RESEARCH ARTICLE

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Association of *LIN28B* Gene Polymorphisms (rs221634, rs221635, rs314276, rs9404590, and rs12194974) with Non-Hodgkin Lymphoma Susceptibility and Clinical/ Pathological Features

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Abstract

Background and Aim: Lymphoma is a common hematopoietic cancer. It has been proposed that *LIN28B* gene and its variations may have function in cancer progression and metastasis. Therefore, the purpose of this investigation has been to examine the correlation among *LIN28B* gene polymorphisms (such as rs221634 A>T, rs221635 T> C, rs314276 C>A, rs9404590 T>G, and rs12194974 G>A) as well as the risk of NHL in an Iranian sample. **Materials and Methods:** In the current case-control research, 175 individuals with Non-Hodgkin Lymphoma along with 175 normal controls participated; polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) methodology has been utilized to the genotype samples. **Results:** Our data demonstrated that rs12194974 and the rs221635 variants have been correlated with higher NHL risk, while rs221634 and rs314276 variants were correlated with lower risk of NHL ($P \leq 0.05$). In addition, we detected an association between rs221634 and treatment with R-CHOP. No substantial correlation has discovered among rs9404590 polymorphism and NHL in any inheritance models ($P \geq 0.05$). **Conclusion:** This was the first investigation evaluating the correlation among *LIN28B* gene polymorphisms as well as the occurrence of NLH. Further studies in different ethnic populations and large-scale sample size are needed to support results.

Keywords: *LIN28B*- polymorphisms- non-hodgkin lymphoma- cancer

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Introduction

Lymphomas are solid tumors with body's lymphatic system origin. Relatively, 10% of all lymphomas belongs to Hodgkin's lymphoma, and also non-Hodgkin lymphoma (NLH) is responsible for 90% of Lymphomas (Ansell, 2015). Due to lymphatic tissue spread throughout the human body, lymphoma can originate almost anywhere. Hodgkin and non-Hodgkin's lymphoma can affect a different lymphocyte type. Mature B, T lymphocytes along with natural killer cells are sources of NHLs (Bassan et al., 2016). According to the varied histological appearances and clinical features, NLH can be classified into 36 subtypes (21 types of B-cells and 15 types of T-cells) (Lewis et al., 2020). There are two most common types of NHL in adults, including large B cell lymphoma or DLBCL (Diffuse large B cell lymphoma) as well as follicular lymphoma (Thandra et al., 2021). Despite growing cases of NHL, the etiology is largely unknown. Studies have shown that NHL risk is influenced by gene variations in immunodeficiency pathways, either positively or negatively (Mashhadi et al., 2021). The 14.6 kb long human *LIN28B* gene (HGNC: 32207) is found on the 6q16.3-q21 locus. This gene consists seven exons, which are highly polymorphic. *LIN28B* gene encodes a miRNA-binding protein, which was initially known as a regulator of growth time in Elegance worm (Rangel-Guerrero et al., 2020). The LIN28 is RNA-binding protein, which can play a key role in cell proliferation. LIN28A and *LIN28B* have two LIN28 paralogs in the human body, while there is only one *LIN28B* gene in the elegans worm, which can be related to many hyperplastic diseases (Li et al., 2017). Lin28 homologues play a significant part in various cell growth's processes and tissue inflammatory response as well as tumorigenesis (Pretzsch et al., 2021). The polymorphisms

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in this gene cause changes in the maturity and height of people, and together with *OCT4, SOX2* and *KLF4* genes can play a role in the pluri-potency of TB stem cells (Gurtan and Sharp, 2013).

It is reported that Let-7 might negatively regulate the *LIN28B* gene because it has complementary sites in 3′UTR of *LIN28B* gene, leading to tumorigenesis by inhibiting Let7 post-transcriptional modifications observed in hepatocellular carcinoma (Sakurai et al., 2012). Guo et al. showed that the expression level of this gene increases in hepatocellular carcinoma (Guo et al., 2006). In addition, it has been determined that *LIN28B* polymorphisms can be effective in the occurrence of cancer. In this regard, Jing et al. reported that *LIN28* gene polymorphisms increased susceptibility to neuroblastoma, in particular, rs221634 polymorphism was known as a risk factor for neuroblastoma (He et al., 2016). Studies conducted on *LIN2828B* gene polymorphisms and hepatoblastoma cancer in Chinese kids reported that rs314276 polymorphism (an intronic locus) increased risk of hepatoblastoma, which is among the largest malignant tumors in kids (Zhang et al., 2018). So far, no study has been carried out regarding the polymorphisms of LIN28 and the risk of developing NLH. Determining the correlation among rs221634 A>T, rs221635 T>C, rs314276 C>A, rs9404590 T>G, and rs12194974 G> has been the goal of this work. In the Iranian sample, a polymorphism increases the likelihood of acquiring NLH cancer. In addition, we investigated the effects of these variants on NHL pathological and clinical features.

Materials and Methods

Patients

In the current case-control research in Zahedan, southeast Iran, 350 people participated, comprising 175 NHL cases and 175 normal controls. The Zahedan University of Medical Sciences Local Ethics Committee accepted the study protocol (IR.ZAUMS.REC.1400.407), and all participants provided written informed consent. Each individual had his or her venous blood drawn into a tube containing ethylenediaminetetraacetic acid (EDTA). When not in use, genomic DNA has been extracted using salting-out procedure and kept at -20°C.

Genotyping

LIN28B Gene Polymorphisms genotyping (such as rs12194974, rs221634, rs221635, rs314276 and rs9404590) has been accomplished utilizing PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) methodology. In Table 1, the primers are displayed. 1 µL of genomic DNA (100 ng/mL), 1 µL of each primer, and 10 µL of master mix, along with 7 µL of double-distilled water (ddH2O) were all included in each 0.2 mL tube used for the PCR reaction. Thus, the following PCR conditions have been chosen for these SNPs: For rs12194974, the following conditions have been utilized: 95°C for 6 min, 35 cycles of 95°C for 30 s, 60°C for 35 s, as well as 72°C for 35 s, and the last extension step of 72°C for 10 min. For rs221634, following conditions must be met: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 56 °C for 35 s, and 72 °C for 35 s, and a final extension step of 72 °C for 10 min. For rs221635, the following steps have been used: 95°C for 5 min; 30 cycles of 95°C for 30 s; 60°C for 30 s; and 72°C for 30 s; and lastly, a 72°C for 5 min as extension step. For rs314276, the following steps have been used: 95°C for 6 min; 30 cycles of 95°C for 30 s; 62°C for 30 s; and 72°C for 30 s; and lastly, a 72°C for 5 min as extension step. For rs9404590, following steps were used: 95°C for 6 min; 30 cycles of 95°C for 30 s; 62°C for 30 s; and 72°C for 30 s; and the last extension step of 72°C for 5 min. Following that, a restriction enzyme indicated in Table 1 was used to digest 10 µL of PCR product. The PCR results were next seen under ultraviolet light after electrophoresis through agarose gels having 0.5 µg/mL of ethidium bromide (Figures 1–5). We randomly re-genotyped about 20% of all samples to test the accuracy of the genotyping, and the genotypes have been 100% concordant.

Statistical analysis

SPSS version 22.0 has been utilized to do the statistical analysis. Thus, independent sample t test and $\chi 2$ test results have been utilized to examine the data. The odds ratios (ORs) as well as 95% confidence intervals (95% CIs) were measured using the logistic regression methodology. A P value of <0.05 has been regarded as statistically substantial.

Results

Participants in this research were 175 healthy individuals (containing 94 males and 81 females; mean age of 42.51 ± 12.26 years), and 175 pathologically diagnosed NHL patients (110 males and 65 females; mean age of 45.36 ± 15.69 years). Age and gender did not substantially vary across groups (P = 0.06 and 0.10, respectively).

Table 2 displays the genotypic and allelic frequencies of *LIN28B* variants (rs12194974, rs221634, rs221635, rs314276, and rs9404590) in NHL patients and controls. The findings showed that rs12194974 G>A variant of *LIN 28B* gene substantially lowered the NHL risk within co-dominant ((P=0.02 GA vs GG; P=0.004 AA vs GG), dominant (P=0.005), recessive (P=0.03), as well as allelic (P=0.002) models. Moreover, rs221634 A>T variant of *LIN 28B* gene substantially raised the NHL risk within co-dominant (P ≤0.001), dominant (P<0.001), recessive (P=0.004), as well as allelic (P<0.001) models. The rs221635 T>C variant substantially lowered the NHL risk in co-dominant (P =0.04, TC vs TT; P = 0.02, CC vs TT), dominant (P=0.01,), and allelic (P=0.02) models. Rs314276 C>A variant substantially raised the NHL risk; co-dominant (P=0.002 CA vs CC; P <0.001, AA vs CC), dominant (P <0.001), recessive (p= 0.004), as well as allelic (P<0.001) models. We detected no remarkable link between rs9404590 T>G variant and NHL occurrence in any inheritance models. The details are given in Table 2.

As presented in Table 3, we estimated the correlation among *LIN28B* polymorphisms and the patients' clinical properties such as age, histology, four stages of disease, treatment (R-CHOP or others), radio therapy, and kind of NHL (primary or recurrent). Our result revealed that

Table 1. The Primers Used for Detection of LIN28B (rs221634, rs221635, rs314276, rs9404590 and rs12194974) Polymorphisms

Polymorphism	PCR primers (5'→3')	Restriction Enzyme	Fragment, bp
rs12194974	F: AGCTCTTGGGGAACAATCGC	BmrI	AA=289
	R: TAGGAAAAGGCAGAGGCACAT		GG=223+66
rs221634	F: TCTCCCACCAGAGAGCTAGA	SspI	TT= 293
	R: GCACTATAATTAACTGGTACC		AA=213+80
rs221635	F: TTCACAACTGCATGTTTCTGACAA	Hin1I	TT= 457
	R: TAATTCACAGACCTGCTGCCC		CC= 256+201
rs314276	F: TGAATTAAAACATGTAGCTGCTGA	SspI	CC=345
	R: TGAAATCGTCTTGAATTGCAACC		AA=258+87
rs9404590	F: ATCAGGACAGTTTGCCCGAC	BglII	GG=283
	R: AAGTGCGGTCAAAGAGAGGG		TT=232+51

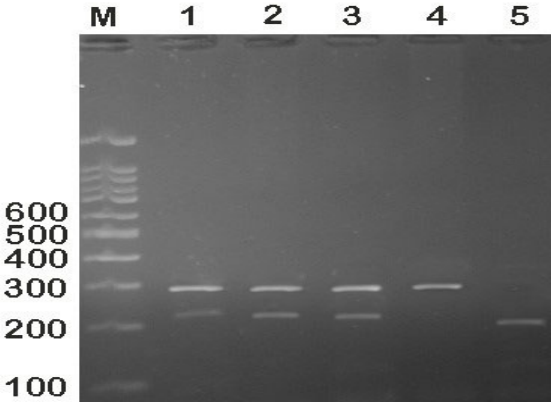

Figure 1. The Electrophoresis Pattern of the Digested Products of the *LIN28B* Gene rs221634 (A/T). 1, 2, 3: AT heterozygous genotype, 4: TT homozygous genotype, 5: AA homozygous genotype.

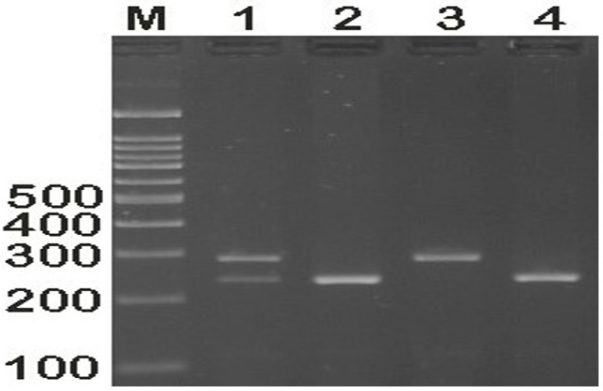

Figure 3. The Electrophoresis Pattern of the Digested Products of *LIN28B* Gene rs9404590. 1: heterozygous TG genotype, 2 and 4: the homozygous TT genotype, and 3: GG genotype.

there has been a substantial correlation among rs221634 and treatment with R-CHOP which is a combination of chemotherapy and a targeted therapy and known as chemoimmunotherapy (P=0.03). There was no considerable link between other studied variants and clinical features in NHL cases.

In addition, the haplotype analysis of *LIN28B* polymorphisms (rs9404590, rs12194974, rs314276, rs22163, rs221635; respectively) was compared between NHL cases and healthy controls. The detected genotypes led to the development of 26 haplotypes (Table 4). In both the patients and the controls, the TTCTG haplotype has been the most prevalent. The outcomes of the haplotype analysis revealed that ACCTG, ATCTG, ATCGG, ATCTA, ACCGA, ACATA, ACCTA, TCCGA and ATCGA haplotypes have been related to decreased NHL risk

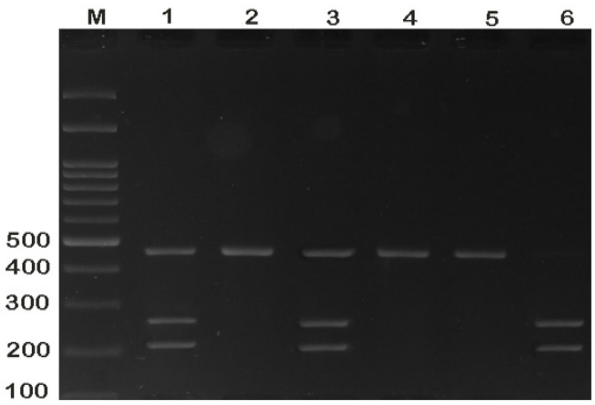

Figure 2. The Electrophoresis Pattern of the Digested Products of *LIN28B* Gene rs221635. 1, 3: the heterozygous TC genotype; 2, 4 and 5: homozygous TT genotype and 6 CC: genotype.

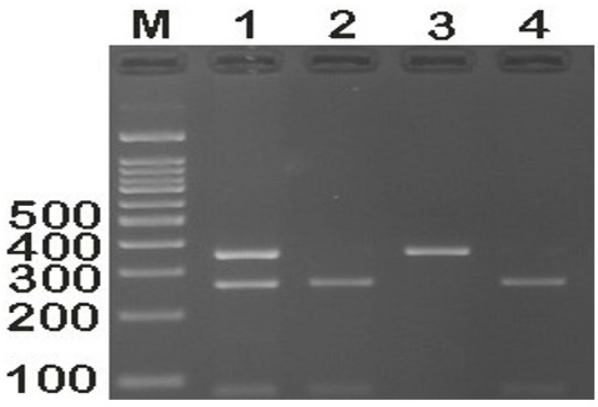

Figure 4. The Electrophoresis Pattern of the Digested Products of *LIN28B* Gene rs314276. 1: CA heterozygous genotype, 2 and 4: AA homozygous genotype, and 3: CC genotype.

Table 2. Association of *LIN28B* (rs221634, rs221635, rs314276, rs9404590 and rs12194974) Polymorphisms and Non-Hodgkin Lymphoma (NHL) Risk

Polymorphism	Case n (%)	Control n (%)	OR (95%CI)	P value
LIN28B (rs12194974)				
Co-dominant				
GG	88 (50.3)	62 (35.4)	1	-
GA	77 (44.0)	91 (52.0)	0.57 (0.38-0.93)	0.02
AA	10 (5.7)	22 (12.6)	0.32 (0.14-0.72)	0.004
Dominant				
GG	88 (50.3)	62 (35.4)	1	-
GA+AA	87 (49.7)	113 (64.6)	0.54 (0.35-0.83)	0.005
Recessive				
GG+GA	165 (94.3)	153 (87.4)	1	-
AA	10 (5.7)	22 (12.6)	0.42 (0.19-0.92)	0.03
Allele				
G	253 (72.3)	215 (61.4)	1	-
A	97 (27.7)	135 (38.6)	0.61 (0.44-0.84)	0.002
LIN28B (rs221634)				
Co-dominant				
AA	36 (20.6)	71 (40.6)	1	-
AT	100 (57.1)	86 (49.1)	2.29 (1.40-3.76)	0.001
TT	39 (22.3)	18 (10.3)	4.27 (2.15-8.50)	<0.001
Dominant				
AA	36 (20.6)	71 (40.6)	1	-
AT+TT	139 (79.4)	104 (59.4)	2.64 (1.64-4.24)	<0.001
Recessive				
AA+AT	136 (77.7)	157 (89.7)	1	-
TT	39 (22.3)	18 (10.3)	2.50 (1.37-4.58)	0.004
Allele				
A	172 (49.1)	228 (65.1)	1	-
T	178 (50.9)	122 (34.9)	1.93 (1.43-2.62)	<0.001
LIN28B (rs221635)				
Co-dominant				
TT	64 (36.6)	43 (24.6)	1	-
TC	90 (51.4)	100 (57.1)	0.60 (0.37-0.98)	0.04
CC	21 (12.0)	32 (18.3)	0.44 (0.23-0.86)	0.02
Dominant				
TT	64 (36.6)	43 (24.6)	1	-
TC+CC	111 (63.4)	132 (75.4)	0.56 (0.36-0.90)	0.01
Recessive				
TT+TC	154 (88.0)	143 (81.7)	1	-
CC	21 (12.0)	32 (18.3)	0.61 (0.34-1.11)	0.13
Allele				
T	218 (62.3)	186 (53.1)	1	-
C	132 (37.7)	164 (46.9)	0.69 (0.51-0.93)	0.02
LIN28B (rs314276)				
Co-dominant				
CC	83 (47.4)	119 (68.0)	1	-
CA	70 (40.0)	49 (28.0)	2.05 (1.29-3.25)	0.002
AA	22 (12.6)	7 (4.0)	4.51 (1.84-11.03)	<0.001
Dominant				
CC	83 (47.4)	119 (68.0)	1	-
CA+AA	92 (52.6)	56 (32.0)	2.36 (1.53-3.64)	<0.001
Recessive				
CC+CA	153 (87.4)	168 (96.0)	1	-
AA	22 (12.6)	7 (4.0)	3.45 (1.43-8.31)	0.004
Allele				
C	236 (67.4)	287 (82.0)	1	-
A	114 (32.6)	63 (18.0)	2.20 (1.55-3.13)	<0.001
LIN28B (rs9404590)				
Co-dominant				
TT	45 (25.7)	57 (32.6)	1	-
TG	96 (54.9)	91 (52.0)	1.34 (0.82-2.17)	0.24
GG	34 (19.4)	27 (15.4)	1.60 (0.84-3.02)	0.15
Dominant				
TT	45 (25.7)	57 (32.6)	1	-
TG+GG	130 (74.3)	118 (67.4)	1.40 (0.88-2.22)	0.16
Recessive				
TT+TG	141 (80.6)	148 (84.6)	1	-
GG	34 (19.4)	27 (15.4)	1.32 (0.76-2.30)	0.32
Allele				
T	186 (53.1)	205 (58.6)	1	-
G	164 (46.9)	145 (41.4)	1.25 (0.92-1.68)	0.15

(P<0.05). The linkage disequilibrium pattern of the five studied polymorphisms of *LIN28B* is shown in

Discussion

In the present case-control research, we studied the relationship among *LIN28B* rs9404590, rs12194974, rs314276, rs22163, rs221635 gene polymorphisms and NHL risk as well as their effects on NHL clinical features in an Iranian population. The findings showed that rs12194974 G>A, and the rs221635 T>C variants of *LIN28B* were correlated with a significant decrease in NHL risk, while rs221634 A>T and rs314276 C>A variants considerably increased the susceptibility to NHL. Given that haplotype included information about several SNPs, and also compared to single SNP analysis, haplotype analysis had more statistical power; therefore,

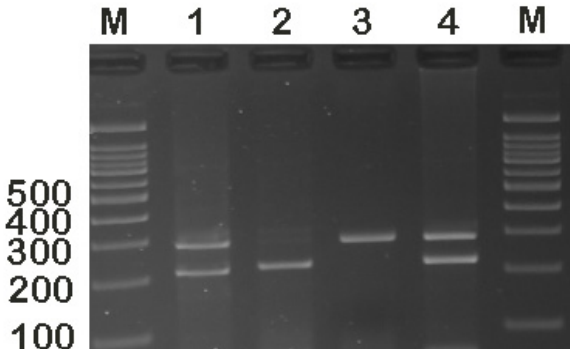

Figure 5. The Electrophoresis Pattern of the Digested Products of *LIN28B* Gene rs12194974, 1 and 4: heterozygous AG genotype, 2: homozygous GG genotype, and 3: AA genotype.

Table 3. Association of *LIN28B* (rs221634, rs221635, rs314276, rs9404590 and rs12194974) Gene Polymorphisms with Demographic and Clinical Characteristics of NHL Subjects

Characteristics of Patients	*LIN28B* rs12194974				*LIN28B* rs221634				*LIN28B* rs221635				*LIN28B* rs314276				*LIN28B* rs9404590			
	GG	GA	AA	P	AA	AT	TT	P	TT	TC	CC	P	CC	CA	AA	P	TT	TG	GG	P
Age, years				0.42				0.14				0.16				0.68				0.75
≤50	54	52	8		22	71	21		36	64	14		53	48	13		29	61	24	
>50	34	25	2		14	29	18		28	26	7		30	22	9		16	35	10	
Histology				0.15				0.8				0.86				0.53				0.45
DLBCL	49	34	3		19	47	20		30	46	10		38	35	13		25	47	14	
Others	39	43	7		17	53	19		34	44	11		45	35	9		20	49	20	
Stage				0.32				0.19				0.82				0.48				0.82
I	51	37	7		26	46	23		34	50	11		43	36	16		28	50	17	
II	13	13	0		3	17	6		7	14	5		13	12	1		6	13	7	
III	7	4	2		3	9	1		4	8	1		8	5	0		4	7	2	
IV	17	20	1		4	25	9		16	18	4		18	15	5		7	23	8	
Treatment				0.91				0.03				0.99				0.33				0.17
R-CHOP	47	40	6		26	46	21		34	48	11		43	35	15		27	53	13	
Others	41	36	4		10	53	18		30	41	10		40	34	7		18	43	20	
Radio Therapy				0.63				0.58				0.15				0.38				0.34
No	76	69	8		31	86	36		59	78	16		71	64	18		37	87	29	
Yes	12	8	2		5	14	3		5	12	5		12	6	4		8	9	5	
Kind				0.99				0.33				0.99				0.74				0.4
Primary	80	70	9		35	89	35		58	82	19		76	64	19		43	85	31	
Recurrent	8	7	1		1	11	4		6	8	2		7	6	3		2	11	3	

Table 4. Haplotype Analysis of *LIN28B* Polymorphisms and NHL Risk

rs9404590	rs12194974	rs314276	rs221634	rs221635	Case (%)	Control (%)	OR (95%CI)	P-value
T	T	C	T	G	46 (13.1)	27 (7.7)	1 [reference]	-
A	C	C	T	G	26 (7.4)	45 (12.8)	0.34 (0.17-0.66)	0.002
A	T	C	T	G	24 (6.8)	31 (8.8)	0.45 (0.22-0.93)	0.03
A	T	C	G	G	20 (5.7)	30 (8.5)	0.40 (0.19-0.82)	0.01
T	C	C	G	G	25 (7.1)	15 (4.2)	0.98 (0.44-2.17)	0.96
T	T	C	G	G	25 (7.1)	13 (3.7)	1.12 (0.45-2.57)	0.77
A	T	C	T	A	13 (3.7)	24 (6.8)	0.31 (0.14-0.72)	0.005
A	C	C	G	G	16 (4.5)	19 (5.4)	0.49 (0.22-1.12)	0.09
A	C	C	G	A	10 (2.8)	19 (5.4)	0.31 (0.12-0.76)	0.009
T	T	A	G	G	17 (4.8)	5 (1.4)	1.99 (0.66-6.02)	0.22
T	T	C	T	A	9 (2.5)	12 (3.4)	0.44 (0.16-1.18)	0.1
A	C	A	T	G	11 (3.1)	9 (2.5)	0.71 (0.26-1.95)	0.52
A	T	A	G	A	13 (3.7)	7 (2.0)	1.09 (0.39-3.07)	0.87
A	C	A	T	A	7 (2.0)	12 (3.4)	0.34 (0.12-0.98)	0.04
A	C	C	T	A	5 (1.4)	13 (3.7)	0.22 (0.07-0.70)	0.007
A	T	A	G	G	13 (3.7)	5 (1.4)	1.52 (0.49-4.75)	0.47
T	C	C	G	A	5 (1.4)	12 (3.4)	0.24 (0.07-0.77)	0.01
T	T	A	T	G	11 (3.1)	5 (1.4)	1.30 (0.40-4.11)	0.67
T	C	C	T	A	6 (1.7)	9 (2.5)	0.39 (0.12-1.22)	0.1
A	T	C	G	A	5 (1.4)	10 (2.8)	0.29 (0.09-0.95)	0.03
T	C	C	T	G	9 (2.5)	5 (1.4)	1.05 (0.32-3.48)	0.93
T	T	C	G	A	6 (1.7)	8 (2.2)	0.44 (0.14-1.40)	0.16
A	T	A	T	G	9 (2.5)	4 (2.5)	1.32 (0.37-4.70)	0.67
A	T	A	T	A	7 (2.0)	5 (1.4)	0.82 (0.24-2.84)	0.76
T	T	A	G	A	8 (2.2)	2 (0.5)	2.35 (0.46-11.87)	0.29
T	C	A	T	G	5 (1.4)	4 (2.5)	0.73 (0.18-2.97)	0.66

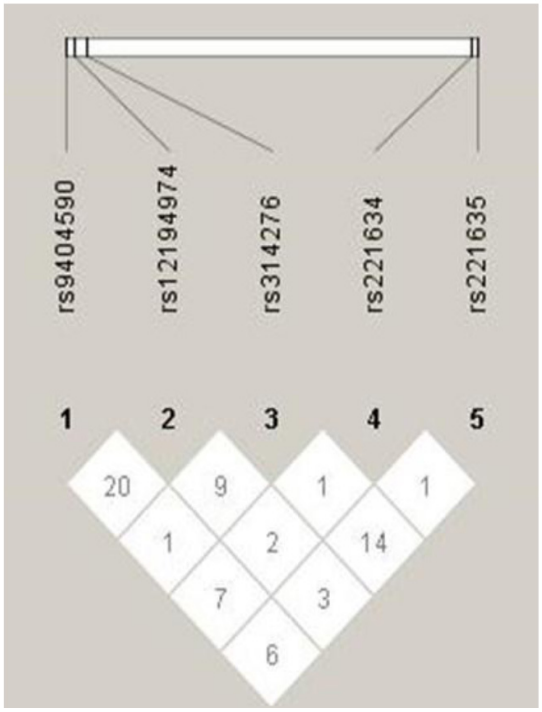

Figure 6. Linkage Disequilibrium Analysis of *LIN28B* Polymorphisms (rs221634 A>T, rs221635 T>C, rs314276 C>A rs9404590 T>G, rs12194974 G>A

in this work, the haplotypes derived from the discovered genotypes were examined. The haplotype evaluation revealed that the frequencies of ACCTG, ATCTG, ATCGG, ATCTA, ACCGA, ACATA, ACCTA, TCCGA and ATCGA haplotypes in controls were higher than those in NHL patients, and this finding proposed their possible role with lower risk of NHL.

Due to multifactorial and polygenic models of susceptibility to Lymphoma, a large number of studies have tried to find new markers. According to our knowledge, the present work is the initial investigation into how *LIN28B* polymorphisms affect NHL risk; however, the association between the variations of this gene and other types of cancers has been reported previously (Permuth-Wey et al., 2011; Yang et al., 2020). Studying the genetic variations can be a fundamental factor in the cancer prevention (Harati-Sadegh et al., 2021). Genotyping technologies led to genome-wide correlation research for human illnesses, especially cancer. *LIN28B*, a direct miRNA let-7 target, is crucial for controlling and maturing stem cells and developing tissues, and it can be one of the potential miRNAs, which may have important function in cancer (Zhang et al., 2013; Zhou et al., 2017).

Functional experiments proposed that that *LIN28B* might inhibit cancer cell apoptosis (Lin et al., 2018). Moreover, increased expression of *LIN28B* protein has

been discovered in colon tumors, head and neck, gastric, breast and ovarian cancers, and oral squamous cell carcinoma, which is linked with patient survival reduction, poor prognosis and tumor recurrence (Denaro et al., 2014; Zhang et al., 2019).

Evidence supports that the origins of the human carcinomas' genetic susceptibility are an enormous number of alleles with poor penetrance and a modest effect size. The crucial components of an individual's propensity to develop cancer are genetic variations in controlling genes associated with apoptosis(Mir et al., 2015; Heidari et al., 2020). As a crucial oncogene, the *LIN28B* gene may have a role in the forming and spreading NHL(Lin et al., 2018). Furthermore, previous reports implied that potential functional SNPs of *LIN28B* may change *LIN28B* gene's function or expression, and elevate cancerous cells proliferation and risk of tumor recurrence(Han et al., 2020). Besides, variations in *LIN28B* lead to losing binding sites of micro-RNAs which may have a crucial role in the suppression of cell growth (Piskounova et al., 2011; Tan et al., 2021).

The role of *LIN28B* single nucleotide polymorphisms (rs221634 A>T, rs221635 T>C, and rs9404590 T>G) in cancer metastasis and prognosis was previously well established in childhood carcinomas, such as Wilms tumor and neuroblastoma (He et al., 2017; Fu et al., 2018). In line with our findings, children in Southern China were shown to have an elevated risk of neuroblastoma for *LIN28B* rs221634 A>T and rs314276 C>A polymorphisms. Moreover, linkage between *LIN28B* gene rs314276 C>A polymorphism as well as the development and survival of patients with colon, ovarian non-small cell lung oral cavity cancers, along with neuroblastoma was confirmed by previous studies. In a further investigation, Yang et al., (2020) stated that *LIN28B* rs94904590 T>G and rs314276 C>A SNPs raised hepatoblastoma risk.

This work may have several limitations as follows. Due to the low frequency of the disease in the research area, the sample size has been modest, and it appears that additional research with higher number of participants is required for precise conclusion. Moreover, it is possible that we were biased in the sampling, because the study was restricted to an Iranian sample. The Hardy-Weinberg disequilibrium law, however, is well fit by the genotype frequencies of two research polymorphisms among our subjects, proving that the subjects were chosen at random. In addition, five potentially functional polymorphisms were investigated and un-functional SNPs were not included in this study, and no quantitative experiments were performed to evaluate gene expression changes. Therefore, confirmation tests including q-PCR, sequencing, and western blotting in larger populations and different ethnicities for *LIN28* gene (various SNPs in both introns and exons loci) and its targets are recommended for the future studies.

In conclusion, for the first time, we found that rs12194974, rs221635, rs221634, and rs314276, might be associated with the susceptibility of NHL in Iranian individuals. To examine polymorphisms different patterns in various populations, additional studies in various ethnicities with larger sample size are required to verify and extend our outcomes. To better understand the relationship between *LIN28B* SNPs and NHL pathological features, additional functional research is required.

Author Contribution Statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [AA], [GB], [AN], [SMH] and [MT]. The first draft of the manuscript was written by [AA and GB] and all authors commented on previous versions of the manuscript. All authors read and approved the final attest that all listed authors meet the authorship criteria and that no other authors meeting the criteria have been omitted.

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MSc student thesis (AA # 10518) at Zahedan University of Medical Sciences. The protocol of the current study was verified by the Ethics Committee of Zahedan Medical Sciences University (Zahedan, Iran). All supporting data have been shown in the manuscript.

Conflict of Interest

None.

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