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Evaluation of Serum Human Kidney Injury Molecule-1 and *N*-Acetyl-β-D-Glucosaminidase as Early Markers of Kidney Injury in Patients with β-Thalassemia Major in Diyala Governorate

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Abstract

Thalassemia is a genetic disease identified by a defect in the production of one or more globin chains. This study was aimed at determining the possibility of using "serum kidney injury molecule-1 (KIM-1)" and "*N*-acetyl-β-D-glucosaminidase (NAG)" in the diagnosis of early renal damage in thalassemia patients. Serum biomarkers (CBC, urea, creatinine, KIM-1, and NAG) were determined in 45 patients with major thalassemia with an age range of 4-45 years who attended Baquba General Hospital. A significant increase in S.KIM-1 and S.NAG levels in patients compared with controls. The level of KIM was positively correlated with that of urea and creatinine in comparison with control. Also, the level of NAG was strongly positively correlated with the level of KIM-1 ($p = 0.000$) and had a moderately positive correlation with urea and creatinine ($p = 0.000$). According to ROC, KIM-1 had a sensitivity of 95.56% and a specificity of 88.89%, while NAG had a sensitivity of 100% and a specificity of 75.6%. This study concluded that S.KIM-1 and S.NAG are highly promising biomarkers for the detection of "acute kidney injury" in an early stage before elevated urea and creatinine in thalassemia patients.

Keywords: Thalassemia, KIM-1, NAG.

تقدير مصل جزيء إصابة الكلى البشرية-1 و N-acetyl-β-D الجلوكوزامينيداز لمرضى الثلاسيميا في محافظة ديالى

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الخلاصة

لثلاسيميا هو مرض وراثي يتم تحديده من خلال خلل في إنتاج سلسلة واحدة أو أكثر من غلوبين. هدفت هذه الدراسة إلى تحديد إمكانية استخدام "جزيء إصابة الكلى المصل-1 (KIM-1)" و"N-acetyl-β-D-glucosaminidase (NAG)" في تشخيص التلف الكلوي المبكر لدى مرضى الثلاسيميا. تم تحديد المؤشرات

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الحيوية في المصل (صورة الدم الكاملة ، واليوريا ، والكرياتينين ، و KIM−1 ، و NAG) في 45 مريضًا يعانون من الثلاسيميا الكبرى مع الفئة العمرية (4−45) عامًا الذين حضروا مستشفى بعقوبة العام. زيادة كبيرة في مستويات S.KIM−1 و S.NAG في المرضى مقارنة مع مجموعة السيطرة. . ارتبط مستوى KIM ارتباطًا إيجابيًا بمستوى اليوريا والكرياتينين مقارنةً بمجموعة السيطرة. أيضًا ، كان مستوى NAG مرتبطًا ارتباطًا إيجابيًا قويًا بمستوى KIM−1 (p = 0.000)) وعلاقة إيجابية مع اليوريا والكرياتينين (p = 0.000). كان لدى KIM−1 حساسية: 95.56٪ ؛ النوعية: 88.89٪ وكان NAG حساسية: 100٪ ؛ النوعية 75.6٪ حسب (ROC). خلصت هذه الدراسة إلى أن S.KIM−1 و S.NAG مؤشرات حيوية واعدة للغاية للكشف عن "إصابة الكلى الحادة" في مرحلة مبكرة قبل ارتفاع اليوريا والكرياتينين في مرضى الثلاسيميا.

1. Introduction

Thalassemia is a genetic disturbance inherited from a person's parents and a blood condition in which hemoglobin synthesis is reduced [1]. The majority of cases of β-thalassemia are caused by mutations in the β-globin gene, which include deletion, insertion, and conversion of one or more nucleotides within the gene sequence [2,3]. Thalassemia symptoms range from none to severe, depending on the type. Mild to severe anemia (low red blood cells or hemoglobin) is common [4]. The tiredness and pale skin are common symptoms of anemia. Also all possible symptoms such as bone problems, an enlarged spleen, yellowish skin, and black urine [4,5]. Alpha and beta-thalassemia are the two most common types. The severity of the two types is determined by how many of alpha globin's four genes or beta globin's two genes are absent [6]. Blood tests, such as a CBC, specific HB tests, and genetic tests, are usually used to diagnose thalassemia [1]. Acute kidney injury can be diagnosed by serum creatinine but is delayed in response to renal injury. Therefore, the diagnosis of acute kidney disease is usually late, and the degree of injury may be underestimated. Serum creatinine is a marker of glomerular filtration; therefore, it is not specific to injury in other areas of the nephron. Creatinine production varies depending on age, sex, and weight (in particular, muscle mass). As a result, there has been a surge in interest in developing novel urine biomarkers, such as renal injury molecule-1, which has been identified as a potential marker of acute kidney injury [7-9]. Following kidney damage (KIM-1) is a substantial increase in cells of the proximal tubules. KIM-1's ectodomain is lost in the lumen and is a urine biomarker of renal damage. Some studies have revealed that shed KIM-1 can be used as a blood biomarker for kidney damage [10]. NAG is a lysosomal enzyme that is extensively secreted in the epithelium of the proximal tubules. Under normal circumstances, it is produced in modest amounts, resulting in a low urinary concentration. When the epithelium of the renal tubules is injured, however, production is increased, resulting in a considerable rise in urine content and activity [11]. Renal injury remains an underappreciated complication in patients with major β-thalassemia. Chronic anemia, iron overload because of repeated blood transfusions, and specific iron chelators are the chief factors in the pathogenesis of renal injury in β-thalassemia [10]. As a result, the purpose of this study was to determine the efficacy of kidney injury molecule-1 (KIM-1) and *N*-acetyl-β-D-glucosaminidase (NAG) as markers of early renal injury in beta-thalassemia major patients with elevated serum urea and creatinine.

2. Materials and methods

2.1. Studied groups

There are 45 patients with major thalassemia (26 males and 19 females), ranging in age from 4 to 45 years. These patients were attending the specialized hematology center in Baquba General Hospital, Diayla, Iraq, and were selected randomly for a period from June to September 2021. Patients were diagnosed by a consultant physician (using the clinical history of the disease, physical examination, and lab tests) to have thalassemia without other overlapping

diseases. The control group was made up of 45 apparently healthy subjects who were the same age and gender as the patients. This study was carried out under the supervision of the hospital's expert and ethics committees and with the consent of the participating patients and controls. Subjects with autoimmune diseases, emergency conditions, and pregnant women were excluded.

2.2. Blood sample

All blood samples were collected intravenously with disposable sterilized syringes, then one part of the blood was collected in an EDTA tube for hematological tests and the other part was centrifuged to obtain the serum that was stored at -20 °C in disposable, plain tubes for ELISA tests.

2.3. Biochemical analysis

All subjects were tested for Hb, PCV, WBC, and platelet counts on a Sysmex SF-3000 automated hematology analyzer. Roche Cobas u 411 analyzes urea and creatinine. NAG was done by a human *N*-acetyl-β-D-glucosaminidase ELISA kit from (Cat. No. MBS700742, USA), and KIM-1 was done by human kidney injury molecules (KIM-1) using an ELISA kit from (Cat. No. MBS264966, USA).

2.4. Statistical analysis

The data was analyzed using SPSS software (version 26). Chi-square, mean, standard deviation, and t-tests were used for comparing results among groups. In addition, the receiver operating curve (ROC) for estimated sensitivity and specificity with positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), and negative likelihood ratio (-LR) is estimated by MedCalc, with results considered significant when the P-value is less than 0.05.

3. Results

The study included 45 cases of thalassemia major, ranging in age from 4 to 45 years. Demographic characteristics according to the gender of the subjects being studied are presented in Table 1, which showed that the male group was more attacked, with about 26 (55.3%) versus 19 (44.2%) cases in the female groups. In addition, this study documented that the number of healthy control female groups was 24 (55.8%) versus 21 (44.7%) in the male groups; these results were statistically non-significant (P-value = 0.2).

Table 1: Crosstabs for the study population according to gender

Study group		Gender		Total	P-value
		Male (%)	Female (%)		
Patient	N	26	19	45	0.2 *N.S
	%	55.3	44.2	50.0%	
Control	N	21	24	45	
	%	44.7	55.8	50.0%	
Total	N	47	43	90	
	%	100.0	100.0	100.0	

*N.S (no significant)

Table 2 shows that 32 cases (66.7%) of thalassemia were in the age group 4-19 years. Only 5 and 8 cases (27.8% and 33.3%) were in the second and fifth decades (20-30 and 35-45 years,

respectively) of the total study count of 45 cases. The differences in the number of cases were statistically highly significant (P-value = 0.003).

Table 2: Crosstabs for the study population according to age

Studied groups		Categorical age (years)			Total	P-value
		4-19	20-34	35-45		
Patient	N	32	8	5	45	0.003 *H.S
	%	66.7	33.3	27.8	50.0	
Control	N	16	16	13	45	
	%	33.3	66.7	72.2	50.0	
Total	N	48	24	18	90	
	%	100.0	100.0	100.0	100.0	

*HS= highly significant.

Table 3 shows that both HB (g/dL) and PCV (%) levels were lower in the blood of thalassemic patients, as indicated by a lower mean value (5.13 ± 1.02 and 19.80 ± 4.64, respectively). The levels of both HB (g/dL) and PCV (%) showed normal levels among healthy control subjects (11.89 ± 2.51 and 38.10 ± 6.41, respectively). These distinctions were statistically significant (P-value = 0.000). In addition, the level of serum iron (mg/dL) was significantly (P = 0.02) higher in thalassemic patients, which was observed by an increased mean value (100.77 ± 30.36) compared with normal levels among healthy control subjects (88.51 ± 17.41).

Table 3: Comparisons between the levels of hematological parameters among patients with thalassemia and healthy controls

Parameter	Study group	No.	Mean	Standard deviation	t-test	*P-value
HB (12-15g/dL)	Patient	45	5.13	1.02	16.68	0.00 (H.S)*
	Control	45	11.89	2.51		
PCV (35-49%)	Patient	45	19.80	4.64	15.5	0.00 (H.S)*
	Control	45	38.10	6.41		
WBC ($4-11\times10^9$/L)	Patient	45	8.26	3.87	2.4	0.00 (H.S)*
	Control	45	6.79	1.12		
Platelet ($150-410\times10^9$/L)	Patient	45	174.04	54.36	6.3	0.00 (H.S)*
	Control	45	282.51	100.09		
Iron (50-170mg/dL)	Patient	45	100.77	30.63	2.3	0.02 (S)**
	Control	45	88.51	17.41		

*H.S = Highly significant **S = Significant

Table 4 shows the basic parameters of renal function that were measured, which include blood urea and serum creatinine. The mean urea and creatinine levels in the patient group were 35.95 ± 7.49, 0.87 ± 0.22mg/dL, respectively, while the control group had 25.88 ± 7.02, 0.69 ± 0.18 mg/dL.

Table 4: Comparison between the levels of renal disease parameters among cases and controls

Parameter	Study group	No.	Mean	Standard deviation	t-test	P-value
Urea (15-44 mg/dL)	Patient	45	35.95	7.49	6.5	0.000 (H.S)*
	Control	45	25.88	7.02		
Creatinine (0.55-1.3 mg/dL)	Patient	45	0.87	0.22	3.9	0.000 (H.S)*
	Control	45	0.69	0.18		

***H.S = Highly significant

Table 5 shows a highly significant increase in serum Kim-1 and NAG levels in patients (1849.33 ± 551.53 pg/mL and 873.06±216.42 mIU/mL, respectively) when compared with that of controls (761.07 ± 655.96 pg/mL and 392.04 ± 241.81 mIU/mL, respectively).

Table 5: Comparison between the levels of KIM (pg/mL) and NAG (mIU/mL) parameters among cases and controls

Parameter	Study group	No.	Mean	Standard deviation	t-test	P-value
KIM-1 (pg/mL)	Patients	45	1849.33	551.53	8.51	0.00 (H.S)*
	Controls	45	761.07	655.96		
NAG (mIU/mL)	Patients	45	873.06	216.42	9.9	0.00 (H.S)*
	Controls	45	392.04	241.81		

*H.S = Highly significant

Table 6 shows that the levels of KIM (pg/mL) were positively correlated with the levels of urea (mg/dL) and creatinine (mg/dL) with r = 0.35, p = 0.001, and 0.21, p = 0.05, respectively. This table also showed that the levels of NAG (mIU/mL) were strongly positively correlated with the levels of KIM (pg/mL) (r = 0.65, p = 0.000) and had a moderately positive correlation with urea (mg/dL) and creatinine (mg/dL) (r = 0.41, P-value = 0.000).

Table 6: Correlation between the levels of the renal disease parameters among cases and controls

Parameter		Urea (mg/dL)	Creatinine (mg/dL)	KIM (pg/mL)
KIM (pg/mL)	Pearson correlation (r)	0.35	0.21	
	P-value	0.001	0.05	
NAG (mIU/mL)	Pearson correlation (r)	0.41	0.41	0.65
	P-value	0.000	0.000	0.000
	N	90	90	90

Currently, ROC analysis is used to calculate the strength of serum assays to reveal KIM-1 and NAG, as shown in Table 7 and Figure 1. These showed that urea (sensitivity: 86.67%, specificity: 68.89%), creatinine (sensitivity: 86.67%, specificity: 55.56%), KIM-1 (sensitivity: 95.56%; specificity: 88.89%), and NAG (sensitivity: 100%; specificity: 75.6%) were the most sensitive and specific.

Table 7: Results of ROC for the parameters urea, creatinine, KIM, and NAG

Variable(s)	AUC	P-value	95% CI	Cut-off value	Sensitivity (%)	Specificity (%)	PPV	NPV	+ve LR	-ve LR
Urea (mg/dL)	0.828	<0.0001	73.2 - 94.9	>28	86.67	68.89	73.6	83.8	2.79	0.19
Creatinine (mg/dL)	0.724	<0.001	73.2 - 94.9	>0.66	86.67	55.56	66.1	80.6	1.95	0.24
KIM (pg/mL)	0.915	<0.0001	84.9 - 99.5	>1265	95.56	88.89	89.6	95.2	8.6	0.05
NAG (mIU/mL)	0.917	<0.001	92.1 - 100	>524	100	75.6	80.4	100	4.09	0.00

AUC, the area under the curve, PPV = positive predictive value; NPV = negative predictive value, LR = likelihood ratio

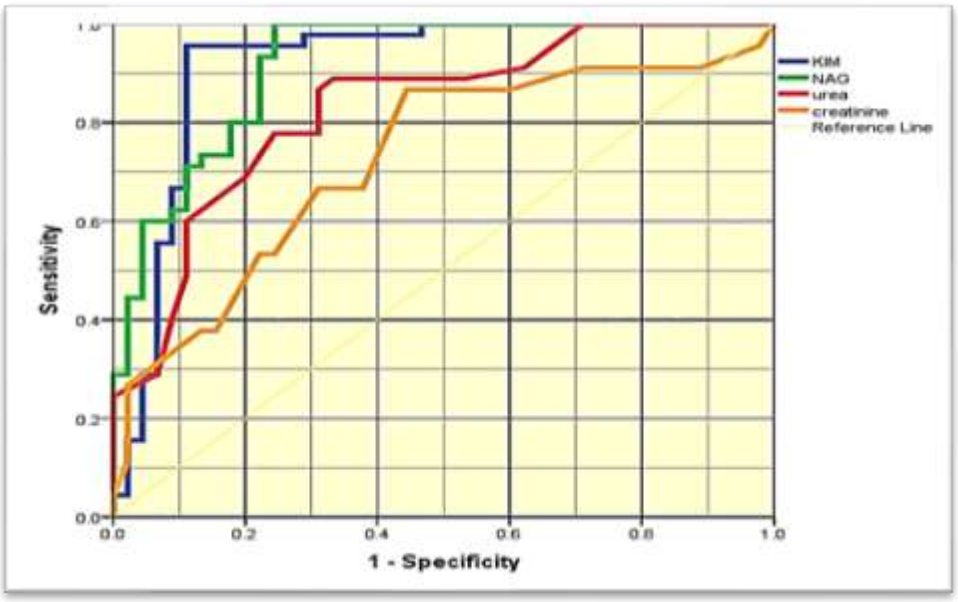

Figure 1: Results of ROC for the parameters urea, creatinine, KIM, and NAG

4. Discussion

Thalassemia is a common genetic disturbance caused by the disappearance or decrease of one or more globin chain syntheses. About 5% of the population around the world were thalassemia carriers, as the WHO reported [11]. We found that thalassemia was more common in people under the age of twenty (n = 32, 66.7%). This result agrees with [3], when it was found that about 57% of the study groups were under the second decade of age. A study done by Abdul-Karim, Abdul-Jalil, and Al-Azawi in 2000 showed the patient's age was often less than 10 years, which can reveal a shortened life expectancy in patients with thalassemia [13]. Also, the present study found that the levels of HB (g/dL), PCV (%), WBC (10^9/l), and iron (mg/dL) were significantly decreased in the blood of thalassemia patients. The result of a significant increase in platelet level (10^9/l) among thalassemic patients is in line with previous studies [13-15], which identified that an irregular HB level causes a reduction in RBCs

production and PCV due to abnormal erythropoiesis. It was also revealed that the levels of WBC (10^9/L), iron (mg/dL), and platelets (10^9/L) were normal among both thalassemia patients and healthy control groups, as indicated by the normal mean score of these tests when compared with the normal value of these tests. This finding contradicts previous research [16], which found increases in WBCs and platelets in patients with β-thalassemia. Other studies showed an increase in WBCs and a decrease in platelets in thalassemic children [17]. This may be attributed to the high breakdown of RBCs, which stimulates erythropoietin secretion from the kidney and induces the bone marrow to produce an excess of different types of hemocytes. Many factors could contribute to renal dysfunction in patients with thalassemia. Hypoxia, iron overload, chronic anemia, discontinuation of iron chelation, and post-splenectomy, for example, can all cause renal damage, resulting in proximal tubule epithelial cell dysfunction and fibrosis. Hemodynamic renal changes may be induced by anemia. Due to repeated blood transfusions, an iron overload will arise and cause oxidative stress to the kidney and direct cytotoxicity. Furthermore, the use of particular iron-chelating drugs is connected with a temporary, non-progressive excess in the levels of serum creatinine [19-21]. In the current study, when the essential and routinely used parameters of kidney function were measured, which include urea and creatinine, there was a statistical difference in patients compared to controls. But the findings were still within the normal limits, which did not indicate renal dysfunction. Our results are similar to those of Aldudak *et al*. and Belsare *et al*., who found no obvious difference in urea and creatinine in the patient population [22,23]. Renal injury, which manifests as tubule and glomerular dysfunctions in thalassemic patients with type β-major, is still one of the undervalued complications that become more visible with the onset of blood transfusions [19,22-23]. The objective of this work is to evaluate the role of KIM-1 and NAG as promising markers for the detection of renal involvement in thalassemia patients. The current study found a highly significant increase in serum KIM-1 and NAG levels in patients. These results were in line with previous studies [25-27], which found that increased levels of KIM-1 and NAG are considered good markers of proximal tubular damage in patients with thalassemia. This is due to β-thalassemia, which causes irregular HB levels as well as a decrease in RBC and PCV production due to abnormal erythropoiesis. This leads to overload of iron from regular transfusion and, in particular, iron chelators are the major causative factors in kidney dysfunction in patients with β-thalassemia as shown in other studies [27,28]. In the present study, we have studied the relationship between serum kidney injury molecules and particular renal parameters like serum creatinine and urea. Moreover, the present study is in agreement with other studies [27,28], which found that KIM-1 was strongly correlated with serum levels of urea and creatinine. In addition, the current study agrees with that done in 2021 by Mahmoud *et al*., who found a significant positive correlation between NAG and KIM-1 in thalassemia patients [19]. Furthermore, the current study contradicts prior studies that revealed that there are numerous grounds to believe that KIM-1 is released into the blood following the injury of the renal proximal tubules. The polarity of the tubular cell is absent during damage, allowing KIM-1 to be discharged directly into the interstitium. Additionally, the increase in permeability of the transepithelial membrane after tubular injury causes tubular contents to flow back into the circulation. In addition, increased microvascular permeability plays a role in the pathogenesis of kidney damage. In renal endothelial cells, the architecture of the actin cytoskeleton is broken down with the loss of adhesion connections from cell to cell and cell to the matrix, and endothelial cells are separated from the basement membrane, allowing KIM-1 to enter the circulation [8]. The resulting AUC for this study is significantly larger than the NAG of another study done by Kaufmann *et al.*, which found that the AUC for NAG (AUC: 0.80, 95% CI: 0.66-0.94, sensitivity: 79%, specificity: 82%) is significantly larger than creatinine (AUC: 0.839 *vs*. AUC: 0.752), while KIM-1 (AUC: 0.63) [31]. This difference was

mostly due to differences in the present study using serum samples compared with other studies using urine samples and differences in regional areas.

5. Conclusion:

Serum KIM-1 and NAG are highly specific and sensitive, making them promising biomarkers for the early detection of "acute kidney injury" in thalassemia patients before elevated urea and creatinine.

6. Recommendation:

A serial assay of serum KIM and NAG should be measured to evaluate the early assessment of "acute kidney injury" in thalassemia patients.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education, and scientific research ministries in Iraq.

Conflict of interest

The authors declare that they have no conflict of interest.

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