Suppression of Growth Differentiation Factor 15 Gene Expression by Curcumin in Patients with Beta-Thalassemia Intermedia

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SUMMARY

Background: β-thalassemia is an inherited disorder caused by defects in the synthesis of the beta-globin chain. One of the significant clinical complications in β-thalassemia intermedia is iron overload toxicity, which may be attributed to reduced levels of hepcidin. This reduction in hepcidin leads to increased absorption of iron in the intestines, ultimately resulting in iron overload. The objective of this study was to assess the impact of curcumin on the expression of growth differentiating factor-15 (GDF-15) and hepcidin genes in patients with beta-thalassemia intermedia.

Methods: This study was designed as a randomized controlled double-blind clinical trial. Prior to and after the intervention period with curcumin, a blood sample of 5 mL was collected from both the placebo and curcumin-treated groups for the assessment of hepcidin and growth differentiating factor-15 gene expression.

Results: This study revealed a significant reduction in the expression of growth differentiating factor-15 in the curcumin group compared to the placebo group during the 3-month treatment period. Furthermore, curcumin supplementation led to a remarkable 10.1-fold increase in the levels of hepcidin in the curcumin group compared to the placebo group.

Conclusions: The results of this study show that curcumin administration increases the mRNA levels of hepcidin in whole blood of thalassemia intermedia patients and supports the idea that curcumin could be a potential treatment to reduce suppression of hepcidin in thalassemias and other iron-loading anemias.


KEYWORDS

β-thalassemia, GDF-15, hepcidin, curcumin, clinical trial

INTRODUCTION

Beta thalassemia intermedia (β-TI) is a specific subtype of beta-thalassemia that is characterized by being non-transfusion-dependent. This means that individuals with...
β-TI do not require regular blood transfusions, which sets it apart from other forms of beta-thalassemia where regular transfusions are typically necessary for management [1-3]. The disease may arise from a combination of one or two mutated alleles and an abundance of redundant genetic variations within the HBB gene [1,4]. Iron overload is a notable factor that can exacerbate the clinical symptoms of β-TI [4-8]. The primary cause of this phenomenon is an increase in iron absorption. Two important regulators of iron intake are hepcidin and growth differentiating factor 15 (GDF15). Hepcidin, a hormone secreted by hepatocellular cells, inhibits iron transportation by binding to ferroportin in the duodenum. Conversely, activation of GDF-15, triggered by ineffective erythropoiesis, anemia, and hypoxia, can disrupt the iron absorption process in the gastrointestinal tract by suppressing hepcidin levels. Ultimately, this leads to iron overload [4-11]. Curcumin, an innocuous herbal compound, has been found to have significant beneficial effects on all types of thalassemia, particularly intermedia. In addition to its well-known characteristics such as being an antioxidant, anti-inflammatory, and cancer-preventive agent, curcumin also possesses iron chelation properties. This means that it can bind to and remove excess iron from the body. This iron chelation ability of curcumin may help modulate proteins involved in iron metabolism, further contributing to its positive effects on thalassemia [12-16].

In 2021, a clinical trial study was conducted to comprehensively review the effects of curcumin on patients with beta-thalassemia intermedia. The results of this study were published in prestigious international journals, generating significant interest among researchers. Subsequently, the study was extended to investigate the effects of curcumin on the genome of beta-thalassemia intermedia patients, building upon the findings of the previous study [11,16].

Therefore, the purpose of this study is the ability of curcumin to suppress the growth-differentiating factor-15 gene expression in beta-thalassemia intermedia patients.

MATERIALS AND METHODS

This study is a double-blind randomized clinical trial conducted at the outpatient thalassemia clinic in Shiraz, Iran. It aims to investigate the potential of curcumin in suppressing the expression of the growth-differentiating factor-15 (GDF15) gene in individuals with beta-thalassemia intermedia. Ethical approval was obtained from the ethics committee of Shiraz University of Medical Sciences, and informed consent was obtained from all participants prior to sample collection. The study enrolled participants randomly, and an IRCT registered code number (IRCT2019090204468N1) was assigned. The intervention involved a three-month treatment period with curcumin. The expression levels of both the GDF15 gene and hepcidin gene were evaluated before and after the treatment. Sample collection was performed at the clinic.

Study population

Inclusion criteria

Thirty patients with β-thalassemia intermedia aged between 20 and 35 years were included in this study. The diagnosis of β-thalassemia intermedia was confirmed by an expert hematologist based on transfusion independence in thalassemia.

Exclusion criteria

Exclusion criteria were: patients with combined diabetes, smoking, hypertension, cardiovascular diseases, active infection, hepatitis C virus, human immunodeficiency virus, positive serum hepatitis B surface antigen, other iron chelating drugs for patients (the exception to Deferasirox), patients who have undergone cholecystectomy, individuals with other hemoglobinopathies or sensitivity to curcumin ingredients, and patients currently undergoing blood transfusion.

Study design

All subjects included in the study were adult males, aged between 20 and 35 years. The patients were divided into two groups: one group received curcumin capsules, while the other group received a placebo. A total of 15 patients were randomized into each group. The curcumin capsules and placebo were provided by Karen Pharmaceutical Company. It is important to note that both groups were only using Deferasirox as an iron chelator. This decision was made to eliminate any interference factor with the type of iron chelator and to assess the impact of the curcumin capsules on the patients’ body iron status. Additionally, the participants in the study were allowed to continue their main treatment for beta-thalassemia intermedia, and they were not restricted from following their doctor’s prescribed interventions for the condition. The curcumin supplementation was provided as an additional intervention alongside their regular treatment. During the three-month study period, the patients were asked to limit their intake of turmeric ingredients in their diet. Additionally, they were monitored closely with follow-up appointments scheduled every two weeks to receive their curcumin capsules and to record their blood pressure and anthropometric measures.

RT-PCR analysis

To evaluate the hepcidin and GDF-15 mRNA expression, reverse transcriptase-polymerase chain reaction (RT-PCR) was done. Total RNA was extracted from human whole blood using the TRIzol LS Reagent (Invitrogen, USA) and then was purified by DEPC water. Purified RNA was stored in the refrigerator at -70°C un-
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til converting RNA to complementary DNA (cDNA). After RNA purification and determining its concentration using the Nanodrop (Thermo-Scientific, USA), cDNA was acquired from 1 µg of RNA using cDNA synthesis kit (Thermo- Fisher, Scientific, USA) and amplified by polymerase chain reaction (PCR) for hepcidin, GDF-15, and beta-glucuronidase (GUSB). Quality control of cDNA production was checked by 1% agarose gel electrophoresis to confirm that just a single PCR product was amplified and there was no corresponding production to primer-dimer pairs. Oligonucleotides used as PCR primers for amplifying hepcidin, GDF-15, and GUSB (as internal control gene) cDNA were as follows:

Gene GDF15:
Forward: 5´-TACGAGGACCTGCTA-3´;
Reverse: 5´-ACHTCTGGGCTGATATC-3´
Gene HAMP:
Forward: 5´-CTGACCAGTGGCTCTTTT-3´;
Reverse: 5´-TGGCTCCAGCTCTGTCC-3´
Gene GUSB:
Forward: 5´-TCTGCTCACACCAATCTT-3´;
Reverse: 5´-GGCTTCTGATACTTCACCA-3´

All of the probes were designed to exon junctions or intron spans in the completely processed message to inhibit the contaminating by genomic DNA. Internal control that did not include cDNA were run in parallel. PCR amplification was executed in triplicate using a 96-well plate format. The PCR cycling conditions were as follows: 95°C for 10 minutes followed by 40 PCR cycles (95°C for 15 seconds, 61°C for 1 minute, and 72°C for 2 minutes).

Statistical analysis
To analyze the results, 2-ΔΔCt method was used. Hepcidin and GDF-15 gene expression fold change was calculated in each group rather than GUSB gene as a control blank gene. Values in the figures are expressed as mean ± SEM. Mann-Whitney U test was applied to detect differences in the participants’ gene expression between curcumin and placebo groups.

RESULTS
The study included 30 patients diagnosed with thalassemia intermedia who were randomly divided into two groups: the curcumin supplement group (n = 15) receiving a daily dose of 1,500 mg of curcumin, and the placebo group (n = 15). Two patients from the control group were excluded from the study due to non-referral. Ultimately, a total of 28 patients successfully completed the study. The clinical characteristics of the study population are reported in another study [16]. There were no significant differences in baseline characteristics of the study groups (age, BMI, GDF-15 expression or hepcidin expression).

Figure 1 compares fold change in the expression of GDF-15 and hepcidin between curcumin and placebo groups. There was a significant decrease in GDF-15 expressions in the curcumin group during the 3-month treatment period compared to the placebo. In addition, curcumin increased the levels of hepcidin 10.1-fold in the curcumin group compared to placebo, although this change was not statistically significant.

DISCUSSION
Hepcidin, a circulatory peptide mainly expressed in the liver, is a key regulator of iron metabolism and hemostasis. Hepcidin inhibits release of iron from enterocytes, hepatocytes, and macrophages into the plasma by promoting the internalization and degradation of a transmembrane iron exporter, ferroportin. Consequently, the presence of hepcidin leads to a reduction in the availability of iron in the plasma. [17]. Hepcidin expression is down-regulated during iron deficiency and hypoxia and up-regulated during iron-overload and inflammation. Although blood transfusion induces iron loading in many patients with thalassemia; studies have shown that hepcidin levels are decreased in individuals with β-thalassemia syndromes due to an erythropoietic mechanism [18,19]. It is known that hypoxia and undefined signals from massive erythroid proliferation and ineffective erythropoiesis downregulate hepcidin production in patients with thalassemia, despite systemic iron overload [18]. Indeed, novel therapies aiming to increase hepcidin expression might prove more effective than iron chelation alone in preventing iron overload toxicity in β-thalassemia syndromes. This iron overload toxicity has a major impact on quality of life, which induces numerous endocrine diseases, hepatic cirrhosis, cardiac failure, and even death [20]. Curcumin has a pronounced effect on iron metabolism [11,15,21, 22]. Previous studies reported the iron-chelator activity of curcumin [21-24]. However, no data is available on the impact of curcumin on hepcidin mRNA expression in thalassemia intermedia patients. We therefore tested whether curcumin affected levels of hepcidin in thalassemia intermedia patients. Our randomized placebo-controlled study demonstrated that daily administration of 1,500 mg curcumin for 12 weeks increased the whole blood mRNA levels of hepcidin 10.1-fold compared to placebo in thalassemia intermedia patients, although this change was not statistically significant. There is limited evidence for the effects of curcumin on expression of hepcidin in thalassemia patients. In the only available report, curcumin (1,000 mg/day) treatment for 12 weeks increased strongly suppressed concentration.
of hepcidin slightly by 12.3% in β-thalassemia major patients, although these changes were not statistically significant [15]. On the other hand, a randomized placebo-controlled study demonstrated that a single intake of 6 grams of curcumin could significantly decrease serum hepcidin levels in healthy volunteers [25]. Chin et al. conducted a study to investigate the impact of a 6-month dietary supplementation with 0.2% curcumin on hepcidin expression in mice. The results of the study demonstrated that, in comparison to the control group, curcumin led to a decrease in liver hepcidin expression. [26]. Jiao et al. reported that curcumin can moderately exhibit iron chelating activity in vivo. Six-month intervention with 2% curcumin in iron depleted mice that received 5 mg iron/kg diet, significantly reduced hepcidin level [22]. Nevertheless, hepcidin level remained unchanged in mice that received iron at ≥ 12 mg/kg diet or those that received lower dose of curcumin [22].

Patients with thalassemia intermedia, who have a milder form of anemia, experience significantly heightened gastrointestinal iron absorption and subsequent iron accumulation in the absence of blood transfusions, this phenomenon is believed to be attributed to the inappropriate suppression of hepcidin through an erythropoietic mechanism [19]. Moreover, the serum from individuals with β-thalassemia has been found to inhibit hepcidin mRNA expression in hepatoma cells [27]. The results suggest that overexpression of GDF-15, which is caused by an expanded erythroid compartment, plays a role in the development of iron overload in thalassemia syndromes by inhibiting hepcidin expression [10]. GDF-15, a member of the transforming growth factor-β (TGF-β) superfamily, showed increased expression and secretion during erythroblast maturation [10]. Currently, there is no available data on the impact of curcumin on GDF-15 expression in humans. Our study showed that curcumin significantly decreased the whole blood mRNA levels of GDF-15 compared to placebo in thalassemia intermedia patients. This observation suggests that curcumin could be a potential treatment to reduce GDF-15 overexpression in thalassemia syndromes.

Our study has several limitations. Firstly, the effects of curcumin on serum hepcidin levels must be assessed in patients which will be our goal for future studies. Secondly, the determination of a safe dose of curcumin was used in this study, which may have resulted in moderate effects on the expression of the hepcidin gene, possibly due to the low oral bioavailability of curcumin. Therefore, new formulations of curcumin with enhanced oral bioavailability should be tested in further studies.
In summary, our findings indicate that curcumin administration increases the mRNA levels of hepcidin in whole blood of thalassemia intermedia patients. This supports the idea that curcumin could be a potential treatment to reduce suppression of the hepcidin gene expression in beta-thalassemia and other iron-loading anemias. This study showed that curcumin may increase hepcidin expression via the GDF15 downregulation. Curcumin’s safety profile and affordability make it an appealing option for global use. However, in order to assess the potential beneficial or adverse effects of curcumin in individuals, further comprehensive studies are needed, particularly in patients with iron-loading anemias and hemoglobinopathy, such as thalassemia intermedia. These studies should focus on determining the optimal dosage and therapeutic regimen of curcumin before proceeding to confirmatory studies.

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All authors have no conflict of interest to declare.

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