Correlation between Irisin Levels and growth hormone in thalassemia major in Baghdad city

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Abstract
The Background and objective In spite of recent advances in iron overload, elevate iron deposition in pituitary gonadotropic cells keep on one of the main complications in thalassemic patients, mainly decrease in hypogonadism, is usually detected during puberty. Early diagnosis and treatment are crucial for normal pubertal development and to reduce the complications of hypogonadism. The risks and benefits of hormonal replacement therapy, especially regarding the thromboembolic event, remain a challenge for providers caring for thalassemic patients.: The aim of this study: To evaluate correlation between irisin levels with GH deficiency (GHD) Methods: There were 138 participants in the research study,48 control persons and 90 β thalassemia major patients. Irisin and GH values were calculated in order to evaluate correlation between them, and calculated levels were estimated using enzyme-linked immunosorbent assay (ELISA) kit. Results: The levels of Irisin and GH in the patients' group and in the normal control found to differ significantly (0.039) and ($p < 0.001$) respectively. Significant moderateNegative correlation was seen between the level of Irisin and GH ($p < 0.003$). Conclusions: We report that GHR leads to an increase in irisin levels, strongly related to a decrease in GH. These data confirm the favorable effects of GHR in children significant moderate negative correlation between irisin and GH in beta thalassemia major make Irisin good early predictable marker for growth development. Growth deficiency resulting from iron deposition in the pituitary gonadotrope is commonly found in thalassemia major patients. Early diagnosis and treatment are crucial for normal pubertal development and to reduce the complications of hypopituitarism.
Keywords: β-thalassemia major, Irisin, GH, pituitary, iron

1. Introduction

Thalassemia refers to a group of inherited diseases character-ized by decreased or absent synthesis of normal globin chains. The direct consequence is an imbalance of the alpha and beta globin chain synthesis that results in anemia from ineffective erythropoi-esis and hemolysis (Cazzola, 2022). The term thalassemia major refers to the severe form that is often associated with life-long transfusion dependent anemia (Gordon et al, 2021).
Thalassemia is a disorder of haemoglobin, in which the production of natural hemoglobin is partially or completely impaired. Alpha- and beta-thalassemia has spread widely from the Mediterranean region to Southeast Asia and the Middle East (Atmakusuma& Lubis, 2021). In Iran, the prevalence of carriers is higher in the north around the Caspian Sea and also near the Persian Gulf in the south (about 10%). Although beta-thalassemia is much more common than alpha thalassemia, alpha-thalassemia is still one of the main health problems in Iran. Iron deficiency and beta-thalassemia carriers are the two main causes of microcytosis, and the distinction between these conditions is of therapeutic importance as well as important implications for thalassemia carrier screening (Saad & Mazin, 2021). Most patients with alpha and beta-thalassemia (minor, intermediate, and major thalassemia) had normal and high serum ferritin levels. Especially in thalassemia major and intermediate iron overload is a major problem with serious consequences. The long-term effect of high serum ferritin in all types of alpha-thalassemia as well as in beta-thalassemia minor, which may sometimes be high, is unknown, (Rujeerapaiboon, et al, 2021).
Irisin, the secreted product of the fibronectin type III domain 5 protein, is a recently identified myokine that was first isolated from muscle tissue in 2012 by Boström et al.Data suggest that irisin may play an important metabolic role in regulating adipose tissue metabolism by converting white to brown adipose tissue and may reduce the risk of obesity and diabetes (). Brown adipose tissue activity is high in the pediatric population, especially in subjects with a lower body mass index (BMI); thus, a reduction in irisin may play a role in the pathogenesis of childhood obesity These data indicate that irisin may be a possible link between skeletal muscle and adipose tissue. Significantly, irisin improves glucose tolerance in mice and circulating irisin levels correlate with glucose tolerance and insulin resistance in humans. Because irisin plays a role in both adipose tissue and glucose metabolism, two targets of GH action, changes in irisin may mediate effects of GH deficiency (GHD) and GH replacement (GHR) on these endpoints (Gordon 2021) . (Al-

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Rayahi, Sanyi & Alhussein, 2019; Wang, et al 2022). (Chen et al, 2018). Specifically, GHD is characterized by altered body composition (reduced muscle mass and increased adiposity) as well as metabolic alterations), and GHR may result in a reversal of these effects

A direct interplay between irisin and GH has been documented in nonmammalian species. Indeed, fish irisin acts directly at the pituitary level to inhibit GH transcript expression via multiple signaling pathways. However, it is not known whether GHD and its replacement can affect irisin levels or whether changes in irisin levels in GHD and replacement are associated with the known changes in body composition and glucose homeostasis. . (Al-Rayahi, Sanyi & Alhussein, 2019; Wang, et al 2022).

The aim of this study was to evaluate circulating irisin levels in a cohort of children with GHD at diagnosis, the changes in them during GHR, and any associations of irisin with body composition and metabolic parameters. Based on data indicating that irisin may be a link between muscle and adipose tissue and possible effects of irisin on glucose homeostasis, we hypothesized that GHD is associated with lower irisin levels, which would increase after GHR, and is associated with changes in body composition and metabolic endpoints. . (Al-Rayahi, Sanyi & Alhussein, 2019; Wang, et al 2022).

Lacking adequate excretory mechanisms, thalassemic patients receiving a blood transfusion (usually 1 mg of iron per 1 mL of blood) inevitably experience significant iron overload. Normally, iron is bound to transferrin and transported to bone marrow and tissue, where transferrin receptor takes up iron and stores it as ferritin. Transferrin saturation is usually maintained at 10–50%, and less than 1% of total body iron is found in the blood. (Mahdi et al, 2020).

As a consequence of iron overload in thalassemic patients, either from blood transfusion or excessive iron absorption, trans-ferrin is fully saturated and non-transferrin-bound iron (NTBI) is found excessively in the blood. Instead of using the transferrin receptor, (Garbowski et al, 2021; Silva et al, 2022).

Iron deposition in the anterior pituitary gland can be demonstrated beginning in the first decade of life, but clinical manifestations are usually not evident until the onset of puberty. At the earlier stage, only a diminished gonadotropin reserve with intact gonadotropin pulse was observed. There may be an asymptomatic phase of pituitary siderosis before hypogonadism occurs. Later, the gonadotropin reserve significantly diminishes, with markedly reduced spontaneous pulsatile gonadotropin activity which may lead to ir-reversible damage of the HPG axis. However, additional studies are still required before the natural history can be conclusively determined. (Mallio et al, 2020; Verberckmoes et al, 2022).

Hepcidin is a protein that in humans is encoded by the HAMP gene. Hepcidin is a key regulator of the entry of iron into the circulation in mammals. During conditions in which the hepcidin level is abnormally high, such as inflammation, serum iron falls due to iron trapping within macrophages and liver cells and decreased gut iron absorption. (Jameel et al, 2019) This typically leads to anemia due to an inadequate amount of serum iron being available for developing red blood cells. When the hepcidin level is abnormally low such as in hemochromatosis, iron overload occurs due to increased ferroportin mediated iron efflux from storage and increased gut iron absorption. (Hassan et al, 2019).

Aim of study

Detect correlation between Irisin and GH in thalassemia major in Baghdad city.

2. Methods

This case-control study was carried out on patients who attended Iraqi center of albatool, Baghdad Teaching Hospital of the period from November 2021 to June 2022.

Patients with beta thalassemia major, were selected in our study. In this study, 138 patients successfully completed the course in this study. All patients (69 male &69 female), whose ages ranged from 19 to 31 years were diagnosed as having beta thalassemia major based on previous medical reports, laboratory tests and clinical examination by consultant hematologist. The results of those patients were compared with (48) healthy age– matched (19-31) years individuals (24 males and 24 females) as a control group to compare with patients.

The control group subjects were selected as healthy individuals without a history of any physiological or pathological disease, current or previous any types of anemia (iron deficiency, sickle cell…etc) and not suffering from hematological or hypertension depending on previous medical reports and laboratory investigation. Venous blood sample (5ml) were taken from each patient in the morning at 6:00 a.m–10:00 a.m just prior to the start of the blood transfusion session. Blood samples of the patients were obtained from arteriovenous fistula (Vascular assay) to ensure that a pre blood transfion sample has been obtained.

Venous blood samples were also taken from the control group by means of disposable syringe. Whole blood was taken in EDTA tubes for Hb estimation while the rest of blood was centrifuged for 10 minutes at 3000 RPM to obtain serum which was stored at (–40oC) until time of assay.

3. Results

Descriptive statistics

The following tests were performed for all patients and control groups and evaluated by ELIZA laboratory methods:ferritin, irisin and GH.

Colorimeter analysis was used for iron detection

All statistical analysis was performed, using

descriptive statistics and independent t-test. Pearson's Chi-square test was used to calculate the association between the variables. P < 0.05 were considered to be statistically significant. All statistical analyses were conducted using SPSS version 22.0 (SPSS Inc, Chicago, Illinois, U.S.A.).

Descriptive statistics mean and standard deviation were used to compare of Iron, Ferritin,IRISIN and GH between patients and control groups . The Spearman correlation test was used to test the correlation BETWEEN Irisin and GH

Table 1 Shows that the levels of Iron, Ferritin, Irisin and GH in the patients group and in the normal control found to differ significantly P value (0.001), (0.002) (0.001) and(0.039) respectively.

Groups Variables	Controls(n=48) Mean ±S. D		Patients(n=90) Mean ± S. D		t-test	P- Value	C.S
Age	51.55± 16.14		53.18± 11.00		0.510	0.612	P>0.05 (NS)
Serum iron	21.3±6		90± 4.14		19.6	0.001	P<0.01 (HS)
Serum ferritin	99± 33		777± 101		18.3	0.002	P<0.01 (HS)
GH (g/dL)	89.9±1.06		22.13±0.41		59.546	0.000	P<0.01 (HS)
S. Irisin(mg/dL)	10.50±0.39		44.6±0.99		31.97	0.033	P<0.05 (S)
Gender	No.	(%)	No.	(%)	X^2=2.052 P =0.152 (NS)		
Male	24	50%	45	50%			
Female	24	50%	45	50%			
Total	48	100%	90	100%			

Table 2 shows that Significant moderate Negative correlation was seen between the level of Irisin and GH (p < 0.003).

Table (4-2): correlation between LH concentration (MMOL/dl) and FSH in thalassemia patient.

Correlations			
		Gh p	irisinp
Gh	Pearson Correlation	1	-.300**
	Sig. (2-tailed)		.004
	N	90	90
Irisin	Pearson Correlation	-.300**	1
	Sig. (2-tailed)	.004	
	N	90	90
**. Correlation is significant at the 0.01 level (2-tailed).			

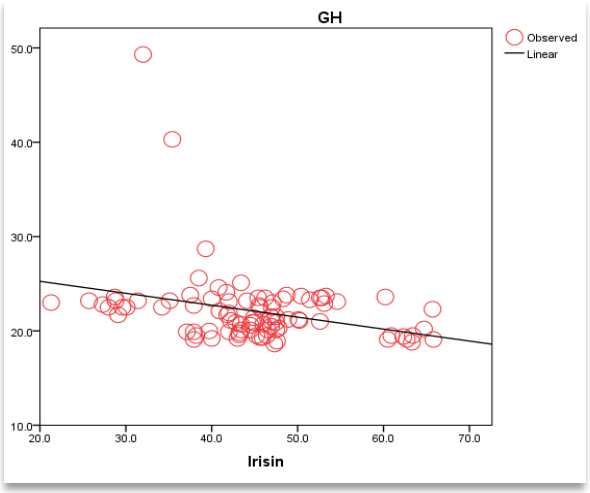

Figure 1 Shows moderate negative correlation between Irisin and GH

4. Discussion

Iron overload is the principal cause of morbidity and mortality in β-thalassemia with or without transfusion dependence. Iron homeostasis is controlled by the hepatic peptide hormone hepcidin. Hepcidin regulates dietary iron absorption, plasma iron intensities, and tissue iron distribution. (Nemeth & Ganz, 2021). A deficiency in this hormone is the main or contributing factor of iron overload in iron-loading anemias such as β-thalassemia. (Hennigar et al, 2021) (Rasool, Hussein, & Taha, 2019).

Table1 displays the range of serum ferritin levels observed in patients. The mean serum ferritin level was 777 (SD± 101) ng/ml. The serum ferritin level increases as the frequency of blood transfusion and the age of the patient increases.

Transfused iron is deposited first within the reticuloendothelial cells prior to parenchymal iron loading within the heart and liver. However, as in primary iron overload, the majority of morbidity and mortality ultimately results from progressive heart and liver failure. Cazzola, M. (2022).

Effective management of iron overload requires frequent evaluation of the body iron stores (Jameel et al, 2019). There is, therefore, a need for quantitative, non-invasive methods for measuring body iron that are safe, accurate and readily available. The iron status of the body in overload conditions can be assessed by different methods. Serum ferritin measurement, although easy to perform frequently, offers variable results, still at present, no other serum test is a better predictor. (Saad, etal (2021).

The liver is the major site of iron overload, containing 70% or more of body iron content. Liver iron correlates closely with total body iron in transfusional iron overload and total body iron. Estimation of direct liver iron concentration is the most accurate method of estimation of iron overload. But in our set up this method was not available. Indirect method with serum ferritin level measurement is reliable, easy to perform, low cost, and had no side effects. In

any event, when serum ferritin is greatly increased, whatever the reason, there is cause for concern and an increasingly aggressive iron chelation treatment should be given. (Nashwan,etal (2022).

This study demonstrates that patients with thalassemia with GHD show lower irisin levels than control subjects and that GHR is able to increase these levels. (Mahdi, Sanyi & Al-Rayahi, 2019).

Very few studies have evaluated irisin in patients' thalassemia with GHD. Taking these aspects into account, the evaluation of circulating irisin could be of interest in these patients.

Contradictory data have been found regarding the production of irisin in human tissues. In some studies, the amount of muscular mass has been reported as the main predictor for circulating irisin, whereas in other studies adipose mass has been found to be the major factor influencing irisin levels. Irisin has been found to be significantly related to body composition and insulin sensitivity, although the role of irisin in obesity and its metabolic consequences are controversial, especially regarding its relationship with insulin resistance and BMI. (Al-Rayahi, Sanyi & Alhussein, 2019; Wang, et al 2022).

In our study, we found lower irisin levels in the GHD group than in control subjects, these data confirmed the relationship between irisin and GH. Indeed,

In thalassemia children, irisin levels have been found to be negatively related to GH. providing evidence regarding the role of irisin in Muscles growth, and in the authors' hypothesis increased irisin levels can be interpreted as an adaptive response that compensates for the decreasing growth hormone and metabolic disturbances associated with low growth in children (Hasan etal 2019). Conversely, Al-Daghri et al. () showed a positive correlation between serum irisin levels and GH

The results of the current study are in agreement with the study by Binay et al., who showed that in thalassemia children irisin levels were negatively correlated with GH that support our result that has been shown in table 1 p value 0.004 highly significant negative correlation between GH and irisin level see figure 1. Indeed, it has been suggested that circulating irisin levels could be an indicator of body fat mass. Similarly, body fat mass was the main independent factor associated with irisin levels in a large cohort of Korean adolescents (Pasiakos 2021) and in patients with hypopituitarism (Silva etal 2022). Recently, in a cohort of children with obesity, lower mean irisin concentrations were found compared with children thalassemia with normal weight, with a significant inverse correlation between irisin and BMI and WC, and children with thalassemiaexhibited lower irisin concentrations than those without metabolic syndrome. The authors of this study concluded that irisin might be a biomarker for metabolic syndrome in prepubertal children (Chen etal 2018), and these data are concordant with our study.

In agreement with other studies, we found that irisin levels were correlated with BMI and WC, without association with metabolic parameters (Malia 2020).

To the best of our knowledge, the only study on the relationship between GHR and irisin levels was performed in patients with Turner syndrome. Wikiera et al. (Mahdi 2020) evaluated the anthropometric and metabolic data of 36 young girls before and after supraphysiological doses of GHR (0.05 mg/kg/d). After GHR, an increase in irisin was observed, with a concomitant positive association between irisin and IGF-I levels. These results are in agreement with those of our study. Indeed, we found a significant increase in irisin levels after 12 months of GHR. In addition, we documented a significant correlation between irisin levels and hormonal parameters (i.e, stimulated GH levels at baseline and IGF-I during GHR). Recent data suggest a relationship between irisin and the GH/IGF-I axis. Indeed, in the skeletal muscle of 15 obese men with reduced GH levels, the local expression of fibronectin type III domain 5 was associated with mRNA expression of IGF-I (Gordon etal 2021), although fasting irisin levels were found to be similar in normal and hypopituitaric adults with GHD (Cazzola 2022).

In our study, no correlation was found between irisin and metabolic parameters. An independent association of irisin with fasting blood glucose levels has been documented in adults (Al-Rayahi 2019) and children (AL tikriti etal 2019). However, we did not find a significant correlation between irisin and glucose levels probably because fasting glucose, HbA1c, and HOMA-IR, even if they increase, almost always remain within the normal range during GHR. In addition, in children with GHD an increase in insulin levels, and consequently in HOMA-IR, or a decrease in ISI, during GHR probably does not indicate a real condition of insulin resistance, and there may be a failure of basal indexes to reliably assess insulin sensitivity. Indeed, the increase in HOMA-IR documented in our study, as well as the trend to decrease in ISI, may represent an expected consequence of GH-induced hyperinsulinemia (Hassan etal 2019). Similarly, we did not find a significant change in lipid profile during GHR or a correlation between irisin and circulating lipids.

To support the controversial involvement of irisin in metabolic disturbances, in a group of children with thalassemia from the southern Italy, Nigro et al. (Srole etal 2021) documented that irisin was significantly higher in obese children than in control children and was inversely correlated with adiponectin but had no direct association with insulin resistance.

In our opinion, the discrepancies among the studies may be related to the lack of evaluation of the physical activity levels of the enrolled subjects, and this point represents the main limitation of our study, in addition to the lack of information about nutritional behavior. Indeed, irisin secretion is dependent on the nutritional status and physical activity of the body (Rujeerapaiboon 2021), and a lifestyle intervention program is associated with a

rise in irisin levels in obese children although other reports on changes in irisin in response to exercise have been discordant. Indeed, initial data about the improvement in irisin synthesis and secretion after physical exercise have not been confirmed by other studies (Atmakusuma 2021). The acute rise in exercise-induced irisin is most likely accounted for by skeletal muscle release, and basal irisin levels could be accounted for by adipose tissue production); this could explain the discrepancies among the studies. In addition, the majority of human studies have produced contradictory results, and data may also be affected by exercise intensity (i.e, acute speed/strength vs. chronic or resistance exercise activity) Rasool 2019). However, no child evaluated in this study practiced agonistic sports, and all subjects performed moderate standard school sports activities, with a frequency of no more than twice a week.

whereas data about the correlation between irisin and GHremain quite controversial, the relationship between irisin and muscle mass seems more robust. Recent reports have questioned the presence of circulating irisin, claiming that the measured human irisin arises from artifacts of poor antibody specificity (Nashwan etal 2021), and the existence of this protein and its role in humans is still a matter of debate (Saad etal 2021).

In summary, circulating irisin levels could represent a metabolic marker of treatment. Indeed, this study confirms the beneficial metabolic effects of GHR in children with GHD, demonstrated by the increase in irisin levels that could reflect the body composition changes that occur during GHR.

Although the irisin levels observed after GHR were significantly increased over baseline, they did not reach the values of control subjects, probably due to the short-term course of treatment. However, because there are no other similar studies on irisin in this population, we believe that these data should be considered as preliminary and whether the association between GH and irisin is casual or not remains to be demonstrated by future studies with larger populations and a longer follow-up period.

5. Conclusions

Strong correlation between hepcidin and FSH in beta thalassemia major make hepcidin good early predictable marker for Hypogonadism. hypogonadism resulting from iron deposi-tion in the pituitary gonadotrope is commonly found in thalassemia major patients. Early diagnosis and treatment are crucial for normal pubertal development and to reduce the complications of hypogonadism.

Ethical Clearance

All of the subjects' groups received information regarding the study's purpose and methodology. They provided informed permission for study participation, and the study was authorized by Research Committee of Baghdad teaching hospital.

Training and Human Development Center.

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