RESEARCH ARTICLE

Birgul Onec [1]
Durdu Mehmet Kos [2]
Gulsah Altun [3]
Mehmet Ali Sungur [4]

[1] Department of Hematology, Department of Internal Medicine, Duzce University Faculty of Medicine, Duzce, Türkiye
[2] Ministry of Health, General Directorate of Public Hospitals, Türkiye
[3] Department of Nephrology, Department of Internal Medicine, Duzce University Faculty of Medicine, Duzce, Türkiye
[4] Department of Biostatistics and Medical Informatics, Duzce University Faculty of Medicine, Duzce, Türkiye

Corresponding Author:
Birgul Onec
mail: birgulonec@gmail.com

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konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

The Relationship of Hepcidin, Soluble Transferrin Receptor, Growth Differentiation Factor-15 And Anemia in Multipl Myeloma

ABSTRACT

Objective: Multiple myeloma (MM) is a malignant hematological disease and anemia is observed in the majority of patients. Hepcidin, Growth differentiation factor-15 (GDF-15), soluble transferrin receptor (sTfR) have been investigated in many forms of anemia, especially in chronic diseases and cancers. However, there are few studies investigating their role in MM. We aimed to determine the relationship between hepcidin, sTfR and GDF-15 levels in MM patients and their clinical features such as anemia parameters, disease stage and overall survival.
Methods: Hepcidin, sTfR and GDF-15 levels, as well as clinical and anemia-related parameters, were analyzed in newly diagnosed MM patients and healthy volunteers.
Results: Although MM patients had significantly lower Hb and Hct levels compared to the control group, none of the GDF-15, hepcidin and sTfR levels showed a significant difference between the groups. Among MM patients, we found that the anemic subgroup had significantly lower hepcidin levels than the non-anemic subgroup. GDF-15, hepcidin and sTfR levels showed weak or moderate, but statistically significant positive correlation with each other, while GDF15 was positively correlated with creatinine and sTfR levels were correlated with many parameters such as LDH, CRP, ferritin, albumin, creatinine, Hb and ISS, all of which weak. None of the levels of GDF-15, hepcidin and sTfR had a significant effect on survival.
Conclusions: We suggested that these mediators may play a role in anemia of MM but there is not a clear relashionship as in chronic disease anemia, there may be different mechanisms according to the characteristics of the patient groups.
Keywords: Multiple Myeloma, Anemia, Hepcidin, Growth Differentiation Factor-15 (GDF-15), Soluble Transferrin Receptor.

Multipl Miyelomda Hepsidin, Solubl Transferrin Reseptörü ve Büyüme Farklılaşma Faktörü-15'in Anemiyle İlişkisi

ÖZET

Amaç: Multipl myelom (MM) malign bir hematolojik hastalıktır ve hastaların çoğunluğunda anemi görülmektedir. Hepsidin, büyüme farklılaşma faktörü-15 (GDF-15), solubl transferrin reseptörü (sTfR) başta kronik hastalıklar ve kanserlerde olmak üzere birçok anemide çalışılmıştır ancak MM'daki anemide rollerini araştıran çalışmalar çok azdır. Multipl myelom hastalarında hepsidin, sTfR ve GDF-15 düzeylerini ve bu düzeylerin anemi parametreleri, hastalık evresi ve toplam sağkalım gibi klinik özelliklerle ilişkisini belirlemeyi amaçladık.
Gereç ve Yöntem: Yeni tanı almış MM hastalarında ve sağlıklı gönüllülerde hepsidin, sTfR ve GDF-15 düzeylerinin yanı sıra diğer klinik ve anemi ile ilişkili parametreler analiz edildi.
Bulgular: Miyelom hastaları kontrol grubuna göre belirgin düşük Hb ve Hct düzeylerine sahip olmalarına rağmen, GDF-15, hepsidin ve sTfR düzeylerinin hiçbiri gruplar arasında anlamlı bir farklılık göstermiyordu. MM hastaları içinde anemik alt grupta, anemik olmayan altgruba göre belirgin düşük hepsidin düzeyleri olduğunu saptadık. GDF-15, hepsidin ve sTfR düzeylerinin birbirleriyle zayıf ya orta düzeyde pozitif yönde korelasyon gösterirken, GDF15 kreatinin ile pozitif yönde köreleydi ve sTfR düzeyleri ise LDH, CRP, ferritin, albümin, kreatinin, Hb ve ISS evresi gibi birçok parametre ile ancak zayıf düzeyde korelasyon gösteriyordu. GDF-15, hepsidin ve sTfR düzeylerinin hiçbiri sağkalım üzerine anlamlı etkiye sahip değildi.
Sonuç: Bu mediyatörlerinin MM anemisinde bir rolü olabileceğini ancak kronik hastalık anemisindeki gibi net bir ilişki olmadığını, hasta gruplarının özelliklerine göre değişken mekanizmalar olabileceğini düşündürmüştür.
Anahtar Kelimeler: Multipl Myelom, Anemi, Hepsidin, Büyüme Farklılaşma Faktörü-15 (GDF-15), Solubl Transferrin Reseptör.

INTRODUCTION

Multiple myeloma (MM) is a malignant plasma cell disease which develops with a clonal increase of plasma cells in the bone marrow. The most important clinical findings defining the symptomatic disease are known as lytic lesions in the bones, deterioration in kidney functions, hypercalcemia and anemia. Anemia is present in 70% of patients at presentation and develops in 97% of patients during its course. (1) Anemia is usually normochromic, normocytic and its etiology includes invasion of bone marrow by tumor cells, inhibition of erythropoiesis due to tumor-microenvironment relationship, decrease in erythropoietin due to renal dysfunction, and inflammation-related factors. Some of the patients have iron deficiency and some have iron overload associated with the inability to use iron, and these iron metabolism disorders have been associated with organ damage and a decrease in overall survival (2). Although anemia in multiple myeloma is similar to anemia of chronic disease with many parameters, its mechanism has not been clarified.

Hepcidin is a circulating peptide hormone synthesized mainly from the liver and excreted in the urine, and is the main regulator of systemic iron balance (3). Hepcidin performs this regulation by coordinating the use and storage of iron, and by preventing the exit of iron to the plasma (3, 4). Recently, molecules from the transforming growth factor-β (TGF-β) family have been shown to be regulators of hepcidin. Growth differentiation factor-15 (GDF-15), also known as MIC-1, PLAB, PTGF-β, PDF or NAG-1, is a TGF-β family member whose production is induced in inflammatory or malignant diseases (5). GDF-15 downregulates the transcription of the HAMP gene encoding hepcidin in hepatic cell lines and downregulates hepcidin in vitro. The fact that increased GDF-15 levels are especially high in aggressive malignancies such as prostate, gastrointestinal, colorectal, and pancreatic cancers suggests that it also could have a role in cancer progression (6-9). Recent studies enounced that the severity of cancer-related anemia is related to GDF-15 levels and its interactions with hepcidin are important in the development of anemia in cancer (5). GDF-15 levels have been shown to be elevated in some anemia types characterized by ineffective erythropoiesis and iron overload, such as thalassemia, congenital dyserythropoietic anemia, and pyruvate kinase deficiency (10-12). On the other hand, another study, focused GDF-15 production in anemic states with different types of erythropoietic dysfunction reported that the hepcidin defect seen in this kind of anemia is associated with sTfR level, not GDF-15 levels (13).

Studies investigating this issue in patients with multiple myeloma are limited. Tarkun et al. reported that myeloma patients had significantly higher GDF-15 levels compared to controls and GDF-15 was correlated with some prognostic markers such as Beta-2 microglobulin and albumin, but could not clearly show its relationship with stage and survival (14). On the other hand, several studies reported elevated hepcidin levels in multiple myeloma (15, 16) but it is emphasized that hepcidin and GDF-15 levels will not be sufficient in erythropoiesis-related diseases, and sTfR measurements may provide clearer results (13). In the light of these studies, it can be thought that sTfR may give better results than hepcidin and GDF-15 in the evaluation of multifactorial anemia in MM disease. In this study, we aimed to measure hepcidin, sTfR and GDF-15 levels in multiple myeloma patients and to determine whether they differ from the control group and to determine their relationship with the depth of anemia, disease stage and known prognostic markers.

MATERIAL AND METHODS

This study was approved by Düzce University Faculty of Medicine Non-Invasive Ethics Committee with the decision dated 20.01.2015 and numbered 2015/110 and was supported by the Scientific Research Project of Düzce University BAP- 2015.04.03.336.

Patients who were newly diagnosed as multiple myeloma according to the diagnostic criteria of the International Myeloma Working Group and started to be followed up at our Internal Medicine and Hematology clinic were were evaluated for the study. Patients who were using drugs that can influence bone marrow or hematological parameters or transfused blood products in last 30 days were excluded from the study.

Considering similar studies in the literature (3,5,6), the sample size determination method and the total number of subjects to be used in the study, the effect size between control and MM group was calculated as 250 pg/ml. In order to determine that the 250-unit difference between the groups was statistically significant, the required minimum sample size was calculated as 26 in each group under the conditions of 90% power and 5% type I margin of error. Calculations were made with PASS v.11 package program. Basically, two groups (Multiple Myeloma and Control group) were compared in the study.

A total of 42 newly diagnosed multiple myeloma patients were evaluated, but the study was completed with 28 patients after the exclusions and with a control group consisted of 28 volunteers who did not have any complaints or disease. After 10 hours of fasting, blood samples were taken from the patient and control groups into empty tubes and immediately centrifuged. The obtained sera were stored frozen at -70°C throughout the patient collection process. After reaching the targeted number of patients and control groups, serum hepcidin, sTfR and GDF-15 levels were studied by

ELISA method (Huma Hepcidin 25 ELISA Kit, Human Soluble Transferrin Receptor1 ELISA Kit, Human Growth Differentiation Factor 15 ELISA Kit, FineTest®, Wuhan, respectively. Fine Biotech Co. Wuhan, China)

Routine tests performed in our hospital were evaluated for the diagnosis and staging of the patients. Urea, creatinine, total protein, albumin, calcium, C reactive protein (CRP), iron, total iron binding capacity (TIBC), ferritin, vitamin B12, folate and hemogram were analized on a daily basis, without delay.

Patients were subsequently grouped into anemic and non-anemic for secondary purposes according to their hemoglobin (Hb) values.

Statistical Evaluation: The distribution of the data was analyzed with the Shapiro-Wilks test, group comparisons were made with the Independent samples t-test for the continuous variables with normal distribution, and with the Mann-Whitney U test for the continuous variables that did not show normal distribution. Relationships between categorical variables were examined with Pearson chi-square or Fisher's Exact tests. Spearman correlation analysis was used to examine the correlation between continuous variables. Life tables and Kaplan-Meier survival analysis were used for survival analysis; groups were compared with Log-rank test. Statistical analyzes were made with the SPSS v.22 package program and the significance level was taken into account as 0.05.

RESULTS

The mean age of the 56 individuals included in the study was 65.70±9.22 (45-84) and there was no significant difference between patient and control groups (66.68±9.87 and 64.71±8.58, p=0.430). The overall male/female ratio was 23(41.1%)/33(58.9%) and were similar in both groups (p= 0.415).

Anemia parameters of both groups are detailed in Table 1. Revealing the anemia of the patient group, median Hb and hematocrit (Hct) levels were lower in the patient group (Hb 9.95 vs 13.40 g/dL and Hct 30.35% vs 40.00%, p<0.001). There were also statistically significant differences in mean corpuscular volume (MCV), red cell distribution width (RDW), platelet (Plt), TIBC, ferritin, vitamin B12 values, but the values were within normal limits. Ferritin was lower in the control group than in the patient group, but transferrin saturation (TS) was not different between the two groups (p=0.705).

Table 1. Hemogram and anemia parameters in patient and control groups

	Group	n	Mean ± SD	Median (min-max)	p
WBC (10^3/uL)	Patient	28	6030.36±2705.66	6100 (2000 - 14400)	0.121 #
	control	28	6700.00±1605.78	6800 (3500 - 9500)	
Hb (g/dL)	Patient	28	10.20±2.15	9.95 (6.70 - 14.49)	<0.001*
	control	28	13.49±1.47	13.40 (10.20 - 15.80)	
Hct (%)	Patient	28	31.00±6.68	30.35 (20.10 - 44.00)	<0.001*
	control	28	39.65±4.97	40.00 (29.10 - 48.00)	
MCV (FL)	Patient	28	90.01±7.52	90.35 (74.30 - 109.10)	0.045*
	control	28	86.23±6.23	86.40 (75.00 - 107.60)	
RDW (%)	Patient	28	17.29±3.94	16.60 (13.50 - 31.80)	0.011*
	control	28	15.06±2.08	14.45 (12.50 - 21.80)	
plt (10^3/uL)	Patient	28	217392.86±118759.74	190500 (50000 - 678000)	0.044 #
	control	28	244500.00±71796.78	234500 (133000 - 502000)	
Ferritin (ng/mL)	Patient	28	441.98±545.59	177.20 (3.87 - 1735.00)	<0.001 #
	control	20	46.89±44.24	41.10 (5.80 - 168.40)	
İron (µg/dL)	Patient	28	75.18±42.55	77.84 (14.00 - 202.00)	0.982*
	control	19	74.93±28.37	75.00 (25.60 - 123.00)	
TIBC (µg/dL)	Patient	28	279.34±61.54	284.50 (156.50 - 430.00)	<0.001*
	control	19	353.60±56.74	345.00 (240.80 - 462.00)	
TS (%)	Patient	28	28.54±18.74	27.91 (3.49 - 90.99)	0.135 #
	control	19	21.19±9.17	22.52 (5.14 - 37.76)	
B12 (pg/mL)	Patient	28	474.39±378.37		<0.001 #
	control	25	253.44±213.97	194.00 (30.00 - 1129.00)	
Folate (ng/mL)	Patient	28	11.70±7.93	8.11 (2.16 - 25.00)	0.671 #
	control	21	10.14±3.85	9.60 (5.12 - 23.40)	

*: Independent samples t test, #: Mann-Whitney U test

(WBC: White blood cell, Hb: Hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, RDW: red cell distribution width, Plt: platelet, MPV: mean platelet volume, PDW: platelet distribution width, TIBC: total iron binding capacity, TS: Transferrin saturation, B12: Vitamin B12)

Biochemical parameters, GDF-15, hepcidin, and sTfR levels in patient and control groups are given in Table 2. As expected, albumin levels were lower (median 3.50 g/dL vs. 4.24 g/dL, p<0.001) and sedimentation rate was higher (56 mm/hr vs. 18.5 mm/hr, p<0.001) in myeloma patients. Estimated glomerular filtration rate (eGFR) was lower and creatinine was higher in the patient group revealing impaired kidney functions. CRP, total protein, calcium, lactate dehydrogenase (LDH), GDF-15, hepcidin and sTfR levels were similar in both groups.

Table 2. Comparison of biochemical parameters, Growth differentiation factor-15, hepcidin and soluble transferrin receptor levels in patient and control groups

	Group	n	Mean ± SD	Median (min-max)	p
Sedim. (mm/h)	Patient	28	61.57±37.58	56.00 (4.00 - 140.00)	<0.001*
	control	12	24.67±19.92	18.50 (5.00 - 77.00)	
CRP (mg/dL)	Patient	27	1.40±2.53	0.64 (0.03 - 12.36)	0.540 #
	control	20	0.86±1.13	0.48 (0.15 - 4.66)	
T. protein (g/dL)	Patient	28	7.71±1.69	7.69 (4.08 - 11.15)	0.166*
	control	19	7.24±0.42	7.40 (6.50 - 7.84)	
Albumin (g/dL)	Patient	28	4.33±4.87	3.50 (1.82 - 29.00)	<0.001 #
	control	22	4.16±0.38	4.24 (3.20 - 4.61)	
Calcium (mg/dL)	Patient	28	9.78±2.00	9.38 (7.68 - 16.59)	0.653*
	control	26	9.60±0.55	9.55 (8.60 - 10.50)	
LDH (U/L)	Patient	28	233.21±131.71	192.00 (109.00 - 663.00)	0.083 #
	control	14	248.83±66.97	236.00 (156.00 - 371.60)	
Creatinine (mg/dL)	Patient	28	2.15±1.90	1.07 (0.47 - 6.51)	0.003 #
	control	28	0.80±0.21	0.81 (0.37 - 1.22)	
eGFR (mL/min/1.73m^2)	Patient	28	54.41±33.54	62.58 (6.88 - 102.90)	<0.001 #
	control	28	88.65±15.53	89.14 (52.50 - 116.80)	
GDF-15 (pg/mL)	Patient	28	1316.21±99.78	1288.20 (1209.74 - 1675.22)	0.057 #
	control	28	1270.39±43.16	1258.60 (1219.75 - 1378.46)	
Hep (pg/mL)	Patient	28	62053.39±7141.18	60697.58 (52382.24 - 77599.32)	0.207 #
	control	28	60489.33±8497.34	56728.30 (51234.98 - 86363.43)	
sTfR (ng/mL)	Patient	28	1230.02±89.10	1206.08 (1107.60 - 1382.03)	0.064 #
	control	28	1209.23±184.66	1178.65 (1112.96 - 2125.02)	

*: Independent samples t test, #: Mann-Whitney U test
(Sedim: Blood sedimentation rate, CRP: C reactive protein, LDH: lactate dehydrogenase, eGFR: estimated glomerular filtration rate, GDF-15: Growth differentiation factor-15, Hep: hepcidin, sTfR: soluble transferrin receptor)

There were 10 (35.7%) patients with stage I, 10 (35.7%) patients with stage II, and 8 patients (28.6%) with stage III according to International Staging System (ISS) and there was no significant difference in terms of GDF-15, hepcidin or sTfR levels between groups of stages. With Durie Salmon Staging, 13 patients were stage 1-2, 15 patients were stage 3A-3B, and there was no significant difference between these stage groups in terms of GDF-15, hepcidin or sTfR levels. The Karnovski score was >50 in 16 (57%) patients. There was no significant difference between low or high Karnovski score and GDF-15, hepcidin or sTfR levels (p>0.05). In order to investigate the differences between anemic and non-anemic myeloma patients, two groups were formed using the median Hb value (9.95 g/dL) in the patient group. As the details are summarized in Table 3, hepcidin level was lower in the anemic group (p=0.043), but there was no significant difference in GDF-15 or sTfR levels.

Table 3. Comparison of growth differentiation factor-15, hepcidin and soluble transferrin receptor levels in anemic and non-anemic patients

	Group	n	Mean ± SD	Median (min-max)	p
GDF-15 (pg/mL)	Hb<9.95 g/dL	14	1313.36±82.34	1296.79 (1221.60 - 1531.91)	0.783 #
	Hb>9.95 g/dL	14	1319.06±117.81	1278.63 (1209.74 - 1675.22)	
Hep (pg/mL)	Hb<9.95 g/dL	14	59157.46±5195.96	58139.87 (52382.24 - 72733.61)	**0.043** #
	Hb>9.95 g/dL	14	64949.32±7800.94	65507.04 (54121.11 - 77599.32)	
sTfR (ng/mL)	Hb<9.95 g/dL	14	1231.86±79.55	1206.08 (1132.60 - 1369.32)	0.662 #
	Hb>9.95 g/dL	14	1228.17±100.75	1205.33 (1107.60 - 1382.03)	

#: Mann-Whitney U test ; (GDF-15: Growth differentiation factor-15, Hep: hepcidin, sTfR: soluble transferrin receptor, Hb: Hemoglobin)

Transferrin saturation was calculated using the iron and total iron binding capacities of the patients. Patients with TS<10% (n=4) were classified as having iron deficiency. Although the group was very small, GDF-15, hepcidin and sTfR levels were not different from those with normal TS in patients with low TS. Similarly, in the analysis performed in the whole group, none of the Hb, ferritin, and TS values were significantly correlated with GDF-15, hepcidin and sTfR levels (Table 4).

Table 4. Investigation of correlations of hemoglobin, ferritin, transferrin saturation values with GDF-15, hepcidin and sTfR levels

		GDF-15	Hep	sTfR
Hemoglobin	r	0.075	0.295	-0.204
	p	0.705	0.127	0.299
Ferritin	r	-0.019	-0.131	0.328
	p	0.925	0.505	0.088
TS	r	-0.169	0.163	0.085
	p	0.391	0.407	0.666

(GDF-15: Growth differentiation factor-15, Hep: hepcidin 25, sTfR: soluble transferrin receptor, TS: Transferrin saturation)

When the correlations were examined, there was a positive moderate correlation between GDF-15 and sTfR (r=0.531, p<0.001) while both GDF-15 and hepcidin (r=0.303, p=0.023) and hepcidin and sTfR levels (r =0.286, p=0.033) showed significant but weak positive correlations. Additionally, GDF15 was positively correlated with creatinine (r=0.426, p=0.001), and sTfR levels were correlated with many parameters such as LDH, CRP, ferritin, albumin, creatinine, Hb and ISS stage, all of which weak. Correlation analyses are detailed in Table 5. When evaluated in terms of survival times, the mean overall survival rate (OS) in the anemic group was 26.2 ±4.5 months, the cumulative probability of survival was 0.786 ±0,110 for the 1st year and 0.524 ±0.168 for the 2nd year. The mean OS in the nonanemic group 44,7±10,8 months, and cumulative probability of survival was 0.67±0.14 both for 1st and 2nd years. Although the mean OS seems better in the non-anemic group, no statistically significant difference was found (p=0.703).

Using the median values of GDF-15, hepcidin and sTfR levels, patients were grouped and the effect of low or high values on survival was investigated. In the group with low GDF-15, mean OS was 34.5±7.9 and median OS was 55.4 months, while the cumulative probability of survival (CPS) at 1 and 2 years was 0.587, versus 45.9±12 .2 and 36.6±18.5 months with CPS rates of 0.836 and 0.597 in the group with high GDF-15. There was no statistically significant difference between the two groups (p=0.684). In the group with low hepcidin levels mOS: 53.3±14.3 months, mdOS:55.4±27.6 months, CPS was 0.762 for the 1st year and 0.653 for the 2nd year, not different from the patient group with high hepcidin levels (mOS: 31.8±7.05 mdOS:36.6±15.4 months, CPS 1st:0.665 and 2nd :0.554, p=0.345). Similarly, there was no statistically significant difference between OS time and CPS rates of groups of patients with low or high sTfR levels (mOS 53.57±14.1 months, mdOS 55.4±25.5 months, CPS 1st year:0.755, CPS 2nd year:0.647 in the group with low sTfR levels vs 32.5±7.2, 36.6±21.9, 0.675, and 0.563, respectively in the group with high sTfR levels, p=0.339).

Table 5. Investigation of the correlations of growth differentiation factor-15, hepcidin, soluble transferrin receptor levels with various clinical and laboratory parameters

		GDF-15	Hep	sTfR
Sedim.	r	-0.217	-0.283	-0.016
	p	0.268	0.144	0.936
CRP	r	0.324	0.160	0.388
	p	0.100	0.426	0.046
Hb	r	0.075	0.295	-0.306
	p	0.705	0.127	0.022
Hct	r	0.056	0.300	-0.204
	p	0.778	0.120	0.299
MCV	r	-0.306	-0.030	-0.069
	p	0.113	0.880	0.728
RDW	r	-0.127	-0.147	-0.085
	p	0.520	0.456	0.667
PLT	r	-0.025	-0.140	-0.287
	p	0.900	0.476	0.139
Ferritin	r	-0.019	-0.131	0.358
	p	0.925	0.505	0.012
İron	r	-0.141	0.111	0.088
	p	0.475	0.572	0.655
TIBC	r	0.008	-0.166	-0.151
	p	0.968	0.398	0.443
B12	r	-0.159	-0.014	0.093
	p	0.420	0.945	0.637
Folate	r	0.293	0.113	-0.073
	p	0.131	0.568	0.711
T. Protein	r	-0.324	-0.279	-0.400
	p	0.093	0.150	0.035
Albumin	r	-0.198	-0.175	-0.421
	p	0.314	0.374	0.026
Calcium	r	-0.077	-0.039	0.018
	p	0.696	0.844	0.928
LDH	r	0.125	0.177	0.467
	p	0.528	0.369	0.012
Creatinine	r	0.426	0.056	0.308
	p	0.001	0.778	0.021
eGFR	r	-0.408	-0.045	-0.355
	p	0.002	0.821	0.007
fLCR	r	-0.089	-0.369	-0.145
	p	0.695	0.091	0.521
GDF-15	r	1,000	0.303	0.531
	p	-	0.023	0,000
Hep	r	0.303	1,000	0.286
	p	0.023	-	0.033
sTfR	r	0.531	0.286	1,000
	p	0,000	0.033	-

(GDF-15: Growth differentiation factor-15, Hep: hepcidin, sTfR: Solubl transferrin receptor, Sedim: Blood sedimentation rate, Hb: Hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, RDW: red cell distribution width, Plt: platelet, MPV: mean platelet volume, PDW: platelet distribution width, TIBC: total iron binding capacity, LDH: lactate dehydrogenase, eGFR: estimated glomerular filtration rate, fLCR: free light chain ratio)

DISCUSSION

Multiple myeloma is a hematological malignancy that develops with clonal increase of plasma cells. Myeloma constitutes 1% of all malignancies and 10% of hematological malignancies. Although the data in our country are not conclusive, it is the second most common hematological malignancy in the United States (17, 18). While the incidence is 2/100,000, approximately 86,000 new cases are detected every

year around the world. Every year, 63,000 people die from MM, which corresponds to 0.9% of cancer deaths worldwide (19,20). It is a disease of the elderly population, as the average age of diagnosis is 69 years. Anemia is present in 70% of patients at presentation and develops in 97% of patients during its course (12). Anemia for the general population is defined by the World Health Organization as an Hb value below 12 g/dL in women and 13 g/dL in men, but the cut-off value for anemia in MM criteria is different and makes comparisons difficult. In order to compare the evaluations with the population, when Hb<12 g/dL anemia was accepted, anemia was found in 82% of our patients (23 of 28 patients) in our study. The median Hb value was 9.95 g/dl, which was below the level (Hb<10 g/dl) accepted by the International Myeloma Study Group as the criterion for symptomatic multiple myeloma. About half of the patients had anemia at the level indicated by the International Myeloma Working Group. In our stdy, when the admission symptoms were examined, it was found that the symptoms that caused the patient to consult a doctor were the symptoms of anemia in 32% of the patients, and MM was detected while investigating for anemia. When those with more than one symptom were taken into account, approximately 60% of the patients had symptoms of anemia.

Since anemia is a common condition in the elderly population, it is important to clarify the characteristics of multiple myeloma-associated anemia and to reveal the underlying mechanisms in the diagnosis and treatment of MM disease. In our study designed for this purpose, we closely examined the anemia parameters of MM patients. In addition to the fact that Hb and Hct were lower in the patient group (p<0.001), which clearly indicates anemia, MCV and RDW were also higher than in the control group. These findings were notable for the anemia of myeloma. While the median MCV was 90 fL in the patient group, it was similar to the median MCV 89.9 in the subgroup with anemia (p:0.388) and the MCV of only three patients was <80 fL. Therefore, patients generally had a normocytic anemia.

Vitamin B12 level was higher than the control group (mean 474.39±378.37 vs 253.44±213.97, p,<0.001) and therefore was not explanatory in terms of anemia. In the literature, there are studies reporting both low and high B12 levels in MM patients, and it is known that B12 level can be falsely measured in relation to paraproteins in the serum (21,22). The folate level was not different between the patients and the control group. Ferritin levels of the patients were significantly higher than the control group (median 177.20 vs 41.10 µg/L, p<0.001). Hypoferritinemia (<15 µg/L) was observed in only one non-anemic patient but hyperferritinemia was seen in 10 (35%) patients with higer values in anemic subgroup. Konig et al. reported 30% of patients with ferritin levels of 400-1000 µg/L and 24% of patients with >1000 levels much higher than our study (2), however, in this study, the patient group also included patients who received multiple treatments. On the other hand, Song et al. reported hyperferritinemia in 44% of their pre-treatment study population (23). Since ferritin is a well-known acute phase reactant, it is thought that it may reflect inflammation rather than iron stores, and transferrin saturation (TS) was examined in patients. Patients with TS<10% (n=4) were classified as having iron deficiency and although the group was very small, GDF-15, hepcidin and sTfR levels were not different in this group from those with normal TS. Similarly, Hb, ferritin, and TS values were not correlated with GDF-15, hepcidin and sTfR levels in the patient group. The number of patients with TS above 45% was only 2 (7%), much lower than that reported by Konig et al (22%). Since there was no significant difference in TS between the patient and control groups (p=0.135), it was thought that hyperferritinemia in the patients did not reflect iron overload.

Although myeloma patients had significantly lower Hb and Hct levels compared to the control group, none of the GDF-15, hepcidin and sTfR levels were significantly different between the MM and control groups. These findings were inconsistent with the study conducted by Tarkun et al. (14), which reported significantly higher GDF-15 levels in myeloma patients compared to controls. On the other hand, when the correlations of GDF15 were examined, except for its significant correlation with both hepcidin and sTfR, similar to the study of Tarkun et al., GDF15 had a positive correlation with serum creatinine level and therefore a negative correlation with eGFR. In our study, however, there was no significant correlation between hemoglobin, albumin, CRP or ISS or Durie Salmon Staging and GDF15. These differences may be due to the fact that both studies were conducted with relatively small groups, or they may be related to unpredictable conditions such as genetic risk factors that were not evaluated in both studies.

In our study, we found that hepcidin was not significantly different from the control group in MM patients, but it was significantly lower in the anemic subgroup among MM patients compared to the nonanemic subgroup. This was in contrast with previous studies suggesting that hepcidin elevation is one of the etiological causes of anemia in MM (15). When their correlations were examined, there was a moderate positive correlation with GDF15 and sTfR, but no correlation with parameters such as Hb, Hct, ferritin, creatinine, CRP or disease stage. It has been reported that hepcidin is increased in multiple myeloma in a few studies (15, 16, 24), but in the study of Haraguchi et al. (25), in which the prohepsidin measurement was used, there was no increase in prohepsidin levels in MM patients. In

that study (25), in the subgroup analyzes the prohepcidin levels were higher in patients with severe kidney damage than those with mild renal dysfunction and the control group. Similar to our results, Haraguchi et al also reported that there was no significant correlation between prohepcidin levels and other clinical parameters and anemia parameters including Hb. Sharma et al. (15) evaluated hepcidin expression in MM cells in their study, based on the high urinary hepcidin levels, and suggested that the increase in hepcidin did not originate from myeloma plasma cells, but rather that the increase in serum IL6 and other cytokines increased hepcidin production from hepatocytes. They reported that they did not see the same effect in the serum of each patient in the cell series they tested, and that no significant hepcidin production increase was observed in up to 30% of the patients. We think that the well-known heterogeneous character of the disease plays a role in whether this increase occurs or not. As Sharma et al. showed using anti-IL6 antibodies, hepcidin induction occurs by different mechanisms, with and without IL-6 dependent, and the contribution of these concomitant inflammatory mechanisms is likely to vary in selected patient groups. For example, although there was anemia level close to ours in the patient group studied by Sharma et al., all of the patients were ISS stage III patients. Hepcidin was not different from controls in a small number of MGUS patients included in the study. Therefore, in studies such as ours, in which patients with different ISS or Durie Salmon Stages are examined, consistent results may not be obtained due to the different mechanisms or levels of accompanying inflammatory processes. For example, in our study, CRP levels were not different from controls, suggesting that our patient group had lower inflammation levels, and there was no relationship between hepcidin and CRP.

In our study, sTfR levels were also not significantly different in MM patients from the control group, but they were positively correlated with many parameters such as GDF15, hepcidin, LDH, CRP, ferritin, albumin, creatinine, Hb and ISS stage, and negatively correlated with eGFR. The correlation between sTfR and GDF15 was strong, and since there are correlations to several clinical parameters, this parameter was thought to be particularly important for MM. The relationship between Hepcidin, sTfR, and GDF-15 was investigated in anemia associated with ineffective erythropoiesis, particularly in vitamin B12 deficiency anemia and thalassemia by Fertrin et al. (13). GDF-15 downregulates the HAMP gene encoding hepcidin, and an increase in GDF 15 may be a reason for the low hepcidin levels in patients with transfusion-related iron overload. In Fertrin's study, hepcidin and GDF15 were not correlated, and hepcidin levels were most strongly correlated with sTfR. These findings revealed that anemia in hematological diseases in which the erythropoietic system is affected in different ways cannot be explained only by the interaction of mediators originating from inflammation. Victor et al. investigated hepcidin and sTfR levels (24), but they found hepcidin levels to be high in the MM group and strongly negatively correlated with sTfR in their study. This may be due to the relatively small number of studies or the fact that Victor et al. worked with a younger patient group. Since there are not enough studies investigating sTfR levels for multiple myeloma, it is necessary to study with larger and more homogeneous groups with clinical information.

CONCLUSION

Although myeloma patients had significantly lower Hb and Hct levels compared to the control group, none of the GDF-15, hepcidin and sTfR levels were significantly different between the MM and control groups. We detected significantly lower hepcidin levels in the anemic subgroup among multiple myeloma patients compared to the nonanemic subgroup. When the correlations were examined, besides the significant correlations of GDF-15, hepcidin and sTfR levels with eachother, GDF15 was positively correlated with creatinine, and sTfR levels were correlated with many parameters such as LDH, CRP, ferritin, albumin, creatinine, Hb and ISS stage, all of which weak. None of the levels of GDF-15, hepcidin and sTfR had a significant effect on survival. These suggested that mediators of chronic inflammation may play a role in anemia in myeloma, but there is not always a clear interaction as in anemia of chronic disease, and there may be mechanisms involving partial response deficiencies and variable responses depending on the characteristics of patient groups. Since myeloma has a very heterogeneous structure, there is a need to continue studies by creating larger and clearer groups in terms of features such as genetic risk factors and clinical stages in order to explain these findings.

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