Evaluation of Serum IL-36, Insulin Resistance and Correlated with Iron Overload Levels in Patients with Beta Thalassemia Major

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Abstract

Background: Patients with chronic transfusion-dependent thalassemia major (β -TM) frequently exhibit iron overload, which may have an impact on insulin resistance and cell function. the cytokines interleukin (IL)-36 Their contributions to type 2 diabetes mellitus (T2DM) were poorly understood. Aim: This study was to assess the association between serum levels of IL-36, serum iron, and ferritin, as well as glucometabolic function and IL-36 levels in thalassemia major (β -TM) patients. Methods: The present case-control study was carried out in Department of Hematology and Transfusion Medicine, thalassemia Unit" at "AL-Zahra'a Teaching Hospital" in Najaf – Iraq, Throughout the period from January 2022 – June 2022 for as patient group treated by Iron chelators. Fasting serum glucose, fasting insulin, Serum iron, ferritin, insulin resistance index and IL-36 were assessed for 60 healthy control participants and 120 TM patients, Age range was 10-12 years. Results: Compared to controls, patients with β -TM had considerably higher serum levels of IL-36. According to correlation analysis, serum IL-36 was positively linked with age, BMI, ferritin, Fe, TS%, Insulin, S.FBG, and HOMO-IR, (r = (0.493**), (0.313**), (0.511**), (0.398**), (0.422**), (0.326**), (0.527**) and (0.440**) nonetheless, there was a significant negative correlation between serum IL-36 levels and TIBC ,UIBC ,Transferrin and HOMO- β (r = (-0.339**), (-0.431**), (-0.339**) and (-0.459**) respectively. the highest value of the AUCROC was found for serum IL-36 concentrations with a threshold value of 32.923 ng/mL (AUCROC = 0.737 [95% CI: 0.666 - 0.808; p<0.001]). The sensitivity and specificity for this variable were 0.675 and 0.683, respectively. Conclusions: Our research revealed that patients with β -TM had elevated serum levels of the inflammatory cytokine IL-36. Furthermore, to improper glucose metabolism, which is common in patients with β-TM who get chelation therapy and several transfusions and is connected to the reduced activity of HOMA-β cells (increased insulin resistance) and aberrant glucose metabolism (HOMA-IR).

Keywords: Thalassemia, IL-36 Insulin resistance and iron status

1. Introduction

A significant hereditary illness called thalassemia major (TM) is frequently managed by recurrent blood transfusions. (Rund and Rachmilewitz 2005). Blood is regularly transfused into β -TM patients have increased survival and improved quality of life; However, chronic iron overload brought on by blood transfusions, which is commonly followed by endocrine problems, especially aberrant glucose metabolism, including diabetes mellitus (DM) and impaired glucose tolerance (IGT), is a side effect.(Saffari, Mahyar et al. 2012), (Tangvarasittichai, Pimanprom et al. 2013). IGT with hyperinsulinemia and impaired insulin sensitivity is a symptom of insulin resistance in these patients, whose origins of abnormalities in glucose metabolism are not entirely known.. (Delvecchio and Cavallo 2010), (Suvarna, Ingle et al. 2006), whereas pancreatic b cell abnormalities brought on by insulin deficit secondary to iron deficiency (Sanctis, Soliman et al. 2013) are thought to be another potential reason for high glucose levels. Additionally, people with -TM who do not have diabetes or who are in a euglycemic state prior to the onset of either glucose intolerance or diabetes have both insulin deficit and insulin resistance. (Simcox and McClain 2013), (De Assis, Ribeiro et al. 2012). Additionally, iron poisoning causes an increase in immunoglobin synthesis and a reduction in cytokine/interleukin response. Immune response is negatively impacted by the accumulated elements responsible for immunity control. (Maes and Carvalho 2018). Additionally, higher level of ferritin may be a cause of impaired fasting blood glucose (IBG) of diabetes (Li, Peng et al. 2014).

Autoimmunity, infections, and allo-immunization are complications of blood transfusion. Increased activity of lymphocytes is caused in part by iron overload, and there may be a correlation between serum ferritin levels and the extent of cytokine production by lymphocytes. (Gharagozloo, Karimi et al. 2013).

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Expression of cytokines in beta cells and autoimmune type 1 diabetes is related high expression of pro- inflammatory cytokines "(IL-1 to IL-38, TNF-α, TGF-β, and interferons)" (Akdis, Aab et al. 2016). In this case control study, we aimed to compare levels of serum IL-36 in children with Beta-Thalassemia further to assess anomalies in glucose metabolism, insulin resistance, and cell function in -TM patients in Iraq.

2. Patients and Methods

180 subjects enrolled in this study which divided to two groups. The first group is made up of 60 healthy persons acting as the control group, second group consist of 120 subjects of Iraqi children with "Beta-thalassemia Major" from Department of Hematology and Transfusion Medicine, At the "Thalassemia Unit" "AL-Zahra'a Teaching Hospital" in (Najaf – Iraq), Throughout the period from January 2022 – June 2022 for as patient group treated by Iron chelators. The age of all studied groups was range from (5-20) years old. Venous blood was collected a proximately 24 hours before transfusion with washed cells to be sure that the circulating blood belongs to the patient and is not the transfused blood in this study. Interleukin-36 (IL-36) has been determined using enzyme linked immunosorbent assay (ELISA) technique using the manufacture instruction as supplied with kit from MELSIN. Using a BIOLABO kit (France), perform a photometric colorimetric test to determine the presence of iron. Iron concentration has been measured by Colorimetric method(Carter 1971). Ferritin has been determined using enzyme linked fluorescent assay (ELISA) technique using the manufacture instruction as supplied with kit from BIOLABO (France).

The insulin ELISA assay is a two-site enzyme immunoassay that employs the direct sandwich method. Using a Glucose kit, a quantitative in vitro diagnostic measurement of fasting serum glucose was conducted using the colorimetric approach. HOMA -calculator was used to assess HOMA-IR and HOMA-%.

HOMA IR = [glucose (in mg/dl) * insulin (μIU/mL)] / 405.

HOMA β %= 20× insulin (μIU/ml)/(Glucose (mg/dL) - 63) (Matthews, Hosker et al. 1985). The study excluded those who had any type of chronic illness.

Exclusion criteria: Patients and controls with any acute illness or pathological injuries, positive for viral hepatitis and HIV were not enrolled in this study.

3. Statistics analysis

IBM's SPSS statistics program, version 25.0, was used to express the data for the current study as Mean + SD. Using a two-tailed unpaired Student's t test, the two groups were compared. In order to determine the relationship between the variables, a Pearson correlation analysis was carried out. A scatter diagram showed the relationship, and an independent t-test was utilized to demonstrate the difference between the group variation that was deemed statistically significant. *P < 0.05, **P <0.01, ***P < 0.001, ****P < 0.0001.

4. Results

Patients and controls did not have any statistical difference for age distribution. Patient data are given in Table 1. β-TM patients had higher serum IL-36 concentrations than those of healthy controls (37.033±7.465 vs 32.041±2.399; p < 0.0001) (Fig. 1). Patients with -TM exhibited considerably greater ferritin levels. (2160.262±935.918 vs. 98.316±19.252; p <0.001), iron (32.263±5.836 vs. 17.818±3.817; p<0.001) Additionally, higher FBS (131.726±15.087 vs. 84.934± 9.713; p<0.001) contrasted with the control group. Additionally, compared to the control group, they had a much higher insulin resistance index (HOMA-IR) (4.457±1.077vs. 1.470±0.463; p<0.01); Patients with β-thalassemia had lower HOMA- β% levels (4.072±0.755 vs 8.845±0.289; p<0.001)

There was a significant positive person correlation in all patients with β-TM between serum levels of IL-36 and age, BMI, ferritin, Fe, TS%, Insulin, S.FBG, and HOMO-IR, (r = (0.493**), (0.313**), (0.511**), (0.398**), (0.422**), (0.326**), (0.527**) and (0.440**) respectively (Fig. 2). the serum levels of and a significant negative association was found between IL-36 and TIBC, UIBC, Transferrin and HOMO- β (r = (-0.339**), (-0.431**), (-0.339**) and (-0.459**) respectively (Fig. 3)

Table (1): general Characteristics of the enrolled patients and control

Parameters	Mean ± SD: (Range) patients (n=120)	Controls (n=60)	*P* - value
Age (years	12.93± 4.660	12.58±4.600	N. S BMI(kg/m2)
18.12 ± 2.146	22.60±2.418	0.00** Interleukin-36 (ng/ml)	37.033±7.465
32.041±2.399	0.00** Ferritin (ng/ml)	2160.262±935.918	98.316±19.252
0.00** IRON (μmol/L)	32.263±5.836	17.818±3.817	0.00** TIBC
(μmol/L)	49.112±14.480	54.456±12.874	0.00** UIBC (μmol/L)
16.848±6.70	37.305±13.249	0.00** Transferrin (g/L)	0.123±0.036
0.138 ±0.032	0.00** TS%	71.767±23.127	34.458 ±12.404
0.00** S.FBG (mg/dl)	131.726±15.087	84.934± 9.713	0.00** Insulin
mlU/mL	13.553±1.929	6.090±1.956	0.00** HOMA-IR
4.457±1.077	1.470±0.463	0.00** HOMA-β%	4.072±0.755
8.845±0.289	0.00**		

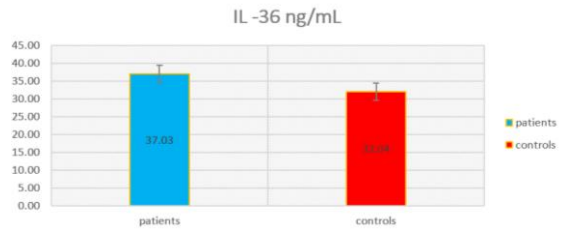


Figure 1: Comparisons of serum Interleukin -36 (ng/ml), levels for the patients and control.

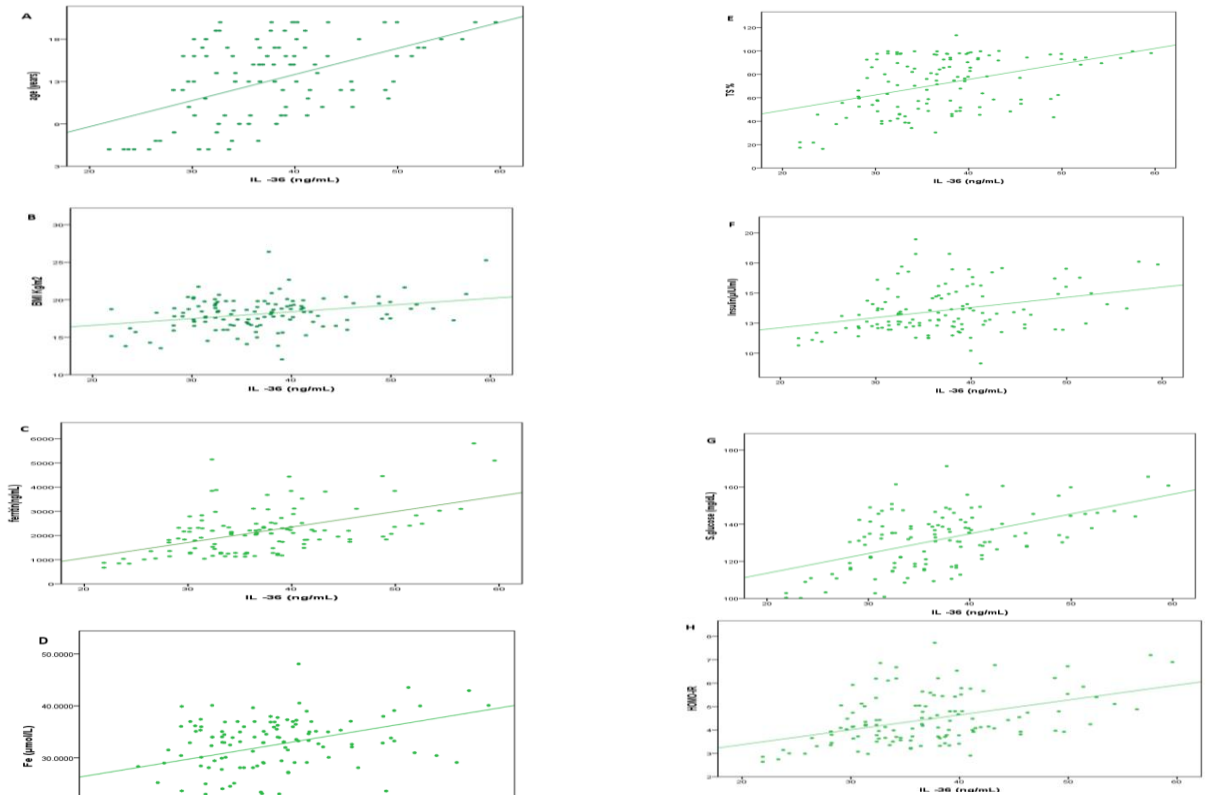
In This study was found the significant difference in Interleukin -36 between the values of patients and control groups From this investigation, it was suggested that assessing the concentration of a new superfamily of cytokines (IL-36) might be regarded as a clinical biochemical criterion in patients with β -TM in Iraqi children. Additionally, this research may have indicated a connection between elevated IL-36 levels and iron excess. The anomalies in iron metabolism may have been caused by increased IL-36 production, which is likely the result of macrophage overstimulation. These alterations in serum cytokine levels in β -TM should be further examined in follow-up data from larger series of β -TM patients before a clinical significance can be assigned.

AUCROC, or the area under the ROC curve, was utilized to evaluate whether any level of serum IL-36 could be used to differentiate between patients with and without iron overload. The highest value of the AUCROC was found for serum IL-36 concentrations with a threshold value of 35.437ng/mL (AUCROC = 0.737 [95% CI: 0.666 - 0.808; p<0.001]). The sensitivity and specificity for this variable were 0.675 and 0.683, respectively (Fig. 4).

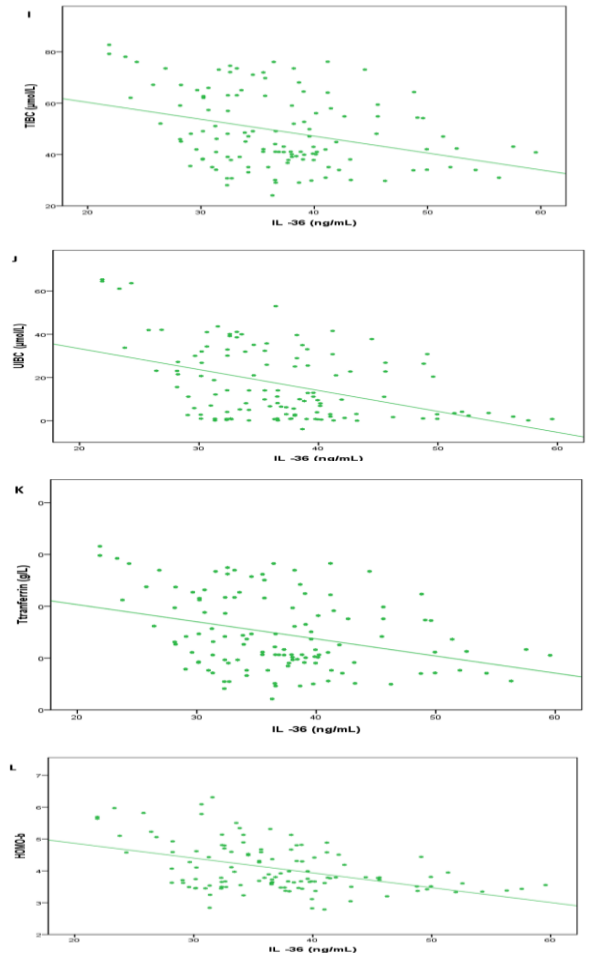
Table (2): Correlation between IL -36 ng/mL and studied parameters in Major Thalassemia patients

Parameters	r	P-Value
Age (years)	0.493**	0.000
BMI (Kg/m²)	0.313**	0.001
Ferritin ng/mL	0.511**	0.000
Fe (µmol/L)	0.398**	0.000
TIBC (µmol/L)	-0.339**	0.000
UIBC (µmol/L)	-0.431**	0.000
TS %	0.422**	0.000
Transferrin (g/L)	-0.339**	0.000
Insulin(µIU/ml)	0.326**	0.000
S.FBG mg/dL	0.527**	0.000
HOMO-IR	0.440**	0.000
HOMO- β	-0.459**	0.000

BMI: body mass index, Data are presented as Mean with Standard Deviation (SD) and NS (non-significant differences at P 0.05). Significant differences are indicated by the following symbols: * at P 0.05, ** at P 0.01.



Figure's 2: positive correlation between serum levels of IL-36 (ng/mL) with (A) age, (B) BMI, (C) ferritin , (D) Iron , (E) TS %, (F) Insulin, (G) S. glucose and (H) HOMA-IR in patients with β -TM



*Figure's 3 : Negative correlation of IL-36 (ng/mL) with (I) TIBC, (J) UIBC, (K) transferrin and (L) HOMA-b in patients with **β** -TM*

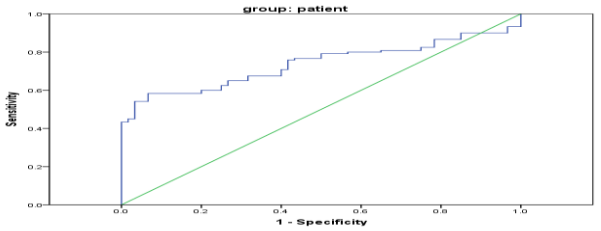


*Figure 4: Receiver-operating characteristic (ROC) curves for serum IL-36 levels in **β**-TM patients*

5. Discussion

Numerous researchers have recently become interested in IL-36 up-regulation in a variety of illnesses. These cytokines are critical for the emergence of inflammatory conditions that affect the skin, joints, blood vessels, heart, and nerves. (Takaishi, Satoh et al. 2018). IL-36 in psoriasis is the subject of numerous studies (Madonna, Girolomoni et al. 2019), inflammatory arthritis (Derer, Groetsch et al. 2014), systemic lupus erythematosus (Chu, Wong et al. 2015), inflammatory bowel disease, such as Crohn's disease and ulcerative colitis (Neufert, Neurath et al. 2020).

However, as far as we are aware, reports on the function of IL-36 cytokines in β -TM are limited. In this study, we measured the serum IL-36 levels in patients with β -TM, and we found that they were significantly greater than those in control participants, proving that IL-36 contributes to the development of diabetes. Inflammatory cytokines interact with the immunological and endocrine systems, altering the structure of pancreatic islet cells and making them (insulin resistant)IR, which ultimately results in the development of T2DM.(Nano, Racanicchi et al. 2013). But according to our data, there is a negative association between IL-36 and HOMA-IR and a positive correlation between the two.

In the present investigation, patients with β -TM experienced aberrant FBG results at a rate that was significantly greater than that of the control group. Diabetes mellitus and impaired fasting glucose levels were common in β -TM patients. The prevalence of this finding abnormality in this investigation is comparable to that in the literature. (Khalifa, Salem et al. 2004), (Chatterjee and Bajoria 2009).

According to reports, a patient's assessment date, the level of chelation, and the transfusion rely on the occurrence of diabetic mellitus (DM) in people with β-TM, and the degree of associated patient compliance. (Chatterjee and Bajoria 2009), as age-related increases in prevalence (De Assis, Ribeiro et al. 2012).

It has been demonstrated that patients with TM have lower HOMA- and higher HOMA-IR levels than healthy persons. In our study, compared to the control group, β-TM patients showed significantly increased HOMA-IR.; but a considerable decline in HOMA-, when comparing the outcomes between β-TM patients.

Impairment in glucose metabolism is most likely caused by iron toxicity in the liver and pancreas as well as insulin dysregulation, which is most likely caused by high iron and ferritin levels. In earlier research, chelation therapy patients with hemochromatosis and β -TM were found to have aberrant glucose metabolism, poor insulin secretion, and insulin resistance. (Tangvarasittichai, Pimanprom et al. 2013), (Saffari, Mahyar et al. 2012). The ability of b cells to release insulin is also thought to be reduced in response to iron overload in animal models. (Chen, Feng et al. 2009).

These studies all point to iron overload's involvement in the etiology of insulin resistance. Patients with β -TM have a heterogeneous illness spectrum, including insulin resistance (resulting from liver damage and oxidative stress on vascular endothelia) and -cell dysfunction (caused by islet cells' direct exposure to iron toxicity). The fact that fasting insulin levels are normal is not surprising because these alterations cause insulin secretion to move in the other direction.

6. Conclusion

Our research revealed that patients with β -TM had elevated serum levels of the inflammatory cytokine IL-36. The current study looked into how inflammatory cytokines interact to cause type 2 diabetes, along with aberrant glucose metabolism, which is common in patients with -TM who get

chelation therapy and numerous transfusions and is connected to the dysfunction of HOMA- cells (increased insulin resistance) and abnormal glucose metabolism (HOMA- IR).

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Declaration of interests

The authors declare no conflict of interests.

funding: none

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