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Determination of Serum Cathepsin G Level in Patients with Multiple Myeloma

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ABSTRACT

Multiple myeloma is a hematological malignancy identified by bone marrow infiltration of clonal plasma cells. It is still not a curable disease under current conditions. Cathepsin G is a serine protease playing a role in inflammation that is present in the azurophilic granules of neutrophils. It is known that there is a relationship between Cathepsin G and chronic inflammatory diseases and tumors. The goal of the study is to define its role in multiple myeloma. In the study, 33 patients newly diagnosed with MM who were never received treatment and 33 control subjects were included. Basic laboratory parameters and Cathepsin G levels were examined both in the myeloma patient group and control group. While the serum CathG level in the control group is 22.84 ng/mL, the serum CathG level of the MM patient group is 10.77 ng/mL. Serum CathG level in the control group is statistically significantly higher than the MM patient group. The aim of the present study is to contribute to the literature in hematological malignancies, to figure out the role of Cathepsin G in multiple myeloma, and to open a door to new treatment options for multiple myeloma, which is an incurable disease, yet.

Keywords: Cathepsin G, Inflammation, Multiple myeloma.

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Introduction

Multiple myeloma (MM) is a hematological malignancy caused by excessive neoplastic reproduction of plasma cells. MM forms 1% of all malignancies and 10% of hematological malignancies. Mean age of diagnosis is 65. Despite mean survival in MM is 5-7 years, it may vary depending on the host factors, tumor load, cytogenetic anomalies, and the response to treatment [1].

In the patient group suitable for autologous stem cell transplantation, 4-year survival rate is over 80%. Among these patients, overall survival is more than 8 years. Overall survival is lower in elder patients (age >75) and it is approximately 5 years. Although there is an increase in general survival rates and response to treatment via new treatment options, MM is still an incurable disease [1]. Therefore, new treatment options are being searched for MM.

Cathepsin G (CathG) is a neutral serine protease found in azurophilic granules of neutrophils which is accepted as one of the inflammation effectors. In addition, it is also found in some non-myeloid cells such as endothelium, smooth muscle cells, brain astrocytes, and fibroblasts. CathG activates metalloproteases and plays a role in chemotaxis by degrading the extracellular proteins. CathG both helps specific immune response by antigen presentation and neutralizes toxins with its antimicrobial properties. It also has antimicrobial properties and neutralizes toxins [2].

CathG involves in the pathogenesis of some diseases related to chronic inflammatory process (such as atherosclerosis, chronic obstructive pulmonary diseases, neuropathies, and tumor). CathG has pro-inflammatory or anti-inflammatory properties depending on the physiological conditions paradoxically, location of CathG secretion, and the nature of the substrate. CathG-mediated proteolysis may cause an increase in inflammatory response or suppression of inflammation. As an example, elastolytic activity of CathG causes atherogenesis in early period. However, CathG inhibits the progress of atherosclerosis by eliminating low-density lipoproteins (LDL). It is thought that CathG, which is multifunctional protease, is of critical importance in maintaining the sensitive balance between tissue preservation and destruction during inflammatory response [2,3].

It is known that neutrophils invade several tumor tissues and affect tumor development [4]. However, the role of neutrophil proteases including CathG in the tumor metastasis is not well understood.

The goal of the study is to determine the role of CathG in MM and to elucidate new agents in myeloma for which new treatment options are being searched.

Materials and Methods

In this study, blood samples taken with approval from Sivas Cumhuriyet University Interventional Clinical Research Ethics Committee in February 2022 (Decision no: 2022-02/01) were used with the second ethics committee approval (Decision no:2023-09/02).

Thirty-three patients with newly diagnosed MM, who were applied to the Department of Internal Medicine, Division of Hematology of Sivas Cumhuriyet University

and who had never received any treatment, were included in the study. The control group consisted of 33 volunteers, who were over 18 years, had no comorbidities, no regular medication, were not pregnant, did not smoke, had no active infection, and had no diagnosed active malignancy or history of malignancy.

In the study, examinations made at the beginning of application were used as routine blood examinations. ISS (International Staging System) was used for determining the stages of the disease. The presence of fractures at the time of diagnosis were decided by examining the positron emission computerized tomography (PET/CT), computerized tomography, and if any, magnetic resonance image reports.

Serum CathG level and laboratory values were analyzed by using the blood samples taken. Bloods taken were centrifuged at 4000 Rpm for 10 minutes and kept in an Eppendorf tube at -80 degrees. Serum cathepsin G level was measured by commercial ELISA kit (Sun Red, China®).

Statistics

Data analysis was done by SPSS 26.0 program and studied with 95% confidence level. Mean, standard deviation (Mean±sd), median (M), and 1st and 3rd quartiles were given for the measurements. In the study, Mann Whitney U test was used for two groups in comparing measurements according to groups, Kruskal Wallis H test for more than two groups, Chi-square test for the relationship of group variables, and Spearman rho correlation test for the relationships between numerical measurements.

Results

In the present study, 33 MM patients applied to the Division of Hematology, Department of Internal Medicine of Sivas Cumhuriyet University Faculty of Medicine, and 33 control subjects were included. Of MM patient group, 39.4% (n=13) were under 65 years and 60.6% (n=20) were over 65 years. In the control group, 66.7% (n=22) were under 65 years and 33.3% (n=11) were over 65 years. In the MM patient group, 39.3% (n=13) were female and 60.6% (n=20) were male and in the control group, 60.6% (n=20) were female and 39.4% (n=13) were male.

A statistically significant difference was found between the MM patient group and control group in terms of age (p=0.006<0.05), BUN (p<0.001), creatinine (p=0.001<0.05), uric acid (p<0.001), total protein (p=0.001<0.05), albumin (p<0.001), phosphorus (p=0.002<0.05), magnesium (p=0.004<0.05), hemoglobin (p<0.001), platelet count (p<0.001), and erythrocyte sedimentation rate (p<0.001) measurements. In MM patient group, age (69), BUN (22.3 mg/dL), creatinine (0.95 mg/dl), uric acid (6.2 mg/dL), total protein (85 g/L), phosphorus (3.99 mg/dL), erythrocyte sedimentation rate (102 mm/h) measurements were higher and in the control group, albumin (46.4 g/L), hemoglobin (14.05 g/dL), platelet count (291 10^9/L) measurements were higher. Other measurements did not indicate any significant difference among the groups (p>0.05) (Table 1).

There was a statistically significant difference between MM patient group and control group in terms of serum CathG (p=0.040 <0.05) measurements. In the control group, CathG measurements were higher than MM patient group (Table 1).

Table 1. Comparison of Clinical Measurements According to the Groups

	MM	Control	Test/p
Age	69 (59.5-77.0)	34 (31-38)	U=8.000/ 0.000*
BUN(mg/dL)	22.3 (17.35-32.5)	12.4 (10.95-13.95)	U=154.5/ 0.000*
Creatine (mg/dl)	0.95 (0.74-1.51)	0.76 (0.64-0.88)	U=293.5/ 0.001*
Uric acid (mg/dL)	6.2 (4.7-7.7)	4.4 (3.6-5.1)	U=180.5/ 0.000*
Total protein(g/L)	85 (67.3-102.15)	74.5 (71.2-75.9)	U=356.0/ 0.016*
Albumin(g/L)	35.6 (30.55-38.5)	46.4 (44.7-48.15)	U=22.0/ 0.000*
LDH (U/L)	169 (145.5-213.0)	167 (153-181)	U=535.5/ 0.908
Ca (mg/dL)	9.1 (8.78-10.32)	9.52 (9.28-9.75)	U= 428.5/ 0.137
P (mg/dL)	3.99 (3.69-4.67)	3.4 (3.17-3.82)	U=273.0/ 0.000*
Mg (mg/dL)	1.93 (1.74-2.05)	2.08 (1.95-2.18)	U=296.5/ 0.001*
WBC (10^6/L)	6672.12±2648.42	6545.45±1520.87	t=0.238/ 0.812
Neu (10^6/L)	3420 (2390-5395)	3460 (2690-4605)	U=537.5/ 0.928
Lymp (10^6/L)	1931.52±696.55	2152.73±493.05	t=-1.489/ 0.142
Hgb (g/dL)	10.48±2.19	14.05±1.65	t=-7.466/ 0.000*
Platelet (10^9/L)	223 (157.5-268.5)	291 (244,5-342)	U=263.5/ 0.000*
ESR (mm/h)	102.0 (42.5-116.5)	6.0 (2.0-17.0)	U=31.0/ 0.000*
Cathepsin G (ng/mL)	10.77 (5.12-23.26)	22.84 (8.17-32.92)	U=384.0/ 0.040*

*p<0.05 significant difference, t/Mann Whitney U test BUN: Blood Urea Nitrogen, LDH: Lactate dehydrogenase, Ca: Calcium, P: Phosphor, Mg: Magnesium, WBC: White Blood Cell, Neu: Neutrophil, Lymp: Lymphocyte, Hgb: Hemoglobin, ESR: Erythrocyte Sedimentation Rate

In MM patient group, the rate of IgG kappa was 30.3% (n=10), the rate of IgG lambda was 24.2% (n=8), the rate of IgA kappa was 6.1% (n=2), the rate of IgA lambda was 18.2% (n=6), the rate of kappa light chain was 12.1% (n=4), the rate of lambda light chain was 6.1% (n=2), and the rate of non-secretory type myeloma was 3% (n=1) (Table 2).

Table 2. Relationship of Clinical Properties with Gender in MM Patient Group

N (%)		Female	Male	Total	p
MM type	IgG kappa	7 (53.8)	3 (15)	10 (30.3)	0,009*
	IgG lambda	3 (23.1)	5 (25)	8 (24.2)	
	IgA kappa	0 (0)	2 (10)	2 (6.1)	
	IgA lambda	0 (0)	6 (30)	6 (18.2)	
	Kappa light chain myeloma	1 (7.7)	3 (15)	4 (12.1)	
	Lambda light chain myeloma	2 (15.4)	0 (0)	2 (6.1)	
	Non-secretory type myeloma	0 (0)	1 (5)	1 (3)	
Stage at diagnosis according to ISS	Stage 1	3 (23.1)	3 (15)	6 (18.2)	0.811
	Stage 2	3 (23.1)	6 (30)	9 (27.3)	
	Stage 3	7 (53.8)	11 (55)	18 (54.5)	
	Yes	10 (83.3)	19 (95)	29 (90.6)	
	Yes	4 (100)	8 (61.5)	12 (70.6)	
Bone involvement on PET at diagnosis	No	1 (7.7)	5 (26.3)	6 (18.8)	0.164
	Yes	12 (92.3)	14 (73.7)	26 (81.3)	
Fracture at diagnosis	No	10 (76.9)	16 (84.2)	26 (81.3)	0.606
	Yes	3 (23.1)	3 (15.8)	6 (18.8)	

At the moment of diagnosis, 54.5% (n=18) of the patients were in stage 3, 27.3% (n=9) in stage 2, and 18.2% (n=6) patients in stage 1. Bone involvement on PET/CT at diagnosis was detected in 81.3% (n=26) of the patients. Fracture at the time of diagnosis was seen in 18.8% (n=6) patients (Table 2). A statistically significant relationship was found between gender and MM sub-types in MM patient group (p=0.023<0.05). While the incidence rate of IgG kappa in women was higher (53.8%), the incidence rate of IgA lambda in men was higher (30.0%). The relationship of other clinical properties with gender was not significant (p>0.05) (Table 2).

In the study, 75% of the patients in the myeloma group received ortezomib+cyclophosphamide+dexamethasone protocol as the first-line therapy, and 2% of the patients were followed up as drug-free. Of the patients, 52% responded positively to first-line therapy and 33.3% of them were subject to autologous stem cell transplantation. Second-line therapy was given to a total of 22 patients; 40.9% of these patients received lenalidomid+dexamethasone protocol and remission was obtained in 44.4%. After second-line therapy, autologous stem cell transplantation was applied to 26.7% of the patients. A total of 12 patients received third-line therapy and the most preferred protocols in the treatment were lenalidomid+dexamethasone and lenalidomid+dexamethazone+ixazomib protocols.

Remission was obtained in 4 patients and 2 of them underwent autologous stem cell transplantation. In MM patient group, the difference between the patients who responded first-, second-, and third-line therapies and the patients who did not respond in terms of CathG measurements was not statistically significant (p>0.05).

In MM patient group, a negative statistically significant relationship was found in terms of lactate dehydrogenase (r=-0.428, p<0.05), WBC (White blood cell) (r=-0.349, p<0.05), absolute neutrophil count (r=-0.370, p<0.05), and hemoglobin (r=-0.353, p<0.05) measurements via CathG measurement (Table 3). The relationship between other measurements was not significant (p>0.05).

Table 3. Relationship of Clinical Measurements and Cathepsin G Measurement in MM Patient Group

	Cathepsin G
Blood Urea Nitrogen	-.179
Creatine	-.160
Uric acid	-.135
Total protein	-.045
Albumin	.022
Lactate dehydrogenase	-.428*
Calcium	-.099
Phosphor	-.227
Magnesium	.138
White Blood Cell	-.349*
Neutrophil	-.370*
lymphocyte	-.113
Hemoglobin	-.353*
Platelet	-.195
Erythrocyte Sedimentation Rate	.194
Beta2 Microglobulin	-.305

**p<0.05 significant relationship, p>0.05 no significant relationship, 0≤r≤0.25 very weak, 0.26≤r≤0.49 weak, 0.50≤r≤0.69 moderate, 0.70≤r≤0.89 strong, 0.90≤r≤1 very strong; Spearman's rho correlation test*

Although serum CathG levels were higher in alive patients than in dead patients in the MM patient group, the difference was not statistically significant (p>0.05) (Table 4).

Table 4. Comparison of Cathepsin G measurements in MM Patient Group according to the Final Situation

	Final situation		
	Exitus	Alive	Test/p
Cathepsin G (ng/mL)	10.24 (4.79-23.05)	11.71(5.22-24.64)	U=129.0/ 0.828

*p<0.05 significant difference, t/Mann Whitney U test

Discussion

Inflammation is defined as one of the necessary factors of cancer; inflammatory cells and secreted factors play a role in almost every step of tumor development and progression. Neutrophils is one of the important factors of tumor-related inflammatory cell infiltration. Their roles in tumor biology have been progressively recognized. Indeed, it is shown that neutrophils have both pro- and anti-tumorigenic properties [5]. Although neutrophils morphologically appear in the same way under a light microscope, it is thought that they have more than one sub-type with different genetic expression profiles. In recent years, neutrophil extracellular traps (NETs) are accepted as a main antimicrobial effector mechanism released by neutrophils as a response to several stimulus. Previous researchs have shown that NETs are involved in many parts of tumor biology, from tumor progression to tumor-related thrombosis [6]. Among them, neutrophil elastase, calprotectin, proteinase 3, matrix metalloproteinase 9, myeloperoxidase, cathepsin G, and bactericidal/permeability-increasing protein take place. Even though the role of NETs in inflammation is well known, its role in tumor progression is not clear [7]. However, there are researchs showing the relationship between NETs and breast, lung, colorectal, pancreas, skin, neurological cancers, and hematological cancers [8-14].

CathG is characteristically stored in the azurophilic granules of neutrophils and released via various stimuli such as the presence of bacteria. CathG is not only related to the resistance to bacterial or fungal infections, but also has diverse physiological functions such as the induction of thrombocyte activation, reshaping of extracellular matrix, induction of leucocyte chemotaxis, activation of inflammatory cytokine release, induction of endothelium-dependent vascular relaxation, anonychia of cardiomyocytes, and endothelial cell damage [15].

Previous researchs have notified that CathG has a role in the forming of metastasis by facilitating angiogenesis and tumor cell dissemination. It is thought that tumor cells form tumor aggregates through homotypic cell-cell interaction as they move away from the main tumor site and tumor emboli and metastases occur. CathG facilitates the formation of tumor aggregates in in vitro breast cancer models. In their study, Yui et al., showed that cell aggregation induced with CathG depended on E-cadherin [16]. Then, in their other study conducted on MCF-7 and MDA MB-231 human breast cancer cell lines, they concluded that CathG induced multicellular aggregate formation in low metastatic and E-cadherin-positive MCF-7 cells; whereas, it decreased the adhesion capacity of high metastatic and E-cadherin-negative MDA MB-231 cells to culture substrates [15]. They argued that CathG secreted by neutrophils infiltrating intra-tumor environments may be an important factor affecting the metastatic capacity of tumor cells and may facilitate dissemination of tumor cell aggregates by acting on E-cadherin-positive tumor cells and inducing detachment from the extracellular matrix, whereas it may inhibit metastasis by suppressing the adhesion capacity between highly metastatic E-cadherin-negative tumor cells and the extracellular matrix. However, they also stated that further studies are needed [15].

There are in vivo evidence showing that CathG plays a role on cancer metastasis. Previous researchs have indicated that high CathG levels in human lung cancers are related to the tumor degree and stage [17].

Wilson et al., indicated that CathG facilitated the formation of osteolytic bone lesions in breast cancer metastasis models. It is thought that high CathG level induced by tumor cells have increased osteoclast activation and thus, the bone lesions have occurred [18].

In the study conducted by Tomoya et al., on MCF-7 human breast cancer cell line, it was shown that cathepsin G inhibited cancer cell contact not over type IV collagen but over fibronectin and had induced cell-cell adhesion [19].

Depending on various researchs in the literature, it has been concluded that CathG facilitates the reshaping of extracellular matrix and potentialize the tumor progress and metastasis [18].

Previous researchs in the literature notified that there was a high level of CathG transcription in the promyelocytic stage of granulocyte development [20]. In their study, Zhang et al., suggested that CathG is highly expressed in myeloid leukemia blasts, and it may be an ideal immunotherapeutic target candidate in patients with acute myeloid leukemia [21].

Conclusion

Upon the literature review, any study on the role of CathG in MM could not be found. In the present study, a statistically significant difference was found between the MM patient group and the control group in terms of serum CathG measurements (p=0.040 <0.05). CathG measurements were higher in the control group than in the MM patient group. This result gives a clue that CathG may have a role in the etiopathogenesis. However, when the literature is examined, most studies conducted on CathG revealed that the presence of CathG was examined in tumor tissue and serum CathG level was not examined.

It is thought that the difference in CathG level in the study may be caused by this reason. Thus, further studies regarding CathG level in multiple myeloma tumor tissue are needed. Any relationship was not found between CathG and general survival; however, a negative and a statistically significant relationship was observed in MM patient group between CathG level and lactate dehydrogenase ($r=-0.428$, $p<0.05$), WBC (White blood cell) ($r=-0.349$, $p<0.05$), absolute neutrophil count ($r=-0.370$, $p<0.05$), and hemoglobin ($r=-0.353$, $p<0.05$) values. These provide clues that CathG may be relatively related to the prognosis.

Consequently, it is considered that CathG plays a role in the etiopathogenesis of MM. However, in vivo, in vitro, and clinical studies are required via further techniques. It is thought that determining the role of CathG in MM will provide new treatment alternatives in the future.

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Conflict of Interest

Authors declared no conflict of interest.

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