The Role of Erythropoiesis on Hepcidin Level in Polytransfused \(\beta\)-Thalassemia Major Patients


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Abstract

Background: Hepcidin peptide hormone is a main controller of iron homeostasis, it becomes elevated in case of iron overload, however despite iron overload in \(\beta\)-Thalassemia major (\(\beta\)-TM) patients a contradictory decrease in Hepcidin occurs. The incompatible Hepcidin level is the main responsible factor causing the iron overload status in iron-loading anemias such as (\(\beta\)-TM) which contributes to organ dysfunction and to iron toxicity.

Aim of Study: To evaluate the conflicting effect of increased erythropoiesis in contrast to the effect of iron overloading on Hepcidin serum level and its correlation with iron status in (\(\beta\)-TM) patients.

Subjects and Methods: For all patients and controls Complete Blood Count (CBC), serum assay of Hepcidin, iron, ferritin, transferrin, HCVAbs, HBs Ag, CRP and serum level were performed.

Results: Hepcidin level was significantly lowered in (\(\beta\)-TM) patients compared to controls with high significant difference \(p=0.00\). There was no correlation between serum Hepcidin and ferritin level, neither was between Hepcidin and serum iron, transferrin, Hb level and reticulocyte count in the study group. Hepcidin/Ferritin ratio showed high significance difference \(p<0.000\) with mean value in the study group 0.056 vs. 4.75 in controls pointing to an incompatible levels of Hepcidin to iron overload status. Hepcidin level was negative correlated with age \(p<0.05\).

Conclusion: Hepcidin was markedly decreased in the study group with no significant correlation between serum Hepcidin and ferritin level as a marker of iron overload in thalassemia major. Hepcidin deficiency is the main contributing factor of iron overload in \(\beta\) thalassemia which results from a strong suppressive effect of the high erythropoietic activity on Hepcidin expression. Hepcidin-ferritin ratio was markedly depressed <1 in all \(\beta\) thalassemia major patients which indicating suppression of Hepcidin out of proportion with the degree of iron loading.


Introduction

HEPCIDIN is an acute phase reactant and hepatic peptide hormone, circulating in blood and excreted in urine. It influences the dietary iron absorption, blood iron levels, and tissue iron release. Hepcidin acts by regulating the concentration of the receptor ferroportin. The Ferroportin is an iron exporter it exports iron into plasma from intestinal cells that absorbs dietary iron and from spleen and liver macrophages that recycle iron from old erythrocytes. Hepcidin binding to ferroportin initiates degradation of the receptor, resulting in stoppage of the iron export process with decreased iron levels in plasma. On the other hand, when Hepcidin concentration is low, the ferroportin exerts its function with increased iron plasma levels [1].

The disruption of Hepcidin homeostasis causes many iron disorders. Homeostatically regulated by iron stores the prolonged excess of Hepcidin levels causes iron-restricted anemia while Hepcidin shortage results in iron overload with iron deposition and damage to vital organs [2].

The \(\beta\)-eta-Thalassemia Major is a genetic chronic hemolytic anemia associated with ineffective erythropoiesis and peripheral hemolysis resulting in severe form of anemia. In \(\beta\)-eta-Thalassemia Major-Hepcidin deficiency results from a strong suppressive effect of the high erythropoietic activity on...
Hepcidin expression [3]. The excessive dietary iron absorption in these patients accompanied by iron accumulation due to repeated blood transfusions leads to a state of severe iron overload which is the major cause of morbidity and mortality in β-thalassemia with or without transfusion dependence [4].

Subjects and Methods

A case-control study was aimed to be carried on 200 Thalassemia major patient, recruited from Thalassemia Blood Transfusion Centre, Children's Hospital, Assiut University Hospitals from March 2016 to June 2017. Blood transfusion had been started before two years old and all of them were on regular blood transfusion every two to four weeks when patients are in severe anemia. It was based on detailed clinical history, examination, Complete Blood Count (CBC) and Hb electrophoresis. For all patients a complete blood count, HCV Abs, HBs Ag, C-reactive protein, serum iron, TIBC, serum ferritin, and serum Hepcidin was performed. Patients on iron chelating therapy, Sickle Cell Beta thalassemia disease, serum C-Reactive Protein (CRP) level >8mg/l, sero-positivity for HBsAg, anti-HCV antibody, thalassemia megaloblastic or aplastic crises were excluded from the study. After applying the exclusion criteria out of 200 patients, 40 cases were included in the study, compared to 20 age-and sex-matched healthy subjects included as controls.

Venous blood samples were collected immediately prior to transfusion in thalassemia major patients. All patients and controls were subjected to the following investigations:

Venous blood samples were collected in EDTA coated tubes and were used for the hematological parameters as CBC and reticulocyte count the CBC was done using (CELL-DYN 3700-Abbott).

For the biochemical parameters, plain tubes were used blood allowed to clot then serum centrifugation at 3000rpm for 10 minutes, the serum was separated and frozen at –20ºC for subsequent usage.

- **HCV Antibodies and HBs Ag**: Was done on AD-VIA Centaur CP system which is an indirect two wash sandwich immunoassay.
- **C-Reactive Protein (CRP)**: Was done using CRP LATEX TEST KIT (Biotec Laboratories Ltd-United Kingdom) which is latex agglutination slide test for in vitro diagnostic use for the qualitative and semi quantitative estimation of CRP in human serum.

- **Serum iron and TIBC**: Was done using (Stanbio Iron and Total Iron Binding Capacity N.0370) which is quantitative colorimetric determination of iron and unsaturated iron binding capacity in serum.
- **Serum ferritin and transferrin**: Was performed on auto analyzer Hitachi 911 (Boehringer Mannheim) which is particle enhanced immunoturbidimetric assay.
- **Serum Hepcidin**: Was done using Human Hepcidin ELISA Kit (Glory Science.co., Ltd. LOT. 201503), the kit uses a double-antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to assay the level of Human Hepcidin in samples. The Kit is for the quantitative level of Hepcidin in the sample, adopt purified human Hepcidin to coat micro titer plate, make solid-phase antibody, then add Hepcidin to wells, combine Hepcidin antibody with labeled horseradish peroxidase (HRP) to form antibody-antigen-enzyme-antibody complex, after washing, add Tetramethylbenzidine (TMB) substrate solution, TMB substrate becomes blue color at HRP enzyme catalyzed, reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wave length of 450nm. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The Hepcidin concentration in each sample is interpolated from this standard curve.

Statistical analysis:

The statistical analysis of data was done by Statistical Package of Social Science (SPSS) Version 16; data were expressed as mean ± standard deviation. Independent *t*-test was done to compare the two groups. Pearson correlation coefficient (*r*) was done to assess correlation among continuous variables. A *p*-value (<0.05) was considered significant.

**Results**

The age for patients ranged (3-15) years (mean 7.62 ± 3.06) years, the total of 40 patient included in the study 26 (65%) were males and 14 (35%) were females.

Complete blood picture in the study group and the controls revealed high significant difference (*p*<0.000) as regard uncorrected WBC, corrected WBC, reticulocyte count and Hb level. On the other side there was no significant difference (*p*>0.05) in platelets count between the study and the controls.
Serum iron mean value in control group was 256.22 vs. 86.80 in the study group with high significant difference (p<0.000). TIBC mean value was 317.42 in study group vs. 392.75 in the control group with significant difference (p=0.05). Serum Ferritin mean value was significantly higher 505.95 in the study group vs. 392.75 in the control group with high significant difference (p<0.000). Ferritin ratio showed high significance difference with high significant difference (p<0.000). Ferritin mean value was significantly higher 505.95 in the study group vs. 392.75 in the control group with high significant difference (p<0.000). Serum Transferrin mean value 188.5 in study group vs. 45.65 in the control group with significant difference (p=0.00). Hepcidin level was significantly lowered in the study group 27.02 vs. 172.55 in the control group with high significance (p<0.000). TIBC mean value 256.22 vs. 86.80 in the study group vs. 45.65 in the control group with high significant difference (p<0.000). Hepcidin production. The management of thalassemia involves avoiding the conflicting effect of increased erythropoiesis in contrast.

Hepcidin level was significantly lowered in the study group 27.02 vs. 172.55 in the control group with high significant difference (p=0.00). Hepcidin/ Ferritin ratio showed high significance difference (p<0.000) with mean value in the study group 0.056 vs. 4.75 in controls pointing to an incompatible levels of Hepcidin to iron overload status.

There was no correlation between serum Hepcidin and ferritin level; neither was between Hepcidin and serum iron, transferrin, Hblevel, reticulocyte count, age and sex in the study group.

Table (1): Complete blood picture in the study and the control groups.

<table>
<thead>
<tr>
<th>Item</th>
<th>Study group ‘n=40’</th>
<th>Control group ‘n=20’</th>
<th>p-value</th>
<th>***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Uncorrected WBC (X10^9) L</td>
<td>13.92±2.70</td>
<td>7.27±2.02</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
<tr>
<td>2- Corrected WBC (X10^9) L</td>
<td>10.45±1.85</td>
<td>7.27±2.02</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
<tr>
<td>3- Platelets (X10^12) L</td>
<td>316.58±93.86</td>
<td>270.20±79.04</td>
<td>p&gt;0.063</td>
<td>n.s</td>
</tr>
<tr>
<td>4- Reticulocyte count %</td>
<td>5.3±1.76</td>
<td>1.17±0.48</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
<tr>
<td>5- Hb (g/dl)</td>
<td>6.06±0.89</td>
<td>12.70±0.43</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
</tbody>
</table>

**: High significant correlation at p<0.01.

Table (2): Serum iron, TIBC, ferritin and transferrin in the study an the control group.

<table>
<thead>
<tr>
<th>Item</th>
<th>Study group ‘n=40’</th>
<th>Control group ‘n=20’</th>
<th>p-value</th>
<th>***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Serum iron ‘µg/dl’</td>
<td>256.22±88.61</td>
<td>86.80±21.49</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
<tr>
<td>2- TIBC ‘µg/dl’</td>
<td>317.42±77.95</td>
<td>392.75±33.83</td>
<td>p&gt;0.02</td>
<td>n.s</td>
</tr>
<tr>
<td>3- Ferritin ‘ng/ml’</td>
<td>505.95±91.75</td>
<td>45.65±17.84</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
<tr>
<td>4- Transferrin ‘mg/dl’</td>
<td>188.50±51.82</td>
<td>323.50±103.26</td>
<td>p&lt;0.000</td>
<td>***</td>
</tr>
</tbody>
</table>

**: High significant correlation at p<0.01.

Table (3): Serum Hepcidin and Hepcidin/Ferritin ratio in the study and the control groups.

<table>
<thead>
<tr>
<th>Item</th>
<th>Study group ‘n=40’</th>
<th>Control group ‘n=20’</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Hepcidin</td>
<td>27.02±13.41</td>
<td>172.55±53.97</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>‘µg/dl’</td>
<td>(16.0-82.0)</td>
<td>(55.0-235.0)</td>
<td></td>
</tr>
<tr>
<td>* Hepcidin/ Ferritin</td>
<td>0.056±0.03</td>
<td>4.75±3.03</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>ratio</td>
<td>(0.03-0.18)</td>
<td>(1.04-10.68)</td>
<td></td>
</tr>
</tbody>
</table>

**: High significant correlation at p<0.01.

Fig. (1): Negative correlation between Hepcidin and age (p<0.05). And there was no significant correlation with ferritin, serum iron and transferrin.

Discussion

β-thalassemia is the most prevalent chronic hemolytic anemia in Egypt (85.1%). A carrier status of 9-10.2% has been detected among 1000 normal random subjects from diverse geographical areas of Egypt [6]. β-thalassemia commonly found in, but not restricted to, Mediterranean countries creating a main public health problem. Iron overload is the commonest complication encountered in patients with ineffective erythropoiesis and peripheral hemolysis resulting in severe anemia that involves chronic blood transfusions [6]. Hepcidin deficiency is the main influencing factor for iron overload status in iron-loading anemias such as β thalassemia. Hepcidin deficiency results due to the suppressive effect of the erythropoietic activity on Hepcidin production. The management of β-thalassemia by tradition involves avoiding the adverse outcomes of disease causing transfusion therapy beside iron chelation therapy.

Hepcidin serum level assessment can be used to monitor patients with iron-loading anemias and identify the patients prone to iron overload complications and iron toxicity.

This study was designed to evaluate the conflicting effect of increased erythropoiesis in contrast...
to the effect of iron overloading on Hepcidin serum level and its correlation with iron status (serum iron, TIBC, ferritin and transferrin) in thalassemia major patients in comparison to healthy controls.

In the current study (β)-thalassemia major patients showed decreased concentrations of Hepcidin due to conflicting effects of erythropoiesis and iron overload. This agreed with Jawad et al., 2016 who reported that Hepcidin level was found to be significantly lowered in thalassemia patients as compared to controls. They also reported no correlation of Hepcidin with serum ferritin and Hb in patients [7].

Haghpanah et al., investigated 88 randomly selected patients with Thalassemia, there was no correlation between serum Hepcidin and ferritin levels in the two groups of patients. This is in agreement with what we reported in our study [8].

Chauhan et al., 2014 also reported no correlation between Hepcidin and ferritin. Ferritin was significantly elevated in (β)-TM children compared to controls. The mean serum Hepcidin/ferritin index in the study group was significantly lower than the control group, indicating inappropriate levels of Hepcidin to iron overload. The inappropriate Hepcidin levels may further contribute to iron overload enhancing iron toxicity. These findings are consistent with our study results [9].

Camberlein et al., 2007 and Kattamis et al., 2006 findings support the theory that the high erythroid drive exerts the dominant effect on Hepcidin production in thalassemia patients over the effect of iron load. This is in agreement with what we reported in our study [10-12]. Also Nemeth et al., 2006 reported that in patients with chronic anemias with hemolysis such as thalassemia who suffer from iron overload the measurements of urinary Hepcidin revealed that Hepcidin levels were markedly decreased, despite patients iron overload as perceived by their elevated serum ferritin levels [13].

Pasricha et al., reported that in (β)-thalassemia major patients, the Hepcidin-ferritin ratio was markedly <1 both pre-and post-transfusion, indicating suppression of hepatic expression that is out of proportion to the degree of iron loading and implying a suppressive effect from erythropoiesis. These findings are in consistent with our study results. But in contrast to our findings, their study reported, pretransfusion Hepcidin was positively correlated with hemoglobin and ferritin [14].

**Conclusion:**

In polytransfused (β-TM) patients the Hepcidin levels dynamically reflect the competing effects of erythropoietic activity and iron overload. However the strong suppressive effect of high erythropoietic activity on Hepcidin expression rules over the iron overload effect, resulting in suppression of Hepcidin out of proportion to degree of iron overload thus enhances iron organ damage and iron toxicity. Hepcidin serum level can be used to monitor patients prone to iron overload complications, also Hepcidin-ferritin ratio allows for tailoring iron chelation therapy in (β-TM) patients.

Hepcidin was markedly decreased in the study group with no significant correlation between serum Hepcidin and ferritin level as a marker of iron overload in thalassemia major. Hepcidin deficiency is the main contributing factor of iron overload in (β) thalassemia which results from a strong suppressive effect of the high erythropoietic activity on Hepcidin expression. Hepcidin-ferritin ratio was markedly depressed <1 in all (β) thalassemia major patients which indicating suppression of Hepcidin out of proportion with the degree of iron loading.

**References**

8. HAGHPANAH S., ESMAEILZADEH M. and HONAR N.: Relationship between serum Hepcidin and Ferritin levels in patients with Thalassaemia Major and Intermedia

دور تكوين كرات الدم الحمراء على مستوى الهيبسديدين في مرضى أنيميا البحر المتوسط الذين يتعرضوا لنقل الدم المتكرر

الهيبيددين عبارة عن هرمون بيتيدات وهو المتحكم الرئيسي في تنظيم الحديد في الجسم، فهو يزيد مستواه في حالات أنيميا زيادة الحديد. ولكن على الرغم من زيادة نسبة الحديد في الجسم في مرضى أنيميا البحر المتوسط (الاكتاسيميا الكبرى)، نجد أن مستوى الهيبسديدين منخفض. عدم تزايد مستوى الهيبسديدين هو العامل الرئيسي لزيادة نسبة الحديد في الجسم في مرضى البحر المتوسط وتحديث مضاعفات كتسامم الحديد وفشل الأعضاء.

لقد أجريت دراسة حالية في مركز نقل الدم أنيميا البحر المتوسط، مستشفى الأطفال الجامع، مستشفى جامعة النسياب، وتم عمل التحاليل الآتية لكل من المرضى وحالات الضابطة: صور دم كاملة، نسبة الهيبسديدين، أجماس مضادة للكروستين و، بروتين س حساس، نسبة الحديد في الدم، وشبيع الحديد، الفرتين والترانسفرين.

وتبين بعد إجراء هذه الدراسة العملية أن مستوى الهيبسديدين في المرضى منخفض جداً مقارنة بنمط الأصحاء مع عدم وجود علاقة بين مستوى الهيبسديدين والفرتين في الدم وكذلك عدم وجود علاقة بين الهيبسديدين ونسبة الحديد والترانسفرين والهيموجلوبين والعدد الشبكي.

في حين تم وجود علاقة ميكسي بين الهيبسديدين والعمر بالنسبة للمرضى.

تقليل الهيبسديدين هو العامل الموثوق إلى زيادة نسبة الحديد في الجسم في مرضى أنيميا البحر المتوسط (الاكتاسيميا الكبرى) التي تنتج عن التأثير المثبت القوي لعملية تكوين كرات الدم الحمراء على تكوين الهيبسديدين. ووجد أيضاً أن نسبة الهيبسديدين إلى الفرتين في المرضى من خفضه أقل من 1؟ مع الاصحة وحتى إذا كان التقليل الهيبسديدين لا يتناسب مع مدرج زيادة الحديد في الجسم.