

Evaluation of Interleukin-10 -1082 G/A Gene Promoter Polymorphism among Hematologic Malignancies in Children

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ABSTRACT

Background: Hematological malignancies are forms of cancer that begin in the cells of the blood-forming tissue, such as the bone marrow. Childhood blood cancers are relatively rare but are still found to be the major cause of death in children aged 1-14years. **Objective:** The aim of the current work was to evaluate IL-10 (1082G/A) gene promoter polymorphism as a risk factor in children with hematological malignancies.

Patients and Methods: This study included a total of 30 patients with newly diagnosed hematologic malignancies and 30 healthy children served as a control, attending at Oncology Unit, Department of Pediatrics, Zagazig University and Department of Pediatrics, Mansoura Cancer center, Mansoura University Hospitals. All patients and controls were subjected to the detection of IL-10-1082G/A gene promoter polymorphism using ARMS-PCR.

Results: There was no significant difference between patients and controls as regards age and sex. Fever was the most common presentation in patients followed by anorexia, pallor, lymph node enlargement, and bone pain. There was no significant difference between patients and controls as regards the genotype of the IL10 (1082) gene.

Conclusion: It could be concluded that no significant association between hematologic malignancies in children and controls as regards the genotype of the IL-10 (1082) gene.

Keywords: Interleukin-10, 1082 G/A gene promoter polymorphism, Hematologic malignancies, children.

INTRODUCTION

Childhood cancer mortality rates were higher 50 years ago, but can now, fortunately, be successfully treated in approximately 80% of cases where there is access to modern treatments and robust supportive care ⁽¹⁾. Hematologic malignancies (HMs) are the most common neoplasms in childhood representing about 45% (30.6% leukemias, 14.2% lymphomas) of all newly diagnosed pediatric cancers. The treatment of childhood leukemia has undergone dramatic change in the last 50 years. Recently, about 90% of children are cured of this once nearly uniformly fatal disease. Lymphomas make up a large category of childhood cancers. Chief among these cancers are the NHLs, which are responsible for 6% of all pediatric cancers ⁽²⁾.

Interleukin-10 (IL-10) is a multifunctional cytokine with both immunosuppressive and anti-angiogenic functions. In consequence, IL-10 can have both tumor-promoting and tumor-inhibiting properties. Raised levels of serum and peri-tumoral IL-10 production have been reported in many malignancies, which have been interpreted in support of a role for IL-10 in tumor escape from the immune response. However, gene studies in several malignancies argue more convincingly for an anti-tumor function of IL-10, possibly via inhibition of pathways of angiogenesis ⁽³⁾.

There are some reports describing elevated levels of IL-10 expression in patients with particular cancers, including malignant melanoma, ovarian cancer, and other carcinomas, lymphoma, and myeloma ⁽⁴⁾. While the mechanisms remain unclear, there is a considerable and growing body of evidence for the antitumor properties of IL-10 and this may result at least in part from inhibition of angiogenesis, possibly by inhibition of production of angiogenic cytokines,

growth factors, and matrix Metalloproteinases (MMPs) and stimulation of the production of inhibitors of angiogenesis. Based on this, several investigators have suggested the therapeutic use of IL-10 in cancer patients, but at present no clinical trials have been performed ⁽⁵⁾.

In recent years a considerable number of genetic polymorphisms have been identified within the IL-10 gene, particularly within the promoter region of the gene. Some of these polymorphisms are associated with differential levels of IL-10 expression. A considerable number of studies have been performed to determine whether IL-10 polymorphisms are associated with susceptibility to many immune-mediated diseases ⁽⁶⁾.

This study was aimed to evaluate IL-10-1082G/A gene promoter polymorphism as a risk factor in children with hematologic malignancies and its association with other well-known risk factors.

PATIENTS AND METHODS

This study included a total of 30 patients with newly diagnosed hematologic malignancies and 30 healthy children served as a control, attending at Oncology Unit, Department of Pediatrics, Zagazig University and Department of Pediatrics, Mansoura Cancer center, Mansoura University Hospitals. This study was conducted between September 2020 to July 2022.

Children were divided into 2 groups: Patients group: It included 30 patients with newly diagnosed hematologic malignancies. They were 16 males and 14 females. Their age ranged from 2 years to 15 years with a mean age of 7.4 years, and **Control group:** It included 30 healthy children. They were 14 males and 16 females. Their age ranged from 3 years to 15 years with

a mean age of 7.9 years. They matched well with patients as regards age and sex.

Inclusion Criteria: Children aged 1-18 years of age, both sexes, newly diagnosed children suffering from any type of hematological malignancies. Diagnosis of the patient with hematologic malignancies was confirmed by histological, pathological means as well as a morphological examination of bone marrow along with immunophenotyping wherever possible.

Exclusion Criteria: Patients less than 1 year and more than 18 years, patients who were unwilling to give consent to participate in the study, patients with an associated blood disease and Patients started their Cancer treatment protocols.

Ethical consent:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University. Written informed consent was taken from the participants' parents. The study was conducted according to the Declaration of Helsinki.

Data collection:

The following entry variables were uniformly recorded:

Demographic variables, i.e., age, sex, maternal educational level, and full medical and family history.

Clinical examination was followed for all children with special emphasis on the presence of purpura, fever, abdominal pain, lymphadenopathy, jaundice, splenomegaly, liver enlargement, mediastinal mass, or CNS manifestations.

Laboratory investigations

All patients and controls were subjected to the detection of IL-10- 1082G/A gene promoter polymorphism using ARMS- PCR.

Timing of blood tests: Blood samples were collected for all patients just after being diagnosed as cancer patients and before the administration of chemotherapy.

Sample collection: blood was collected into an EDTA tube and frozen at -70 °C until the time of assay.

DNA Isolation: Genomic DNA was extracted from whole blood-EDTA samples using Wizard Genomic DNA Purification Kit (Promega Co., WI, USA)

according to the manufacturer's instructions.

Genotyping

IL-10 (1082 G/A was analyzed by sequence-specific primers polymerase chain reaction (SSP-PCR) as an ARMS-PCR (amplification mutation (refractory system.) using four primer mixtures For 1082 G/A, primer G (sense): 5'-CTACTAAGGCTTCTTTGGGAG-3` primer; A (sense):

5'-ACTACTAAGGCTTCTTTGGGAA-3` and antisense: primer (antisense):

5'-CAGTGCCAACCTGAGAATTTGG-3` were used.

As internal control the following primers sense: 5` – GCCTTCCCAACCATTCTTA-3`` and antisense: 5` - TCACGGATT CTGTTGTGTTTC-3` were used. The reaction was done in two tubes one for each allele, the final volume for each PCR reaction was 25µl. The PCR mixtures consisted of DreamTaq Green PCR Master Mix (2*) (Fermentas, Thermo Fisher Scientific Inc.), 10 pmol of each allele-specific primer, 10 pmol of antisense primer, 3.5 pmol of each control primer, and 100 ng of DNA.

PCR cycling conditions consisted of 94 °C for 2 min [1 cycle], followed by 96 °C for 25 s, 70° C for 45 s, and 72° C for 20 s [5 cycles]; followed by 96° C for 25 s, 65 °C for 50 s, and 72° C for 45 s [11cycles]; and finally 96 °C for 25 s, 55° C for 60 s, and 72° C for 2 min [15 cycles]. The control primer resulted in an amplicon of 429 bp and the 1082 primers resulted in an amplicon of 258 bp. By 2% agarose gel, the size of PCR products was determined relative to the migration of a 100 bp step ladder (Fermentas).

Statistical analysis

All data were analyzed by Microsoft Excel 2010 and SPSS software (IBM SPSS Statistics, version 21). General characteristics of patients were presented in terms of percentage, mean, and standard deviation. Chi square and T-test were used. A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1 shows that there was no significant difference between patients and controls as regards age and sex.

Table (1): Demographic data of studied groups.

	Patients, No. 30		Controls, No. 30		Test	P
	Mean± SD (Range)		Mean± SD (Range)			
Age (years)	7.4± 3.0 2-15		7.9± 2.6 3-15		t= 0.71	0.4
Gender	N	%	N	%		
Male	16	53.3	14	46.7	X ² = 0.27	0.6
Female	14	46.7	16	53.3		

Table 2 shows that fever was the most common presentation in our patients followed by anorexia, pallor, lymph node

enlargement, and bone pain.

Table (2): Clinical presentations in our patients.

Clinical Presentations	N	%
Fever	27	90.0
Anorexia	24	80.0
Pallor	23	76.7
Lymph node enlargement	17	56.7
Hepatosplenomegaly	16	53.3
Bone pain	16	53.3
Abdominal mass	13	43.3
Cachexia	11	36.7
Bleeding	10	33.3
Purpura	9	30.0
Arthritis	6	20.0
Jaundice	5	16.7

Table 3 shows that there was no significant difference between patients and controls as regards the genotype of the IL-10 (1082) gene.

Table (3): Genotype of IL-10 (1082) gene in patients and controls

Genotype	Patients, No. 30		Controls, No. 30		X ²	P
	N	%	N	%		
GG (wild homozygous)	4	13.3	5	16.7	0.71	0.6
AG (heterozygous)	21	70.0	23	76.7		
AA (mutant homozygous)	5	16.7	2	6.7		

DISCUSSION

The study included 30 patients with hematological malignancies with relative male predominance (male: female ratio: 1.14). This came in hand with **Smith et al.** (7) who reported male to female ratio of different hematologic malignancy types ranged from 1.19 to 3.3:1. Another review about hematologic malignancies reported increased incidence among males with a male-to-female ratio equal to 2.79:1 (8). In an Indian report, male to female ratio of hematologic malignancies was 2.2:1 (9). Similar findings were reported in the **Moroccan study** (10). **Manzour et al.** (11) reported a 2: 1 male to female ratio in all types of hematological malignancies except for ALL as the ratio was 1.2: 1). Study and control groups were matched in age and sex distribution so there was no statistically significant difference between both groups regarding age and sex.

In the current study, the mean age for hematologic malignancies was 7.4±3.0 years and ranged from 2 to 15 years. Similarly, **Rodriguez-Abreu et al.** (12) reported that hematological malignancies could occur at any age and 35% of leukemia cases occurred in an age ranged from 0 to 14 years. Also, **Al-Kahiry** (8) reported that 33% of hematological malignancies in children occurred from 0 to 14 years with a peak from birth to the age of 2 years and then fall. **Manzour et al.** (11) also showed the same age range for all types of hematological malignancies in children except for

patients with Hodgkin lymphoma who had a higher age range (9.0±4.0 years).

The most frequent presenting symptom was fever followed by anorexia and pallor then lymph nodes, spleen, and liver enlargement. **Hedge et al.** reported fever and pallor as the commonest presenting symptoms and the spleen was the commonest site for extramedullary involvement. Similarly, **Crist et al.** (13) reported that constitutional manifestations secondary to bone marrow infiltration were the commonest presenting symptoms. Childhood hematological malignancy presenting with bone pain and spine involvement is a recognized clinicopathological complex that mimics a wide range of orthopedic conditions. Bone pain was the common presentation in the current study (53.3% of the patients). Other studies reported different percent of malignancy children presented with bone pain and musculoskeletal disorders as **Teo et al.** (14) found that 71% of the patients presented with bone and spine aches. While **Barbosa et al.** (15) found bone pain in 66% of the. Some studies reported non-specific features as presenting symptoms like fatigue, bleeding, and fever (16). Other less frequent features like splenomegaly and hepatomegaly seen in our patients have also been described in a study among aviators in India (17). On the other hand, approximately none of our patients presented with CNS leukemia manifestations contrary to (18).

In the current study, there was no significant difference between patients and controls as regards the genotype of the IL-10 (1082) gene. A few epidemiological studies have investigated the association between IL-10 polymorphisms and the risk of different cancer types. In agreement with our study, **Michaud *et al.*** ⁽¹⁹⁾ reported that there was no apparent relationship of the IL-10 gene promoter polymorphisms with the risk of nasopharyngeal carcinoma and prostate cancer respectively. On the contrary **Yao *et al.*** ⁽²⁰⁾ reported IL-10 gene promoter – 1082 A/G polymorphism was associated with oral cancers when compared to controls.

Seifart *et al.* ⁽²¹⁾ reported that the IL-10– 1082 genotypes carrying the G allele appeared with higher frequency in the small cell lung cancer (SCLC) group.

Shin *et al.* ⁽²²⁾ also reported that the IL-10 gene promoter GCC haplotype was shown to be associated with increased hepatocellular carcinoma risk as compared with the ATA haplotype in Koreans. Another meta-analysis by **Zhang *et al.*** ⁽²³⁾ indicated that haplotype in IL-10 promoter was involved in the development of cancer.

CONCLUSION

It could be concluded no significant association between hematologic malignancies in children and controls as regards the genotype of the IL-10 (1082) gene.

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