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RESEARCH ARTICLE - MEDICAL TECHNIQUES

Correlation between Neutrophil Gelatinase Associated with Lipocaline and Beta-2 Microglobulin with Other Renal Markers in Iraqi Patients with Multiple Myeloma

Inas Nazeeh Abed[1], Usama Salman Al-timari[2], Hiba Abid Al-Hussein Hassan[3*], Abbas Thamer Dwayyikh[4]

[1] Medical Laboratory Science Technologist, Medical City, Baghdad Teaching Hospital, Baghdad, Iraq

[2] Department of Medical Laboratory Technology, Al-Nisour University College, Baghdad, Iraq

[3] College of Health & Medical Technology - Baghdad, Middle Technical University, Baghdad, Iraq

[4] Specialist Medical Laboratory Technologist, Medical City, National Center for Teaching Laboratories (NCFTL), Baghdad, Iraq

[*] Corresponding author E-mail: hebaabdul8@gmail.com

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Abstract

Multiple myeloma, often known as myeloma, is a kind of bone marrow cancer. The bone marrow is a spongy tissue in the middle of some bones that creates the body's blood cells. The current study is designed to find the correlation between Neutrophil gelatinase-associated lipocalin and ß2-Microglobulin with other renal markers among patients with multiple myeloma and healthy people. One hundred twenty patients with multiple myeloma and sixty healthy control were attending the National Center for Teaching Laboratories (NCFTL), Baghdad Hospital Advisory (BHA), and Baghdad Teaching Hospital. For the period from November 2021 to March 2022. Information taken from patients involved age, genus, drugs used, and other medical details. Multiple myeloma disease was diagnosed using symptoms, biochemical testing, x-ray, and clinical examination by specialists. Then serum urea, creatinine, total protein, globulin, and albumin are done by Autoanalyzer. Furthermore, Neutrophil gelatinase-associated lipocalin and ß2-Microglobulin were done by ELISA technique. The present study found that the male patients in the age range (60-69) and (50-59) years had a higher percentage (30%, 20% and 36%,16.7%) than female patients in both studied groups. The multiple myeloma disease patients with urea and creatinine above normal had a highly significant increase (p= 0.000) in the mean ± SD of urea, creatinine, total protein, globulin, Beta 2 microglobulin, and Neutrophil gelatinase-associated lipocalin (57.83±30.92 mg/dl, 2.09±1.38 mg/dl, 77.95±13.08 g/l, 42.52±11.16 g/l, 39.14±16.20, 452.50±188.83) respectively. In addition, patients with urea and creatinine normal had a highly significant increase (p= 0.000) in the mean ± SD of creatinine, total protein, Globulin, Beta 2 microglobulin, and Neutrophil gelatinase-associated lipocalin (0.80±0.20 mg/dl, 73.07±11.75 g/l, 40.34±10.67 g/l, 43.28±18.78, 432.77±198.45) respectively compared with the healthy controls.

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1. Introduction

Multiple myeloma (MM) is a communal plasma cell malignancy that is associated with significant renal impairment (RI) and has a high mortality rate. Kidney injury can limit treatment options and lead to poor outcomes, although it is still curable in certain people. Traditional renal impairment indicators, which are the most widely available, have substantial drawbacks, such as delayed onset after renal damage, many interfering factors, and reduced sensitivity to modest changes in glomerular filtration [1]. Lipocalin-2 (Lcn2), as well identified as Neutrophil Gelatinase-Associated Lipocalin (NGAL), is a lipoprotein involved in inflammation, ligand transport, iron transport, and preservation. As a marker of early renal tubular damage, Neutrophil Gelatinase-Associated Lipocalin in blood and urine rises within 2 hours following acute kidney injury (AKI). Neutrophil Gelatinase-Associated Lipocalin is more sensitive and detects earlier than creatinine. Treatment with diuretics does not affect the level of Neutrophil Gelatinase-Associated Lipocalin in the urine or serum [2, 3]. ß2-Microglobulin (ß2-M) is a protein produced by many cells and myeloma cells. It can be used to determine the illness stage, as high levels may indicate advanced disease. Useful for early prognosis of multiple myeloma and diagnosis of renal tubular diseases. Diagnosis of renal tubular diseases [4]. Multiple myeloma is the second most frequent hematological malignancy in high-income countries, accounting for 1% of all neoplastic disorders [5]. Due to this cancer's clinical and genetic heterogeneity, treatment outcomes and prognoses can vary [6]. Renal impairment with a serum creatinine > 2.0 mg/dl linked to plasma cell dyscrasia is one of the hallmark characteristics of MM. MM is the most frequent monoclonal gammopathy that causes kidney injury [6].

Nomenclature			
MM	multiple myeloma	ELISA	Enzyme-linked immunoassay
AKI	Acute kidney injury	GFR	Glomerular filtration rate

2. Materials & Methods

2.1. Patients and control

During the period November 2021 to March 2022, the current investigation was carried out at three major medical facilities in Baghdad: National Center for Teaching Laboratories (NCFTL), the Baghdad Hospital Advisory (BHA), and the Baghdad Teaching Hospital. Every patient was given a particular formula questionnaire to fill out, which included descriptive information name, age, genes, medicines used, and other medical data were all included in the questionnaire. Inclusion criteria were based on a negative enzyme-linked immunosorbent assay (ELISA) test for serum neutrophil gelatinase-associated lipocalin and serum B2Microglobulin for patients with (urea and creatinine normal) and patients with (urea and creatinine above average). Multiple myeloma diseases were done by the symptoms, biochemical tests, x-ray, and medical checks by way of specialists. In this study, multiple myeloma patients have a long term with drugs used. Sixty control groups from the medical staff and patient's relatives (group I) and one hundred twenty patients presented with multiple myeloma divided into two groups firstly :(group II) patients with a normal concentration of urea and creatinine; Secondly (group III) patients with an abnormal concentration of urea and creatinine), and. Patients were selected regarding age and genes. They are (40) males and (20) females in each group, with their ages ranging from (40 to 80) years. The hospital's Ethics and Research Committee approved the study, which was overseen by a consultant, and patients and controls gave their consent to the sampling.

2.2. Biological samples

A blood sample of around 8 mL was obtained from each participant in this investigation via vein puncture with disposable syringes then divided into 6ml and 2ml of blood and put into a gel tube for centrifuged at 3500xg for the time of about 10 min then the serum was divided into two tubes: 6ml of serum for biochemical tests (B.urea, S.creatinine, S.Total protein, serum albumin, and S. globulin) by Autoanalyzer, 2ml of serum was then placed in Eppendorf tubes and stored at -20 C° until it was time to measure serum Neutrophil Gelatinase-Associated Lipocalin and B2Microglobulin using the ELISA technique (commercial kits were purchased from My BioSource in the United States).

2.3. Statistical analysis

Data were revised, coded, and analyzed using the "Statistical Package of Social Science (SPSS) version 26.0.

1. For presentation of data using:
   - Tabular presentation of data (Complex frequency distribution table).
   - Mathematical presentation method (Mean and Standard Deviation).
2. For analysis of data using:
   - Independent sample t-test.
   - Simple Correlations (r) Coefficient.

The comparison of significant (p-value) in any test was considered as:
A P-Value of greater than 0.05 (P>0.05) was non-statistically significant (NS).
P-Value of less than 0.05 or equal (P≤0.05) was statistically significant (S).
P-Value of less than or equal to 0.01 (P≤0.01) was highly statistically significant (HS).

3. Result & Discussion

The baseline characteristics of the studied groups according to age and gender with the comparison of significance were observed in Table 1. Table 1 revealed that the male patients in the age group (60-69) and (50-59) years are predominant with a higher percentage (30%, 20% and 36%,16.7%) than female patients in all studied groups with a significant difference in gender of the healthy group (p= 0.00) and no significant difference was seen in gender with group III (P= 0.433). In addition, a significant difference in gender in group II (P=0.017).

Table 1 Distribution of Studied groups according to Age and gender

Age groups (Years)	Studied groups: Group I N=(60) gender		GroupII N=(60) gender		Group III N=(60) gender	
	Male (n=40)	Female (n=20)	Male (n=40)	Female (n=20)	Male (n=40)	Female (n=20)
(40-49)	6(10.0%)	6(10.0%)	8(13.3%)	2(3.3%)	0(0.0%)	0(0.0%)
(50-59)	18(30.0%)	2(3.3%)	12(20.0%)	8(13.3%)	10(16.7%)	8(13.3%)
(60-69)	10(16.7%)	12(20.0%)	18(30.0%)	4(6.7%)	22(36.7%)	10(16.7%)
(70-79)	6(10.0%)	0(0.0%)	2(3.3%)	6(10.0%)	8(13.3%)	2(3.3%)
P-Value	P=.000 (HS)		P=.017 (S)		P=.433 (NS)	

The present study shows the male patients more than the female patients which agreement with another study done by Carrero *et al.*, 2018 which found that men's kidney function deteriorates faster than women's, presumably due to men's unhealthy habits and the protective effects of estrogens or the destructive effects of testosterone [7]. Also, the current study agrees with other studies of Canadian Cancer Statistics 2009, Alexander DD, Mink PJ, Adami HO which found that a higher percentage with multiple myeloma disease over the age of 60 years [8]. In

addition, the more occurrence of acute kidney injury in elderly individuals can be potentially attributed to the resulting: A) comorbidities that collect with age may facilitate AKI (e.g., renovascular disease, congestive heart failure); B) comorbidities may necessitate processes, drugs, or surgery that function as kidney stressors and nephrotoxins; C) the kidney undergoes age-dependent structural and functional variations over time. Also, In the case of pathophysiological experiments, the latter results in a lower GFR at baseline and a lower kidney reserve, making older adults more prone to acute stress and more likely to develop clinically meaningful AKI, which is similar to the prevalence rate [9].

Table 2 shows a comparison in urea, creatinine, total protein, albumin, globulin, B_2Microglobulin, and Neutrophil Gelatinase-Associated Lipocalin between the studies groups which include group I, group II and group III. The group III had a highly significant increase (p= 0.000) in the mean ± SD of urea, creatinine, total protein, Globulin, Beta 2 microglobulin, and Neutrophil Gelatinase-Associated Lipocalin (57.83±30.92 mg/dl, 2.09±1.38 mg/dl, 77.95±13.08 g/l, 42.52±11.16 g/l, 39.14±16.20, 452.50±188.83) respectively. In addition, group II had a highly significant increase (p= 0.000) in the mean ± SD of creatinine, total protein, Globulin, Beta 2 microglobulin, and Neutrophil Gelatinase-Associated Lipocalin (0.80±0.20 mg/dl, 73.07±11.75 g/l, 40.34±10.67 g/l, 43.28±18.78, 432.77±198.45) respectively compared with the group I (0.67±0.15624 mg/dl, 66.63±4.66 g/l, 26.77±3.25 g/l, 14.35±7.49, 165.80±90.77) respectively. While the highly significant decrease in the mean ± SD of S. Albumin (35.4230±3.62170) g/l in group III. While non-significant in the mean ± SD of S.urea (p=0.243)(29.82±11.53 mg/dl) in group II patients when compared with group I.

Table 2 Comparison of the biochemical parameters between studied groups

parameters	Group II N=(60)	Mean ±Std. GroupI N=(60)	Group III N=(60)
Serum urea(mg/dl)	29.82±11.53 P= .243 (NS)	32.00±8.57	57.83±30.92 P=.000 (HS)
Serum Creatinine(mg/dl)	0.80±0.20 P=.000 (HS)	0.67±0.15624	2.09±1.38 P=.000 (HS)
Serum Total protein (g/l)	73.07±11.75 P=.000 (HS)	66.63±4.66	77.95±13.08 P=.000 (HS)
Serum Albumin (g/l)	32.79±5.95 P=.000 (HS)	40.31±4.64	35.4230±3.62170 P=.000 (HS)
Serum Globulin (g/L)	40.34±10.67 P=.000 (HS)	26.77±3.25	42.52±11.16 P=.000 (HS)
B2Microglobulin(mg/dl)	43.28±18.78 P=.000 (HS)	14.35±7.49	39.14±16.20 P=.000 (HS)
Neutrophil Gelatinase-Associated Lipocalin (pg/ml)	432.77±198.45 P=.000 (HS)	165.80±90.77	452.50±188.83 P=.000 (HS)

The finding of this study approve that the upper levels of urea and creatinine in multiple myeloma plasma may be related to impaired renal function in MM progression and impede toxin secretion as shown in another study [10]. Also, the current study agrees with the study done by Andronesi *et al.*, 2019 who found that total protein and globulin were increased in a group of patients due to the damage to the kidney [11]. Furthermore, The study by Salman, Salah, and Abass, 2020 show a decrease in albumin because to the bone disorder is the most common complication in the MM, the damage that occurs in the bone result from stimulation of osteoclast formation and activation that occurs in the area of the bone that is closed to myeloma cell. Besides, to increase the bone resorption, a decrease in bone formation have been reported and this attributed to the suppression effect of myeloma cell on osteoblast cell and so inhibit bone formation [12]. According to a study by Van Donge in 2021, patients with multiple myeloma had higher levels of the proteins B2Microglobulin and Neutrophil Gelatinase-Associated Lipocalin, which are the smallest molecular weight proteins and are filtered through the glomerulus and reabsorbed through the proximal tubules. While increased serum concentrations of these markers indicate glomerular damage, an increase in urinary concentrations implies tubular failure [13]. Regarding to the correlation coefficients (r) in the Table 3 showed a positive correlation with highly significance at P<0.01 between Neutrophil Gelatinase-Associated Lipocalin with Serum urea mg/dl, Serum creatinine mg/dl, Serum total protein g/l and Serum globulin g/l with correlations (r) (0.210, 0.214, 0.235, 0.387) respectively. However highly positive significance correlation was seen between B2Microglobulin and Serum globulin g/l (r =0.272; p<0.01). Finally, no significant correlations (r) between β_2Microglobulin with Serum urea mg/dl, Serum creatinine mg/dl and Serum total protein g/l (r=0.128, r = 0.144 and r =0.083) respectively.

Table 3 Correlations (r) between Neutrophil Gelatinase-Associated Lipocalin and B2Microglobulin with biochemical markers

Parameters	Neutrophil Gelatinase-Associated Lipocalin		β_2Microglobulin	
	r	P-Value	r	P-Value
Serum urea(mg/dl)	0.210**	.005	0.128	.088
Serum Creatinine (mg/dl)	0.214**	.004	0.144	.050
Serum Total protein (g/l)	0.235**	.001	0.083	.266
Serum Albumin (g/l)	- 0.329**	.000	- 0.404**	.000
Serum Globulin (g/L)	0.387**	.000	0.272**	.000

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

The results shown in Table 3 are consistent with previous literature that shows Neutrophil Gelatinase Associated Lipocalin over conventional biomarkers of acute kidney injury like serum creatinine and urea nitrogen is that the levels often increase long before any variations in serum creatinine or urea nitrogen. Moreover, Neutrophil Gelatinase-Associated Lipocalin may also be an early, sensitive indicator of acute kidney injury in patients with multiple myloma [14]. This information may be consistent with current evidence from a study on plasma Neutrophil Gelatinase-Associated Lipocalin as an early indicator of renal impairment, which found that serum Neutrophil Gelatinase-Associated Lipocalin is highly connected with creatinine in circumstances where the correlation coefficient is 0.4 [15]. Furthermore, the present study agrees with previous studies evaluated that plasma Neutrophil Gelatinase-Associated Lipocalin levels showed positive correlations with higher serum creatinine (r = 0.510; P <0.0001) [16,17].

Additionally, the finding of this study agree with study done by Suzuki, 2012 who observed that the serum globulin level was positively correlated with renal impairment ($r = 0.21$, $p < 0.001$) and negatively correlated with albumin level ($r = -0.34$, $p < 0.001$) [18].

4. Conclusions

Serum Neutrophil Gelatinase-Associated Lipocalin and B2Microglobulin were found to be elevated in patients with multiple myeloma earlier than elevated of serum urea and creatinine when the kidney injury. So, they are more correlated with diagnosis of early acute kidney injury (AKI) in patients with multiple myeloma than other biochemical markers such as (urea, creatinine, total protein, albumin, and globulin).

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