The Relationship Between Ghrelin and Iron Metabolism In Beta Thalassemia Major Patients

Keywords
ghrelin, iron metabolism, hepcidin, HIF2α, Beta thalassemia major

Abstract

Introduction

Studies in beta thalassemia major (β-TM) patients have shown that the responses of HIF2α, hepcidin and ferroportin molecules to high iron levels are impaired. In recent years, studies conducted in patients with iron deficiency anemia have investigated the relationship between ghrelin hormone and iron metabolism. In this study, we aimed to contribute to the etiopathogenesis of this disease by examining the changes in ghrelin hormone levels in patients with β-TM.

Material and methods

Fifty-two β-TM and 23 controls were included in our study. Blood counts, routine biochemical parameters, HIF2α, hepcidin and ghrelin levels were studied in blood samples taken from the volunteers.

Results

Erythrocyte indexes, serum bilirubin, iron, unsaturated iron binding capacity, total iron binding capacity and ferritin levels showed significant differences between two groups ($p<0.05$) . There was no significant difference between two groups for serum HIF2α and hepcidin levels. When two groups were compared, ghrelin levels were found to be significantly higher in patients ($p<0.05$). When the correlation between parameters was examined in all subjects, a weak positive correlation was found between ghrelin and HIF2α ($r=0.263$) ($p<0.05$) and a significant positive correlation was found between ghrelin and ferritin ($r=0.417$) ($p<0.05$).

Conclusions

Our study showed that there is a positive correlation between ghrelin and ferritin levels. Elevated ghrelin levels in patients with β-TM may have an important role in regulating impaired iron metabolism. Molecular level studies are needed to determine synthesis pathways.

The Relationship Between Ghrelin And Iron Metabolism In Beta Thalassemia Major Patients

Introduction

Beta thalassemia major (β-TM) is a congenital hemoglobinopathy with complications affecting many organs. Chronic anemia due to ineffective erythropoiesis, hypoxia, frequent blood transfusions, and high serum iron levels despite iron chelation treatment are the most common problems seen in these patients. As it is known, the ferroportin-hepcidin pathway has an important role in the regulation of iron metabolism. Ferroportin-1 molecule is a protein that provides the release of iron molecule from tissues such as intestine and liver into blood according to the body's needs. Hepcidin and hypoxia induced factor 2 alpha (HIF2α) molecules are involved in the regulation of ferroportin-1 molecule levels. In the literature of iron metabolism, it is stated that hepcidin hormone increases in periods when serum iron level is high, and it causes a decrease in iron level by providing the degradation of ferroportin [1]. HIF2α molecule increase serum iron level by increasing the gene expression of the ferroportin molecule, especially in the intestinal cells [2]. There are studies showing that high iron levels in beta thalassemia patients are related to increased iron absorption due to impaired hepcidin-ferroportin pathway and chronic hypoxia, especially from the small intestines [3].

Ghrelin hormone is synthesized in many organs, especially in fundus of stomach, hypothalamus, pituitary, thyroid, small intestine, liver, kidney, heart, pancreas, and gonads. Regulating appetite, releasing growth hormone, anti-insulinergic effect, and vasodilation are stated as physiological effects of the ghrelin hormone [4, 5]. Studies conducted in recent years have emphasized the relationship between serum ghrelin and iron levels. In most of these studies, the effects of ghrelin on iron metabolism were investigated in iron deficiency anemia [6, 7], There are few studies examining the interaction with ghrelin hormone in patients with high iron levels (hemoglobinopathy, etc.). The effects of ghrelin hormone on gonadal development in beta thalassemia patients were examined and it was emphasized that gonadal developmental failure may occur due to ghrelin hormone deficiency [8]. There are very few studies on ghrelin hormone and iron metabolism in the literature. In a study, it was reported that ghrelin peptide increased the expression of ferroportin-1 molecule in the liver and spleen [9].

In this study, we investigated the relationship between serum ghrelin, hepcidin, HIF2α and ferritin levels in patients with β-TM. We aimed to contribute to the etiopathogenesis of this disease by examining the changes in ghrelin hormone levels in patients with β-TM.

Materials and Methods

Fifty-two (24 males, 28 females) patients with β-TM and 23 (9 males, 14 females) healthy controls were included in this study. The volunteers included in the study group did not have any chronic diseases such as diabetes mellitus, hypertension, thyroidal and renal diseases. In addition, iron deficiency anemia was accepted as an exclusion criterion in the control group. Venous blood samples were taken from all volunteers in the morning after 10-12 hours of fasting. The blood samples were taken from the patient group before transfusion. Each patient is on a regular transfusion program (every 2-4 weeks). The hemogram parameters (RBC-red blood cells, Hb-hemoglobin, Hct-hematocrit, MCV-mean corpuscular volume) from the blood samples were measured with the XN 1000 autoanalyzer (Sysmex Corp. Japan) using impedance and flow cytometry methods. Measurements of serum iron, total iron binding capacity (TIBC), and unsaturated iron binding capacity (UIBC), total bilirubin and direct bilirubin levels were performed spectrophotometrically with a fully automatic AU 5800 analyzer (Beckman Coulter Inc., USA). Serum ferritin levels were measured by chemiluminescence method on a fully automatic DxI 800 analyzer (Beckman Coulter Inc., USA). Serum ghrelin, HIF2α, hepcidin levels were measured by ELISA method using the USCN ELISA kit (Cloud-clone corp Wuhan, China).

Statistical Analysis

Descriptive statistics were presented with mean, standard deviation, median, and range of distribution values. The Kolmogorov-Smirnov test was used to determine the distribution of the collected data for each variable considered in the study. In the analysis of the difference between the data of the patient and control groups, the Unpaired sample t test was used for the data that fit the normal distribution, and the Mann-Whitney U test was used for the data that did not fit the normal distribution. Spearman test was used for correlation analysis between the two groups. $p<0.05$ was considered statistically significant.

Results

A total of 75 subjects, including 52 patients with β-TM [24 men (46.1%)], 28 women (53.8%))and 23 healthy controls [(9 men (39.1%), 14 women (61.9%)] were included in this study. The mean age of the participants was 29.73 ± 6.78 years in the patient group and 33.26 ± 8.22 years in the control group. There was no significant difference between the groups in terms of age and gender. When the erythrocyte indexes of the patient group and the control group were compared; in the patient group RBC levels were found to be significantly higher, Hb, Hct and MCV levels were found to be significantly lower ($p<0.05$). In the comparison of the biochemical parameters of the patient group and the control group, serum iron, ferritin, total bilirubin, and direct bilirubin levels were significantly higher in the patient group ($p<0.05$), while UIBC and TIBC levels were significantly lower in the patient group ($p<0.05$) (Table 1).

In the comparison of serum ghrelin, HIF2α and hepcidin parameters of the patient and the control groups; ghrelin levels were found to be significantly higher in the patient group ($p<0.05$). There was no significant difference in HIF2α and hepcidin levels between the patient and control groups (Table 2). When the correlation between ghrelin-HIF2α, ghrelin-hepsidin, and HIF2α-hepcidin were examined, a weak positive correlation was found between ghrelin-HIF2α ($r=0.263$) ($p<0.05$) in all subjects (Table 3) (Figure 1), no significant correlation was found between these parameters in the control group (Table 3) and a weak positive correlation was found between ghrelin-HIF2α ($r=0.285$) ($p<0.05$) in the patient group (Table 3). When the correlation between ghrelin-iron, ghrelin-UIBC, ghrelin-TIBC, and ghrelin-ferritin were examined, a positive correlation was found between ghrelin-ferritin ($r=0.417$) ($p<0.05$) in all subjects (Table 4) (Figure 2). Furthermore, a weak positive correlation was found between HIF2α-UIBC ($r=0.291$) ($p<0.05$) in the patient group. No significant correlation was found between other parameters in the patient and control groups. The correlation analysis of ghrelin, HIF2α and hepcidin parameters with iron, UIBC, TIBC and ferritin parameters are shown in Table 4.

Discussion

In β-TM, RBC indexes show hypochromic microcytic anemia. Consistent with the other studies in the literature, hypochromic microcytic anemia was found in the patient group of our

study as well [10-13]. Increased erythrocyte production and hemolysis in thalassemia patients result in increased hemoglobin breakdown products. In some studies, the bilirubin results of thalassemia patients were found to be high [14–16]. In our study, total bilirubin and direct bilirubin levels were found to be higher than the control group, which is consistent with the literature. Iron overload occurs in patients with β-TM due to repeated blood transfusions, ineffective erythropoiesis, progressive iron absorption from the gastrointestinal tract, and reticuloendothelial iron recycling. Regular transfusions can double the rate of iron accumulation [17]. Consistent with other studies in the literature, in our study, the serum iron level of the patient group was found to be statistically significantly higher than the control group [18–20]. Ferritin levels were found to be high due to iron load in thalassemia [21–26]. In our study, serum UIBC and TIBC levels were found to be significantly lower and ferritin levels were found to be significantly higher in the patient group, consistent with the literature.

Hepcidin expression increases in iron overload and inflammation, and decreases in iron deficiency and hypoxia states [26-28]. However, hepcidin production was found to be low despite iron overload in thalassemia studies. The possible reason for this is the inhibition of hepcidin gene expression by factors such as erythroferron (ERFE) [29–31]. ERFE is synthesized by erythroblasts upon erythropoietin (EPO) stimulation. ERFE is released into the circulation and attenuates hepcidin signal in response to iron. When anemia causes hypoxia, other mediators such as Platelet-Derived Growth Factor-BB (PDGF-BB) released by different cell types also suppress hepcidin [32]. It is known that low serum hepcidin level increases intestinal iron absorption and decreases iron stores in macrophages, leading to iron overload. Many studies have shown that low serum hepcidin level in patients with β-TM can lead to increased levels of iron absorption and iron overload. As a therapeutic target, hepcidin may help manage iron overload in β-TM patients [17, 26]. Because some studies in the literature have shown that a moderate increase in hepcidin expression in β-thalassemic mice limits iron overload, reduces the formation of reactive oxygen species and improves anemia [33, 34]. In our study, serum hepcidin levels in the β-TM patient group did not show a statistically significant difference than control group. When we examined the correlation between hepcidin and iron parameters, no significant correlation was found in both groups. Contrary to expectations, the reason why hepcidin was not suppressed in the patient group may be the blood transfusion which keeps the patients' Hb at optimum levels and the administration of a controlled iron chelation therapy. The measurement of hepcidin at normal values, even in samples taken before transfusion, may be an indication that high hepcidin levels have been

reached after treatment. This may reflect the effectiveness of treatment in our patient group. Under normal conditions, high hepcidin levels, which we expect in case of iron overload, can be used as an indicator of effectiveness of response to treatment.

HIF2α has an important role in enterocyte iron uptake and is the primary regulator of EPO production. [35]. HIF1α is activated in response to acute hypoxia, while HIF2α is associated with chronic hypoxia [36]. Intestinal HIF2α is a critical regulator of iron absorption in cases of iron deficiency, erythropoiesis, and hepcidin deficiency [37, 39]. Intestinal HIF2α is critical in patients with β-thalassemia, as anemia has been shown to induce intestinal hypoxia and excessive iron absorption significantly contributes to iron overload. In the literature, intestinal HIF2α has been shown to be elevated in diseases with iron overload [37, 38]. In our study, when serum HIF2α levels in the thalassemia major patient group were compared with the HIF2α levels in the control group, no significant difference was observed. This may be because of hepcidin levels were not suppressed in patient group. This may make us to think that the ongoing treatments of the patient group are effective. In addition, the mean Hb levels of our patients were measured as 8.9 g/dL. These Hb levels may not have caused an increase in the synthesis of HIF2α. When we examined the correlation between HIF2α and iron parameters, no significant correlation was found in the control group. In the patient group, we found a significant correlation between HIF2α-UIBC. The reason for this may be that UIBC decreases as a result of increased iron demand despite iron load in β-TM patients and that increased iron demand is a stimulus for HIF2α.

There are studies in the literature investigating the relationship between ghrelin and anorexia seen in iron deficiency anemia (IDA) [40–42]. It has been shown that both ghrelin and iron content decrease in IDA and significant positive correlation was found between two parameters. In addition, it has been shown that both ghrelin and iron levels are significantly lower in prepubertal children with IDA compared to controls. Another study showed that hepcidin and serum ghrelin, which are the main regulators of systemic iron homeostasis, increased with iron treatment in children with IDA, suggesting that iron treatment has an important role in hepcidin and ghrelin synthesis. [9, 41, 42]. These findings suggest that iron has a role in ghrelin synthesis.

Investigation of ghrelin levels in patients with beta thalassemia is important to learn the place of ghrelin in iron metabolism. In the literature, there is a study investigating the effect of ghrelin on puberty and reproductive function in beta thalassemia patients. In this study, it is

stated that serum ghrelin levels were lower compared to the control group. Researchers stated that low ghrelin level is related to growth and development retardation during puberty in beta thalassemia patients [8]. In another study, Karamifar et al. investigated the leptin and ghrelin levels and their relation to short and lean body structure in β-TM and thalassemia intermedia patients. Ghrelin levels were found to be significantly higher in β-TM patient group compared to the control group. They stated that the reason for this may be a compensatory response to growth retardation or a partial resistance that causes an increase in ghrelin levels [43]. Unfortunately, we could not find any study in the literature investigating ghrelin levels in beta thalassemia patients, except for these two studies. In our study, we found that serum ghrelin levels were significantly higher in the patient group (Table 2). On the contrary, Kashanian et al. found lower levels of ghrelin in their study [8]. On the other hand, the study by Karamifar et al. found higher levels of ghrelin in the patient group, which supports our study [43]. The different results between these two studies may be due to the difference in age distribution of the beta thalassemia patients included in the study.

Although we found that there are many studies in the literature about changes in HIF2α, hepcidin, ferroportin levels in iron absorption in patients with beta thalassemia, we could not find any study examining the relationship between ghrelin and iron metabolism. Investigating the relationship between ghrelin and iron metabolism, Lou et al. conducted their studies in cell culture in mice. This study showed that ghrelin increased the expression of GHSR1α, GOAT, hepcidin, ferritin light chain and ferroportin1 in the spleen of mice [9]. This study, which showed that ghrelin increased ferroportin expression, made us to think that ghrelin has a role in iron metabolism. As known, HIF2α plays a role in the synthesis of ferroportin. HIF2α and ferroportin levels have an important role in iron absorption in patients with beta thalassemia.

In this study, we tried to contribute to the metabolism of iron absorption in patients with beta thalassemia major by examining the relationship between HIF2α, hepcidin and ghrelin levels. When we examined the correlation between ghrelin and iron parameters (iron, UIBC, TIBC and ferritin) in our study, no significant correlation was found in the control group. In the patient group, we found a significant positive correlation between serum ghrelin and ferritin (Table 4). This finding made us to think that there is a relationship between ghrelin synthesis and ferritin levels. When we compare our findings with the literature, there are many publications about iron deficiency anemia in which ferritin and ghrelin levels increase with

iron treatment. The low ghrelin levels in iron deficiency anemia and the increase in ghrelin levels with subsequent treatments suggested that iron is required for ghrelin synthesis.

Conclusion

We think that hepcidin, HIF2α and ghrelin can be examined in more details by cell culture studies using intestinal cells to explain the iron absorption metabolism in patients with β-TM. Also further clinical studies are needed in larger patient groups.

Working Limitations

The patient group participating in our study is an adult group between the ages of 18-45 and under treatment. Therefore, hepcidin, HIF2α, ghrelin levels may not be sufficient to show the main effects in β-TM disease. Our study was made from blood samples taken before blood transfusion planned according to the treatment protocol in patients with β-TM. Therefore, the data on hepcidin HIF2α and ghrelin levels coincide with the period when patient values are the most irregular. Since blood was not taken from the same people again after the treatment, we cannot comment on the change of these parameters with the treatment. Since our study was based on serum samples, we could not demonstrate the interaction of hepcidin, HIF2α and ghrelin at the cellular level.

Ethics

Ethics Committee Approval: The study was approved by the Health Sciences University Antalya Training and Research Hospital Clinical Research Ethics Committee (No: 10/18).

Informed Consent: This study was approved by the local ethics committee in accordance with the principles of the 2008 Declaration of Helsinki. 'Informed consent was obtained from all subjects and/or their legal guardian(s).

Conflict of interest: The authors declare no competing interests.

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Hospital

Data Availability: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Abstract

Objective: Studies in beta thalassemia major (β-TM) patients have shown that the responses of HIF2α, hepcidin and ferroportin molecules to high iron levels are impaired. In recent years, studies conducted in patients with iron deficiency anemia have investigated the relationship between ghrelin hormone and iron metabolism. In this study, we aimed to contribute to the etiopathogenesis of this disease by examining the changes in ghrelin hormone levels in patients with β-TM.

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Conclusion: Our study showed that there is a positive correlation between ghrelin and ferritin levels. Elevated ghrelin levels in patients with β-TM may have an important role in regulating impaired iron metabolism. Molecular level studies are needed to determine synthesis pathways.

Keywords: Beta thalassemia major, iron metabolism, ghrelin, HIF2α, hepcidin

PARAMETERS	CONTROL n=23 Median (Minimum-Maximum)	PATIENT n=52 Median (Minimum-Maximum)
RBC (10^6/mm^3)	3.31 (2.64-4.23)	4.65 (4.22-6.28)*
Hb (g/dL)	14 (8-16.6)	8.9 (7.6-11.6)*
Hct (%)	41.4 (29.4-50.8)	26.9 (21.5-35.5)*
MCV (μm^3)	88.7 (66.1-94.2)	82.6 (70.1-86.9)*
Iron (µg/dL)	89 (32-610)	212 (39-395)*
UIBC (µg/dL)	249 (13-413)	34 (0-269)*
TIBC (µg/dL)	348 (248-623)	249 (62-473)*
Ferritin (µg/L)	30.7 (5-122.8)	370.2 (89-10783)*
Total Bilirubin (mg/dL)	0.63 (0.31-1.48)	2.28 (0.86-7.07)*
Direct Bilirubin(mg/dL)	0.10 (0.04-0.34)	0.47 (0.15-0.96)*

Table 1: Comparison of CBC and biochemical parameters of patient and control groups

Mann-Whitney U test was used to compare the groups. Statistical significance was defined as *P*-value<0.05.

RBC-red blood cells, Hb-hemoglobin, Hct-hematocrit, MCV-mean corpuscular volume, UIBC-unsaturated iron binding capacity, TIBC-total iron binding capacity.

PARAMETERS	CONTROL n=23 Median (Minimum-Maximum)	PATIENT n=52 Median (Minimum-Maximum)	P Value
Ghrelin (pg/mL)	198.38 (49.32-263.33)	216.83 (60.36-273.53)	0.018*
HIF2α (ng/mL)	0.72 (0.15-1.87)	1.03 (0.04-4.26)	0.078
Hepcidin (pg/mL)	105.29 (29.38-597.04)	107.96 (7.18-1139.72)	0.629

Table 2: Comparison of Ghrelin, HIF2α and Hepcidin parameters of the patient and control groups

Mann-Whitney U test was used to compare the groups. Statistical significance was defined as P-value<0.05

HIF2α –hypoxia induced factor 2 alpha

PARAMETERS	ALL SUBJECTS n=75 r	CONTROL n=23 r	PATIENT n=52 r
Ghrelin-HIF2α	0.263*	0.108	0.285*
Ghrelin-Hepcidin	-0.085	-0.023	-0.128
HIF2α-Hepcidin	0.120	-0.061	0.163

Table 3: Correlation analysis between Ghrelin, HIF2α and Hepcidin parameters.

Spearman correlation test was used in the correlation analysis between groups. Statistical significance was defined as * P-value<0.05

HIF2α –hypoxia induced factor 2 alpha

PARAMETERS	ALL n=75 r	CONTROL n=23 r	PATIENT n=52 r
Ghrelin-Iron	0.207	-0.32	0.10
Ghrelin-UIBC	-0.151	0.13	0.10
Ghrelin-TIBC	-0,041	0.27	0.094
Ghrelin-Ferritin	0.417*	0.012	0.411*
HIF2α- Iron	-0.004	-0.288	-0.086
HIF2α-UIBC	0.089	-0.051	0.291*
HIF2α-TIBC	-0.076	-0.166	-0.008
HIF2α-Ferritin	0.188	-0.230	0.099
Hepcidin- Iron	-0.068	0.118	-0.212
Hepcidin-UIBC	-0.147	-0.288	-0.085
Hepcidin-TIBC	-0.188	-0.186	-0.172
Hepcidin-Ferritin	0.115	0.136	0.121

Table 4: Correlation analysis of Ghrelin, HIF2α and Hepcidin parameters with Iron, UIBC, TIBC and Ferritin parameters.

Spearman correlation test was used in the correlation analysis between groups. Statistical significance was defined as * P-value<0.05

UIBC-unsaturated iron binding capacity, TIBC-total iron binding capacity, HIF2α-hypoxia induced factor 2 alpha

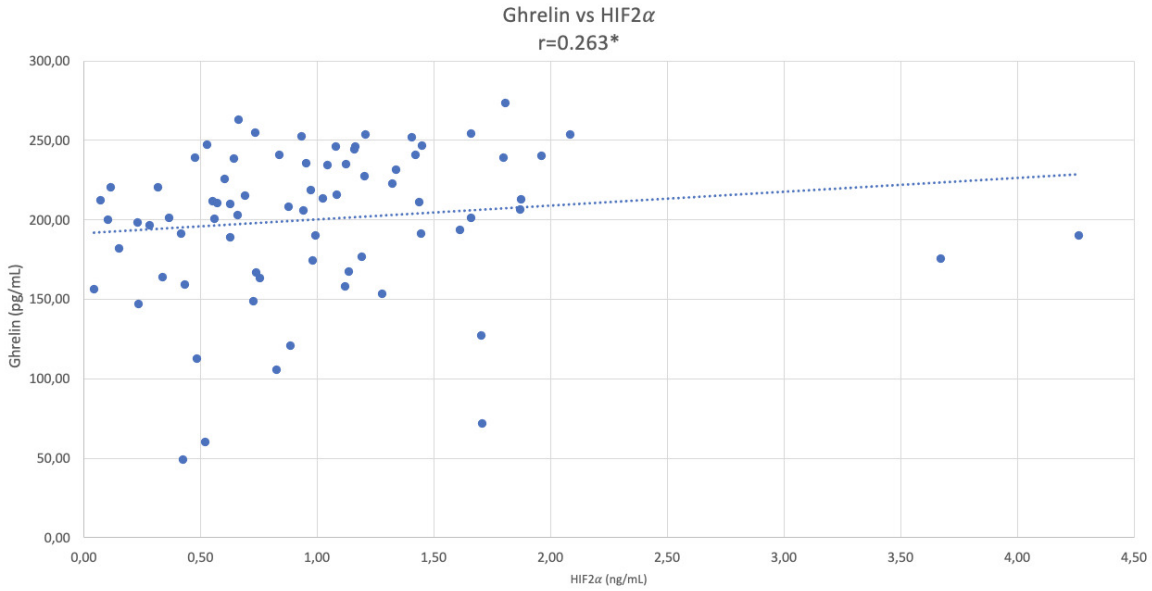

Figure 1: Correlation analysis between Ghrelin and HIF2α in all subjects. Spearman correlation test was used in the correlation analysis between groups. Statistical significance was defined as * P-value<0.05 HIF2α –hypoxia induced factor 2 alpha

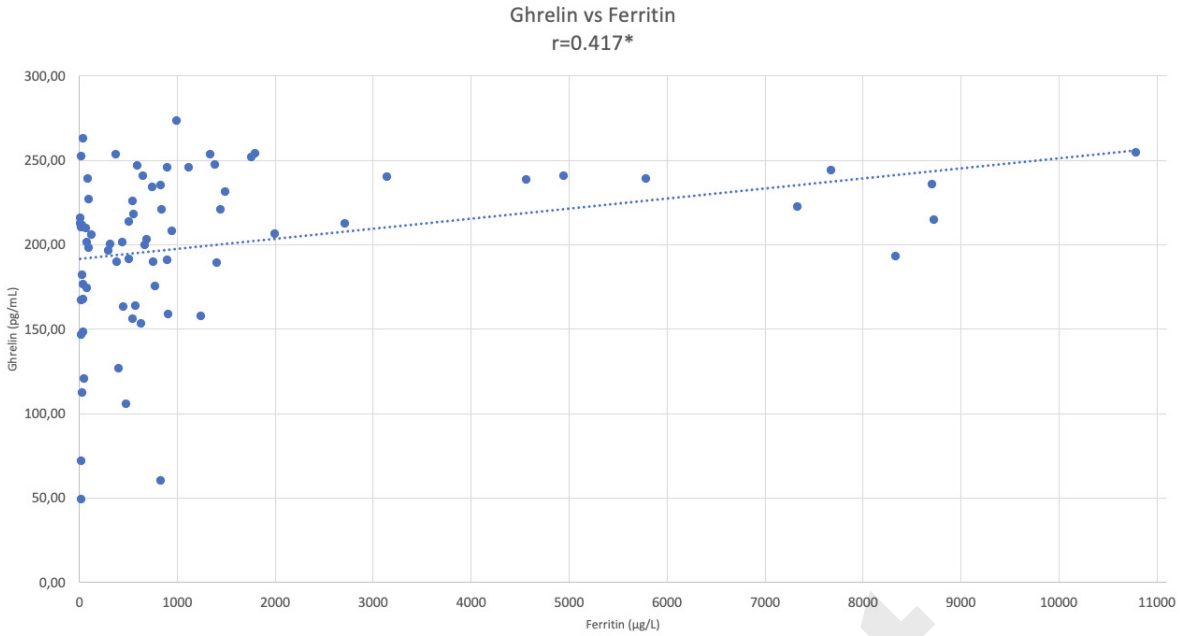

Figure 2: Correlation analysis between Ghrelin and Ferritin in all subjects. Spearman correlation test was used in the correlation analysis between groups. Statistical significance was defined as * P-value<0.05