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Original Research Article

Study of Serum Hepcidine Levels in Beta Thalassemia Major Patients

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Abstract:

Introduction: Thalassemia is a group of hereditary single gene disorders of haemoglobin chains. Excess iron in vital organs is known to cause impaired organ function and increased rates of morbidity and mortality. The regulation of iron by hepcidin is of clinical importance in thalassemia patients, as anemia often occurs along with iron overload. Our aim was to determine serum hepcidin level in beta thalassemia patients and healthy controls and to compare serum hepcidin level in beta thalassemia major patients and healthy controls.

Material & Methods: This was a case–control study. Total 35 diagnosed patients of β-thalassemia major were taken as cases, and 35 healthy, age and sex matched individuals were included as controls after taking informed consent. Samples were taken for determination of serum Hepcidin levels along with serum iron, serum ferritin and total iron binding capacity.

Observation and Results: 35 beta thalassemia major patients and 35 age and sex matched healthy controls were included in the study. Both groups comprised of 21 boys and 14 girls. Serum hepcidin level was found significantly low in Thalassemia patients as compared to the controls.

Discussion: Iron overload in β-thalassemia patients is a major cause of mortality and morbidity leading to a marked cellular damage and organ dysfunction. The increase in serum ferritin in β-thalassemia patients is mainly due to the suppression of hepcidin caused by ineffective erythropoiesis which then increases iron absorption.

Conclusion: Determination of hepcidin concentration is a useful indicator for high risk of iron toxicity in patients of beta thalassemia.

Keywords: Thalassemia, Hepcidin, Iron etc.

Introduction

Thalassemia is a group of hereditary single gene disorders which is caused by deficient or absent synthesis of haemoglobin chains. There is mutation in the β-globin chain resulting in β thalassemia. The World Health Organization (WHO) recognizes thalassemia as the world's most prevalent genetic disorder and it occurs in 4.4/10,000 live births globally [1].

Iron overload is the main cause of mortality and morbidity in patients with β thalassemia major. This harmful iron over load results in many complications like growth retardation, delayed sexual maturation and later on involvement of liver, heart and endocrine glands. Excess iron in vital organs is known to cause impaired organ function and increased rates of morbidity and mortality [2]. In transfusion-dependent thalassemia (TDT) patients, iron overload mainly occurs as a result of transfusions. In comparison, iron overload in cases of non-transfusion-dependent thalassemia (NTDT) can occur from increased intestinal absorption despite receiving occasional transfusions [3].

The regulation of iron by hepcidin is of clinical importance in thalassemia patients, as anemia often occurs along with iron overload. Hepcidin as a therapeutic target might help the management of iron overload in thalassemia patients [4,5]. Hepcidin is a peptide hormone produced in the

liver that plays a crucial role in iron homeostasis. Serum iron levels must be tightly regulated to ensure an adequate supply is available for hemoglobin synthesis during erythropoiesis, without allowing iron overload to occur in the body. Hepcidin decreases the level of iron by reducing dietary absorption and inhibiting iron release from cellular storage. Hepcidin production increases when iron levels rise above the normal range of 65 to 175 mcg/dL in males and 50 to 170 mcg/dL in females. Also, hepcidin is an acute-phase reactant, one of many molecules whose plasma concentration changes in response to inflammation. During states of acute or chronic inflammation, levels of hepcidin and other acute-phase reactants increase, leading to a decrease in serum iron levels as hepcidin levels rise. Hepcidin plays a vital role in iron homeostasis in humans, regulating iron absorption from the intestine and its recycling by macrophages. Hepatocytes are primarily responsible for the synthesis of hepcidin.

The final hepcidin protein has 25 amino acids, produced by the hepatocytes and regulates intestinal iron absorption and its distribution throughout the body. It is, therefore, emerging as an important diagnostic marker. Erythroferrone is a hormone produced by erythroblasts during erythropoiesis. It down-regulates the hepcidin gene expression. Hepcidin gene is also down regulated during hypoxic conditions. Both erythroferrone and hypoxia signal a demand for iron to construct new hemoglobin molecules [6,7,8].

When hepcidin levels become elevated, iron remains in its intracellular storage form, bound to the molecule ferritin. Hepcidin forms a connection between the immune system and the hematologic system.

Once released into circulation from hepatocytes, hepcidin regulates plasma iron levels through interactions with ferroportin-1. Ferroportin is an iron export transmembrane protein present in the macrophages and the enterocytes. When hepcidin binds to ferroportin, it causes the cell to target the hepcidin-ferroportin complex for lysosomal degradation. The cell types most affected by this interaction are duodenal enterocytes and reticuloendothelial macrophages. Duodenal enterocytes absorb dietary iron, and reticuloendothelial macrophages store iron recovered from degraded erythrocytes in the bone marrow, liver, and spleen. The degradation of ferroportin blocks iron absorption from enterocytes and iron mobilization from the macrophages [9,10]. We undertook this study with the aim to determine serum hepcidin level in beta thalassemia patients and healthy controls and to compare serum hepcidin level in beta thalassemia patients and healthy controls. This study will help in monitoring thalassemia patients and establishing the role of serum hepcidin in thalassemia.

Materials and Methods

This was case–control study conducted in the department of biochemistry after Ethical committee approval. Total 35 diagnosed patients of β-thalassemia major were taken as cases, and 35 healthy age and sex matched were included as controls, after taking informed consent. Samples were taken for determination of serum Hepcidin levels along with serum iron, serum ferritin and total iron binding capacity.

Inclusion Criteria:

1. Diagnosed cases of thalassemia major.
2. Subject in the age group of 5 – 15 years.
3. Verbal assent from the children in age group of 7 – 15 years in the presence of parents along with written informed consent from parents.
4. For the age group 5 – 6 years, written informed consent from parents.
5. Both male and female were included.

Exclusion criteria

1. Thalassemia Intermedia and Thalassemia minor
2. Hemolytic anemia
3. Bone diseases
4. Liver or Renal dysfunction
5. Cardiovascular dysfunction
6. H/O infection, surgery

A complete blood count (CBC) test was performed immediately with peripheral blood smears, and the samples were then stained with Wright–Giemsa stain. Hb was measured according to the sodium lauryl sulfate (SLS)-Hb method using an XN1000 SYSMEX machine. Serum hepcidin concentration was measured using a competitive enzyme-linked immunosorbent assay (cELISA) kit. Serum iron is determined by colorimetric method and serum ferritin by immune-turbidimetry method.

Observation and Results:

35 beta thalassemia major patients and 35 age and sex matched healthy controls were included in the study. Both groups comprised of 21 boys and 14 girls. Serum Iron and ferritin levels of control and test groups were compared along with their hemoglobin level (Table 02). Serum hepcidin level was found significantly low in Thalassemia patients as compared to the controls. Increased iron load in thalassemia causes decreased levels.

Table 1: Age wise distribution

Age group	Boys	Girls
05-07 years	03	02
08-10 years	04	03
11-13 years	04	04
11-12 years	05	02
14-15 years	05	03
Total	21	14

Table 2: Values of Haemoglobin, Serum Iron & Ferritin levels

Age group	Mean Hb. (Patients) gm%	Mean Hb (control) gm%	Mean S. Iron (Patients) (µg/dl)	Mean S. Iron(control) (µg/dl)	Mean S. Ferritin (Patients)ng/ml	Mean S. Ferritin (control)ng/ml
05-07 years	5.8	9.6	296.7	102.4	658	33.5
08-10 years	7.2	9.9	366.3	109.8	697	45.9
11-13 years	6.1	12.1	285.8	115.6	784	42.6
11-12 years	5.9	12.5	358.4	120.2	801	36.2
14-15 years	6.2	13.1	295.6	121.0	822	48.4
Mean value	6.24 gm%	11.44gm%	322.8 µg/dl	113.8 µg/dl	752 ng/ml	41.3 ng/ml

Table 3: Distribution Of Serum Hepcidin levels

Age group	Mean S. Hepcidin (Patients) ng/ml	Mean S. Hepcidin (control) ng/ml
05-07 years	2.02	7.48
08-10 years	1.78	7.93
11-13 years	2.16	8.86
11-12 years	2.01	9.02
14-15 years	1.52	8.71
Mean S. Hepcidin	1.9 ng/ml	8.4 ng/ml

Discussion:

Thalassemia complication arises not only due to ineffective erythropoiesis, but also due to iron overload due to increased gastrointestinal iron absorption. Iron overload in β-thalassemia patients is a major cause of mortality and morbidity leading to a marked cellular damage and organ dysfunction [11,12,13]. Excess iron deposition is associated with cardiac hypertrophy and dilatation, and it also damages thyroid, parathyroid and adrenal glands ((30,31, 32).

Hepcidin is a key regulator of iron homeostasis produced by hepatocytes and regulating intestinal iron absorption. The increase in serum ferritin level in β-thalassemia patients is mainly due to the suppression of hepcidin caused by ineffective erythropoiesis which then increases iron absorption [14]. The median serum hepcidin levels in the present study were lower than those reported in healthy adults.

Multiple previous studies have reported lower serum hepcidin levels in β-thalassemia patients with iron overload [15]. This outcome supports the claim that hepcidin down-regulation induced by thalassemia can lead to iron overload.

Another in vivo model revealed that the iron metabolism gene (Hfe) effectively down-regulated hepcidin in a mouse model of BTI (th3/+), while increasing incidences of anemia and iron overload [16]. On the other hand, th3/+ mice with increased hepcidin levels as a result of overexpression of hepcidin gene (Hamp1) showed limited iron overload and improved circumstances of anemia [17]. The percentages of patients requiring regular blood transfusion were significantly different among the three groups.

In addition, iron overload may have occurred from regular blood transfusions notably, serum hepcidin levels decreased due to high erythroid signals [18]. A previous study reported an impairment in normal β chain production in HbE/β-thalassemia patients.

The results also demonstrated that the decrease in HbA level was associated with a significant decrease in RBCs, Hb, PCV, MCV, MCH and MCHC levels.

Conclusion:

In our study we found that serum hepcidin levels were lower in patients when compared to controls. Determination of hepcidin concentration is a useful indicator for high risk of iron toxicity in patients of beta thalassemia. Hepcidin plays a central role in iron transport and utilization and is, therefore, an important marker of iron bioavailability along with its role in innate immunity through inflammatory cytokines. Phlebotomy is the mainstay of treatment for iron overload states, but a hepcidin agonist

could help alleviate the symptoms from the deficient natural hepcidin.

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