Association between the Interleukin-1 Receptor Antagonist (IL1RN) Variable Number of Tandem Repeats (VNTR) Polymorphism and Lymphoma

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ABSTRACT

Introduction: Lymphoma is a common hematopoietic cancer. Immunosuppression is one of the main risk factors for the development of lymphoma. The interleukin (IL)-1 receptor antagonist IL1RN, which binds to the IL-1 receptor, moderates a variety of immune responses related to IL-1. We aimed to assess the impact of IL1RN variable number of tandem repeats (VNTR) polymorphism on lymphoma risk in an Iranian population sample.
Materials and Methods: DNA was extracted from peripheral blood of 120 subjects with non-Hodgkin Lymphoma (NHL), 50 subjects with Hodgkin's lymphoma (HL), and 186 unrelated healthy individuals. IL1RN VNTR polymorphism was detected using polymerase chain reaction.
Results: Our findings revealed that the IL1RN VNTR polymorphism was associated with protection against NHL (P≤0.001, OR: 0.30, 95% CI: 0.18-0.53). The IL1RN 2 allele significantly decreased the risk of NHL (p = 0.023, OR = 0.66, 95%CI = 0.46–0.93). In addition, we found that IL1RN 1/2 was associated with a lower risk of HL (p ≤0.001, OR = 0.24, 95%CI = 0.12–0.50).
Conclusion: Our results suggest that the presence of IL1RN VNTR polymorphism is associated with a decreased risk of lymphoma in an Iranian subpopulation in southeast Iran.

Keywords: Interleukin-1 receptor antagonist; Variable number of tandem repeats (VNTR); Polymorphism; Lymphoma

INTRODUCTION

Non-Hodgkin's Lymphoma (NHL) represents a heterogeneous group of lymphoid system malignancies, and is one of the most common hematopoietic cancers constituting about 43% of all hematologic malignancies and 90% of lymphomas[1]. According to the GLOBOCAN database, NHL is the tenth and twelfth most common cancer in males and females worldwide, respectively, with 509,590 new cases and

248,724 deaths in 2018[2]. Although the exact pathogenesis of NHL is mostly unknown, cumulative evidence proposed that both genetic and environmental factors are involved in lymphoma development[3,4]. Of these, immune dysfunction has emerged as one of the most important causes of lymphoma[4,5]. Indeed, patients with acquired immune deficiency syndrome (AIDS) and post-organ transplant immune deficiencies have increased risks of developing NHL[6]. Interleukin-1 family is proinflammatory cytokines involved in inflammation and the immune response[7], and are encoded by the IL-1 gene cluster located on chromosome 2q14 [8]. The IL-1 family comprises of the IL-1α, IL-1β, and IL-1 receptor antagonists [9]. Several studies have proposed a key role for IL-1 in the development, progression, angiogenesis, and metastasis of cancer [10-12]. IL1RN is a member of the IL-1 superfamily that functions as a competitive antagonist of the cell surface IL-1 receptor, thereby moderating a variety of IL-1 related immune and inflammatory responses[13]. IL1RN is produced by several cell types such as immune cells, epithelial cells, and adipocytes [14].

The IL1RN gene has an 86 bp variable number of tandem repeats (VNTR) polymorphism in the second intron. Associations between this polymorphism and some cancers have been reported in previous studies[11,15-17], but with controversial results. In the present study, we aimed to determine whether an association exists between the presence of the IL1RN VNTR polymorphism and lymphoma risk in an Iranian population sample.

MATERIALS AND METHODS

This case control study included 120 patients with NHL (20-90 years of age; 74 males and 46 females), 50 patients with HL (5-71 years of age; 34 males and 16 females), and 186 healthy subjects (21-75 years of age; 86 males and 100 females). The patients were selected from individuals admitted to the Ali ibn Abi Talib Hospital regional referral hospital for cancer cases in the southeast of Iran (Sistan & Baluchistan province). The diagnosis of lymphoma was confirmed by histology and pathology tests. The control group did not have any kind of cancers and consisted of a population permanently residing in and native to the Southeast of Iran. The study was performed with the approval of the ethics committee of the Zahedan University of Medical Sciences (IR.ZAUMS.REC.1397.296) and informed consent was obtained from all participants. Peripheral blood samples were taken from all patients and healthy subjects and stored at -20 until DNA extraction. Genomic DNA was extracted from blood samples by the 'salting out' method. The quality of the extracted DNA was assessed using NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Genotyping of VNTR polymorphisms of IL-1RN was achieved by polymerase chain reaction (PCR) with 5`-CTCAGCAACACTCCTAT-3` forward primer and 5`- TCCTGGTCTGCAGGTAA-3` reverse primer. PCR was performed in a final volume of 20 μl containing 10 μl of 2X Prime Taq Premix (Genet Bio, Daejeon, Korea), 7 μl ddH2O, 1 μl of each primer (10 μM) and 1 μl genomic DNA (~100 ng/ml), according to the following protocol: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 25 s, extension at 72 °C for 30 s, and final extension at 72 °C for 10 min. The PCR products were separated on a 2% agarose gel.

Statistical Analysis

Data statistics were analyzed using SPSS 20 software (SPSS Inc., Chicago, IL, USA). The associations between IL1RN variants and lymphoma risk were estimated by calculation of the odds ratio (OR) and 95% confidence intervals (CI) under different genetic models. Differences were considered to be statistically significant when $P<0.05$.

RESULTS

The study group included 120 patients with NHL, 50 patients with HL and 186 healthy individuals. No significant differences were observed between the groups regarding sex and age (P=0.092 and P=0.083). Based on the

differences of 86-bp VNTR, five types of alleles can be distinguished: IL1RN1 (4 repeats, 420-bp), IL1RN2 (2 repeats, 240-bp), IL1RN3 (5 repeats, 498-bp), IL1RN4 (3 repeats, 326-bp) and IL1RN5 (6 repeats, 595-bp) [9]. In the current study, we observed various combinations of four alleles (IL1RN*1, *2, *3 and *4) (Figure 1). Table 1-3 shows the genotype and allele frequencies of IL1RN VNTR polymorphisms observed in patients and control subjects. The results indicate that the IL1RN 1/2 genotype is associated with a decreased risk of NHL (P≤0.001, OR: 0.30, 95% CI: 0.18-0.53). Furthermore, the IL1RN 2 allele significantly decreased the risk of NHL (p = 0.023, OR = 0.66, 95%CI = 0.46–0.93) compared to other alleles (Table 1). As shown in Table 2, genotype frequency analysis revealed that only IL1RN 1/2 is significantly different between HL patients and healthy controls (p ≤0.001, OR = 0.24, 95%CI = 0.12–0.50). However, no significant differences were found between HL and controls regarding allele frequency (P=0.13). Lastly, no significant differences regarding genotype and allele frequency were observed between NHL and HL patients (P=0.74, 0.84 respectively).
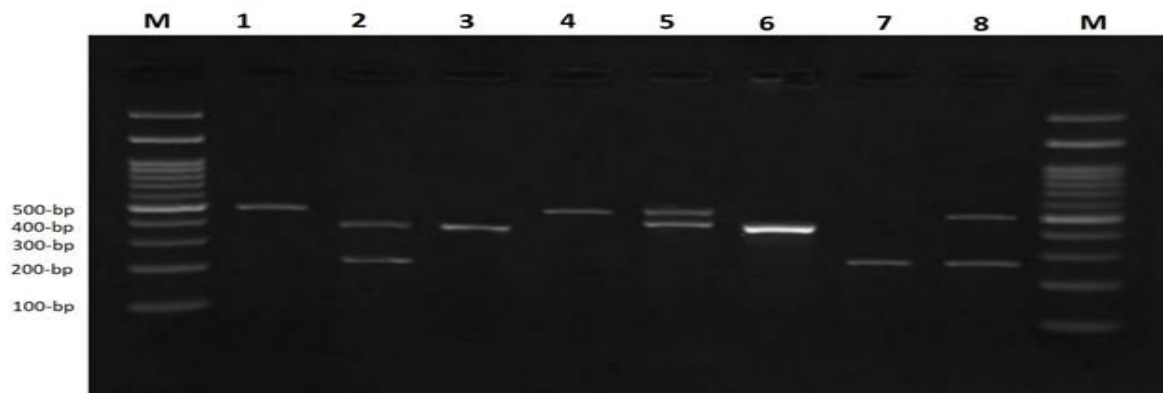

Figure 1: Electrophoresis pattern of PCR product for detection of *IL1RN* VNTR polymorphism
M: DNA Marker; Lane 1 and 4: ILRN 3/3; Lane 2: ILRN1/2; Lanes 3 and 6: ILRN 1/1; Lane 5: ILRN 1/3; Lane 7: ILRN 2/2; Lane 8: ILRN 2/3.

Table 1: Genotype and allele frequencies of the IL1RN VNTR polymorphism in NHL and healthy subjects (control)

IL1RN Genotypes	NHL n (%)	Normal n (%)	OR (95% CI)	p
IL1RN 1/1	44(36.7)	33(17.8)	1.0	-
IL1RN 1/2	55(45.8)	135(72.6)	0.30(0.18-0.53)	≤0.001
IL1RN 1/3	9(7.5)	10(5.4)	0.67(0.24-1.84)	0.610
IL1RN 1/4	2(1.7)	1(0.5)	1.50(0.13- 17.25)	0.788
IL1RN 2/2	7(5.8)	6(3.2)	0.87(0.26-2.84)	0.935
IL1RN 2/3	1(0.8)	0(0.0)	-	-
IL1RN 3/3	2(1.7)	1(0.5)	1.50(0.13- 17.25)	0.788
Alleles				
IL1RN 1	154(64.2)	212(57.0)	1.0	-
IL1RN 2	70(29.2)	147(39.5)	0.66(0.46-0.93)	0.023
IL1RN 3	14(5.8)	12(3.2)	1.60(0.71-3.64)	0.333
IL1RN 4	2(0.8)	1(0.3)	2.75(0.24-30.63)	0.78

Table 2: Genotype and allele frequencies of the IL1RN VNTR polymorphism in HL and healthy subjects (control)

IL1RN Genotypes	HL n (%)	Normal n (%)	OR (95% CI)	*p*
IL1RN 1/1	20(40.0)	33(17.8)	1.0	-
IL1RN 1/2	20(40.0)	135(72.6)	0.24(0.12- 0.50)	≤0.001
IL1RN 1/3	6(12.0)	10(5.4)	0.99(0.31-3.14)	0.781
IL1RN 1/4	0(0.0)	1(0.5)	-	-
IL1RN 2/2	4(8.0)	6(3.2)	1.10(0.27- 4.37)	0.826
IL1RN 3/3	0(0.0)	1(0.5)	-	-
Alleles				
IL1RN 1	66(66.0)	212(57.0)	1.0	-
IL1RN 2	28(28.0)	147(39.5)	0.61(0.37- 0.99)	0.062
IL1RN 3	6(6.0)	12(3.2)	1.60(0.58- 4.44)	0.524
IL1RN 4	0(0.0)	1(0.3)	-	-

Table 3: Genotype and allele frequencies of the IL1RN VNTR polymorphism in NHL and HL

IL1RN Genotypes	NHL n (%)	HL n (%)	OR (95% CI)	*p*
IL1RN 1/1	44(36.7)	20(40.0)	1.0	-
IL1RN 1/2	55(45.8)	20(40.0)	1.25(0.59- 2.60)	0.684
IL1RN 1/3	9(7.5)	6(12.0)	0.68(0.21-2.17)	0.730
IL1RN 1/4	2(1.7)	0(0.0)	-	-
IL1RN 2/2	7(5.8)	4(8.0)	0.79(0.20-3.02)	0.98
IL1RN 2/3	1(0.8)	0(0.0)	-	-
IL1RN 3/3	2(1.7)	0(0.0)	-	-
Alleles				
IL1RN 1	154(64.2)	66(66.0)	1.0	-
IL1RN 2	70(29.2)	28(28.0)	1.07(0.63-1.81)	0.901
IL1RN 3	14(5.8)	6(6.0)	1.00(0.36-2.71)	0.798
IL1RN 4	2(0.8)	0(0.0)	-	-

DISCUSSION

Over recent decades the incidence of cancers, including hematological malignancies such as NHL and HL, has progressively increased[1]. Immune deregulation remains one of the most well-defined risk factors reported to be associated with the development of NHL, the most prevalent hematologic cancer type[4]. In the current study, we were the first to investigate the association between the IL1RN VNTR polymorphism and the risk of lymphoma in an Iranian population. Our results showed that the IL1RN VNTR polymorphism was correlated with a significantly decreased risk of lymphoma in our study population. The IL1RN 1/2 genotype decreased the risk of NHL as well as HL. In agreement with our results, the IL-1RN *2 genotype has been proposed to be associated with reduced breast cancer prevalence, and the IL1RN 2 allele was found to decrease the risk of breast cancer with a borderline significance[18]. Similarly, the presence of the IL-1RN 2 allele was associated with a significantly decreased risk of lung cancer in Hu study and therefore suggested to be protective[19]. In that same study, IL1RN levels were higher in the serum from patients with lung and colorectal cancer, gynecological carcinoma, and HL (as compared to serum from control subjects), which implies a critical role for this protein in the formation and development of tumors[19]. In contrast, Ibrahimi et al showed that 1/2 and 2/4 genotypes of IL1RN are correlated with colorectal cancer (CRC) susceptibility, and that allele 2 of IL-1RN is associated with CRC risk[20]. Furthermore, no significant differences in IL-1RA gene polymorphism were reported between hepatocellular carcinoma patients and control subjects[21]. To the best of our knowledge, there are no reports regarding the association of the ILRN VNTR polymorphism and lymphoma. However, a pooled analysis of three studies showed that IL1RN rs2637988 was correlated with an increased risk of NHL[22], probably by increasing predisposition to shorter immune responses[23]. Conversely, several other polymorphisms in IL-1RN (rs3181052, rs452204 and rs315919) are associated with a decreased risk of esophageal cancer in a Northwest Han Chinese population[11].

A growing body of evidence strongly suggests that chronic inflammation may be a potential risk factor for a variety of cancers. Cytokines play important roles in inflammation and immune responses, and can influence cancer susceptibility as well [20]. IL-1 is a key inflammatory cytokine that has been shown to induce tumor growth, angiogenesis, invasiveness and metastasis in various cancers[24]. IL-1RN can alter IL-1 expression levels and compete for binding spots on the IL-1 receptors, consequently modulating the effectiveness of IL-1 signaling and IL-1 related immune and inflammatory responses[11]. Despite the convincing results, our study has several limitations, which include the analysis of a relatively small sample size and the gene environmental interactions. Another limitation is the lack of information on the expression levels of IL-1RN in serum of lymphoma patients and control groups.

In summary, our findings suggest that the IL-1RN VNTR polymorphism is associated with a decreased risk of lymphoma in an Iranian subpopulation. Future studies with larger sample sizes and different populations should be performed to validate our findings.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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