statistically by "Chi-square" test, χ2=0.963, degrees of freedom=2, p= 0.618 data were obtained. . Since P was >0.05, no significant relationship could be established between the patient group and the healthy control group in terms of *BAFF* gene c.-871C >T polymorphism (Table 4).

When the *DICER1* gene was evaluated statistically for the c.+3473A>G polymorphism with the Yates Chi-square test, the results obtained were as follows, respectively:

1. There was no statistical significance between patients with DLBCL (n=49) and patients with other B- NHL(n=11) in terms of the c.+3473A>G polymorphism of the *DICER1* gene (χ2yates=1,397, s.d=1, p=0.237 and p>0.05) .

2. There was no statistical significance between patients with DLBCL (n=49) and the healthy control group (n=30) in terms of *DICER1* gene c.+3473A >G polymorphism (χ2yates=0.066, s.d=1, p=0.791 and p>0.05).

3. There was no statistical significance between the patients with other B-NHL (n=11) and the healthy control group (n=30) in terms of the c.+3473A>G polymorphism of the *DICER1* gene (χ2 yates=1.518, s.d=1 , p=0.217 and p>0.05).

Statistical evaluation of *DICER1* gene c.+3473A>G polymorphism according to patient subgroups and control group is shown in Table 5.

Statistical evaluation of the *BAFF* gene c.-871C >T polymorphism between patients with DLBCL (n=49) and healthy controls (n=30) using the chi-square test χ2=0.693, degrees of freedom= 2 and p=0.707 results were obtained. Since P>0.05, there was no statistical significance between patients with DLBCL and the healthy control group in terms of *BAFF* gene c.-871C>T polymorphism (Table 6).

The exon 11 and exon 25 regions of the *DICER1* gene were evaluated for the presence of mutation between the patient group (n=60) and the healthy control group (n=15). No mutation was found in exon 25 in the sequence analysis evaluations of the patient group and the healthy group. Heterozygous c.1827C >T genetic change was detected in the exon 11 region of 1 patient with MALT lymphoma from the samples belonging to

Table 3: Statistical evaluation of patient and control group results of DICER1 gene c.+3473A>G polymorphism

Polymorphism	Patient group n=60 n (%)	Control group n=30 n (%)	Total
Wild Genotype_AA	44 (64.7%)	24 (35.3%)	68 (80%)
Homozygote Genotype_GG	0 (0%)	0 (0%)	0 (0%)
Heterozygote Genotype_AG	16 (72.7%)	6 (27.3%)	22 (20%)
Total	60 (66.6%)	30 (33.3%)	90 (100%)

DICER1: Dicer 1, Ribonuclease III

Table 4: Statistical evaluation of patient and control group results of BAFF gene c.-871C>T polymorphism

Polymorphism	Patient group n=60 n (%)	Control group n=30 n (%)	Total
Wild Genotype_CC	20 (66.7%)	10 (33.3%)	30 (33.3%)
Homozygote Genotype_TT	11 (57.9%)	8 (42.1%)	19 (21.1%)
Heterozygote Genotype_CT	29 (70.7%)	12 (29.3%)	41 (45.6%)
Total	60 (66.6%)	30 (33.3%)	90 (100%)

BAFF: B cell activation factor

Table 5: Statistical evaluation of DICER1 gene c.+3473A>G polymorphism according to patient subgroups and control group

Patient subgroups and control group	Wild Genotype_AA	Homozygote Genotype_GG	Heterozygote Genotype_AG	Total
DLBCL	38 (77.5%)	0 (0%)	11 (22.5%)	49 (54.5%)
Other B-cell non-Hodgkin lymphoma	6 (54.5%)	0 (0%)	5 (45.5%)	11 (12.2%)
Control group	24 (80%)	0 (0%)	6 (20%)	30 (33.3%)
Total	68 (75.6%)	0 (0%)	22 (24.4%)	90 (100%)

DICER1: Dicer 1, Ribonuclease III, DLBCL: Diffuse Large B-cell Lymphoma

Table 6: Statistical evaluation of BAFF gene c.-871C>T polymorphism by patient subgroups and control group

Patient subgroups and control group	Wild Genotype_CC	Homozygote Genotype_TT	Heterozygote Genotype_CT	Total
DLBCL	15 (30.6%)	10 (20.4%)	24 (48.9%)	49 (54.5%)
Other B-cell non-Hodgkin lymphoma	5 (45.5%)	1 (9%)	5 (45.5%)	11 (12.2%)
Control group	10 (33.3%)	8 (26.7%)	12 (40%)	30 (33.3%)
Total	30 (33.3%)	19 (21.1%)	41 (45.6%)	90 (100%)

BAFF: B cell activation factor, DLBCL: Diffuse Large B-cell Lymphoma

Table 7: Statistical evaluation of the sequence analysis results of the *DICER1* gene exon 11 and exon 25 regions

Patient and control groups	Exon 11 Region of *DICER1* gene (n)	Exon 25 Region of *DICER1* gene (n)
B-NHL **Malt Lymphoma** **Other-NHLs**	**Heterozygous c.1827C >T (1)** **Normal (59)**	**Normal (60)**
Control group	**Normal (15)**	**Normal (15)**
Total	**75**	**75**

DICER1: Dicer 1, Ribonuclease III, B-NHL: B-cell Non-Hodgkin Lymhoma, NHL: Non-Hodgkin Lymhoma

the patient group. When the patient group and the healthy control group were analyzed with the “Fisher” test in terms of genetic changes observed in the exon 11 region, a p=1 value was obtained. Since P>0.05, there was no statistical significance between both groups (Table 7).

DISCUSSION

It has been shown in previous studies that the *DICER1* gene, whose role in the development of B-NHL was investigated within the scope of the study, has many mutations at both the germline and somatic levels. There are 133 germline mutations and 95 somatic mutations reported on the *DICER1* protein construct (7). The results of the studies conducted to date have shown that mutations and single nucleotide polymorphisms that may cause errors in the normal function of the *DICER1* gene may play a role in the formation of a number of malignancies, including pleuropulmonary blastoma (PPB), ovarian cancer, nasopharyngeal cancer, breast cancer and T-cell lymphomas (8). In addition, it is known that miRNAs have roles in the regulation of hematopoiesis and the developmental stages of B cells, and non-Hodgkin B-cell lymphomas originate significantly due to the differentiation of B cells (9). In the results of the study conducted by Li et al. in 2014, it was shown that there is a positive correlation between the c.+3473A>G polymorphism of the *DICER1* gene in patients with T-cell lymphoma and the overall survival of these patients (10). Again, in the results of the study conducted by Li et al. in patients with T-cell lymphoma in 2012, it was observed that there was a significant increase in 5-year survival of patients carrying the c.+3473A>G polymorphism of the *DICER1* gene (8). Murray et al. defined a germline mutation in the 11th exon of the *DICER1* gene of a patient with familial PPB in the results of a study they conducted in 2014, and this mutation was found to have a pathogenic effect since it resulted in a frameshift in the relevant gene sequence (11). When the same patient was evaluated in terms of somatic mutations in the *DICER1* gene; A somatic hotspot mutation expressed as c.5425G>A[p.Gli1809Arg] has been found in exon 25 of the *DICER1* gene (11). When they analyzed the serum samples of patients with PPB with mutations in the *DICER1* gene, they found that miR 125a-3p, miR-125b-2-3p, let 7a-3p, let-7b-3p and six other miRNA levels were approximately 40 times higher than the healthy control group (11).

The *BAFF* gene is expressed by immune system cells, including monocytes, macrophages, dendritic cells, a subset of T lymphocytes, and some of the immune system cells such as B lymphocytes (12). In addition, the *BAFF* gene plays an important role in the selection, homeostasis, and transformation of B cells into malignant cells (13). In the literature, it has been shown that the *BAFF* gene is abnormally expressed in malignant B cells of B-NHL patients. It has been understood that this situation protects the malignant cells from apoptosis by spontaneous or drug stimulation and provides the activation of NF-kB, a transcription factor belonging to the Rel gene family, via autocrine or paracrine pathways (14, 15). It has been reported that exogenous *BAFF* upregulates the c-Myc gene, which stimulates B cell proliferation, downregulates the cell proliferation inhibitor p53, and increases the expression of the B cell differentiation inhibitor, BCL6, in some NHLs (14). B-cell tumors were found to express little or no membrane-dependent *BAFF*, but it was reported that serum *BAFF* levels were significantly higher in most patients with NHL whose serum *BAFF* levels were compared with healthy controls, and higher serum *BAFF* levels were associated with aggressive disease and poor response to therapy (14). Novak et al. examined the expression of *BAFF* and *BAFF* receptors in biopsy samples of tumor tissue from patients with B-cell NHL in 2004 and reported that there is a relationship between the increase in *BAFF* gene expression and the aggressiveness of the tumor tissue (16). In 2012, Zhai et al. investigated the *BAFF* gene c.-871C>T(rs9514828) polymorphism in patients with T-cell lymphoma. They found that those with homozygote genotype for c.-871C>T(rs9514828) polymorphism had a significantly higher 5-year survival rate than those with wild type or heterozygote genotype (17).

The results of these studies investigating *DICER1* gene and *BAFF* gene polymorphisms and *DICER1* gene mutations showed that single nucleotide polymorphisms and mutations in *DICER1* and *BAFF* genes may result in changes in gene expression, and this may contribute to the formation of B-cell lymphomas. Considering the studies in the literature, we aimed to determine the role of *DICER1* and *BAFF* genes in the development of B-cell non-Hodgkin lymphomas.

In our study, when the DNA samples of the patient group and control group were analyzed for the c.+3473A>G polymorphism of the *DICER1* gene, no individual carrying the GG genotype was found in the patient and control groups. When the study results were compared between healthy and patient groups with GA and AA genotypes, no statistically significant relationship could be established (p>0.05). The absence of a significant difference between the two groups suggested that different genetic mechanisms may also be involved in the formation of B-cell non-Hodgkin lymphoma. It was thought that the small number of the population participating in the study may be one of the reasons why no significant difference could be found between the results.

In our study, when the patient group and the healthy control group were analyzed for the presence of a mutation in the 11th exon of the *DICER1* gene, a heterozygote c.1827C>T [p.Asp609Asp]-rs150087634 - change was found in a patient with MALT lymphoma. As a result of this base change, the encoded amino acid (aspartic acid) does not change, so this mutation is silent. When the exon 25 region of the *DICER1* gene in the same patient and control group was examined for the presence of mutation, no mutation was found in the exon 25. Our findings were found to be similar to the results obtained by Lee et al. in hematological tumors.

In our study, when the patient and control groups were compared in terms of c.-871C>T polymorphism in the *BAFF* gene, no difference was found between the patient and control groups (p>0.05). Although the contribution of this polymorphism seen in the *BAFF* gene to the development of B cells and B-cell lymphoma was emphasized in previous studies, the c.-871C>T polymorphism in the *BAFF* gene in our study group was not statistically significant in the patient and control groups. The reason why this difference did not occur is thought to be due to the sample size of our study group.

In summary, the polymorphic regions of c.+3473A>G(rs3742330) in the *DICER1* gene and c.-871C>T(rs 9514828) in the *BAFF* gene were examined in patients with B-cell NHL and healthy control groups. There was no significant relationship between patients and subgroups of patients. When the exon 11 and exon 25 regions of the *DICER1* gene were investigated for the presence of mutations, no statistically significant relationship could be established between the patient and control groups (p>0.05). Conducting the study in a larger sample and adding the expression profiles of the genes in question will make the results more meaningful. In this context, the study is planned to be expanded and continued.

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