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RESEARCH ARTICLE

Genotyping of the *BCL2* Gene Polymorphism rs2279115 Shows Associations with Eukemia Tendencies in the Iraqi Population

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Abstract

The purpose of this study is to assess the SNP (rs2279115; - 938 C>A) polymorphism in the *BCL-2* gene of Iraqi leukemic patients. The B-cell lymphoma 2 (*BCL-2*) gene is considered to be related with leukemia improvement. The single nucleotide polymorphism "(SNP; -938 C>A)" of *BCL-2* was discovered a few years ago. In accordance with the SNP's (rs2279115) function against leukemia, we evaluated the distribution frequency of the allele in addition to the relationship of the genotype with clinicopathological features. This study involved a case-control design in which the *BCL-2* gene promoter polymorphism with the rs2279115 variant was genotyped in a total of 230 individuals, including 120 leukemia patients and 110 healthy subjects, to predict the variation in leukemic Iraqi patients. The patient group was enrolled from the Medical Euphrates Center for Oncology in the Najaf province that is involved in the WHO strategy of leukemia. From whole categories of blood, the DNA was extracted and genotyped using the BccI enzyme and the RFLP- PCR technique. The occurrences of the CC, AC, and AA alleles of the promoter *BCL-2* gene polymorphism (C-938A) in leukemia patients were 38 (32%), 55 (46%), and 27 (22.5%), respectively, while control they were 46 (42%), 54 (49.1%), and 10 (9.1%), respectively in the control group (P<0.01). It was shown that the existence of the A allele in the SNP (rs2279115) in the *BCL-2* gene promoter was related to an increased risk for the development of leukemia in the Iraqi population. The minor allele (A) in (rs2279115) of *BCL-2* gene was significantly elevated (p<0.01) in leukemia patients (22.5%) as compared with healthy individuals (9.1%). Accordingly, the homozygous genotype AA (OR=3.27, CI 95% 1.41-7.60, P= 0.01) significantly augmented the risk of leukemia development by more than three times when compared with those of reference wild-type AA. The results of this study indicated that the AA genotype of BCL-2 (-938C>A) was associated with a predisposition to leukemia in the Iraqi population.

Keywords: Leukemia, BCL-2, SNP, Cancer, Iraq.

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Abbreviations: SNP, single-nucleotide polymorphism; WHO, World Health Organization; RFLP-PCR, restriction fragment length polymorphism polymerase chain reaction; BMI, body mass index; *BCL2*, B-cell lymphoma 2; OR, odds ratio.

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INTRODUCTION

During our lives, more than one-third of people will develop some type of disease or malignancy. In Western regions, this is the primary cause of death after cardiovascular disease (Davidson, 1953). Heterogeneous causes involving environmental and genetic toxins promote cancer development that involves abnormal cell growth with the opportunity to expand to new locations in the body (American Cancer Society, 2011). Leukemia is one kind of cancer that originates in the bone marrow, in the blood-forming tissues of the body that prevents typical blood function by abnormal cell division (Buffler et al., 2005.). The development of types of leukemia depends on the category of the affected blood cell. Some forms of leukemia are very familiar in children, like acute lymphocytic leukemia (ALL). Other types that arise frequently in adults are chronic myeloid leukemia (CML), as well as acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) (American Cancer Society, 2014). Differentiated features of AML that account for most cancer-related deaths are due to the monoclonal spread of myeloid blasts in blood and other tissues (He et al., 2014).

It is imperative to maintain tissue balance through restricted cell turnover (Moazami-Goudarzi et al., 2016). Apoptosis is a process that involves a chain of biochemical reaction signals for intrinsic or extrinsic processes. Relatives of the BCL2 protein play a central role in regulating apoptosis through these intrinsic or extrinsic pathways on the outer mitochondrial membrane *(Zhang T, Saghatelian A, 2013).* Regulation of proliferation and apoptosis homeostasis is carried out through pro-apoptotic members such as "*BCL2*-associated X protein (BAX)" and anti-apoptotic component like *BCL2* itself which keeps cells from cell death programming (Bachmann et al., 2011).

The BCL2 proto-oncogene is firstly recognized in B-cell lymphoma rather than in a regulator of apoptosis process (Hockenbery et al., 1990). It also contributes to the healing of corrupted DNA and cell cycles (Deng and Gao, 2003; Zhang et al., 2007; Adams et al., 1999; Hara et al., 2008). The *BCL2* gene has six exons and two different functional promoters localized on chromosome 18q21.33, and the first promoter is affected by negative regulation of the second promoter (Young, 1993).

The expression of *BCL2* gene is dependent on endogenous and environmental stimuli that regulate the results of transcription and post-transcriptional levels. (Young, 1993; Donnini et al, 2004). At the transcriptional level, the regulation of *BCL2* gene is carried out by positive or negative elements existing within the promoter, encoding the section and 32 -UTR (Ma et al., 2007).

MATERIALS AND METHODS

Study subjects

In the current work, 120 leukemic patients in addition to 110 individuals as a control group were genotyped. All details of patients with leukemia were obtained from the Middle Euphrates Center for Oncology in Al-Sader Medical City in Najaf, Iraq. Leukemic patients were newly diagnosed, with histological confirmation and were not previously treated with radio- or chemotherapy. The classification of histology for all patients was performed by the center's senior pathologists. Both subject groups under study were from Najaf and its surrounding regions. There were no criteria restrictions of their age or gender for participation.

Exclusion criteria involved all cases of previous cancer diagnoses and metastasis from other organs or previous treatments by radiotherapy or chemotherapy. Healthy individuals were cancer-free with no family history of cancer.

Genotyping BCL2

The genomic DNA was extracted from peripheral blood drawn both from patients and control subjects. Approximately 3ml of blood was collected in a Vacutainer Potassium-salt EDTA tube based on the protocol from the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA, Cat# A5081). The concentration and purity of the extracted DNA were measured by using a Biodrop spectrophotometer (England) based on absorbance at 260 and 280 nm. Extracted DNA specimens were stored at -20°C for the genotyping analysis. *BCL2* genotypes at the promoter region (-938) SNP were evaluated by restriction fragment length polymorphism polymerase chain reaction technique (RFLP- PCR). The primers used for amplification of the target fragment were designed depending on the sequence of rs2279115. All primer sequence orders are in direction of 5' to

3' forward *BCL2* (TTA-TCC-AGC-AGC-TTT-TCG-G') and reverse *BCL2* (GGC-GGC-AGA-TGA-ATT-ACA-A) with a 252bp amplicon size. The PCR reaction was carried out with a 25µl total volume reaction containing 1µl of genomic DNA (100 ng/µl), which was amplified with 10 mM of forward and reverse primers, 0.2mM each dNTP, 2.0 mM MgCl2, 1.0 and a U Taq DNA polymerase with a 1X reaction buffer (Promega, Cat#M7502). PCR program conditions included initial melting at 95°C for 5 minutes followed by 30 cycles of amplification with a denaturation step of 25 seconds at 95°C, an annealing step of 30 seconds at 58°C, an elongation step of 20 seconds at 72°C, and one level of elongation of 7 minutes at 72°C.

A volume of 10 µl of PCR product reaction from each sample was incubated with BccI Enzyme (NEB, England) for 1 hour for digestion. To visualize the digested and undigested products, 2% agarose was used in gel electrophoresis, and images were taken under an ultraviolet light transilluminator.

Statistical Analysis

BCL2 promoter (SNP -938C>A) genotyping based on an allele frequency was assessed by a statistical online web program (http://www.orge.org/software/hwe-mr-calc.shtml) for the Hardy-Weinberg Equilibrium (HWE). Significant levels were considered as $p<0.05$.

RESULTS

Leukemia patients and healthy subjects were all from Iraq. There was no significant statistic variation between the patients and healthy subjects groups in the their biochemical characteristic that involved age, gender, and residency, as presented in Table 1. A Chi-square (χ^2) test was applied to verify whether all subjects were compatible with the Hardy-Weinberg equilibrium (HWE). Both patient and healthy groups were harmonious with the HWE. The χ^2 values were 0.68 and 1.1, respectively.

The amplicon of the promoter region of the variant *Bcl2* gene (rs2279115; - 938 C>A) that produced by digestion of BccI restriction enzyme was under asingle band (252bp) for CC wild-type, the AC allele produced 252 bp and 154bp fragments, and the AA allele produced 154bp and 98bp fragments.

As shown in Table 2, the *BCL-2* genotype homozygote CC allele was linked to a decreased risk of leukemia by 3.27 times as in the homozygote AA. Moreover, the genotype homozygote AA was associated with an increased risk of leukemia by 2.9-times as in the shared genotypes AC+CC alleles. Random selection of various samples from leukemia patients was carried out to demonstrate the results of the PCR product and genotyping (Fig. 1).

Table 1. Distribution of known clinicopathological variables of leukemia patients and control groups

		Control n=110	Patients n=120	P-Value
Age (years)	≤35>35	32	47	N.S
		78	73	N.S
Gender				
Male		58	65	N.S
Female		52	55	N.S
Leukemia Type				
ALL		-	0	
AML		-	64	
CLL		-	23	
CML		-	33	
Residency				
An-Najaf		76	81	N.S
Samawa		16	22	N.S
Dewanea		18	17	N.S
		Control n=110	Patients n=120	P-Value
Age (years)	≤35>35	32	47	N.S
		78	73	N.S
Gender				
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Residency				
An-Najaf		76	81	N.S
Samawa		16	22	N.S
Dewanea		18	17	N.S

Table 2. Genotyping distribution of *BCL2* (rs2279115; - 938 C>A) in leukemic and healthy subjects

Genotype	No. of individuals (%)		Non-adjusted[2]	
Allele	Leukemic Patients[1]	Healthy Individuals[1]	P-Value	OR
CC reference	38 (32)	46 (42)	1	
AC	55 (46)	54 (49.1)	0.01	1.23(0.70- 2.18)
AA	27 (22.5)	10 (9.1)	0.01	3.27(1.41-7.60)
AA+AC	82(68)	64(58.2)	0.01	1.550.9-2.66
CC+AC	93(77.5)	100(91)	1	
AA	27 (22.5)	10 (9.1)	0.005	2.91.33-6.32
2(AA)+AC	109(90)	74(67)	0.03	1.781.059-3.003
Total	120	110		

[1] χ^2 for HWE of leukemia patients and healthy individuals groups is 0.68 and 1.1 respectively (both $p > 0.05$).
[2] Logistic regression model, non-adjusted.

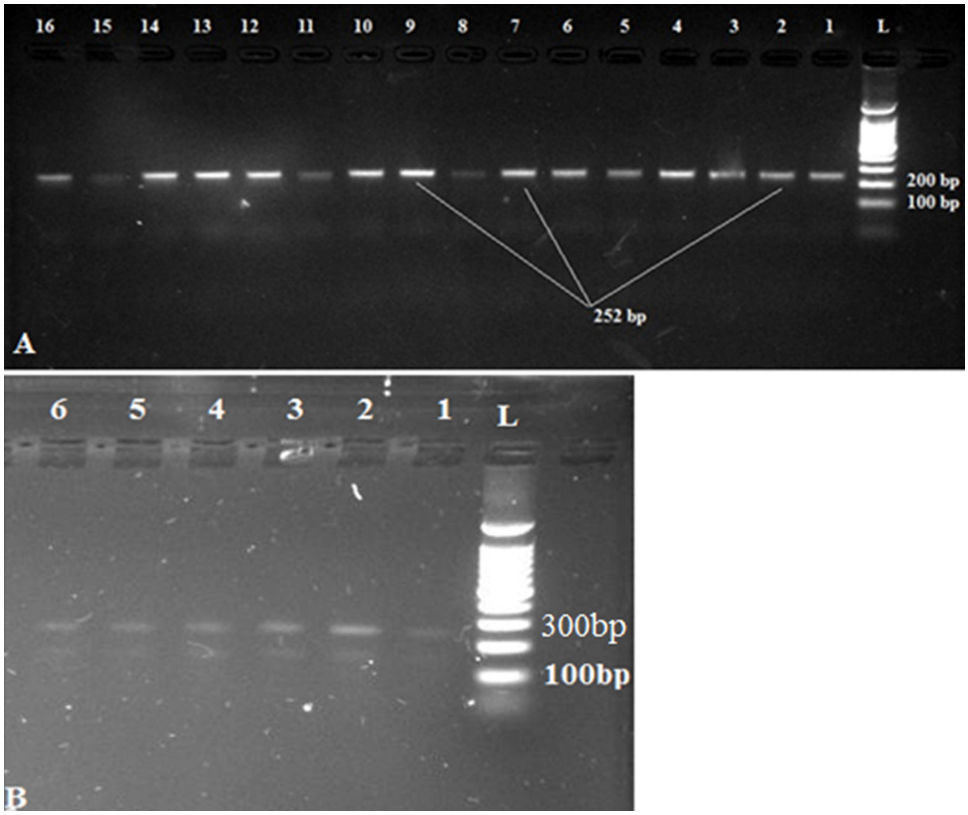

Fig. 1. Genotyping polymorphism of BCL-2 (-938C > A) with PCR product, A; and RFLP-PCR fragments, B. The wild type CC allele of the BCL-2 promoter region produced a single 252-bp fragment, the AC allele produced 252bp and 154bp fragments, and the AA allele produced 154bp and 98bp fragments.

DISCUSSION

The purpose of this study was to identify a polymorphism in the promoter region (-938C>A) of the BCL-2 gene. Many types of cancers are affecting the health of the global population and leukemia is considered to be one of the most damaging blood cancers. Depending on what kind of blood cell is affected, different types of leukemia (ALL, AML, CLL, CML) can develop (Arber et al., 2018). Due to the past three decades of war against Iraq, many types of cancers involving leukemia have been reported; especially following the last war in 2003 and the next seven years of occupations when many kinds of weapons were used (Al-hashimi & Wang, 2013). Most leukemic Iraqi patients were subjected to many crises in the last three decades. Therefore, it is essential to understand the correlation between the promoters of *BCL-2* gene polymorphism and leukemia development, as unfortunately there is no information on this in Arab, particularly Iraqi populations, specifically as regards to the role of gene polymorphisms as predictors for leukemia. The *BCL-2* gene is identified as an antiapoptotic regulatory protein that may serve as an inhibitor of proliferation (Bachmann et al., 2011). Hence, many studies examine the role of *BCL-2* gene variants in altering gene expression and/or protein function that could affect the sensitive equilibrium of pathways regulating cell death. This could represent a potential biomarker that would offer the best treatment option with drugs targeting *BCL-2* (Zhang and Ming, 2008). The purpose of this work was to detect the risk association between a BCI-2 gene polymorphism and leukemia in Iraqi subjects. The Hardy-Weinberg equilibrium (HWE) was measured. The single nucleotide polymorphism (rs2279115) was consistent with HWE (p>0.05). In other words, this SNP is associated with the development of leukemia in the cases under study. This finding was also seen in Asian inhabitants, but not in Caucasian populations (Zhang et al., 2014). *BCl-2* gene variant polymorphism (rs2279115), genetic power, and HWE primarily rely on the sample magnitude, project design, and allele frequency in all groups (Evans & Purcell, 2012). An intended SNP of *BCL-2* gene genotyping exhibits strong links with leukemia, as the presence of the minor A allele in (rs2279115) was three times more prevalent in leukemia homozygote and recessive patterns when compared with reference wild-types, as this reduces apoptosis and the improves proliferation rate. The failure of apoptosis due to enhanced expression of the anti-apoptotic protein *BCL-2* culd thus promote malignant cell growth (Meka et al., 2015).

CONCLUSIONS

A *BCL-2* gene polymorphism (rs2279115) is correlated with the occurrence of leukemia in Iraqi population. The presence of the (AA) homozygous genotype of *BCL-2* (-938C > A) is associated with a three-fold increase in the likelihood of leukemia development. On the other hand, carriers of the heterozygous (AC) allele have about a 50 % risk level of (AA) genotype, as compared with reference genotypes (CC).

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