Leptin and ghrelin serum concentrations in thalassemia major and intermedia patients and normal subjects

Hamdollah Karamifar 1, Maryam Bahmanyar 2, Vincenzo De Sanctis 3, Mehran Karimi 4
1 Pediatric Endocrine Department – Shiraz University of Medical Sciences – Shiraz (Iran)
2 Pediatric Department – Shiraz University of Medical Sciences – Shiraz (Iran)
3 Department of Reproduction and Growth – Pediatric and Thalassaemia Unit – Ferrara (Italy)
4 Hematology Research Center – Shiraz University of Medical Sciences – Shiraz (Iran)

Abstract

Endocrine dysfunctions related to iron overload, such as delayed puberty, short stature and hypogonadism, lead to major problems in thalassaemic patients. Leptin, a polypeptide with 167 amino acids produced by white adipose tissue, is a hormone which reduces appetite and increases energy consumption by affecting the central nervous system. Ghrelin, a peptide hormone produced by the stomach, stimulates growth hormone release via growth hormone secretagogue receptor.

To evaluate leptin and ghrelin serum levels in thalassemia, 50 normal subjects, 50 β-thalassaemia major patients and 50 thalassaemia intermedia patients were randomly selected. Mean leptin concentration was 2.6 ± 1.2 μg/L in patients with β-thalassaemia major, and 2.8 ± 2.4 μg/L in patients with β-thalassaemia intermedia. These values appeared to be significantly lower than controls (9.2 ± 2.9 μg/L) (p < 0.001). Mean ghrelin concentrations were 1042.1 ± 275.9 pg/mL and 989.3 ± 275.5 pg/mL in β-thalassaemia major and intermedia groups, respectively. This value in thalassaemia major appeared to be significantly higher compared to the control group (876.9 ± 384.3 pg/mL) (p < 0.01).

There was a positive correlation between serum leptin concentration and body mass index (BMI) in thalassaemia major and intermedia. Leptin levels were significantly lower in thalassaemia major patients with short stature compared to controls, but no correlation was found between ghrelin levels and short stature in any of the three groups. These results suggest that one of the endocrinopathies affecting thalassaemic patients is adipose tissue dysfunction and it may be that low leptin levels play a role in the endocrine dysfunction in these patients. These findings need to be confirmed in further studies.

Key words: leptin, ghrelin, thalassemia.
Introduction

Presently, low weight and short stature in thalassaemic patients have become a major healthcare problem, as thalassaemia is the most common genetic disorder worldwide. Thalassaemia major (TM) manifests as a progressive hemolytic anemia caused by a defect in both beta globin genes. This severe anemia – hemoglobin (Hb) is usually between 3 to 7 mg/dL – leads to severe hepatosplenomegaly and growth disorders and most patients will depend on recurrent transfusions by the age of two. In thalassaemia intermedia (TI), patients carry a mutation in beta globin genes but are still capable of maintaining Hb between 6 to 10 mg/dL, so that they will not need recurrent transfusions, except in case of infections or surgery (1, 2). Endocrine disorders such as short stature, delayed puberty and hypogonadism, caused by iron overload in thalassaemic patients are major problems indicating endocrine system dysfunction (3).

Leptin is a 16 kD polypeptide with 167 amino acids. This hormone is secreted by adipose tissue and has a major role in long term maintenance of body weight. It can reduce the appetite and increase energy consumption by affecting the hypothalamus. Leptin inhibits neuropeptide Y which is an appetite stimulator. It also leads to gamma MSH expression which also reduces the appetite via hypothalamus (4-8).

Ghrelin is a 28 amino-acid peptide secreted from the stomach which leads to growth hormone (GH) release. Ghrelin serum concentration increases before food intake and decreases after that (9). Ghrelin's effect on appetite, but not on GH release, depends on intact vagus outflow (10-12). Many hormones regulate serum level of ghrelin such as PYY3-36, which reduces ghrelin and suppresses appetite.

The aim of this study is to find a possible relationship between leptin and ghrelin levels and complications of thalassaemia major and intermedia such as short stature and low weight, in order to find a potential therapy – such as recombinant leptin hormone – to improve the health status of these patients.

Methods and materials

The study was performed from January 2008 to July 2009 at the Namazi Hospital of Shiraz, Iran. The study population consisted of 50 patients with TM referred to the Thalassaemia Department of Shahid Dastgheib Hospital of Shiraz, and 50 patients with TI referred to the Motahari Clinic of Shiraz, selected by using random cluster sampling methods. Fifty healthy children (matched for age and gender), selected randomly among the students of four educational zones of Shiraz city, served as the control group. All patients and healthy children had normal liver function tests. Written informed consents were taken for the study from all parents.

Height was measured using a stadiometer, and weight was measured by Seca scale. Body mass index (BMI) was calculated using the formula (13):

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\text{BMI} = \frac{\text{wt (kg)}}{\text{Ht (m)}^2}
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Patients were referred to Namazi Hospital Research Center for collection of blood samples. The most recent results of the patients' hemoglobin and serum ferritin were recorded. Fasting blood samples (5 ml) were collected at 8 AM in Namazi Endocrine Research Center. The samples were centrifuged and the sera were maintained at -70 C. for tests.

Leptin serum concentration was measured via radioimmunoassay using Leptin Kit (DRG Instruments GmbH, Germany) and ghrelin serum concentration was determined via ELISA using Ghrelin Kit from the same company.

Variables in this study included gender, age, height, weight, BMI, leptin concentration, ghrelin concentration, Hb and serum ferritin. The study was approved by Research Council of Shiraz University of Medical Sciences.

Chi square test was performed for investigating relationships between qualitative variables. To study the relation between ghrelin and leptin with BMI in the three groups controlling for age, partial correlation test was used.

One way ANOVA test was used to compare a quantitative variable in more than two groups and Least Statistical Difference was used to compare couple tests. BMI was studied in the three groups using ANOVA, considering age as a bias factor. All statistical analyses were performed by SPSS 15 Software and p value < 0.05 was considered as significant.
Results

The thalassaemia major group (Group 1) included a total of 50 patients: 24 female and 26 male with an average age of 14.2 ± 4.2 years. There were 50 patients in thalassaemia intermedia group (Group 2): 21 female and 29 male with an average age of 14.0 ± 4.8. The average age of 50 normal controls (Group 3) (20 female and 30 male) was 15.5 ± 2.0 years (Table 1). There was no statistically significant difference in mean age or gender among the studied groups.

Of the total of 150 children in this study, 19 patients (38%) in the first group, 17 patients (34%) in the second group and 10 subjects (20%) in the control group were underweight (BMI < 5th percentile) and there was a significant difference in the percentage of underweight subjects in groups 1 and 2 in comparison with group 3 (p < 0.05) (Table 1). Short stature (height for age < 5th percentile) was present in 58% of Group 1, 30% in Group 2 and 14% in the control group. The differences were statistically significant (p < 0.001) (Table 1). Mean leptin serum level was 2.6 ± 1.2 µg/L in Group 1, 2.8 ± 2.4 µg/L in Group 2 and 9.2 ± 2.9 µg/L in Group 3. As shown by one way variance analysis, leptin levels in groups 1 and 2 were significantly lower than in Group 3 (p < 0.001) (Table 2). Mean ghrelin serum level was 1042 ± 275.9 pg/mL in Group 1, which was significantly higher (p < 0.01) than in the control group (876.9 ± 384.3 pg/mL). In thalassaemia intermedia (Group 2), mean serum ghrelin level was 989.89 ± 275.5 pg/mL which, according to one way variance analysis, was not significantly different from Groups 1 or 3.

Covariance analysis was performed for comparison of BMI among the three groups controlling for age. Mean BMI was 17.0 ± 2.7 in Group 1, 17.8 ± 2.6 in Group 2 and 19.2 ± 2.7 in Group 3. The difference between Groups 1 and 3, and also that between Groups 2 and 3, was statistically significant (p < 0.001); therefore, BMI in TM and TI patients was significantly lower than in healthy controls (Table 2). A significant correlation was found between serum leptin level and BMI controlled for age in all groups (p < 0.004, p < 0.002, p < 0.001 respectively): with decreasing BMI, serum leptin level also decreased. No significant correlation was found between ghrelin serum level and BMI using partial correlation tests.

The relation between leptin serum level and short stature was shown to be significant in Group 1 (TM) using T-test (p < 0.03), indicating that among TM patients (Group 1), those with short stature had a lower leptin level. No correlation was found between serum leptin level and short stature in the other two groups. No significant relation was found between ghrelin serum level and short stature in any of the three groups. Mean serum ferritin level was 1955.76 ng/ml in Group 1, 688.72 ng/ml in Group 2, and 98 ng/ml in group 3, that is, significantly higher in TM compared to TI and the control group (p < 0.001).

Discussion

As seen in the distribution of short stature among the study groups (Table 1), short stature in thalassaemic patients is more prevalent than in normal subjects. In addition, mean serum leptin level in thalassaemia major and intermedia patients is significantly lower than in healthy children (Table 2). Moreover, in major thalassaemia patients with short stature, mean leptin serum level was significantly lower than in normal controls and a signifi-
cantly relationship was observed between short stature and leptin serum level. In a study in 1999 to investigate leptin serum concentration, 162 thalassaemia major patients were compared to 138 healthy controls. Mean leptin serum level in male subjects was 2.69 ± 1.23 in the thalassaemic group and 6.86 ± 2.71 in the control group. In female subjects levels were 6.37 ± 2.9 in the thalassaemic group and 9.37 ± 5.2 in the control group. In both cases the difference was found to be statistically significant (p < 0.0005 and p < 0.05, respectively) (14). In another study in Athens on 40 major thalassaemia patients (15) it was found that leptin serum level was lower in these patients. The results of these previous studies (14-15) indicate a relationship between leptin serum levels and BMI and a lower leptin level among thalassaemia patients in comparison with a normal population. Our study has also confirmed this fact. It seems that adipose cells of thalassaemia patients are not able to produce adequate leptin which might be due to deposition of iron in these cells. Therefore, the defect in adipose tissue function in thalassaemic patients can be considered as an endocrine system dysfunction, although it seems other factors may interfere in the decrease of serum leptin level in thalassaemic patients. As a patient is more underweight with less fat tissue, the ability to produce leptin production would be lower (14).

According to other researches, there are different contributing factors to short stature in thalassaemia, including hypothyroidism, hypoparathyroidism (16, 17), adrenal insufficiency (18) and pancreatic dysfunction (19). In our study there was direct correlation between short stature and serum leptin levels in TM patients. We believe that low leptin may be a factor of short stature in these patients but further studies are needed to investigate the possible relationship. Serum ghrelin levels in the TM group were higher compared to controls which might be due to a compensatory response to growth retardation or a partial resistance to ghrelin that leads to its increased level (20). The results of this study did not show any relation between ghrelin serum concentration and short stature, which is consistent with previous studies. In a study, in 2006, in Turkey by Camurdan MO et al. on 17 children with constitutional growth retardation, 19 with familial short stature and 11 normal subjects, serum concentrations of ghrelin, IGF-1 and IGFBP-3 were measured. The study showed that serum ghrelin levels in children with familial short stature were higher than in controls (20). But according to a previous research, height and weight are independent to ghrelin level (20). So the Authors postulated that the negative relation found between height and ghrelin level is because of a compensatory increase in ghrelin level in response to short stature. In China, Zou CC et al. performed another study on 117 patients with short stature due to growth hormone deficiency, 81 with idiopathic short stature and 125 normal children as controls. The aim of the study was to explore serum ghrelin concentration and polymorphism of the ghrelin/obestatin gene (21). The results indicated that in patients with growth hormone deficiency ghrelin serum level was significantly lower than in the control group, which suggests a probable important role for ghrelin in growth hormone secretion and growth control. In this study, sexual maturation was not investigated; this factor, as well as others, should be considered in future researches.

References


