ORIGINAL ARTICLE

Correlation between Neutrophil gelatinase-associated lipocalin and ß2-Microglobulin with serum protein electrophoresis in multiple myeloma patients

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Abstract

Objective: To find the correlation between neutrophil gelatinase-associated lipocalin and beta2-microglobulin in patients with multiple myeloma.

Method: The case-control study was conducted from November 2021 to March 2022, after approval from ethics review committees of three major medical establishments in Baghdad, Iraq. There were randomly selected multiple myeloma patients of either gender with normal concentration of urea and creatinine in group A, multiple myeloma patients of either gender with increased concentration of urea and creatinine in group B and healthy controls in group C. Neutrophil gelatinase-associated lipocalin and beta2-microglobulin were assessed using enzyme-linked immunosorbent assay technique. Data was analysed using SPSS 26.

Results: Of the 180 subjects, there were 60(33.3%) in each of the 3 groups, with each group having 40(66.5%) males and 20(33.3%) females, and overall age ranging 40-79 years. Protein electrophoresis in group A patients was significantly higher (p=0.001) in alpha1, beta and gamma globulin. Among group B patients, a highly significant increase *(p*=0.001) was noted in alpha1, alpha2, beta and gamma globulin. Beta2-microglobulin was a more sensitive and specific marker for renal injury than neutrophil gelatinase-associated lipocalin in myeloma patients(*p*<0.05).

Conclusion: Beta2-microglobulin and neutrophil gelatinase-associated lipocalin were both more sensitive markers than serum urea and creatinine in the diagnosis of acute kidney injury in multiple myeloma patients.

Keywords: Lipocalin, Creatinine, Multiple myeloma, Microglobulin, Urea, Electrophoresis, Globulins, Albumins, Enzyme, Immunosorbent assay, Kidney injury.

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Introduction

Multiple myeloma (MM) is a communal plasma cell cancer that causes high mortality and is frequently linked to severe renal impairment (RI). Renal damage can limit therapeutic options and lead to poor outcomes, but it is treatable in some people. The most widely used RI markers have significant flaws, including delayed onset after renal injury, multiple interfering variables, and lower sensitivity to minor alterations in glomerular filtration.[1] The kidneys filter urea, a waste product of protein breakdown in the liver, from the bloodstream and into the urine. The urea test determines how effectively the kidneys are functioning. Creatinine (Cr) is a waste product produced by muscles that are filtered from the bloodstream and excreted in the urine by the kidneys. If the kidneys are injured and unable to filter Cr) out, it will build up in the circulation, as evidenced by elevated Cr levels on a blood test.[2] Serum protein electrophoresis (SPE) is a test that measures the amount of monoclonal (M) protein produced by myeloma cells in the blood. The presence of M-protein in the blood confirms the diagnosis of MM. Higher M-protein levels usually indicate that the disease is more aggressive.[3] Neutrophil gelatinase-associated lipocalin (NGAL) is a lipoprotein implicated in inflammation, the transport of tiny molecular hydrophobic ligands, and the transport and preservation of iron. NGAL levels in blood and urine rise within 2h after acute kidney injury (AKI). NGAL is more sensitive than Cr, and detects it early. Treatment with diuretics does not affect the level of NGAL in the urine or the blood.[4, 5] Many cells in the body, including myeloma cells, produce the protein beta2-microglobulin (ß2-M). Although this protein does not cause any difficulties on its own, it can be used to determine the stage of illness, as high levels may signal advanced disease. It is useful in assessing the prognosis of MM patients and in the diagnosis of renal tubular disease.[6] MM is the second most prevalent haematological malignancy in high-income countries (HICs), accounting for 1% of all cancers.[7] Variable responses to therapy and prognosis result from the clinical and biochemical heterogeneities of this cancer. RI and serum Cr >2.0mg/dl linked to plasma cell dyscrasia are two of the distinguishing characteristics of MM. The most frequent monoclonal gammopathy that causes kidney disease is MM.[8]

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The current study was planned to find the correlation between NGAL and ß2-M in MM patients.

Patients and Methods

The case-control study was conducted from November 2021 to March 2022, after approval from ethics review committees of the National Centre for Teaching Laboratories (NCFTL), the Baghdad Hospital Advisory (BHA), and the Baghdad Teaching Hospital (BTH), Baghdad, Iraq. Using random sampling technique, the sample was raised, with MM patients of either gender with normal concentration of urea (15-45mg/dl) and Cr (0.5-1.2mg/dl) in group A, MM patients of either gender with increased concentration of urea (>45mg/dl) and Cr (>1.2mg/dl) in group B, and healthy controls from among medical staff and patients' families in group C. Individuals with chronic conditions and MM patients who had been on medicines for a long time were excluded. All groups were matched for gender distribution and age 40-80 years.

After taking informed consent from the subjects, biochemical testing, protein electrophoresis, X-ray and clinical examination by specialists were used to diagnose MM. From each participant, 10ml blood was taken by single-use syringes and divided into two tubes: 6ml of serum for biochemical tests for urea and Cr using an automated analyser (Cobas C311), and 2ml of serum for serum protein electrophoresis using a serum protein kit (SAS-1) and quantitation of serum proteins by agarose gel electrophoresis. The serum was then divided into 2mL Eppendorf tubes and stored at -20°C until it was time to assay serum NGAL and ß2-M using the enzyme-linked immunosorbent assay (ELISA) technique (MyBioSource, USA).

Data was analysed using SPSS 26 Data was expressed as frequencies and percentages or as mean and Standard deviation, as appropriate. Receiver operating characteristic (ROC) curve, independent sample t-test, paired sample t-test, simple correlations (r) coefficient, and simple linear regression were used where necessary. P<0.05 was considered statistically significant and p<0.01 was considered highly significant.

Results

Of the 180 subjects, there were 60(33.3%) in each of the 3 groups, with each group having 40(66.5%) males and 20(33.3%) females, and overall age ranging 40-79 years. Male gender and age groups 50-59 years and 60-69 were significant (Table 1).

Group B patients had a highly significant increase (p=0.001) in the mean urea, Cr, ß2-M, and NGAL values, while group A patients had a highly significant increase (*p*= 0.001) in the mean Cr, ß2-M and NGAL values compared to group C controls (Table 2).

Table-1: Distribution of the subjects according to age and gender.

	Studied groups					
Age groups (Years)	**Group C healthy control (n=60)**		**Group A patients (n=60)**		**Group B patients (n=60)**	
	Gender		**Gender**		**Gender**	
	Male (n=40)	**Female (n=20)**	**Male (n=40)**	**Female (n=20)**	**Male (n=40)**	**Female (n=20)**
(40-49)	6(10%)	6(10%)	8(13.3%)	2(3.3%)	0(0.0%)	0(0.0%)
(50-59)	18(30%)	2(3.3%)	12(20.0%)	8(13.3%)	10(16.7%)	8(13.3%)
(60-69)	10(16.7%)	12(20%)	18(30.0%)	4(6.7%)	22(36.7%)	10(16.7%)
(70-79)	6(10%)	0(0.0%)	2(3.3%)	6(10.0%)	8(13.3%)	2(3.3%)
***p*-value**	*p*=0.000		*p*=0.017		*p*=0.433	

Table-2: Inter-group comparison of biochemical parameters.

			Mean ±SD		
Parameter	**Group A patients**	***p*-value**	**Group C healthy controls**	***p*-value**	**Group B patients**
Blood urea (15-45) (mg/dl)	29.82±11.53		32.00±8.57		57.83±30.92
		0.243		0.000	
Serum Creatinine (0.5-1.2) (mg/dl)	0.80±0.20		0.67±0.15624		2.09±1.38
		0.000		0.000	
ß 2-M(mg/dl)	43.28±18.78		14.35±7.49		39.14±16.20
		0.000		0.000	
NGAL (pg/ml)	432.77±198.45		165.80±90.77		452.50±188.83
		0.000		0.000	

SD: Standard Deviation; ß2-M: Beta 2-macroglobulin; NGAL: Neutrophil gelatinase-associated lipocalin.

Table-3: Inter-group comparison of protein electrophoresis.

Parameter	Mean ±SD Group I patients	*p*-value	Group III healthy control	*p*-value	Group II patients
SPE albumin (35-50) (g/l)	32.91±5.86	0.000	40.23±3.52	0.000	34.93±3.853
SPE alpha1 (1.00-4.00) (g/l)	3.65±1.07	0.000	2.35±0.86	0.000	3.81±0.98
SPE alph2 (6.00-12.00)(g/l)	8.53±1.98	0.131	8.02±1.69	0.000	9.88±2.33
SPE beta (6.00-12.00) (g/l)	10.77±4.92	0.001	8.63±1.06	0.000	9.41±1.73
SPE gamma (7.00-15.00) (g/l)	16.61±10.38	0.000	10.43±1.72	0.000	19.87±9.15

SD: Standard Deviation; SPE: Serum protein electrophoresis.

Table-4: Correlations (r) between NGAL and ß2M with proteins electrophoresis

Parameters	NGAL		ß2M	
	r-value	*p*-value	*r*-value	*p*-value
SPE albumin(g/l)	- 0.373**	0.000	- 0.443**	0.000
SPE alpha1(g/l)	0.372**	0.000	0.403**	0.000
SPE alph2(g/l)	0.108	0.184	0.145	0.050
SPE beta(g/l)	0.027	0.717	0.081	0.279
SPE gamma(g/l)	0.293**	0.000	0.113	0.133

**. Correlation is significant at the 0.01 level (HS).

ß2M: Beta 2-macroglobulin, NGAL: Neutrophil gelatinase-associated lipocalin, SPE: Serum protein electrophoresis.

Table-5: Receiver Operating Characteristic Curve (ROC) analysis of the correlation of neutrophil gelatinase-associated lipocalin (NGAL) and beta 2-macroglobulin (ß2M) with serum urea (mg/dl) and serum creatinine (mg/dl).

Test Result Variable(s)	Area Under Curve (AUC)	Sig. (*p*-value)	Cut-off	Sensitivity	Specificity
NGAL(pg/ml)	0.946	0.000	>182.5	0.983	0.20
B2M(mg/dl)	0.951	0.000	>20.8	0.90	0.100
Serum Creatinine (mg/dl)	0.854	0.000	>0.86	0.68	0.100
Serum urea(mg/dl)	0.719	0.000	>40.60	0.63	0.20

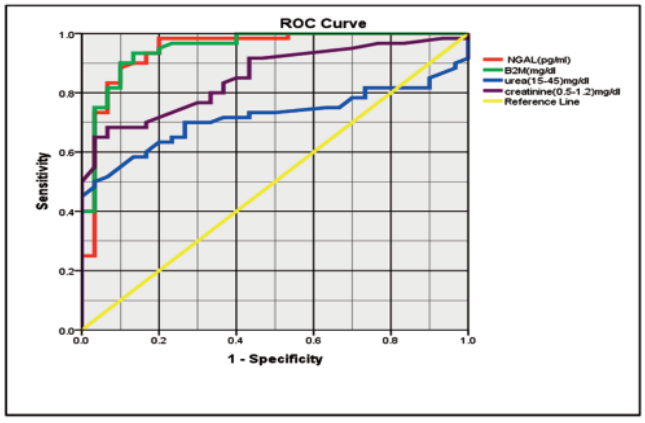


Figure 3: Receiver operating characteristic curve (ROC) analysis of the correlation of neutrophil gelatinase-associated lipocalin (NGAL) and beta 2-macroglobulin (ß2-M) with serum urea (mg/dl) and erum creatinine (mg/dl).

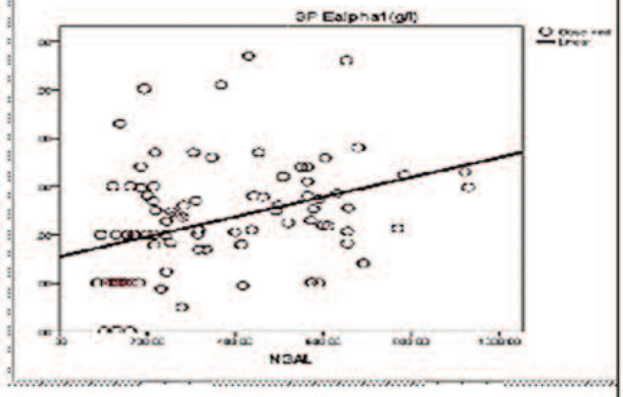
SPE in group B patients showed a highly significant increase (p=0.001) in mean alpha1, alpha2, beta and gamma globulin values, while group A patients showed a highly significant increase (p=0.001) in the mean alpha1, beta, and gamma globulin values compared to group C controls (Table 3). Also, group A and group B patients showed a highly significant decrease in the mean albumin values compared to group C, and SPE alpha2 value was non-significant (p=0.131) in group A patients.

Correlation coefficients of NGAL with SPE alpha1 and SPE gamma g/l (r=0.372; r = 0.293) were positively significant (p>0.01) (Figure (1A-B), but correlation of NGAL with SPE albumin g/l (Figure 1C) (r=0.373) was inversely significant (p=0.001) in myeloma patients. Inverse correlation was found between ß2-M and SPE albumin g/l (r=-0.443; p=0.001) (Figure 2A), while positive significant correlation was found with SPE alpha1 g/l (r=0.403; p=0.001) (Figure 2B). Other parameters, such as SPE alpha2, and beta2, did not correlate with NGAL and B2M, and SPE gamma g/l did not correlate with ß2-M (Table 4).

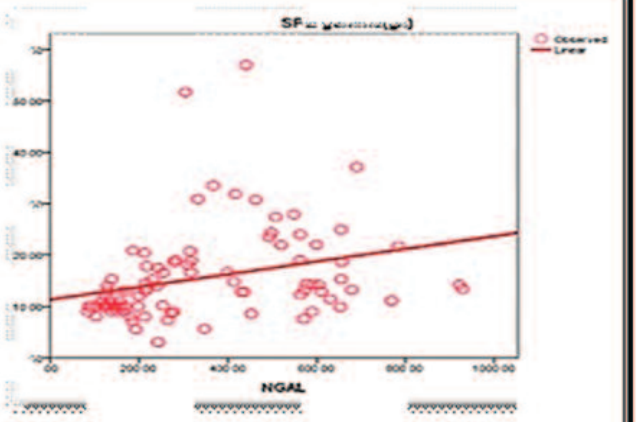
ROC analysis of the correlation of NGAL and ß2M with serum urea and serum Cr were also done (Table 5, Figure 3).

Discussion

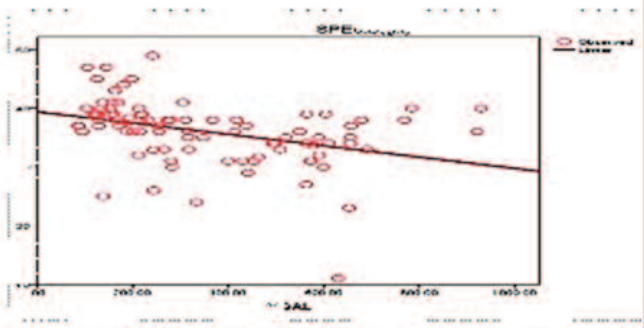
The current study showed AKI being more common among males, which agrees with another study. This is presumably a result of men's unhealthy lifestyles, oestrogen's protective benefits, or testosterone's destructive effects. [9] Also, old age was a significant factor in AKI incidence, which was in line with literature.[10] Age-related comorbidities may contribute to the greater prevalence of AKI, like renovascular disease and congestive heart failure (CHF). The comorbidities may require procedures, medicines or surgeries, which act as nephrotoxins and renal stresses, and the kidney alters structurally and functionally with age. The latter makes elderly patients more susceptible to acute stress and AKI. As a result, elderly patients have a lower glomerular filtration rate (GFR) at baseline and a lower kidney reserve



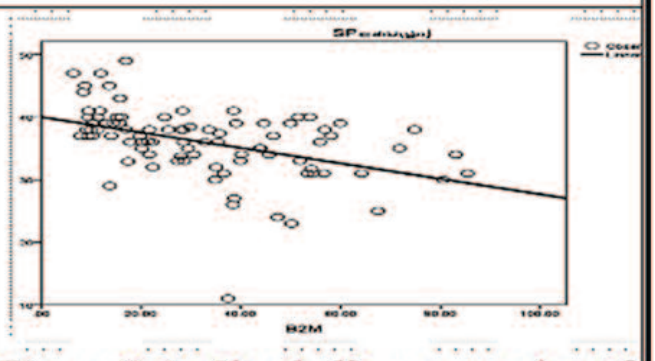
Figure(1a)- Simple liner regression of NGAL with SPEalpha1(g/l)



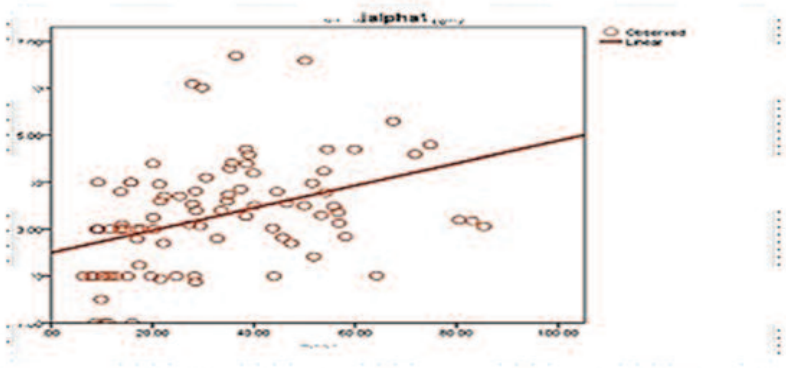
Figure(1b)- Simple liner regression of NGAL with SPE gamma(g/l)



Figure(1c)- Simple liner regression of NGAL with SPEalb(g/l)



Figure(2a)- Simple liner regression of B2M with SPEalb(g/l)



Figure(2b)- Simple liner regression of B2M with SPEalpha1(g/l)

ß2M: Beta 2-macroglobulin, NGAL: Neutrophil gelatinase-associated lipocalin, SPE: Serum protein electrophoresis.

in the face of pathophysiological challenges.[11]

The finding agrees with a study which reported that kidney involvement was a common symptom of MM and was caused mostly by a high tumour load along with the development of myeloma cast nephropathy, and tumour lysis syndrome.[12] Furthermore, the current findings agreed with a study which reported that ß2M and NGAL were elevated in MM patients. The glomerulus filters low molecular weight proteins, like ß2M and NGAL, which are then reabsorbed by the proximal tubules. Increasing serum concentrations of these indicators suggest glomerular injury, but increased urine amounts indicate tubular injury.[13]

The present study agreed with a study that discovered that an increase in alpha1, alpha2, beta and gamma globulin and a decrease in albumin is caused by the proliferation of a single, typically malignant plasma cell clone that produces either a single class of intact immunoglobulins, heavy chains, light chains, or both, and a drop in albumin.[3]

The resulting abnormally high amounts of these proteins in the blood may be due to the presence of monoclonal proteins of various types, like immunoglobulins (antibodies) and free light chains, are produced by myeloma cells in MM. These proteins may be eliminated by the kidneys, depending on their size. The effects of proteins or light chains on the kidneys can be harmful. These proteins are called para proteins or M-proteins. MM is the most common cause of paraproteinemia, which is responsible for alpha and gamma globulin protein levels increases when there is inflammation. Furthermore, NGAL as a small molecule is freely filtered by the glomerulus. Similarly, β2M is filtered by the glomeruli and is reabsorbed by the proximal tubule. NGAL and β2M are thus synthesised systemically in response to kidney damage in myeloma patients suffering from kidney damage.[14,15] In addition, the current finding agrees with a study which indicated that as the stage progressed, the variables of SPE albumin decreased significantly, whereas M-protein concentration increased significantly.[16] When the liver produces less albumin, or when it is lost or degraded, the level of albumin drops. The immune system produces globulins in the liver. Because immunoglobulins migrate to the gamma region of the serum protein spectrum, much of the therapeutic attention is concentrated there.[14] Another study found that NGAL levels may be elevated early in the asymptomatic stages of the spectrum of monoclonal gammopathy.[1]

NGAL may also serve as an early, accurate indicator of acute renal damage in MM patients.[17] One study found that NGAL has AUCs (0.69-0.75) with a lack of specificity in AKI.[18] The current study showed slightly higher AUC than a previous study.[19] Furthermore, the current results were in agreement with certain other studies.[20]

Differences in findings might have been owing to different study populations, the methodology of assessment, and maybe the treatment received.

In the current study, the sample size was not calculated which was a limitation as it may have influenced the power of the study.

Conclusion

NGAL and β2M were found to be more sensitive markers than other renal markers, such as blood urea and serum Creatinine, in the diagnosis of AKI in MM patients. The two proteins did not appear to be related to disease severity, but they appeared to be useful in identifying individuals at the initial stage of renal problem.

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Conflict of Interest: None.

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References

1. Woziwodzka K, Małyszko J, Koc-Żórawska E, Żórawski M, Dumnicka P, Jurczyszyn A, et al. Renal Impairment Detectors: IGFBP-7 and NGAL as Tubular Injury Markers in Multiple Myeloma Patients. Medicina (Kaunas) 2021;57:1348. doi: 10.3390/medicina57121348.
2. Li L, Dong M, Wang XG. The Implication and Significance of Beta 2 Microglobulin: A Conservative Multifunctional Regulator. Chin Med J (Engl) 2016;129:448-55. doi: 10.4103/0366-6999.176084.
3. Tripathy S. The role of serum protein electrophoresis in the detection of multiple myeloma: an experience of a corporate hospital. J Clin Diagn Res 2012;6:1458-61. doi: 10.7860/JCDR/2012/4213.2532.
4. Guo L, Zhu B, Yuan H, Zhao W. Evaluation of serum neutrophil gelatinase-associated lipocalin in older patients with chronic kidney disease. Aging Med (Milton) 2020;3:32-9. doi: 10.1002/agm2.12098.
5. Matsa R, Ashley E, Sharma V, Walden AP, Keating L. Plasma and urine neutrophil gelatinase-associated lipocalin in the diagnosis of new onset acute kidney injury in critically ill patients. Crit Care 2014;18:R137. doi: 10.1186/cc13958.
6. Fairfield H, Falank C, Avery L, Reagan MR. Multiple myeloma in the marrow: pathogenesis and treatments. Ann N Y Acad Sci 2016;1364:32-51. doi: 10.1111/nyas.13038.
7. Candido S, Maestro R, Polesel J, Catania A, Maira F, Signorelli SS, et al. Roles of neutrophil gelatinase-associated lipocalin (NGAL) in human cancer. Oncotarget 2014;5:1576-94. doi: 10.18632/oncotarget.1738.
8. Zhao R, Xie Y, Yang B, Wang C, Huang Q, Han Y, et al. Identification of metabolic biomarkers to predict treatment outcome and disease progression in multiple myeloma. Am J Cancer Res 2020;10:3935-46.
9. Martin WP, Conroy C, Naicker SD, Cormican S, Griffin TP, Islam MN, et

al. Multiplex Serum Biomarker Assays Improve Prediction of Renal and Mortality Outcomes in Chronic Kidney Disease. Kidney360 2021;2:1225-39. doi: 10.34067/KID.0007552020.

10. Alexander DD, Mink PJ, Adami HO, Cole P, Mandel JS, Oken MM, et al. Multiple myeloma: a review of the epidemiologic literature. Int J Cancer 2007;120(Suppl 12):40-61. doi: 10.1002/ijc.22718.
11. Morris BJ, Willcox DC, Donlon TA, Willcox BJ. FOXO3: A Major Gene for Human Longevity--A Mini-Review. Gerontology 2015;61:515-25. doi: 10.1159/000375235.
12. Andronesi AG, Tanase AD, Sorohan BM, Craciun OG, Stefan L, Varady Z, et al. Incidence and risk factors for acute kidney injury following autologous stem cell transplantation for multiple myeloma. Cancer Med 2019;8:3278-85. doi: 10.1002/cam4.2187.
13. van Donge T, Staub E, Atkinson A, Gotta V, van den Anker J, Risch L, et al. Age appropriate reference intervals for eight kidney function and injury markers in infants, children and adolescents. Clin Chem Lab Med 2020;59:373-82. doi: 10.1515/cclm-2020-0781.
14. O'Connell TX, Horita TJ, Kasravi B. Understanding and interpreting serum protein electrophoresis. Am Fam Physician 2005;71:105-12.
15. Smertka M, Wroblewska J, Suchojad A, Majcherczyk M, Jadamus-Niebroj D, Owsianka-Podlesny T, et al. Serum and urinary NGAL in septic newborns. Biomed Res Int 2014;2014:717318. doi: 10.1155/2014/717318.
16. Mohammed N, SaiBaba KSS, Yadagiri.B, Gundeti S, Raju SB. Biochemical Characterization of Multiple Myeloma Patients across ISS Stages – A Data Base Workup from a Tertiary Care Hospital in India. Asian Pac J Cancer Care 2019;4:77-82. DOI:10.31557/APJCC.2019.4.3.77.
17. Chae H, Ryu H, Cha K, Kim M, Kim Y, Min CK. Neutrophil gelatinase-associated lipocalin as a biomarker of renal impairment in patients with multiple myeloma. Clin Lymphoma Myeloma Leuk 2015;15:35-40. doi: 10.1016/j.clml.2014.07.014.
18. McMahon KR, Zappitelli M. Biomarkers in AKI. In: Deep A, Goldstein S, eds. Critical Care Nephrology and Renal Replacement Therapy in Children. Cham, Switzerland: Springer, 2018; pp 43–62. Doi: 10.1007/978-3-319-90281-4_4
19. Rajpal R, Dowling P, Meiller J, Clarke C, Murphy WG, O'Connor R, et al. A novel panel of protein biomarkers for predicting response to thalidomide-based therapy in newly diagnosed multiple myeloma patients. Proteomics 2011;11:1391-402. doi: 10.1002/pmic.201000471.
20. Fei F, Ma T, Zhou X, Zheng M, Cao B, Li J. Metabolic markers for diagnosis and risk-prediction of multiple myeloma. Life Sci 2021;265:118852. doi: 10.1016/j.lfs.2020.118852.