URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN—AN EARLY BIOMARKER FOR RENAL INJURY IN PATIENTS WITH BETA-THALASSEMIA MAJOR

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ABSTRACT

INTRODUCTION: The iron overload and the development of secondary hemosiderosis in patients with β-thalassemia major (β-TM) lead to organ damages, including kidney disorders from early childhood. Contemporary urinary markers such as neutrophil gelatinase-association lipocalin (NGAL), β2-microglobulin (β2-MG), and N-acetyl-β-D-glucosaminidase (NAG) could be a useful tool for clinicians in diagnosis of early tubular lesions.

AIM: The aim of this article is to make an assessment of contemporary urine biomarkers β2-microglobulin, neutrophil gelatinase-associated lipocalin, and N-acetyl-beta-D-glucosaminidase in the diagnosis of early renal injury in patients with β-TM.

MATERIALS AND METHODS: The current study was conducted by examining 44 patients with β-thalassemia major and 30 controls. All participants were tested for NGAL, β2-MG, and NAG in the first sample morning urine using ELISA method.

RESULTS: The results show statistically significant differences between the two examined groups in urinary NGAL.

CONCLUSION: Urinary NGAL indicates subclinical kidney injury when the tubular reabsorption of molecules is impaired.

Keywords: *β-thalassemia major (β-TM), β2-microglobulin (β2-MG), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-beta-D-glucosaminidase (NAG)*

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INTRODUCTION

Patients with β-thalassemia major (β-TM) are characterized by co-morbidity and poor quality of life. Iron overload and the development of secondary hemosiderosis from early childhood lead to organ damages, including renal disorders. Some of patients develop low-grade proteinuria and albuminuria, while others develop tubular disorders-hyper-

calciuria, hyperphosphaturia, hyperuricosuria, aminoaciduria, glucosuria (1,2,3). Elevated levels of uroproteins β2-microglobulin (β2-MG), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-beta-D-glucosaminidase (NAG), Tamm Horsfall protein have been reported (4).

Several factors are responsible for impaired renal function in patients with β-TM: shortened erythrocyte life, iron deposits in tissues, and iron chelation therapy (5,6).

The increased erythrocyte turnover and the need for frequent hemotransfusions results in the deposition of hemosiderin in the glomeruli, proximal tubules and interstitium with subsequent renal damage. Hashemieh et al. (2017) report that renal hemosiderosis is prevalent in patients with β-TM (7). The accumulated free intracellular iron is toxic for the tissues. It generates reactive oxygen species (ROS), which: damage cell membranes by lipid peroxidation; lead to destruction of cell organelles (mitochondria); disrupt mitochondrial respiratory chains, stimulate the development of fibrosis. ROS increase the risk of neoplasia such as hepatoma, and infections. Apoptosis of the cell occurs (Fig. W1) (8).

Chronic anemia and hypoxia reduce peripheral vascular resistance, increase renal plasma flow and intraglomerular pressure. Hyperfiltration with macromolecule transudation occurs, as well as low-grade proteinuria. The proteinuria is one of the risk factors of renal impairment. It activates genes responsible for the synthesis of fibrogenic cytokines. One of these cytokines is TGF-β1, which is directly related to the pathogenesis of tubulointerstitial fibrosis (10).

Chelators can lead to impaired renal function from a transient slight increase in serum creatinine to acute renal impairment (11,12). Tanji et al. (2001) report that deferasirox increases serum creatinine as a result of renal plasma reduction and glomerular filtration in some thalassemic patients. The effects of deferasirox on the expression of cyclooxygenase 2 (COX-2), an enzyme in the synthesis of prostaglandins, have been investigated by the authors. They state that COX-2 expression and prostaglandin synthesis, mediators of inflammation, are increased upon treatment with deferasirox (13). Chelators affect the arachidonic acid cascade and the production of prostaglandins with subsequent imbalance between vasodilators and vasoconstrictors. Tubuloglomerular feedback is activated, production of ade-
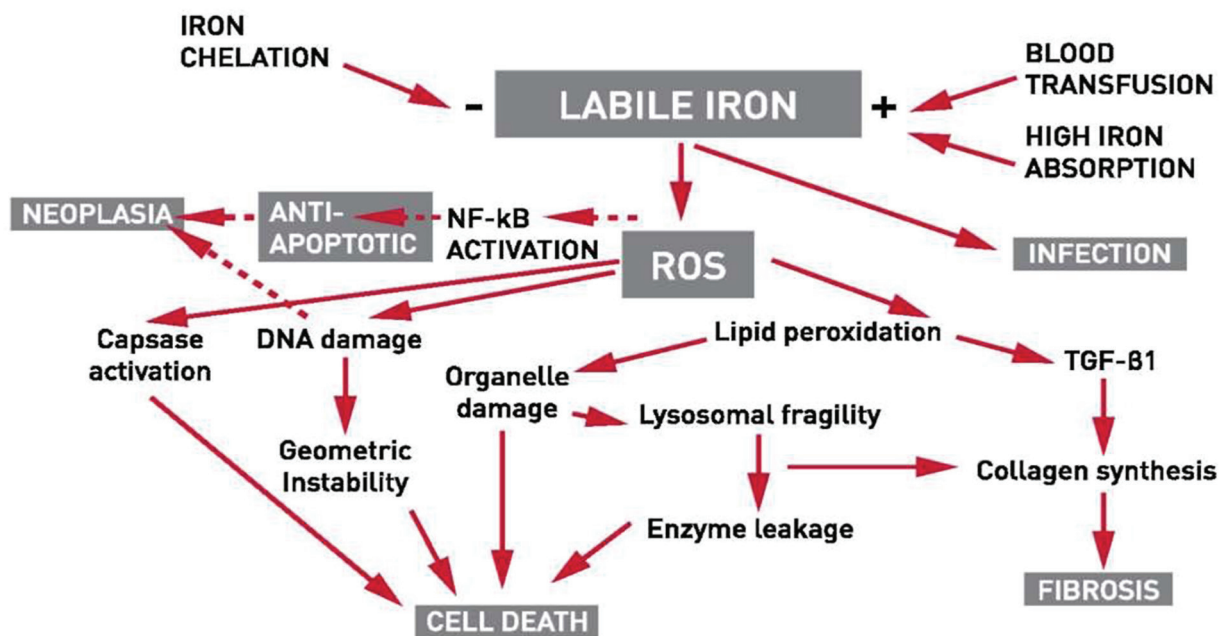

Fig. 1. Pathological mechanism and consequences of iron overload (9)

Anemia

↓

•**Glomerular dysfunction:**

•↓ vascular resistance → ↑ renal blood flow → glomerular hyperfiltration→ glomerular disease→ ↓ glomerular filtration rate.

Hypoxia

↓

• **Tubulointerstitial dysfunction:**

•Epithelial – mesenchymal transdifferentiation.

•Accumulation of extracellular matrix

•Activation of fibroblasts.

•Progressive fibrosis → decrease in diffusion capacity of oxygen.

The Nephron in ß Thalassemia

Iron overload

↓

• **Tubulointerstitial dysfunction:**

• Direct iron cytotoxicity on tubules and glomerular permeability→ enzymuria + proteinuria.

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• Moderate tubular atrophy and interstitial fibrosis.

•**Glomerular dysfunction:**

•Mild to moderate glomerulosclerosis.

•↓ nitric oxide (NO) availability→ decrease in blood flow →↓Glomerular filtration rate

Iron chelation

↓

• **Tubular dysfunction:**

•Vacuolization of proximal tubular epithelium.

• Apoptosis of tubule cell

•Decrease in intracellular energetics and mitochondrial function→decrease tubular absorption of solutes

•**Glomerular dysfunction:**
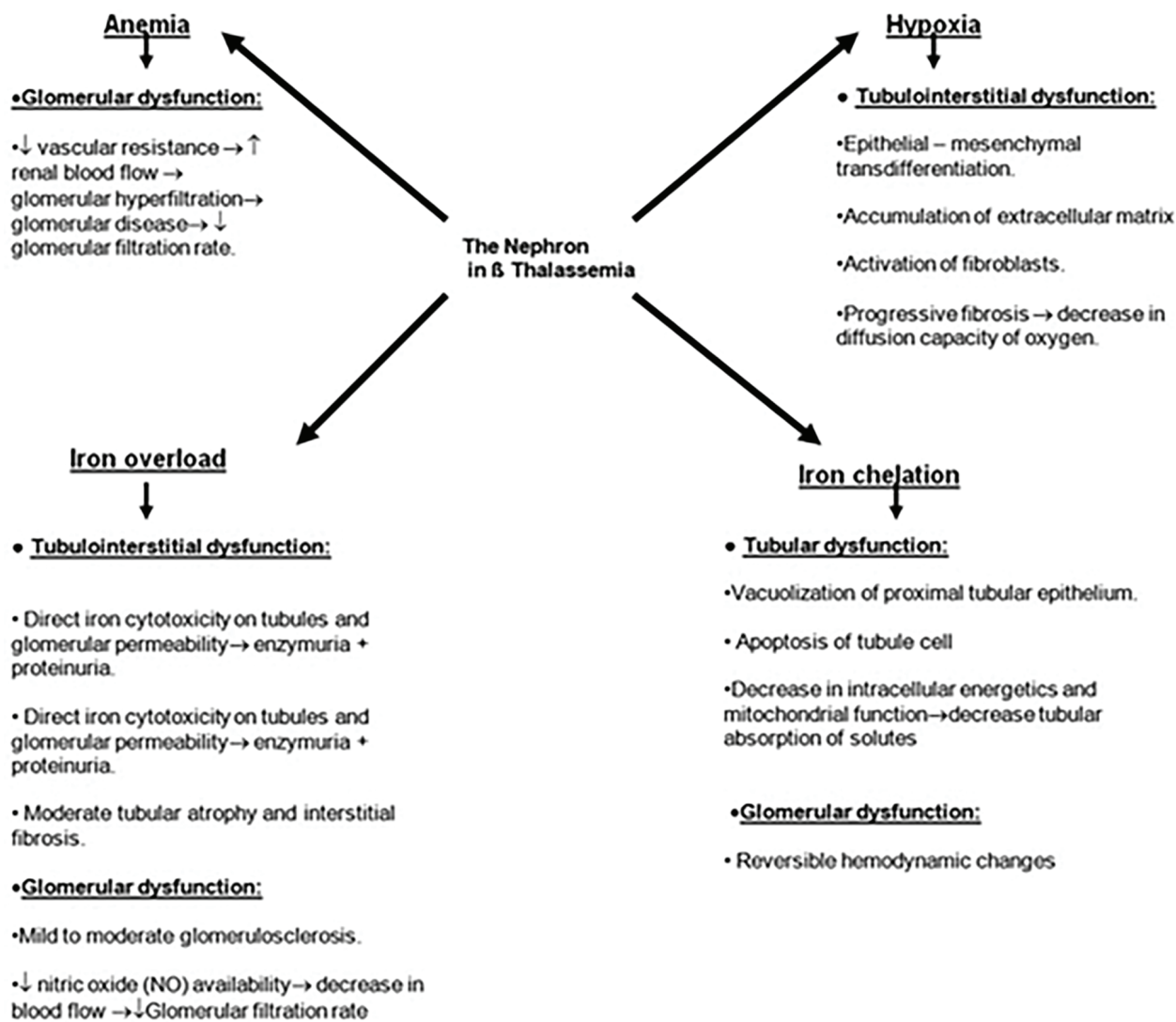
• Reversible hemodynamic changes

Fig. 2. β-thalassemia and the nephron (14)

nosine and adenosine triphosphate is increased. This results in vasoconstriction of afferent pre-glomerular arterioles with subsequent reduction of glomerular filtration (5).

It is known that chronic kidney disease (CKD) very often is not diagnosed before the onset of an end-stage renal disease (ESRD) due to the lack of significant complaints of patients. The therapeutic options are limited in cases of advanced renal impairment. The practice of early examination and treatment can slow down the progression of CKD. This determines the growing interest of clinicians to look for new, non-invasive and sensitive markers of renal dysfunctions.

Neutrophil gelatinase-associated lipocalin is a protein with molecular mass of 25 kDa. It was described for the first time in 1993 (15,16). In a state of stress (infection, inflammation, ischemia, neoplastic proliferation) there is a pronounced expression in the damaged epithelial cells in both the kidney and the colon, as well as in the liver and the lungs (17). Neutrophil gelatinase-associated lipocalin is filtered through the glomerulus and is largely reabsorbed in the proximal tubule. Neutrophil gelatinase-associated lipocalin excretion is a result of renal injury affecting reabsorption in proximal tubule and/or inducing de novo synthesis of NGAL in the distal tubule.

Neutrophil gelatinase-associated lipocalin is a renal troponin, an early biomarker and predictor of

renal impairment in cases of sepsis, administration of nephrotoxic drugs, contrast-induced nephropathy, urinary infections, glomerulonephritis, diabetic nephropathy, lupus nephropathy, and transfusion-dependent anemias (18,19,20). Velat Sen et al. (2015) indicate NGAL as a potential marker in screening or renal dysfunctions in patients with β-TM (4).

N-acetyl-β-D-glucosaminidase is a lysosomal enzyme in the proximal tubules of kidneys and can be used as a marker of proximal tubular damage and nephrotoxity. It does not filter through the glomerular basement membrane due to its high molecular weight of about 130-140 kDa. Urinary NAG testing is a rapid, non-invasive method for determining renal tubular function (21).

Beta 2-microglobulin is a protein with molecular mass of 11.58 кDa which is found in the cell membrane of all eukaryotic cells. It is completely filtered and in 99.9% is reabsorbed in the proximal tubule (22).

AIM

The aim of this study is to make an assessment of the contemporary urine biomarkers β2-MG, NGAL, and NAG in the diagnosis of early renal injury in patients with β-TM.

MATERIALS AND METHODS

A total of 44 patients with β-TM aged between 7 and 57 years of whom 18 children (12 girls and 6 boys) and 26 adults (14 women and 12 men) participated in our study. The diagnosis of β-TM was confirmed by hemoglobin electrophoresis.

Inclusion criteria: Patients with β-TM with signed informed consent were eligible for the study.

Exclusion criteria: Patients with β-TM and diabetes mellitus, chronic glomerulonephritis, uncontrolled arterial hypertension, acute urinary infections, pregnancy, cancer disease, taking nephrotic drugs, and application of iodine-based contrast materials in the last month were not eligible.

The control group consisted of 30 healthy participants aged between 7 and 45 years of whom 13 were children (9 girls and 4 boys) and 17 were adults (12 women and 5 men). None of them reported about hereditary anemia, requiring regular blood transfusions. They did not have cardiovascular, renal, endocrine and/or cancer disease, did not abuse alcohol and narcotic substances. During the study the women were not pregnant, did not breastfeed, did not take contraceptive drugs, and were not menstruating. The study was conducted in the period between September 2018 and August 2019.

Measurement of the uroproteins β2-MG, NGAL, and NAG of patients and healthy controls was performed by first sample morning urine centrifuged at 3000 rpm, after that the supernatant was separated and frozen at a temperature –20°C until the start of study. The biomarkers were measured with immunosorbent assay (ELISA).

Statistical Analysis

Descriptive and analytical methods were used. The significance level for differences between the studied groups was set to p≤0.05.

The strength of the correlation between the variables is based on the Pearson coefficient (r). The levels are determined as statically significant for values of r >0.5 and <r=0.7; for 0.7 <r=0.9 as strong and for r>0.9—very strong.

RESULTS

We selected the reference group according to requirements of IFCC (International Federation of Clinical Chemistry) and developed reference ranges of the laboratory parameters studied and presented in the dimensions indicated by us: β2-microglobulin/creatinine ratio mg/mol, NGAL/creatinine ratio ng/mol., NAG/creatinine ratio U/mol (Table 1).

Table 1. Reference ranges of the studied urinary biomarkers/creatinine ratios of healthy controls

Biomarker	Minimum	Maximum	Mean	SD
β2-MG/creatinine	0	8.3650	2.531000	± 2.1676115
NGAL/creatinine	0	0.0190	0.002233	± 0.0037268
NAG/creatinine	0.0002	0.1800	0.018363	± 0.0353029

The correlations between parameters β2-MG/creatinine, NGAL/creatinine, NAG/creatinine ratios determined by the Mann-Whitney U-Test are presented at Table 2.

DISCUSSION

For the first time in Bulgaria a study was done investigating tubular disorders in patients with β-TM. Contemporary, non-invasive, sensitive and

Table 2. Mann-Whitney U-Test

Biomarker	Controls	Patients	U test/Z	P
β2-MG/creatinine	2.5310 ± 2.1676	3.393 ± 2.7238	-1.59	0.116
NGAL/creatinine	0.0022 ± 0.0037	0.0106 ± 0.040	2.25	0.028
NAG/creatinine	0.0183 ± 0.0353	0.0283 ± 0.0706	-0.89	0.375

Table 3. Pearson correlation

	NGAL/creatinine	NAG/ creatinine	β2-MG/creatinine
NGAL/Creatinine			
Pearson Correlation	1	- 0.011	**0.297**
P		0.944	**0.050**
N	44	44	44
NAG/ Creatinine			
Pearson Correlation	-0.011	1	-0.231
P	0.944		0.132
N	44	44	44
B2 -MG/ Creatinine			
Pearson Correlation	**0.297**	- 0.231	1
P	**0.050**	0.132	
N	44	44	44

A positive correlation has been demonstrated between β2-MG/creatinine and NGAL/creatinine ratios (p=0.050, r=0.297) (Table 3, Fig. 3).
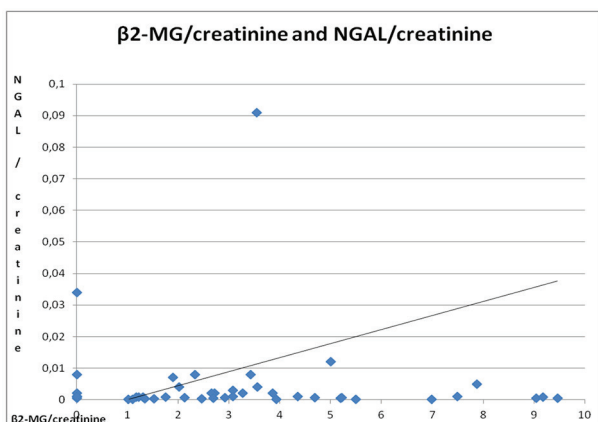

Fig. 3. Correlation between β2-MG/creatinine and NGAL/creatinine ratios

specific urinary biomarkers were measured, which provides additional information about the tubulopathies in thalassemic patients. There was a statistically significant difference in the NGAL/creatinine ratio between patients and controls. Our results confirmed those in the world literature reporting significantly high levels of urinary NGAL in patients with β-TM (4).

Neutrophil gelatinase-associated lipocalin like β2-MG indicates very early damages of the proximal tubule when the tubular reabsorption of soluble substances is impaired. The urine NGAL can be used as a reliable marker for subclinical renal injury in thalassemic patients. N-acetyl-β-D-glucosaminidase is a slightly later marker in diagnosing of tubulopathies. Their detection in the urine indicates cell lysis.

We found early disorders of tubular function before the onset of cell death, which makes the study

relevant, further supplementing existing bodies of knowledge on the matter with additional supporting data.

CONCLUSION

The development of the renal inflammatory process affects primarily the tubule cells. This could be the result of the damage done on various levels by oxidative stress. The urinary molecules β2-MG and NGAL are an evidence of disorders in the reabsorption of substances. More severe impairments in the tubules manifest during high levels of NAG. Knowing the pathophysiology of tubulopathies in patients with β-TM and their diagnosis using non-invasive ways will give clinicians more opportunities to predict the onset of the early renal damage in order to counteract with new therapeutic schemes in the early stages of the disease.

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