RESEARCH ARTICLE

Neutrophil gelatinase-associated lipocalin as a biomarker for detection of early renal impairment in Iraqi patients with multiple myeloma

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Abstract

Objective: To evaluate the serum neutrophil gelatinase-associated lipocalin levels in multiple myeloma patients as an indicator of disease progression.

Method: The case-control study was conducted at the Clinical Biochemistry Department of the National Centre of Haematology, Mustansiriyah University, Baghdad, Iraq, from October 2020 to July 2021, amd comprised diagnosed multiple myeloma patients and healthy controls of either gender aged 40-60 years. Neutrophil gelatinase-associated lipocalin and renal functions were measured for all patients in addition to obtaining a complete history and physical examination. Data was analysed using MedCalc.

Results: Of the 60 sujects, 30(50) were cases with mean age 64±2.1 years and 30(50%) were controls with mean age 60 ±3.2 years. There were 18(68%) males among the cases and 17(55%) among the controls. In the cases group, mean levels of creatinine, urea and neutrophil gelatinase-associated lipocalin were 1.62±0.85mg/dl, 55.56±28.05mg/dl and 389.39±116.12pg/mL, respectively. The corresponding values for the controls were 0.9518±0.1623mg/dl, 30.17±8.47mg/dl and 120.82±68'52pg/ml. The neutrophil gelatinase-associated lipocalin cut-off level >190pg/ml in multiple myeloma patients was strongly associated with renal impairment ($p<0.001$).

Conclusion: Neutrophil gelatinase-associated lipocalin was found to be an accurate indicator of early kidney disease in patients with de novo multiple myeloma.

Key Words: Lipocalin, Creatinine, Myeloma, Kidney, Hematology, Urea, Tumour

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Introduction

The plasma cell cancer, known as multiple myeloma (MM), is currently incurable but treatable, and is distinguished by a constellation of features, including renal impairment, which complicates about 20-40% of patients recently diagnosed with MM.[1] The major causes behind MM are hypercalcaemia, precipitation of monoclonal immunoglobulin (IG) in renal tubules, infiltration by myeloma cells, and nephrotoxic drugs.[2]

Recently, the discovery of novel therapeutics for MM has improved the prognosis through improvement in progress and overall survival. The diagnosis of renal failure in patients with MM is characterised by serum creatinine >2.00mg/dl or creatinine clearance (CC) <60ml/min.[3] A recent study proposed a cut-off of CC <40ml/min as renal impairment criterion.[4] However, these parameters are not sensitive enough to elicit the diagnosis of renal impairment in MM, which, in turn, leads to delayed diagnosis and treatment. Recent studies showed that monoclonal gammopathies of renal significance, which include all kidney diseases that are triggered by monoclonal IGs that are secreted by either B cells or plasma cells, carry increased morbidities. In addition, early treatment given to eradicate plasma cell clones or beta cells is fundamental, especially to protect renal function. For that reason, it is important to identify novel biomarkers that detect early-stage renal impairment.[5-7] Neutrophil gelatinase-associated lipocalin (NGAL) consists of glycoprotein that appears in many molecular structures and is expressed in different tissues. The kidney is one of the tissues having the ability to filter and reabsorb NAGL. Additionally, the affected tubular cells are able to produce it locally.[8] Also, neutrophils are able to release it which limits their use in other severe disorders causing inflammation and sepsis.[9,10] There are several biomarkers that have been tested to detect renal impairment in MM, including NGAL, cystatin C, ribonucleic acid-binding protein (RBP), and N-acetyl-glycosaminidase.[11-13] NGAL, as a biomarker, has specific characteristics, including rapid elimination from renal circulation, high sensitivity in acute and chronic conditions, a short half-life, and the fact that it can be detected within a few hours.[8]

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The current study was planned to evaluate serum NGAL levels in MM patients as an indicator of disease progression.

Patients and Methods

The case-control study was conducted at the Clinical Biochemistry Department of the National Centre of Haematology (NCH), Mustansiriyah University, Baghdad, Iraq, from October 2020 to July 2021. After approval from the institutional ethics review committee, the sample was raised using consecutive sampling technique from among those visiting NCH. Those included were newly-diagnosed, symptomatic MM patients of either gender aged 40-60 years who had no comorbidities. MM was diagnosed in line with the International Myeloma Working Group (IMWG) guidelines.[14,15] MM patients with severe renal impairment on dialysis were excluded. Matched for gender and age, healthy controls were enrolled randomly from among those on a routine visit.

After taking informed consent from all the participants, a venous blood sample of approximately 6ml was drawn from each subject using disposable syringes. It was placed in a gel tube for centrifugation at 3500 x g for 5 minutes. Then, 2mL of serum was used for urea and creatinine analysis by a semi-autoanalyzer, and 2ml was placed in Eppendorf tubes and stored at -20°C till NGAL was measured using the enzyme-linked immunosorbent assay (ELISA) (My BioSource, United States).

Data was analyzed using MedCalc. Data was expressed as frequencies and percentages as well as mean ± standard deviation (SD) and standard error (SE) of the mean, as appropriate. Student`s t-test was used to investigate the significance of the results. The cut-off value, sensitivity, and specificity of NAGL were evaluated using the receiver operator characteristic (ROC) curve. P<0.05 was considered significant.

Results

Of the 60 subjects, 30(50) were cases with a mean age of 64±2.1 years, and 30(50%) were controls with a mean age of 60±3.2 years. There were 18(68%) males among the cases and 17(55%) among the controls. In the cases group, mean levels of creatinine, urea and NGAL were 1.62±0.85mg/dl, 55.56±28.05mg/dl and 389.39±116.12pg/mL, respectively. The corresponding values for the controls were 0.9518±0.1623mg/dl, 30.17±8.47mg/dl and 120.82±68'52pg/ml (Table 1).

Based on ROC curve, NGAL >190pg/ml was strongly associated with renal impairment (p<0.001) (Table 2).

Table-1: Demographic characteristics, renal function tests, serum NGAL and ROC analysis in patients and controls..

Characteristics	Patients (NO=30)	Control NO=30		p-value
Age (years)	64±2. 1	60 ±3. 2		0.05
Gender				
Male	18 (68%)	17 (55%)		0.08
Female	12 (32%)	13 (45%)		
BMI mean and percentage %				
>25	22.1 (12%)	No=15	22.9 (12 %)	0.07
25-29.9	27.91 (23%)	No=8	26.53 (22 %)	
More than 30	32.03 (65%)	No= 7	33.02 (66 %)	
Renal Function Test				
Urea (mg/dl)	55.56 ± 28.05	30.17 ± 8.47	T=3.3	
	Range (27.51-83.6)	Range (21.7-38.64)	P=0.03	
Creatinine (mg/dl)	1.62 ± 0.85	0.9518± 0.1623	T=4.9	
	Range (0.77-2.47)	Range (0.78-1.11)	0.002	
Biomarker				
NGAL (pg/ml)	389.39±116.12	120.82±68.52	T=17.12	
	Range (273.27-505.51)	Range (52.3-189.34)	P<0.001	

Correlation between NGAL and Duration of Disease	Parameters
Pearson correlation coefficient (r)	-0.03
P-value	0.8
Covariance	-8.7
Sample size (n)	30
Statistic	-0.16

ROC: Receiver Operator Curve, BMI: Body mass index, NGAL: Neutrophil gelatinase-associated lipocalin.

Table-2: NGAL cut-off value as per receiver operator characteristic (ROC) curve.

NGAL cut off value (pg/ml)	Sensitivity	Specificity	AUC	P-value
>190.373	100	100	1	<0.001

NGAL: Neutrophil gelatinase-associated lipocalin, AUC: Area under curve.

Discussion

The current study evaluated serum NGAL levels in newly diagnosed MM patients, and MM's relationship with the patients' renal function. From a clinical perspective, the definition of renal impairment in patients with MM is measured by serum creatinine and blood urea, which are considered cornerstones in defining the glomerular filtration rate (GFR), and, in turn, the status of renal impairment. The presence of renal impairment in the criteria of symptomatic MM, involving increase in calcium level, renal impairment, anaemia and bone lesion, has already been validated and mentioned in the Salmon and Durie Classification (SDC).[16] Nevertheless, the levels of serum creatinine differ from one patient to another based on different non-renal factors, including age, gender and muscle mass. These different levels of serum creatinine and the subsequent overestimation of GFR might lead to unsuitable management for patients with MM, in general,

and those with renal impairment, in particular. Novel biomarkers of renal injury in MM have recently included serum cystatin C, urinary activin A and NGAL.[5, 17, 18]

In patients with MM, NGAL level has been studied and linked to several aspects, like disease activity, staging system and M protein levels, which makes it an important tool as a biomarker of eliciting disease burden and detecting renal impairment.[5,19] In patients with mild to moderate renal impairment, the levels of NAGL were recorded in high concentrations in the current study, which is in line with a study in which urine NGAL was found to be a sensitive indicator for the detection of renal impairment in patients with monoclonal gammopathies with undetermined significance (MGUS).[20] A study concluded that serum NGAL levels were increased in soldering MM (SMM), MGUS, and MM possibly because of the dual effect of cystatin C and NGAL, which may cause active renal injury and loss of functional nephrons.[5]

In another study, a panel of renal indicators were tested in 3 groups: the first group was of MM patients with chronic kidney disease (CKD), the second group was of MM patinets with normal kidney function, and the third group had healthy controls. The study showed increased NGAL levels in the urine and serum of the first group compared to the other groups.[21]

The current study showed that NGAL had high sensitivity and specificity for detecting renal injury in newly-diagnosed MM with a cut-off value of 190pg/dl. The result is similar to that obtained in an earlier study which stated that serum NGAL in patients with MM with renal impairment had a slightly higher positive predictive value (PPV) than that of urine, which might be better in differentiating this distinctive group of patients. Yet, the urinary NGAL is still considered an indicator of renal impairment as it has a significant relationship with estimated GFR (eGFR).[21]

Limitation: The current study had a small sample size, and the sample size was not calculated which could have affected the power of the study.

Conclusion

NGAL was found to be an effective indicator that may demonstrate an early kidney disease in patients with de novo MM.

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