IMMUNOLOGICAL INVESTIGATIONS
2017, VOL. 46, NO. 1, 10–21
http://dx.doi.org/10.1080/08820139.2016.1208219

Taylor & Francis
Taylor & Francis Group

Effect of Cytokine Genes in the Pathogenesis and on the Clinical Parameters for the Treatment of Multiple Myeloma

H. Haydaroglu[a], S. Oguzkan Balcı[b], S. Pehlıvan[c], K. Ozdılli[d], E. Gundogan[a], V. Okan[a], A. F. Nursal[e], and M. Pehlıvan[a]

[a]Department of Adult Hematology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey; [b]Department of Medical Biology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey; [c]Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [d]Department of Hematology-Oncology, Faculty of Medicine, Medipol University, Istanbul, Turkey; [e]Department of Medical Genetics, Faculty of Medicine, Giresun University, Giresun, Turkey

ABSTRACT

In this study, we aimed to explore the association among gene variants of five cytokines, tumor necrosis factor alpha (TNF-α), transforming growth factor beta-1 (TGF-β1), interferon gamma (IFN-γ), interleukin-6 (IL-6), and interleukin-10 (IL-10), and clinical parameters and prognosis in patients with multiple myeloma (MM) treated with novel therapeutic drugs in Turkish population for the first time except TNF-α. We analyzed five cytokine genes in 113 cases with MM and 113 healthy controls. Cytokine genotyping was performed by the polymerase chain reaction–sequence-specific primer method (PCR-SSP). AG genotype associated with high expression in TNF-α gene (–308) variant was found to be significantly higher ($p = 0.019$), and GG genotype associated with low expression in TNF-α gene (–308) variant was significantly lower in MM group as compared with controls ($p = 0.012$). IFN-γ (+874) variant TT genotype was increased ($p = 0.037$), and AA genotype was decreased ($p = 0.002$) in MM group in contrast to controls. IFN-γ (+874) T allele was higher in MM patients compared with controls (OR = 1.985, $p = 0.000$), while A allele was significantly lower (OR = 0.5037, $p = 0.0005$). Multivariate analysis revealed that factors associated with 5-year overall survival (OS) were only IPI III (RR = 1.630, $p = 0.018$) and thrombocytopenia (RR = 2.207, Cox p = 0.021), while 5-year event-free survival (EFS) was associated with IPI III (RR = 1.524, $p = 0.022$), thrombocytopenia (RR = 2.902, $p = 0.002$), APSCT treatment (RR = 1.729, $p = 0.035$), and female gender (RR = 0.435, $p = 0.002$) with negative prognostic values. Our results suggested that TNF-α gene (–308) AG genotype and IFN-γ (+874) TT genotype and T allele may have a role on MM, while other cytokines were not associated with the risk of MM.

KEYWORDS

Cytokine gene expression; multiple myeloma; prognostic factors

Introduction

Multiple myeloma (MM) is the B cell lymphoproliferative disease that is characterized by a single plasma cell produced monoclonal immunoglobulin (Ig) or Ig fragments (M protein) and neoplastic proliferation of plasmacytoid cell clones (Durie, 1986; Lodh et al., 2012). It is characterized with overproduction of monoclonal immunoglobulin, osteolytic bone

CONTACT Dr. Handan Haydaroglu, MD handanh@gantep.edu.tr/tezhandan@hotmail.com Department of Hematology, Faculty of Medicine, Gaziantep University, 27310 Sahinbey, Gazıantep, Turkey.

lesions, hypercalcemia, anemia, and renal failure (Dancester et al., 1959). Although aggressive treatments approach in MM, it is still not a disease that can be cured. So, it is important to elucidate the etiology of the disease (Durie, 1986). The etiology of MM was not fully understood but, it was reported that age, gender, race and ethnicity, underlying immune system disorder, complex genetic and epigenetic abnormalities may play roles in the etiology of the MM (Durie, 1986; Lodh et al., 2012). Signaling system, apoptosis, cell cycle, and bone marrow microenvironment disorders play roles in the pathogenesis of myeloma (Cifci et al., 2011). Myeloma cell adhesion to bone marrow stromal cells (BMSCs) is required for growth and survival of MM tumor cells and various cytokines take part in this process (Hideshima et al., 2004).

Interleukin 6 (IL-6), one of the major cytokines active in adhesion, is produced primarily by BMSCs and less by myeloma cells. IL-6 binds to interleukin 6 receptor (IL-6R) with IL-6 signaling. IL-6R, which is produced by myeloma cells, is formed of an alpha (gp80) and a signal transducer beta (gp130) chain, and this complex binds to the signal transducer membrane protein gp130, which activates Janus kinases/signal transducer-activator of transcription (JAKs/STAT) and a GTPase/mitogen-activated protein kinase (RAS/MAPKs) pathway. JAKs/STAT plays a role in myeloma cell survival and inhibition of apoptosis, and RAS/MAPK is active in proliferation. IL-6 is a key growth and survival factor for myeloma cells, as well as a major morbidity factor for patients with MM. Increased IL-6 level is associated with the disease-related renal involvement, anemia, thrombocytosis, bone reabsorption and pro-thrombotic state (Joshua, 1988). Interleukin-10 (IL-10) is synthesized in myeloma cells and IL-10 increases proliferation of plasma cells (Kovacs, 2010). Additionally, IL-10 causes decreased expression of surface molecule B7.1 (CD 80) of the dendritic cell presenting antigens to Th2 and thus leads to development of functionally defective dendritic cells and increased tendency for infections (Brown et al., 2001). However, some studies have reported that gene variants of IL-6 and IL-10 do not have influence on growth and proliferation of MM cells (Mazur et al., 2005). Transforming growth factor beta-1 (TGF-β1) synthesized by myeloma cells induces IL-6 secretion, which conducts tumor cell proliferation by paracrine effect, from BMSCs (Hayashi et al., 2004). TGF-β1 also takes role in tumor progression by inducing the stroma and angiogenesis (Heinrich et al., 1995). Several studies reported that TGF-β1 has negative effect on terminal differentiation of osteoblasts and bone mineralization and that increased levels influence growth of bone lesions in MM (Matsumoto & Abe, 2011). Human tumor necrosis factor alpha (TNF-α) gene is located in the MHC locus at region 6 on chromosome 6p21.3 (Black et al., 1997). TNF-α is one of the most important cytokines in the inflammation. It effects both tissue destruction and repairmen (Wajant et al., 2003). Not only mechanisms reducing the tendency to neoplastic transformation but also the mechanisms creating neoplastic transformation tendency are stimulated by effect of TNF-α. While some studies showed that high levels of TNF-α suppresses the tumor angiogenesis in malign tissues, the other studies showed that TNF-α may act as an endogenous tumor growth factor (Waterston & Bower, 2004). In most studies, it was shown to be a strong association between TNF-α and hematologic and nonhematologic malignancy (Wajant et al., 2003; Dogra et al., 2013). Some references describe interferon-gamma (IFN-γ) as an anti-apoptotic cytokine, while others suggest it is a tumor proliferation inhibitor. IFN-γ synthesizes B-cell activator factor (BAFF) and its

receptor, thus induces release of anti-apoptotic substances by activating nuclear factor kappa B (NF-κB) signaling pathway, and inhibits spontaneous apoptosis. IFN-γ has the most potent inhibitor effect on proliferation of myeloma cells. It performs this effect by IL-6-dependent inhibition (Palumbo et al., 1995).

In this study, it was aimed to investigate 8 variants in 5 different cytokine genes in 113 MM patients and 113 healthy controls to compare the correlations of these results with clinical data and determine their effects on total overall survival (OS) and event-free survival (EFS) life durations.

Patients and methods

Patients

Total 113 patients who were diagnosed with MM and treated in Gaziantep University Medical Faculty Hematology Clinic and 113 healthy controls were enrolled to this study. The patients were followed up in our clinic between 2001 and 2015. Of 113 patients with MM, 64 were male and 49 were female. The age of these patients ranged from 31 to 81 years and median age was 60 years. Also, 113 healthy controls were enrolled to this study, 56 of the controls were female, 57 were male and the median age was 58 years (32–85 years). Staging was assigned according to the Durie and Salmon (DS) and the International Staging System (ISS) criteria (Anderson et al., 2013). Written informed consent was received from all participants. The study was approved by the local ethics committee (280/20.08.2013).

Genotyping analysis

DNA samples were isolated from peripheral blood both in the healthy control group and patient group (Miller et al., 1988). Single nucleotide polymorphisms (SNPs) of the TNF-α (–308), TGF-β1 (codon 10 and codon 25), IL-10 (–1082, –819, and –592), IL-6 (–174), and IFN-γ (+874) gene were analyzed by polymerase chain reaction–sequence-specific primer method (PCR-SSP) (Karaoglan et al., 2009).

Statistical analysis

All data were analyzed using SPSS, version 14.0, for Windows (SPSS, Inc., Chicago, IL, USA). Categorical data were analyzed using Pearson's χ^2 analysis. Odds ratio (OR) and 95% confidence interval (95% CI) were also calculated. OR (95% CI) was adjusted by age and sex. The data were analyzed for appropriateness between the observed and expected genotypes as well as for Hardy–Weinberg equilibrium (HWE) as described elsewhere. All analyses were two-tailed, and differences were interpreted as statistically significant when $p < 0.05$.

Results

Clinical features of the MM patients are given in Table 1. The median follow-up time was 30.4 months (range 6.9–169.7), mortality ratio was 55%, and EFS was found to be 65 months (Table 1). Eighty-nine patients were designated as the stage III (78%) and 17

Table 1. Clinical features and treatment regimens of MM patients.

		Multiple myeloma			Control	
		Median	n^a	%	n^a	%
Age		60 (31–81)			58	32–85
Gender	Female/male		49/64	44/56	56/57	49/51
Ig subtype	κ/λ		73/40	65/35		
	G/A		74/23	66/20		
	Light chain		16	14		
Stage (Salmon–Durie)	II/III		17/89	15/78		
	A/B		83/30	74/26		
IPI	I		32	28		
	II/III		38/43	34/38		
ECOG	>1		44	39		
Hemoglobin	g/dl	9.9 (6.0–14.8)				
Leukocyte	ml	6450 (1290–17,300)				
Platelets	10^3/ml	204 (26–700)				
C-reactive protein	mg/dl	6 (3–193)				
LDH	IU/L	336 (126–2540)				
b2mikroglobulin	mg/L	4.45 (1.5–28.7)				
Albumin	g/L	3.35 (1.6–5.2)				
First-line treatment (n^b)	imid ± Bort ± APSCT	42 ± 59 ± 48				
EFS*		65				
Mortality		55				
Median follow-up time (month)	30.4	(6.9–169.7)				

n^a = 113; n^b = 100; BODEC, bortezomib, dexamethasone, cyclophosphamide; TD, thalidomide, dexamethasone; LD, lenolidomid, dexamethasone; APSCT, autologous peripheral blood stem cell transplantation; ECOG, performance status; LDH, lactic dehidrogenase; IPI, International Prognostic Index; EFS, event-free survival.
* = median.

patients as the stage II (15%) according to the Salmon–Durie staging system at diagnosis. Of 113 patients with MM, 32 patients were IPI I, 38 patients were IPI II, and 43 patients were IPI III. The mean hemoglobin levels, leucocyte counts, thrombocyte counts, c-reactive protein (CRP), lactate dehidrogenase (LDH), B2-microglobuline, and albumin levels were 9.9 g/dl (range 6–14.8 g/dl), 6450 mm^3 (range 1290–17,300 mm^3), 204 × 10^3 (range 26–700 × 10^3), 6 mg/dl (range 3–193 mg/dl), 336 IU/L (range 126–2540 IU/L), 4.45 mg/L (range 1.5–28.7 mg/L), 3.35 g/L (range 1.6–5.2 g/L), respectively, and the other clinical features of MM patients are shown in Table 1.

Patients who were followed up for at least 6 months after the first-line treatment were analyzed for response status and OS. In MM patients as the first-line treatment, 42 patients received imid (RD)-based regimens, 59 received Bortezomib-based regimens (BCD), and 48 patients underwent APSCT. Patients received complete response, partial response, and very good partial response were included in the study. Sixty-eight patients with MM had relapsed during follow-up. Lenolidomide and dexamethasone were given to 30 MM patients who were relapsed under Bortezomib treatment, 8 cycles of BCD protocol (Bortezomib, 1.3 mg/m^2 intravenous; cyclophosphamide, 50 mg po once a day, dexamethasone, 40 mg po) were given to 24 MM patients relapsed under imid-based treatment, and EDAP protocol (cisplatin, dexamethasone, cytarabine) were given to 14 MM patients relapsed under Bortezmib and Lenolidomide treatment. Table 2 shows genotype distributions for patients and controls. TNF-α (–308), TGF-β1 (codon 10 and codon 25), IL-10 (–1082, –819, and –592), IL-6 (–174), and IFN-γ (+874) gene variants of 113 MM patients and comparative results with the control group are summarized in Table 2. No significant differences were detected between the MM group and healthy controls with

Table 2. Comparison of frequencies of TNF-α, TGF-β1, IL-10, IL-6, and IFN-γ gene variants between patients with MM and healthy controls.

		MM		Control				
	Genotype	n^a	%	n^a	%	OR (B)	95% CI	*p*
TNF-α (–308)	GG	83	73.5	98	86.7	0.423#	0.213–0.840#	**0.012#**
	AG	29	25.6	15	13.3	0.435*	0.218–0.871*	**0.019***
	AA	1	0.9	–	0	1.009#	0.992–1.027#	1.000#
	G allele	195		211		1.515	0.7592–3.023	0.311
	A allele	21		15		0.6601	0.3308–1.317	0.311
TGF-β1 (codon 10)	CC	19	16.8	15	13.3	0.538*	0.229–1.265*	0.155*
	TC	66	58.4	58	51.3	0.655*	0.357–1.203*	0.173*
	TT	28	24.8	40	35.4	0.757#	0.364–1.577#	0.577#
	C allele	104		88		1.337	0.9196–1.943	0.1535
	T allele	122		138		0.7480	0.5146–1.087	0.1535
TGF-β1 (codon 25)	GG	94	83.2	89	78.7	0.597#	0.220–1.621#	0.312#
	GC	16	14.2	23	20.4	1.462*	0.717–2.980*	0.296*
	CC	3	2.6	1	0.9	0.299*	0.030–2.953*	0.301*
	G allele	204		201		1.153	0.6296–2.113	0.7579
	C allele	22		25		0.8671	0.4733–1.588	0.7579
IL-10 (–1082)	AA	34	30.1	43	38.1	1.427#	0.821–2.481#	0.261#
	AG	66	58.4	52	46.1	0.598*	0.267–1.339*	0.211*
	GG	13	11.5	18	15.8	0.895*	0.247–3.237*	0.865*
	A allele	134		138		0.9288	0.6372–1.354	0.7732
	G allele	92		88		1.077	0.7386–1.569	0.7732
IL-10 (–819)	CC	60	53.1	56	49.6	0.684*	0.241–1.941*	0.476*
	CT	46	40.7	47	41.6	0.742*	0.257–2.141*	0.581*
	TT	7	6.2	10	8.8	0.868#	0.515–1.463#	0.690#
	C allele	166		159		1.166	0.7731–1.758	0.5301
	T allele	60		67		0.8578	0.5688–1.294	0.5301
IL-10 (–592)	CC	60	53.1	56	49.6	0.684*	0.241–1.941*	0.476*
	CT	46	40.7	47	41.6	0.742*	0.257–2.141*	0.581*
	TT	7	6.2	10	8.8	0.868#	0.515–1.463#	0.690#
	C allele	166		159		1.166	0.7731–1.758	0.5301
	T allele	60		67		0.8578	0.5688–1.294	0.5301
IL-6 (–174)	GG	64	56.6	58	51.3	0.683*	0.246–1.894*	0.464*
	GC	37	32.8	48	42.4	1.537*	0.871–2.710*	0.138*
	CC	12	10.6	7	6.2	1.239#	0.733–2.091#	0.505#
	G allele	165	164			1.023	0.6757–1.548	0.9158
	C allele	61	62			0.9779	0.6461–1.480	0.9158
IFN-γ (+874)	TT	32	28.3	18	15.9	2.085#	1.089–3.990#	**0.037#**
	TA	57	50.5	47	41.6	1.410*	0.693–2.871*	0.344*
	AA	24	21.2	48	42.5	3.360*	1.551–7.281*	**0.002***
	T allele	121		83		1.985	1.363–2.892	**0.0005**
	A allele	105		143		0.5037	0.3457–0.7337	**0.0005**

Note. Bold values show the statistically significant expression level of cytokines in Multiple Myeloma patients.
[a]*n* = 113, *OR (95%CI) was adjusted by age and sex, #Fisher's exact test.

respect to the distributions and numbers of genotypes and alleles in TGF-β1 (codon 10 and 25), IL-6 (–174), and IL-10 (–1082, –819, –592). In TNF-α gene (–308) variant group, while GG subgroup was significantly lower in MM patients (OR = 0.423, $p = 0.012$), AG genotype was significantly higher in MM patients compared with the control group (OR = 0.435, $p = 0.019$). There was no significant difference in G and A alleles when compared with control group and MM patients ($p > 0.05$). High expression of AG genotype of TNF-α gene in the patient group suggests carrying this genotype has a role in MM development, while presence of GG genotype associated with low expression appears to protect against MM. IFN-γ (+874) gene variant TT genotype was found to be significantly higher (high expression) in MM patients compared with controls (OR = 2.085, $p = 0.037$), while AA genotype was significantly lower (OR = 3.360, $p = 0.002$). IFN-γ (+874) variant allele

assessment showed T allele was significantly higher in MM patients compared with healthy controls (OR = 1.985, $p = 0.0005$), while A allele was significantly lower (OR = 0.5037, $p = 0.0005$). High expression of IFN-γ gene TT genotype and T allele in MM patients suggests an association regarding predisposition for the disease, while AA genotype and A allele appear to protect against MM development. Evaluation of TGF-β1 (codon 25) variant revealed a deviation from HWE in the patient group, while no deviation was observed in the control group ($P^{HWE} = 0.038$, $C^{HWE} = 0.714$). IL-10 (–1082) variant evaluation showed a deviation from HWE in the patient group but not in the control group ($P^{HWE} = 0.025$, $C^{HWE} = 0.731$). There were no deviations from HWE in both the patient and control group.

Among the 113 patients included in the study, 13 were followed up for less than 6 months, therefore 100 patients were analyzed for 5-year OS and 5-year EFS. The 5-year OS was 55.9 months and 5-year EFS was 39.1 months in these 100 patients. On univariate analyses, while three factors that predicted for better OS were IPI I at diagnosis ($p = 0.008$), ECOG < 1 ($p = 0.01$), platelet count ≥100,000 ($p = 0.005$) and the four factors that predicted for better EFS were IPI I at diagnosis ($p = 0.012$), male gender ($p = 0.022$), platelet count ≥100,000 ($p = 0.016$), and applying APSCT as first-line treatment ($p = 0.045$) (Table 3).

The two factors – gender and APSCT – found to significantly affect 5-year EFS on univariate analysis were evaluated using multivariate analysis (Cox proportional hazard model backward) and male gender ($p = 0.002$) and APSCT ($p = 0.035$) remained statistically significant. Platelet count and IPI I/II/III, which are factors significantly associated with both 5-year OS and 5-year EFS, were evaluated using multivariate analysis (Cox proportional hazard model backward); IPI I remained significant for both 5-year OS ($p = 0.018$) and 5-year EFS ($p = 0.022$), and platelet count ≥100,000 remained significant for 5-year OS ($p = 0.021$) and 5-year EFS ($p = 0.002$) (Table 4).

Discussion

MM, an incurable B-cell malignancy, is characterized by an excess of monotypic plasma cells in the bone marrow secreting monoclonal immunoglobulins. It accounts for approximately 10% of hematological malignancies and 1% of cancer deaths in Western countries (Purdue et al., 2011). Cytokines regulate the immune responses and many other biological processes. SNPs of cytokines and receptors affect cytokine expression levels and thus play a substantial role in many diseases.

TNF-α is one of these important cytokines. TNF–308G/A polymorphisms encoding high TNF-α levels may be important in the susceptibility or severity of diseases. High TNF production may promote the condition leading to the development of cancer like non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia, autoimmune diseases as rheumatoid arthritis, ankylosing spondylitis, and many infectious diseases (Elahi et al., 2009). The role of SNPs of TNF-α in the development of MM was shown in several studies. TNF-α (–308 and –238) gene variants and cytokine levels were examined in 81 relapsed refractory MM patients treated with thalidomide and a larger group of 255 MM patients and the control group. TNF-α (–308) GG genotype carrier MM patients were found with higher TNF-α levels ($p = 0.006$) which suggested its contribution in development of the disease (Neben et al., 2002). In another study, 210 MM patients treated with thalidomide and 218 healthy controls were evaluated for

Table 3. Univariate analysis (Logrank test) of prognostic factors in 100 patients with MM.

			5-year OS	Logrank	5-year EFS	Logrank
		N	Median* (%)	*p*-Value	Median* (%)	*p*-Value
All patients		100	55.9 (42)		39.1 (26)	
Gender	Female	43	51.5		30.5	
	Male	57	59.2	0.226	50.5	**0.022**
Age	<65	69	57.7		39.5	
	≥65	31	49.6	0.097	39.1	0.324
Stage (Salmon–Durie)	II	23	55.3		39.1	
	III	77	55.9	0.502	39.1	0.135
	A	75	55.9		40.7	
	B	25	54.0	0.235	34.5	0.750
IPI (ISS)	I	26	81.3		53.2	
	II	39	56.9	36.1		
	III	35	51.5	0.008	27.2	**0.012**
Ig subtypes	κ	65	55.9		40.7	
	λ	35	58.5	0.766	39.1	0.626
	G	63	50.5		40.7	
	A	22	49.6		30.5	
	Light chain	15	50.5	0.503	28.8	0.768
ECOG	≤1	63	51.5		39.1	
	>1	37	58.5	0.010	39.1	0.235
Platelet (×103/L)	<100	14	17.5		17.5	
	≥100	86	58.5	0.005	39.5	**0.016**
LDH (IU/L)	<480	81	54.0		38.8	
	≥480	19	59.2	0.413	53.2	0.459
CRP (mg/L)	<5	39	49.6		35.0	
	≥5	61	59.2	0.291	40.7	0.689
First-line treatment	Bortezomib	59/41	50.5/59.2	0.393	39.5/38.8	0.869
	±imid	52/48	59.2/42.0	0.084	45.8/26.9	0.128
	±APSCT	48/52	59.5/51.5	0.060	39.5/26.9	**0.045**
TNF-α (–308)	GG[a]	70	58.5		39.5	
	GA/AA[c]	30	55.3	0.702	36.1	0.322
TGF-β (codons 10, 25)	TCGC, TTGC, CCGC, CCCC, CCGG, TCCC[ab]	26	50.5		24.6	
	TTGG, TCGG[c]	74	57.7	0.602	45.3	0.210
IL-10 (–1082, –819, –592)	GCC/ACC, GCC/ATA, ACC/ACC,ACC/ATA, ATA/ATA[ab]	88	55.9		39.1	
	GCC/GCC[c]	12	58.5	0.409	29.1	0.410
IL-6 (–174)	CC[a]	10	55.3		45.8	
	GG/GC[c]	90	55.9	0.555	39.1	0.108
IFN-γ (+874)	AA/TA[ab]	72	54.0		39.5	
	TT[c]	28	59.2	0.445	39.1	0.726

Note. Bold values indicate statistically significant values.
OS, overall survival; EFS, event-free survival; ECOG, performance status; LDH, lactic dehydrogenase; IPI, International Prognostic Index; ISS, International Staging System.
[a]Low production.
[b]Intermediate production.
[c]High production.
*Median (month).

Table 4. Multivariate analysis of 100 MM patients (Cox proportional hazard model backward).

	OS			EFS		
	(B) relative risk	95% CI	*p*	(B) relative risk	95% CI	*p*
Gender M/F				0.435	0.256–0.739	0.002
IPI I/II/III	1.630	1.089–2.439	0.018	1.524	1.062–2.187	0.022
Platelet (×103/L) <100/≥100	2.207	1.125–4.328	0.021	2.902	1.463–5.756	0.002
First-line treatment ± APSCT				1.729	1.039–2.876	0.035

OS, overall survival; EFS, event-free survival; IPI, International Prognostic Index; APSCT, autologous peripheral blood stem cell transplantation.

TNF-α (–238, –308) gene polymorphism, and TNF-α (–308) GA genotype ($p = 0.017$) and G > A variant ($p = 0.02$) were found significantly low in MM patients. It was concluded that GA + AA genotype carrier state and G > A variant were protective against MM (Du et al., 2009). In an Hungarian population of 94 MM patients, 141 control, were controlled for TNF-α (–308) A allele which was found significantly reduced in the MM patient group ($p = 0.027$) (Kadar et al., 2008), and similarly in a British population of 181 MM patients and 233 controls, A variant was found to be significantly low in the MM patients (Gareth et al., 2005) and it was concluded that A allele was protective against development of MM. In our study, it was determined that TNF-α (–308) gene GG genotype was significantly low ($p = 0.012$) and GA genotype was significantly high ($p = 0.019$) in MM. It suggests that GA genotype carrier state is a predisposing factor for development of MM, while GG genotype is protective against MM. However, TNF-α (–308) GG genotype was not found to be significantly associated with response to first-line treatment, 5-year OS, or 5-year EFS ($p > 0.05$).

There are studies investigating the relation between TGF-β1 (codon 10 and 25) gene variants and prostate cancer and EBV-related hematologic diseases in the literature. In the study done by Omrani et al. (2009), it was detected that TGF-β1 (codon 10) TT genotype and T allele carriers have 1.67-fold higher risk for developing prostate cancer, while Li et al. (2008) reported a positive correlation did not exist between TGF-β1 (codon 10 and 25) variants and both TGF-β1 levels and clinical-pathological parameters in gastric cancer patients. There are limited number of studies examining the relation between MM and TGF-β1 levels and TGF-β1 (codon 10 and 25) variants. Kyrtsonis et al. (1998) assessed TGF-β1 levels in 35 MM patients including some with thrombocytopenia at various stages and 44 control group patients with other hematological malignancies including 17 patients with thrombocytopenia. Low TGF-β1 levels were found in all thrombocytopenic patients regardless of presence of malignancy, which confirmed that TGF-β1 is produced by platelets. Additionally, high TGF-β1 levels were found in myeloma patients with immune paresis, which suggests TGF-β1 may have a role in immunosuppression in MM patients. Banu et al. (2011) detected higher TGF-β1 (codon 10) gene TT variant in MM patients in their study on 80 MM patients and 100 healthy controls, and reported subjects who are carriers of this genotype may have predisposition to MM. Wang et al. (2008) studied TGF-β1 levels and SNP on codons 10, 25, and 263 in monoclonal gammopathy (MG) patients and the control group. Gene variants were not detected on TGF-β1 codon 25 and codon 263. The highest plasma TGF-β1 levels were found in codon 10 TT genotype. Codon 10 (C > T) allele frequency was compared to M protein type and amount, and a significance was not detected ($p > 0.05$). In our study, both the comparison between MM patients and the control group for TGF-β1 (codons 10 and 25) genotypes and allele variants and the evaluation of relation with clinical parameters revealed no significant findings ($p > 0.05$). A relation was not found between TGF-β1 (codons 10 and 25) variants and response to first-line treatment, 5-year OS and 5-year EFS ($p > 0.05$). Evaluation of TGF-β1 (codon 10) variant did not show a deviation from HWE in both the patient and control groups. Evaluation of TGF-β1 (codon 25) variant revealed a deviation from HWE in the patient group, while no deviation was observed in the control group ($P^{HWE} = 0.038$, $C^{HWE} = 0.714$).

Studies investigating the relation between IL-10 gene (–1082, –819, and –592) variants and various malignancies are available in the literature. Cil et al. evaluated the relation between papillary thyroid carcinoma (PTC) and IL-10 (–1082), IL-6 (–174), and TNF-α (–308) gene variants in 190 PTC patients and 216 healthy controls and found significantly higher IL-10 (–

1082) GG genotype (p = 0.049) and IL-10 (–1082) G allele (p = 0.009) in the patient group compared with the control group. It was concluded that IL-10 (–1082) variant GG genotype and G allele carrier state could cause disposition to PTC (Cil et al., 2014). Mazur et al. (2005) compared IL-6 (–174 GC) and IL-10 (–1082 AG, –819 CT, –592 AC) promotor site SNPs in MM patients and controls and detected a significant relation between variants and disposition to the disease. Banu et al. found significantly higher rates of IL-10 (–1082) GG and IL-10 (–592) CC genotypes in MM patients in their study comparing 80 MM patients and 100 healthy controls and detected higher cytokine levels in MM patients with this genotype. They stated that these variants may cause predisposition to the disease and may be related to clinical and biological parameters (Banu et al., 2011). Zheng et al. investigated IL-10 (–1082) gene variant in 73 MM and 27 monoclonal gammopathy of undetermined significance (MGUS) patients and healthy controls. It was found that IL-10 (–1082) GG genotype and G allele carrier status rates were higher in MM patients and these polymorphisms may cause predisposition to MM (Zheng et al., 2001). In our study, both the comparison between MM patients and the control group for IL-10 gene (–1082, –819, and –-592) genotype and allele variants and the evaluation of relation with clinical parameters revealed no significant findings ($p > 0.05$) (Tables 2 and 3). Evaluation of IL-10 gene (–819 and –592) variant revealed no deviation from HWE in both the patient and control groups, while deviation in HWE was observed for IL-10 gene (–1082) variant in the patient group (P^{HWE} = 0.025, C^{HWE} = 0.731).

In MM biology, the discovery of IL-6, providing the growth and survival of myeloma cells, has been a turning point. Several studies investigating the relation between IL-6 (–174) variant and various cancer cells have been performed, some of which have found a significant relation, while others have not. Jiao et al. (2014) studied 6202 lung cancer patients and 7067 healthy controls, Mandal et al. (2014) studied 164 prostate cancer patients and 140 healthy controls in a Caucasian population and found a statistically significant relation between IL-6 (–174) variant and the diseases. IL-6 serum levels are known to increase in myelodysplastic syndrome (MDS) and MM. Aladzsity et al. (2009) studied 102 MDS and 100 MM patients and 99 healthy control to define whether gene polymorphisms in IL-6 (174 G > C) and its receptors were associated with cytokine levels, although no statistically significant findings were obtained ($p > 0.05$). Increased IL-6 levels are established as poor prognostic factors in MM. IL-6 (174 G > C) variant and frequency of C allele were investigated in 54 MM patients and 60 healthy controls from Brazil, and high C allele and low IL-6 secretion was detected in the control group, suggesting this allele could be protective against MM. Since genotype distributions of the patient and control groups in the study were similar, it was concluded that genotype differences in IL-6 (–174) variant did not create disposition to MM (Duch et al., 2007). Association between IL-6 (–174, –572, and –597) and IL-6R variants and the diseases were studied in 136 MM, 14 plasmocytoma patients, and healthy controls, and no statistically significant relation was detected (Cozen et al., 2006). In another study, IL-6 (–174 GC) variant and MM were not found to be associated (Mazur et al., 2005). Banu et al. (2011) detected high incidence of IL-6 (–174) GG genotype in MM patients and reported increased cytokines in patients with this variant which resulted in increased tendency for the disease. In our study, both the comparison between MM patients and the control group for IL-6 gene (–174) genotype and allele variant and the evaluation of relation with clinical parameters revealed no significant findings ($p > 0.05$). IL-6 (–174) variant was not associated with response to first-line therapy, 5-year OS, or 5-year EFS according to univariate analysis ($p > 0.05$).

Du et al. (2013) found significantly higher IFN-γ (+874) AT and AA genotypes in patients with esophagus cancer, while Wang et al. (2011) found significantly higher AA genotype (OR = 2.22, $p = 0.012$) and A allele ($p = 0.009$) in patients with cervix cancer compared with control groups. Gonullu et al. (2007) assessed the association of TNF-α, TGF-β1, IL-10, IL-6, and IFN-γ variants with breast cancer in 38 patients and 24 healthy controls and found that IL-6 (–174) GC genotype and IL-10 (–1082, –819, –592) GCC/ATA haplotype were predisposing factors for breast cancer. The majority of the available studies investigating the relation between IFN-γ (+874) variant and cancer are related to breast or cervix cancer. Among the solid cancers, positive relation between cancer and IFN-γ (+874) AA genotype has been established particularly for breast and cervix cancers. Limited reports are available regarding the relation between IFN-γ (+874) variant and hematologic malignancies. Stern et al. (2010) assessed IFN-γ (+874) AT variant and disease relation in 236 patients who developed secondary NHL following solid organ transplantation such as liver, kidney, or heart, and found no significant relation for disease predisposition. A study from Romania assessed 80 MM patients and 100 healthy controls and found significantly higher rate of IFN-γ (+874) AA genotype in the patient group and suggested this genotype could cause tendency for the disease (Banu et al. 2011). In our study, TT genotype (OR = 2.085, $p = 0.037$) and T allele (OR = 1.985, $p = 0.0005$) were found significantly higher in MM patients compared with controls, while AA genotype (OR = 3.360, $p = 0.002$) and A allele (OR = 0.5037, $p = 0.0005$) were significantly lower. IFN-γ gene (+874) TT genotype and T allele were found to be related with disposition to the disease, while AA genotype and A allele had protective effects.

When we compare clinical features and factors that affect prognosis (age, gender, stage (Salmon–Durie and IPI), Ig subtype, ECOG, platelet count, LDH, and CRP levels, 5-year EFS, and 5-year OS), patients with IPI-I have longer OS compared with patients with IPI III and II ($p = 0.008$), ECOG < 1 at diagnosis ($p = 0.01$), and also patients with higher platelet count (≥100 × 10^3/ml) have better OS ($p = 0.005$), and patients with IPI I at diagnosis ($p = 0.012$), male gender ($p = 0.022$), platelet count ≥100,000 ($p = 0.016$), applying APSCT as first-line treatment ($p = 0.045$) were the factors that predicted for better EFS in our study. Among the study results, it may be interesting that female gender had negative impact on EFS. We live in southeastern region of Turkey and we collected samples in this area. Unfortunately, in this region, women have worse clinical status and lower education level. Women are less self-caring and they get less attention from their husbands. Therefore, females are normal to have a negative impact on EFS.

Conclusions

In this study, cytokine polymorphisms and the relationship between cytokine polymorphisms and clinical parameters in MM were investigated in Turkish population for the first time, except TNF-α. In conclusion, increased rate of AG genotype highly expressed in TNF-α (–308) variant in MM patients suggests these variants may be effective in the etiopathogenesis of MM, while low expression of GG genotype may suggest this genotype is protective for MM. High rate of TT genotype and T allele in IFN-γ (+874) variant in MM patients suggests this variant may have a role in the etiopathogenesis of MM, while low expression of AA genotype and A allele may be protective for MM. Additionally, evaluation of clinical parameters and cytokine gene

variants suggest patient's ECOG and IPI at baseline, gender, and platelet count have prognostic value in MM.

References

Aladzsity I, Kovács M, Semsei A, et al. (2009). Comparative analysis of IL6 promoter and receptor polymorphisms in myelodysplasia and multiple myeloma. Leuk Res 33, 1570–1573.

Anderson KC, Alsina M, Bensinger W, et al. (2013). Multiple myeloma, version 1.2013. J Natl Compr Canc Netw 11, 11–17.

Banu C, Moise A, Arion CV, et al. (2011). Cytokine gene polymorphisms support diagnostic monitoring of Romanian multiple myeloma patients. J Med Life 4, 264–268.

Black RA, Rauch CT, Kozlosky CJ, et al. (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 385, 729–733

Brown RD, Pope B, Murray A, et al. (2001). Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-beta1 and interleukin-10. Blood 98, 2992–2998.

Cifci S, Yilmaz M, Pehlivan M, et al. (2011). DNA repair genes polymorphisms in multiple myeloma: no association with XRCC1 (Arg399Gln) polymorphism, but the XRCC4 (VNTR in intron 3 and G-1394T) and XPD (Lys751Gln) polymorphisms is associated with the disease in Turkish patients. Hematology 16, 361–367.

Cil E, Kumral A, Kanmaz-Ozer M, et al. 2014. Interleukin-10-1082 gene polymorphism is associated with papillary thyroid cancer. Mol Biol Rep 41, 3091–3097.

Cozen W, Gebregziabher M, Conti DV, et al. (2006). Interleukin-6-related genotypes, body mass index, and risk of multiple myeloma and plasmacytoma. Cancer Epidemiol Biomarkers Prev 15, 2285–2291.

Dancester CP, Hussain OAN, Jackson WPU. (1959). Clinical features of multiple myeloma. A review of the clinical manifestations and laboratory investigations in 40 cases. Postgrad Med J 35, 662–667.

Dogra S, Khullar G. (2013). Tumor necrosis factor-α antagonists: Side effects and their management. Indian J Dermatol Venereol Leprol 79(Suppl), S35–S46.

Du J, Yuan ZG, Zhang CY, et al. (2009). Effect of TNF-alpha gene polymorphism on outcome of thalidomide-based regimens for multiple myeloma. Zhonghau Xue Ye Xeu Za Zhi 30, 649–653.

Du W, Ye W, Chen M, et al. (2013). Association research between polymorphism of IFN-gamma and IL-10, environmental risk factors, and susceptibility to esophageal cancer. Wei Sheng Yan Jiu 42, 770–776.

Duch CR, Figueiredo MS, Ribas C, et al. (2007). Analysis of polymorphism at site -174 G/C of interleukin-6 promoter region in multiple myeloma. Braz J Med Biol Res 40, 265–267.

Durie BG. (1986). Staging and kinetics of multiple myeloma. Semin Oncol 13, 300–309.

Elahi MM, Asotra K, Matata BM, Mastana SS. (2009). Tumor necrosis factor alpha -308 gene locus promoter polymorphism: an analysis of association with health and disease. Biochim Biophys Acta 1792, 163–172.

Gareth JM, Peter JA, Fiona KM, et al. (2005). Haplotypes in the tumour necrosis factor region and myeloma. Br J Haematol 129, 358–365.

Gonullu G, Basturk B, Evrensel T, et al. (2007). Association of breast cancer and cytokine gene polymorphism in Turkish women. Saudi Med J 28, 1728–1733.

Hayashi T, Hideshima T, Nguyen AN, et al. (2004). Transforming growth factor beta receptor I kinase inhibitor down-regulates cytokine secretion and multiple myeloma cell growth in the bone marrow microenvironment. Clin Cancer Res 10, 7540–7546.

Heinrich MC, Dooley DC, Keeble WW. (1995). Transforming growth factor beta 1 inhibits expression of the gene products for steel factor and its receptor (c-kit). Blood 85, 1769–1780.

Hideshima T, Bergsagel PL, Kuehl WM, Anderson KC. (2004). Advances in biology of multiple myeloma: clinical applications. Blood 104, 607–608.

Jiao F, Xu D, Li Q, et al. (2014). Lack of association between -174G>C and -634C>G polymorphisms in interleukin-6 promoter region and lung cancer risk: a meta-analysis. Tumour Biol 35, 5021–5027.
Joshua DE. (1988). Biology of multiple myeloma-host-tumour interactions and immune regulation of disease activity. Hematol Oncol 6, 83–88.
Kadar K, Kovacs M, Karadi I, et al. (2008). Polymorphism of TNF-alpha and LT-alpha genes in multiple myeloma. Leuk Res 32, 1499–1504.
Karaoglan I, Pehlivan S, Namiduru M, et al. (2009). TNF-alpha, TGF-beta, IL-10, IL-6 and IFN-gamma gene polymorphisms as risk factors for brucellosis. New Microbiol 32, 173–178.
Kovacs E. (2010). Interleukin-6 leads to interleukin-10 production in several human multiple myeloma cell lines. Does interleukin-10 enhance the proliferation of these cells? Leuk Res 34, 912–916.
Kyrtsonis MC, Repa C, Dedoussis GV, et al. (1998). Serum transforming growth factor-beta 1 is related to the degree of immunoparesis in patients with multiple myeloma. Med Oncol 15, 124–128.
Li X, Yue ZC, Zhang YY, et al. (2008). Elevated serum level and gene polymorphisms of TGF-beta1 in gastric cancer. J Clin Lab Anal 22, 164–171.
Lodh M, Goswami B, Gupta N, et al. (2012). Assessment of oxidative stress and inflammatory process in patients of multiple myeloma. Indian J Clin Biochem 27, 410–413.
Mandal S, Abebe F, Chaudhary J. (2014). 174G/C polymorphism in the interleukin-6 promoter is differently associated with prostate cancer incidence depending on race. Genet Mol Res 13, 139–151.
Matsumoto T, Abe M. (2011). TGF-β-related mechanisms of bone destruction in multiple myeloma. Bone 48, 129–134.
Mazur G, Bogunia-Kubik K, Wrobel T, et al. (2005). IL-6 and IL-10 promoter gene polymorphisms do not associate with the susceptibility for multiple myeloma. Immunol (Lett) 96, 241–246.
Miller SA, Dykes DD, Polesky HF. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16, 1215.
Neben K, Mytilineos J, Moehler TM, et al. (2002). Polymorphisms of the tumor necrosis factor-alpha gene promoter predict for outcome after thalidomide therapy in relapsed and refractory multiple myeloma. Blood 100, 2263–2265.
Omrani MD, Taghipour-Bazargani S, Salari-Lak S, Bagheri M. (2009). Association of codon 10 polymorphism of the transforming growth factor beta 1 gene with prostate cancer and hyperplasia in an Iranian population. Urol Int 83, 329–332.
Palumbo A, Bruno B, Boccadoro M, Pileri A. (1995). Interferon-gamma in multiple myeloma. Leuk Lymphoma 18, 215–219.
Purdue MP, Lan Q, Menashe I, et al. (2011). Variation in innate immunity genes and risk of multiple myeloma. Hematol Oncol 29, 42–46.
Stern M, Opelz G, Dohler B, Hess C. (2010). Natural killer-cell receptor polymorphisms and posttransplantation non-Hodgkin lymphoma. Blood 115, 3960–3965.
Wajant H, Pfizenmaier K, Scheurich P. (2003). Tumor necrosis factor signaling. Cell Death Differ 10, 45–65.
Wang H, Gao C, Xu L, et al. (2008). Laboratory characterizations on 2007 cases of monoclonal gammopathies in East China. Cell Mol Immunol 5, 293–298.
Wang Q, Zhang C, Walayat S, et al. (2011). Association between cytokine gene polymorphisms and cervical cancer in a Chinese population. Eur J Obstet Gynecol Reprod Biol 158, 330–333.
Waterston A, Bower M. (2004). TNF and cancer: good or bad? Cancer Therapy 2, 131–148.
Zheng C, Huang D, Liu L, et al. (2001). Interleukin-10 gene promoter polymorphisms in multiple myeloma. Int J Cancer 95, 184–188.