RESEARCH ARTICLE

DOI: 10.4274/tjh.galenos.2024.2023.0469
Turk J Hematol 2024;41:83-90

Do Alarmins Have a Role in Multiple Myeloma?

Alarminlerin Multipl Miyelomda Rolü Var mı?

Ayfer Gedük[1], Merve Gökçen Polat[1], Esra Terzi Demirsoy[1], Berrin Öztaş[2], Baldan Huri Eryılmaz[3], Emel Merve Yenihayat[1], Hayrunnisa Albayrak[1], Haşim Atakan Erol[1], Özgür Mehtap[1], Pınar Tarkun[1], Abdullah Hacıhanefioğlu[1]

[1]*Kocaeli University Faculty of Medicine, Department of Hematology, Kocaeli, Türkiye*
[2]*Kocaeli University Faculty of Medicine, Department of Biochemistry, Kocaeli, Türkiye*
[3]*Kocaeli University Faculty of Medicine, Department of Internal Medicine, Kocaeli, Türkiye*

Abstract

Objective: Calprotectin (CLP), S100A6, and high mobility group nucleosome-binding protein 1 (HMGN1), known as alarmins, are involved in the pathogenesis of many tumors. In this study, we aimed to investigate the relationships of serum CLP, S100A6, and HMGN1 levels with the clinical and laboratory findings of patients with multiple myeloma (MM) and their roles in the pathogenesis of MM.

Materials and Methods: We measured the serum CLP, S100A6, and HMGN1 levels of 55 newly diagnosed patients and 32 healthy controls using the sandwich enzyme-linked immunosorbent assay method. The medical records of the patients were also reviewed.

Results: Serum CLP, S100A6, and HMGN1 levels were significantly decreased in MM patients compared to the control group (p=0.012, p=0.001, and p=0.030, respectively). Receiver operating characteristic analysis was used to determine diagnostic cut-off values for serum CLP, S100A6, and HMGN1 of <98 ng/mL (area under the curve [AUC]: 0.663, 95% confidence interval [CI]: 0.554-0.761, p=0.009), <1174.5 pg/mL (AUC: 0.706, 95% CI: 0.598-0.799, p=0.001), and <440.18 pg/mL (AUC: 0.640, 95% CI: 0.530-0.740, p=0.03), respectively. CLP levels were found to be statistically significantly higher in patients with light chain MM (91.58±22.57 ng/mL) compared to heavy chain MM (79.42±15.83 ng/mL) (p=0.03). A negative correlation was observed between CLP and M protein, immunoglobulin G, globulin, and beta-2 microglobulin (correlation coefficients: -0.361, -0.370, -0.279, -0.300, respectively; p=0.024, p=0.06, p=0.04, p=0.0033).

Conclusion: In this study, we found that serum CLP, S100A6, and HMGN1 levels were statistically lower in patients with newly diagnosed MM compared to the control group. These results suggest that CLP may bind to the paraprotein produced by heavy chain MM in the blood, causing its blood levels to be low. Additionally, low levels of HMGN1, which is involved in DNA repair, suggest that HMGN1 may contribute to the complex genetic abnormalities found in cases of MM.

Keywords: S100A8/9, Calprotectin, S100A6, HMGN1, 1q21 gain/amplification

Öz

Amaç: Alarminler olarak bilinen calprotektin (CLP), S100A6 ve high mobility group nucleosome-binding protein 1 (HMGN1), birçok tümörün patogenezinde rol almaktadır. Bu çalışmada, multipl myelom (MM) hastalarında serum CLP, S100A6 ve HMGN1 düzeylerinin klinik ve laboratuvar bulgularıyla ilişkisini ve MM patogenezindeki rolünü araştırmayı amaçladık.

Gereç ve Yöntemler: Yeni tanı almış 55 MM hastası ve 32 sağlıklı gönüllünün serum CLP, S100A6 ve HMGN1 düzeyleri ELISA yöntemiyle ölçüldü. Hastaların medikal kayıtları tarandı.

Bulgular: Hastaların tanıda bakılan CLP, S100A6 ve HMGN1 seviyeleri kontrol grubuna göre istatistiksel olarak anlamlı derecede düşük bulundu (sırasıyla p=0,012, p=0,001, p=0,030). Alıcı çalışma karakteristikleri (receiver operating characteristic) analizinde MM için CLP <98 ng/mL (eğri altında kalan alan [area under the curve, AUC]: 0,663, %95 güven aralığı [GA] 0,554-0,761, p=0,009), S100A6 <1174,5 pg/mL (AUC: 0,706, %95 GA: 0,598-0,799, p=0,001), HMGN1 için ise <440,18 pg/mL (AUC: 0,640, %95 CI: 0,530-0,740, p=0,03) tanısal cut-off değeri olarak belirlendi. CLP seviyesi, hafif zincir MM hastalarında (ortalama ± standart sapma; 91,58±22,57), ağır zincir MM hastalarına (79,42±15,83) göre istatistiksel olarak anlamlı derecede yüksek bulundu (p=0,03). CLP ile M protein, IgG, globülin ve beta 2 mikroglobulin arasında negatif korelasyon gözlendi (sırasıyla korelasyon katsayısı: -0,361; -0,370; -0,279; -0,300, p=0,024, p=0,06, p=0,04, p=0,0033).

Sonuç: Çalışmamızda yeni tanı almış MM hastalarında CLP, S100A6 ve HMGN1 serum seviyeleri tanısal anlam taşıyacak düzeyde düşük bulunmuştur. Bu sonuçlar, CLP'nin kanda ağır zincir MM tarafından üretilen paraproteine bağlanabileceğini ve dolayısıyla kan seviyelerinin düşük bulunduğunu göstermektedir. Ayrıca DNA tamirinde rol alan HMGN1'nin düşük düzeyleri, HMGN1'in MM'de bulunan komplex genetik anormalliklere katkı sağlayabileceğini düşündürtmektedir.

Anahtar Sözcükler: S100A8/9, Kalprotektin, S100A6, HMGN1, 1q21 kazanımı/amplifikasyonu

Address for Correspondence/Yazışma Adresi: Esra Terzi Demirsoy, M.D., Kocaeli University Faculty of Medicine, Department of Hematology, Kocaeli, Türkiye
Phone : +90 262 303 80 03
E-mail : esraterzi@gmail.com ORCID: orcid.org/0000-0001-7083-9379

Received/Geliş tarihi: December 17, 2023
Accepted/Kabul tarihi: February 29, 2024

Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by the clonal proliferation of plasma cells. The incidence of MM is approximately 160,000 cases/year worldwide [1]. In the last two decades, significant progress has been made in understanding the pathophysiology of MM. With the introduction of new-generation agents, the survival of MM patients has improved significantly. However, MM is still recognized as an incurable disease [2,3]. Therefore, the identification of new pathways and biomarkers involved in the pathogenesis of MM is extremely important for the identification of new therapeutic targets.

Alarmins are present intracellularly in granules, nuclei, or cytosol and they are rapidly released as a result of degranulation, cell damage/death, or immune induction. In the extracellular compartment they behave as cytokines and act as early warning signals for the immune system, promoting innate and adaptive immune responses. Alarmins can be classified into several categories including antimicrobial peptides and proteins, heat shock proteins, nucleotides/metabolites, certain degradation products of the extracellular matrix, nuclear binding proteins (e.g., HMGB1 and high mobility group nucleosome-binding protein [HMGN1]), and ion-binders (e.g., S100A6, A8, and A9) [4].

The incidence of chromosome 1q21 gain and amplification in MM increases with the occurrence of relapse and is approximately 40% [5]. In many studies, acquisition and especially amplification of 1q21 have been found to be associated with poor prognosis independently of other serious risk factors [6,7]. Most of the genes belonging to the S100 protein family (S100A1-16) are encoded in the 1q21 region. The S100 protein family, a subgroup of calcium-binding EF-hand type proteins, consists of members that tend to form homodimeric and/or heterodimeric complexes with each other. They are involved in many processes such as cell proliferation, differentiation, apoptosis, inflammation, and calcium homeostasis. They bind to various receptors such as toll-like receptor 4 and receptor for advanced glycation end products (RAGE), and they activate the Janus kinase/signal transducer and activator of transcription, nuclear factor kappa B (NF-κB), and mitogen-activated protein kinase (MAPK) pathways [8,9,10]. Increased expression of S100 family members has been identified in various cancer types and has been correlated with tumor cell proliferation, invasion, metastasis, and angiogenesis [11,12,13,14]. Calprotectin (CLP) has a heterodimeric structure consisting of S100A8 and S100A9 subunits [15]. S100A6 is another member of the S100 protein family. In the literature, there are few studies directly investigating the correlation between MM and CLP or S100A6.

HMGN1 is a protein located in the cell nucleus that plays a role in the regulation of DNA repair, transcription, and replication [16,17]. It has important functions in host defense and tissue repair [18]. Rat experiments suggest that HMGN1 may be used as a therapeutic agent in the treatment of malignancies, as well as for tumor-suppression effects, and may have a potential role in vaccine applications [19]. Our literature review did not reveal any studies investigating the correlation between HMGN1 and MM.

In this study, we aimed to determine serum CLP, S100A6, and HMGN1 levels in patients with newly diagnosed MM and to investigate their possible roles in MM pathogenesis by evaluating their relationships with clinical findings.

Materials and Methods

In this study, 55 patients with MM newly diagnosed in the Department of Hematology of the Medical Faculty of Kocaeli University and 32 healthy volunteers participated. The diagnosis of MM was determined according to the criteria of the International Myeloma Study Group [20]. Patients with rheumatological disease, active infection, or concurrent malignancy were excluded. Staging was performed according to the International Staging System (ISS) and Revised ISS criteria. Bone marrow biopsy was performed for all patients at the time of diagnosis. Positron emission tomography was used to evaluate bone involvement. The medical records of the patients were reviewed.

Written informed consent was obtained from all participants. Ethical approval was obtained from the Kocaeli University Medical Faculty Ethics Committee on October 21, 2021 (project no: 2021/285, decision no: GOKAEK-2021/18.06).

Blood samples obtained from the patients and control group were centrifuged at 3500 rpm for at least 15 min. The obtained serum was stored at -80 °C for the duration of the study. The S100A6, CLP, and HMGN1 levels of these samples were determined by sandwich enzyme-linked immunosorbent assay (ELISA) method (cat. no: FINE TEST EH1923, cat. no: ELABSCIENCE E-EL-H2357, and cat. no: FINE TEST EH2476, respectively). The sensitivity levels of these tests are 37.5 pg/mL, 0.94 ng/mL, and <0.188 pg/mL, respectively. Both the intra-assay and inter-assay coefficient of variation % values of all three tests were below 10%.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA). Demographic variables were analyzed using descriptive analyses. Numerical variables were presented as mean ± standard deviation or median (minimum-maximum or 25^{th}-75^{th} percentiles). Categorical variables were summarized as numbers (percentages). The normality of distribution of continuous variables was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical

parameters were analyzed using chi-square and Fisher exact tests. Pairwise comparison analyses were performed to determine the sources of differences. Associations between numerical variables were determined by Spearman correlation analysis. Receiver operating characteristic (ROC) analysis was used to determine area under the curve (AUC), sensitivity, specificity, and cut-off values. All statistical analyses were carried out with 5% significance and a two-sided p value of <0.05 was considered statistically significant.

Results

In this study, the findings of 55 MM patients and 32 healthy volunteers as a control group were analyzed. The two groups were similar in terms of age, sex distribution, and comorbidities (p=0.328, p=0.64, and p=0.359, respectively) (Table 1). The median age of the patients was 63 years (range: 40-81 years) and 38.2% were female. Laboratory and clinical features of the patients are shown in Table 2.

The median (25^{th}-75^{th} percentiles) CLP, S100A6, and HMGN1 levels of the MM patients were respectively as follows: 80.7 ng/mL (69.4-97.1), 932 pg/mL (727.2-1120.5), and 439.4 pg/mL (338.1-517.5). In the control group, the CLP, S100A6, and HMGN1 levels were respectively 93.9 ng/mL (78.9-130.9), 1201.2 pg/mL (861.8-1536.7), and 488 pg/mL (443.5-558.3). Thus, the CLP, S100A6, and HMGN1 levels of the patients at the time of diagnosis were found to be statistically significantly lower than those of the control group (p=0.012, p=0.001, and p=0.030, respectively) (Figure 1).

In the ROC analysis, the CLP cut-off value of <98 ng/mL was diagnostic for MM (p=0.009). For this cut-off value, the sensitivity and specificity were 78% and 50%, respectively. A cut-off value of <1174.5 pg/mL for S100A6 was found to be diagnostic with sensitivity of 89.09% and specificity of 53.1% (p=0.001). In the analysis performed for HMGN1, a cut-off value of <440.18 pg/mL was determined (p=0.03). For this cut-off value, the sensitivity and specificity were 52.7% and 78.1%, respectively. The ROC curves are shown in Figure 2.

CLP levels were found to be statistically significantly higher in patients with light chain MM (mean ± standard deviation: 91.58±22.57) compared to patients with heavy chain MM (79.42±15.83) (p=0.03). Values of CLP, S100A6, and HMGN1 levels are summarized in Table 3 according to clinical and laboratory findings.

Only 9 patients had chromosome 1q21 gain and amplification analysis performed, and 2 of them were positive. The median S100A6 and CLP levels were determined as 657.4 pg/mL (248.9-1065.8) and 93.5 ng/mL (82.07-105) for the positive cases and 966.3 pg/mL (756-1160.3) and 76.2 ng/mL (67.2-89.3) for the negative cases, respectively.

The correlations between CLP, S100A6, and HMGN1 levels and clinical and laboratory values are summarized in Table 4.

Table 1. Demographic data and comorbidities of all participants.

	Patient group, n=55	Control group, n=32	p
Age, years	63	64	p=0.328
Sex	21 women (38.2%)	15 women (46.9%)	p=0.64
Presence of comorbidities	30 (54.5%)	15 (46.8%)	p=0.359

Discussion

The roles of MAPK and NF-κB, which are activated by the RAGE pathway, in which CLP is a ligand, in the pathogenesis of MM are known [21,22,23]. However, there are limited studies in the literature on the role of CLP in the pathogenesis of MM. S100A9 was previously shown to increase the secretion of cytokines such as tumor necrosis factor alpha, interleukin-6, and interleukin-10 from myeloid-derived suppressor cells, which play an important role in the survival and proliferation of myeloma cells [24]. Lin et al. [25], in their study on cell lines and rats, suggested that CLP contributes to MM progression

Table 2. Laboratory and clinical features of the patients.

Feature	n (%)	Feature	n (%)
Stage (ISS)*		**Lytic bone lesions*****	
I	10 (19.6)	None	11 (21.2)
II	17 (33.4)	<4	10 (19.2)
III	24 (47)	4-10	11 (21.2)
R-ISS**		>10	20 (38.4)
I	6 (18.2)	**Pathological fractures*****	
II	18 (54.5)	Present	11 (21.2)
III	9 (27.3)	Absent	41 (78.8)
Type of MM		**Plasmacytoma*****	
IgA kappa	5 (9)	Present	10 (19.2)
		Absent	42 (80.8)
IgA lambda	3 (5.5)	**Comorbidities**	
IgG kappa	18 (32.7)	CAD	5 (9.1)
IgG lambda	14 (25.5)	HT	8 (14.5)
Lambda light chain disease	5 (9.1)	DM + HT	7 (12.7)
Kappa light chain disease	8 (14.5)	None	25 (45.5)
Non-secretory	2 (3.6)	Others	10 (18.2)

*: Data of 4 patients are missing; **: data available for 33 patients; ***: data of 3 patients are missing; ISS: International Staging System; R-ISS: Revised ISS; CAD: coronary artery disease; HT: hypertension; DM: diabetes mellitus.

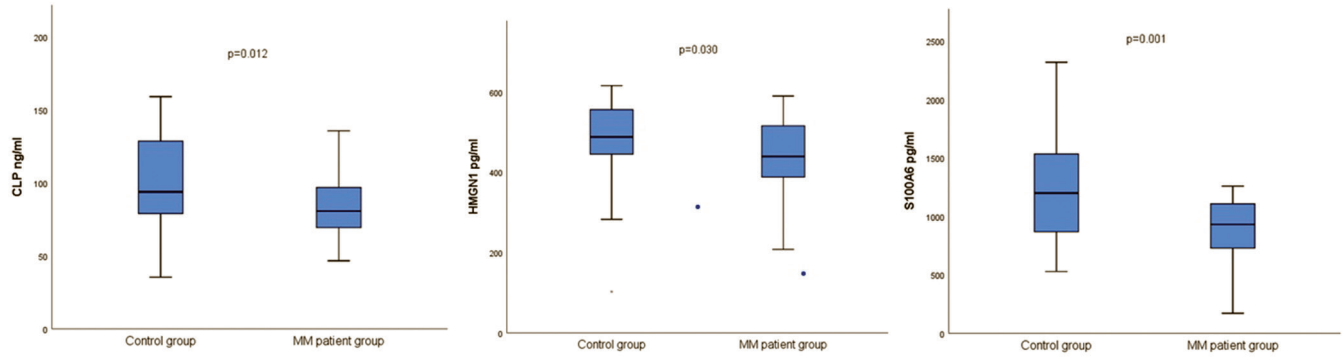

Figure 1. Calprotectin, HMGN1, and S100A6 levels of the control group and the patients at the time of diagnosis.
CLP: Calprotectin; HMGN1: high mobility group nucleosome-binding protein 1.

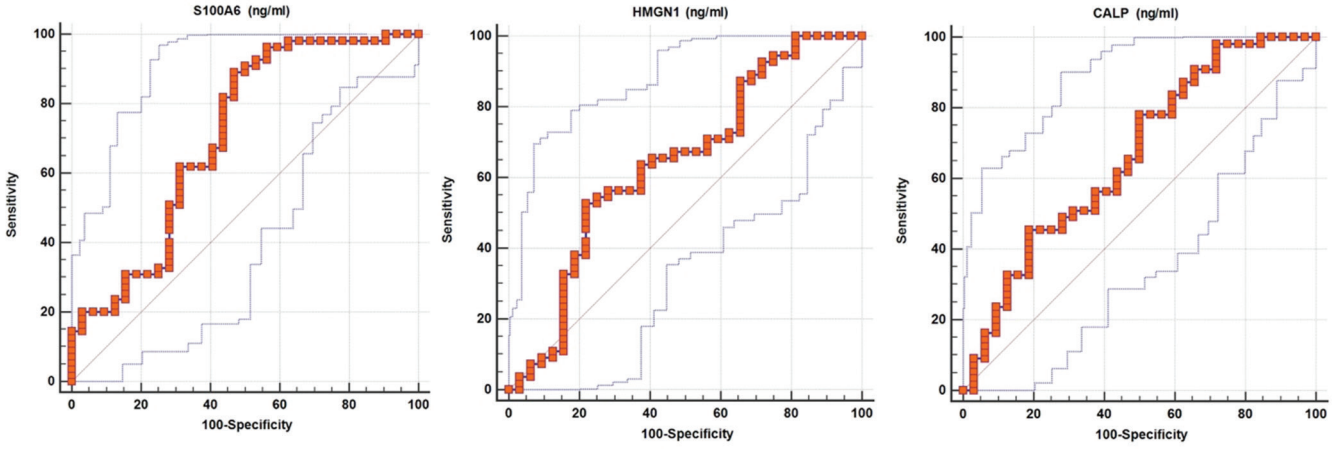

Figure 2. Receiver operating characteristic curves for cut-off values of S100A6 (AUC: 0.706, 95% CI: 0.598-0.799), high mobility group nucleosome-binding protein 1 (AUC: 0.640, 95% CI: 0.530-0.740), and calprotectin (AUC: 0.663, 95% CI: 0.554-0.761) in diagnosing multiple myeloma.
CLP: Calprotectin; HMGN1: high mobility group nucleosome-binding protein 1.

by increasing megakaryopoiesis and indirectly angiogenesis. They also found that CLP levels were higher in the bone marrow of MM patients than in peripheral blood, but they did not compare those results with healthy controls [25]. In another study, fecal CLP levels were found to be significantly higher in MM patients compared to a healthy control group. When newly diagnosed MM patients and treated MM patients were compared, fecal CLP levels were found to be higher in the newly diagnosed group [26]. In our study, CLP levels were examined in serum instead of feces, as in studies conducted on solid malignancies and rheumatological diseases. Serum CLP levels were found to be low enough to have diagnostic significance in the MM patient group compared to the healthy control group. A significant negative correlation was found between CLP levels and serum M protein, immunoglobulin G, and globulin levels. In addition, serum CLP levels were found to be statistically significantly higher in patients with light chain MM compared to heavy chain patients. These results suggest that CLP may bind to the paraprotein produced by heavy chain MM in the blood, causing its blood levels to be low. The negative correlation between beta-2 microglobulin, which is a prognostic factor for MM, and CLP level suggests that low CLP levels may be associated with poor prognosis. However, this hypothesis needs to be confirmed by survival analyses.

In a recent study conducted with relapsed refractory MM patients, increased *S100A6* gene expression and high protein levels in CD138-positive cell samples were found. The association of increased gene expression with advanced-stage disease and decreased overall survival was shown in the same study [27]. In our study, serum S100A6 levels were significantly lower in patients with newly diagnosed MM compared to healthy controls. The low serum levels of S100A6 despite its increased intracellular level may be due to increased clearance, limited release into the extracellular space, or rapid reuptake by binding to its cellular receptors. It is also known that the secretion of S100A6, found in the cytoskeleton, from neutrophils is very limited and conditional, unlike CLP [28]. In addition, the

Table 3. Calprotectin, S100A6, and high mobility group nucleosome-binding protein 1 (HMGN1) levels summarized according to clinical and laboratory findings.

	Calprotectin (ng/mL), median (min-max)		S100A6 (pg/mL), median (min-max)		HMGN1 (pg/mL), median (min-max)	
Sex						
Female	76.93 (556.02-111.48)	p=0.390	893.4 (181.33-1980.7)	p=0.291	436.75 (363.72-582.06)	p=0.96
Male	82.7 (46.72-135.82)		981.39 (136.33-1260)		445.68 (207.90-590.12)	
Comorbidities						
Present	77.9 (52.7-117.9)	p=0.417	950.9 (777.4-1056.2)	p=0.800	436.49 (271.3-588.39)	p=0.642
Absent	80.7 (46.7-135.8)		932 (172.06-1260.06)		440.18 (207.9-590.12)	
Type of MM						
Light chain	90.01 (58.02-135.82)	**p=0.030**	1065.8 (172-1231)	p=0.192	449.1 (370.3-590.1)	p=0.88
Heavy chain	77.18 (46.72-111.48)		923.5 (136.3-1980.7)		436.2 (207.9-588.39)	
ISS						
I	78.36 (62-102.23)	p=0.825	979.7 (172-1980)	p=0.725	437.3 (207.9-582.06)	p=0.677
II + III	81.24 (46.72-135.82)		954.56 (136.33-1260)		438.11 (271.30-590.1)	
R-ISS						
I	75.17 (62-90.01)	p=0.228	979.76 (172.06-1980.7)	p=0.845	405.28 (207.90-524.51)	p=0.217
II + III	85.24 (52.7-135.82)		923.53 (243.46-1251)		436.24 (271.3-590.12)	
Lytic bone lesions						
<4	83.75 (60.93-111.48)	p=0.507	1003 (172.06-1260)	p=0.825	436.24 (360.72-588.39)	p=0.900
>4	77.18 (46.72-135.82)		972.46 (181.33-1980.73)		439.8 (207.90-590.1)	
Pathological fractures						
Present	95.5 (60.9-135.8)	p=0.170	896.33 (538.05-1010.91)	p=0.190	494 (207.90-590.12)	p=0.710
Absent	80.78 (46.72-117.98)		985.66 (842.64-1047.56)		436.7 (271.3-588.3)	
Plasmacytoma						
Present	76.93 (52.7-105)	p=0.591	987.06 (181.33-1251)	p=0.703	494 (367.27-582.06)	p=0.061
Absent	81.71 (46.72-135.82)		972.46 (172.06-1980.7)		434.54 (207.9-590.1)	

ISS: International Staging System; MM: multiple myeloma; R-ISS: Revised ISS; min: minimum; max: maximum.

Wnt/β-catenin pathway is activated in MM and S100A6 increases intracellular β-catenin by interacting with calcyclin-binding protein/Siah-1-interacting protein [29,30]. As a result, S100A6 may be primarily involved in intracellular pathways in the pathogenesis of MM.

Since analysis was not performed for a sufficient number of patients, the effects of chromosome 1q21 gain and amplification on CLP and S100A6 could not be evaluated.

Genetic analyses have revealed that both the progression of myeloma precursor conditions to MM and MM disease progression are associated with clonal evolution as a result of accumulating mutations [31,32]. HMGN1 has global roles in the repair of DNA lesions and local roles in the transcriptional control of proto-oncogenes and tumor-suppression genes. It has been shown that proto-oncogenes and pro-metastatic genes such as c-fos, *BCL3*, and *N-cadherin* are upregulated in HMGN1-negative cell lines [33,34]. In our study, serum HMGN1 levels were found to be significantly lower in MM patients compared to the control group. If low serum HMGN1 levels are a reflection of low intracellular levels, it can be suggested that HMGN1 may contribute to the genetic abnormalities seen in MM. However, the presence of autoantibodies against HMGN1 in the blood in some autoimmune diseases has been shown in the literature, and the low levels we detected in MM patients may be of immune origin [35,36,37]. The immunomodulatory effects observed in studies evaluating HMGN1 combinations in cancer treatment suggest that HMGN1 may be a promising molecule in the treatment of MM, in which the microenvironment plays an important role in the pathogenesis [19,38,39,40].

Study Limitations

The limitations of this study include its relatively small cohort, lack of consecutive sampling, and not being supported by immunohistochemistry and in vitro cell line models. However, the use of the easily accessible ELISA method provides advantages in terms of reproducibility and verifiability.

Table 4. Correlations between S100A6, calprotectin, and high mobility group nucleosome-binding protein 1 (HMGN1) levels and clinical and laboratory values.

		1	2	3	4	5	6	7	8	9	10	11
1- S100A6	r	1.000										
	p											
2- Calprotectin	r	**0.256***	1.000									
	p	**0.017**										
3- HMGN1	r	**0.534****	**0.357****	1.000								
	p	**0.000**	**0.001**									
4- Beta-2 microglobulin	r	-0.063	**-0.300***	-0.088	1.000							
	p	0.661	**0.033**	0.538								
5- CRP	r	0.173	0.114	0.185	**0.289***	1.000						
	p	0.205	0.407	0.177	**0.040**							
6- M protein	r	-0.056	**-0.361***	-0.045	**0.439****	0.068	1.000					
	p	0.733	**0.024**	0.784	**0.008**	0.683						
7- Age	r	-0.087	-0.060	-0.167	0.259	0.264	**0.353***	1.000				
	p	0.422	0.582	0.123	0.066	0.051	**0.027**					
8- IgG	r	-0.018	**-0.370****	-0.176	**0.289***	0.119	**0.701****	**0.483****	1.000			
	p	0.897	**0.006**	0.204	**0.042**	0.392	**0.000**	**0.000**				
9- IgA	r	-0.075	0.092	0.129	-0.209	0.057	-0.249	-0.257	**-0.528****	1.000		
	p	0.586	0.502	0.349	0.141	0.680	0.126	0.059	**0.000**			
10- Globulin	r	-0.145	**-0.279***	-0.027	**0.318***	0.098	**0.906****	**0.342***	**0.679****	-0.157	1.000	
	p	0.295	**0.041**	0.847	**0.024**	0.481	**0.000**	**0.011**	**0.000**	0.257		
11- Plasma cell ratio	r	0.062	-0.086	0.032	0.172	0.079	**0.405***	0.090	0.022	-0.262	0.111	1.000
	p	0.669	0.551	0.826	0.249	0.585	**0.014**	0.534	0.879	0.066	0.449	

*: Significant at the level of 0.05; **: significant at the level of 0.01; CRP: C-reactive protein; IgG: immunoglobulin G; IgA: immunoglobulin A.

Conclusion

In this study, we found that serum levels of CLP, S100A6, and HMGN1 were significantly lower in patients with newly diagnosed MM. Further studies using molecular, genetic, and immunohistochemical methods with larger and consecutive samples are needed to clarify the roles of CLP, S100A6, and HMGN1 in the pathogenesis of MM.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Kocaeli University Medical Faculty Ethics Committee on October 21, 2021 (project no: 2021/285, decision no: GOKAEK-2021/18.06).

Informed Consent: Written informed consent was obtained from all participants.

Authorship Contributions

Surgical and Medical Practices: A.G., E.T.D., M.G.P., B.Ö.; Concept: A.G., E.T.D., M.G.P., P.T., Ö.M., A.H.; Design: A.G., E.T.D., M.G.P., P.T., Ö.M., A.H.; Data Collection or Processing: A.G., E.T.D., M.G.P., B.H.E., H.A., H.A.E., E.M.Y.; Analysis or Interpretation: A.G., E.T.D., M.G.P., B.Ö.; Literature Search: A.G., E.T.D., M.G.P.; Writing: A.G., E.T.D., M.G.P.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by the Kocaeli University Scientific Research Projects Coordination Unit (project number: TSA-2022-2836).

References

1. Ludwig H, Novis Durie S, Meckl A, Hinke A, Durie B. Multiple myeloma incidence and mortality around the globe; interrelations between health access and quality, economic resources, and patient empowerment. Oncologist 2020;25:e1406-e1413.
2. Braunlin M, Belani R, Buchanan J, Wheeling T, Kim C. Trends in the multiple myeloma treatment landscape and survival: a U.S. analysis using 2011-2019 oncology clinic electronic health record data. Leuk Lymphoma 2021;62:377-386.
3. Minnie SA, Hill GR. Immunotherapy of multiple myeloma. J Clin Invest 2020;130:1565-1575.
4. Nie Y, Yang D, Oppenheim JJ. Alarmins and antitumor immunity. Clin Ther 2016;38:1042-1053.
5. Marzin Y, Jamet D, Douet-Guilbert N, Morel F, Le Bris MJ, Morice P, Abgrall JF, Berthou C, De Braekeleer M. Chromosome 1 abnormalities in multiple myeloma. Anticancer Res 2006;26:953-959.
6. Giri S, Huntington SF, Wang R, Zeidan AM, Podoltsev N, Gore SD, Ma X, Gross CP, Davidoff AJ, Neparidze N. Chromosome 1 abnormalities and survival of patients with multiple myeloma in the era of novel agents. Blood

Adv 2020;4:2245-2253.

7. Hanamura I, Stewart JP, Huang Y, Zhan F, Santra M, Sawyer JR, Hollmig K, Zangarri M, Pineda-Roman M, van Rhee F, Cavallo F, Burington B, Crowley J, Tricot G, Barlogie B, Shaughnessy JD Jr. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood 2006;108:1724-1732.
8. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, Geczy CL. Functions of S100 proteins. Curr Mol Med 2013;13:24-57.
9. Kuberappa PH, Bagalad BS, Ananthaneni A, Kiresur MA, Srinivas GV. Certainty of S100 from physiology to pathology. J Clin Diagn Res 2016;10:ZE10-ZE15.
10. Heizmann CW. S100 Proteins. In: Offermanns S, Rosenthal W (eds). Encyclopedia of Molecular Pharmacology. Cham, Springer, 2021.
11. Sinha P, Okoro C, Foell D, Freeze HH, Ostrand-Rosenberg S, Srikrishna G. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. J Immunol 2008;181:4666-4475.
12. Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. Nat Rev Cancer 2015;15:96-109.
13. Moon A, Yong HY, Song JI, Cukovic D, Salagrama S, Kaplan D, Putt D, Kim H, Dombkowski A, Kim HR. Global gene expression profiling unveils S100A8/A9 as candidate markers in H-Ras-mediated human breast epithelial cell invasion. Mol Cancer Res 2008;6:1544-1553.
14. Liu K, Zhang Y, Zhang C, Zhang Q, Li J, Xiao F, Li Y, Zhang R, Dou D, Liang J, Qin J, Lin Z, Zhao D, Jiang M, Liang Z, Su J, Gupta VP, He M, Yang X. Methylation of S100A8 is a promising diagnosis and prognostic marker in hepatocellular carcinoma. Oncotarget 2016;7:56798-56810.
15. Pröpper C, Huang X, Roth J, Sorg C, Nacken W. Analysis of the MRP8-MRP14 protein-protein interaction by the two-hybrid system suggests a prominent role of the C-terminal domain of S100 proteins in dimer formation. J Biol Chem 1999;274:183-188.
16. Yang D, Postnikov YV, Li Y, Tewary P, de la Rosa G, Wei F, Klinman D, Gioannini T, Weiss JP, Furusawa T, Bustin M, Oppenheim JJ. High-mobility group nucleosome-binding protein 1 acts as an alarmin and is critical for lipopolysaccharide-induced immune responses. J Exp Med 2012;209:157-171.
17. Gerlitz G, Hock R, Ueda T, Bustin M. The dynamics of HMG protein-chromatin interactions in living cells. Biochem Cell Biol 2009;87:127-137.
18. Nanduri R, Furusawa T, Bustin M. Biological functions of HMGN chromosomal proteins. Int J Mol Sci 2020;21:449.
19. Wei F, Yang D, Tewary P, Li Y, Li S, Chen X, Howard OM, Bustin M, Oppenheim JJ. The alarmin HMGN1 contributes to antitumor immunity and is a potent immunoadjuvant. Cancer Res 2014;74:5989-5998.
20. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, Kumar S, Hillengass J, Kastritis E, Richardson P, Landgren O, Paiva B, Dispenzieri A, Weiss B, LeLeu X, Zweegman S, Lonial S, Rosinol L, Zamagni E, Jagannath S, Sezer O, Kristinsson SY, Caers J, Usmani SZ, Lahuerta JJ, Johnsen HE, Beksac M, Cavo M, Goldschmidt H, Terpos E, Kyle RA, Anderson KC, Durie BG, Miguel JF. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538-e548.
21. Geduk A, Oztas B, Eryılmaz BH, Demirsoy ET, Menguc MU, Unal S, Mersin S, Polat MG, Aygun K, Yenihayat EM, Albayrak H, Erol HA, Balcı S, Mehtap O, Tarkun P, Hacihanefioglu A. Effects of AGEs, sRAGE and HMGB1 on clinical outcomes in multiple myeloma. Indian J Hematol Blood Transfus 2023;39:220-227.
22. Vrábel D, Pour L, Ševčíková S. The impact of NF-κB signaling on pathogenesis and current treatment strategies in multiple myeloma. Blood Rev 2019;34:56-66.
23. Chatterjee M, Jain S, Stühmer T, Andrulis M, Ungethüm U, Kuban RJ, Lorentz H, Bommert K, Topp M, Krämer D, Müller-Hermelink HK, Einsele H, Greiner A, Bargou RC. STAT3 and MAPK signaling maintain overexpression of heat shock proteins 90alpha and beta in multiple myeloma cells, which critically contribute to tumor-cell survival. Blood 2007;109:720-728.
24. De Veirman K, De Beule N, Maes K, Menu E, De Bruyne E, De Raeve H, Fostier K, Moreaux J, Kassambara A, Hose D, Heusschen R, Eriksson H, Vanderkerken K, Van Valckenborgh E. Extracellular S100A9 protein in bone marrow supports multiple myeloma survival by stimulating angiogenesis and cytokine secretion. Cancer Immunol Res 2017;5:839-846.
25. Lin C, Garcia-Gerique L, Bonner EE, Mastio J, Rosenwasser M, Cruz Z, Lawler M, Bernabei L, Muthumani K, Liu Q, Poncz M, Vogl T, Törngren M, Eriksson H, Vogl DT, Gabrilovich DI, Nefedova Y. S100A8/S100A9 promote progression of multiple myeloma via expansion of megakaryocytes. Cancer Res Commun 2023;3:420-430.
26. Khosravi P, Abroun S, Kaviani S, Masoudifar S, Farahani HS. Calprotectin as new potential clinical marker for multiple myeloma. PLoS One 2023;18:e0282841.
27. Liu M, Wang Y, Miettinen JJ, Kumari R, Majumder MM, Tierney C, Bazou D, Parsons A, Suvela M, Lievonen J, Silvennoinen R, Anttila P, Dowling P, O'Gorman P, Tang J, Heckman CA. S100 calcium binding protein family members associate with poor patient outcome and response to proteasome inhibition in multiple myeloma. Front Cell Dev Biol 2021;9:723016.
28. Singh P, Ali SA. Multifunctional role of S100 protein family in the immune system: an update. Cells 2022;11:2274.
29. Sukhdeo K, Mani M, Hideshima T, Takada K, Pena-Cruz V, Mendez G, Ito S, Anderson KC, Carrasco DR. β-catenin is dynamically stored and cleared in multiple myeloma by the proteasome-aggresome-autophagosome-lysosome pathway. Leukemia 2012;26:1116-1119.
30. Schneider G, Filipek A. S100A6 binding protein and Siah-1 interacting protein (CacyBP/SIP): spotlight on properties and cellular function. Amino Acids 2011;41:773-780.
31. Bolli N, Maura F, Minvielle S, Gloznik D, Szalat R, Fullam A, Martincorena I, Dawson KJ, Samur MK, Zamora J, Tarpey P, Davies H, Fulciniti M, Shammas MA, Tai YT, Magrangeas F, Moreau P, Corradini P, Anderson K, Alexandrov L, Wedge DC, Avet-Loiseau H, Campbell P, Munshi N. Genomic patterns of progression in smoldering multiple myeloma. Nat Commun 2018;9:3363.
32. Hu Y, Chen W, Wang J. Progress in the identification of gene mutations involved in multiple myeloma. Onco Targets Ther 2019;12:4075-4080.
33. Lim JH, Catez F, Birger Y, West KL, Prymakowska-Bosak M, Postnikov YV, Bustin M. Chromosomal protein HMGN1 modulates histone H3 phosphorylation. Mol Cell 2004;15:573-584.
34. Rubinstein YR, Furusawa T, Lim JH, Postnikov YV, West KL, Birger Y, Lee S, Nguyen P, Trepel JB, Bustin M. Chromosomal protein HMGN1 modulates the expression of N-cadherin. FEBS J 2005;272:5853-5863.
35. Ayer LM, Sénecal JL, Martin L, Dixon GH, Fritzler MJ. Antibodies to high mobility group proteins in systemic sclerosis. J Rheumatol 1994;21:2071-2075.
36. Ayer LM, Rubin RL, Dixon GH, Fritzler MJ. Antibodies to HMG proteins in patients with drug-induced autoimmunity. Arthritis Rheum 1994;37:98-103.
37. Morse JH, Barst RJ, Fotino M, Zhang Y, Flaster E, Fritzler MJ. Primary pulmonary hypertension: immunogenetic response to high-mobility group (HMG) proteins and histone. Clin Exp Immunol 1996;106:389-395.
38. Chen CY, Ueha S, Ishiwata Y, Yokochi S, Yang D, Oppenheim JJ, Ogiwara H, Shichino S, Deshimaru S, Shand FHW, Shibayama S, Matsushima K. Combined treatment with HMGN1 and anti-CD4 depleting antibody reverses T cell exhaustion and exerts robust anti-tumor effects in mice. J Immunother Cancer 2019;7:21.

39. Han Z, Yang D, Trivett A, Oppenheim JJ. Therapeutic vaccine to cure large mouse hepatocellular carcinomas. Oncotarget 2017;8:52061-52071.

40. Chen CY, Ueha S, Ishiwata Y, Shichino S, Yokochi S, Yang D, Oppenheim JJ, Ogiwara H, Deshimaru S, Kanno Y, Aoki H, Ogawa T, Shibayama S, Matsushima K. Combining an alarmin HMGN1 peptide with PD-L1 blockade results in robust antitumor effects with a concomitant increase of stem-like/progenitor exhausted CD8+ T cells. Cancer Immunol Res 2021;9:1214-1228.