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Original article

Determination of concentration of light chains in serum and urine in patients with multiple myeloma

Izeta Aganović-Mušinović[1], Lukša Pranjić[2]*, Alma Sofo-Hafizović[3], Sanida Ljaljević[4]

[1] Department of Immunology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
[2] Student, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
[3] Clinic for Hematology, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina
[4] Clinic for Eye Diseases, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina

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Abstract:

Introduction: Multiple Myeloma (MM) is a malignant disease of the hematopoietic system, which is classified as malignant tumor of mature B-lymphocytes according to the classification of WHO. Multiple Myeloma is characterized by an uncontrollable monoclonal proliferation of bone marrow plasma cells. The main goal of this study was to present the importance of determining κ and λ chains in serum and urine, as well as their ratio in patients with Multiple Myeloma according to various stages of disease and to compare them with the control group.
Methods: The research was conducted at the Clinic for Hematology, University Clinical Center Sarajevo (UKCS). The study included 82 patients of both genders (41 male, 41 female). The control group included 20 healthy volunteers, both genders. The conducted research was a clinical, cross-sectional, descriptive study.Methods of work were Immunoelectrophoresis - for determining Bence-Jones proteins in urine. Determining the concentrations of free light chains in patients with Multiple Myeloma was done using the nephelometric method in serum and urine.
Results:Patients with Multiple Myeloma had a statistically significantly higher ratio value of κ/λ chains in serum 3,9 (0,57-22,48) when compared to the control group 1,35 (1,15-1,79) (p=0,025), as well as, significantly higher ratio value of λ chains in urine 0,007 (0,003-0,003) in comparison with the control group 0,0 (0,0-0,0) (p<0,001). Patients with Multiple Myeloma had a statistically significantly higher ratio value of κ/λ chains in urine 18,49 (0,27-73,29) in comparison with the control group of volunteers 0 (0-0) (p<0.001).
Conclusions: The results of our research have shown that the ratio of κ/λ chains in the serum of patients with MM had a significantly higher value 3,9 (0,57-22,48) in comparison with the κ/λ chains in the serum of the control group of volunteers 1,35 (1,15-1,79) (p=0,025).
Key words: Multiple Myeloma, light chains, heavy chains, Immunoelectrophoresis

***Corresponding author**
Lukša Pranjić
Faculty of Medicine,
University of Sarajevo,
Čekaluša 90, 71000 Sarajevo,
Bosnia and Herzegovina.
Email: pranjicluksa@hotmail.com

Introduction

Multiple myeloma (MM) is a malignant disease of the bloodstream system classified into a group of malignant neoplasms of mature B lymphocytes according to the division of the World Health Organization (1). Multiple myeloma is characterized by uncontrolled monoclonal proliferation of plasma cells in the bone marrow (2). Since malignant plasma cells generally retain the ability of synthesis and immunoglobulin secretion, one of the major features of the disease is the finding of monoclonal protein (M protein), (whole molecules of immunoglobulin and/or light chains) in serum and/or urine (3). Depending on the stage of the disease with the monoclonal protein, the disease is marked by a different degree of bone, osteolytic changes, anemia, hypercalcemia, kidney damage, hypervisic syndrome and blood clotting disorder. There are two phases in the pathogenesis of multiple myeloma. The first phase is the appearance of monoclonal plasma cells that are due to the antigenic stimulation of plasmablasts (possibly infection or general inflammation), lymphocytes B that have undergone a differentiation process in the germinal lymph node and where the relocation of immunoglobulin genes is completed and migrated to the bone marrow where the differentiation in the long-lived plasma cells ends. Antigen stimulation leads to proliferation of cells, which may result in new changes. The diagnosis of MM is based on the presence of the monoclonal plasma cells excess in the bone marrow, monoclonal immunoglobulins in serum or urine and associated organ or tissue damage such as hypercalcemia, renal failure, anemia or bone lesions (4,5). In about 60% of patients, the M-component is IgG, in 20-25% IgA, and significantly less IgM, IgD or IgE (6). In the other 15-20% of patients, only a cappa or just a lambda chain is obtained by a plasma cell that, due to its relatively small molecular weight, is easily found in the urine and is known as the Bence-Jones protein. In these patients, BJ proteniuria is not associated with the

M component (light chain disease). However, about 80% of malignant plasma cells synthesize a complete immunoglobulin molecule as well as a large amount of light chains so that the M-component is present in the serum, as well as the BJ protein in the urine (7, 8). The clinical picture of MM is initially nonspecific. The most common symptoms are weakness (82%), bone pain (58%), fatigue (32%), and weight loss (24%). There are also symptoms that occur more rarely such as increased tendency to bleeding on the skin and mucous membranes (13%), hyperviscocity, recurrent infections (13%), different degrees of renal damage (20%). Often in clinical practice, acronym of CRAB - from C - calcium, R - renal (renal insufficiency), A - anemia, B - bone lessions - is used for the description of the most common laboratory and / or clinical manifestations: hypecalcemia, kidney damage, anemia and bone lesions that define the symptomatic myeloma (9, 10). Diagnosis of MM is established according to strictly defined rules proposed by the World Health Organization, and the classification is by Salomon-Durie (Graipp) (11, 12, 13).

Since the MM is characterized by the production of any κ or λ chains, we aimed to determine their significance in the individual occurrence either in serum or urine in relation to the stage of the disease and to determine the ratio of κ / λ chains in serum and urine as a potential early marker of the change in the phase of MM illness.

Materials and methods

Subjects

The study included 82 patients of both sexes (41 women and 41 men). The research was conducted at the Clinic for Hematology, University Clinical Center Sarajevo (UKCS). The conducted research was a clinical, cross-sectional, descriptive study.

A group of patients with multiple myeloma patients included 62 patients who were hospitalized and diagnosed in the Clinic for Hematology at the University Clinical Center Sarajevo. The diagnosis of the disease has been established in accordance with the guidelines of the World Health Organization (WHO), based on anamnestic data, an objective examination of a hematologist specialist and laboratory findings. The control group included 20 healthy subjects of both sexes, without clinical and biochemical signs and symptoms of the disease, who voluntarily agreed to participate in the study.

The selection of partitipants for inclusion in the study was done after detailed information of potential partitipants on the significance and methods of research, filling out the questionnaire and giving their written consent. The survey questionnaire contained general information such as demographic data, habits (smoking, drinking coffee) and special information on the duration of the disease, on previous diseases and medicines, or the treatment that was used earlier.

Exlusion criteria for the study were: the presence of another malignant disease, addiction to narcotics, age under 18 years, treatment for some other malignant disease in the past 5 years.

For the determination of Bence-Jones proteins in urine immunoelectrophoresis was used. Determination of light and heavy chain serum concentrations was done by nephelometric method. Determination of light and heavy chain urine concentrations was done by nephelometric method. Nephelometric determination of light and heavy chain concentrations in the urine and in the serum of multiple myeloma patients was performed on Dade Behring Nephelometer.

Statistical analysis

Normal distribution of variables was examined by Kolmogorov Smirnov test. The results were statistically analysed so that for each parameter the median and interquartile range was determined. Man Whitnay U test was applied to test the differnce in variables that were not normally distributed. A Kruskal-Wallis test was used to determine the statistically significant difference in mean values of variables between multiple groups. The correlation coefficient between the variables was determined by the Spearman method. The value of <0.05 was taken as statistically significant. Analyzes were made using the statistical program SPSS Version 17.

Results

The results are presented as a median and interquartil range (25-75 percentiles). P-value is in the ratio to the control group of subjects.

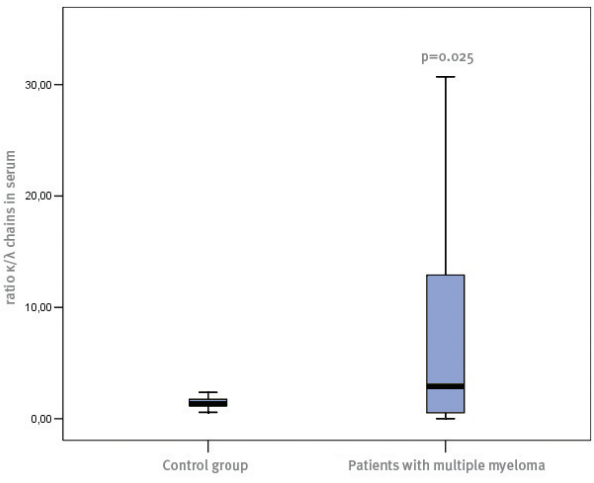

Figure 1. The ratio of serum κ / λ chains in patients with multiple myeloma and in the control group of subjects

Patients with multiple myeloma had statistically significantly higher values of the κ / λ chains ratio in serum 3.9 (0.57-22.48) compared to control group 1.35 (1.15-1.79) (p = 0.025).

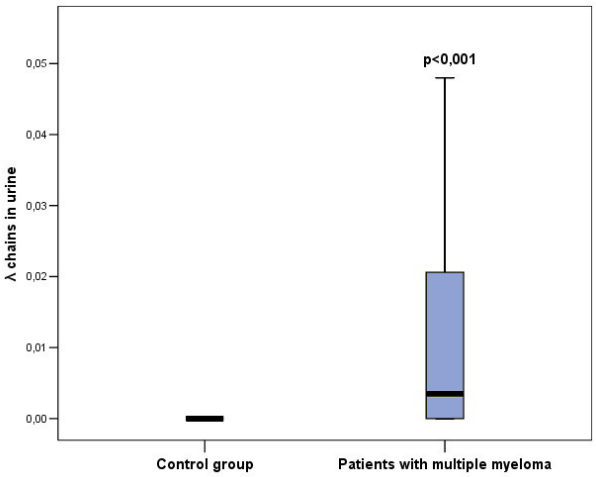

Figure 2. The level of λ chains in urine in patients with multiple myeloma and in control group of subjects

Patients with multiple myeloma had statistically significantly higher values of λ chains in urine 0.007 (0.003-0.003) compared to control group 0.0 (0.0-0.0) (p <0.001)

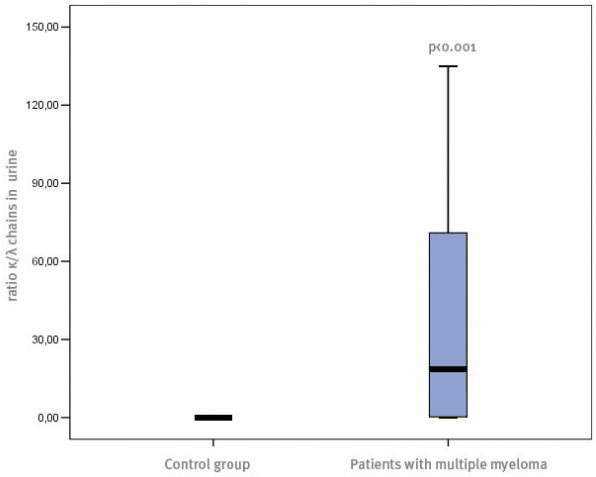

Figure 3. The ratio of κ / λ chains in the urine in patients with multiple myeloma and in the control group of subjects

Patients with multiple myeloma had statistically significantly higher values of κ / λ chains ratio in urine 18.49 (0.27-73.29) compared to control group 0 (0-0) (p<0.001) Serum κ chain values, serum λ chain values and urinary chain λ values did not differ significantly between multiple myeloma patients and control group. ROC curve analysis showed that the values of serum κ chains, serum λ chains, and λ chains in urine did not show significant (potential) biomarker importance.

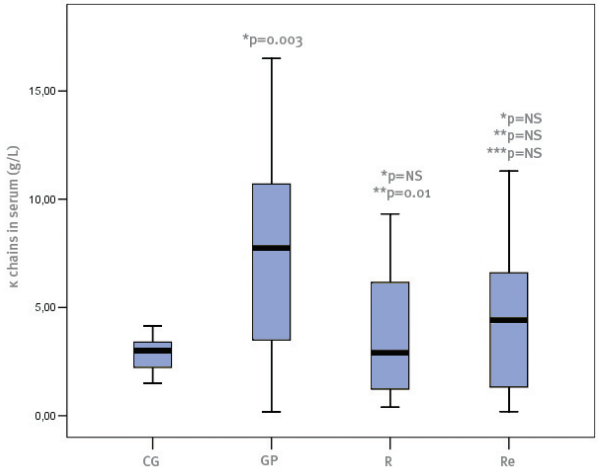

Figure 4. Level of serum κ chains in patients with multiple myeloma and in the control group of subjects

Legend: CG- control group; GP- group at presentation; R- remission group; Re- relapse group; p is in the ratio to the control group of subjects
* in ratio to the control group of subjects
** in ratio to the group of newly diagnosed patients with MM
*** in ratio to the group of patients with MM in remission (plateau phase) of the disease

The serum κ chain values in patients with newly discovered MM was 7.74 g/L (3.22 g/L - 11.15 g/L) and was statistically significantly higher in relation to the control group 3 g/L (2,19 g/L - 3.4 g/L; p = 0.003) and in relation to the group of patients with multiple myeloma in remission phase 2.9 g/L (1.22 g/L - 6.27 g/L; p = 0.01). There was no significant difference in serum κ chain values in patients with relapse multiple myeloma 4.4 g/L (1.6 g/L-6.75 g/L) compared to the control group (p = NS) and also in patients with multiple myeloma with other stages of the disease.

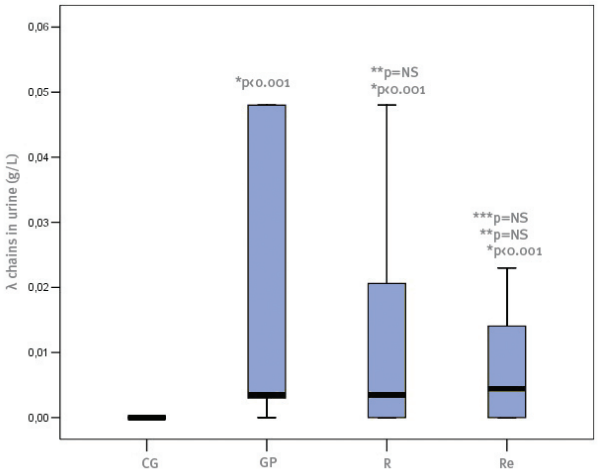

Figure 5. The level of λ chains in urine in patients with multiple myeloma and in the control group of subjects

Legend: CG- control group; GP- group at presentation; R- remission group; Re- relapse group; p is in the ratio to the control group of subjects
* in ratio to the control group of subjects
** in ratio to the group of newly diagnosed patients with MM
*** in ratio to the group of patients with MM in remission (plateau phase) of the disease

Compared to the value of λ chains in urine of the the control group 0 (0.00 - 0.0 g/L), the value of λ chains in urine of patients with newly discovered multiple myeloma 0.0046 g/L (0.0034 g/L - 0.99 g/L; p <0.001), in patients with multiple myeloma in remission phase 0.00985 g/L (0.0035 g/L - 0.0329 g/L; p <0.001) and in patients with multiple myeloma in relapse phase 0.00975 g/L (0.0041 g/L - 0.034 g/L; p <0.001) was statistically significantly higher. There was no significant difference in the concentration of λ chains in urine between patients with multiple myeloma divided by disease phase (p = NS).

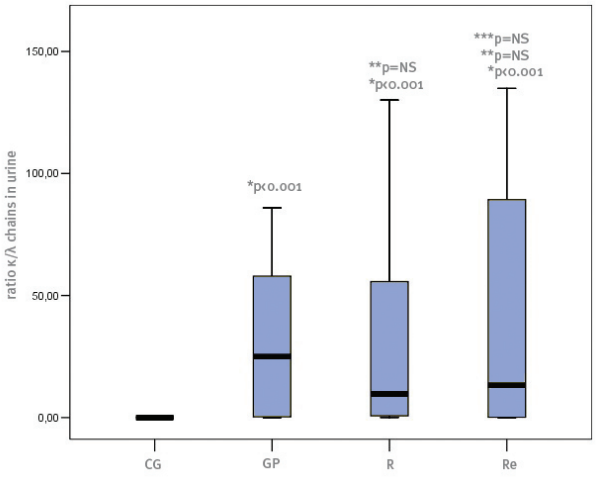

Figure 6. The ratio of κ / λ chains in urine in patients with multiple myeloma and in the control group of subjects

Legend: CG- control group; GP- group at presentation; R- remission group; Re- relapse group; p is in the ratio to the control group of subjects
* in ratio to the control group of subjects
** in ratio to the group of newly diagnosed patients with MM
*** in ratio to the group of patients with MM in remission (plateau phase) of the disease

The value of κ / λ chains ratio in urine in the control group 0 (0.0-0.0) was statistically significantly lower in relation to the value of κ / λ chains ratio in the urine of newly detected patients with multiple myeloma 25.14 (0.22- 59.99, p <0.001), patients with multiple myeloma in remission 9.76 (0.50-65.68), and in relation to the values in patients with multiple myeloma in relapse 13.19 (0.11-100.68). There was no significant difference in the values of κ / λ chains ratios in urine between patients with multiple myeloma divided by the stage of the disease (p = NS). The serum λ chains value, serum κ / λ chains ratio value and λ chains value in urine did not statistically significantly differ between patients with multiple myeloma divided by the disease phase and the control group of subjects.

Statistically significant correlation was determined between λ chains in serum with κ chains in serum (rho= 0.477; p=0.03); κ chains in urine and λ chains in serum (rho=0.606; p=0.004) and highly negative correlation between λ chains in serum with the ratio of κ / λ chains in serum (rho=-0.648; p=0.004). A statistically significant negative correlation between age and κ chains value in urine was observed in the control group (rho=-0.522; p=0.018), while with other parameters of the multiple myeloma, the age was not significantly correlated (p = NS).

Statistically significant correlation was determined between κ chains in serum with ratio of κ / λ chains in serum (rho= 0.851; p=0.000001); κ chains in serum with κ chains in urine (rho=0.437; p=0.0003); κ chains in serum with the ratio of κ / λ chains in urine (rho=0.605; p=0.000003); between λ chains in serum and urine (rho=0.505; p=0.000002); κ chains in urine with the ratio of κ / λ chains in serum (rho=0.419; p=0.0006) as well as between the ratio κ / λ chains in serum and urine (rho=0.667; p=0.0000002). Sta-

Table 1. Correlation between different MM parameters and correlation between MM parameters and age in the control group

Parameters		κ chains in serum (g/L)	λ chains in serum (g/L)	ratio κ/λ chains in serum	κ chains in urine (g/L)	λ chains in urine (g/L)	ratio κ/λ chains in urine	Age (years)
κ chains in serum (g/L)	rho	-	0,477	0,248	0,347	.	.	0,066
	p=		0,033	NS	NS	.	.	NS
λ chains in serum (g/L)	rho	0,477	-	-0,648	0,606	.	.	-0,147
	p=	0,033		0,001	0,004	.	.	NS
ratio κ/λ chains in serum	rho	0,248	-0,648	-	-0,368	.	.	0,244
	p=	NS	0,001		NS	.	.	NS
κ chains in urine (g/L)	rho	0,347	0,606	-0,368	-	.	.	-0,522
	p=	NS	0,004	NS		.	.	0,018
λ chains in urine (g/L)	rho	.	.	.	.	-	.	.
	p=	.	.	.	.		.	.
ratio κ/λ chains in urine	rho	.	.	.	.	.	-	.
	p=	.	.	.	.	.		.
Age (years)	rho	0,066	-0,147	0,244	-0,522	.	.	-
	p=	NS	NS	NS	0,018	.	.	

Table 2. Correlation between different MM parameters and correlation between MM parameters and age in the patient groups

Parameters		κ chains in serum (g/L)	λ chains in serum (g/L)	ratio κ/λ chains in serum	κ chains in urine (g/L)	λ chains in urine (g/L)	ratio κ/λ chains in urine	Age (years)
κ chains in serum (g/L)	rho	-	-0,384	0,851	0,437	-0,290	0,605	0,113
	p=		0,002	0,000001	0,0003	0,022	0,000003	NS
λ chains in serum (g/L)	rho	-0,384	-	-0,737	-0,153	0,505	-0,373	-0,181
	p=	0,002		0,000001	NS	0,000002	0,017	NS
ratio κ/λ chains in serum	rho	0,851	-0,737	-	0,419	-0,427	0,667	0,096
	p=	0,000001	0,000001		0,0006	0,0005	0,000002	NS
κ chains in urine (g/L)	rho	0,437	-0,153	0,419	-	0,363	0,888	0,020
	p=	0,0003	NS	0,0006		0,003	0,000001	NS
λ chains in urine (g/L)	rho	-0,290	0,505	-0,427	0,363	-	-0,425	-0,128
	p=	0,022	0,000002	0,0005	0,003		0,006	NS
ratio κ/λ chains in urine	rho	0,605	-0,373	0,667	0,888	-0,425	-	0,068
	p=	0,000003	0,017	0,0000002	0,000001	0,006		NS
Age (years)	rho	0,113	-0,181	0,096	0,020	-0,128	0,068	-
	p=	NS	NS	NS	NS	NS	NS	

tistically significant positive correlation was determined between κ chains in urine and λ chains in urine (rho=0.365; p=0.003) and κ chains in urine with the ratio of κ/ λ chains in urine (rho=0.888; p=0.000001). Statistically significant negative correlation was determined between κ and λ chains in serum; κ chains in serum and λ chains in urine; λ chains in serum and the ratio of κ / λ chains in serum; κ / λ chains in urine and λ chains in serum; κ / λ chains in urine and λ chains in urine (rho=-0.384; p=0.002; rho=-0.290; p=0.022; rho=-0.737; p=0.000001; rho=-0.373; p=0.017; rho=-0.427; p=0.0005; rho=-0.425; p=0.006 respectively)
There was no statistically significant correlation between age and disease parameters in patients with multiple myeloma (p = NS)

Discussion

The results of our study showed that the serum κ / λ chains ratio in MM patients had significantly higher values of 3.9 (0.57-22.48) than serum κ / λ chains ratio in the control group 1, 35 (1.15-1.79) (p = 0.025).
Hutchinson et al. (14) investigated the "justifiability" of serum Free Light Chains (FLC) in diagnosing multiple myeloma in patients with severe renal failure. The study comprised 142 subjects. All patients with MM had an abnormally high ratio of serum κ / λ chains, which is consistent with our results. Drayson et al. (15) who investigated the concentration of FLC and the ratio of serum κ / λ chains in patients with non-secretory MM, came to a similar conclusion. Katzmann and al. (16), in their study, aimed at determining the contribution of urine screening for monoclonal gammopathies, concluded that patients with MM have an abnormally elevated serum κ / λ chains ratio, which is in line with our research.
Katzmann et al. (17) came to similar results to ours, where for the differentiation between patients with MM and the control group of subjects the greatest importance was the value of λ chains concentration in the urine, and then the ratio of κ / λ chains in urine, certainly also because, as they did not appear even in the trace in the control group of the subjects. The third place in importance was the ratio of serum κ / λ chains. Their study, aimed at determining the contribution of urine screening for monoclonal gammopathies, involving 428 patients, concluded that the determination of serum light chain and their relationship was sufficient for screening. However, in order to eliminate the urine test it was necessary to perform three different tests (determination of serum κ / λ chains ratio, serum protein electrophoresis and serum immunofixation) since none of the tests were individually positive in more than 93.5% of cases, while in all 428 patients monoclonal protein was found in urine. Of this, 426 patients required therapy, which is in line with our research, and the determination of urinary FLC has been shown to be a very simple, reliable and practical method with high specificity and sensitivity as a marker of early disease relapse.
Something different came from Kang et al. (18) who investigated the utility of serum and urine FLC determination as a tumor marker of multiple myeloma. Their research has shown that the highest sensitivity has shown the light κ / λ chains ratio in serum and urine, which is not entirely consistent with our research. In almost all patients, they found abnormally high concentrations of light chains in serum and urine, and they can be used as biomarkers for MM diagnosis, which is consistent with our research.

In our study, the results showed that patients with MM had statistically significantly higher concentration of λ chains in the urine compared to the control group of subjects. In the control group, the presence of λ chains was not noticed, and hence the concentration value should be increased in the studied patients by the stages of the disease and in general.

Song et al. (19) investigated the concentrations of serum lightweight proteins and their significance in the light chains multiple myeloma. The aim was to determine the clinical significance of serum light chains. They tested 37 newly diagnosed patients with MM light chains. In this case, 17 patients were κ light type, and 20 patients were λ light type. They have come to the conclusion that all patients exhibited abnormally elevated values of κ and λ chains during diagnosis. The correlation of sFLC with uFLC and renal impairment was analysed. The concentrations of light chains were determined both in the serum and in the urine, both before and after chemo. They have come to the conclusion that all patients showed abnormally elevated values of κ and λ chains during diagnosis. Serum and urinary light chains were not correlated. In 12 patients with very good partial remission and normal urinary light chain concentration, the serum light chains concentration was abnormally elevated in 8 patients. Accordingly, it has been concluded that the serum light chains are more suitable for monitoring the response to therapy than the urinary λ light chains, which is inconsistent with our research. In our study, of 62 subjects we had 44 patients with MM κ type and 18 patients with MM λ type. All patients had abnormally elevated values of κ and λ chains during diagnosis as in Song's study. Our study showed that patients with MM had a statistically significantly higher value of the κ / λ chains ratio in urine 18.49 (0.27-73.29) relative to the control group of subjects 0 (0-0) (p <0.001) that had negative correlation with concentration of λ chains in urine (rho=-0.425; p=0.006) and positive correlation with concentration of κ chains in urine (rho=0.888; p=0.000001)(Table 2).

Mead et al. (20) measured the serum light chains concentration in patients with MM that produces monoclonal intact immunoglobulin proteins. The concentration was measured in 493 patients. Abnormally high concentrations were observed in 96% of patients with newly detected MM. FLC concentrations fell in response to treatment. This is in line with our research, which has also shown that the FLC concentration can be used as a biomarker in monitoring the course of the disease.

Our study showed that the value of κ chains values in the serum with newly detected MM was statistically significantly higher in relation to the control group and compared to the group of patients with MM in the remission phase. We did not find a significant statistical difference in the κ chains values in the serum of patients with MM in the relapse phase compared to the control group, as well as in the group of patients with MM in other stages of the disease.

Compared to the control group, the value of λ chains in urine 0 (0.0.0-0.0 g/L) was statistically significantly higher in patients with newly discovered MM 0.0046 g/L (0.0034 g/L -0.00 g/L; p <0.001), in MM patients in remission phase 0.00985 g/L (0.0035 g/L - 0.0329 g/L; p <0.001) and in patients with MM in relapse phase 0.00975 g/L (0.0041 g/L - 0.034 g/L; p <0.001). There was no significant difference in the concentration of λ chains in urine among patients with multiple myeloma divided by disease phase (p = NS).

Malysz et al. (21) in their 117-patient study concluded that FLC concentrations were significantly higher in random urine samples, both in newly diagnosed and in post-therapy MM patients, which is consistent with our research .

Our results are in line with the research by Abracham S.R. et al. (8) who investigated the correlation of serum FLC quantification with urinary Bence-Jones protein in MM light chains and found that the concentration of λ chains in urine was significantly increased in patients with MM.

The values of λ chains in the control group were in trace, so that each value observed during the course of the disease was significant.

The value of κ / λ chains ratio in urine in the control group 0 (0.0-0.0) was statistically significantly lower in relation to the value of κ / λ chains ratio in the urine of newly detected patients with multiple myeloma 25.14 (0.22-59.99, p= 0.001), patients with multiple myeloma in remission 9.76 (0.50-65.68), and in relation to the value of patients with multiple myeloma in relapse of the disease 13.19 (0.11-100.68). There was no significant difference in the ratio of κ / λ chains in the urine between patients with multiple myeloma divided by the stage of the disease (p = NS).

Yang et al. (22) analyzed the serum and urinary monoclonal proteins of 72 patients with MM and found that in all patients, the ratio of κ / λ chains in the urine was significantly elevated relative to the reference values, which agrees with the results of our research (Table 2).

A statistically significant negative correlation between the age and κ chains in urine was observed in the control group (rho=-0.522; p=0.018), while with other parameters of the multiple myeloma, the age was not significantly correlated (p = NS). Similar results came from Herkner KR et al. (23) that followed the κ and λ chain concentrations, as well as their ratio in 1543 healthy pediatric patients. They concluded that the concentration of κ chains in the serum changes with the age of the subjects and showed a linear increase in κ

/ λ chains ratios with an increase in patient age, which we did not find in our study. Bradwell et al. (24) determined the concentration of κ and λ chains and their interaction with 444 inhabitants of Spain, and found that there was no statistically significant difference in the biomarker MM concentrations in the subjects of the age of up to 70 years, but that in patients older than 70 years there was statistical a significant increase in biomarker MM parameters, which is partially inconsistent with our results.

Conclusion

Our study did not establish a statistically significant correlation between age and disease parameters in patients with MM. The results of our research are partially agreed with the research of Kyrtsonis et al. (25) who tested patients aged 21-90 years. Their research showed that the concentration of κ and λ chains grew with increasing age. The highest increase was observed in patients over the age of 80 years, which is not in line with our research. There is no dependence on age when it comes to the ratio of κ and λ chains, since we have observed only negative correlation between the urine κ chains concentration and the age (rho=-0.522; p=0.018) within the control group of subjects (Table 1).

Our study showed that there was no statistically significant correlation between age and disease parameters in patients with newly detected MM (p = NS). There was no statistically significant correlation between age and disease parameters in patients with MM in the remission phase (p = NS).

Declaration of interest

The authors declare no conflicts of interest.

References

[1] Elias Campo, Steven H. Swerdlow, Nancy L. Harris, Stefano Pileri, Harald Stein, and Elaine S. Jaffe.The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011; 117(19): 5019–5032.

[2] Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. Lancet. 2009;374(9686):324-39.

[3] Macauda A, Calvetti D, Maccari G, Hemminki K, Försti A, Goldschmidt Het al. Identification of miRSNPs associated with the risk of multiple myeloma. Int J Cancer. 2016; 15;139(8):1703-14.

[4] Joan Bladé, Laura Rosiñol, Carlos Fernández de Larrea. How I treat relapsed myeloma. Blood 2015; 125:1532-1540

[5] Ramón Vélez, Ingemar Turesson, Ola Landgren, Sigurdur Y Kristinsson and Jack Cuzick Incidence of multiple myeloma in Great Britain, Sweden, and Malmö, Sweden: the impact of differences in case ascertainment on observed incidence trends. BMJ Open. 2016; 6(1): e009584.

[6] Riedel DA, Pottern LM. The epidemiology of multiple myeloma. Hematol Oncol Clin North Am. 1992; 6(2):225-47.

[7] Perrotta C, Staines A, Codd M et al. Multiple Myeloma and lifetime occupation: results from the EPILYMPH study. Journal of Occupational Medicine and Toxicology 2012;7:25

[8] Abraham S.R., Clark J.R, Bryant C.S., Lymp F.S., Larson T., Kyle A.R. et al. Correlation of Serum Immunoglobulin Free Light Chain Quantification with Urinary Bence Jones protein in Light Chain Myeloma. Clinical Chemistry 2002; 45 (4): 655-657.

[9] Anderson KC, Carrasco RD. Pathogenesis of Myeloma. Annu. Rev, Pathol. Mech.Dis. 2011;6:249-74

[10] Mateos MV, Landgren O. MGUS and Smoldering Multiple Myeloma: Diagnosis and Epidemiology. Cancer Treat Res. 2016;169:3-12.

[11] International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Haematol. 2003;121(5):749-57.

[12] Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. ancer. 1975;36(3):842-54

[13] Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, Bladé J i sur. International staging system for multiple myeloma. J Clin Oncol. 2005; 20;23(15):3412-20.

[14] Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, Basnayake K, Harding S, Bradwell AR, Mead G. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol. 2008; 22:9-11.

[15] Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H, Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. Blood. 2001;97(9):2900-2.

[16] Katzmann JA, Dispenzieri A, Kyle RA, Snyder MR, Plevak MF, Larson DR, Abraham RS, Lust JA, Melton LJ 3rd, Rajkumar SV. Elimination of the need for urine studies in the screening algorithm for monoclonal gammopathies by using serum immunofixation and free light chain assays. Mayo Clin Proc. 2006;81(12):1575-8.

[17] Katzmann JA, Abraham RS, Dispenzieri A, Lust JA, Kyle RA. Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. Clin Chem. 2005; 51(5):878-81.

[18] Kang SY, Suh JT, Lee HJ, Yoon HJ, Lee WI. Clinical usefulness of free light chain concentration as a tumor marker in multiple myeloma. Ann Hematol. 2005;84(9):588-93

[19] Song P, An ZM, Zhou XG, Li F, Wang LP, Zhao Q, Yu YP, Zhai YP. [Test of Serum Free Light Chain and Its Clinical Significance in Light Chain Multiple Myeloma]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2015;23(5):1357-61.

[20] Mead GP, Carr-Smith HD, Drayson MT, Morgan GJ, Child JA, Bradwell AR. Serum free light chains for monitoring

multiple myeloma. Br J Haematol. 2004;126(3):348-54.

[21] Malysz J, Zhu J., Creer MH, Dolloff NG, Bayerl MG, Talamo G. Retrospective study of quantitative free light chain levels in random urine of patients with multiple myeloma. Journal of HematologicalMalignancies, 2013; 3(2): 8-15.

[22] Yang JJ, Zhang GS, Chen XR. [Analysis of monoclonal protein in 72 cases of multiple myeloma]. Hunan Yi Ke Da Xue Xue Bao. 2001; 26(2):152-4.

[23] Herkner KR, Salzer H, Böck A, Mühl A, Tsaka T, Steger H, Pollak A, Schatten C, Popp W, Huber-Spitzy V, et al. Pediatric and perinatal reference intervals for immunoglobulin light chains kappa and lambda. Clin Chem. 1992; 38(4):548-50.

[24] Bradwell AR Hevylite: Concepts. 6th International Symposium of Clinical Applications of Serum Free Light Cahin Analysis (plus Hevylite), Bath, UK, 2010.

[25] Kyrtsonis MC, Vassilakopoulos TP, Kafasi N, Sachanas S, Tzenou T, Papadogiannis A, Galanis Z, Kalpadakis C, Dimou M, Kyriakou E, Angelopoulou MK, Dimopoulou MN, Siakantaris MP, Dimitriadou EM, Kokoris SI, Panayiotidis P, Pangalis GA. Prognostic value of serum free light chain ratio at diagnosis in multiple myeloma.Br J Haematol. 2007;137(3):240-3.