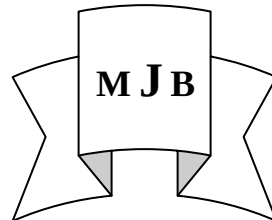


An Evaluation Study of the Renal System in Patients with Beta Thalassemia

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Abstract

Beta-thalassemia is a hemolytic anemia of genetic origin, it results from a decreased or absent synthesis of beta globin chains that normally constitute a part of the adult hemoglobin (HbA, which is $\alpha_2\beta_2$). This genetic defect will lead to a compensatory ineffective erythropoiesis, severe anemia, and increased erythrocytes turnover. Thalassemia syndrome is usually accompanied by a wide spectrum of complications arising from the disease itself as well as the methods used to alleviate it.

This study aims to investigate the presence of renal dysfunctions in children and adolescent with transfusion dependent (TD) β T, without any overt renal diseases, using both conventional and early markers of glomerular and tubular dysfunctions this will help the medical staff for proper management with less morbidity and mortality.

This study were lasted from (February till September/ 2011) conducted in the Hereditary Bleeding Disorders Center in Hilla city, on (Eighty one) β -thalassemic patients who enrolled in this work and control group include (twenty) apparently healthy subjects. Patients in this study were divided in to three groups according to the age : group I was (3-10) years, group II was (11-18) years and group III was (19-25) years and healthy subjects with an age range (3-25) years.

Result shows significant increase of serum creatinine, serum urea and serum uric acid in patient groups compared with control group ($p < 0.05$). However, significant increase was observed in group III than those of group I as comparison between the patient groups ($p < 0.05$).

The present study found that urinary NAG were highly significant increase of NAG in urine samples of Patients groups in comparison with control group at ($p < 0.01$), this increment will be concomitantly with aging of age group ($p < 0.05$). As it appear as early detection markers of tubular damage.

Lastly hematuria in all of patient studied groups were 22 (27.5%), pyurea were 28 (34%) and protein urea were 20 (24.69%) demonstrated that in patients with beta thalassemia, hematuria is more common in older age groups. Furthermore, protein urea and pyurea seems to be a common accompanying finding in patients with beta-thalassemia.

The changes in data that showed in this study with β -thalassemia patients may refer to a multiple renal abnormalities with progression of the age and that's may be due to multiple blood transfusion, iron overload or chelation therapy.

الخلاصة

مرض التلاسيميا نوع بيتا هو فقر دم تحلي من أصل وراثي، و ينتج من نقص أو انعدام تكوين سلاسل الكلوبين نوع بيتا و التي تكون جزء من هيموغلوبين الإنسان البالغ الطبيعي مما يؤدي إلى بناء كريات دم حمراء تعويضي غير مؤثر، فقر دم شديد، زيادة أستيقلاب كريات الدم الحمراء، زيادة امتصاص الحديد. يكون مرض التلاسيميا عادة مصحوب بطيف واسع من التعقيدات الناجمة من المرض ذاته و كذلك الطرق المستخدمة لعلاجها.

تهدف هذه الدراسة : معرفة وتحديد ما إذا كان مريض التلاسيميا لديه اعتلال في الجهاز البولي ، ومن ثم إجراء الفحوصات المختبرية اللازمة والتي من خلالها نبين أسباب ذلك إن كانت لخلل في الجهاز البولي كمضاعفات للمرض الأساسي أو لأسباب أخرى ، وبعد ذلك

تقديم الاقتراحات للكادر الطبي المختص في مركز أمراض الدم الوراثية / مستشفى بابل للولادة والأطفال عن الإجراءات الممكنة للتقليل من مضاعفات المرض .

الدراسة سُجِّلَتْ في هذه الدراسة مِنْ (فبراير/شباط حتى سبتمبر/أيلول / ٢٠١١) المرضى المشاركون في هذه الدراسة تم أخذهم من مركز أمراض الدم الوراثي في مستشفى بابل للنسائية و الأطفال، بعدد كلي قدره (٨١) التلاميذ ليكونوا فريق المقارنة الثاني إلى جانب الفريق الأول و(٢٠)المكون من أشخاص أصحاء. المرضى الذين سَجَّلُوا في هذه الدراسة تم تقسيمهم الى مجاميع حسب الأعمار: المجموعه الأولى كانت (٣-١٠) سَنَة، المجموعه الثانية (١١-١٨) سَنَة و المجموعه الثالثة كَانَتْ (١٩-٢٥) سَنَة، والأشخاص أصحاء كانوا بمدى عُمُر (٣-٢٥) سنوات.تمت مقارنتها بالأشخاص الأصحاء أولاً ، إضافة الى مقارنات تم عملها بين مجاميع الدراسة نفسها. تم أخذ عينة دم (٥مل) من كافة المشاركين للحصول على المصل المستخدم في حساب المقاييس المذكورة.

أظهرت النتائج في مقارنة المرضى مع الأصحاء تم ملاحظة فرق معنوي في مستوى (واليورينا بالدم ، والكرياتين ، وحامض اليوريك) و كذلك وجد تغيرات معنوية بين المجاميع المرضية أنفسهم حسب العمر .

اما بالنسبة الى فحص البول فقد اظهرت النتائج ،زياده في وجود انزيم (NAG) في البول خصوصا في الأعمار المتأخرة، علما وجود انزيم (NAG) بنسب كبيره في البول يدل على وجود أصابه في ألأنابيب الكلويه كما أثبتت الدراسات السابقة. وايضا وجود (Hematuria)بنسب عاليه في الأعمارالكبيره بإضافة الى ذلك فقد وجود الاليومين بنسب كبيره في البول خصوصا في الأعمار المتأخرة ، أظهرت النتائج وجود(WBC) بنسب عاليه في البول في الأعمار الكبيرة.

الاستنتاجات والتوصيات،كل التغيرات التي اظهرت في النتائج تدل على إصابة الكلية خصوصا في الأعمار المتأخرة من المرض ويعزى ذلك إما إلى المرض نفسه او عمليات نقل الدم المتكررة،بالتالي ترسب الحديد او العلاج المستخدم.كما يوصى بأجراء الأختبارات ألأزمه للكشف المبكر عن إصابة الكلية للتقليل من مضاعفات المرض.

Introduction

Thalassemia syndromes were among the first genetic diseases to be understood at the molecular level [1], it present to be affect about 1.5%, i.e. 200 million peoples of the world population are carriers of β -thalassemia gene [2].The term "thalassemia" refers to a group of blood diseases characterized by decreased synthesis of one of the two types of polypeptide chains (α or β) that form the normal adult human hemoglobin molecule (HbA, $\alpha_2\beta_2$), resulting in decreased filling of the red cells with hemoglobin, and anemia [3]. The result is a chronic hemolytic anemia from the first year of life that, in most homozygous cases is quite severe and fatal unless repeated blood transfusions are commenced early [4].

A little information is available about renal involvement in this disease. In the recent years, few studies have demonstrated proteinuria, and excess

urinary secretion of markers of tubular damage such as N-acetyl beta -D-glucosaminidase (NAG) in patients with beta-thalassemia [5]. NAG is a proximal renal tubular protein that is excreted in the urine during tubular damage ,glomerular damage can also occur in these patients due to recurrent infections and the repeated use of deferoxamine(desferal), resulting in a decreased ability of the kidneys to clear immune complexes [6].

Aim of Study

The fallowing study were aimed to:-

- 1- Investigate the presence of glomerular and/or tubular dysfunctions in children and adolescent with transfusion dependent (TD), without any overt renal diseases, using both conventional and early markers of glomerular and tubular dysfunctions.
- 2- The determination of biochemical indices of renal function might help

prevention of serious kidney damage before any clinical symptom is observed.

Materials and Methods

Patients and Controls

The study was conducted in the Hereditary Bleeding Disorders Center in Babylon maternity and pediatric teaching hospital of Hilla city, from Eighty one β -thalassemia patients were enrolled in this study from (February till September/ 2011). Total of patients with thalassemia were (81) included in this study whose age ranged (3-25) years, the mean age = (14.17633) years. These patients divided in to three groups according to age group I were (3-10) year, with mean $\pm$ SD (6.52 $\pm$ 1.417) year's group II were (11-18) years with mean $\pm$ SD (14.08 $\pm$ 2.399) year and group III were (19-25) year, with mean $\pm$ SD (21.95 $\pm$ 2.400) year. Control group include twenty apparently healthy subjects with an age range (3-25) years; and mean $\pm$ SD was (13.20 $\pm$ 6.005) years

Methods

Determination serum creatinine:-

procedure recommended by the company Randox, United Kingdom by using spectrophotometer and read at 520 nm [7].

Determination serum uric acid:

According to procedure recommended by the uric acid kit from Biolabo, France [8] by using spectrophotometer and read at 580nm. **Determination serum urea:** Procedure recommended by the urea kit from the Biomerieux Company, France by using spectrophotometer and read at 580 nm, [9].

Microscopic Urinalysis:-Microscopic examination of the urine sediment generally follows macroscopic urinalysis [10]. **Urine total protein and albumin:** Total protein is measured in urine by using turbid metric method was

measured photo metrically by using spectrophotometer at 600 nm wave length as well as urine albumin is measured by the same steps [7].

N-aceyl-B-D-Glucosaminidase (NAG) Assay in urine:-The N-aceyl-Beta-D glucosaminidase (NAG) assay kit is for determination of NAG in patient urine samples. The assay is for investigation use or expert only [11].

The data were analyzed by using of computer SPSS statistics 17program. Analysis Of Variance (ANOVA) test was used to examine the differences between different groups [12].

Result

A-Serum creatinine, Blood urea and

Serum uric acid:-

1-Serum creatinine:-

The mean of serum creatinine that shown in table (1), the mean $\pm$ SD of control group was (45.95 $\pm$ 2.395 mmol/L), while in patient groups were (46.03 $\pm$ 3.836 mmol/L) in group I, (46.88 $\pm$ 3.090 mmol/L) in group II and (48.23 $\pm$ 4.800 mmol/L) in group III respectively . In Comparison of the serum creatinine of the patient groups with the healthy controls show significant increase between patient group III and control group at (p < 0.05) on other hand, patient groups show no significant difference with the control group at (p> 0.05). A comparison within patient groups show significant increase between group III and group I at (p < 0.05) with no significant difference between group II and group III at (p> 0.05).

2- Serum Urea:-Mean of serum urea that show in table (1)these result project the mean $\pm$ SD of control group was (4.31 $\pm$ 0.821 mmol/L), while in patient groups were projected as (4.82 $\pm$ 0.992 mmol/L) of group I, (5.01 $\pm$ 1.575

mmol/L) of group II and (5.62±0.665 mmol/L) of group III.

In Comparison of the serum urea of patients groups with the healthy controls show highly significant increase between patient group III and control group at ($p < 0.01$). Also, significant increase were present between patient group II and control group at ($p < 0.05$), while, in patient group I comparison no significant than control group at ($p > 0.05$). Patients within groups comparison show high significant increase between group III and group I at ($p < 0.01$), and neither no significant difference between group II and group III, and no significant difference between group I and group II at ($p > 0.05$).

Table 1 Changes in serum creatinine serum, urea and serum uric acid for β -thalassemic patient and control

Parameters	Serum Creatinine(μ mol/l)			
	Group I	Group II	Group III	Control
Group I(46.03±3.836)	—	N.S	*	N.S(45.95±2.39)
Group II(46.88±3.090)	N.S	—	N.S	N.S
Group III(48.23±4.800)	*	N.S	—	*
M±SD Serum Urea (mmol/l)				
Group I (4.82±0.992)	—	N.S	**	N.S(4.31±0.821)
Group II(5.01±1.575)	N.S	—	N.S	*
Group III(5.62±0.665)	**	N.S	—	**
M±SD Serum uric acid (umol/L)				
Group I (281.21±75.579)	—	N.S	*	N.S(263.90±5.319)
Group II(303.92±80.243)	N.S	—	N.S	N.S
Group III(324.23±71.697)	*	N.S	—	*
* Significant differences at ($p < 0.05$).				
** Significant differences at ($p < 0.01$).				
N.S Non- Significant differences at ($p > 0.05$).				

3- Serum uric acid:-Gained data of serum uric acid in table (1) show mean±SD of serum uric acid of control group was (263.90±5.319 μ mol/L) While, in group I was (281.21±75.579 μ mol/L), group II was (303.92±80.243 μ mol/L) and group III was (324.23±71.697 μ mol/L). Comparison about these levels of serum uric acid show significant increase in patient group III with control group at ($p < 0.05$) only. While, no significant difference between group I and group II, and between group II and group III at ($p > 0.05$), as well as significant increase well established between group I and group III at ($p < 0.05$).

B- Result of N-aceyle-B-D-Glucosaminidase (NAG) in urine:-

The result in table (2) show mean $\pm$ SD of (NAG) in urine of control group was (7.882 $\pm$ 3.331 IU), while patients groups were (9.529 $\pm$ 4.534IU), (12.757 $\pm$ 4.593 IU) and (17.488 $\pm$ 4.711 IU) in group I, group II and group III respectively. Result comparison of the level of NAG in urine between the patient groups and the healthy control

group that revealed a high significant increase in both of group II and group III compared with control group at ($p < 0.01$), while, in group I and control subject study show no significant deference at ($p > 0.05$). Meanwhile, a comparison Within patient groups show highly significant deference between all these patient groups according to the age at ($p < 0.01$).

Table 2 Changes in Urinary NAG of β -thalassemic patient and control:-

Parameters	M $\pm$ SD Urinary NAG (IU)			
	Group I	Group II	Group III	Control group (7.882 $\pm$ 3.331)
Group I(9.529 $\pm$ 4.534)	—	**	**	N.S
Group II(12.757 $\pm$ 4.593)	**	—	**	**
Group III(17.488 $\pm$ 4.711)	**	**	—	**
* Significant differences at ($p < 0.05$).				
** Significant differences at ($p < 0.01$).				
N.S non-significant differences at ($p > 0.05$)				

C-Result of Microscopic Urinalysis:-

1- **Result of hematuria:-**From the of all thalassemic studied patients (81) only 22 (27.5%) appeared to show hematuria as demonstrated in table (8)and figure(1).However, those 22 patients were divided in to N=10 (45.4%) of patient group III , N= 7(31.8%) of patient group II and N= 5 (22.7%)of patient group I. in table(3) show group I was the lowest percentage from other patient groups.

2- **Result of pyuria:-** result of present of pus in urine (pyurea) show about 28 (34%) of all β -thalassemic patients. The presences of pus cell in the urine show in

table (3) and figure (2) were divided as; N=13(46.4%) in group patient III, (N=11(39.2%)in group patient II and finally N=4(14.2%) as the lower number and percent in group I comparable than of the other two groups.

3- **Result of proteinuria:-**Twenty patients (24.69%) out of eighty one patients of β -thalassemia were present to show protein urea as in figure (3).And were divided as[N=9(45%)] in group III, [(N= 7(35%)]in group II and[N= 4(20%)] in group I of the whole β -thalassemic studied patients as show table(3) .

4- Results of cast & crystals in urine:-In table(3) show number of the cast and the crystals in urine and show the percentage of the all patient groups .Result revealed those patients have cast in urine about (26),whom categorized according to age groups as; [N=7(26.9%)] in group I,

[N=8(30.7%)]in group II and [N=11(42.4%)]. While, about (34) patient have crystals in urine whom categorized according to age groups as; [N=15 (44.1%)]in group I, [N=8(23.5%)] in group II and [N=11(32.3%)].

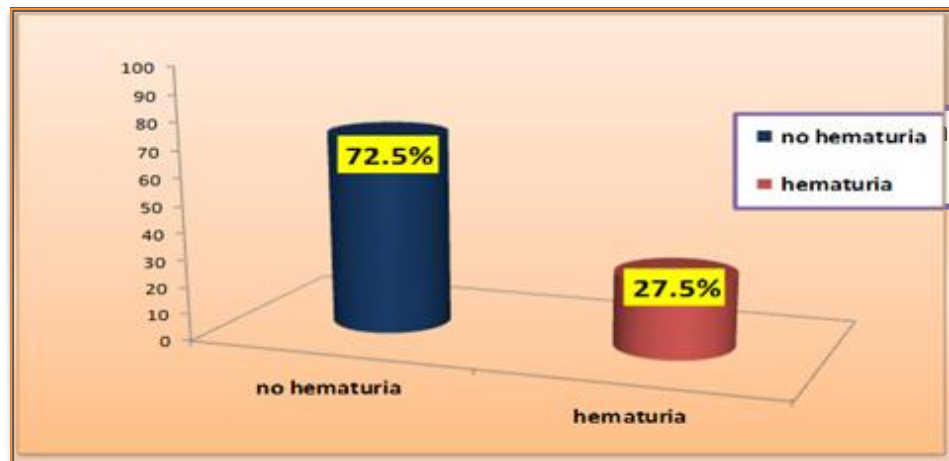


Figure 1 percentage of hematuria and no hematuria in all β -thalassemic patients group

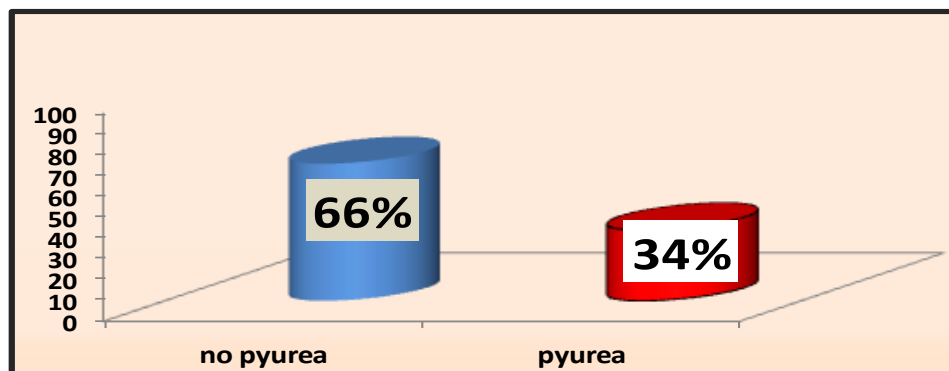


Figure 2 Percentage of pyuria and no pyuria in all β -thalassemic patients group

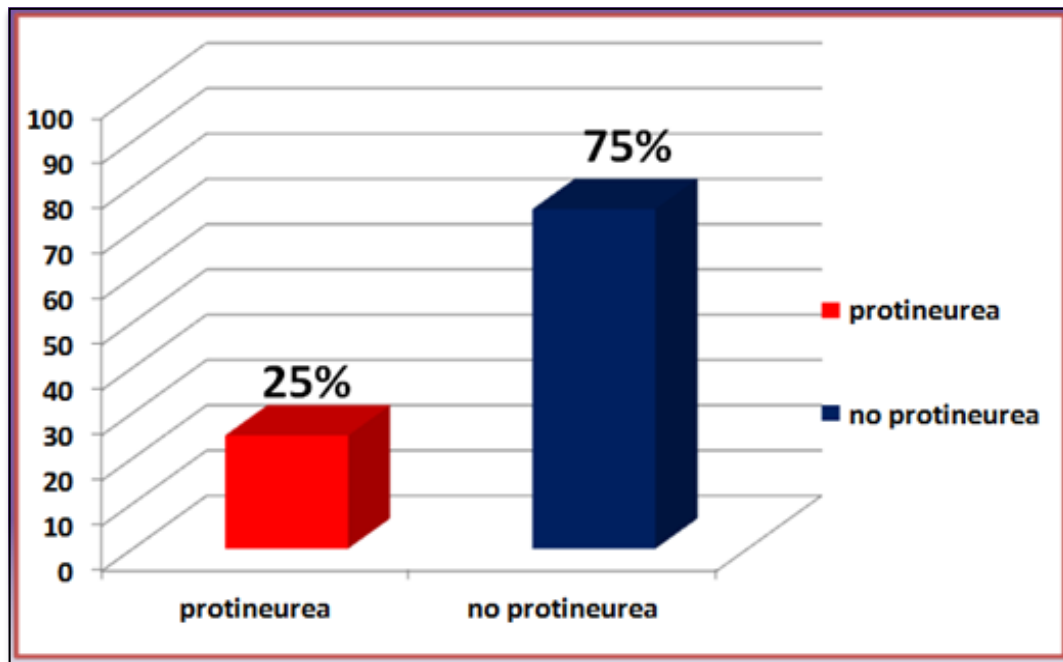


Figure 3 Percentage of proteinuria and no proteinuria in all β -thalassemic patients groups.

Table 3 Results of number and percentage of hematuria, pyuria, Proteinuria, cast and crystal in all β -thalassemic patient groups

Patient Group(year)	Hematuria	pyuria	Proteinuria	cast	crystal
Group I (3-10)	5 (22.7%)	4(14.2%)	4(20%)	7(26.9%)	11(32.4%)
Group II (11-18)	7 (31.8%)	11(39.4)	7(35%)	8(30.7%)	8(23.5%)
Group III (19-25)	10(45.5%)	13(46.4)	9(45%)	11(42.4%)	15(44.1%)
Total	22 (100%)	28(100%)	20(100%)	26(100%)	34(100%)

Discussion

1. Serum creatinine, uric acid and urea Mean serum level of Cr of patients were significantly higher than controls and found a significant change between patient groups according to age, similarly to [13] who found a

positive correlation between age and Cr.

Shortened red cell lifespan and excess iron causes functional and physiological abnormalities in various organ systems, in β -thalassemia patients have a high prevalence of renal tubular abnormalities [6]. The

severity correlated with the degree of anaemia, being least severe in patients on hypertransfusion and iron chelation therapy, suggesting that the damage might be caused by the anaemia and increased oxidation induced by excess iron deposits [14].

In other report in patient with and without chelation, diminished GFR, elevated serum creatinine and tubular dysfunctions and elevated serum uric acid these high frequency of glomerular and tubular dysfunctions in β -thalassemic patients which could be attributed to oxidative stress and DFO therapy [15]. The increased serum and excretory levels of UA can be explained by rapid erythrocyte turnover in combination with decreased reabsorption of filtered UA from damaged renal tubules [16]. Elevated of serum urea may be due to renal tubular acidosis had also been reported in patients with thalassemia in previous studied [15].

Severely affected patients are treated by blood transfusion every 3-4 weeks, which results in iron overload in various tissue including the liver, heart and endocrine tissue. The kidneys are another site of iron accumulation in thalassemia. Unlike in the other organs, it is unclear whether kidney affection results solely from intravascular hemolysis, chronic transfusion or as a complication of iron chelation therapy [17]. In β -thalassemia, impaired biosynthesis of β -globin leads to accumulation of unpaired α -globin chain. Iron overload, usually observed, generates oxygen-free radicals and peroxidative tissue injury [18].

Although severe anemia and chronic hypoxia are believed to play a role in renal involvement in β -thalassemia, lipid peroxidation is currently the most favored hypothesis,

according to this hypothesis, the imbalance in synthesis of hemoglobin (Hb) leads to excess unpaired globin chain and high intracellular content of non-Hb iron. The unstable (Hb) subunits are known to generate free oxygen radical species that starting a chain of oxidative events which leading to disintegration of denatured globin chains, hem, and iron, which bind to different membrane proteins and altering their normal structure and functions, in addition, the excess free iron is known to be a catalyst of lipid peroxidation via the Fenton reaction [14]. Fenton reaction are antioxidant reserve productions of super oxide radical in some diseases leads to release of iron from ferritin, further more ferritin iron can be released either by a reductive process. These free iron catalyst subsequent reaction lead to production of hydroxyl radicals (OH) [19].

Renal toxicity: In animal studies deferasirox has been shown to cause renal tubular epithelial cell damage. It is recommended that serum creatinine be hacked in duplicate prior to initiating therapy with deferasirox, and then monthly. A non-progressive increase in serum creatinine has been noted in 33 % of patients on deferasirox, in those with well controlled iron stores, a chance of renal toxicity is more as their liver iron concentration is less. The drug should be withdrawn if there is a progressive increase in serum creatinine [20]. Increase of serum Cr, blood urea and serum UA in patient with β -thalassemia well agreed with data reported by [21] and [6].

2. N-aceyl-B-D-Glucosaminidase (NAG) in urine:-

The present study revealed that excretion of urinary NAG was markedly higher in the group of patients than in the control group while, within patient groups the results show significant difference according to age, this finding will appear to be a close relation with another study showed significant relationship between urinary NAG and the age of the patient that may be due to duration of deferoxamine therapy or duration of receiving blood transfusions [6].

N-acetyl beta-D-glucosaminidase is a widely distributed lysosomal enzyme contained in the tubular epithelial cells and released in the urine as a result of tubule toxicity of proteinuria in the early stages of idiopathic membranous nephropathy, glomerular hypertrophy, focal segmental glomerulosclerosis and minimal change disease, Since NAG is not of plasmatic origin and is not filtered through the glomeruli, the increase of urinary NAG in urine is due to tubular dysfunction and considered to be a sensitive and reliable index of proximal tubular toxicity and a possible predictor of proteinuria [22]. Another study demonstrating proteinuria, aminoaciduria, low urine osmolality, and excess secretion of the proximal tubule damage markers, such as N-acetyl-beta-D-glucosaminidase (NAG) activity in such patients [16].

On the other hand, deferoxamine (DFO) therapy has been proved to be nephrotoxic and induce dose-dependent proximal tubular dysfunction by an unknown mechanism [15]. Split of NAG in patient excretion can be considered to be indicator for the tubular toxification and a may regard as predictor for proteinuria, aminoaciduria and eventual renal impairment of these

patients [6]. Hence, urinary excretion of NAG was sensitive markers of proximal tubular damage (23). The result of this study of urinary NAG found to be agreement with other studies such as [24, 25, 6, 23 and 15].

3. Hematuria, pyurea and protein urea:-

The investigated results of this work showed that the prevalence of hematuria in patients with beta-thalassemia younger than 10 years.

Cetin (2003) reported that 14.6% of patients with β -thalassemia showed significant signs of renal tubulopathy, such as decreased tubular reabsorption and tubular proteinuria [26]. In other report from Iran on beta-thalassemic patients for detection of early kidney dysfunction that shown prevalence of proteinuria and hematuria [6]. All the thalassemic patients had evidence of tubular damage that directly correlated with the amount of transfused iron [27]. Subclinical tubular dysfunction due to iron overload in children and adults and symptomatic hyperchloremic metabolic acidosis in an adult patient secondary to deferasirox [28].

Albuminuria in could be attributed mainly to destruction of glomerular filtration membrane which proven by decreased in GFR as well as tubulopathy [15]. Glomerular diseases may also develop; immunoglobulin A nephropathy was reported in a patient with thalassemia. Mohkam(2008) found protein urea in 89.3% of β T patients[6]. Another report found in these study large number of patients on DFO therapy showed impaired GFR(58.82%), microalbuminurea (47.10%) and proteinurea (47.10) [15].

Presence of pus in urine or termed as (pyuria), as a result as, high susceptibility to Urinary Tract Infections (UTI) [29] and high number of Bacterial isolates were found in urine which mean that urine of thalassemic patients were more favorable for bacterial growth may be due to that the observed immune disorder represents mostly a secondary immune system defect and primary problem [30]. Whole presenting findings above were generally agreement with another reports republished by [31, 15 and 32].

3. Casts and crystals

The present study found casts in urine in some patient, with β -thalassemia. Casts are formed in the renal tubule as a consequence of the precipitation of Tamm Horsfall protein. Tamm Horsfall protein, which is the most abundant protein in normal urine, is a glycoprotein excreted by the renal tubule, although hyaline and granular casts may not always indicate pathology, but other casts are abnormal and suggest renal disease [33]. Meantime, present study in a concomitant with cast existence urine analysis showed crystals in urine in some patient of β -thalassemia, large numbers of calcium oxalate crystals found in the urine often are an important clue to the early clinical diagnosis of this toxicity [33].

Conclusion

- There is an elevated serum creatinine , blood urea and serum uric acid as a result of glomerular and tubular dysfunctions in β -thalassemic patients which could be attributed to oxidative stress and DFO therapy.
- The study found urinary NAG excretion in urine and it as a reliable

index of the tubular damage in β -thalassemic patients.

- The study demonstrated that, hematuria is more common in older age groups of β -thalassaemic patients. Furthermore, proteinuria seems to be common accompanying finding in patients with beta-thalassemia.
- The study conclude the presence of proteinuria, hematuria and increase of urine NAG can be explained by renal tubule damage, while hematuria with abnormal RBC, mass proteinuria and the increase of serum creatinene may be due to the involvement of renal glomerulus.

Recommendations

- 1- A preventive program were be needed to include family counseling and implemented as a part of prevention of the disease in high risk families.
- 3- Super-transfusion with neo-cytes (young red cells), might be effective in decreasing the rate of iron accumulation in homozygous beta-thalassemia.
- 4-As renal dysfunction may not be detected by routine tests, use of early markers is recommended. Urinary NAG excretion can be considered a reliable index of the tubular toxicity and a possible predictor of proteinuria proteinuria and eventual renal impairment in these patients.

References

- 1-Melody, J. and Cunningham, M.D.(2008).Update on Thalassemia: Clinical Care and Complications PediatrClin N Am 55 447–460.
- 2-Prabhu, R.; Prabhu, V. and Prabhu, R.S.(2009).Iron overload in β -thalassemia-a review.J. Bioac. Tech.;1(1):p20-31.
- 3-Capellini, M.; Elftheriou, A.C.; Piga, A.; Porter, J. and Taher, A. (2008).Guicelies for the clinical management of thalassemia,2nd revised edition,Team up

- creations limited,Cyprus:p20-136.
- 4-Lukens, J.N.(1999).The thalassemia's and related disorders, in: Quantitative disorders of hemoglobin synthesis,10th edition ,Lippincotts Williams and Wilkins:p1405-1448.
- 5-Sadeghi, B.S.; Hashemi, M. and Karimi, M.(2008).Renal tubular function in patients with beta thalassemia major in Zahedan, southeast Iran. Singapore Med J; 49:410-12.
- 6-Mohkam, M.; Shamsian, B.S.; Gharib, A.; Nariman, S. and Arzanian, M.T. (2008).Early markers of renal dysfunction in patients with beta-thalassemia major.Pediatr Nephrol.; 23:971-6.18).
- 7-Burits, C.A. and Ashwood, E.R.(1999).Tietiz textbook of clinical Chemistry .4th .ed. W.B., Saunders Comp. USA, 2 (1500-1503).
- 8-Fossatl, P.; Prencipe, L. and Bertl, G.(1995).Use of 3.5- dichloro-2-Hydroxybenzene sulfonic acid / 4 Amino phenazone chromogenic system in direct enzymatic assays of uric acid in serum and urine .Clin . chem. 26:227-231.
- 9- Chevillon, I.; Larrose , C. and Moreau, N . (1998).conservation desechantllons de sang avantanalyse des parameters biochimiquesles plus courants. Ann .boil .clin (paris) .vol .56 .p. 200-240.
- 10- Haber, M.H.(1988).Quality assurance in urinalysis.Clin Lab Med;8:431.
- 11- Price, R.G.; Whiting, P.H.; Jung, K. Mattheimer, and Burchardt, H. (1992).Urinary Enzymes, Springer-Verlag, BarlinEds:, 203-221.
- 12-Daniel, W.(1999). Biostatistics: A foundation for Analysis in the Health Sciences. 7th.edition. John Wiley. Philadelphia, p 83.
- 13-Helin, I.; Axenram, M. and Grubb, A.(1998).Serum cystatin C as a determinant of glomerular filtration rate in children. ClinNephrol, 49(4):221-225.
- 14-Sumboonnanonda, A.; Malasit, P.; Tanphaichitr, V.S.; Ong-ajyooth, S.; Petrarat, S. and Vongjirad, A.; (2003), Renal tubular dysfunction in alphathalassemia.PediatrNephrol 18:257–260
- 15-Hamed, E.A. and ElMelegy, N.T.(2010). Research l functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study Italian Journal of Pediatrics, 36:39
- 16-Aldudak, B.; Karabay, P.; Bayazit, A.; Noyan, A.; Ozel, A.; Anarat, A.; Sasmaz, I.; Kilin, C.Y.; Gali, E.; Anarat, R. and Dikmen, N.(2000).Renal function in pediatric patients with β thalassemia major.PediatrNephrol 15:109-112.
- 17-Traez, M.J.; Alam, J. and Nath, K.A.(2007).Physiology and pathophysiology of heme, implication for kidney disease. J Am SocNephrol: 18; p414.
- 18-Kassab, C.A.; Laradi, S. and Ferchichi, S. (2003).Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia.Clin ChimActa; 338:79-86.
- 19-Salma, A.R.A. and Iqbal, H.D.(2011). Some Biochemical Parameters In Iraqi Patients With Thalassemia And Related With Dm1 Int. J. Chem. Res., v01(5), 46-56 ISSN: 2249-032
- 20-Herschko, C.; Konjin, A.M. and Nick, H.P. (2001).A new potent, oral chelator:Evaluation in hypertransfused rats with selective radio-iron probes of hepatoellular and reticulo-endothelial iron stores and iniron-loaded rat heart cells in culture. Blood ICL 670; 97:1115-1122.)
- 21-Jafari, H.M; Vahidshahi, K.; Kosaryan, M.; Karami, H.; Reza, M.M. and Ehteshami, G.(2011).Major β – thalassemia, use of desferiexamine and renal proximal tubular damage IBratissLekListy; 112 (5)p278-281.
- 22-Voskaridou, E.; Terpos, E.; Michail, S.;

- Hantzi, E.; Anagnostopoulos, A.; Margeli, A.; Simirloglou, D.; Loukopoulos, D. and Papassotiriou, I.(2006).Early markers of renal dysfunction in patients with sickle cell/ beta-thalassemia. *Kidney Int* 69:2037–2042
- 23-Economou, M.; Printza, N.; Teli, A.; Tzimouli, V.; Tsatra, I.; Papachristou, F. and Athanassiou, M.M. (2010).Renal Dysfunction in Patients with Beta-Thalassemia Major Receiving Iron Chelation Therapy either withDeferoxamine and Deferiprone or with Deferasirox. *ActaHaematol*, 123(3):148-152.
- 24-Cunningham, M.J.; Macklin, E.A.; Neufeld, E.J.; Cohen, A.R.(2004).Complication Complications. *Pediatric Clinics of North America*
- 25-Kearney, S.L.; Nemath, E. and Neufeld, E.J.(2007).Urinaruhipcidine in Congenital chronic anemia .*Pediatr Blood Cancer* ;48(1):57-36.
- 26-Cetin, T.; Oktenli, C. and Ozgurtas, T.(2003).Renal tubular dysfunction in beta-thalassemia minor. *Am J Kidney Dis*; 42:1164-1168.
- 27-Smolkin, V.; Halevy, R.; Levin, C.; Mines, M.; Sakran, W.; Ilia, K. and Koren, A.(2008).Renal function in children with β -thalassemia major and thalassemia intermedia. *PediatrNephrol*, 23:1847-1851.
- 28-Papanikolaou, G.; Tzilianos, M.; Christakis, J.L.; Bogdanos, D.;Tsimirika ,K. and Farlane .M. J. (2005).Hepcidin in iron overload disorders. *Blood*; 105: 4103-5.
- 29- Nowrouzian, F.L.; Fireman, V.; Adlerberth, I.; and Wold, A.E.(2007). Reduced phase switch capacity and functional adhesion expression of type-1 fimbriatedE. coli from immunoglobulin A deficient individuals. *Infect. Immun.* 75:932-940.
- 30- Kenneth, N.(2002).Mechanisms of bacterial pathogenesis: Colonization and invasion Deferoxamine in Thalassemia.*Calcif. Tissue Int.* 57: 229.
- 31-Muncie, H.L. and Campbell, J.(2009).Alpha and beta thalassemia. *Am FamPhysician.*; 80:339-44.
- 32-Fallahzadeh, M.H.; Fallahzadeh, M.k.; Mehdi, S.; Shervin, R.; Ali, D. and Fallahzadeh, M.A.(2010). Hematuria in Patients with Beta-Thalassemia Major .*Iranian Journal of Kidney Diseases (I.J.K.D)*; 4:133-6.
- 33-Robert, W. and Schrier, M.D.(2007).Diseases of the Kidney & Urinary Tract, 8th. Edition Copyright © Lippincott Williams & Wilkins.