Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.234154.2873 Volume 30, Issue 4, July 2024
Manuscript ID ZUMJ-2103-2158
DOI 10.21608/ZUMJ.2021.65606.2158

Original Article

Evaluation of Novel Biomarkers for Early Detection of Acute Kidney Injury in Children with B-Thalassemia Major

Mohamed Abd Elrazek Mostafa Mohamed[1], Mervat AbdAllah Hesham[2], Naglaa Ali Khalifa[3], Mohamed Refaat Beshir[2]

1- Pediatric Department, Elkoren Central Hospital, Sharkia, Egypt
2- Pediatric Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
3- Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Corresponding author:
Mohamed Abd Elrazek Mostafa Mohamed.
E-mail: m_a.razek@yahoo.com

Submit Date 28-02-2021
Accept Date 09-05-2021

ABSTRACT

Background: Improved survival in patients with β-thalassemia has enabled the appearance of several clinical morbidities, involving renal problems. Incredibly important is the early detection of persons at higher risk of getting renal failure. This work aimed to compare the sensitivity of KIM-1, NGAL and microalbuminuria in early detection of kidney injury in thalassemic children. **Methods:** A cross sectional study was conducted in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals on thirty thalassemic patients during their regular follow-up visits and thirty age- and sex-matched healthy children as a control group from June to December 2017. All thalassemic patients were subjected to full medical history. Periodic thorough clinical examination was done with special emphasis on disease-related complications. Routine laboratory investigations were performed in addition to serum concentrations of Neutrophil Gelatinase-Associated Lipocalin (NGAL) protein (ng/ml), urine concentration of kidney injury molecule-1 (KIM-1) and microalbuminuria. **Results:** A highly statistically significant increase in the levels of urinary KIM-1, serum NGAL and albumin in urine among cases compared to controls was found. We found that the sensitivity of KIM-1 biomarker in prediction of kidney injury among thalassemia patients was 93.3%, higher than sensitivity of NGAL and microalbuminuria biomarkers. **Conclusions:** In early identification of acute renal failure in thalassemia patients, urinary KIM-1 is more sensitive than serum NGAL and microalbuminuria.

Keywords: β-thalassemias; Biomarkers; Kidney; Children..

INTRODUCTION

Beta-thalassemias (β-thalassemias or β-TM) seem to be a group of inherited blood disorders due to impaired or lacking hemoglobin beta-chain synthesis, triggering different phenotypes varying from severe to clinically asymptomatic anemia. Improved survival in patients with β-thalassemia has enabled the appearance of several clinical morbidities, involving renal problems [1].

Incredibly important is the early detection of persons at higher risk of getting renal failure because it can allow specific steps to delay the development of renal disease, thus reducing the occurrence of end-stage renal failure as well as death. Traditional biomarkers like serum creatinine or blood

urea nitrogen (BUN) are often used to diagnose kidney failure but that has remained unchanged for several years. Even so, as fast, and sensitive diagnostic indicators of renal injury, those biomarkers have some limitations [2].

In such a way, new biomarkers are intended to allow earlier renal dysfunction to be recognized. Several new early renal impairment biomarkers are already identified during last years, with the use of genetic tools [3].

Urine N-acetyl-b-d-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and liver-type protein-binding fatty acid (L-FABP) are perhaps the most potential biomarkers. KIM-1 and NGAL are considered markers that used to detect early tubular dysfunction caused by various kidney diseases [4].

Several reports have shown that the earliest indicator of renal impairment in which the weakened kidneys cause amounts of albumin to leak into the urine is microalbuminuria (MA). An early warning sign of negative outcomes for both kidney and heart disorders are microalbuminuria or microalbumin/creatinine ratio [5].

The aim of our study is to compare the sensitivity of KIM-1, NGAL and microalbuminuria in early detection of kidney injury in thalassemic children.

METHODS

Technical design: This cross-sectional study was conducted in the outpatient clinic of Hematology Unit of Pediatric department and Clinical Pathology department at Zagazig University Hospitals on thirty thalassemic patients during their regular follow-up visits and thirty age- and sex-matched healthy children as a control group from June to December 2017. Inclusion criteria included children of both sexes with ages ranged between 3 and 17 years and diagnosed clinically with β-thalassemia major while exclusion criteria involved children out the mentioned age group or with concomitant diseases that could affect kidney functions or potentially lead to renal damage, such as diabetes, rheumatic heart disease, thyroid disease, hepatic diseases, sepsis, or consumption of nephrotoxic drugs or diuretics. We also excluded patients with a history suggestive of recurrent urinary tract infections, a family history of hereditary renal diseases, any active infection, renal stones, hydronephrosis, urinary reflux or end-stage renal disease.

Methods: All thalassemic patients were subjected to full medical history. Periodic thorough clinical examination was done with special emphasis on disease-related complications. Laboratory investigations were performed involving complete blood count, serum ferritin level, renal functions, serum concentrations of Neutrophil Gelatinase-Associated Lipocalin (NGAL) protein (ng/ml), urine concentration of kidney injury molecule-1 (KIM-1) by ELISA method and microalbuminuria by Cobas C 6000 analyzer.

Administrative considerations: Written informed consent was obtained from all participants after clear explanation of the study and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (Institutional Research Board “IRB”). The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis:

Once data was collected, a code sheet was developed. Organization, tabulation, presentation, and analysis of data were performed using SPSS V21 (Statistical Package for Social Studies version 21). Quantitative data was described using number and percent. Quantitative data was described using number and percent. Quantitative data was presented as mean, range and standard deviation (SD). Parametric tests were applied for normally distributed data. Correlations were done by using Pearson’s correlation coefficient for normally distributed variables value. Comparison between groups was done using Chi-sequence test for qualitative variable.

RESULTS

Table (1) shows the demographic characteristics of the studied patients. This table shows no statistically significant difference among both studied cases and control group regarding age and sex. The clinical data among studied thalassemia cases is demonstrated in Table (2). 63.3% of the studied thalassemia cases had positive family history, 93.3% presented with pallor and jaundice, while only 50% presented with splenomegaly and 40% presented with hepatomegaly. Splenectomy is present in 36.7% of cases. The onset of thalassemia among studied cases was ranged from 3 months to 18 months with mean of 8.63 ± 3.74. Renal complaint was absent in 63.3 % of thalassemic children, while loin pain was present in 20%, dysuria in 10% while both loin pain and dysuria were present in 6.7% of cases. Table (3) demonstrates the laboratory data among both studied cases and controls. We found a high statistically significant difference among both cases and controls regarding all lab data recorded, except serum creatinine which presented in close levels among both groups, but slightly higher among cases. The difference in the specific laboratory data among both studied cases and controls was cleared in Table (4). A highly statistically significant increase in the levels of urinary KIM-1, serum NGAL and albumin in urine among cases compared to controls was found. Table (5) clears the Pearson`s correlation between specific novel biomarkers and laboratory data and onset of disease among thalassemic patients. This table shows a high statistically significant negative correlation between the three biomarkers and hemoglobin (HB), red blood cells (RBCs) and platelets count of the studied cases. While there was a high statistically significant positive correlation with white blood cells (WBCs), serum ferritin and serum urea. Also, there was positive significant correlation between KIM-1 and onset of disease. There was non-significant correlation between three biomarkers and serum creatinine. Table (6) reveals the reliability data of early biomarkers in prediction of kidney affection among thalassemia patients. We found that the sensitivity of KIM-1 biomarker in prediction of kidney injury among thalassemia patients was 93.3%, higher than sensitivity of NGAL and micro-albuminuria biomarkers (represent 83.3% for each of them), while specificity in exclusion of negative cases was 73.3% in KIM-1, versus 80% and 76.7% specificity of both NGAL and micro-albuminuria.

Table (1): Demographic data of the studied population.

Variables	Cases n = 30		Controls n= 30		x^2 \ t-test*	P-value
Sex	N	%	N	%		
Male	14	46.7	14	46.7	0.000	1.0
Female	16	53.3	16	53.3		
Age /years Mean ± SD	9.73±6.59		8.12±6.74		1.45*	0.151

* Data are presented as mean ± SD, median (Range), or number (%).

Table (2): Clinical data among studied thalassemia cases.

Variables	N (n=30)	%
Age of onset of disease /months **Mean ± SD** **Range**	8.63 ± 3.74 3 -18 months	
Family history **+ve** **-ve**	 19 11	 63.3 36.7
Pallor	28	93.3
Jaundice	28	93.3
Mongoloid face	12	40
Short stature	10	33.3
Hepatomegaly	12	40
Splenomegaly	15	50
Splenectomy	11	36.7
Renal complaint **No** **Loin pain only** **Dysuria only** **Dysuria and loin pain**	 19 6 3 2	 63.3 20 10 6.7

* Data are presented as mean ± SD, median (Range), or number (%).

Table (3): Laboratory data among both studied cases and controls.

Variables	Cases=30		Control=30		t-test	P-value
	Mean ± SD	range	Mean ± SD	range		
HB (g/dL)	7.3 ± 1.51	5.79-8.81	11.8 ± 0.77	11.03-12.57	14.24	0.000**
RBCs (10^6/µL)	3.49 ± 0.703	2.78-4.19	4.68 ± 0.47	4.12-5.15	7.68	0.000**
Platelet count (10^9/L)	257 ± 75.12	181-332	299.3 ± 72.44	226.68-371.74	2.22	0.03*
WBCs (10^3 /µL)	11.5 ± 3.5	8-15	6.46 ± 2.04	4.42-8.5	6.87	0.000**
Serum ferritin (µg/L)	2637.3 ±1089.9	1547.4-3727.2	32.88 ± 6.15	26.73-39.03	13.14	0.000**
Serum creatinine (mg/dl)	0.68 ± 0.18	0.5-1.4	0.64 ± 0.15	0.49-0.79	0.929	0.357
Serum urea (mg/dl)	27.6 ± 6.17	21.43-33.77	20.24 ± 4.01	16.23-24.25	5.5	0.000**

**P-value <0.001 is high significant *P-value <0.05 is significant.

Table (4): Difference in specific laboratory data among both studied cases and controls.

Variables	Case=30		Controls=30		t-test	P-value
	Mean ± SD	Range	Mean ±SD	Range		
KIM (ng/mL)	4.13 ± 0.53	3.6-4.66	3.08 ± 0.53	2.55-3.61	7.55	0.000**
NGAL (ng/mL)	192.6 ± 55.7	136.9-248.3	102.6 ± 30.9	71.7-133.5	7.76	0.000**
Micro-albuminuria (Mg/24h)	25.33 ± 9.27	16.06-34.6	13.5 ± 5.78	7.72-19.28	5.93	0.000**

**P-value <0.001 is high significant.

Table (5): Pearson`s correlation between specific novel biomarkers and laboratory data and onset of disease among thalassemic patients.

Variables	KIM-1		NGAL		Micro-albuminuria	
	R	P-value	r	P-value	r	P-value
HB (g/dL)	-0.642	0.000	-0.707	0.000**	-0.616	0.000
RBCs (10^6/µL)	-0.603	0.000	-0.680	0.000**	-0.466	0.000
Platelet count (10^9/L)	-0.272	0.04	-0.354	0.03*	-0.416	0.001
WBCs (10^3/µL)	0.559	0.000	0.615	0.000**	0.451	0.000
Serum ferritin (µg/L)	0.643	0.000	0.688	0.000**	0.595	0.000
Serum creatinine (mg/dl)	0.240	0.07	0.09	0.537	0.188	0.150
Serum urea (mg/dl)	0.669	0.000	0.676	0.000**	0.5	0.000

**P-value <0.001 is high significant *P-value <0.05 is significant.

Table (6): Reliability data of early biomarkers in prediction of kidney affection among thalassemia patients.

	Cutoff	AUC	Sensitivity	Specificity	P
KIM	3.43	0.926	93.3%	73.3%	0.000**
NGAL	139	0.928	83.3%	80%	0.000**
Micro-albuminuria	18.5	0.889	83.3%	76.7%	0.000**

**P-value <0.001 is high significant *P-value <0.05 is significant.

DISCUSSION

Thalassemia seems to be a chronic disease that influences several organs in different manners. Several reports on renal impairment have shown glomerular filtration rate (GFR) abnormalities and different grades of renal

tubular dysfunction in children with β-TM. Biomarkers are significant predictive entities which permit injury and proper functioning to be differentiated. For early recognition of acute renal injury, a few new urine biomarkers were already evaluated. Impaired renal function might be mirrored by NAG, NGAL, KIM-1, L-FABP and IL-88 upregulation [4].

Our study was done on 30 thalassemic patients and 30 control, each group contains 14 males and 16 females, the onset of the disease in patients was between 3-18 months signifying that β- TM presented early in life.

The results of the present study revealed positive family history of the disease in nearly two thirds (63.3%) of the studied cases, this goes in line with **El-Dakhakhny** who discovered that more than half (57%) of the included subjects had relatives affected by thalassemia [6].

Almost of our patients (93.3%) were presented with moderate to severe degree of pallor and jaundice and (40%) of them were presented with mongoloid face while (33.3%) with short stature. Haidar and colleagues concluded that there had been a gradual decrease in growth velocity in patients with β - thalassemia from the beginning of childhood across all of adolescence, particularly those who suffered extreme bone problems during DFX treatment [7].

As regards of Hb concentration in patient, it was ranged between 5.7 – 8.9 ml/dl. while in control group ranging between 11 and 12.6 mg/ dl. **Doddamani** and colleagues found that the patients' mean Hb level dropped dramatically around 4.7-7.44 between cases. and 11.33-13.47 between controls, respectively [8].

During our study we found no significant difference in serum creatinine concentration between case and control groups and the mean level of serum creatinine in our patients was within the normal range (0.68 ± 0.180 mg/dl and0.64 ± 0.15 mg/dl respectively).

In comparison, **Jafari** and colleagues observed a marked decline in serum creatinine levels in β-TM patients relative to normal controls and clarified this finding by a reduced body mass index, normally found in β-TM patients, owing to developmental delays and reduced muscle mass [9].

Our findings revealed that serum urea concentration in patient group was significantly higher than that of control group (27.6 ± 6.17 mg/dl, 20.24 ± 4.01 mg/dl respectively). In contrast **Aldudak** and his colleagues reported that there was no substantial difference among cases and controls concerning serum urea (26.6 ± 7.2 mg/dl in cases and 27.3 ± 7.9 mg/dl in controls). In β-TM patients, the cause of elevated serum uric acid levels is attributed to fast erythrocyte degradation and excessive uric acid urinary excretion [10].

We also found a high significant difference in serum ferritin level between cases and controls (2637.3 ± 1089.9 ng/dl and 42.88 ± 8.15ng/dl respectively). **Tantawy** and colleagues found similar findings, that revealed that serum ferritin was significantly associated with the tubular dysfunction markers tested, and this could offer a proof for the proposed belief in free iron involvement in proximal tubular dysfunction [11].

There was a highly significant difference between cases and controls regarding levels of urinary KIM-1 in our study. This is in contrast to **Sen** and colleagues who found no difference between the urinary KIM-1 levels of β-TM patients and the control group and indicated that the chronic existence of β-TM kidney damage, likely without interstitial renal damage, may explain the normal KIM-1 urinary values of their patients [12].

Our results also demonstrated a high significant difference between cases and controls regarding level of serum NGAL (192.6±55.7 in cases, and 102.6±30.9 in controls). This agrees with **Patsaoura** and colleagues who found that serum NGAL in cases was (139.1±86.1) and in controls (51.2±11.8) [13].

Marked increase in protein concentration in urine in thalassemia patient was found in our results. MA in our study was 25.3 ± 9.2 mg/24h in cases and 13.5±5.7mg/24h in controls. **Doddamani** and colleagues found

the similar finding (27.8 ±16.12 µg alb/mg cr in cases, and 8.3±6.2 µg alb/mg cr and proposing the existence of MA in β-TM patients showing increased emission of free radicals and lipid peroxidation [8].

Our study cleared a high statistically significant negative correlation between the three biomarkers and Hb and RBCs of the studied cases. While there was a high statistically significant positive correlation, with serum ferritin and serum urea. In addition, we found a positive significant correlation between KIM-1 and the onset of disease. There was also a non-significant correlation between the three biomarkers and serum creatinine.

On the other hand, in their cross-sectional analysis, **Sen** and colleagues indicated a lack of association among urinary markers and values of Hb, Hct and ferritin and concluded that it could be correlated with very early tubular dysfunction, such that elevation occurred only in NAG and NGAL but not in KIM-1 and L-FABP or urinary electrolytes [12].

CONCLUSIONS

Our evidence indicates the presence of renal impairment in β-TM children. In early identification of acute renal failure in thalassemia patients, urinary KIM-1 is more sensitive than serum NGAL and MA. In addition, patients with beta thalassemia must be controlled during their lives to identify early renal dysfunction. Longitudinal studies are required to examine the true frequency, pathways, and implications of kidney disorders in β-TM patients. The use of early markers such as KIM-1 and NGAL, that may be cost-effective for early identification of kidney failure in β-TM patients, is recommended because early kidney dysfunction cannot be identified by routine tests.

Declaration of interest

The authors report no conflicts of interest.

Funding information

None declared

REFERENCES

1. **Mallat NS, Mallat SG, Musallam KM and Taher AT.** Potential mechanisms for renal damage in beta-thalassemia. J Nephrol. 2013;26(5):821-8.
2. **Bagshaw SM and Gibney RT.** Conventional markers of kidney function. Crit Care Med. 2008;36(4): S152-8.
3. **Barrera JO and Bobadilla NA.** Are recently reported biomarkers helpful for early and accurate diagnosis of acute kidney injury? Biomark. 2012;17(5):385-93.
4. **Devarajan PR.** Emerging urinary biomarkers in the diagnosis of acute kidney injury. Expert Opin Med Diagn. 2008;2(4):387-98.
5. **Zhang ZH, Lu BA, Ni HO, Sheng XI and Jin NI.** Microalbuminuria can predict the development of acute kidney injury in critically ill septic patients. J Nephrol. 2013;26(4):724-30.
6. **Eldakhakhny AM.** Quality of life of school age thalassemic children at Zagazig City. J Am Sci. 2011;18(3):364-33.
7. **Haidar R, Musallam KM and Taher AT.** Bone disease and skeletal complications in patients with β thalassemia major. Bone. 2011;48(3):425-32.
8. **Doddamani PA, Suma MN, Ravi MD, Prashant AK, Vishwanath PR and Nagalakshmi CS.** Importance of assessment of microalbuminuria in β-thalassemia major patients. Int J Health Allied Sci. 2012;1(4):235.
9. **Jafari HM, Vahidshahi KI, Kosaryan MO, Karami HI, Rez MA and Ehteshami SA.** Major beta-thalassemia, use of desferiexamine and renal proximal tubular damage. Bratisl Lek Listy. 2011;112(5):278-81.
10. **Aldudak BI, Bayazit AK, Noyan AI, Özel AO, Anarat AM, Sasmaz IA, et al.** Renal function in paediatric patients with β-thalassemia major. Pediatr nephrol. 2000;15(1):109-12.
11. **Tantawy AA, Elbablawy NA, Adly AA and Ebeid FS.** Early predictors of renal dysfunction in Egyptian patients with β-thalassemia major and intermedia. Mediterr J Hematol Infect Dis. 2014;6(1):232-48.
12. **Şen VE, Ece AD, Uluca ÜN, Söker MU, Güneş AL, Kaplan İB, et al.** Urinary early kidney injury molecules in children with beta-thalassemia major. Renal fail. 2015;37(4):607-13.
13. **Patsaoura AI, Tatsi EL, Margeli AL, Kanavaki IN, Delaporta PO, Kyriakopoulou DI, et al.** Plasma neutrophil gelatinase-associated lipocalin levels are markedly increased in patients with non-transfusion-dependent thalassemia: Lack of association with markers of erythropoiesis, iron metabolism and renal function. Clin biochem. 2014;47(12):1060-4.

Citation:

Mostafa, M., Hesham, M., Khalifa, N., Besher, M. Evaluation Of Novel Biomarkers For Early Detection Of Acute Kidney Injury In Children With B-Thalassemia Major. *Zagazig University Medical Journal*, 2024; (2282-2288): -. doi: 10.21608/zumj.2021.65606.2158