

Estimation of Serum Cystatin-C as early marker of Kidney Dysfunction in correlation with Serum Ferritin among β -Thalassemia Major Patients

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Abstract

The Cystatin C is early predictor for evaluation the kidney functions. The Serum Cystatin C is a cysteine proteinase inhibitor, that the nucleated cells produce it at stable rate. It is filtered through the glomerular filtration membranes of kidneys, and the filtration rate will be unaffected by external factors. A group of recessively inherited hemoglobin disorders called Major Beta-thalassemia and considered the most common genetic disorder all over the world, which could be noticed by the reduction in of β -globin chain synthesis.

Objective: To study Serum Cystatin-C estimation as early marker of renal function in relation with other parameters in beta thalassemia patients.

Materials and Method: A case-control study was executed on thalassemia patients between February and April 2019 at Al-Zahra'a Teaching Hospital in Najaf/Iraq.

Results: the results in this study showed there are significant differences ($p \leq 0.05$) between major beta thalassemia patients and control group in regarding to hematological parameters. The result of biochemical parameters showed there are variations between patients and control where there were significant statistical increases in our thalassemic patients comparing to the control about Cystatin-C (1.27 ± 0.33 and 0.7 ± 0.24 ng/ml respectively).

Conclusion: the cystatin -C is useful marker at major beta thalassemia patients that suffering from renal dysfunction.

Keyword: cystatin -C, Ferritin, liver enzymes.

Introduction

A group of recessively inherited hemoglobin disorders called Major Beta-thalassemia and considered the most common genetic disorder all over the world, which could be noticed by the reduction in of β -globin chain synthesis⁽¹⁾. Most of the types come from severe anemia that requires even transfusion of the blood. For fifth decades of life, the grouping of chelation treatment and transfusion has lengthened the

life of thalassemia cases^(2,3). The totally insufficient production of hemoglobin Hb A ($\alpha_2\beta_2$) in human adult is the Thalassemia disease, which is produced from the absence or decrease in the synthesis of alpha α - or beta β - globin chain and thalassemia is categorized into α and β thalassemia based on those chain disorders. Important medical indications of these patients are severe anemia, hemolysis, and ineffective erythropoiesis^(4,5). The autosomal receding genetic anemia is Beta-thalassemia major (β -TM) in which there is a lack in creating of 1 globulin chain resulting hemoglobin (Hb) molecule. Generally, in the first year of life, blood transfusion is needed for patients of beta thalassemia⁽⁶⁾. For instance, renal difficulties are one of the new unrecognized difficulties appeared with the progresses in chelating

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and treatment agents of β -TM patients ⁽⁷⁾. Numerous researches proved that common difficulties among β -TM patients are low urine osmolarity, aminoaciduria, proteinuria, dysfunction, and proximal tubular ^(8,9). All human nucleated cells can secrete and synthesize the Cystatin-C, which is a low-molecular-weight non-glycosylated protein, and has the ability to inhibit cysteine protease ⁽¹⁰⁾. Cystatin-C is not reabsorbed back into the serum or secreted by the renal tubules due to its sensitivity as a biomarker for glomerular filtration rate (GFR). It is better than creatinine clearance in the renal function impairment diagnosis, because muscle mass, diet, sex, and height could not affect it ⁽¹¹⁾. Cystatin-C is a cysteine protease inhibitor that is secreted into the blood after it is synthesized from all human cells. In comparing to the creatinine clearance, the benefit of measuring Cystatin-C is that height, sex, diet, and muscle mass could not affect it ⁽¹²⁾.

Materials and Method

A case-control study conducted between February to April 2019 in the Thalassemia Center at the Teaching Hospital of Al-Zahra'a in Najaf/Iraq. A group of 40 β -thalassemia major intermedia patients (10 females and 30 males) have been enrolled in this research, as well as 40 healthy control, ranged between 4 and 20 years old. Both control and patients were matched in gender and age. 5ml of blood was taken from beta-thalassemia patients as well as from healthy control for *biochemical tests*.

The patients are employed for measuring of the following parameters:

1. *Determination of Human CST3 (Cystatin C) concentration in serum by using ELISA kit performed by (Abcam, USA).*
2. *Determination of Creatinine concentration in serum using automated chemistry instrument from LANDWIND Company.*
3. *Determination of urea concentration in serum using automated chemistry instrument from LANDWIND Company.*
4. *Determination of ALP concentration in serum using automated chemistry instrument from LANDWIND Company.*

5. *Determination of AST concentration in serum using automated chemistry instrument from LANDWIND Company.*

6. *Determination of ALT concentration in serum using automated chemistry instrument from LANDWIND Company.*

7. *Determination of hematological parameters (complete blood count) by using automated hematological analyzer instrument from MINDRY Company.*

Statistical Analysis

Statistical Package for Social Sciences (SPSS) program version 24 has been used for data analysis. T-independent test was used to find the variances between patients and control, the results were expressed as (Mean \pm SD). Pearson correlation coefficient (r) were calculated to evaluate the correlation between parameters. A p-value of (<0.05) has been considered significant and (<0.001) has been considered highly significant.

Results and Discussion

Hematological Parameters of Study Groups

Our study included 40 beta thalassemia major patients their age ranging between 4-20 years (30 males and 10 females), and the control group included 40 healthy participants their ages and sex mated with patients group. There were statistically highly significant differences between beta thalassemia major patients and control as regarded with hematological parameters (CBC) included in this study, sever anemic presentation were seen in patients indicated by the levels of hemoglobin (7.7 ± 1.2) (13.8 ± 1.1) and RBCs count (3.83 ± 1.01) (5.22 ± 1.4) in comparison with the control. The hypochromic microcytosis observable in patients than control with MCH and MCV values (17.2 ± 2 fl) and (62.3 ± 6 pg) in patients compared to (30.7 ± 2.6 fl) and (84.7 ± 5.4 pg) in controls, with p-value 0.001 for both. In addition, a remarkable significantly increases in platelets count reported in patients (378 ± 80.3) as compared with control (246 ± 82.7) with p-value 0.001. Furthermore, a highly important leukocytosis also realized in patients than control (11.12 ± 1.18) and (7.04 ± 1.8) respectively (table 1).

Table 1. Hematological parameters of study groups

Parameters	Patients (n=40)	Control (n=40)	P-value
	Means \pm SD	Means \pm SD	
Hb g\dl	7.7 \pm 1.2	13.8 \pm 1.1	<0.001**
MCV(fl)	17.2 \pm 2	30.7 \pm 2.6	<0.001**
MCH (pg)	62.3 \pm 6	84.7 \pm 5.4	<0.001**
RBCX 1012/L	3.83 \pm 1.01	5.22 \pm 1.4	<0.001**
WBCX 109/L	11.12 \pm 1.18	7.04 \pm 1.8	<0.001**
PLT 109\ L	378 \pm 80.3	246 \pm 82.7	<0.001**

** : highly significant

The beta-thalassemic patients enrolled in the present study showed a significantly changed specially with red blood cell mass (RBC count and Hb) in association with RBCs indices (MCV and MCH) attendant to a significantly observable microcytosis, the plain anemic appearances have been seen in the patients where hemoglobin levels as comparing with the controls. The RBC related differences are also related to an important secondary leukocytosis and thrombocytosis, these results agrees with Hagag et al. ⁽¹³⁾, and Ayyash & Sirdah ⁽¹⁴⁾. Arshad et al. ⁽¹⁵⁾, described that thalassemic patients have different problems related with less Hb level, because of the reduction of erythrocyte numbers and lessened values of RBC indices (MCV, MCH, MCHC, HCT). So, those patients undergo anemia resultant in less oxygen content in blood. The study carried out by Shanthi et al. ⁽¹⁶⁾, concluded that the decreasing in the RBCs count, PCV and levels of Hb, that detected in beta thalassemia patients are because of continuous breakdown of erythrocyte and increased early degradation, as the presence of abnormal globin molecule leads to erythrocytes ruptured before maturation. Regarding to leukocytosis and thrombocytosis in our thalassemia patients due to the severe anemia that complemented by hyper cellular (thrombocytosis and leukocytosis) that causing encouragement of erythropoietin hormone which acts on bone marrow to increase proliferation of

blood cells, or resulting from the activation of immune system by receiving blood from various donors ⁽¹⁷⁾.

Biochemical Parameters of Study Groups

The result of biochemical parameters showed a variation in the serum level (Cystatin-C, ferritin, creatinine, urea and liver enzymes includes AST, ALT and ALP) in beta thalassemia major patients as compare with control. When compared to control, there were statistically significantly increase in the our thalassemic patients regarding Cystatin-C (mean=1.27 \pm 0.33 and 0.7 \pm 0.24 ng\ml respectively), Ferritin (mean=3480 \pm 232 and 90.6 \pm 43.5ng\ml respectively), Creatinin (mean=1.02 \pm 0.4 and 0.5 \pm 0.16 mg\dl respectively), Urea (mean=25.9 \pm 4.8 and 17.4 \pm 3.9mg\dl respectively),AST (mean=67.47 \pm 26.3 and 23.14 \pm 8.1U\l respectively), ALT (mean= 75.79 \pm 28.6 and 21.49 \pm 8.81 U\l respectively), and ALP (mean=289.78 \pm 113.43 and 97 \pm 52.12 U\l respectively) table (2). There was statistically positive correlation between creatinine and cystatin (fig.1) (r=0.569) and urea (fig.2) (r=0.137), the result agrees with Behairy et al. ⁽⁶⁾. In accordance with our results, Elbedewy et al., concluded that beta thalassemia major patients suffer from glomerular and tubular dysfunction due to poor chelation therapy and inadequate transfusion ⁽¹²⁾.

Table 2: Biochemical parameters of study groups.

Parameters	Patients (n=40)	Control (n=40)	P-value
	Means ± SD	Means ± SD	
Cystatin C (ng/mL)	1.27±0.33	0.7±0.24	<0.001**
Ferritin ng/ml	3480± 232	90.6± 43.5	<0.001**
Creatinine (mg/dl)	1.02±0.4	0.5±0.16	<0.001**
Urea (mg/dl)	25.9±4.8	17.4±3.9	<0.001**
AST U/L	67.47±26.3	23.14±8.1	<0.001**
ALT U/L	75.79±28.6	21.49±8.81	<0.001**
ALP U/L	289.78±113.43	97±52.12	<0.001**

** : highly significant

The DFO, iron overload, chronic hypoxia, and long-standing anemia implicated in the mechanisms of tubulopathy in patients with β-thalassemia major (6). In the current study, there were highly significance increases in the levels of urea, creatinine, ferritin, cystatin shown by thalassemic patients. These results were in agreement with Ali and Mohmoud, as they reported considerably higher levels of serum ferritin and serum creatinine in thalassemic group than controls (18). Serum Cystatin-C is supposed to be a more potent endogenous marker of GFR than creatinine as it is believed to be produced at a constant level by all nucleated cells, generously

filtered by the glomeruli, minimally bound to proteins, and completely reabsorbed and metabolized in the proximal tubule (12). Our result show increase in serum level cystatin when compared with control. Some study found a positive association between serum ferritin and serum cystatin Papassotiriou et al. (19). Serum ferritin in our result at high levels this indicate to iron overload and many studies concluded that cirrhosis of liver is associated with increase in serum ferritin levels (20), this accordance with the result of our study that show highly significant increases in the liver enzymes (AST, ALT and ALP) when compared with control group.

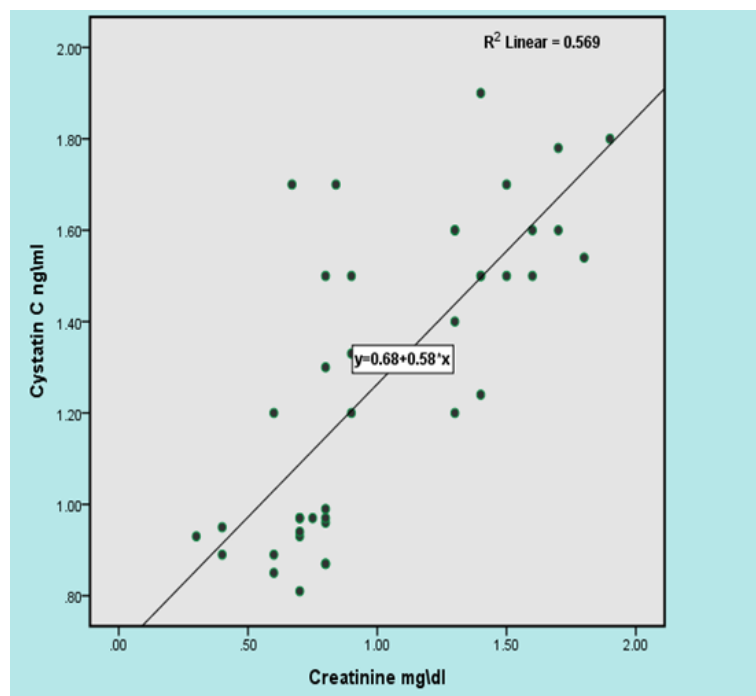


Figure 1: Correlation between Cystatin-C and creatinine in beta thalassemia patients.

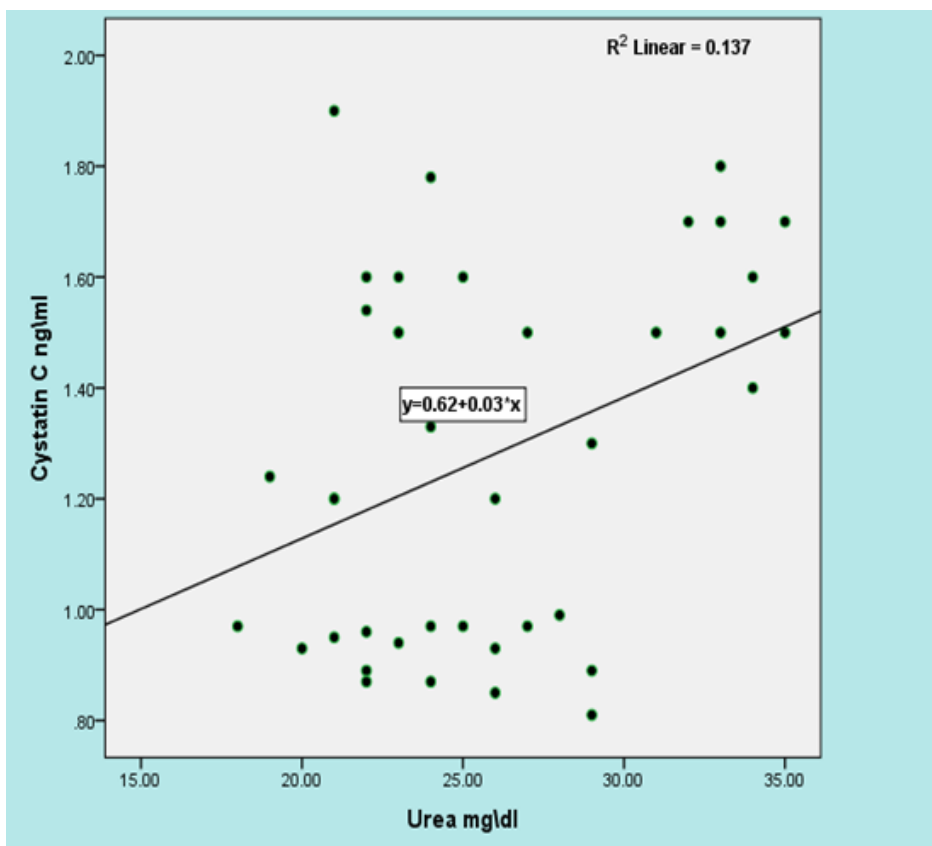


Figure 2: Correlation between Cystatin-C and Urea in beta thalassemia patients

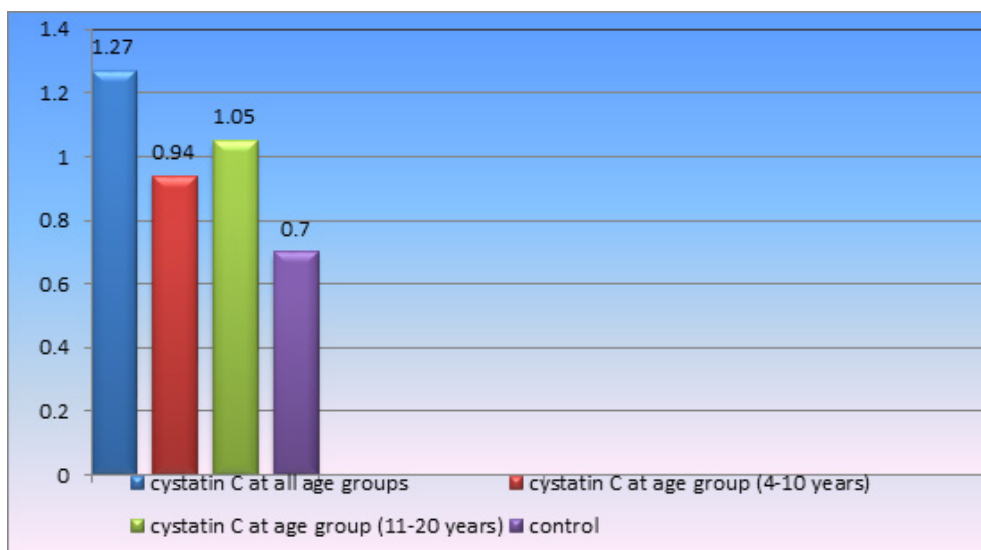


Figure 3: The Cystatin –C concentration distribution at age groups.

Conclusion

In this paper, we concluded that Cystatin C could be used as early predictor for major beta-thalassemia patients, which can be developed for renal dysfunction in the future.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of

both environmental and health and 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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