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Comparative Study of Deferiprone and Silymarin versus Deferiprone and Placebo as Iron Chelators in Children with Beta Thalassemia with Iron Overload

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Abstract

Background: Beta thalassemia is an inherited hemoglobin disorder resulting in chronic hemolytic anemia requiring life-long blood transfusion that cause iron overload. Silymarin plays a role as an iron chelator in iron overloaded patients.

The aim of this work was to compare the iron chelating efficacy of combination therapy of oral Deferiprone and silymarin with oral Deferiprone and placebo.

Patients and methods: This study was conducted on 40 children with beta thalassemia major under follow up at Hematology Unit, Pediatric Department, Tanta University Hospital in the period between October 2012 and October 2013 with their serum ferritin levels more than 1000 ng/ml and they was divided in two groups. Group I: Received oral Deferiprone and silymarin for 6 months. Group II: Received oral Deferiprone and placebo for 6 months.

Results: In the current study, there were no significant differences in the initial serum ferritin, serum iron and TIBC levels between group I and group II but after regular chelation therapy, serum ferritin and serum iron were significantly lower and TIBC was significantly higher in group I than group II. No statistically significant difference in serum creatinine, blood urea, ALT, AST and serum bilirubin levels between Group I and Group II before and after chelation therapy.

Conclusion: From this study we concluded that, Deferiprone in combination with silymarin are better iron chelators in iron-loaded thalassemic patients than Deferiprone and placebo.

Keywords: Thalassemia; Silymarin; Deferiprone; Iron overload

Introduction

Thalassemias are a heterogeneous group of inherited anemias that collectively represents the most common monogenic disorders [1]. β- thalassemias are characterized by absent or reduced synthesis of β- globin chains of hemoglobin, caused by mutations of β-globin gene cluster [2] resulting in reduced hemoglobin in Red Blood Cells (RBCs), decreased RBCs production and anemia [3]. In Egypt, β-thalassemia is the commonest cause of chronic hemolytic anemia (85%) and it represents a major public health problem. It is particularly common in populations of Upper Egypt and peoples of Delta and Red Sea Hill Region [4,5].

The most common treatment for thalassemia is blood transfusion which is necessary to provide the patients with healthy red blood cells containing normal hemoglobin. Repeated blood transfusion leads to iron overload [6].

The iron loading in thalassemia depends on the volume of blood transfused and the amount accumulated from gut absorption [7]. In β-thalassemia increased gastrointestinal iron absorption is mediated by down-regulation of hepcidin and up-regulation of ferroportin. Hepcidin regulates iron transport across the gut mucosa, thereby preventing excess iron absorption and maintaining normal iron levels within the body. Ferroportin is a transmembrane protein that transports iron from the inside of a cell to the outside it [8]. As each unit of packed cells contains approximately 200 mg of iron so a patient who receives 25 units per year accumulates 5 gram of iron per year in the absence of chelation. Add to this the increased intestinal iron absorption. For that reason, patients must undergo chelation therapy [9] that has a major impact on the treatment of thalassemia [10] as excess iron accumulates in the body and is deposited in body organs as heart, liver and endocrine glands causing organ damage [11].

Deferiprone is a bidentate oral iron chelator that began clinical trials in UK in 1980. It was first licensed for use in thalassaemia in India, followed by European Union and other countries outside US and Canada, in 1990s [12]. According to official European Licensing Agency (EMEA), Deferiprone could be used as a second line drug for removing iron in patients who are unable to use Desferrioxamine (DFO) or in whom DFO therapy has proven ineffective [13]. Some studies show no difference between deferiprone and desferrioxamine [14]. Deferiprone daily dose that had been evaluated most thoroughly is 75 mg/kg/day, given in three divided doses [15].

Silymarin is an herbal remedy used for treatment of liver and gall bladder disorders. Silymarin is a flavonolignan complex isolated from Silybin marianum. It has a strong antioxidant, hepatoprotective,

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and iron chelating activities [16]. There are some studies designed to investigate the therapeutic activity of silymarin in patients with thalassemia major under conventional iron chelation therapy [17,18].

Patient and Methods

This prospective study was conducted on 40 children with beta thalassemia major under follow-up at Hematology Unit, Pediatric Department, Tanta University Hospital having serum ferritin level more than 1000 ng/ml in the period between October 2012 and October 2013 and was performed after approval from research ethical committee center in Tanta University Hospital and obtaining an informed written parental consent from all participants in this research.

Study Design

Thalassemic patients included in the study were divided into two subgroups; Group I and Group II by simple random allocation, Group I received combination of daily oral Deferiprone 75 mg/kg/day divided into three doses [15] and oral silymarin in the form of Legalon tablets 140 mg, one hour before each meal (3 times daily) for 6 months [19] while group II received daily oral Deferiprone 75 mg/kg/day divided into three doses and placebo.

Inclusion criteria

Children with β-thalassemia with serum ferritin >1000 ng/ml who did not receive any iron chelation therapy before the start of this study.

Exclusion criteria

Children with β- thalassemia with serum ferritin <1000 ng/ml or who received any iron chelation therapy before the start of this study or who uses the drugs in irregular manner during this study.

All the children in both groups were subjected to the following:

1. Complete history taking with especial account on onset of thalassemia, chelation therapy, frequency of blood transfusion.
2. Thorough clinical examination with especial account on: pallor, jaundice, mongloid facies, splenomegaly, hepatomegaly and splenectomy.
3. Investigations including:

- Complete blood count.
- Hemoglobin electrophoresis.
- Liver functions including bilirubin level, Alanine Transferase (ALT) and Aspartate Transferase (AST).
- Renal function tests including blood urea and serum creatinine.
- Assessment of serum iron status including serum ferritin, serum iron and iron binding capacity. Assessment of liver and renal functions and serum iron status were done two times in studied patients; one time before the start of chelation therapy and one time after 6 months of chelation therapy.

Specimen collection and handling

Four ml of venous blood were collected using sterile needles through gentle venipuncture after sterilization of site of puncture by alcohol, and collected samples were divided into; 2 ml in a plain glass tube that were allowed to clot for 4 minutes and then centrifuged to separate serum which was used for estimation of serum iron, ferritin and TIBC [20-23] one ml was delivered on 20 µL EDTA solution for complete blood count including reticulocyte count and differential count which was done on leishman stained peripheral blood smear with evaluation using ERMA PCE-210 N cell counter [24] one ml was added to 2 ml hemolysate for Hb electrophoresis [25].

Determination of serum iron: The iron dissociated from transferrin-iron complex by a solution of guanidine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex (according to procedure recommended by the serum iron from Biomaghreb company) [21].

Determination of serum total iron binding capacity (TIBC): An excess of iron is added to the serum to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate (according to procedure recommended by the serum total iron binding capacity from Biomaghreb company) [22].

Serum ferritin test: Serum level of ferritin by ELIZA [DRG® Ferritin ELISA (EIA-4292)] [23].

Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS Version 16 (Tables 1 and 2) [26].

Results and Discussion

Thalassemia's are one of the most common genetic disorders worldwide. It is the commonest cause of chronic hemolytic anemia in Middle East [27]. Silymarin is a flavonoid agent with antioxidant and free radical scavenging abilities. Silymarin also acts as an iron chelator by binding Fe (III). Despite the iron chelating activity of silymarin suggests its possible application in chelation therapy of iron overload; the biological effects of silymarin are different from other iron chelators, probably due to antioxidant activity of silymarin, which causes pro-oxidant effect via iron-catalyzed oxidation with subsequent generation of reactive oxygen species [19].

This study was carried out on 40 children with β-thalassemia major under follow up in Pediatric Hematology Unit, Tanta University Hospital in the period between October 2012 and October 2013. Patients included in the study were divided into two subgroups (group I and group II); group I received combination of Deferiprone and silymarin while group II used Deferiprone and placebo for six months.

	Group I (No=20)	Group II (No=20)	Chi-Square X^2	P - value
Age (months) Range Mean ± SD	40-60 50.35 ± 6.14	42-60 50.10 ± 6.11	0.26	0.79
Sex Males Females	10 10	9 11	0.100	0.752
Age of onset of thalassemia (months) Range Mean ± SD	6-36 11.5 ± 4.3	6-36 10.45 ± 4.13	0.63	0.97
Frequency of blood transfusion Every 2 week Every 3 week Every 4 week Every 6 week	4 cases 10 cases 5 cases 1 case	5 cases 5 cases 9 cases 1 case	2.921	0.404
Hemoglobin (gm /dl) Range Mean ± SD	7-8 7.3 ± 0.32	7-8.8 7.72 ± 0.60	0.96	0.635

differences between group I and group II regarding age.

Table 1: Clinical data of studied thalassemic patients.

	Group I (No=20)	Group II (No=20)	t value	p value
Serum creatinine (mg/dl) Before (mean ± SD) After (mean ± SD) t3 value p3 value	0.63 ± 0.11 0.59 ± 0.07 1.24 0.22	0.62 ± 0.10 0.61 ± 0.07 1.55 0.13	t1. 0.07 t2. 2.0	p1. 0.94 p2. 0. 48
Blood urea (mg/dl) Before (mean ± SD) After (mean ± SD) t3 value p3 value	35.30 ± 6.25 36.95 ± 5.44 2.31 0.32	33.75 ± 6.4 35.45 ± 5.47 1.93 0.68	t1. 1.06 t2. 0.98	p1. 0.29 p2. 0.33
ALT(U/L) Before (mean ± SD) After (mean ± SD) t3 value p3 value	26.25 ± 3.64 27.50 ± 3.16 0.90 0.73	26.30 ± 2.71 28.20 ± 3.63 2.83 0.11	t1. 0.84 t2. 1.42	p1. 0.93 p2. 0.17
AST(U/L) Before (mean ± SD) After (mean ± SD) t3 value p3 value	22.65 ± 4.51 23.7 ± 3.77 0.50 0.62	23.75 ± 5.85 24 ± 9.4.05 0.47 0.64	t1. 0.84 t2. 1.14	p1. 0.40 p2. 0.26
Total bilirubin (mg/dl) Before (mean ± SD) After (mean ± SD) t3 value p3 value	3.19 ± 0.40 3.23 ± 0.51 0.54 0.44	3.15 ± 0.43 3.24 ± 0.47 0.53 0.35	t1. 0.82 t2. 0.09	p1. 0.41 p2. 0.92
Ferritin (ng/ml) Before (mean ± SD) After (mean ± SD) t3 value p3 value	1901 ± 563.38 989.5 ± 178.57 6.53 0.000	1885.2 ± 510.54 1260 ± 212.26 5.40 0.000	t1 0.41 t2. 3.99	p1. 0.68 p2 0.000
Iron (ug/dl) Before (mean ± SD) After (mean ± SD) t3 value p3 value	236.40 ± 34.68 156.55 ± 21.42 0.7.91 0. 000	227.7 ± 35.49 172 ± 40.24.51 5.72 0.000	t1. 2.20 t2. 6.33	p1. 0.40 p2. 0.04
TIBC (ug/dl) Before (mean ± SD) After (mean ± SD) t3 value p3 value	192.45 ± 10.53 275.20 ± 10.08 24.18 0.000	191.85 ± 9.81 265.9 ± 9.19 21.72 0.000	t1. 0.29 t2. 2.33	p1. 0.77 p2. 0.03

Table 2: shows no statistically significant difference in serum creatinine, blood urea, serum bilirubin, ALT and AST between Group I and Group II before and after chelation therapy. There were no statistically significant difference in serum ferritin, serum iron and TIBC levels between group I and group II before start of chelation therapy while there was statistically significant difference between group I and group II after chelation therapy with lower serum ferritin and serum iron and higher TIBC in group I than group II. t1 comparison between group I and group II before chelation therapy, t2 comparison between group I and group II after chelation therapy and t3 comparison between the same group (as group I or II before and after chelation therapy).

Table 2: Renal and hepatic functions and iron status in studied patients before and after chelation therapy.

In the current study, there were no significant differences in the initial serum ferritin, serum iron and TIBC levels between group I and group II but after regular chelation therapy, serum ferritin and serum iron were significantly lower and TIBC was significantly higher in group I than group II. This is in agreement with [17] who assessed the efficacy of silymarin and desferroxamine compared with desferroxamine alone in removing excess iron in 48 patients with beta thalassemia and found that, serum ferritin and serum iron were significantly lower and TIBC was significantly higher in patients who received silymarin and desferroxamine than patients who received desferrioxamine alone, [28] who studied the beneficial effects of silymarin in thalassemic patients and suggests that silymarin in combination with desferrioxamine can be safely and effectively used in the treatment of iron-loaded thalassemic patients and [18] who compared (silymarin and Exjade) with (Exjade and placebo) in 40 patients with thalassemia major and found that, serum ferritin and serum iron were significantly lower and TIBC was significantly higher in patients who received silymarin and Exjade than patients who received Exjade and placebo and they concluded that combined therapy of silymarin and Exjade or silymarin and desferroxamine depleted iron stores more successfully than either Exjade or desferroxamine alone and they recommended to use combination of silymarin and desferrioxamine or silymarin and Exjade in treatment of iron overload in children with thalassemia major [17,18,28].

In disagreement with the previous studies; [29] found no significant changes in liver iron concentration after silymarin use and they recommended evaluating this drug by longer course of treatment to clarify its effects on reduction of liver iron concentration.

Variation in results could be explained by different mode of evaluating iron reduction (serum ferritin in our study versus liver iron concentration in [29] study and variation in severity of iron over load in both studies.

In this study, there were no significant differences in pre and post values of renal and liver function tests in group I before and after chelation therapy. This data is in agreement with [17,18,28] who demonstrated that; thalassemic patients with severe iron overload can be safely treated with a combination of silymarin and desferrioxamine or Exjade with no detectable abnormalities in complete blood count, liver or renal functions due to silymarin use.

Stickel and Schuppan [30] found that an average daily dose of

silymarin (420 mg/day for 41 months) was found to be non-toxic, relative to placebo, in clinical trials [31]. Drug-drug interaction and liver toxicity by interference with co-drugs by induction or inhibition of cytochrome- P450 is a major concern for the use of silymarin [32]. Studies were performed to investigate the potential for hepatotoxicity, cytochrome-P450 isoenzymes induction and inhibition on dry extract from S. marianum. The results indicated that interference or hepatotoxicity of the dry extract from S. marianum at the recommended daily dose of 420 mg/day of silymarin (equivalent to 210 mg silybin) is unlikely and is considered safe [33].

Conclusion

From this study we concluded that, Deferiprone in combination with silymarin are better iron chelators in iron-loaded thalassemic patients than Deferiprone and placebo.

Recommendations

Extensive multicenter studies in large number of patients with longer duration of follow up and more advanced methods of assessment of iron status is recommended to clarify the exact role of silymarin in reduction of iron over load in children with beta thalassemia.

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