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Journal of Bioscience and Applied Research

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## IL-17F and IL- 23 gene polymorphism in patients with acute myeloid leukemia, an Egyptian study.

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### Abstract

**Background:** Acute myeloid leukemia (AML) is a highly fatal disease occurring due to proliferation and accumulation of myeloid progenitor cells. Th17 cells had been claimed by many studies to play a role in the development of AML. **Aim Of work:** This work aimed to detect possible role of IL 17 F and IL 23 gene polymorphisms in pathogenesis of AML ,relation to prognosis and response to treatment. **Subjects , materials and methods:** This study was done on 68 patients with newly diagnosed AML (as a patient group) ,together with 56 matched healthy volunteers( control group). IL 17 F and IL 23 gene polymorphisms were genotyped by real time polymerase chain reaction(Real time PCR). **Results:** No significant differences were detected between patients and controls in respect to IL 17 genotype distribution , there was statistically significant differences between patients and controls regarding IL 23 genotype distribution. No statistically significant relation was found between interleukin 17 and interleukin 23 and any of the bad prognostic markers. **Conclusion:** We concluded that IL23 gene polymorphism could be considered as independent risk factor in the pathogenesis of AML ,while we could not prove that IL 17 gene polymorphism has a role in the development of AML.

**Keywords:** IL-17F ; IL 23; gene polymorphism; AML.

## Introduction

AML is a malignant bone marrow disease where there is arrest of development of hematopoietic precursors at early stage. AML is a complex and heterogeneous disease in all respects, including cytologic features, stage of differentiation, antigen expression, cytogenetic findings, gene expression, growth regulation, and response to treatment. The biology of AML is also complex, with multiple pathogenetic phenomena and pathways. AML is differentiated from other hematological diseases by presence of 20% or more blasts in the bone marrow (Jemal A et al,2002) .

Pathogenesis of AML includes a group of molecular alterations that changes transformation of cells. These alterations involves control of cell proliferation, differentiation, self-renewal, survival and cell cycle .Normal regulatory mechanisms are damaged by leukemic changes, and studying of the mechanism of these changes is of great importance in applying new treatment protocols( Licht JD and Sternberg DW ,2005).

Although cause of AML is multifactorial, it is well known that the environment and genetic factors have important role in pathogenesis of AML(Scheijen B and Griffin J.D 2002, Baldus CD et al 2003, Downing J.R. 2001).

Uptill now, few information is well established about the mechanisms involved in the pathogenesis of AML. T helper type 17 cells are unique T Cells subset that play an important role in the pathogenesis of several diseases, including cancers. Some researchers have reported that the levels of Th17 and related cytokines differ in normal cells from AML malignant cells, assuming that Th17 may be involved in the pathogenesis of AML(Li P 2012).

Interleukin (IL)-23 is a proinflammatory cytokine which consists of a p19 subunit and a p40 subunit (Li P 2012,Del Vecchio M et al 2007). IL-23 is mainly produced by antigen-presenting cells due to stimulation by microbes or immune stimuli and included in immune response regulation against infections and tumor development( Trinchieri G 2003).

Recently , it has been known that IL-23/IL-23R has direct antitumor activities in hematologic malignancies. IL-23 reduces endogenous and

exogenous tumor growth through inhibition of proliferation of tumors and apoptosis induction (Hunter CA 2005,Cocco C et al 2011).

The *IL-23R* gene is located on chromosome 1p31 encoding one subunit of the IL-23R( Paraham et al 2002, Cocco C et al 2010).

The aim of this work was to study possible role of IL 17 F and IL 23 gene polymorphisms in pathogenesis of AML ,relation to prognosis and response to treatment.

## Subjects , materials and methods

This study was done on 68 patients having *de novo* acute myeloid leukemia at time of initial diagnosis before induction of therapy (as a patient group). Patients were diagnosed and selected among cases referred to medical oncology department at Kasr Al-Aini hospital, Cairo University in the period from June 2015 to July 2016 . Diagnosis of AML depended on morphological and phenotypic data.

In addition, 56 matched apparently healthy subjects participated in the study as well (control group). Patients selected among cases referred to Kasr AL-Aini .

An informed consent accompanied by detailed explanation of the procedure and its outcome was taken from the participants before the study. Approval of the committee of Ethics , local institutional research board approval was also taken prior to the study.

Patients and controls were assessed for clinical and laboratory findings. Patients were examined for cytochemical, immunophenotypic and cytogenetic studies to establish diagnosis and to divide patients according to their subtypes. Patients involved in the study were treated according to the protocol of nuclear medicine and oncology department, Cairo University, using ongoing induction(Preisler et al 1994) and consolidation( Mayer RJ et al 1994) . regimens for treatment of adult AML cases. Significant association to relapse free survival and overall survival were estimated for studied genes at a median follow-up of 18 months.

## Collection and processing of samples

IL 17 F and IL 23 gene polymorphisms were analyzed using real time RT-PCR. Three ml of peripheral blood and three ml of bone marrow samples were collected in

sterile ethylenediaminetetraacetic acid (EDTA) vacutainer tubes, from AML cases and control group. One microgram of total RNA was converted into cDNA using random hexamers (High-capacity cDNA kit; Applied Biosystems ). An aliquot of the cDNA was used for quantitative PCR amplification by Gene Amp 7500 Sequence Detection System (Applied Biosystems), using 50 µl reaction mix containing 5µl cDNA, 25 µl TaqMan Universal PCR master mix, 2.5µl primer-probe mix using the following primers and probes:

For IL17, forward 5'-TCCCAAAGGTCCTCAGATTACT-3' , reverse 5'-TTTGCTCCAGATCACAGA-3'. For -actin, forward 5'-GGATGCAGAAGG AGA TCACTG-3' and reverse 5'-CGATCCACA CGGAGTACTTG-3'.

The expression levels of IL17 were normalized to the internal reference gene -actin. Conditions for real-time PCR for IL17 was 1 cycle of 95°C for 10 minutes, then 40 cycles each consisting of 95°C for 15 seconds, 60°C for 1 minute, 95°C for 15 seconds, 60°C for 1 minute, 95°C for 15 seconds, and 60°C for 15 seconds. Each sample was analyzed in duplicate. A pooled sample of cDNA from all normal controls was chosen as a calibrator.

### Statistics

A pre-designed SPSS (Statistical Package for Social Science Version 17) file was used for analysis of data . The tests used were unpaired t - test, with 95 % confidence intervals (95% CI), Mann-Whitney Test was used to compare nonparametric data .Odds ratio to access the risk conferred by a particular allele and genotype. A p -value<0.05 was considered to be statistically significant.

### Results

The current study was conducted on 68 AML patients, as well as 56 age and sex matched healthy volunteers . Patients involved in the study were 31 males (45.6%) and 37 females (54.4%). Their ages were 18 to 70 years with a mean of  $40.07 \pm 1.61$ . Control group were 22 males (39.3%) and 34 females (60.7 %). Their ages were 18 to 66 years with a mean of  $39.93 \pm 12.04$  years. No significant differences were detected between the patient and control groups regarding age ( $P = 0.950$ ) or sex ( $P = 0.480$ ).

Clinical data of patients are collected in table 1.

**Table 1:** Clinical characteristics of patients

Laboratory data of the studied groups are collected in table 2.

Liver , kidney functions,coagulation profile are normal in all patients and controls .CSF was free in all patients,X ray showed abnormal findings in 2 patients(2.9%). Adverse reactions after transfusion were found in 17 patients (25%).Sixty three patients (92.6% ) were positive for HLA-DR,all cases were positive for CD13 and CD 33 while were negative for CD 19 and Glycophorin A.

**Table 2:** laboratory data of patients and controls

Results of genotype distribution were indicated in table 3.

**Table 3:** Results of IL 17 and IL 23 genotype distribution in patients and controls.

It was found that no statistically significant differences between patients and controls regarding IL 17 genotype distribution ( p value =0.1744) ,while there was statistically significant differences between patients and controls regarding IL 23 genotype distribution ( p value =0.0292).

Risk Results for Patients group were summarized in table 4 and 5 (according to the bad prognostic markers which are : old age more than 60 years,presence of organomegaly or lymphadenopathy,high TLC ,PB blasts more than 50% and BM blasts more than 70%).

**Table 4 :** Risk results in patients in relation to IL 17

No statistically significant relation was found between interleukin 17 and any of the bad prognostic markers.

**Table 5 :** Risk results in patients in relation to IL 23

No statistically significant relation was found between interleukin 23 and any of the bad prognostic markers

### Discussion:

Recently, researches suggested that th17 cells may have a role in AML pathogenesis. CD4+ cells is characterized by by interleukin (Il)-17a and Il-17F production, which which have strong homology, and surface expression of the Il-23 receptor (Il-23R) .This work aimed to study possible role of IL 17 F and IL 23 gene polymorphisms in pathogenesis of AML ,relation to prognosis and response to treatment .

No statistically significant differences were observed between patients and controls as regards IL 17 genotype

distribution ( p value =0.1744) ,while there was statistically significant differences between patients and controls regarding IL 23 genotype distribution ( p value =0.0292).

The results of this study were in contrast to the results reported by (Tomasz Wróbel et al 2014) who observed that of the studied polymorphisms , only the IL-17F heterozygous polymorphism predisposed to AML . IL-17F G variant and its homozygosity were more detected among patients than controls. That was the first study to report such information.

Also the study done by (Yixiang Han 2014 ) reported that Th17-associated cytokines were increased in PB and BM of patients with AML if compared with controls and this was associated with elevated frequencies of Th17 cells. Interleukin-17 and IL-22, which are secreted by Th17 cells, suppress the differentiation and expansion of Th1 cells( Dunn GP et al 2006 , Prabhala RH et al 2010).They observed that IL-17A and IL-22 can play an important antitumor effect.( Dunn GP et al 2006 ) . Therefore, IL-17 may play a pro-tumor effect on IL-17R-negative AML cells (Kryczek I et al 2009,Tosolini M et al 2011).They concluded that high frequencies of Th17 cells in AML together with high levels of IL-17 support differentiation of Th17 cells and suppressing immune responses, therefore could have a crucial role in inventing new therapeutic modalities in AML .

(Kawaguchi et al.,2006) observed that IL-17 expression and activity may be suppressed in carriers of the rare G allele .( Espinoza et al.,2011) found that stimulated T cells from healthy individuals having the 197A allele produced more IL-17 than those without the 197A allele (Wu et al.,2009) found higher frequencies of Th17 cells in peripheral blood from untreated AML patients,if compared with healthy controls . They found that increased IL-17 concentrations accompanied the increased Th17 cell frequencies were decreased when patients reached CR postchemotherapy.

(Abousamra et al.,2013) studied AML and ALL patients and found that circulating Th17 cells were significantly higher in acute leukemias patients than in healthy controls.

**Conclusion:** we concluded that IL23 gene polymorphism could be considered as independent risk factor in the pathogenesis of AML ,while we could not prove that IL

17 gene polymorphism has a role in the development of AML.However further studies conducted on larger sample size and in combination with other markers should be considered.

**Sources of support:** None.

**Funding source:** None.

**Acknowledgement :**None to declare

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**Table 1:** Clinical characteristics of patients

Clinical characteristics	Number of patients (68) %
-Constitutional symptoms	24 (35.3%)
-Signs	
-Hepatomegaly	24 (35.3%)
-Splenomegaly	22 (32.4%)
-Lymphadenopathy	16 (23.5%)

**Table2:** laboratory data of patients and controls

Laboratory data	Patients (No=68)	Controls (No=56)	P value
-Total leucocytic count/ mm <sup>3</sup>			
Range	2.8 - 356.7	2.3 - 30	
Median	56.75	7	0.001[S]
-Hemoglobin (gm %)			
Range:	3.4-13.4	11.1-16.2	0.001[S]
Mean±SD	8.24 ± 1.86	12.46 ± 1.03	
-Platelets ×10 <sup>3</sup> mm <sup>3</sup>			
Range:	10 – 160	139 - 465	
Median	44.5	245.5	0.001[S]
-PB blasts			
Range	13 - 96		
Median	46.5		
-BM blasts			
Range	0 - 99		
Median	58.5		
-FAB subtypes(no%)			
M0	2 (2.9%)		
M1	13 (19.1%)		
M2	22 (32.4%)		
M3	5 (7.4%)		
M4	12 (17.6%)		
M5	11 (16.2%)		
M6	1 (1.5%)		
M7	2 (2.9%)		

\*Not significant [NS] (p value >0.05), \*\* significant[S] (p value <0.05), \*\*\* highly significant [HS] (P value < 0.001).

**Table 3:** Results of IL 17 and IL 23 genotype distribution in patients and controls

	Genotype	Patients (no=68)	Controls (no=56)	P value
IL 17	Normal (N (%))	64 (94%)	56 (100%)	0.1744(NS)
	Hetero (N (%))	4 (6%)	0 (0%)	0.1744(NS)
IL 23	Normal (N (%))	43 (62.7%)	46 (82.1%)	0.0292(S)
	Hetero (N (%))	25 (37.3%)	10 (17.9%)	0.0292(S)

**Table 4 :** Risk results in patients in relation to IL 17

Parameters	Groups		IL 17		P value	OR (CI 95%)
			Hetero (n=4)	Normal (n=63)		
Age	Age more than 60 years	N%	0(0%)	4(6.3%)	0.603	1.068 (1.001-1.139)
	Age Less than or equal to 60 years	N%	4(100%)	59(93.7%)		
Hepatomegaly	Present	N%	1(25%)	23(36.5%)	0.642	0.580 (0.057-5.902)
	Absent	N%	3(75%)	40(63.5%)		
Splenomegaly	Present	N%	1(25%)	20(31.7%)	0.778	0.717 (0.070-7.326)
	Absent	N%	3(75%)	43(68.3%)		
Lymphadenopathy	Present	N%	0(0%)	16(25.4%)	0.248	1.085 (1.002-1.176)
	Absent	N%	4(100%)	47(74.6%)		
TLC	High	N%	4(100%)	50(79.4%)	0.312	0.926 (0.859-0.998)
	Low	N%	0(0%)	13(20.6%)		
PB Blasts	Above 50%	N%	2(50%)	30(47.6%)	0.926	1.100 (0.146-8.303)
	Less or equal to 50%	N%	2(50%)	33(52.4%)		
BM Blasts	Above 70%	N%	1(25%)	25(39.7%)	0.559	0.507 (0.050-5.149)
	Less or equal to 70%	N%	3(75%)	38(60.3%)		



**Table 5 :** Risk results in patients in relation to IL 23

Parameters	Groups		IL 23		P value	OR (CI 95%)
			Hetero (n=25)	Normal (n=42)		
Age	Age more than 60 years	N%	2(8%)	2(4.8%)	0.588	1.739 (0.229-13.189)
	Age Less than or equal to 60 years	N%	23(92%)	40(95.2%)		
Hepatomegaly	Present	N%	9(36%)	15(35.7%)	0.981	1.012 (0.361-2.842)
	Absent	N%	16(64%)	27(64.3%)		
Splenomegaly	Present	N%	7(28%)	14(33.3%)	0.649	0.778 (0.263-2.298)
	Absent	N%	18(72%)	28(66.7%)		
Lymphadenopathy	Present	N%	7(28%)	9(21.4%)	0.542	1.426 (0.455-4.470)
	Absent	N%	18(72%)	33(78.6%)		
TLC	High	N%	19(76%)	35(83.3%)	0.463	0.633 (0.186-2.156)
	Low	N%	6(24%)	7(16.7%)		
PB Blasts	Above 50%	N%	11(44%)	21(50%)	0.634	0.786 (0.291-2.125)
	Less or equal to 50%	N%	14(56%)	21(50%)		
BM Blasts	Above 70%	N%	9(36%)	17(40.5%)	0.716	0.827 (0.297-2.301)
	Less or equal to 70%	N%	16(64%)	25(59.5%)		