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Relationship of *LEP, LEPR* Variants, and *LEP* Methylation with Multiple Myeloma and Prognosis

Yasemin Oyacı[1,2], Ayşe Feyda Nursal[3], İstemi Serin[4], Sacide Pehlivan[2], Mustafa Pehlivan[5]

[1]İstanbul University Institute of Graduate Studies in Health Sciences, İstanbul, Turkey
[2]İstanbul University, İstanbul Faculty of Medicine, Department of Medical Biology, İstanbul, Turkey
[3]Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey
[4]Ağrı İbrahim Çeçen University, Ağrı Training and Research Hospital, Clinic of Hematology, Ağrı, Turkey
[5]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Hematology, İstanbul, Turkey

ABSTRACT

Introduction: Leptin (LEP) and LEP receptor (LEPR) play roles in cancer progression. We evaluated *LEP*-2548G/A and *LEPR* 668 A/G variants in patients with multiple myeloma (MM). In addition, the methylation status of CpG sites at 31 and 51 nucleotides (nt) according to the transcription start region of the *LEP* gene was examined.

Methods: DNA was extracted from the peripheral blood of study participants who were healthy controls and patients with MM. These variants were analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The methylation at -31 and -51 nt in the *LEP* was performed using the methylation-specific PCR method. The 2-year progression-free survival (PFS) and 2-year overall survival (OS) were evaluated according to prognostic factors.

Results: There was no significant difference in the genotype distributions of LEPR 668A/G and LEP-2548G/A between the control and patient groups ($p>0.05$). We found that -31 and -51 nt regions of the *LEP* gene were unmethylated in the patient group compared with the control group ($p=0.051$ and $p=0.001$, respectively). The -31 nt methylation was unchanged in 15 patients (78.94%). PFS and OS were higher in these patients than in the others. In multivariate analysis, the methylated/unmethylated ratio at -31 nt methylation was associated with a poor prognosis ($p=0.020$).

Conclusion: To our knowledge, our study is the first to examine these variants and their methylation status in Turkish patients with MM. Our results showed that *LEP* gene -31 nt unmethylation was associated with PFS and OS. These results need to be confirmed in different ethnic and larger sample groups.

Keywords: Multiple myeloma, leptin, leptin receptor, PCR-RFLP, MS-PCR

Introduction

Multiple myeloma (MM) is a hematological malignancy that occurs when abnormal plasma cells grow uncontrollably and invade the bone marrow (BM) (1). The average age at diagnosis is 60 years old (2). MM accounts for 1% of all malignancies and 10% of all hematological cancers (3). The incidence of MM is highest in the African population, whereas the lowest rates are observed in the Asian and Mediterranean populations (4). Although advancements in MM treatment have raised the 5-year survival rate to almost 50%, MM is still regarded as incurable. Although the exact cause of MM remains unknown, in previous studies, age, male gender, obesity, exposure to ionizing radiation, and a history of monoclonal gammopathy of undetermined significance (MGUS) have been mentioned as risk factors (5). Many studies have shown that genetic factors play an essential role in the etiology of MM. Genetic factors, particularly those pertaining to immune response genes, increase the likelihood of MM development.

Leptin (LEP), a hormone, is primarily produced by white adipose cells in the body. By affecting energy expenditure and food intake, it has a significant effect on maintaining body weight homeostasis (6). In addition, LEP has various other functions in the body, such as influencing insulin resistance, cell proliferation, oxidative stress, cancer cell inflammation, apoptosis, and immune regulation (7). LEP is found in human peripheral blood, cord blood, and BM blood. Various studies have shown that high levels of *LEP* may affect the onset and progression of many malignancies. LEP exerts its biological effect by binding to and activating leptin receptors (LEPR). The *LEP* gene is on chromosome 7q31.3. The leptin receptor

Address for Correspondence: İstemi Serin MD, Ağrı İbrahim Çeçen University, Ağrı Training and Research Hospital, Clinic of Hematology, Ağrı, Turkey
Phone: +90 532 317 23 93 **E-mail:** serinistemi@hotmail.com **ORCID ID:** orcid.org/0000-0003-1855-774X

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encoded by the *LEPR* gene is a member of the class 1 cytokine receptor family. LEPR plays a role in the pathogenesis of many malignant cancers (8). *In vitro* and *in vivo* studies have demonstrated the critical role of LEP/LEPR signaling in the growth, maturation, and functionality of hematopoietic progenitor cells and mature blood cells (9). The *LEP* and *LEPR* genes have single-nucleotide polymorphisms (SNPs). The variant -2548G/A (rs7799039) is situated in the promoter region of the *LEP* gene, whereas the variant 668A/G (rs1137101) of the *LEPR* gene is located in the exon 6 region of the *LEPR* gene (10).

Based on this information, in this study, we evaluated whether *LEP*-2548G/A and *LEPR* 668 A/G variants cause susceptibility in patients with MM. In addition, the methylation of CpG sites located at -31 and -51 nucleotides (nt) according to the transcription start region of the *LEP* gene was examined.

Methods

Study population

Nineteen patients (7 females and 12 males, median age: 60 years) diagnosed with MM were included in the study. Staging was assigned according to the Durie and Salmon and the International Staging System criteria (11). The clinical characteristics and treatment protocols of the patients were examined. The 2-year progression-free survival (PFS) and 2-year overall survival (OS) were evaluated according to prognostic factors. The healthy control group consisted of 93 healthy individuals (49 females, 44 males, median age: 45 years). The control group was selected from volunteers without any malignancies or chronic diseases. All participants received written explanations of the study objectives and methods.

The Local Ethics Committee approved all procedures involving human subjects during this study, which was conducted in accordance with the Declaration of Helsinki's criteria.

The study was approved by the İstanbul University, İstanbul Faculty of Medicine Local Ethical Committee (approval number: 62734, date: 29/05/2020).

Genotyping

Peripheral blood was used to extract DNA samples from the groups. The *LEP*-2548G/A and *LEPR* 668A/G variants were analyzed using the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The primer sequence used for *LEP*-2548G/A F:5'-TTTCCTGTAATTTTCCCGTGAG-3' and R:5'-AAAGCAAAGACAGGCATA AAAA- 3', the primer sequence for *LEPR* 668A/G was F: 5'-GCCTAATCCAGTATTTTATATCTG-3' R: 5'GCCACTCTTAATACCCCCAGT. HhaI restriction enzyme for *LEP*-2548G/A and MspI enzyme for *LEPR* 668 A/G were used (12,13). *LEP* gene -31 and -51 nt methylation was performed using the methylation-specific PCR (MS-PCR) method previously described by García-Cardona et al. (14).

Statistical Analysis

SPSS, version 20.0 for Windows (SPSS, Inc., Chicago, IL, USA) was employed to examine all data. Using Pearson's χ^2 analysis, categorical data were evaluated. In addition, the 95% confidence interval (CI) and odds ratio (OR) were computed. OR (95% CI) was age- and sex- adjusted. The log-rank test was used to compare the patient group's 2-year PFS and OS based on prognostic variables. The threshold for statistical significance was set at a p-value of less than 0.05 in all two-tailed analyses.

Results

LEP-2548G/A, *LEPR* 668 A/G variants, and -31 and -51 nt of the *LEP* gene were examined in patients with MM and controls. The clinical characteristics and treatment regimens of the groups are listed in Table 1.

Table 2 summarizes the distributions of genotypes for these variants in groups. *LEP-2548G/A* and *LEPR-668A/G* genotype distributions did not differ significantly between the patient and control groups ($p>0.05$). The methylation status of the -31 and -51 nt regions of the *LEP* gene significantly differed between patients and controls. There was no methylation in the -31 and -51 nt regions of the *LEP* gene in the

Table 1. Clinical features and treatment regimens of the groups

	MM patients, n=19 (%)	Controls, n=93 (%)
Age (median)	60 (41-82)	45 (18-81)
Gender, n (%)		
Female/male	7/12 (37/63)	49/44 (53/47)
Ig subtypes, n (%)		
k/L	12/7 (63/37)	
G/A	12/5 (63/26)	
Light chain	11 (58)	
Stage (Salmon-Durie)		
I-II	11 (58)	
III	8 (42)	
A/B	15/4 (79/21)	
ECOG		
>1	6 (32)	
Hb (gr/dL)	11 (7.8-17)	
Leukocytes (µL)	7580 (3680-11330)	
Platelets (10^3/µL)	178 (71-567)	
CRP (mg/dL)	3.7 (2.2-234)	
LDH (IU/L)	185 (52-902)	
Beta 2-microglobulin (mg/L)	5.4 (1.4-29.4)	
Albumin (gr/L)	3.5 (1.9-4.7)	
Treatment		
VCD, APBSCT, and LD	12 (63)	
VCD ± LD	7(37)	
OS: 2 years (%)	61	
PFS: 2 years (%)	60	
Mortality	6 (32)	
Mean follow-up (month)	20.5 (5.5-34.2)	

MM: Multiple myeloma, Ig: Immunoglobulin, ECOG: Eastern Cooperative Oncology Group performance status, Hb: Hemoglobin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, VCD: Bortezomib, cyclophosphamide, and dexamethasone, APSCT: Autologous peripheral blood stem cell transplantation, LD: Lenalidomide and dexamethasone, OS: Overall survival, PFS: Progression-free survival

patient group compared with the control group (p=0.051 and p=0.001, respectively).

The patient group's 2-year PFS and 2-year OS were compared according to prognostic factors. The results are shown in Table 3. The mean 2-year PFS was 22 months, and the mean 2-year OS was 15.2 months. -31 nt methylation was unchanged in 15 patients (78.94%). PFS and OS were higher in these patients than in others (p=0.019 and p=0.048).

In multivariate analysis, the methylated/unmethylated ratio at -31 nt methylation was associated with a poor prognosis (p=0.020) (Table 4).

Table 2. The genotype distributions of *LEP-2548G/A* and *LEPR-668A/G* and methylation status of the *LEP* gene

	MM patients, n=19 (%)	Controls, n=93 (%)	OR Exp (B)	95% CI	p
***LEP*-2548G/A**					
Genotypes					
G/G	9 (47.4)	35 (37.6)	1.491&	0.552-4.028&	0.450&
G/A	5 (26.3)	46 (49.5)	2.406*	0.679-8.523*	0.174*
A/A	5 (26.3)	12 (12.9)	0.662*	0.158-2.782*	0.574*
***LEPR* 668A/G**					
Genotypes					
A/A	8 (42.1)	60 (64.5)	0.930*	0.083-10.467*	0.953*
A/G	10 (52.6)	27 (29.0)	0.360*	0.032-4.070*	0.409*
G/G	1 (5.3)	6 (6.5)	0.806&	0.091-7.105&	0.100&
-31 nt methylation					
Methylated	15 (78.9)	86 (92.5)	4.734*	0.995-22.513*	**0.051***
Unmethylated	4 (21.1)	7 (7.5)			
-51 nt methylation					
Methylated	10 (52.6)	90 (96.8)	52.246*	7.103-384.532*	**0.001***
Unmethylated	9 (47.4)	3 (3.2)			

*: OR (95% CI) was adjusted by age and sex, &Fisher's exact test, OR: Odds ratio, CI: Confidence interval

Table 3. Univariate analysis (log-rank test) of prognostic factors in 19 patients with MM

		n	2-year PFS (%)	Log-rank (p)	2-year OS (%)	Log-rank (p)
		19	60		61	
Gender	Female/male	7/12	88/47	0.252	85/48	0.259
Age	<65/≥65	12/7	51/85	0.261	47/83	0.285
Stage (Salmon-Durie)	II/III	11/8	71/36	0.490	70/48	0.577
	A/B	15/4	60/75	0.939	60/67	0.875
ECOG	≤1/>1	13/6	60/67	0.937	60/67	0.903
CRP (mg/L)	<5/≥5	3/13	80/55	0.465	80/51	0.407
First-line treatment	VCD + APSCT + LD	12	76		76	
	VCD ± LD	7	32 (9 months)*	**0.030**	34 (15.2 month)*	**0.034**
LEP 668A/G	A/A	8	56		56	
	A/G	10	65		62	
	G/G	1	100	0.836	100	0.769
LEP -2548G/A	A/A	5	100		100	
	G/A	5	60		60	
	G/G	9	56	0.294	56	0.433
-31 nt methylation*	Decreased	4	0 (4.3 month)*		0 (14.8 month)*	
	No change	15	78	**0.019**	76	**0.048**
-51 nt methylation*	Decreased	7	62		63	
	Increased	3	66		66	
	No change	9	66	0.876	66	0.831

*After first-line treatment, MM: Multiple myeloma, PFS: Progression-free survival, OS: Overall survival, ECOG: Eastern Cooperative Oncology Group performance status, CRP: C-reactive protein, VCD: Bortezomib, cyclophosphamide, and dexamethasone, APSCT: Autologous peripheral blood stem cell transplantation, LD: Lenalidomide and dexamethasone

Table 4. Multivariate analysis of patients with MM (cox proportional hazard model backward)

	OS			PFS		
	Exp (B) relative risk	95% CI	p	Exp (B) relative risk	95% CI	p
First-line treatment (APSCT +/-)	0.154	0.125-0.964	**0.046**	0.069	0.006-0.732	**0.027**
-31 nt methylation (M/UM)	0.189	0.034-1.039	0.055	0.065	0.007-0.651	**0.020**

MM: Multiple myeloma, OS: Overall survival, PFS: Progression-free survival, CI: Confidence interval, APSCT: Autologous peripheral stem cell transplantation, M: Methylated, UM: Unmethylated

Discussion

Epidemiological and family studies have determined that the risk of MM and its precursor disease, MGUS, is associated with genetic predisposition (15). One of the known and confirmed risk factors for MM and MGUS is family history. In first-degree relatives of patients with MM, the risk of MM increases two to four times, and the risk of MGUS increases two to three times (16). The initiation and development of MM are accompanied by genetic alterations, such as chromosomal translocations, and structural variants, such as deletions, duplications, and insertions. Deleterious single-nucleotide variations are also frequently observed. These may result in the progression of the illness and treatment resistance (17). Some SNPs have also been shown to play a role in MM susceptibility.

The primary element of the BM microenvironment is crucial for the emergence and development of MM. The BM adipose tissue, which constitutes 50-70% of the BM volume (18). Adipose tissue functions as an endocrine organ that contributes to the release of certain adipocytokines in addition to serving as a store of energy (19). Some studies have shown that changing levels of adipokines secreted from fat tissue play a role in the development of various types of cancer (20). LEP also plays an important role in energy balance and metabolism functions as well as receptors related to adaptive immunity, angiogenesis, and the hematopoietic process (21). LEP levels increase in people with excess fatty tissue that are at increased risk of cancer. This suggests that LEP may play a role in the development of cancer (22). According to certain research examining LEP's impact on hematological cancers, LEP somewhat promotes myeloma cell line proliferation and lessens chemotherapy-induced myeloma cell line apoptosis (23-25). In a meta-analysis where 1269 MM patients and 2158 controls were evaluated, the circulating LEP levels of MM patients were found to be higher than those of controls (26). Esheba et al. (27) and Alexandrakis et al. (28) also supported these results (29). However, there are also studies showing the opposite (30,31). There are studies relating genetic variants of LEP and LEPR to the susceptibility of various malignant tumors (32). *LEP*-2548G/A and *LEPR* 668A/G are the most studied variants. The *LEP*-2548G/A is close to the specificity protein-1 transcription factor binding site located in the *LEP* promoter region. This variant is thought to affect *LEP* gene transcription and expression as well as LEP synthesis and secretion from adipose tissue (12). *LEPR* 668A/G is located in the leptin-binding region in the extracellular domain of the gene (12). This variant, which disrupts LEP binding to its receptor, results in an A to G substitution in exon 6, nucleotide 668, and codon 223 of the start codon (33). Bieńkiewicz et al. (34) reported that the *LEP*-2548A/G A/G genotype may reduce the risk of endometrial cancer formation, whereas the A allele may be a risk factor. The LEP-2548A/G polymorphism was linked to an increased overall risk of cancer, according to a meta-analysis (35). A meta-analysis suggested that *LEPR* 668G/A may play a role in the development of breast cancer in East Asians (36). In addition, it was reported that the *LEPR* 668G/A G/G genotype was related to an increased LEP profile among obese breast cancer females (33). Another study found that *LEPR* 668G/A was not associated with cancer in a study of 35,936 subjects in 44 case-control studies (37). There are not many studies examining these variants in hematological malignancies. These two variants are associated with non-Hodgkin lymphoma (38). Lin et al. (39) showed the same result for *LEP*-2548G/A (39).

In this study, we aimed to investigate whether the *LEP*-2548A and *LEPR*-668G/A variants predispose to the development of MM. DNA methylation pattern is an epigenetic change that affects gene expression. Therefore, we also examined the *LEP*-31 and -51 nt methylation status of the *LEP* gene. We found that *LEP-2548G/A* and *LEPR 668A/G* genotype distributions were not associated with MM development. We found that -31 and -51 nt regions of the *LEP* gene were unmethylated in the patient group compared with the control group. In multivariate analysis, we found that the methylated/unmethylated ratio at -31 nt methylation was related to a poor prognosis.

Study Limitations

This study has several limitations. First, the sample group was small. Additionally, only two variants in this pathway were evaluated. Other variants in these genes may also play a role in the development of MM. However, it is also an advantage of the study that *LEP*-2548G/A and *LEPR* 668A/G variants and the methylation status of -31 and -51 nt in the *LEP* gene are examined for the first time.

Conclusion

To the best of our knowledge, this is the first study to assess these variations and the methylation status of this region in Turkish individuals with MM. Although the current study's findings suggest that *LEP* and *LEPR* polymorphisms could not be a risk factor for the onset of MM, further research is necessary to validate and expand on these findings in larger populations and other ethnic groups.

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Ethics Committee Approval: The study was approved by the İstanbul University, İstanbul Faculty of Medicine Local Ethical Committee (approval number: 62734, date: 29/05/2020).

Informed Consent: It wasn't obtained.

Authorship Contributions: Concept - Y.O., A.F.N., İ.S., S.P., M.P.; Data Collection or Processing - Y.O., A.F.N., İ.S., S.P., M.P.; Analysis or Interpretation - Y.O., A.F.N., İ.S., S.P., M.P.; Literature Search - Y.O., A.F.N., İ.S., S.P., M.P.; Writing - Y.O., A.F.N., İ.S., S.P., M.P.

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References

1. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med 2011; 364: 1046-60.
2. Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. Lancet 2009; 374: 324-39.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
4. Tang CH, Liu HY, Hou HA, Qiu H, Huang KC, Siggins S, et al. Epidemiology of multiple myeloma in Taiwan, a population based study. Cancer Epidemiol 2018; 55: 136-41.
5. Alexander DD, Mink PJ, Adami HO, Cole P, Mandel JS, Oken MM, et al. Multiple myeloma: a review of the epidemiologic literature. Int J Cancer 2007; 120(Suppl 12): 40-61.
6. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Helal AN, Chouchane L. Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma. BMC Cancer 2006; 6: 38.
7. Ray A, Cleary MP. The potential role of leptin in tumor invasion and metastasis. Cytokine Growth Factor Rev 2017; 38: 80-97.
8. Lin TC, Hsiao MY. Leptin and Cancer: Updated Functional Roles in Carcinogenesis, Therapeutic Niches, and Developments. Int J Mol Sci 2021; 22: 2870.
9. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol 2000; 68: 437-46.
10. Gotoda T, Manning BS, Goldstone AP, Imrie H, Evans AL, Strosberg AD, et al. Leptin receptor gene variation and obesity: lack of association in a white British male population. Hum Mol Genet 1997; 6: 869-76.
11. Anderson KC, Alsina M, Bensinger W, Biermann JS, Cohen AD, Devine S 2013; 11: 11-7.
12. Bains V, Kaur H, Badaruddoza B. Association analysis of polymorphisms in LEP (rs7799039 and rs2167270) and LEPR (rs1137101) gene towards the development of type 2 diabetes in North Indian Punjabi population. Gene. 2020; 754: 144846.
13. Sabi EM, Bin Dahman LS, Mohammed AK, Sumaily KM, Al-Daghri NM. -2548G>A LEP Polymorphism Is Positively Associated with Increased Leptin and Glucose Levels in Obese Saudi Patients Irrespective of Blood Pressure Status. Medicina (Kaunas) 2022; 58: 346.
14. García-Cardona MC, Huang F, García-Vivas JM, López-Camarillo C, Del Río Navarro BE, Navarro Olivos E, et al. DNA methylation of leptin and adiponectin promoters in children is reduced by the combined presence of obesity and insulin resistance. Int J Obes (Lond) 2014; 38: 1457-65.
15. Greenberg AJ, Rajkumar SV, Vachon CM. Familial monoclonal gammopathy of undetermined significance and multiple myeloma: epidemiology, risk factors, and biological characteristics. Blood 2012; 119: 5359-66.
16. Clay-Gilmour AI, Hildebrandt MAT, Brown EE, Hofmann JN, Spinelli JJ, Giles GG, et al. Coinherited genetics of multiple myeloma and its precursor, monoclonal gammopathy of undetermined significance. Blood Adv 2020; 4: 2789-97.
17. Wiedmeier-Nutor JE, Bergsagel PL. Review of Multiple Myeloma Genetics including Effects on Prognosis, Response to Treatment, and Diagnostic Workup. Life (Basel) 2022; 12: 812.
18. Veldhuis-Vlug AG, Rosen CJ. Clinical implications of bone marrow adiposity. J Intern Med 2018; 283: 121-39.
19. Masarwi M, DeSchiffart A, Ham J, Reagan MR. Multiple Myeloma and Fatty Acid Metabolism. JBMR Plus 2019; 3: 10173.
20. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: role in cancer promotion. Horm Mol Biol Clin Investig 2015; 21: 57-74.
21. Howard JM, Cathcart MC, Healy L, Beddy P, Muldoon C, Pidgeon GP, et al. Leptin and adiponectin receptor expression in oesophageal cancer. Br J Surg 2014; 101: 643-52.
22. Nalabolu MR, Palasamudram K, Jamil K. Adiponectin and leptin molecular actions and clinical significance in breast cancer. Int J Hematol Oncol Stem Cell Res 2014; 8: 31-40.
23. Reseland JE, Reppe S, Olstad OK, Hjorth-Hansen H, Brenne AT, Syversen U, et al. Abnormal adipokine levels and leptin-induced changes in gene expression profiles in multiple myeloma. Eur J Haematol 2009; 83: 460-70.
24. Liu Z, Xu J, He J, Liu H, Lin P, Wan X, et al. Mature adipocytes in bone marrow protect myeloma cells against chemotherapy through autophagy activation. Oncotarget 2015; 6: 34329-41.
25. Pamuk GE, Demir M, Harmandar F, Yesil Y, Turgut B, Vural O. Leptin and resistin levels in serum of patients with hematologic malignancies: correlation with clinical characteristics. Exp Oncol 2006; 28: 241-4.
26. Liu R, Gao D, Lv Y, Zhai M, He A. Importance of circulating adipocytokines in multiple myeloma: a systematic review and meta-analysis based on case-control studies. BMC Endocr Disord 2022; 22: 29.
27. Esheba NE, Shahba A, El Shora O. Assessment of leptin and resistin levels in non-obese multiple myeloma patients and their relation with Ig level and disease stage. J Egypt Natl Canc Inst 2014; 26: 61-6.
28. Alexandrakis MG, Passam FH, Sfiridaki A, Pappa CA, Moschandrea JA, Kandidakis E, et al. Serum levels of leptin in multiple myeloma patients and its relation to angiogenic and inflammatory cytokines. Int J Biol Markers 2004; 19: 52-7.
29. Moschovi M, Trimis G, Vounatsou M, Katsibardi K, Margeli A, Damianos A, et al. Serial plasma concentrations of adiponectin, leptin, and resistin during therapy in children with acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2010; 32: 8-13.
30. Dalamaga M, Karmaniolas K, Panagiotou A, Hsi A, Chamberland J, Dimas C, et al. Low circulating adiponectin and resistin, but not leptin, levels are associated with multiple myeloma risk: a case-control study. Cancer Causes Control 2009; 20: 193-9.
31. Hofmann JN, Liao LM, Pollak MN, Wang Y, Pfeiffer RM, Baris D, et al. A prospective study of circulating adipokine levels and risk of multiple myeloma. Blood 2012; 120: 4418-20.
32. Zhang S, Jiang J, Chen Z, Wang Y, Tang W, Liu C, et al. Investigation of LEP and LEPR polymorphisms with the risk of hepatocellular carcinoma: a case-control study in Eastern Chinese Han population. Onco Targets Ther 2018; 11: 2083-9.
33. Atoum MF, Hamaid Alparrey AA. Association of Leptin Receptor Q223R Gene Polymorphism and Breast Cancer Patients: A Case Control Study. Asian Pac J Cancer Prev 2022; 23: 177-82.
34. Bieńkiewicz J, Romanowicz H, Malinowski A, Smolarz B. Association of Single Nucleotide Polymorphism -2548 G/A (rs12112075) of leptin gene with endometrial cancer and uterine leiomyomas. Eur J Obstet Gynecol Reprod Biol 2017; 218: 113-8.

35. Tang W, Kang M, Liu C, Qiu H. Leptin rs7799039 (G2548A) polymorphism is associated with cancer risk: a meta-analysis involving 25,799 subjects. Onco Targets Ther 2019; 12: 2879-90.

36. Liu C, Liu L. Polymorphisms in three obesity-related genes (LEP, LEPR, and PON1) and breast cancer risk: a meta-analysis. Tumour Biol 2011; 32: 1233-40.

37. Rong G, Tang W, Wang Y, Qiu H, Chen S. Investigation of leptin receptor rs1137101 G>A polymorphism with cancer risk: evidence from 35936 subjects. Biosci Rep 2019; 39: BSR20182240.

38. Skibola CF, Holly EA, Forrest MS, Hubbard A, Bracci PM, Skibola DR, et al. Body mass index, leptin and leptin receptor polymorphisms, and non-hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev. 2004; 13: 779-86.

39. Lin HY, Shi H, Li CY, Chen QC, Huang TB, Liu PC, et al. LEP and LEPR polymorphisms in non-Hodgkin lymphoma risk: a systematic review and pooled analysis. J BUON. 2015; 20: 261-8.