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ORIGINAL ARTICLE

Association of IL-10 gene promoter polymorphisms and non-Hodgkin lymphoma in Egyptian patients, relation to susceptibility, correlation with survival

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Abstract The pathophysiology of non-Hodgkin's lymphoma is still unknown. Many cytokines, including interleukin-10 (IL-10), play a role in the perpetuation of the disease. The aim of the study was to investigate the association of IL-10 gene promoter polymorphisms with non-Hodgkin lymphoma and to correlate with survival. Fifty patients with diffuse large B-cell lymphoma as well as 50 age- and sex-matched apparently healthy volunteers were genotyped for biallellic IL-10 gene promoter polymorphisms at positions −1082(A/G) and −3557(T/A) using polymerase chain reaction–restriction fragment length polymorphism. There were highly statistically significant differences between the two studied groups regarding results of IL-10 1082A/G polymorphism, for homozygous (GG) and heterozygous (AG) genotypes (p value <0.0001) but no statistically significant differences regarding homozygous (AA) genotype (p value= 0.7583). IL-10 3575T/A polymorphism revealed highly statistically significant differences between the two groups regarding homozygous (TT; p value <0.0001) and heterozygous (TA) genotypes (p value=0.0007), but no significant difference found regarding homozygous (AA) genotype (p value=0.1622). We did not find any associations between bad prognostic factors and any of the genotypes or alleles frequencies. Our results also reported that there was no impact of these polymorphisms on survival of lymphoma patients. IL-10 1082A/G and 3557T/A polymorphisms could be claimed as independent risk factors for susceptibility to lymphoma, regardless of any associated bad prognostic factors and without impact on overall survival.

Keywords Non-Hodgkin lymphoma · Interleukin-10 · Promoter polymorphisms · RFLP

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Introduction

Non-Hodgkin's lymphomas (NHL) are heterogeneous group of lymphoproliferative disorders. Diffuse large B-cell lymphomas comprise the majority of NHL with different genetic variations. The course of disease depends on various biological and clinical parameters (Bogunia-Kubik et al. 2008).

Immune alteration is a major risk factor for NHL, but the specific immune mechanisms involved still remain unresolved. The mechanisms underlying differences in immune response between individuals are complex and include inherited genetic variation and cumulative antigenic exposure to infectious and other environmental challenges that give rise to immunological memory (Wang et al. 2006, 2007; Rothman et al. 2006; Lan et al. 2006).

Lymphoid development, differentiation, and T-helper (Th)1/Th2 balance (i.e., cellular vs. humoral immunity)

are regulated in part by key cytokines including interleukin (IL)-1, IL-2, IL-6, IL-10, tumor necrosis factor (TNF), and lymphotoxin. Furthermore, deregulated concentrations of several cytokines (e.g., IL-6, IL-10, and TNF) have been detected in patients with lymphoma and were associated with an adverse prognosis (Rothman et al. 2006).

IL-10 is an important anti-inflammatory cytokine, mainly produced by monocytes, macrophages, Th2, normal and neoplastic B-cells. Many studies have shown that IL-10 may be involved in the pathogenesis of lymphoid disorders. It is well known that production of many cytokines, including IL-10 is related to their promoter/gene polymorphisms. Serum IL-10 levels have also been shown to reflect response to therapy in patients who had detectable IL-10 levels at diagnosis (Cunningham et al. 2003; Mosmann 1994).

The IL-10 gene is located on chromosome 1q32. Single nucleotide polymorphisms in the promoter region of the IL-10 locus form haplotypes that are associated with levels of IL-10 production (Gibson et al. 2001; Turner et al. 1997; Westendorp et al. 2001).

A number of studies have reported that IL-10 −1082G and IL-10 −3575T alleles are associated with high IL-10 production (Oswald et al. 1992). Several reports have described association of elevated IL-10 expression levels with certain cancers, for example ovarian carcinomas (Loercher et al. 1999), melanoma (Sato et al. 1996), and lymphoma/myeloma (Salmaggi et al. 2000). Elevated IL-10 level can occur for multiple reasons with very different implications. IL-10 can be produced by tumor cells themselves, possibly suppressing antitumor responses. In other cases, IL-10 could be produced by activated cells involved in a host antitumor reaction, and thus it could be an indicator of a potent inflammatory response rather than immunosuppression. Thus, in the absence of production data for a broader panel of cytokines and other immune response parameters, it is difficult to interpret the significance of elevated IL-10 level in many such studies (Moore et al. 2001).

Previous studies have evaluated that polymorphisms in IL-10 3575T→A and IL-10 1082A→G are associated with an increased risk of non-Hodgkin's lymphomas (Rothman et al. 2006; Cunningham et al. 2003; Lech-Maranda et al. 2004).

The aim of our study was to investigate the association of IL-10 gene promoter polymorphism with non-Hodgkin lymphoma and correlate it with survival of these patients and assess its role in altering susceptibility to lymphoma.

Subjects and methods

The study was carried out on 50 patients with diffuse large B-cell lymphoma (DLBCL) in the period between April 2009 and November 2010 among cases referred to nuclear medicine and oncology unit at Kasr El Eini School of Medicine, Cairo University, after giving their written informed consents and having local ethical committee approval. The extent of the disease was categorized according to the Ann Arbor classification (Rosenberg 1977) and International Prognostic Index (IPI; Myers et al. 1993). Performance status was assessed using the Eastern Cooperative Oncology Group criteria (Oken et al. 1982).

All patients included in the study received anthracycline-containing regimens consisting of cyclophosphamide, adriamycin, vincristine, prednisone (CHOP) or high-dose CHOP (Coiffier et al. 1989) according to the age and number of IPI factors. No patients received rituximab as a part of the first-line regimen. Complete remission (CR) was defined by the disappearance of all the disease manifestations and normalization of all the laboratory values. Overall survival (OS) was determined from the onset of treatment until the last follow-up evaluation or death from any cause.

The patients were 27 males and 23 females. Their age ranged from 22 to 73 years. Fifty age- and sex-matched apparently healthy adults were included as control group after giving written informed consents. They were 19 males and 31 females. Their ages ranged from 18 to 77 years. All patients and controls were analyzed for clinical and laboratory findings, including full history taking, clinical examination, routine laboratory investigations including complete blood picture with differential white cell count, erythrocyte sedimentation rate, complete liver and kidney functions, serum uric acid, serum LDH, and beta 2 microglobulin and abdominal ultrasound for detection of organomegaly and lymphadenopathy. The patients were subjected as well to immunophenotyping to exclude other lymphoproliferative disorders, lymph node biopsy to define the type of non-Hodgkin lymphoma, bone marrow biopsy and immunohistochemistry for staging, and CT abdomen and pelvis for proper diagnosis and staging.

Genotyping for detection of interleukin-10 polymorphisms (IL-10 −1082A/G and IL-10 −3575T/A) by restriction fragment length polymorphism (RFLP) was performed for all the subjects participating in the study.

Detection of interleukin-10 polymorphisms (IL-10 −1082A/G and IL-10 −3575T/A) by polymerase chain reaction (PCR)–RFLP: 3 ml of venous blood were withdrawn from all the subjects in a sterile etheylenediaminetetraacetic acid vacutainer. DNA was extracted from the whole blood using DNA extraction kit QIAamp Blood Kit (Cat. No. 51106; Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions.

For IL-10 −1082A/G polymorphism, amplification of a 139-bp fragment spanning position −1082 was done by PCR using forward primer (5′-CTC GCT GCA ACC CAA

CTG GC-3′) and reverse primer (5′-TCT TAC CTA TCC CTA CTT CC; Rasouli et al. 2008). PCR was carried out in 25-μl reaction volume containing 100 ng genomic DNA, 0.5 μmol/l of each primer, 200 μmol/l of each dNTP, 10× PCR buffer, and 2.5 U of Taq DNA polymerase (Taq PCR Master Mix Kit by Qiagen, catalog number 51104). The following thermo cycler program was performed: initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s (denaturation), 58°C for 45 s (annealing), and 72°C for 60 s (extension) and final elongation at 72°C for 10 min. Digestion with Mnl I (Fermentas, INC, USA) was done by incubation at 37°C overnight .The digested fragments were separated on 2.5% agarose gel electrophoresis stained with ethidium bromide. The electrophoretic pattern was visualized under UV light then photographed using a Polaroid camera with a red-orange filter. The genotype was considered GG when clear sharp bands were observed at the specific molecular weights of 106.33 bp and was considered AA at 139 bp. The size of the amplified product was read with the use of a DNA ladder of different molecular weights (Fermentas, NoLimits™ 100 bp DNA Fragment, catalog number SM1441).

For IL-10 −3575T/A, amplification of a 231-bp fragment spanning position −3557 was done using forward primer (5′-GGT TTT CCT TCA TTT GCA GC-3′) and reverse primer (5′-ACA CTG TGA GCT TCT TGA GG-3′; Moraes et al. 2003). PCR was carried out carried out in a 25 μl reaction using 12.5 μl master mix(Taq PCR Master Mix Kit by Qiagen, catalog number 51104) containing 2.5 mM of MgCl and 25 mM of each dNTP. One microliter (0.3 mM) of each primer and 100 ng genomic DNA were added to the reaction mixture. DNA was denatured at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and a final cycle of extension of 72°C for 7 min. The digestion was carried out with one unit of the Tsp509I per tube at 65°C for 3 h (New England Biolabs, Beverly, MA, USA). The genotype was determined by the size of the fragments generated (231 bp for the A allele, 121+110 for the T allele). The digested fragments were separated on 2.5% agarose gel electrophoresis stained with ethidium bromide. The results were documented using a Polaroid camera with a red-orange filter.

Statistics

A pre-designed Statistical Package for Social Science (Version17) file was used for data entry and analysis. Results were analyzed using paired *t* test with 95% confidence intervals (95% CI) and odds ratio to access the risk conferred by a particular allele and genotype. A *p* value <0.05 was considered to be statistically significant. Association with overall survival and classical prognostic factors was tested by multivariate analysis, association with survival was presented in the form of Kaplan–Meier curve, and also haplotype and diplotype were tested for association with classical prognostic factors by multivariate analysis.

Results

The current study was carried out on 50 patients with DLBCL as well as 50 age- and sex-matched apparently healthy volunteers (as a control group). Patients included in our study were 23 females (46%) and 27 males (54%). Their age ranged from 22 to 73 years with a mean value of 47.2±13.9 years. Control group were 31 females (62%) and 19 males (38%). Age ranged from 18 to 77 years with a mean value of 45.6±15.3 years. There were no statistically significant differences between the two groups regarding sex (*p* value=0.160) or age (*p* value=0.585).

Clinical characteristics of patients are summarized in Table 1 (Kube et al. 2008).

Performance status values ranged from 0 to 3 with a mean of 1.46±0.78.

Studying of the laboratory data of both groups revealed highly statistically significant differences regarding the total leucocytic count and hemoglobin level (*p* value <0.0001), but there were no statistically significant differences regarding platelet count (*p* value=0.452), performed liver functions (*p* value=0.0929), or kidney functions (*p* value=0. 174). Laboratory data of patients and controls are summarized in Table 2.

Some additional laboratory tests and procedures were performed for the patients to DLBCL, assess staging and prognostic state. The results are presented in Tables 3 and 4.

After three cycles of chemotherapy, 37 patients (74%) achieved complete remission while 13 patients (26%) achieved only partial remission, 13 patients (26%) needed red blood cells and platelet transfusion while 37 patients (74%) did not need either red blood cells or platelet transfusion. Drug adverse reactions as myelosuppression, infections, or toxicity developed in 28 patients (56%). Concerning the patients group, 41 patients (82%) achieved CR, while seven patients (14%) were resistant during the follow up period of 18 months. Death was the adverse outcome in two patients (4%). The duration of relapse-free survival ranged from 0 to 18 months with a mean value of 11.4±6.9 years.

Concerning the results of PCR–RFLP for IL-10 1082A/G polymorphism in the patients group, seven patients (14%) were homozygous for wild allele (AA), 30 patients (60%) were heterozygous for mutant allele (AG), and 13 patients (26%) were homozygous for mutant allele (GG). For the control group, five cases (10%) had AA genotype,

Table 1 Clinical characteristics of patients

Clinical characteristics	Number of patients (50)	Percentage (%)
Swelling (LN enlargement)	32/50	64
Change of voice	3/50	6
Pathological fractures	5/50	10
Shortness of breathe	4/50	8
Gastric pain and vomiting	4/50	8
Headache and confusion	2/50	4
Smoking status		
Smoker	39/50	78
Non-smoker	11/50	22
B symptoms		
Present	35/50	70
Absent	15/50	30
Ann Arbor staging:		
I	9/50	18
II	23/50	46
III	14/50	28
VI	4/50	8
Number of extra nodal sites involved		
No extra nodal sites	13/50	26
One extra nodal site	30/50	60
Two extra nodal sites	2/50	4
>2 Extra nodal sites	5/50	10
Bulky tumor		
Absent	38/50	76
Present 10 cm or greater	12/50	24
International prognostic[a]		
Index:		
Low/intermediate low	38/50	76
Intermediate high/high	12/50	24

[a] *IPI*, International prognostic: low (IPI=1), intermediate low (IPI=2), intermediate high (IPI=3), high (IPI=4, 5)

seven cases (14%) had AG genotype, while 38 cases (76%) had GG genotype. Relations between patients and controls regarding A allele (AA+AG) and G allele (GG+AG) frequencies were also studied.

Highly statistically significant differences were found between the patients and controls groups regarding the results of PCR–RFLP for IL-10 1082A/G polymorphism for GG and AG genotypes (p value <0.0001), but no statistically significant difference was found between the two studied groups regarding AA genotype (p value=0.7583). This could confirm that the presence of this polymorphism is a factor lowering susceptibility to lymphoma. A allele was found to be significantly higher among patients than controls (p value=0.0001), but no statistically significant difference with G allele frequency (p value=0.7583).

Table 5 shows genotype and allele frequency of IL-10 (1082A/G) in patients and controls groups.

Figure 1 show the results of PCR-RFLP of IL-10 (1082A/G) in patients and control groups.

For IL-10 1082, AA genotype, one band at 139 bp; AG genotype, three bands at 139,106 and 33 bp; GG genotype, two bands at 106 and 33 bp.

Concerning the results of PCR–RFLP for IL-10 3557T/A polymorphism in the patient group, ten patients (20%) were homozygous for wild allele (TT), 33 patients (66%) were heterozygous for the mutant allele (TA), and seven patients (14%) were homozygous for the mutant allele (AA). For the control group, 33 cases (66%) were homozygous for the wild allele (TT), 15 cases (30%) heterozygous for the mutant allele (TA), while two cases (4%) were homozygous for the mutant allele (AA). Highly statistically significant differences were found between the two studied groups regarding the TT genotypes (p value <0.0001) and TA genotypes (p value 0.0007), but no statistically significant difference was found between the two groups regarding AA genotype (p value= 0.1622). This could support that the presence of this polymorphism is a risk factor for increasing susceptibility to lymphoma. T allele (TT+TA) and A allele (AA+TA) frequencies were analyzed and we found statistically significant higher frequency of mutant A allele among patients (p value=0.0001), but no difference as regard T allele frequency (p value=0.1622).

Table 6 shows genotype and allele frequency of IL-10 (3557T/A) in patients and control groups.

Figure 2 show the results of PCR-RFLP of IL-10 (3557T/A) in patients and control groups.

For IL 3557, AA genotype, one band at 231 bp; TA genotype, three bands at 231, 121, and 110 bp; TT genotype, two bands at 121 and 110 bp.

Relations of IL-10 (1082A/G and 3557T/A) polymorphisms to bad prognostic factors [old age, presence of B symptoms, Ann Arbor stage III and IV, presence of bulky tumor, high serum LDH, and ß2 microglobulin] and impact of these polymorphisms on survival of lymphoma patients were also analyzed. Our results revealed that there were no statistically significant associations between different IL-10 (1082A/G and 3557T/A) genotypes and bad prognostic factors (Table 7). This supports the theory of claiming these polymorphisms as independent risk factors for developing lymphoma without relation to any of the bad prognostic markers. We also found that there was no impact of studied polymorphisms on survival of lymphoma patients (Figs. 3 and 4).

Table 7 shows relations of IL-10 (1082A/G) and (3557T/A) polymorphisms to prognostic factors.

Figure 3 shows Kaplan–Meier overall survival estimate according to genotype of IL-10 (1082A/G).

Table 2 Laboratory data of patients and controls

Laboratory data	Patients (50)	Controls (50)	*P* value
Total leucocytic count/mm^3			
Range	2.1–14.4	6–15	<0.0001 [HS]
Mean±SD	6.5±3	9.8±2.4	
Hemoglobin (gm%)			
Range	8.5–16	11.4–16	<0.0001 [HS]
Mean±SD	11.5±1.66	13.2±1.4	
Platelets×10^3 mm^3			
Range	96–614	168–394	0.452 [NS]
Mean±SD	292.6±135.3	276.5±66.6	
Liver functions			
Normal	46/50 (92%)	39/50 (78%)	0.0929 [NS]
Impaired	4/50 (8%)	11/50 (22%)	
Kidney functions			
Normal	48/50 (96%)	39/50 (78%)	0.174 [NS]
Impaired	2/50 (4%)	11/50 (22%)	

NS not significant (p value >0.05), *S* significant (p value <0.05), *HS* highly significant (p value <0.01)

Figure 4 shows Kaplan–Meier overall survival estimate according to genotype of IL-10 (3557T/A).

Table 8 Cox models for haplotypes and diplotypes of IL-10 1082A/G and IL-10 3575T/A polymorphisms adjusted for bad prognostic factors.

No statistically significant correlations were found between any of the haplotypes or diplotypes of the IL-10 1082A/G or 3557T/A polymorphisms to bad prognostic markers supporting our previous results that polymorphisms of interleukin 10 at positions 1082A/G and 3557T/A could be claimed as independent risk factors for susceptibility to lymphoma with no impact of any associated bad prognostic markers.

Discussion

Non-Hodgkin's lymphomas (NHL) are heterogeneous group of lymphoproliferative disorders. In the North America and Europe, B-cell lymphomas comprise the majority of NHL. The course of disease depends on various biological and clinical parameters as well as therapeutic decisions at the beginning of treatment. Biologic mechanisms leading to the development of NHL are not clearly understood. There are several reports suggesting a potential role of IL-10 in the pathophysiology of NHL that influences B-cell growth and development (Benjamin et al. 1992). Many studies have shown that IL-10 may be involved in the pathogenesis of lymphoid disorders (Moore et al. 2001). It is well known that production of many cytokines, including IL-10, is related to their promoter/gene

Table 3 The results of immunophenotyping of the patients group

Flow cytometry	Positive (%)	Negative (%)
CD20	50/50 (100)	50/50 (100)
CD3	50/50 (100)	50/50 (100)
CD5	29/50 (58)	21/50 (42)

CD20, CD3, CD5 were considered positive when more than of 20% of analyzed cells are positive

Table 4 Results of laboratory tests and procedures performed for the patients

Laboratory tests and procedures	Results
Serum LDH level (mg/dl)	
Range	210–564
Mean±SD	308.1±75.4
Serum ß 2 microglobulin (mg/L)	
Range	2–6
Mean±SD	3.54±1.11
Serum albumin level (mg/dl)	
Range	2.5–3.9
Mean±SD	3.38±0.38
Lymph node biopsy	
Positive DLBCL	50/50 (100%)
Negative DLBCL	0
Bone marrow biopsy	
Positive DLBCL	4/50 (8%)
Negative DLBCL	46/50 (92%)
CSF analysis	
Positive infiltration	0
Free	50 (100%)
X-ray and CT	
Positive infiltration	35 (70%)
free	15 (30%)

Table 5 Genotype and allele frequency of IL-10 (1082A/G) in patients and control groups

Genotype frequency	Patients (%)	Controls (%)	*P* value
AA	7 (14)	5 (10)	0.7583 [NS]
AG	30 (60)	7 (14)	<0.0001 [HS]
GG	13 (26)	38 (76)	<0.0001 [HS]
Allele frequency			
A allele (AA + AG)	37 (74)	12 (24)	0.0001 [S]
G allele (GG + AG)	43 (86)	45 (90)	0.7583 [NS]

Table 6 Shows genotype and allele frequency of IL-10 (3557T/A) in patients and controls groups

Genotype frequency	Patients (%)	Controls (%)	*P* value
TT	10 (20)	33 (66)	<0.0001 [HS]
TA	33 (66)	15 (30)	0.0007 [S]
AA	7 (14)	2 (4)	0.1622 [NS]
Allele frequency			
A allele (AA + TA)	40 (80)	17 (34)	0.0001 [S]
T allele (TT + TA)	43 (86)	48 (96)	0.1622 [NS]

polymorphisms. Therefore, polymorphisms within cytokine genes involved in pathomechanism of NHL may be related to susceptibility and/or clinical course of the disease (Bogunia-Kubik et al. 2008).

The aim of the study was to assess association of any of IL-10 (1082A/G, 3575T/A) genotypes prevailing in Egyptian patients with susceptibility to DLBCL and to determine the association between these polymorphisms and overall survival of lymphoma patients.

Our results were consistent with Berglund et al. (2005) who reported that the frequency of the IL-10_{-1082G} allele was not significantly different in patients with DLBCL versus the control group (0.43 vs. 0.46; P=0.38). Furthermore, they did not find any difference in overall survival between patients with IL-$10_{-1082AG/GG}$ genotype and patients with IL-$10_{-1082AA}$ (P=0.50). No difference in survival was shown between patients with IL-$10_{-1082AG/GG}$ and IL-$10_{-1082AA}$genotype when considering only patients younger than 60 years of age or when patients with or without B symptoms or elevated S-LDH levels were analyzed separately. Moreover, no difference in outcome was evident for patients with or without the IL-10_{-1082G} allele when separately analyzing de novo DLBCL and cases with previous history of low-grade lymphoma.

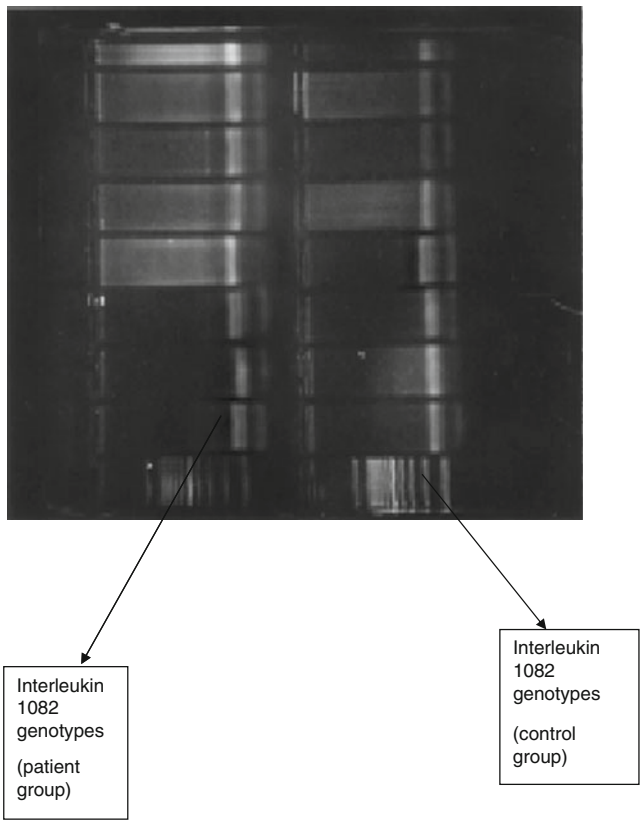

Fig. 1 Show the results of PCR–RFLP of IL-10 (1082A/G) in patients and control groups. For IL 1082, AA genotype, one band at 139 bp; AG genotype, three bands at 139, 106 and 33 bp; GG genotype, two bands at 106 and 33 bp

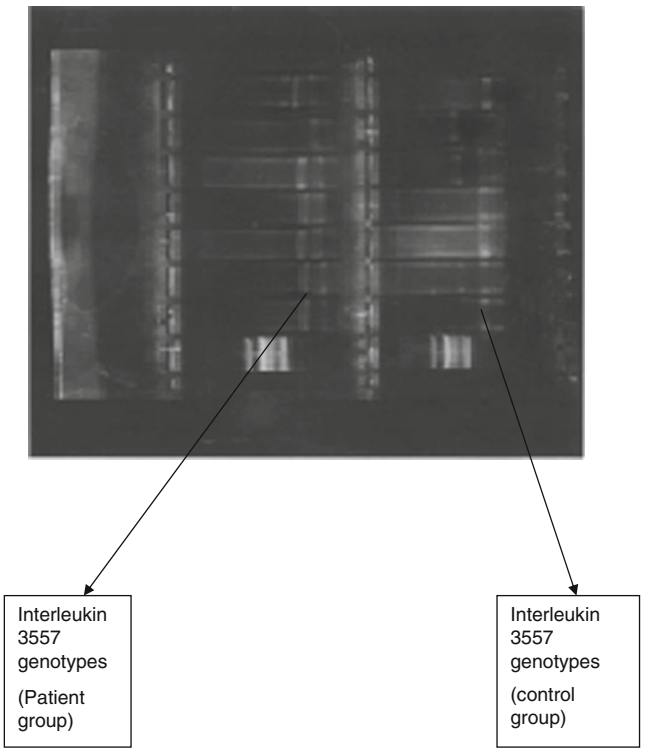

Fig. 2 Show the results of PCR–RFLP of interleukin 10(3557 T/A) in patients and control groups. For IL 3,557, AA genotype, one band at 231 bp, TA genotype, three bands at 231, 121, and 110 bps; TT genotype, two bands at 121 and 110 bp

Table 7 Shows relations of IL-10 10(1082A/G) and 10(3557T/A) polymorphisms to prognostic factors

1082A/G frequency	3557T/A frequency	Hazard ratio	*P* value
GG 26%	TT 20%	1.268	0.530
AA 14%	AA 14%	0.942	0.624
AG 60%	TA 66%	1.080	0.583

Frequency refers to the estimated frequency of the genotype. HR indicates the hazard ratio obtained by the multivariate Cox model adjusted for old age, presence of B symptoms, Ann Arbor stage III and IV, presence of bulky tumor, high serum LDH and ß2 microglobulin

Our results were in contrast to the results of Lech-Maranda et al. (2004) who reported that the frequency of IL-10$_{-1082G}$ allele was found to be higher in 199 patients with DLBCL as compared with 112 control subjects (0.47 versus 0.39, P=0.043) when compared with patients carrying the IL-10$_{-1082AA}$ genotype, patients with the IL-10$_{-1082G}$ allele (IL-10$_{-1082GG/GA}$ genotypes) had higher 5-year freedom from progression (FFP; 60% versus 40%, P=0.013) and OS (63% versus 33% P=0.0009). No associations were found between IL-10$_{-1082G}$ allele and prognostic variables such as presence of B symptoms, Ann Arbor stage III–IV, presence of bulky tumor, high serum LDH, and high ß2 microglobulin. No associations were found between the prognostic variables, FFP, or OS intervals and IL-10$_{-1082GG}$ genotype.

Domingo-Domènech et al. (2007) reported that patients with the IL-10$_{-1082GG}$ genotype had a better overall survival but the genotype was not an independent prognostic factor, because IL-10$_{-1082G}$ was previously found to be associated

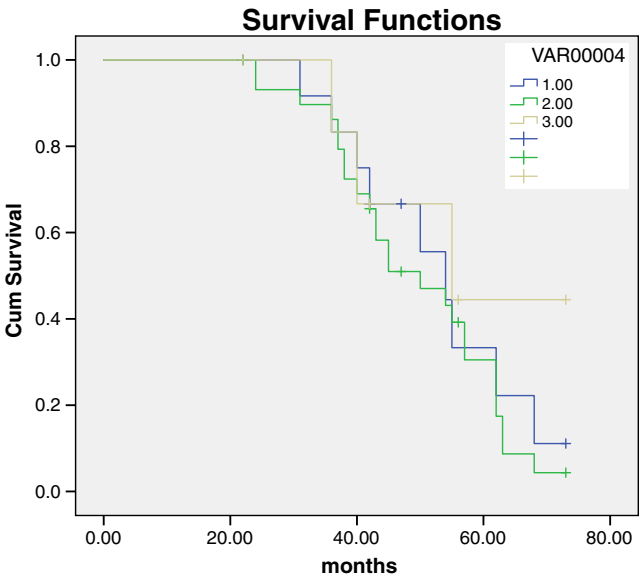

Fig. 3 Kaplan–Meier overall survival estimate according to genotype of IL-10 (1082A/G). *Blue*, *green*, and *yellow lines* denotes AA, AG , and GG genotypes, respectively

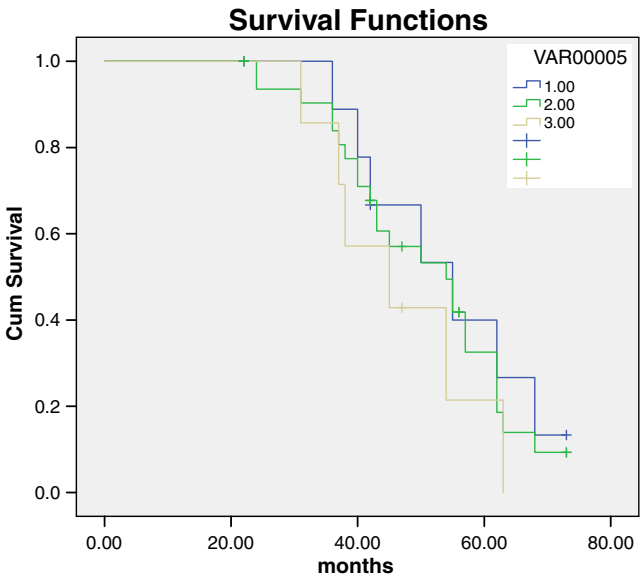

Fig. 4 Kaplan–Meier overall survival estimate according to genotype of IL-10 (3557T/A). *Blue*, *green*, and *yellow lines* denotes TT, TA,and AA genotypes, respectively

with high IL-10-producing capability. Hulkkonen et al. (2001) suggested that increased IL-10 production within tumor microenvironment might be of protective value and conversely that low IL-10-producing capability makes individuals susceptible to more aggressive course of the disease. For example, IL-10 was found to increase T-cell cytotoxicity, to inhibit tumor angiogenesis, and to antagonize the action of proinflammatory cytokines (Lech-Maranda et al. 2004) but IL-10 genotypes associated with a lower production of this anti-inflammatory Th2 cytokine (1082 AA), were found as factors of an unfavorable course of NHL (Bogunia-Kubik et al. 2008).

Concerning the results of PCR–RFLP for IL-10 3557T/A polymorphism, TT wild genotype was highly significantly reported among controls than patients (p value <0.0001) while heterozygous (TA) genotype was significantly higher among patients (p value=0.0007). We also found that A allele frequency was higher among patients (p value= 0.0001) but no difference was found between patient and control groups as regard T allele frequency (p value= 0.1622). When we studied the relations between IL-10 3557T/A polymorphism and previously mentioned prognostic risk factors or relation with survival, we found no significant associations between IL-10 3557T/A genotypes and prognostic factors and there was no impact of IL-10 3557T/A genotypes on survival.

Our results reinforce prior evidence of an association between IL-10 and risk of DLBCL by Wang et al. (2007) and Purdue et al. (2007). More recently, Fernberg et al. (2010) reported that IL-10 AA homozygotes were at a statistically nonsignificant increased risk of NHL overall,

Table 8 Cox models for haplotypes and diplotypes of IL-10 1082A/G and IL-10 3575T/A polymorphisms adjusted for bad prognostic factors

Haplotype							
IL-10 1082A>G		IL-10 3575T>A		Frequency	HR	95%CI	*P* value
A		T		80	1.5	5.1–28.5	0.2113
A		A		77	0.226	11.8%–23.5	0.6346
G		T		86	0.0831	11.1–15.1	0.7732
G		A		83	0.283	10.2–22	0.5944
Diplotype							
IL-10 1082A>G	IL-10 3575T>A	IL-10 1082A>G	IL-10 3575T>A	Frequency	HR	95%CI	*P* value
A	T	G	A	81.5	0.0166	13.6–19.5	0.8975
A	T	A	T	80	0.0625	12.1–17	0.8026
A	T	G	T	83	0.283	10.2–22	0.5944
G	A	G	A	83	0.0709	11.1–16.1	0.7901
G	T	G	A	84.5	0.0191	12.7–18.6	0.8901
G	T	G	T	86	0.0831	12.1–15.1	0.7732

Frequency refers to the estimated frequency of the genotype. HR indicates the hazard ratio obtained by the multivariate Cox model adjusted for old age, presence of B symptoms, Ann Arbor stage III and IV, presence of bulky tumor, high serum LDH and ß2 microglobulin

but stronger associations were estimated for risk of DLBCL (95% CI 1.08–1.85) and mantle cell lymphoma (95% CI 1.04–3.00)

Within a large multicenter study by the InterLymph consortium (Rothman et al. 2006) including 3,568 cases with NHL and 4,018 controls from Europe and North America, subjects with IL-10 −3575T→A polymorphism, TA, or AA variant was associated with an excess risk of NHL overall, and DLBCL in particular (95% CI 1.06–1.40). IL-10 inhibits production of TNF and other proinflammatory cytokines by macrophages. Diminished IL-10 production by IL-10 T3575A polymorphism results in less efficient regulation of proinflammatory cytokines which could possibly increase NHL risk. Moreover, decreased expression of IL-10 may play a role in tumor growth and progression by diminishing its postulated tumor suppressing effects (Karin and Greten 2005).

To further evaluate the association of IL-10 (1082, 3575) polymorphisms to susceptibility to DLBCL, haplotypes were reconstructed. In total, four distinct haplotypes were found. No differences were identified by the haplotype multivariate analysis. Haplotypes were also analyzed by Rothman et al. (2006) who reported that AG haplotype was associated with increased risk of diffuse large B-cell lymphoma. In contrast, the TG haplotype was not associated with risk of diffuse large B-cell lymphoma. The two haplotypes AG and TG differed significantly in risk to develop diffuse large B-cell lymphoma (p=0.007). The higher risk for diffuse large B-cell lymphoma was restricted to the haplotype containing IL-10 −3575T→A and IL-10 −1082A→G (i.e., AG haplotype) rather than the TG haplotype, suggesting that IL-10 −3575T→A is more important than IL-10 −1082A→G in determining risk of non-Hodgkin lymphoma.

For more detailed analysis of associations between IL-10 −3575T→A and IL-10 −1082A→G polymorphism and risk of NHL, diplotypes of IL-10 1082A/G and IL-10 3575T/A polymorphisms adjusted for prognostic factors were reconstructed. We did not find any significant associations between different diplotypes and prognostic factors.

Our study examined a relatively small number of patients and requires confirmation using a larger sample population in a prospective study. This is particularly necessary as lymphoma is a highly heterogeneous disease with a largely unknown etiology.

Conclusion

From the previous results, we conclude that each of IL-10 1082A/G and IL-10 3575T/A polymorphisms separately may be considered as independent prognostic risk factor for lymphoma as lower expression of mutant allele (G) of IL-10 1082A>G, while higher expression of mutant allele (A) of IL-10 3575T>A was associated with increasing susceptibility to NHL. However, there was no effect of combined polymorphisms in the form of haplotypes or diplotypes on susceptibility to NHL. Also, there was no impact of those polymorphisms on survival of lymphoma patients.

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