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GENETIC LINKED TO LEUKEMIA: ROLE OF POLYMORPHISMS IN CANDIDATE GENES INTERCONNECTED TO LEUKEMIA

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ABSTRACT

Objective: To evaluate the overall progress and contribution of candidate gene association to the genetic susceptibility to Leukemia. **Materials and methods:**Study population included patients with cancer from Babil province - Iraq. Whole blood 60 cases at consultant clinic in Babil province from October 2014 to January 2015. Out of the 60 cancer patients, there were 37 male and 23 female. The patient's age range was from 25 to 70 years. Nominated mutations in CYP2E1 (G-1259C, in linkage disequilibrium with C-1019T), in MPO (G-463A) and in NQO1 (C609T and C465T) were investigated by allele-specific oligonucleotide. **Results:** The frequencies among ALL patients differed from those of the control group. In particular, we found that carriers of the CYP2E1*5 variant were more frequent among cases (11.7% vs. 0% in controls), suggesting that the predicted higher level of CYP2E1 might be associated with an increased risk of **LEUKEMIA**. Similarly, it's revealed that NQO1 variants (NQO1*3) were associated with an increased risk of A **LEUKEMIA**, particularly due to a higher prevalence of heterozygous individuals among cases relative to controls. **Conclusion:** It's revealed that genetic polymorphisms in both NQO1 and CYP2E1 play a role in the development of **LEUKEMIA**. This is in accordance with the involvement of these variants in other types of leukemia.

KEYWORDS: Leukemia, CYP2E1, MPO, NQO!, polymorphism, PCR, Iraq.

INTRODUCTION

The observation that cancers can run in families has been made repeatedly for at least 100 years. Although such historical evidence is largely anecdotal, several families have been described that were impressively large and/or had an unusual and well-defined tumor phenotype. For most of the relatively brief time during which cancer genetics has been a recognized discipline, these sorts of families have formed the mainstay of the research effort.[1] During the last few decades, extensive effort has been invested in identifying sources of genetic susceptibility to cancer.[2] Both the International Human Genome Sequencing Project and the International Hap Map Project have generated a very large amount of data on the location, quantity, type, and frequency of genetic variants in the human genome.[3] Facilitated by continuing technological advances that allow faster and cheaper genotyping results, a large and increasing number of observational studies investigating the association between variants in candidate genes and cancer risk have emerged.[4,5] Metastasis is a complex process in which cancer cells break away from the primary tumor and circulate through the bloodstream or lymphatic system to other sites in the body. At new sites, the cells continue to multiply and eventually form additional tumors comprised of cells that reflect the tissue of origin. The ability of tumors, such as pancreatic cancer, to metastasize contributes greatly to their lethality. Many fundamental questions remain about the clonal structures of metastatic tumors, phylogenetic relationships among metastases, the scale of ongoing parallel evolution in metastatic and primary sites, how the tumor disseminates, and the role that the tumor microenvironment plays in the determination of the metastatic site.[6] All tumors accumulate somatic mutations during their development. Most common cancers are associated with diverse cancer genes that are mutated at a low frequency. One of the most striking observations from large cancer databases is the genetic heterogeneity among cancers and even within individual cancer types. However, it appears that a limited number of cellular pathways are central to tumor cell biology.[7,8] Comprehensive catalogs of somatic mutations are being compiled for various cancer types to better understand the mechanisms that underlie this disease.

MATERIALS AND METHODS

Study population included patients with cancer from Babil province - Iraq. Whole blood 60 cases at consultant clinic in Babil province from October 2014 to January 2015. Out of the 60 cancer patients, there were 37 male and 23 female. The patient's age range was from 25 to 70 years.

Genomic DNA Purification Kit, The Wizard® Genomic DNA Purification Kit is designed for isolation of DNA from white blood cells, tissue culture cells and animal tissue, plant tissue, yeast, and Gram positive and Gram negative bacteria. The Wizard® Genomic DNA Purification Kit is based on a four-step process. The first step in the purification procedure lyses the cells and the nuclei. For isolation of DNA from white blood cells, this step involves lysis of the red blood cells in the Cell Lysis Solution, followed by lysis of the white blood cells and their nuclei in the Nuclei Lysis Solution.

Thermal Cycling, Place the tubes in the thermal cycler, and run the recommended program. The preferred protocols for use with the Viriti thermal cyclers are provided below. It may be necessary to optimize the program with other thermal cyclers. In our study, the Program for the Viriti conventional PCR Preheat the thermal cycler to 94°C before placing tubes inside.

Cycling Profile

94°C for 2 minutes, then

94° for 1 minute

57°C for 30 seconds

72°C for 1 minute

For 35 cycles, then:

72°C for 5 minutes

4°C soak.

Agarose Gel Electrophoresis, For optimal visualization of the amplification products, Manufacture Company recommend using a 4% NuSieve® 3:1 Plus TBE buffer precast gel. Alternatively, cast a 4% NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.5µg/ml ethidium bromide.

TABLE 1: FEATURES OF THE PCR ALLELE-SPECIFIC OLIGONUCLEOTIDE ASSAYS TO IDENTIFY CYP2E1, MPO AND NQO1 VARIANTS

Locus	Mutation	PCR primers	bp.
CYP2E1	G-1259C	F:gtggacgcttagGCCCCTTCTTGGTTCAGGAGA R:gtggacgcttagTTCATTCTGTCTTCTAACTGG	**346**
MPO	G-463A	F:CGGTATAGGCACACAATGGTGAG R:GCAATGGTTCAAGCGATTCTTC	**350**
NQO1	C-465T	F:GCAGTGGTTTGGAGTCCC R:CAAACACCCCTGCATCAG	**175**

Genotyping

DNA was isolated from buccal epithelial cells, peripheral blood in remission as described in previous study **9**. Nominated mutations in CYP2E1 (G-1259C, in linkage disequilibrium with C-1019T), in MPO (G-463A) and in NQO1 (C609T and C465T) were investigated by allele-specific oligonucleotide. The oligonucleotides and assay conditions used for this analysis are given in Table 1.

The PCR conditions consisted of an initial melting temperature of 94°C (5 minutes) followed by 35 cycles of melting (94°C, 30 seconds), annealing (65°C, 60 seconds), and extension (72°C, 60 seconds), and a final extension for 10 minutes at 72°C. The amplified product was subjected to restriction enzyme analysis with BsrDI for 16 hours at 57°C, according to the manufacturer's instructions. The PCR products were separated by 2% agarose gel electrophoresis.

RESULTS

DNA variants in CYP2E1, NQO1 and MPO genes were determined by PCR assays. The distribution of the alleles and genotypes in patients with leukemia compared with controls are reported in Tables 2 and FIGURE 1. In controls, the frequencies of the polymorphisms tested were in agreement with those reported for other populations of European descent.[10, 11]

TABLE 2: INCIDENCE OF CYP2E1, NQO1 AND MPO VARIANTS AMONG LEUKEMIA PATIENTS AND CONTROLS

Variant	Patient (60)		Control (25)		OR
	No	%	No	%	
CYP2E1*5	7	11.7	0	0	2.8 (1.2–6.5)
NQO1*3	16	26.7	1	4	1.3 (0.7–2.3)
MPO*2	23	38.3	7	28	1.1 (0.8–1.5)

The frequencies among ALL patients differed from those of the control group (Table 2). In particular, we found that carriers of the CYP2E1*5 variant were more frequent among cases (11.7% vs. 0% in controls), suggesting that the predicted higher level of CYP2E1 might be associated with an increased risk of **LEUKEMIA**. Similarly, it's revealed that NQO1 variants (NQO1*3) were associated with an increased risk of A **LEUKEMIA**, particularly due to a higher prevalence of heterozygous individuals among cases relative to controls. In accordance with this finding, a significant decrease in NQO1*1/*1 individuals among children with **LEUKEMIA** pointed to a protective role of the wild-type form of this enzyme.

The frequency of MPO*2 carriers did not differ between cases and controls, indicating that this variant alone does not modify the risk of patients with **LEUKEMIA**.

DISCUSSION

Incidences of CYP2E1, NQO1 and MPO alleles in patients of patients with **LEUKEMIA** and in a control group from same geographic area. The relative homogeneity of this population, hereditarily, sociocultural in addition to the way of life is predictable to simplify genetic epidemiology studies by at least decreasing the number of mystifying factors related to genetic and cultural heterogeneity, which able to stimulus ecological contact by individual genetics.[12]

It was found that patients carrying CYP2E1*5 were at larger threat of **LEUKEMIA** and this associations have been reported for adult cancers of the nasopharynx, breast and lungs.[13] Conversely, the importance of allele efficient is not clear yet. Other researches have designated its association with increased transcriptional activity[14], whereas others did not confirm the relationship of this allele and CYP2E1 activity.[15]

Previous interpretations of higher frequency of the NQO1*3 allele in therapy-related myeloid leukemia[16] as well as in a subset of infant leukemia patients support our results suggesting that NQO1 variants might play a role in leukemogenesis. NQO1*3 was also found to be associated with an elevated risk of nonhematologic malignancy, like urologic and basal cell carcinomas.[17]

On the other hand, there was no evidence of involvement of MPO variants in **LEUKEMIA** susceptibility. Interestingly the wild-type allele of this enzyme was reported to be correlated with high level of MPO expression in normal and malignant myeloid lineage.[18, 19]

In conclusion, it's revealed that genetic polymorphisms in both NQO1 and CYP2E1 play a role in the development of **LEUKEMIA**. This is in accordance with the involvement of these variants in other types of leukemia.[20]

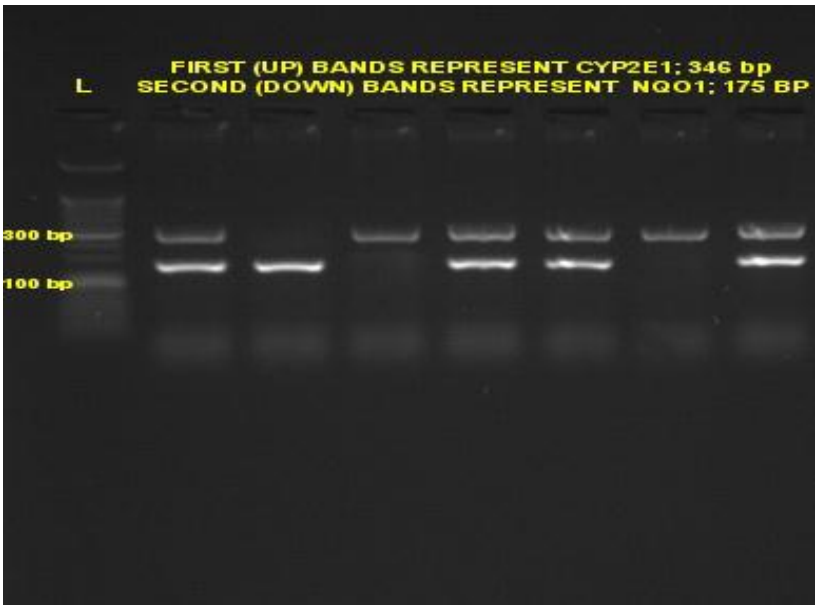

Figure1: Gel electrophoresis of CYP2E1 and NQO1 PCR products. Lane No. 1 shows the DNA marker; Lane No. 2-7 shows the 346 bp for CYP2E1 (up) and 175 bp for NQO1 (down) PCR products.

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