RESEARCH ARTICLE

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Prognostic Impact of *IL17A* Gene Polymorphismson Egyptian Patients with Multiple Myeloma

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Abstract

Introduction: Multiple myeloma (MM) is a B-cell lymphoproliferative disease in which the bone marrow microenvironment plays an important role in pathogenesis. The T helper (Th-17) cell plays an important role in the development of cancer by releasing pro-inflammatory cytokines such as *IL-17A* and *IL-17F*. Th-17 cells have been studied in a variety of solid tumors, as well as few hematological malignancies, including acute myeloid leukemia, non-Hodgkin lymphoma, and monoclonal gammopathy of unknown significance. **Aim:** Our study aimed to assess the association between *IL-17A* polymorphism and MM risk and other MM characteristics in Egyptian patients. Patients & Methods: a prospective study involving 77 patients with MM (mean age 54.6 years; males 53.2%; females 46.8%) and a healthy control group of same age and gender. It was performed at the Mansoura University Oncology Center (OCMU). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach was used to detect *IL17A* 197 G/A (rs2275913) genotypes in genomic DNA from MM patients and healthy controls. **Results:** The *IL-17A* polymorphism may not be associated to myeloma predilection in the Egyptians as a whole. There was also no significant correlation in statistical study between gender and the *IL-17A* polymorphism. (p 0.14), a number of clinical and laboratory characteristics, including hypercalcemia (p 0.28), hypoalbuminemia (p 0.49), renal impairment (p 0.13), high LDH (p 0.62), osteolytic bone lesions (p 0.26), and pathological fracture (p 0.96), are also present. Nevertheless, no statistically significant difference in the OS of MM patients was detected for the *IL-17A* polymorphism (p 0.83). **Conclusion:** Our research demonstrated that *IL-17A* polymorphism may not be linked to multiple myeloma susceptibility in our population and did not influence its different clinical and laboratory features. *IL-17A* polymorphism had no effect on OS in MM patients.

Keywords: *IL-17A*- PCR-RFLP- polymorphism- Multiple myeloma

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Introduction

Multiple myeloma (MM) is one of lymphoproliferative disorders, characterized by malignant plasma cell (PCs) proliferation, bone lytic lesions, and serum and/or urine monoclonal gammopathy (Cowan et al., 2022).

A major proportion of the pathogenesis of MM is played by the bone marrow (BM) microenvironment. According to reports, the relationship between PCs and stromal cells controls PC homing, growth, survival, and resistance to chemotherapy (Dalton, 2003). Also, BM microenvironment shows many different cytokines, and proangiogenic factors, as interleukin 17 (*IL-17*), which is a proinflammatory cytokine secreted from T helper 17 cells (Th -17) (Terpos et al., 2018).

Through the interaction of genetics, environment, and the consequent microbiota populations at barrier surfaces, *IL-17* production and signaling are controlled. The functions of *IL-17* that drive the antimicrobial response and encourage tissue regeneration are increased by injury or infection. If the *IL-17* response is abnormally amplified because of chronic stimulation, the normal antimicrobial and repair functions convert to pathological inflammation, and tissue remodeling, which promotes tumorigenesis (Majumder and McGeachy, 2021). It has been demonstrated that *IL-17* plays a critical role in the development and maintenance of MM by promoting the proliferation of PCs and inhibiting immune responses

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in the tumor micro-environment (Prabhala et al., 2010). We did this study to assess the relationship between the Egyptian patients' *IL-17A* polymorphism and risk for MM and other MM features.

Materials and Methods

Patients

This study was conducted on 77 patients with de novo multiple myeloma, admitted to the Hematology and Oncology Unit, Oncology Center Mansoura University (OCMU), in the period from January 2018 to January 2019). Fourteen healthy volunteers' individuals of similar age and sex were used as the control group. IMWG diagnostic criteria was used for MM patients (Rajkumar et al., 2014). Our work has obtained IRB of the relevant university approval (Code Number: R. 19. 12. 705). Informed consent was obtained from patients and controls for their approval to use their samples for the study.

All patients are given a thorough clinical examination. The diagnosis was based on a full hemogram, Chemistry (Creatinine, Albumin, Calcium, LDH), Bone Marrow (BM) test, haematomorphology, and immunophenotyping analysis. The following immunophenotyping panels were used for Multiple myeloma diagnosis: Cells were stained with fluorescein conjugated SmmAb against CD38, CD138, CD45, CD19, CD20, CD27, CD56, CD117, Anti-kappa, Anti-lambda. The samples were analyzed via a NAVIOS EX 10 flow- cytometer device. Monoclonal antibodies were purchased from Beckman Coulter.

IL-17A genotyping

The QIAamp DNA mini kit was used to extract genomic DNA from whole blood (Qiagen, Germany). The polymerase chain reaction-restriction fragment length polymorphism technique was used to determine IL-17A-197 G/A (rs2275913) genotypes (PCR-RFLP). A total volume of 25 ul for each PCR reaction contained: 12.5 ul DreamTaq green PCR master mix (Thermo Fisher Scientific), 0.1 ul IL-17A forward primer 5'-CAGAAGACCTACATGTTACT-3', 0.1 ul IL17A reverse primer 5'-GTAGCGCTATCGTCTCTCT-3', 11.3 ul nuclease free water, and 1 ul of DNA. The amplification conditions were 95°C for 2 minutes, followed by 34 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 1 minute, followed by a final 72°C for 10 minutes. The 368 bp PCR result was subsequently digested with Thermo Fisher Scientific's XnmI restriction enzyme and observed on a 2% agarose gel. The following genotypes were discovered: GG (344 bp), GA (344, 213, 131 bp), and AA (213, 131 bp) Figure (1).

Statistical analysis:

SPSS 16 (Statistical Program for Social Scientists) was utilized to examine the data. Statistical significance is detected as a two-tailed (p) value 0.05. The frequency distribution methods are done along the determination of the number of cases and percentages for qualitative descriptive statistics. The mean, median, and range were employed for quantitative variables' descriptive statistics. Using the Chi Square Test, associations between categorical variables are examined. In cases where the Chi square presumptions were broken, Fisher's exact test was applied. Kaplan - Meier technique was employed to compute analyses of survival.

Results

Among the 77 MM patients, 41 (53.2%) were men and 36 (46.8%) were women. At diagnosis, their median age was 54.6 years (range, 32-78). The median haemoglobin level was 9.2 g/dl (rang, 4.3 - 14.4), and 63 (81.8%) of the patients had anemia. The mean serum creatinine was 2.88 mg/dl (range, 0.6-15), 55.8% of patients presented with elevated serum creatinine. While mean serum calcium was 9.2mg/dl (range, 6.1- 15), 16 (20.8%) patients were presented with hypercalcemia.

The immunoglobulin subtype was IgG; 86%, and IgA; 14%. Bence Jones protein was positive in17 (22.4%) patients. Based on the International Staging System (ISS), 43.4% of patients are categorized as stage I, 18.4% as stage II, and 38.2% as stage III.57 (74%) of patients presented with osteolytic bone lesions and 10 (13%) had a pathological fracture. 15 (19.5%) patients were associated with plasmacytoma (Table 1). Comparing the genotype and allele frequency distributions, there were no statistically significant differences between the MM patients and the healthy controls (Table 2).

The association between *IL-17A* polymorphisms (AA genotype and the GG/GA genotypes) and the

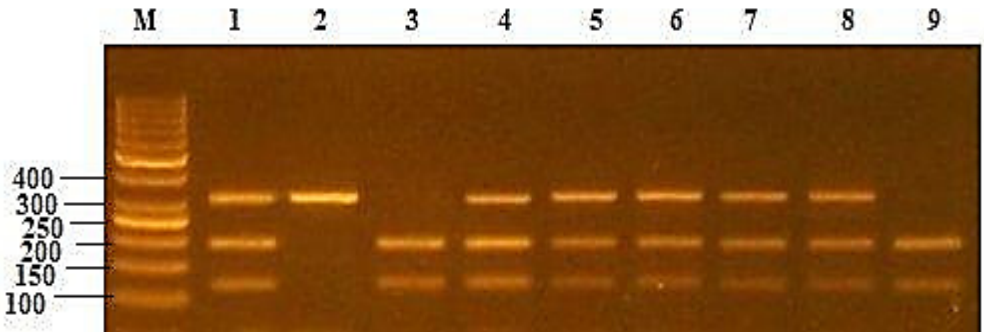

Figure 1. *IL17A*-197 G/A (rs2275913) Genotypes were Separated Using Agarose Gel Electrophoresis. Lane M: contain a 50 b p DNA ladder. Lanes 1 and 4 - 8: show GA genotype (344, 213 and 131 b p). Lanes 3 & 9 show the AA genotype (213 & 131 b p). Lane 2: represents the GG genotype (344 b p)

Table 1. Clinical and Laboratory Characteristics of Multiple Myeloma Patients

Variables			
Age	Mean 54.6 ,Range (32 – 78)		
Gender	Male:41 (53.2%)		
	Female: 36 (46.8%)		
Labboratory charactersitics			
HB (gm/dL)	Mean:9.2- Range (4.3 – 14.4)		
PLT (x10^9/L)	Mean:184 – Range (8-453)		
WBC(x10^9/L)	Mean:7.3 - Range (0.7 – 24)		
ANC(x10^9/L)	Mean : 4.4		
ALC(x10^9/L)	Mean: 2.1		
AMC(x10^9/L)	Mean: 0.6		
Albumin (mg/dl)	Mean 3.3 - Range (1.9 – 5)		
1stESR	Mean: 74.8 - Range (7 – 163)		
LDH (IU)	Mean: 366- Range (106 – 2481)		
Serum Creatinine	Mean: 2.88- Range (0.6 – 15)		
Serum Calcium	Mean: 9.2- Range (6.1 – 15)		
Multiple myeloma characteristics			
BMA plasma cell	Median 40%		
BMB plasma cell %	Median 80%		
Bone osteolytic lesions	57 (74%)		
Pathological fracture	10 (13%)		
Plasmacytoma	15 (19.5%)		
SPEP (monoclonal gammopathy)	66 (85.7%)		
Ig assay	IgG: 86%		
BJP positivity	IgA: 14%		
Hypercalcemia	17 (22.4%)		
Anemia	16 (20.8%)		
Elevated serum Creatinine	63 (81.8%)		
Hypoalbuminemia	43 (55.8%)		
Elevated LDH value	40 (55.6%)		
Cytogenetics	negative	not done	positive
17p deletion	59.70%	40.30%	0%
t (14 – 16)	14.30%	85.70%	0%
14q rearrangement	18.20%	68.80%	13%
ISS			
I	43.40%		
II	18.40%		
III	38.20%		

HB, hemoglobin; PLT, Platelet; WBC, White blood cell; ALC, absolute lymphocytic count; ANC, absolute neutrophil count; AMC, absolute monocyte count; LDH, lactate dehydrogenase; BMA, bone marrow aspiration; BMB, bone marrow biopsy; SPEP, Serum protein electrophoresis; BJP, Bence-Jones proteins; ISS, International Staging System

clinical characteristics of MM patients was statistically insignificant; Gender (p 0.14), High calcium level (p 0.28), hypo-albuminemia (p 0.49), impaired kidney function (p 0.13), elevated lactate dehydrogenase (p 0.62), osteolytic disorders (p 0.26), pathologic fractures (p 0.96), plasma-cytoma (p 0.42). Moreover, among MM patients, there was no statistically significant link between *IL-17A* polymorphism and overall response (p 0.7) or OS (p 0.83) Table 3, Figure 2.

Table 2. Genotype and Allele Frequency Distribution in MM Patients and Healthy Controls. Genotype and allele frequency distribution in MM patients and healthy controls.

	Case	Control		P
AA (n/%)	38 (82.6)	8 (17.4)	46 (100)	0.592
GA and GG (n/%)	39 (86.7)	6 (13.3)	45 (100)	
	77	14	91	
Allele frequencies				
A allele	109	21	130	0.649
G allele	45	7	52	
	154	28	182	

Discussion

IL-17 is a proinflammatory cytokine secreted from T helper 17 cells (Th-17). It is involved in the etiology of many chronic inflammatory disorders, including cystic fibrosis, chronic obstructive pulmonary disease, asthma, and inflammation-associated cancer (Chen et al., 2003, Yang, 2008). Moreover, there was an increased level of Th-17 in the bone marrow from patients with myeloma compared with the marrow in pre neoplastic gammopathy (Dhodapkar et al., 2008, Wang et al., 2015) and in acute myeloid leukemia patients (Wu et al., 2009 and Yu et al., 2014). On other hand, Th -17 was significantly decreased in newly diagnosed chronic myeloid leukemia patients compared with healthy controls (Chen et al., 2015). Also, Th-17 cells have been investigated in different solid tumors, including lung cancer (Armstrong et al., 2019), gastric cancer (Rezalotfi et al., 2019), and ovarian cancer (Bilska et al., 2020).

SNPs in the *IL-17* gene have been linked to cancer risk. According to (Dai et al., 2016), the rs2275913 polymorphism significantly increases cancer risk, particularly in stomach malignancies. In addition, (Lu et al., 2016) discovered that rs2275913 is linked to the risk of cancer in Asian populations and non-gastrointestinal malignancies. Polymorphism in colorectal cancer cases was linked to disease severity, the prevalence of distant metastases, and overall survival, according to (Aleksandrova et al., 2022).

It has been demonstrated that IL-17 plays a critical role in the development and maintenance of MM by promoting the proliferation of PCs and inhibiting immune responses in the tumor micro-environment (Prabhala et al., 2010). This work aimed to evaluate *IL-17A* polymorphism (rs2275913) in Egyptian patients in relation to MM risk and several MM characteristics.

Our finding that there are no statistical noticeable variations in the *IL-17A* 197 G/A polymorphism between MM patients and healthy controls implies that the *IL-17A* polymorphism does not predispose the Egyptian population to MM. Furthermore, this study found no statistically significant link between the *IL-17A* polymorphism and numerous multiple myeloma characteristics such anaemia, hypercalcemia, elevated creatinine, or responsiveness to medication.

Results of Kasamatsu et al., (2018) study coping

Table 3. Clinical Features of All Patients based on *IL17A* Polymorphism.

		AA genotype (n/%)	GA and GG genotype (n/%)	P
Gender	Male	17 (44.7)	24 (61.5)	0.14
	Female	21 (55.3)	15 (38.5)	
HB level	< 10gm/dl	31 (81.6)	32 (82.1)	0.957
	> 10gm/dl	7 (18.4)	7 (17.9)	
Serum creatinine	High creatinine	18 (47.4)	25 (64.1)	0.139
	Normal value	20 (52.6)	14 (35.9)	
Serum albumin	< 3.5mg/dl	18 (51.4)	22 (59.5)	0.493
	> 3.5 mg/dl	17 (48.6)	15 (40.5)	
Serum calcium	Hypercalcemia	6 (15.8)	10 (25.6)	0.287
	Normal value	32 (84.2)	29 (74.4)	
LDH level	> ULN	12 (33.3)	14 (38.9)	0.624
	Normal value	24 (66.7)	22 (61.1)	
Osteolytic bone lesions	Yes	26 (68.4)	31 (79.5)	0.268
	No	12 (31.6)	8 (20.5)	
Plasmacytoma	Yes	6 (15.8)	9 (23.1)	0.42
	No	32 (84.2)	30 (76.9)	
Pathologic fracture	Yes	5 (13.2)	5 (12.8)	0.96
	No	33 (86.8)	34 (87.2)	
SPEP	M-band	34 (89.5)	32 (82.1)	0.352
	No m - band	4 (10.5)	7 (17.9)	
BJP	Positive	9 (24.3)	8 (20.5)	0.69
	Negative	28 (75.7)	31 (79.5)	
ISS	Stage I	20 (52.6)	13 (34.2)	0.268
	Stage II	6 (15.8)	8 (21.1)	
	Stage III	12 (31.6)	17 (44.7)	
Response (65 patients)	Responsive disease	20 (62.5)	22 (66.7)	0.7
	Non responsive disease	12 (37.5)	11 (33.3)	

HB, hemoglobin; LDH, lactate dehydrogenase; SPEP, Serum protein electrophoresis; BJP, Bence-Jones proteins; ISS, International Staging System

with our finding as regard no noticeable variations in the genotype and allele frequencies of *IL-17A* −197 G/A between MM patients, and the healthy controls group, and the absence of significant difference in overall survival of MM patient regarding *IL-17A* polymorphism, however, they found a significant association with lower

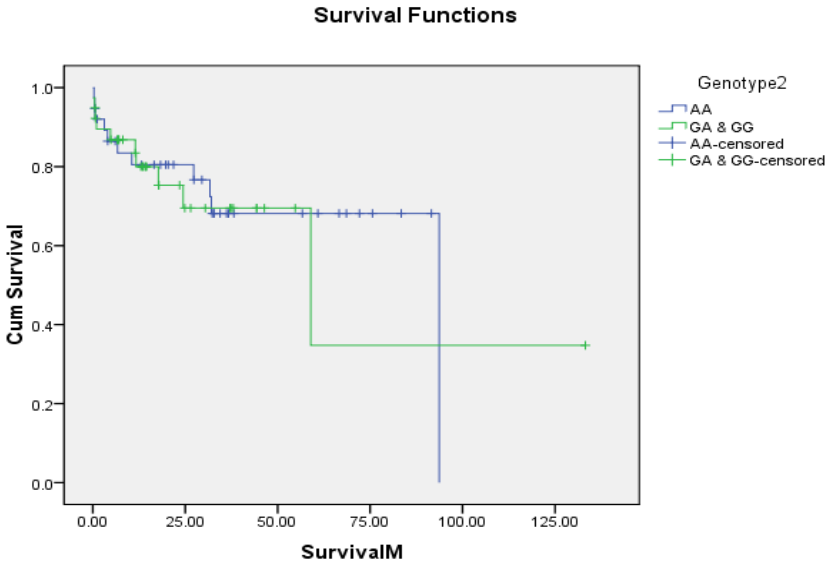

Figure 2. OS as Regarding *IL-17A* Polymorphism (p 0.83)

hemoglobin level.

In this study, there is a limitation in the form of low number of healthy controls and not all patients proceed to base line cytogenetic testing to correlate between the IL-17A polymorphism and cytogenetics. Hence, to confirm our findings, additional studies with larger sample numbers are required. In conclusion, in this research, IL-17A polymorphism had no effect on the various clinical characteristics of MM or its susceptibility to the disease. Moreover, IL-17A polymorphism had no impact on OS survival in MM patients.

Author Contribution Statement

Metwaly Ibrahim Mortada, Doaa Shahin, Nashwa abousamra, Eman A. Soliman and Mayada A. Ghannam equally participated in conception, interpretation of laboratory data and practical work. Shaimaa El-Ashwah, F.E.I. Ghobrial- and M. A. Elbaiomy collecting clinical data and statistical analysis. Nermeen A. Niazy, data processing and statistical study. All authors assisted with the research structure, wrote and edited the final manuscript version, and reviewed and approved the final manuscript.

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Ethical approval

The investigating hospital's institutional research committee Institutional Review Board (IRB), Mansoura Faculty of Medicine, number (R. 19. 12. 705) and the 1964 Helsinki Declaration and its later amendments or comparable ethics standards authorised this study.

Conflict of interest

The authors state that they have no conflicts of interest.

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