The Impact of Genetic Variants in IL-10 and IL-12p40 on the Susceptibility to Non Hodgkin Lymphoma

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Abstract

Background: Non-Hodgkin lymphomas (NHLs) are a diverse group of mature lymphoid neoplasms with a wide range of cellular and histological presentations. Numerous environmental and genetic factors affect the incidence of these neoplasms; however the exact causes are beyond the current knowledge.

Objective: to investigate the association of genetic polymorphisms in two single nucleotide polymorphisms, which are IL-10-1082A/G and IL-12p401188A/ C, with the incidence NHLs among Iraqi patients.

Materials and Methods: Whole blood samples were collected from 55 confirmed patients with NHLs and from 40 family-unrelated, age-matched apparently healthy individuals to represent the control group. DNA was extracted from blood samples and allele specific polymerase chain reaction (AS-PCR) technique was used for genotyping of the two SNPs using specific primers.

Results: The SNP IL-10-1082A/G appeared in three genotypes: AA, AG and GG which represented 36.36%, 45.45% and 18.18%% respectively in NHL patients compared to 32.5%, 35% and 32.5%% respectively with insignificant neither between genotypes nor alleles. Similarly, the SNP IL-12p401188A/C had three genotypes which were AA, AC and CC. These genotypes represented 32.73%, 38.18% and 29.09% respectively among NHL patients and 52.5%, 37.5% and 10% respectively among controls with significant difference for the homozygous mutant genotype (OR=4.667, 95%CI=1.319-16.512, P=0.017).

Conclusion: IL-10-1082A/G polymorphism has no effect on the incidence of NHLs, while allele C of the SNP IL-12p401188A/C could be considered as a risk factor for these neoplasms.

Key words: Non-Hodgkin's lymphoma, single nucleotide polymorphism, IL-10-1082A/G, IL-12p40 1188A/C.

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Introduction

Non-Hodgkin lymphomas are a diverse group of mature lymphoid neoplasms with a wide range of cellular, histologic presentations, cells of origin and etiologies [1]. Numerous environmental and genetic factors have been documented to be associated with the incidence of NHLs, however the exact causes are beyond the current knowledge [2]. Regardless of causes,

the integrity of the immune system represents the cornerstone in the resistance or progression of the disease. Grulich et al. reported many disorders of this system such as immune deficiency and autoimmune disease like rheumatoid arthritis and systemic lupus erythematosis to be predispose to NHLs. Immune deficiency is usually (but not always) manifested by an insufficient quantity of one or more of the immune components. However, in many cases such components are present in sufficient quantities but they cannot achieve their duties due to defect in their synthesis resulting from genetic disorder [3]

Interleukin 10 (IL-10) and interleukin 12 are among the main players cytokines of the immune system. Interleukin-10, first recognized for its ability to inhibit activation and effecter function of T lymphocyte, monocytes, and macrophages, is a multifunctional cytokine with diverse effects on most hemopoietic cell types. The principal routine function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. It plays a key role in differentiation and function of T regulatory cell, which has a certral role in controlling immune responses [4].

Interleukin-12 (IL-12) is also a multifunctional cytokine acting as a key regulator of cell-mediated immune responses through the differentiation of naïve CD4+ T cells into type 1 helper T cells (Th1) producing interferon-γ [5]. Factors influencing the functions of these cytokines are expected to influence the efficiency of the immune system especially the cellular arm, and subsequently alter the individual susceptibility to NHLs.

Single nucleotide polymorphism (SNP) is a variation occurring commonly within a population in which a single nucleotide in a certain gene differs between members of a biological species or paired chromosomes (6). Huge number of SNPs are present in almost every gene including IL-10 and IL-12 genes, but certain SNPs have been found to influence the biological activity of these cytokines. Not only do these SNPs affect the levels of gene expression of the cytokine but also they can alter the binding capacity of this cytokine with its receptor and subsequently the cytokine's activity. Hence, it is reasonable to assume that certain SNPs in IL-10 and IL-12 gene may influence the individual susceptibility to NHLs.

To the best of our knowledge, there is no previous study in Iraq which involved such issue. Thus, this study aimed to investigate the association two SNPs (IL-10-1082A/G and IL-12p401188A/ C) with the incidence NHLs among Iraqi patients.

Materials and Methods

Study Subjects

A prospected study consisted of 55 patients with confirmed NHLs during the period from January 2015 to June 2015 from teaching hospital of pediatric, Baghdad teaching hospital and hematology center/ Al-Mustansiriya University, Baghdad, Iraq. Family unrelated, apparently healthy 35 individuals from Al-Kadhimyia teaching hospital were selected to represent the control group. The mean ages of patients and control were 33.45 and 36.49 years respectively. Informed consents from patients as well as control were taken.

DNA Extraction and Genotyping

Three ml of venous blood was collected from each participant in EDTA tube. DNA was extracted from blood samples using ready kit (gSYNCTM DNA Mini Kit Whole Blood Protocol/ Geneaid/ Korea) according to the manufacturer's instructions. Primers used for both genes are shown in table (1). The PCR conditions for IL-10 gene were an initial denaturation for 5 minutes at 95oC, followed by 35 cycles of denaturation at 94oC for 30 seconds, annealing at 63oC for 30 seconds and extension at 72oC for 30

seconds, and final extension at 72°C for 5 minutes. PCR conditions for IL-12p40 gene were an initial denaturation for 5 minutes at 95°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C or 30 seconds and extension at 72°C for 1 minutes, and final extension at 72°C for 7 minutes. The primers of internal control were used in the amplification of both genes

Table (1): Primer sets sequences and their corresponding genes

Genes	Primers 5'→3'	Fragments	Reference
IL-10	Consensus F: GTAACGTTCTGTGGCTGGAGTC Wild: AACACTACTAAGGCTTCTTTGGGT**A** Variant: AACACTACTAAGGCTTCTTTGGGT**G**	161 bp	[7]
IL-12p40	Consensus F: ATCTTGGAGCGAATGGGC R1: TTGTTTCAATGAGCATTTAGCATCT R2: GTTTCAATGAGCATTTAGTATCG	780 bp	[8]
TLR2 (internal control)	F: CCTGGCAAGTGGACCATTGAC R: GGCCACTCCAGGTAGGTCTT	254 bp	[9]

A ready 50 µl PCR master mix (Bioneer/Korea) was used for amplification for both genes. Template DNA (10 µl) from each sample and primers (5 µl from each) were added to each master mix tube. The mixture then put in shaker and spinner for 10 cycles for better mixing. After mixing, the mastermix tubes were transferred to the thermocycler (MyGenie 32 thermal block/Bioneer/Korea) which is previously programmed with the above protocol according to the gene to be amplified. The amplified products were determined by comparison with a commercial 1000 bp ladder (Kappa Biosystem/USA).

Statistical Analysis

The Statistical Package for the Social sciences (SPSS, version 14) was used for statistical analysis. Risk association between the genotype and NHLs susceptibility was estimated by the calculation of adjusted odd ratio and 95% confidence intervals using bivariate logistic regression. For this analysis, subjects who were homozygous for the wild type allele were considered as reference, and polymorphisms as dependent variables. Chi square test ($\chi 2$) was used to determine the significant difference between each two alleles. A p-value □ 0.05was considered statistically significant.

Results

Allele Specific PCR

This study investigated the association of two polymorphisms (IL-10-1082A/G and IL-12p40 1188A/C) with incidence of NHL. A total of 55 NHL patients and 40 apparently healthy control individuals were recruited for this study. Figure (1) shows the results of allele specific PCR for the SNP IL-10-1082A/G in NHL patients and controls.

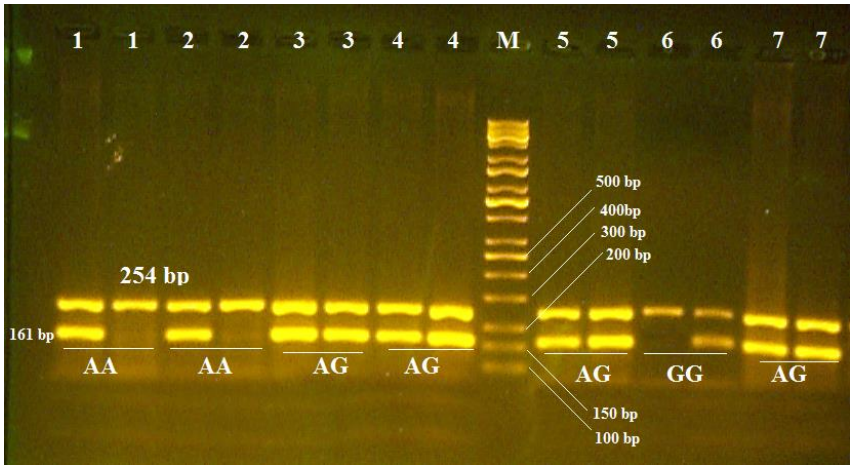

Figure (1): IL-10-1082A/G genotype patterns in NHL patients after genotyping using AS-PCR visualized under U. V light after staining with ethidium bromide. M: 100 bp DNA marker. The 780 bp represents the amplification of IL-10-1082A/G, while the 254 bp represents the amplification of internal control (TLR2 gene).

The SNP IL-10-1082A/G had three genotypes; AA, AG, and GG. In NHL patients, these genotypes account for 20 (36.36%), 25 (455.46%), and 10 (18.18 %) respectively among NHLs patients compared to 13(32.5%), 14(35%), and 13(32.5%) respectively, in control group with insignificant differences neither for heterozygous genotype (OR=2.0, 95%CI=0.679-5.892, P=0.209) nor for homozygous mutant genotype (OR=2.321, 95%CI=0.810-6.650, P=0.117).

Analysis of allele frequencies of this SNP revealed insignificant differences in the frequency of A allele between NHL patients and control (59.09% and 50% respectively), as well as frequency of G allele (40.91% and 50% respectively, OR=1.444, 95%CI=(0.809-2.580, *P*=0.214) as shown table (2).

Table (2): Genotypes and alleles of SNPs IL-10-1082A/G and IL-12p40 1188A/C

Variables	Cases N=55	Control N=40	*P*-value	OR(95%CI)
IL-10-1082A/G				
AA	20(36.36%)	13 (32.5%)	0.27	1.0
AG	25(45.46%)	14(35%)	0.209	2.0(0.679-5.892)
GG	10(18.18%)	13(32.5%)	0.117	2.321(0.810-6.650)
Alleles			0.214	
A	65(59.09%)	40(50%)		1.0
G	45(40.91%)	40(50%)		1.444 (0.809-2.580)
IL-12p40 1188A/C				
AA	18(32.73%)	21 (52.5%)	0.056	1.0
AC	21(38.18%)	15(37.5%)	0.293	1.633(0.655-4.074)
CC	16(29.09%)	4(10%)	0.017	4.667(1.319-16.512)
Alleles			0.007	
A	57(51.82%)	7(71.25%)		1.0
C	53(48.18%)	23(28.75%)		2.304 (1.250-4.249)

N: number, **OR:** odds ratio, **CI:** confidence interval

Figure (2) shows the results of allele specific PCR for the SNP IL-12 1188A/C in NHL patients and controls respectively.

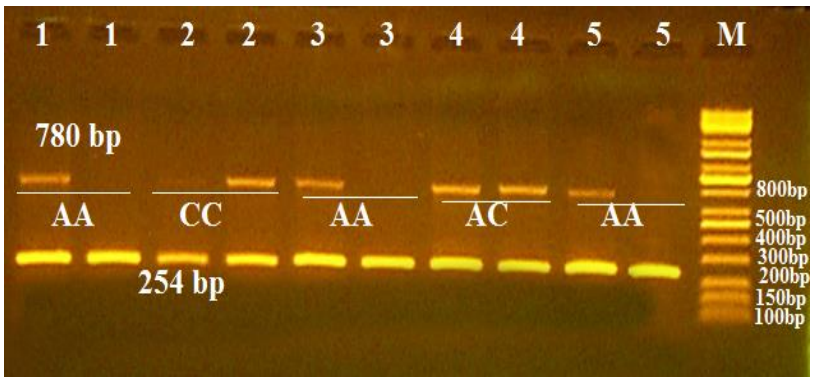

Figure (2): Various *IL-12p40* 1188A/C genotype patterns in controls after genotyping using AS-PCR visualized under U. V light after staining with ethidium bromide. M: 100 bp DNA marker. The 780 bp represents the amplification of *IL-12p40* 1188A/C, while the 254 bp represent the amplification of internal control (TLR2 gene).

Similar to IL-10, IL-12p40 1188A/C had three genotypes which were AA, AC and CC. These genotypes represented 32.37%, 38.18% and 29.09% respectively in NHL patients and 52.5%, 37.5% and 10% respectively among controls with significant difference for the homozygous mutant genotype CC (OR=4.667, 95%CI=1.319-16.512, P=0.017). At allelic level, the frequency of C allele in NHL patient was 48.18% compared with 28.75% in controls with significant difference (OR=2.304, 95%CI=1.250-4.249) (table 4-2).

Discussion

The current study revealed no effect of the SNP IL-10-1082A/G on the susceptibility to NHLs among Iraqi patients. These results are in accordance with that of Talaat *et al.* [10] who did not find any association between two SNPs (-1082 and -819) in the promoter region of IL-10 gene with the incidence of DLBCL among Egyptian population. Also the current results confirmed the recent investigation conducted by Lim et al. [11] who evaluated the effect of IL-10-1082A/G polymorphism in three major races of the Malaysian population on the susceptibility to NHL, and found no association at all .

However, the present results disagree with many other studies. Cunningham et al. [12] reported that the frequency of the low-IL-10 producing AA genotype at position -1082 was significantly higher in patients with aggressive NHLs compared to the controls. Almost the same results were obtained by Bogunia-Kubik *et al.* [13]. S*trikin*gly, in Egyptian population, Ahmed et al [14] linked allele G of this SNP with significantly high risk of developing NHLs. Furthermore, a recent meta-analysis study involved 7794 NHL cases and 8584 controls recorded significant increase risk of NHL associated with higher distribution of G allele of IL-10-1082A/G polymorphisms (OR=1.22, 95% CI = 1.08-1.39).

Thus it seems that many factors among which different races, sample size and statistical method may influence the result

On the other hand, the SNP IL-12p40 1188A/C had significant effect on the incidence of NHLs both in genotype and allele level. These results disagree with previous study by Yang et al. [15] who found A allele but not C which associates with different cancers among which NHL.

The SNP IL-12p40 1188A/C is located in the 3'UTR of the gene. This region although does not encode for a protein, it can influence the amount of translated protein through several mechanisms including effects on mRNA stability as well as on transcriptional activity [16]. Thus the SNP affects the gene silences and could regulate the level of IL-

12B mRNA expression [17]. Peresi *et al.* [18] found that AA genotype of this SNP is associated with lower plasma level of IL-12 in normal control. Furthermore, 1188A variant was shown to be correlated with reduced levels of IL-12p40 subunit of IL-12, while 1188C variant associated with increase this subunit of the cytokine [19, 20]. Thus, it is supposed to be profound of IL-12 associated CC genotype and robust cell-mediated immune response, while the current result revealed a contrast relationship (C allele which is associated with high levels of IL-12 is more prevalent in NHLs than controls)

The first explanation for this discrepancy is that the increased production of IL-12 does not mean that IL-12p70 is overproduced. Rather, the induction involves only the IL-p40 subunit. The homodimers of this subunit antagonizes IL-12p70 activity by binding to the β1 subunit of the IL-12 receptor [21]. Therefore, the increased production of this subunit, in fact, causes reduction in the activity of IL-12 and hence reduces the efficiency of cell-mediated response and increases the susceptibility to the malignancy .

The second explanation referred to the effect of IL-12p40 homodimers on the activity of IL-23 as these homodimers have high affinity to IL-23 receptor and hence abolish IL-23 role in immune response [22]. The main role of IL-23 involves the stimulation of Th17 cells to produce IL-17 [23] which has an important role in the immunity against NHLs [23].

These data strongly suggest that the SNP IL-10-1082A/G in the promoter region of IL-10 gene has no effect on the susceptibility to NHLs among Iraqi patients while allele C of the SNP IL-12p40 1188A/C in the 3'UTR of IL-12p40 gene is a risk factor for NHLs among those patients. However, a study with larger NHLs patients and control
is needed to confirm these results

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