RESEARCH ARTICLE

The Effect of IL-10 Polymorphism in Patients with Non Hodgkin’s Lymphoma in Iraq.

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Abstract

This study aims to examine the association of single nucleotide polymorphisms (SNPs) of IL-10-1082A/G with the incidence of NHLs in patients in Iraq. Fifty-five confirmed patients with NHLs and 40 family-unrelated, age-matched apparently healthy controls were used for blood and DNA extraction. Allele specific polymerase chain reaction (AS-PCR) technique was used for genotyping of the IL-10 SNP. IL-10 production levels in sera of patients and healthy individuals were measured by using ELISA. The SNPIL-10-1082A/G appeared in three genotypes: AA, AG and GG. These genotypes represented 36.36%, 45.45% and 18.18%, respectively, in NHL patients compared to 32.5%, 35% and 32.5%, respectively, in healthy controls, respectively. There was no significant correlation between neither genotypes nor alleles and NHL incidence. IL-10 levels in NHL patient sera was significantly higher ($t=2.606, P=0.011$) than that of healthy control but is neither linked to IL-10 genotypes nor with allele distribution frequency. These results demonstrated that IL-10-1082A/G polymorphism has no significant effect on the incidence of NHLs, therefore other risk factors could be examined further. These suggest that multiple factors contribute to the susceptibility of NHLs in Iraqi patients.

Introduction:

Malignant lymphomas including Non-Hodgkin lymphoma are a diverse heterogeneous group of mature lymphoid neoplasms with a wide range of cellular, histologic presentations, cells of origin and etiologies (Kim, 2014). 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 (Hartge and Smith, 2007). Among these causes, the immune system represents the cornerstone in the resistance or progression of the disease.

Cytokines are soluble proteins secreted by activated lymphocytes and macrophages and have a wide range of functions in hematopoiesis and immune responses (Grulich, 2007). Interleukin 10 (IL-10) is among the main player cytokines of the immune system. IL-10 is a well-known anti-inflammatory cytokine inhibiting the effector function of T lymphocyte, monocytes, and macrophages (Moore et al., 2001, Vieira et al., 1992). It is shown to be involved in the pathogenesis of lymphoid malignancies (Lossos and Morgensztern, 2006, Cortes and Kurzrock, 1997).

A single nucleotide polymorphism (SNP) in a multiple regions of IL-10 gene was shown to influence the biological activity of this cytokine that eventually may determine NHL susceptibility and clinical outcomes (Lech-Maranda et al., 2004). Hence, it is reasonable to assume that certain SNPs in IL-10 gene such as -1082A/G may influence the individual susceptibility to NHLs. To the best of our knowledge, there is no previous studies in Iraq on this issue.
Materials and Methods:
Study Subjects:
In this study, 55 patients with confirmed NHLs were recruited during the period over January 2015 to January 2016 from four teaching hospital in Baghdad city. Family unrelated, apparently healthy 35 individuals were randomly 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:
EDTA blood was collected from each participant. DNA was extracted using ready kit (gSYNC™ DNA Mini Kit Whole Blood Protocol, Geneaid, Korea) according to the manufacturer's instructions. Primers used for IL-10 gene are shown in Table (1). The PCR conditions were an initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec and extension at 72°C for 30 sec, and final extension at 72°C for 5 min. The primers of internal control of Toll-like receptor were used in the amplification of IL-10 genes. A ready 50 µl PCR master mix (Bioneer/Korea) was used for amplification for IL-10 gene. 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 analyzed 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 $< 0.05$ was considered statistically significant.

Results:
IL-10 Polymorphism in NHL patients and controls by using allele specific PCR:
Results of allele specific PCR for the SNP IL-10-1082A/G in NHL patients and controls were illustrated in Table (1). The SNP IL-10-1082A/G had three genotypes; AA, AG, and GG. In NHL patients, these genotypes account for 20 (36.36%), 25 (45.46%), and 10 (18.18 %) respectively among NHL 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, $P=0.214$) (Table 2).

Serum levels of IL-10 as measured by ELISA:
A randomly selected 48 serum samples from NHL patients and 40 samples from healthy controls were used for measuring the levels of IL-10 in patients’ and controls by using an ELISA kit. The levels of IL-10 in the sera from NHL patients were significantly higher (458.31±241.126 pg/ml) than control group (323.46±242.344 pg/ml) ($t=2.606$, $P=0.011$) (Figure 2).

IL-10 levels among the three genotypes AA, AG and GG in sera from NHL patients revealed that those having AG genotype had higher levels of IL-10 (512.03± 205.65 pg/ml), followed by GG genotype (489.86±317.55 pg/ml) and then AA genotype as being the lowest (381.08±223.14 pg/ml). However, the differences were insignificant among the three genotypes (F=1.54, $P=0.225$) (Figure 3).

Discussion:
The current study revealed that there was a significant increase in the levels of IL-10 in NHL patients compared to healthy group. This increase had no association neither with the SNP IL-10-1082A/G genotype neither with its allele frequency. Taken together, SNP IL-10 -1082A/G had no significant correlation with the susceptibility to NHLs among Iraqi patients. These results are similar to those obtained by Talaat et al. (2014) 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. These results has also confirmed the recent investigation conducted by Lim et al.
(2015) 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.

Results of this study however vary with many other studies, where 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 (Cunningham et al. (2003) and Bogunia-Kubik et al. (2008). On the contrary, in Egyptian population, Ahmed et al. (2014) 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 reported a 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 the influence of IL-10 -1082A/G SNP is still controversial and may indicate that many factors among which different races, sample size and statistical method may influence the result.

IL-10 levels were found to be elevated especially in aggressive NHLs and dropped after chemotherapy (Cortes et al., 1997; Guney et al., 2009; Conroy et al., 2013). In general, the elevation of IL-10 could be attributed to three main postulates associated with NHLs. Firstly, studies about the role of IL-10 polymorphism revealed conflicting results. It has been shown that allele A of IL-10-1082A/G binds more strongly to the transcription factor and causes less production of IL-10 compared to allele G (Ahmed et al., 2014). Although, this study demonstrated a slight increase of IL-10 associated with G allele compared to A allele. However, other IL10 -819C>T and -592C>A polymorphism had no close association with non-Hodgkin lymphoma (NHL) susceptibility, but a meta-analysis of 11 studies revealed an association with decreased risk to diffuse large B-cell lymphoma (DLBCL) (Zhang et al., 2015). IL10 3575T/A genotypes has been shown to contribute to risk of NHL (Wang et al., 2007). On the other hand, a little association of IL-10 -3575T/A polymorphism and risk to cancers (Zhu et al., 2015). Because of these controversial results, more careful further studies are required with a larger sample size. It has been found that 61% of the malignant cells obtained from NHL patients produce detectable amount of IL-10 protein by using immunohistochemistry and RT-PCR (Voorzanger et al., 1996). In addition, all patients that have detectable levels of IL-10 in culture also had elevated serum levels of IL-10 in tumour cells as well as those recruited to the lymphoma micro-environment (Upadhyay et al., 2015). Indeed, there is a tendency for elevation of serum IL-10 in NHL patients, as seen in this study. To less extent, the third factor is the association of EBV infection, as a risk factor for NHL, with the human IL-10 gene where the latter has an extensive homology with BCRF1 gene of EBV (viral-IL10) (Vieira et al., 1991) which it may influence the elevation of the total level of IL-10 (Boulland et al., 1998). Therefore, a distinguishable detection at the level of gene expression can provide an answer for this synergistic effect.

Figure 1. Genotyping of IL-10-1082A/G alleles’ distribution in patients with NHL by using allele-specific (AS) PCR. Amplicons electrophoresed on a 1.5% agarose gel stained with ethidium bromide and then visualized under a U.V. transilluminator and photographed. M, 100 bp DNA marker. The 161 bp represents the amplification of IL-10-1082A/G, while the 254 bp represents the amplification of the internal control of toll-like receptor-2 (TLR-2) gene.
IL-10 A/G polymorphism of patients’ samples run side-by-side and shown in lane order (1, 3, 5, 7, 9, 11 and 13) for IL-10A alleles and in lanes (2, 4, 6, 8, 10 and 14) for IL-10G alleles.

**Figure 2.** IL-10 levels in sera from NHL patients was significantly higher ($p=0.011$) compared to healthy controls, as measured by ELISA.

**Figure 3.** The relationship between the levels of IL-10 in sera of NHL patients and the three genotypes (AA, AG and GG) of the IL-10-1082A/G polymorphism.
Table 1. Nucleotide sequences of primer sets and their corresponding genes.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers' sequences 5’→3’</th>
<th>Fragments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>IL-10</em></td>
<td>Consensus Reverse: GTAACTITTCTGGCTGAGT</td>
<td>161 bp</td>
<td>Bhayal <em>et al.</em>, 2012</td>
</tr>
<tr>
<td></td>
<td>Wild-Forward: AACTACTAAGGCTTCTTTGGGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variant-Forward: AACTACTAAGGCTTCTTTGGGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TLR2</em> (internal control)</td>
<td>F: CCTGGCAAGTGGACCATTGAC</td>
<td>254 bp</td>
<td>Chen <em>et al.</em>, 2012</td>
</tr>
<tr>
<td></td>
<td>R: GAGCCACTCCAGGTTAGGTC</td>
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TLR: Toll-like receptor used as an internal PCR control.

Table 2. Genotypes and alleles distribution of SNP *IL-10*-1082A/G in NHL patients and control individuals.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases N=55</th>
<th>Control N=40</th>
<th>P-value</th>
<th>OR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>AA 20(36.36%)</td>
<td>13 (32.5%)</td>
<td>0.27</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>AG 25(45.46%)</td>
<td>14(35%)</td>
<td>0.209</td>
<td>2.0(0.679-5.892)</td>
</tr>
<tr>
<td></td>
<td>GG 10(18.18%)</td>
<td>13(32.5%)</td>
<td>0.117</td>
<td>2.321(0.810-6.650)</td>
</tr>
<tr>
<td>Alleles</td>
<td>A 65(59.09%)</td>
<td>40(50%)</td>
<td>0.214</td>
<td>1.0</td>
</tr>
</tbody>
</table>
|           | G 45(40.91%) | 40(50%) | 1.444 (0.809-2.580) |}

Abbreviations: N, number; OR, odds ratio; CI, confidence interval, P, calculated probability (p-value < 0.05 was considered statistically significant).

**Conclusion:**

No conclusive concurrence of *IL-10* -1082A/G SNP on susceptibility of patients with NHL, but there is an increase of *IL-10* levels in malignant cells induced by NHL. These suggest that NHL is a multifactorial heterogeneous in nature and required further studies.

**Acknowledgement:**

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**References:**