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Correlation between Hepcidin and FSH in β-Thalassemia Major in Baghdad City

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ABSTRACT— 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. Hypogonadism, mainly hypogonadotropic 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. To assessment correlation between hepcidin and FSH in β-thalassemia major. There were 138 participants in the research study,48 control persons and 90 β thalassemia major patients. Hepcidin and follicular simulating hormone (FSH) values were calculated in order to evaluate correlation between them and calculated levels were estimated using enzyme-linked immunosorbent assay(ELISA) kit. The levels of Hepcidin and FSH 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 hepcidin and FSH ($p < 0.003$). Significant moderate negative 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.

KEYWORDS: β-thalassemia major, hypogonadism, hepcidin, FSH

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 [1]. The term thalassemia major refers to the severe form that is often associated with life-long transfusion dependent anemia [2].

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 [21]. 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 [20].

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 [19].

Hypogonadism is the most frequently reported endocrine complication, affecting 70–80% of thalassemia major patients. Hypogonadism is likely to be caused by iron deposits in the gonads, pituitary gland or both. However [3] hypogonadotropic hypogonad-ism resulting from iron deposition in the pituitary gonadotrope is more commonly found. Gonadal iron deposition in ovaries or testes occurs less frequently, as the majority of amenorrheic In normal individuals, iron homeostasis is controlled mainly by iron absorption, not excretion [4].

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 [5].

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 [6], [7].

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 [8], [9].

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 [10].

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 [11].

Aim of study

Detect correlation between hepcidin and FSH 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

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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 (–40^{o}C) until time of assay.

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

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, Hepcidin and FSH between patients and control groups. The Spearman correlation test was used to test the correlation BETWEEN HEPCIDIN and FSH

3. RESULTS

3.1 Descriptive statistics

Table 1 Shows that the levels of Iron, Ferritin, Hepcidin and FSH 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	***Controls(n=48)***		***Patients(n=90)***				
Variables	**Mean ±S.D**		**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)
FSH (g/dL)	28.80±0.413		9.129±0.4620		20.8730	0.001	P<0.01 (HS)
S. hepcidin (mg/dL)	3.72±0.04		7.05±0.11		28.405	0.039	P<0.05 (S)
Gender	No.	(%)	No.	(%)			
Male	24	50%	45	50%			
Female	24	50%	45	50%	X^2=2.052	P =0.152	(NS)

Total	48	100%	90	100%

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

Correlations

		FSHP	hepcidinP
FSHP	Pearson Correlation	1	0.313**
	Sig. (2-tailed)		0.003
	N	90	90
hepcidinP	Pearson Correlation	-0.313**	1
	Sig. (2-tailed)	0.003	
	N	90	90

**. Correlation is significant at the 0.01 level (2-tailed).

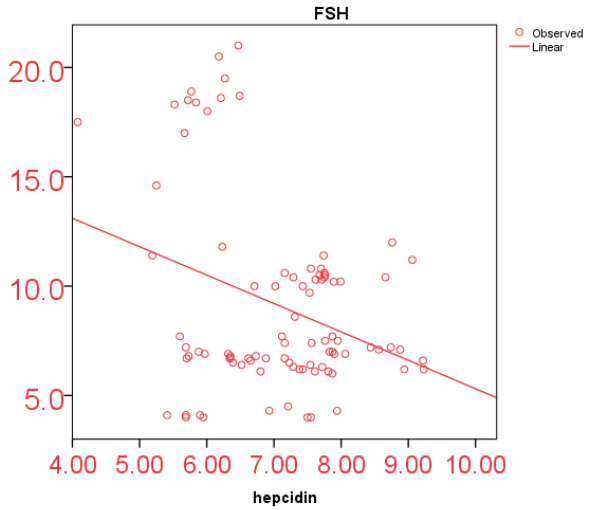

Figure 1 Shows moderate negative correlation between hepcidin and FSH.

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 [12]. A deficiency in this hormone is the main or contributing factor of iron overload in iron-loading anemias such as β-thalassemia [13], [23].

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 [1].

Effective management of iron overload requires frequent evaluation of the body iron stores [10]. 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 [24].

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 [22].

Hepcidin is a key iron regulator hormone, table 1.1 has been shown increase Mean ±S.D hepcidin (3.72±0.04) mg/dl and decrease in FSH levels (28.80±0.413) mg/dl in blood of patients with thalassemia comparison with their controls(7.05±0.11) (9.12±0.46),respectively.

By other hand, our results has been shown strong negative correlation between hepcidin and FSH (reciprocal relationship) Our study that showed in table 2 and figure 1 increase in hepcidin hormone lead to decrease in FSH hormone and this result has been agreed with other previous study like [14]. that explained there is indirect effect of hepcidin and FSH in beta thalassemia major.

Hepcidin increase due to elevate level of iron in blood due to repeated blood transfusion in thalassemia patient and this lead to trigger hepatocyte to produce hepcidin hormone which transport into gut to inhibit iron absorption by binding iron export channel ferroportin which is located in basolateral plasma membrane enterocyte and inhibit ferroportin cause Iron sequestration in cell [15], [16].

But in thalassemia patient repeat iron transport by blood transfusion remain positive feedback continuously for hepatocyte to produce more and more hepcidin and maintain hepcidin in high level [17].

when iron concentration gets too high in pituitary gland and can cause serious problem in our body as defect in reproduction system for example delay in puberty and irregular in menstrual cycle in women and low sex drive in men and delay in growth in children [18].

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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