Original article

Microalbuminuria in hemoglobinopathy patients who are taking Deferasirox

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Abstract

Background: Chelation therapy which is needed to prevent or reverse iron overload may also affect renal function in patients with hemoglobinopathy. The early indicator as well as predictor of nephropathy and glomerular damage among patients with sickle cell disease and thalassemia is microalbuminuria (MA).

Objective: This study was aimed to estimate the frequency of MA in patients with thalassemia and sickle cell syndromes who were taking deferasirox and to find if any relationship between the level of MA and other parameters like age, gender, type of hemoglobinopathy, serum creatinine and ferritin levels.

Materials and methods: This is a clinical study in hemoglobinopathy patients that taking deferasirox (oral iron chelator) in Center For Hereditary Blood Diseases (HBDC) in Basrah during the period between April 2013 and February 2014. The informations were took from patients by a questionnaire form and urine samples were collected from each patient for measurement of microalbuminuria by Enzyme-linked immunosorbent assay method (ELISA) and blood samples for biochemical tests including serum creatinine and ferritin.

Results: In this study, 100 patients were 38 males and 62 females with mean of age was 25.74±10.59 years. MA detected in 31 patients and it was more among males (36.8%, 37.0 mg/ml) as compared to (27.4%, 26.0 mg/ml) of female and more in sickle cell syndrome (35%, 35.0 mg/ml) as compared to (25%, 24.0 mg/ml) of thalassemia patients and more in patients with age <30 years (35.5%, 35.5 mg/ml) as compared to patients with age ≥ 30 years (16.7%, 22.0 mg/ml). There was no significant relationship between MA and serum creatinin or serum ferritin levels but significant relationship was found between MA level and age.

Conclusion:

In conclusion, the study might reflect the relatively low prevalence rate of MA among non-protienuria, deferasirox-taking patients with hemoglobinopathies in Basrah. Microalbuminuria affected by many factors including age, gender, diagnosis and other factors.

Key words: Microalbuminuria, Hemoglobinopathies

Introduction

Hemoglobinopathies are recognized as one of the most common inherited disease worldwide and it is causing a major health burden in Basrah city. The term hemoglobinopathies includes all genetic globin disorders but the main two groups are β-thalassemia disease due to inherited defect in the beta globin chain synthesis and sickle cell disease due to structural defect in hemoglobin SS (Hb SS) molecule (patient has inherited two hemoglobin S genes, one from each parent). [1,2]

Hemoglobinopathies were originally characteristic of the tropic and subtropics but are common worldwide due to migration.[3] The estimated prevalence of carriers of any hemoglobin gene variant is higher in South-east Asian region 45.5%, followed by African region 44.4%, Eastern Mediterranean region 21.7% and is lowest in European region 3.3% of population.[4] Although there is a difference in the worldwide prevalence according to the type of hemoglobin disorder, but both sickle cell and thalassemia syndromes are widely spread throughout Mediterranean Basin and Arab Peninsula from Yemen through Saudi Arabia to Iraq.[5] Accurate Information about hemoglobinopathy prevalence in Iraq is lacking with frequency variation in different Geographical areas but Basrah is endemic in both thalassemia and sickle cell syndrome (SCS) and first report by Alkasab et al (1981) showed an overall HbS prevalence was 13.3% of the studied cases.[6,7]

Renal complications and nephropathy are known complications in patients with thalassemia and sickle cell disease. Chronic anemia and iron overload are common mechanisms for renal complications in hemoglobinopathy but other pathologic changes could result in tubular and glomerular function disturbances.[8-12] Chelation therapy which is needed to prevent or reverse iron overload may also affect renal function in patients with hemoglobinopathy. Deferasirox, the newer oral iron chelator, can cause increase in serum creatinine, proteinuria, and even renal failure.[13,14] The presence of chronic kidney disease is significantly shorten the survival of patients with hemoglobinopathy because of association with very high mortality and accelerated cardiovascular disease.[15,16] Furthermore, several studies suggested that the

risk for death is increased independently in patients even with less severe renal dysfunction.[17,18]

The early indicator as well as predictor of nephropathy and glomerular damage among patients with sickle cell disease and thalassemia is microalbuminuria (MA).[19,20] The aim of study to estimate the frequency of MA in patients with thalassemia and sickle cell syndromes who were taking deferasirox and to find if any relationship between the level of MA and other parameters like age, gender, type of hemoglobinopathy, serum creatinine and ferritin levels.

Subjects, materials and methods:

The study was conducted at Center For Hereditary Blood Diseases HBDC during the period between April 2013 and February 2014, Basrah is southern governorate in Iraq and endemic in Hemoglobinopathy.[6,7] The Study subjects consisted of 100 adult patients (38 male and 62 female) (60 with sickle cell disease and 40 with thalassemia disease) who were taking deferasirox (Exjade ®, Novartis company, tablets 125, 250 and 500 mg) (dose 20-40 mg/kg, orally) during different intervals of treatment (chronic disease) and having no frank proteinuria on general urine examination (GUE). They were apparently healthy and were recruited as they attended to the outpatient clinic.

The patients had been excluded with age less than 16 years, patients had proteinuria (frank nephropathy), and patients that were took hydroxyurea treatment.

Urine samples were collected from each patient for measurement of microalbumin by ELISA method[21] and blood samples for biochemical tests including serum creatinine (creatinine kit)[22]and serum ferritin (ferritin kit)[38].

Measurement of Microalbumin in urin by ELISA method:[21]

Procedure:Preparation of reagents:

-Wash Buffer (NaN3 <0.1%).

-Sample Buffer (NaN3 <0.1%).

- Conjugated enzyme solution (polyclonal rabbit anti-human albumin, 15 ml and Proclin 300 <0.5%).

- Substrate solution TMB (3,3´,5,5´-Tetramethyl-benzidine, 15 ml).

- Stop Solution (1 M sulfuric acid).

-Preparation of samples

-Undiluted urine sample.
-If the concentration of samples are very high. We can be diluted of samples with buffer and dilutions concentration taken during calculation.

-Steps of procedure:

1-Put 20 Ml of calibrators, undiluted samples and controls in to the wells.

2-Put to each well 100 Ml of conjugated enzyme solution.

3-Wait for 30 min at room temperature.

4-Remove the contents of wells and wash with 300 Ml of wash sol. 3 times.

5-Add 100 Ml of substrate solution TMB in to each well.

6- Wait for 15 min at room temperature.

7-Put to each well 100 Ml of stop solution and leaved it for 5 min.

8-Read the results at the optical density (450 nm).

Calculation:

-For quantitative results, We ploted the calibrator optical density against the calibrator concentration to find a calibration curve. The concentration of samples may then be measured from the calibration curve. (Cut-off (0 - 25 µg/Ml))

Measurement of Creatinine by creatinine kit in serum:[22]

Creatinine in alkaline medium produces ayellow-orange color solution with picric acid.

Procedure:

R1:Sodium hydroxide (150 mmol/L) and Disodium phosphate (6.4 mmol/L).

R2: Picric acid (4 mmol/L) and Sodium dodecyl sulfate (0.75 mmol/L)

Pipette in 1 mlpathlenth cuvette	**Blank (optional)**	**Standard**	**Assay**
Reagent R1	0.5 ml	0.5 ml	0.5 ml
D.W	100 Ml		
Standard		100 Ml	
Specimen (Note 1)			100 Ml
Incubate the samples at the room temperature, then add:			
Reagent R2	0.5 ml	0.5 ml	0.5 ml

Mix well. Wait 30 sec, measure absorbance A1 at 490 nm versus D.W or blank. Exactly 2 min. after the first reading measure absorbance A2.

$$\text{Calculation: Serum: result} = \frac{(A2-A1)\text{Assay}}{(A2-A1)\text{Standard}} \times \text{Standard concentration}$$

Measurement of Ferritin by ferritin kit in serum:[38]

Procedure:Preparation of reagents:

1-Congugate enzyme reagent (monoclonal antiferritin)

2-Standerad reagent (human spleen or liver ferritin in serum of bovine with preservatives).

3-Tetramethylbenzidine (TMB) reagent.

4-Diluted hydrochloric acid (stop solution).

Preparation of samples: serum should be obtained from a whole blood specimen without lipemic (milky), hemolytic (bright red) or turbid samples.

Steps of procedure:

1-Put 20µL of samples and standards into appropriate wells.

2- Add 100µL of Conjugate Enzyme Reagent into each well.

3-Mix gently for 30 seconds for complete mixing.

4- Incubate for 45 minutes at room temperature (18-25°C).

5- Wash the wells 5 times with deionized or distilled water.

6- Put 100 µL TMB Reagent into each well with gently mix.

7- In the dark Incubate at room temperature for 20 minutes.

8- Add of Stop Solution (100Ml) into each well.

9- Read optical density at 450nm within 15 minutes.

Calculation:

A standard curve was plotted between the absorbance for each standard against its concentration in ng/mL on graph paper, where the concentrations on the horizontal axis (x) and the absorbance on the vertical axis(y). We measured the corresponding ferritin concentration in ng/mL from the standard curve.

Statistical analysis:

The Mann-Whitney U test of SPSS (version 18) is used to find the relation between two different subjects in the experiment, when the assumptions of the t-test have been violated (the data is not normal distribution). The Mann-Whitney U test (independent two sample test) used to find the relation between MA levels and age, gender and diagnosis, there is only one significant relationship between MA levels and age. Also this test used to find correlation coefficient and linear regression between MA levels with age, serum creatinine and

serum ferritin, there is only one significant correlation and linear regression between MA levels and age.

Results:

Of the 100 studied patients, 38 were males (14 with MA (36.8%)) and 62 were females (16 with MA(27.4%)) and the mean of age for total patients was 25.74±10.59 years and the mean of age for patients with MA was 22.9±8.23 years. Microalbuminuria detected in 31 patients and it was more among males 36.8% as compared to 27.4% of female and more in SCS 35% as compared to 25% of thalassemia patients and more in patients with age <30 years (35.5%) as compared to patients with age ≥ 30 years (16.7%) as shown in table 1.

Microalbuminuria was detected significantly at higher levels 35.5 mg/ml among patients younger than 30 years as compared to 22.0 mg/ml among patients older than 30 years as shown in table 2 and figure 1.

Although MA was detected at higher level 37.0 mg/ml among males than 26.0 mg/ml among females, but it was statistically not significant as shown in table 3.

Also there was no significant difference in the level of MA, but it was more among patients with sickle cell syndrome (35.0 mg/ml) as compared to (24.0 mg/ml) among patients with thalassemia as shown in table 4.

On Further statistical analysis using log microalbuminuria level in relation with age, serum creatinine and serum ferritin levels, table 5. There was no significant relationship between log MA level and each of serum creatinine and serum ferritin levels with the only significant relationship was found between log MA level and age.

Table 1: General characteristics of patients

Characteristics of patients	Total patients	Patients with MA
Male	38	14 (36.8%)
Female	62	17 (27.4%)
Mean of ages (years)	25.74±10.59	22.9±8.23
< 30 years	76	27 (35.5%)
≥ 30 years	24	4 (16.7%)
Types of hemoglobinopathies		
Thalassemias	40 (13 male &27 female)	10 (25%) (4 male & 6 female)
Sickle cell disease	60 (25 male &35 female)	21 (35%) (10 male & 11 female)
Total	100	31 (31%)

Table 2.Microalbuminuria levels (mg/ml) according to the age group of patients.

Age, median (IQR)		**Mann-Whitney U test**	
< 30 year	≥ 30 year	Z value	P value
35.5 (21.0 – 90.0) mg/ml	22.0 (12.0 – 40.0) mg/ml	-2.36	0.019

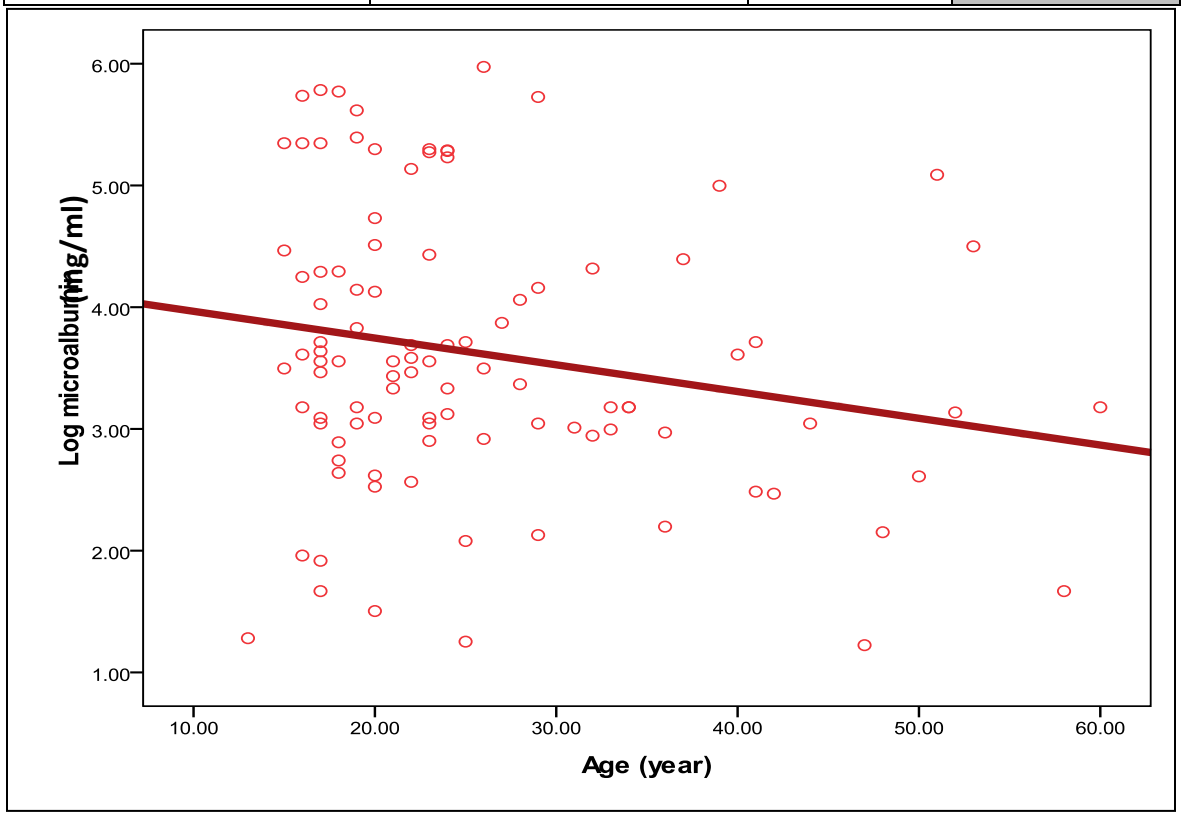

Figure 1.The relationship between Log microalbumin (mg/ml) and age of patients

Table 3.Microalbuminuria levels (mg/ml)according to gender.

Gender, median (IQR)		**Mann-Whitney U test**	
Male	**Female**	Z value	P value
37.0 (21.7 – 153.5) mg/ml	26.0 (18.2 – 73.6) mg/ml	-1.46	0.142

Table 4.Microalbumin levels (mg/ml) in patients' urine with thalassemia and sickle cell syndrome.

Diagnosis, median (IQR)		**Mann-Whitney U test**	
Thalasemia	**Sickle cell syndrome**	Z value	P value
24.0 (16.1 – 62.8) mg/ml	35.0 (21.0 – 107.6) mg/ml	-1.41	0.157

Table 5.The relationship between log microalbuminuria levels and age, serum creatinine and ferritin in patients.

Parameters	**Correlation coefficient**	**P value**
Log microalbuminuria Age	- 0.203	0.043
Log microalbuminuria Serum creatinine	- 0.090	0.373
Log microalbuminuria Serum ferritin	- 0.030	0.565

Discussion:

Microalbuminuria which is common finding in hemoglobinopathies and it is of a particular importance in patients taking the newer iron chelator (deferasirox) which can

be a nephrotoxic [23,24,25] and the MA was measured as an early predictor of nephropathy.[19,20,26]100 patients (60 sickle cell disease and 40 thalassemia disease) were took in this study to estimate the frequency of occurrence of MA among patients.

In this study the MA was detected in 31% of the studied patients, and it was more occurring among patients with SCD 35% as compared to 25% of patients with thalassemia disease as table 1. Up to the knowledge based on reviewing with other studies and previous reports. There don't find a comparative figures in the same study but generally the MA and renal dysfunction were relatively more prevalent and more severe among sickle population with HbSS than in non HbSS hemoglobinopathies, probably because the protective effect of the major genetic modifier which is fetal hemoglobin (HbF) level in non HbSS hemoglobinopathies[27] due to HbF is protective gene that reduce incidence of vasoocclusion, one factor that increased prevalence of nephropathy associated with sickle cell disease.[37]

Microalbuminuria may be affected by many factors including age, gender, diagnosis and other factors.[28] Anyhow, in most cases of sickle nephropathy starting insidiously in the very young age with glomerular hyperfiltration and leading to MA in late childhood or early adulthood and progressed slowly over time.[27] Therefore there was a strong correlation between the prevalence of MA and age as shown by other studies.[28,29,30] On the other hand, MA was commoner finding in female as compared to male gender.[26,31] In contrast, this study showed that the MA was significantly more prevalent in patients younger than 30 years as compared to older age group. There is not a true correlation and might be explained by the small sample size and that the majority of the studied patients were younger than 30 years. The results also showed that MA was more among male gender and probably because SCS more among males (25 out of 38 males with SCD (65.8%) than thalassemia syndrome (13 out of 38 males with thalassemia (34.2%)) and nepherotoxicity more associated with SCS.[29, 32]

The present study failed to show any significant relation between the occurrence of MA and the levels of serum creatinine and serum ferritin both in patients with SCS and patients with thalassemia syndromes. Some studies appeared that MA (preclinical marker of renal damage) was significant increased with renal damage in sickle cell

disease while serum creatinine levels without significant differences [33,34] but other studies showed that sickle nephropathy correlated with both MA and serum creatinine.[35, 36]

Although the present study had some limitations; for example the small sample size, no control group and no creatinine clearance could be measured at time of the study. However, The conclusion of this study could reflect the relatively low prevalence rate of MA among non-proteinuria, deferasirox-taking patients with hemoglobinopathies in Basrah. Microalbuminuria may be affected by many factors including age, gender and diagnosis.

The recommendation to treat the patients with MA with antiprotienuric agents to protect against progression to frank proteinuria, and also we recommend for a more comprehensive case-control study to evaluate the nephrotoxic effect and other safety profile of deferasirox among our patients.

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Conclusion:

The study might reflect the relatively low prevalence rate of MA among non-protienuria, deferasirox-taking patients with hemoglobinopathies in Basrah. Microalbuminuria affected by many factors including age, gender, diagnosis and other factors.

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