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Serum Salusin-α and Salusin -b levels with some Biochemical Parameters in Patients with Major Thalassemia

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ABSTRAC

Thalassemias are a heterogeneous group of genetic disorders in which the production of normal hemoglobin partially or completely is suppressed because of defective synthesis of one or more globin chain. It is sometimes called Mediterranean anemia. Many biochemical changes in the blood accompany this disease. In this research, some biochemical parameters were measured in thalassemic patients and compared with healthy control group. These parameters include serum Iron, ferritin , TIBC, transferrin, transferrin saturation percentage, Salusin-α , salusin-β, insulin ,and lipid profile. The aim of this study was to Measuring the level of cytokine(salusin- α and salusin -b) lipid profile, blood sugar level and resistance to insulin in serum of thalassemia patients and Find interconnectivity between cytokine(salusin- α and salusin -b), iron , lipid profile, blood sugar level and insulin resistance. The results of the research showed that there is a significant increase ($P<0.05$) in BMI,serum iron, ferritin ,UIBC, TS%,FBG, Insulin , HOMA-β, TG, cholesterol, HDL-C, LDL-C, and salusin -b in thalassemic patients group in comparison with healthy control group. A significant decrease ($P<0.05$) in HOMA-IR and salusin- α . There is no significant difference ($P>0.05$) in Age,TIBC,Transferrin, and VLDL-C.

Key words : *Major thalassemia, salusin alpha, salusin beta, HOMA-IR.*

Introduction

The thalassemias are inherited anemias caused by mutations that affect the production of the globin chain , the protein elements of the hemoglobin. Thalassemias produce a enormous public health problems in many parts of the world [24] . These hereditary anemia caused by reduced or absent production of one type of globin chain either α or β globin chain. These disorders range from asymptomatic to severe

anemia that can cause significant morbidity and mortality [15].

Major morbidity and mortality can result from iron overload , liver dysfunction and failure ,pan- endocrine failure , heart failure and fatal cardiac arrhythmias , which establish the main concerns in weakly chelated patients [18]. Thalassemias are classified according to which specific globin chain(s) is/are produced in a reduced amount, which may lead to an inequality in globin chains synthesis, unproductive erythropoiesis, hemolysis, and ultimately to a variable degree of anemia. The chief types of Thalassemias are the a, b, db, d, and gd. The a and b thalassemias are the most common types, and b thalassemia is the most important and broadly spread type which causes severe anemia in the homozygous and compound heterozygous states([27,26,9]. The typical treatment of severe beta-thalassemia is at this time based on medical treatment, and a blood transfusions, iron chelation and spleenectomy, permitting an increased being alive and improvement of the patients' quality of life [23].

In 2003, salusins was defined as a new class of vasoactive peptides by Shichiri et al . Salusin-alpha (Sal-α) and salusin-beta (Sal- β) are peptides composed of 28 and 20 amino acids, correspondingly. Preprosalusin is mostly excreted from vascular smooth muscle cells and endothelial cells. [20] Salusins are synthesized and released from the renal system, central nervous system and vascular system of human [22] While salusins are expressed from coronary atherosclerotic clots , expression of Sal- α is detected at a lower ratio when associate to that of Sal-β [25] .In an in vivo study, it was exposed that Sal- β was synthesised by fibroblasts in the vascular smooth muscle cells of the media layer and by the media layer of aorta during coronary artery bypass graft surgeries in patients

with coronary artery disease [2] . Salusins have differently effect on haemodynamic activities [6]. In the present study, detection of low salusin-alpha and high salusin-beta levels in major Thalassemia patiens suggest the possible effect of decreased salusin-alpha and increased salusin-beta levels in rapid reactionary responses. We detected significant differences between groups regarding salusin levels on TM. This supports the idea that low levels of salusin- alpha and high levels of salusin-beta levels differ significantly in patients with Major Thalassemia to healthy people. In this study, we detected that salusin levels were increased an average of 2 hours after onset of chest pain. [16].

Materials and Method

A case-control study was conducted in "Thallassemia Unit" at "AL-Zahra'a Teaching Hospital" in Najaf city-Iraq, during the period from September to December 2018. The present study included of Sixty Iraqi thalassemia major patients .Their ages ranged 1-28 years old and Thirty apparently healthy subjects were selected as control group. Their age ranges were similar to that of patients. None of these subjects was anemic or has an obvious systemic disease or any chronic diseases. The Body Mass Index was measured by dividing weight in kilograms by length of individual in square meter: BMI = (weight in kg) / (height in meters2). [13] .Fasting venous blood samples were collected from patients and control group and the serum was separated and stored at – 20 0C until they were for analysis. Serum Iron was estimated by using colorimetric method [19] , TIBC were estimated by using colorimetric method . The Ferritin were determined by ELISA assay (Elabscience,USA). The percentage of transferrin saturation was calculated by using the formula: Transferrin (g/L) =Serum iron (μmol/L) / (3.98 X TS%) (Morgan, Dean, & Davies, 2002). Fasting investigation of Serum glucose,and lipid profile (TC, TG, LDLC and HDL-C) levels were estimated by the colorimetric method for the quantitative in vitro diagnostic measurement using a kit. Insulin were measured determined by ELISA assay (CALBIOTCH Company,USA) Insulin resistance index (Homeostatic model assessment-insulin resistance, HOMA-IR) was estimated as follows: HOMA IR = [glucose (in mg/dl) * insulin (μU/ml)] / 405 HOMA β %=360× insulin/ (Glucose - 63). [8]. Salusins (Sal -α and sal -β) were determined by ELISA assay (Elabscience,USA).

Statistical Analysis

Statistical analysis was performed using two statistical software, the Statistical Package of Social Science (SPSS ver. 21) and Graphpad Prism ver.5. Continuous variables were expressed as mean ± standard deviation (SD). Significant differences were assessed using Paired t-test and independent t-test for variables with equal and unequal frequencies respectively. Bivariate correlations were assessed using standardized Pearson coefficients. The p values obtained of less than 0.05 and 0.01 were considered as statistically and highly statistically significant respectively.

Results and Discussion

The anthropometric and biochemical variables of the Thalassemia patients and healthy control group was summarized their statistical significance in Table 1. The present study demonstrated Non significant difference in age of thalassemia patients when compared to those of control group. The results of BMI revealed a significant decrease ($p<0.05$) in groups of thalassemia patients compared with healthy group

On the other hand, found a significant increase in serum iron , ferritin ,UIBC, TS%,FBG,Insulin , HOMA-β ,TG, cholesterol,HDL-C, LDL-C, and salusin -b in patients with Major Thalassemia compared with healthy group but significant lower in in HOMA-IR and salusin- α, ($p›0.001$) while TIBC, Transferrin ,and VLDL-C non-significant.

In Table (2) found a Correlation between Salusin α and studied variables in Major Thalassemia patients. Age,TIBC, and ,UIBC, have a positive correlation , and significant with P value ($P<0.05$). while Salusin β, FBG and Transferrin are a negative correlation and significant.

In Table (3) found a Correlation between Salusin β and studied variables in Major Thalassemia patients Salusin α are a negative correlation and significant with p value ($P<0.05$).

Homeostatic model assessment (HOMA) is a moderately simple method of estimating insulin resistance and beta-cell function by using the fasting plasma measurements of glucose and insulin [17] Patients with Thalassemia major present decreased HOMA-B and elevated HOMA-IR levels compared with healthy people [21] or displayed decreased HOMA-B and elevated HOMA-IR levels after follow-up [14] Similar to previous studies, we demonstrated decreased HOMA-B in

patients without diabetes. Studies in patients with thalassemia and hereditary hemochromatosis who had chronic iron overload showed that glucose dysregulation occurred as a result of insulin resistance followed by β-cell dysfunction [12] Excessive iron causes insulin resistance and subsequently, pancreatic β-cell apoptosis and insulin deficiency [4].

Table(1):The anthropometric and biochemical variable of major thalassemia patients and control

Parameter	Patients (n=58) Mean±SD	Control (n=30) Mean±SD	P-value
Age(Yrs)	17.28± 5.758	17.97± 6.990	N.S
BMI (kg/m2)	17.67± 3.73	29.11± 14.46	0.000**
Ferritin(ng/ml)	3472.22±2356.84	123.70± 48.05	0.000**
IRON (µmol/L)	35.68± 8.19	23.83± 6.096	0.000**
TIBC (µmol/L)	63.56± 19.01	68.81± 12.61	0.175 NS
UIBC	27.88± 15.03	44.98± 12.47	0.000**
TS%	58.71± 13.34	35.52± 11.78	0.000**
Transferrin(g/L)	0.16± 0.05	0.17± 0.03	0.175 NS
FBG(mg/dl)	123.79±26.01	86.41±10.45	0.000**
Insulin (mlU/mL)	10.20± 4.79	5.27±2.17	0.000**
HOMA-IR	2.82± 1.92	1.84±1.34	0.014*
HOMA-β%	66.74±10.48	89.86±13.24	0.001**
TG(mg/dL)	86.01±6.24	83.01± 8.73	0.000**
Cholesterol(mg/dL)	122.07± 12.05	136.53± 20.09	0.000**
HDL-C(mg/dL)	24.62± 2.43	47.47± 4.05	0.000**
LDL-C(mg/dL)	82.38± 11.76	91.91± 16.23	0.002**
VLDL.C	16.88± 5.08	17.62± 2.96	0.464 NS
Salusin α(pg/mL)	54.11± 7.87	57.59± 7.41	0.048*
Salusin β(pg/mL)	48.65± 6.69	44.38± 7.36	0.009**

BMI: body mass index FBG: fasting blood glucose HOMA-IR: hemostasis model assessment-insulin resistance, HOMA-β%: hemostasis model assessment-beta cell percentage. TG: triglyceride, HDL-C: High-density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol. Data represented as Mean ±SD: standard deviation, NS= nonsignificant differences at (P>0.05). *=significant differences at (P≤ 0.05), **=significant differences at (P≤ 0.01).

Salusin α is associated with renal dysfunction in patients with chronic renal failure [11]. It was concluded that renal disorders are not rare in patients with beta-thalassemia major , iron and oxygen radicals may play a key role in the progression of chronic renal failure, the fact that may be related to the changes in kidney excretion of different substances . Patients with Hemoglobinopathies including thalassemia would develop albuminuria and this may affect the blood concentration of albumin. [7]

Salusin is expressed not only in peripheral tissues but also in the central nervous system including neuronal cells of the hypothalamo-pituitary tract [22] .Intravenous administration of salusin- α or salusin- β was shown to cause a rapid decrease in arterial pressure; however, the depressor response of salusin- β was greater than that of salusin- α . Moreover, these depressor responses were concomitant with a decrease in heart rate [20].

Table (2): Correlation between Salusin α and studied variables in Major Thalassemia patients.

Variables	R	P-Value
BMI	0.042	0.826
age	0.351	0.057
Salusin β(pg/mL)	-0.848**	0.000
Insulin(µIU/mL)	-0.024	0.898
HOMA-IR	-0.258	0.169
HOMA-β	0.026	0.893
Ferritin (ng/mL)	-0.303	0.104
IRON (µmol/L)	-0.001	0.995
TIBC (µmol/L)	0.454*	0.012
UIBC	0.458*	0.011
TS%	0.294	0.118
Transferrin (g/L)	-0.454*	0.012
HDL.C	0.158	0.405
VLDL.C	0.115	0.544
LDL.C	0.175	0.355
CHO	0.158	0.404
TG.C	0.119-	0.531
FBG	-0.386*	0.035

P- Value £ 0.05 = significant, r : Pearson correlation. *=significant differences at (P≤ 0.05), **=significant differences at (P≤ 0.01).

Salusin-beta has been shown to have effects on heart through cholinergic mechanisms and cause hypotension, bradycardia, and cardiac dysfunction without affecting systemic vascular resistance [10]

Thalassemia patients developed heart complications represent significant morbidity and remain the leading cause of mortality in transfusion. Cardiac structure and function in thalassemia are mainly affected by two competing factors: iron overload and increased cardiac output (CO). The cardiac iron deposition results in a decrease of left ventricular function. The anaemia together with marrow expansion leads to volume overload and increased CO that then demands increased contractility adding additional stress to the heart (Starling's Law). Cardiac dysfunction in thalassemia manifests with congestive cardiac failure (CCF), arrhythmias and ultimately.

Premature deaths, in some cases, because of the difficulty in accepting the chelation treatment, which was cumbersome, but also occurred even in some patients who accepted the chelation therapy well.[5 , 1])

Table (3): Correlation between Salusin β and studied variables in Major Thalassemia patients

Variables	r	P-Value
BMI	-0.079	0.679
age	-0.264	0.158
Salusin α(pg/mL)	-0.848**	0.000
Insulin(µIU/mL)	0.037	0.845
HOMA-IR	0.280	0.138
HOMA-β	-0.111	0.558
Ferritin (ng/mL)	0.211	0.262
IRON (µmol/L)	0.103	0.588
TIBC (µmol/L)	-0.354	0.055
UIBC	-0.307	0.099
TS%	0.119	0.531
Transferrin (g/L)	0.354	0.055
HDL.C	-0.268	0.158
VLDL.C	-0.128	0.500
LDL.C	-0.284	0.128
CHO	-0.264	0.162
TG.C	0.142	0.454
FBG	0.313	0.092

P- Value £ 0.05 = significant, r : Pearson correlation. *=significant differences at (P≤ 0.05), **=significant differences at (P≤ 0.01).

New results for major thalassemia patients in salusins levels in the current study found that serum salusin alpha levels are significantly lower and salusin beta are significantly higher in patients with major thalassemia compared to healthy as a control group.

Conclusion

In conclusion, the current study have demonstrated that salusins, newly identified bioactive peptides with potent hemodynamic activities, Measurement of serum, Salusin α and Salusin β levels may provide a novel approach to identify patient with major Thalassemia. , our results demonstrated the decrease in serum salusin-α levels in patients with major thalassemia .further,

this study confirmed previous finding reporting that its decrease plays a role in the development and progression of heart disease and renal system . The present study suggests that salusin- α may be a novel therapeutic candidate for the treatment mainly effect the cardio vascular system and further insulin and Salusin β levels increase in serum of major Thalassemia patients.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, University of Kufa, Iraq and all experiments were carried out in accordance with approved guidelines.

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