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Interleukin-17 Gene Polymorphism Is Protective Against the Susceptibility to Adult Acute Myeloid Leukaemia in Egypt: A Case-Control Study

Maha Elsissy*, Ahmed Abdelhafez, Manal Elmasry, Doaa Salah

Laboratory Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt

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***Correspondence:** Maha Elsissy. Laboratory Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: mahaelsissy@yahoo.com

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Abstract

BACKGROUND: Th17 cells are blamed for being accused in the pathogenesis of acute myeloid leukaemia. Th17 cells are CD4+ cell subtype. They produce IL-17A and IL-17F.

AIM: This study aims to trace the relation between IL-17A and IL-17F polymorphisms and AML incidence and to define the connection between IL-17 polymorphisms and its serum level.

METHODS: A group of 100 acute myeloid leukaemia patients and 100 age and sex-matched healthy subjects (controls) were enrolled in the present work. Restriction fragment length polymorphism- polymerase chain reaction (PCR-RFLP) was done to detect IL-17A (rs2275913; G197A) and IL-17F (rs763780; A7488G). Serum IL-17 level was assessed by Enzyme-linked immunosorbent assay analysis (ELISA) in both patients and controls.

RESULTS: IL-17F, IL-17A mutant genotypes and alleles showed no significant relation with acute myeloid leukaemia incidence. Also, ELISA results proved that serum IL-17 did not vary between acute myeloid leukaemia patients and healthy subjects.

CONCLUSION: Interleukin-17 gene polymorphisms did not consider a risk for acute myeloid leukaemia.

Introduction

IL-17 is an essential pro-inflammatory cytokine released by memory CD4 positive T cells, that includes six members (IL-17 A to F) binding to five receptors. IL-17 is important in innate as well as in acquired immune reactions [1]. IL-17 is promoted by microbial metabolites; it enhances tumour enlargement through angiogenic functions [2].

Previous studies proved that altered values of IL-17 were involved in gastrointestinal and female genital system cancers [3], [4].

The IL-17A (rs2275913; G197A) polymorphism is blamed for being involved in autoimmune diseases, chronic inflammatory diseases, and malignancies. It induces the secretion of inflammatory chemokines as well as the release of cytokines stored in macrophages and neutrophils [5].

Interleukin-17F [rs763780; A7488G], is a second member of the IL-17 family. It has an important role in neutrophil functions by secreting different types of both cytokines and chemokines. IL-17F rs763780 polymorphism suppresses the wild IL-17F activities and contributes to increasing susceptibility to tumours. Previous studies proposed

the role of IL-17A and IL-17F gene polymorphism in the development of different types of tumours [6], [7].

The present work aimed to state the relationship between the mutant genotypes of IL-17A (rs2275913; G197A) & IL-17F [rs763780; A7488G] and AML susceptibility, as well as, ELISA estimation of IL-17 in Egyptian AML patients.

Material and Methods

The present research was done on 100 adult Egyptian (de novo) AML cases; samples were collected in the period between May 2014 to December 2016 from the Department of Medical Oncology, Kasr Al-Aini Hospital.

Patients were 64 males and 36 females with age range: 21-71 years and a mean of 47 years. One hundred age, sex and ethnic-matched healthy individuals were in-rolled in the present protocol as control subjects.

Informed consents were signed by all study groups before their participation in the research. The study has been approved by the committee of the Clinical Pathology department as well as the Internal Medicine department, Kasr Al Aini, Cairo University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Diagnosis of AML was based on morphological examination of bone marrow aspirate. Immunophenotyping and Cytogenetic studies were assessed for subtyping of the disease.

The demographic data of the patients were summarised (Table 1).

Table 1: The demographic data of AML patients

Item		Number (100)	
Sex	Male	64	
	Female	36	
Age	< 60	79	
	≥ 60	21	
Hepatomegaly		23	
Splenomegaly		35	
Lymphadenopathy	Cervical	54	
	Axillary	6	
	Inguinal	13	
	Submandibular	50	
	Abdominal	3	
	Mesenteric	2	
	Par aortic	2	
Medical history		32	
Family history		9	
FAB	M1/M2	40	
	M3	44	
	M4/M5	11	
	M6	3	
	M7	2	
Outcome	Died	41	
	Others	CR*	15
		PR**	29
		Relapse	15

CR*-complete remission; PR*-partial remission.

The diseased group was classified morphologically according to the French- American-British (FAB) classification.

Assessment of Interleukin-17A [rs2275913; G197A] and Interleukin-17F [rs763780; A7488G] polymorphisms by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique:

Three millilitres of blood were withdrawn aseptically from the study groups on EDTA. Human DNA extraction was done using commercial Kit (*Fermentas Life Sciences),* according to the manufacturer's instructions. Genetic detection of the SNPs was done as previously stated [8].

As regard to IL-17A (rs2275913; G197A*)***,*** the following primers Forward 5´-AACAAGTAAGAATGAAAAGAGGACATGGT-3´ Reverse 5´ - CCCCCAATGAGGTCATAGAAGAATC - 3´ were used. For IL-17F **[**rs763780; *A7488G]***,** genotyping was performed by using Forward primer 5´-ACCAAGGCTGCTCTGTTTCT-3´ Reverse primer 5´-GGTAAGGAGTGGCATTTCTA-3´.

All PCR reactions were tested in f 25 µl volume; containing Master Mix (GeneON), forward and reverse primers, distilled water, and genomic extracted DNA.

The thermocycler program applied for IL-17A (rs2275913; G197A) was 33 cycles at 96°C for 5 min, followed by 60 s at 95°C, 60 s at 65°C, 50 s at 72°C with a terminal extension step at 72°C for 7 min. ***[9]***

The thermocycler program applied for IL-17F [rs763780; A7488G] was 35 cycles at 94°C for 3 min, then 30 s at 94°C, 30 s at 60°C, 30 s at 72°C with a final extension step at 72°C for 7 min [9].

The amplified product of IL-17A (rs2275913; G197A) was digested with XagI (*Fermentas Life Sciences)*. The wild-type GG produced two bands of 68 and 34 bp, while the polymorphic GA hetero-type produced three fragments of 102, 68 and 34 bp and the AA polymorphic homotype produced one band at 102 bp.

The PCR product of IL-17F [rs763780; A7488G] was digested with NIaIII *(Fermentas Life Sciences)*; and yields one band in the case of wild (AA) 143 bp, three bands for AG variant 143, 80 and 63 bp, and two bands for the GG variant 80 and 63 bp.

Polymorphic genetic analysis was duplicated twice on 42 samples to ensure quality control. These samples were blindly interpreted by different experts, who showed 100% accordance in results.

Enzyme-linked immunosorbent assay (ELISA) for serum IL-17:

Serum samples were withdrawn from both study groups. IL-17 levels were measured by Enzyme-linked immunosorbant assay (ELISA) (R&D Systems, USA). Calibrations and results were repeated twice. Least detectable concentration of IL-

17 was < 15 pg/ ml.

Statistical analysis

Candidates' information was analysed by the statistical package SPSS version 21. Results were illustrated by the mean and standard deviation for quantitative variables and relative frequencies for a group's variables. Comparison between genotype of both study groups was performed using binary logistic regression. Odds ratio (OR) with 95% confidence intervals was calculated. Comparison of quantitative variables was made using unpaired student t-test. Chi-square (χ^2) test was calculated to compare groups' data. If the expected frequency is less than 5, Exact test was used. *P* value < 0.05 was taken as statistically significant. Data of ELISA were analysed by Mann-Whitney U test.

Results

All patients and controls have been tested for IL-17F [A7488G] and IL-17A (rs2275913; G197A) polymorphisms, using PCR-RFLP technique.

The frequency of the polymorphisms under research in AML patients and controls is illustrated in Table II. Mutants subtypes prevalence of IL-17A [G197A], and IL-17F [A7488G] in controls was in accordance with the Hardy–Weinberg equilibrium (P > 0.05).

The frequency of different polymorphic genotypes and alleles of the studied SNPs are illustrated in Table II. Statistical analysis showed that IL-17F [rs763780; A7488G] polymorphic genotype, as well as the polymorphic allele (G allele), was detected in 29/100 (29%) and 37/100 (18.5%) of patients respectively while it was detected in 43/100 (43%) and 62/100 (31%) respectively in controls. The incidence of IL-17F polymorphic genotype, as well as the polymorphic allele (G allele), was significantly lower in AML patients when compared to controls (p = 0.039) and (p = 0.004) respectively.

Otherwise, no significant relation was found between the patients' groups regarding sex and laboratory data (data not shown).

No statistical relation was found between AML patients owning the normal or the mutant genotypes of IL-17A (rs2275913; G197A) as regards their sex, clinical data, and laboratory findings (data not presented). Despite the higher frequency of the IL-17A (rs2275913; G197A) heterozygous genotype observed in patients compared to controls, yet no statistical relationship was proved (p-value 0.077) (Table 2).

Table 2: Frequency of IL-17F [rs763780; A7488G] and IL-17A (rs2275913; G197A) genotypes in patients and controls

Gene	Group		Odd ratio	95% confidence interval	P-value
	Controls (n = 100)	Patients (n = 100)			
	N (%)	N (%)			
IL-17F [rs763780; A7488G]					
Wild type (AA)	57 (57%)	71 (71%)	Reference		
Heterotype (AG)	24 (24%)	21 (21%)	0.702	0.335-1.389	0.309
Homotype (GG)	19 (19%)	8 (8%)	0.338	0.138-0.829	0.015
Mutant allele (G)	62 (31%)	37 (18.5%)	0.505	0.317-0.805	0.004
polymorphic genotype (AG/GG)	43 (43)	29 (29)	0.541	0.301-0.973	0.039
IL-17A (rs2275913; G197A)					
Wild type (GG)	30 (30)	31 (31)	Reference		
Heterotype (GA)	10 (10)	23 (23)	2.226	0.908-5.454	0.077
Homotype (AA)	60 (60)	46 (46)	0.742	0.394-1.396	0.354
Mutant allele (A)	130 (65%)	115 (57.5)	0.729	0.486-1.091	0.124
Polymorphic genotype (GA/AA)	70 (70)	69 (69)	0.954	0.522-1.742	0.898

There was no significant relationship between the combined IL-17F [rs763780; A7488G] & IL-17A [rs2275913; G197A] polymorphism and the incidence of AML between patients and controls (Table 3).

Table 3: Combined effects of interleukin IL-17F [rs763780; A7488G]and IL-17A (rs2275913; G197A) genotypes

Combined Genotypes	Controls (n = 100) No. (%)	Cases (n = 100) No. (%)	p-value
Dual IL-17F [rs763780; A7488G] and IL-17A [rs2275913; G197A] polymorphism	27 (27%)	18 (18%)	0.231

IL-17 level was positively detected in 14 /100 AML patients (range, mean ± SD: 1.37–19.10, 8.8 ± 7.19 pg/ml), and 2 /100 in the control group (of level 1.4 pg/ml). No significant relationships were observed regarding IL-17 levels and AML incidence (data not shown).

Discussion

Th17 cells with their secretory cytokines are considered essential controllers in inflammatory and autoimmune disorders. They represent a curious research point. Many studies proposed the potential effect of Th17 cells on solid tumours but their role in haematological malignancies is still under investigations [10].

In the present research, the association of AML with IL-17A and IL-17F gene polymorphic genotypes was assessed, as well as, ELISA estimation of IL-17 in AML patients. The results proved that IL-17F and IL-17A gene polymorphism did not correlate with AML incidence. These findings were first reported in Egyptian patients. Also, the study concluded that the serum level of IL-17 is not elevated in patients group.

Regarding IL-17F; our results were in contrast to [9] and [11] who stated that IL-17F [rs763780; A7488G] polymorphism was proved to be correlated with a predisposition to AML in Poland and China respectively. IL-17F G homozygous genotype was significantly more frequently observed among patients than healthy individuals.

The study did not prove a significantly elevated level of IL-17 in AML patients. Our finding was in agreement with an earlier study conducted by Kawaguchi et al., who proposed that the IL-17 expression and activity may be affected in polymorphic G allele owners [12].

IL17-A had no impact on AML incidence; as there was no significant relation between cases and controls, regarding polymorphic genotype and mutant allele (p-value 0.898 and 0.124) respectively. This finding was in agreement with [13].

Espinoza et al. proved that T lymphocytes from normal individuals revealed an elevated level of IL-17 protein after provoking A allele [14]. However, we were not able to prove the significant differences concerning IL-17A polymorphism and IL-17 level (data not shown). Also, we did not find a significant relationship between the IL-17 levels in AML patients and healthy controls, as observed in previous studies [11], [15].

Wu et al. found that an increase in IL-17 concentration is associated with an elevation in Th17 cell number in the patient group. It is inhibited in cases with complete response undergoing therapy [8]. Abusamra et al. proposed that the number of circulating Th17 cells significantly increased in ALL and AML patients compared with controls. These patients showed a decrease number of ofTh17 cell number when reaching complete remission after therapy [10]. On the other hand, Tian et al. indicated that compared with normal controls, IL-17 level decreased obviously in acute lymphoblastic leukaemia patients [16].

Variation in results may be due to the difference of patient ethnic groups, the correlation between IL-17F polymorphic genotypes. Also, plasma IL-17 values might be correlated to racial variations [11].

To our knowledge, the published data on the role of the IL-17F and IL-17A polymorphisms in AML are limited [9]*;* thus, our report is considered a novel finding not previously studied in Egyptian population. The authors did not contribute Il-17 polymorphism to the disease incidence in Egyptian cases with AML. Respectively, these results should be re-assessed in a more extended study, including multicentric patients.

In conclusion, although Interleukin-17 is as important in the pathogenesis of inflammation and oncogenesis, yet we did not find a significant association between IL-17 polymorphism and increased susceptibility to acute myeloid leukaemia incidence. Further studies are indeed needed to prove our study conclusion.

Ethical approval

The study has been approved by the ethics committee of the Clinical Pathology department as well as the Internal Medicine Department, Kasr Al Aini, Cairo University, Egypt.

Contributor's statement

All authors are qualified for authorship. Each author should have participated sufficiently in work to take responsibility for appropriate portions of the text.

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