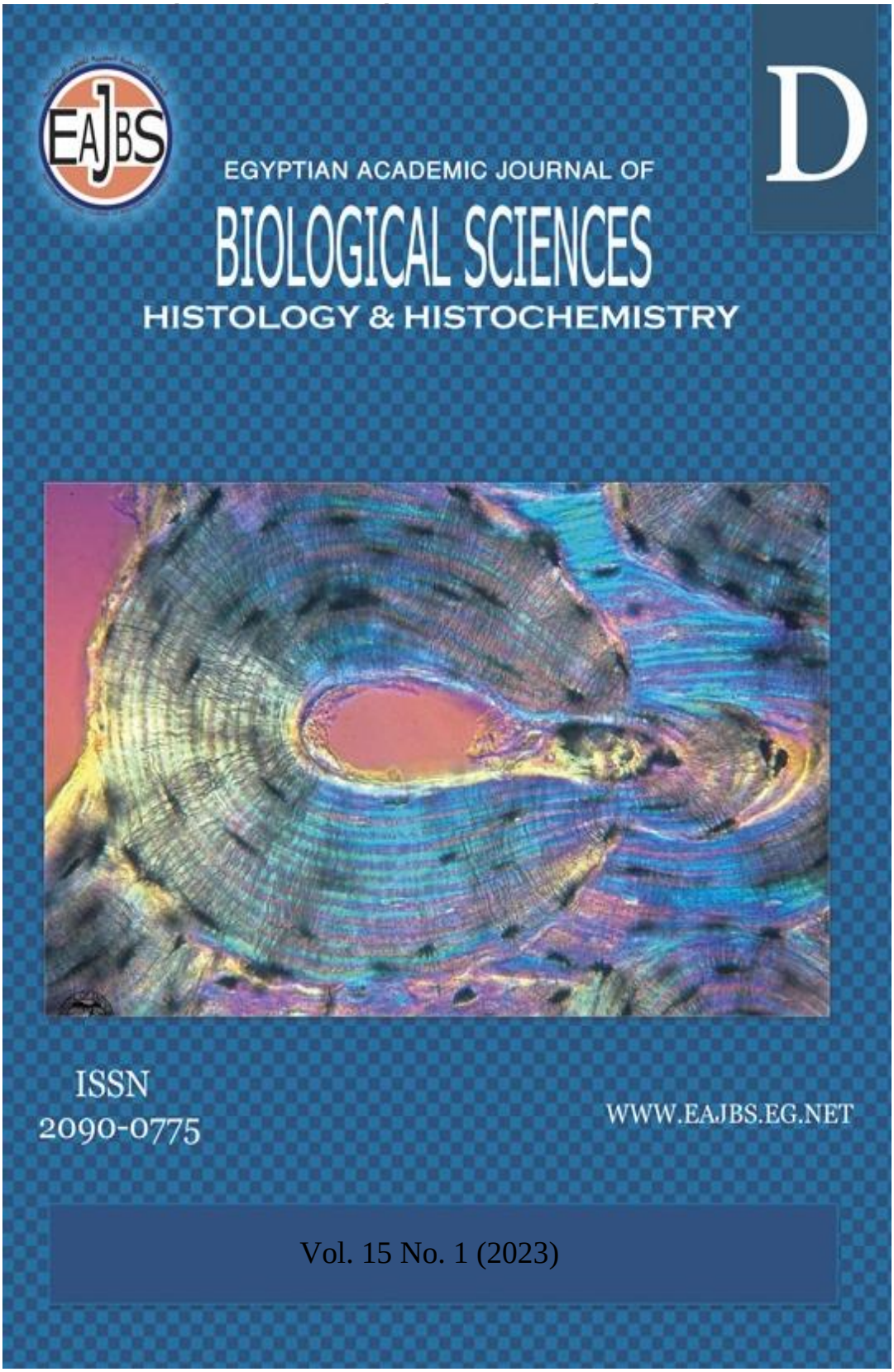
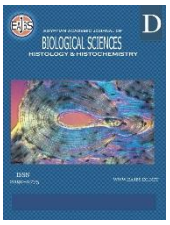


Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.15(1) pp127-138(2023)*
DOI: 10.21608/EAJBSD.2023.295455

Egypt. Acad. J. Biolog. Sci., 15(1): 127-138 (2023)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



Association Between Serum Levels of Adropin and Insulin Resistance in Patients with Beta-Thalassemia Major

Ammar K. Madlool[1], Mohammed k. kahlol[1], Seyed O. R. Siadat[2], Hanaa A. Ali[1], Rawaa A. Ali[3], Azhar M. Brbber[4], Aiman J. Abbas[5], Ayat S. Awad[1], Mohammed S. S. Hasan[6] and Mohauman M. M. AL- Rufaie[1]

1-Department of Chemistry, Faculty of Science, University of Kufa, Najaf 54001, Iraq.
2-Protein Research Center of Shahid Beheshti University, Iran
3-Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51001, Iraq.
4-Ministry of Education, Muthanna Education Directorate, Iraq.
5-College of Pharmacy, University of Kufa, Najaf 54001, Iraq.
6-AL-Sadr Medical City/Najaf 54001, Iraq.
E.Mail : ammar.kareem@saqid.edu.iq , muthanahana74@gmail.com
muhaimin.alrufaie@uokufa.edu.iq

ARTICLE INFO
Article History
Received:22/3/2023
Accepted:16/4/2023
Available:22/4/2023

***Keywords*:**
β-thalassemia major, Adropin, Insulin resistance, Iron overload.

ABSTRACT

Background: Beta-thalassemia is among the most common groups of recessivel inherited disorders worldwide of hemoglobin production which produce by th reduction or absence of the globin chain. Adropin is a protein that plays a important role in metabolic and energy hemostasis and insulin resistance. t examinate the serum level of serum adropin in patients with β-thalassemia majo and evaluate of correlation between adropin with insulin resistance and oth clinical biomarkers. Materials and Methods: hundred and twenty patients with β thalassemia major and sixty healthy control groups were involved in the presen study. The patients were classified into males (n = 55) and females (n = 65 whereas control groups were divided into two subgroups, males (n = 29) an females (n = 55). Serum adropin level, BMI, CRP, ferritin, glucose, insulin HOMA-IR, HOMA-β, iron, TIBC, UBIC, TS and transferrin for both patient an control groups were estimated. Results: Serum adropin, HOMA-β, TIBC, UIB and transferrin demonstrated a significant decrease in patients with β-thalassem major as compared with healthy individuals (0.72 ± 0.24 vs.1.23 ± 0.25, P<0.001 (3.86 ± 1.72 vs. 6.56 ± 2.42, P<0.001), (55.01 ± 5.85 vs 68.15 ± 12.26, P<0.001 (21.02 ± 7.22 vs 44.15 ± 12.29, P<0.001) and (39 ± 4.13, 48.32 ± 8.69, P<0.00 respectively, whereas BMI, CRP, ferritin, glucose, insulin, HOMA-IR, iron and T exhibited significant higher value than control groups. The correlation betwee serum adropin demonstrated a significantly negative correlation with age (*r* = 0.807), BMI (*r* = -0.421), CRP (*r* = -0.197), ferritin (*r* = -0.504), serum glucose (= -0.635), insulin (*r* = -0.418), HOMA-IR (*r* = -0.551), iron (*r* = -0.571) and TS (= -0.605), while revealed a significantly positive correlation with HOMA-β (*r* 0.364), TBIC (*r* = 0.296), UIBC (*r* = 0.553) and transferrin (*r* = 0.266).
Conclusion: The present study showed that the serum level of adropin in patien with β-thalassemia major was significantly lower than in control groups. Thes findings suggest that adropin may be a potential biomarker for predicting the ris of complications since the decreasing of serum adropin might play an importa role in the development of diabetes mellitus, cardiovascular diseases, kidne dysfunction, rheumatoid arthritis, and inflammatory bowel diseases in thes patients.

INTRODUCTION

One or more of the globin chains are reduced or absent during the synthesis of hemoglobin in the case of beta(β)-thalassemia, a group of genetic hematological illnesses that can cause a variety of phenotypes ranging from severe anemia to clinical asymptomatic individuals(Galanello and Origa 2010). β-thalassemia can be classified into three types, β-thalassemia major (β-TM), β-thalassemia intermediate and β-thalassemia minor (carrier)(Ali, Mumtaz *et al.*, 2021). Usually beginning in the first two years of life, β-thalassemia major begins with severe anemia and a need for frequent red blood cell (RBC) transfusions (Thein 2018) (Fucharoen and Weatherall 2012). Several studies have reported the resulting complications from β-TM since ineffective erythropoiesis is one cause of these complications, but another is iron excess brought on by increased gastrointestinal iron absorption and blood transfusions(Moradi and Ghaderi 2013). Because of the accumulation of iron in the spleen, liver, heart and endocrine organs, iron overload causes significant cellular damage and malfunction in these organs (Porter 2009) (Farmakis, Porter *et al.*, 2022). Disturbances in the homoeostasis of serum lipids and carbohydrates as well as oxidative stress are the most common diseases brought on by iron overload in β-TM (Noetzli, Mittelman *et al.*, 2012). In several earlier research, patients with -TM were found to have carbohydrate dysfunction, including glucose intolerance, hyperglycemia, reduced beta cell activity, and insulin sensitivity (Luo, Bajoria *et al.*, 2019) (De Sanctis, Soliman *et al.*, 2013) and According to some studies, oxidative stress is brought on by a weakening of the antioxidant defense system and an increase in the formation of reactive oxygen species caused by iron(Sajadi Hezaveh, Azarkeivan *et al.*, 2019).

Adropin is one of the biomarkers that can be impacted by -TM. Animals and humans both have the hormone adropin in their circulatory systems. Kumar and associates discovered it for the first time in 2008. (Kumar, Trevaskis *et al.*, 2008). It is made up of 43 amino acids and is produced by the proteolytic cleavage of precursors with 76 amino acids (Zhang, Chen *et al.*, 2020). Adropin, hypothesized to be a unique hormone for the energy homeostasis-associated (Enho) gene, is encoded for by this gene (Butler, Tam *et al.*, 2012). Although it is primarily produced by the liver and brain, peripheral tissues such as the lungs, heart, digestive tract, renal medulla, muscles, and breast cancer cells can also produce it (Butler, Zhang *et al.*, 2019) (Ali, D'Souza et al. 2022)In addition to these significant metabolic effects, adropin can also improve non-metabolic features such as endothelial function modulation(Jasaszwili, Billert *et al.*, 2020) (Ye, Zhang *et al.*, 2021). The results of a study done on mice point to the significant function of adropin, which controls the physiological processes of fatty acid oxidation and glucose metabolism. According to this study, adropin treatment for obese mice on a diet increased glucose tolerance, decreased insulin resistance, and promoted the use of carbohydrates in oxidative processes(Gao, McMillan *et al.*, 2015) (Skrzypski, Kołodziejski *et al.*, 2022).

MATERIALS AND METHODS

The case-control study included hundred and twenty with β-thalassemia major and sixty apparently healthy volunteers. The samples were collected throughout the period from January 2022 to July 2022. The ages of patients in this study range from 5-20 years which is identical to healthy controls. The β-thalassemia major disorder was registered in the "Thalassemia Unit" in "Al Zahra Teaching Hospital" AL-Najaf, Iraq.

The study was approved by the regional ethical committee of the University of Kufa, Faculty of Science. The patients which undergo β-

thalassemia major were diagnosed and recognized by clinical symptoms, and hematological and hemoglobin electrophoresis analysis. All patients were given detailed information on the study aims and risks and they gave consent before being enrolled. Diabetes mellitus, infection and inflammation, heart diseases and autoimmune diseases were excluded from the study. A questionnaire was designed to obtain information on a detailed history of the present thalassemia, history of thalassemia, family history, weight, height, age, gender and other anthropometric parameters calculated on all enrolments. 5 ml of all fasting healthy and patient samples were drowned from venous by using a disposable needle and plastic syringes before treatment of the patients by blood transfusion. It was left for 10-15 minutes for clotting and then centrifuged (at 5000 Xg) for 5 minutes in order to separate serum from other components of blood. The Serum was distributed into five Eppendorf tubes and stored at (-70Cº) until the time of analysis. Adropin, insulin and ferritin were examined by a sandwich enzyme-linked immune sorbent assay (ELISA) technique using the manufacturer's instruction as supplied with a kit from MELSIN. The enzymatic colorimetric method used to determine glucose using BIOLABO kit (France). Insulin resistance was calculated by using homeostasis model assessment (HOMA-IR) score that employs the formula: fasting insulin concentration (µIU/l) glucose (mmol/l)/22.5. Individuals with HOMA-IR > 2.7 were accepted as insulin resistant. And calculated HOMA-β by formula HOMA -β =360× Insulin /(Glucose-63)% (Aravind, Poornima et al. 2005).

Anthropometric measurements of Body Mass Index (BMI) were also calculated by a special equation, as the ratio of weight in (Kg) to height squared (m^2), by unit kg/m^2 (Khanna, Peltzer *et al.,* 2022).

Statistical Analysis:

In this study, statistical analysis of the data has been done by the SPSS 26.0 (Statistical Package for Social Sciences) package program. Plasma concentrations of biomarkers, adropin, CRP, ferritin and serum glucose, insulin, HOMA-IR and HOMA-β have a normal distribution. Through findings, the descriptive statistical methods during statistical analysis are mean SD, independent t-test (uses to compare between biomarkers and the significance level is considered acceptable as $P<0.05$.) and the Pearson correlation test (uses to determine the relationship between variables). Receiver operating characteristic (ROC) is also estimated by calculating the area under the curve (AUC) and cut-off value for adropin.

RESULTS

Table 1 was conducted between β-thalassemia major patients (TM) (n=120) and healthy individuals (n=60). Fifty-five of the patients with β-thalassemia major were males (45.84%), while sixty-five of the total patients were females (51.66%). Sixty healthy controls were examined in this study which was classified into subgroups, 29 males (48.33%) and 31 females (51.66%). The study included the investigation of body mass index (BMI) and age for TM and healthy participants. The mean age and BMI for β-TM demonstrated (13.06 ± 4.65) years and (18.12 ± 2.26) Kg/m^2 respectively, whereas the mean age and BMI for healthy persons revealed (12.57 ± 4.65) years and (20.52 ± 3.31) Kg/m^2 respectively. The comparison study of both age and BMI has been examined. Age exhibited a non-significant difference between patients and controls ($P=0.504$), while a statistically significant difference in BMI was detected as compared between the patient and control groups ($P<0.05$).

The investigated biomarkers through this study involve adropin, CRP, ferritin, glucose, insulin, HOMA-IR, HOMA-β, iron, (TIBC), (UIBC), transferrin saturation (TS) and transferrin. Table 1 displays the data for these biomarkers for the -thalassemia

major patient and control groups. The -TM and control groups have undergone a comparison study for the aforementioned metrics. This study showed that -TM significantly differed from control groups in terms of CRP, ferritin, serum glucose, insulin, HOMA-IR, iron, and transferrin saturation (P 0.001), whereas -TM significantly differed from control groups in terms of adropin, HOMA-, TIBC, UIBC, and transferrin (P 0.001).

Table 1: general Characteristics of the enrolled patients and control.

Mean ± SD (Range)			
Parameters	**patients (n=120)**	**Controls (n=60)**	***P* value**
Age yeas	13.06 ± 4.65	12.57 ± 4.65	0.504
BMI Kg/m^2	18.12 ± 2.26	20.52 ± 3.31	0.000
CRP mg/dl	3.30 ± 1.63	1.72 ± 0.60	0.000
Ferritin ng/ml	3541.59 ± 1675.92	106.03 ± 27.13	0.000
Glucose mg/dl	128.35 ± 17.51	86.87 ± 8.96	0.000
Insulin (µIU/ml)	11.67 ± 2.82	7.10 ± 1.76	0.000
HOMA-IR	3.76 ± 1.25	1.54 ± 0.48	0.000
Iron µmol/L	34 ± 4.01	23.99 ± 5.64	0.000
T S	62.50 ± 9.81	36.19 ± 11.54	0.000
HOMA-β	3.86 ± 1.72	6.56 ± 2.42	0.000
TIBC µmol/L	55.01 ± 5.85	68.15 ± 12.26	0.000
UIBC µmol/L	21.02 ± 7.22	44.15 ± 12.29	0.000
Transferrin µmol/L	39 ± 4.13	48.32 ± 8.69	0.000
Adropine ng/ml	0.72 ± 0.24	1.23 ± 0.25	0.000

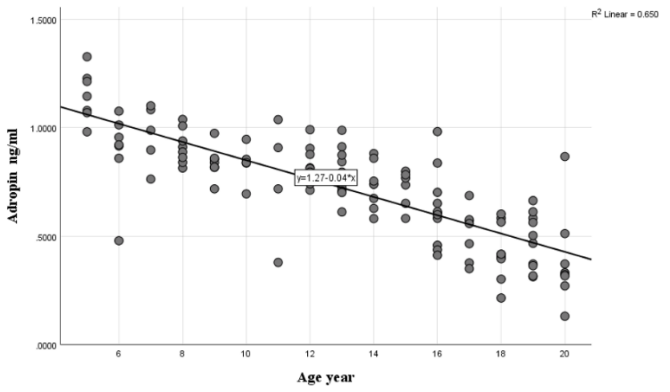
The correlation relationships between adropine and other parameters have been assessed in this stud as shown in Table 2. Through appearing results in this report, we noticed that there is a significantly negative correlation between the level of serum adropine and age, BMI, CRP, ferritin, serum glucose, insulin, HOMA-IR, iron and TS, where the value of correlation between serum adropine and mentioned biochemical parameters are (r = -0.807, P = 0.000), (r = -0.421, P = 0.000), (r = -0.197, P = 0.031), (r = -0.504, P = 0.000), (r = -0.635, P = 0.000), (r = -0.418, P = 0.000), (r = -0.551, P = 0.000), (r = -0.571, P = 0.000) and (r = -0.605, P = 0.000) respectively as explained in Figure 1, whereas adropine has a significant positive association with HOMA-β (r = 0.364, P = 0.000), TIBC (r = 0.296, P = 0.000), UIBC (r = 0.553, P = 0.000) and transferrin (r = 0.266, P = 0.000) as illustrated in Figure 2.

Table 2: Correlation between serum level of adropin with clinical biomarkers in patients with β-thalassemia major.

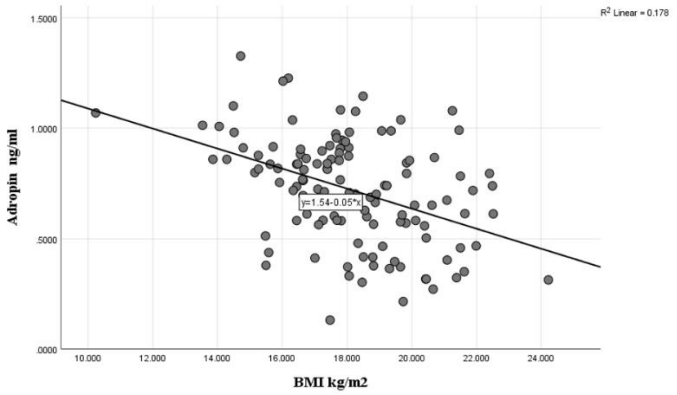
Parameters	r	*P* value
Age	**-0.807****	**0.000**
BMI Kg/m^2	0.421**	0.000
CRP mg/dL	-0.197*	0.031
Ferritin ng/mL	0.504**	0.000
Glucose mg/dL	-0.635**	0.000
Insulin (µIU/mL)	-0.418**	0.000
HOMA-IR	-0.551**	0.000
Iron µmol/L	-0.571**	0.000
T S%	-0.605**	0.000
HOMA-β	0.364**	0.000
TIBC µmol/L	0.296*	0.018
UIBC µmol/L	0.553**	0.000
Transferrin µmol/L	0.296*	0.018

The receiver operating characteristic curve (ROC) of adropine also has been studied, since the area under the ROC curve (ROCAUC) exhibited 0.929 as shown in Figure 3. Adropine showed relatively better performance (accuracy and stability) for the diagnosis and stratification of β-thalassemia major with the cut-off value of 0.892 ng/ml (P<0.001). The sensitivity and specificity for these variables are 0.90 and 0.758, respectively.

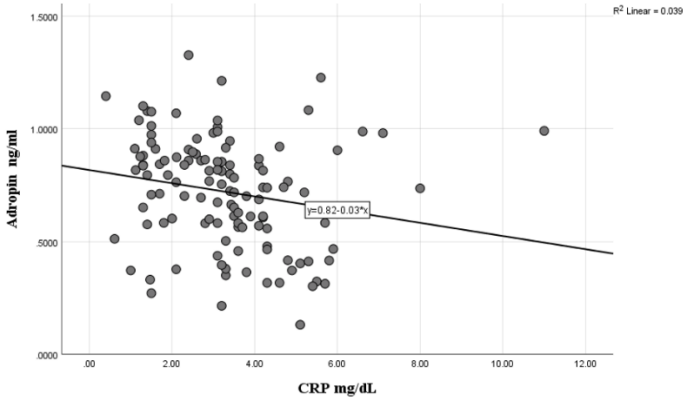
A


R² Linear = 0.650
y=1.27-0.04*x
Adropin ng/ml
Age year

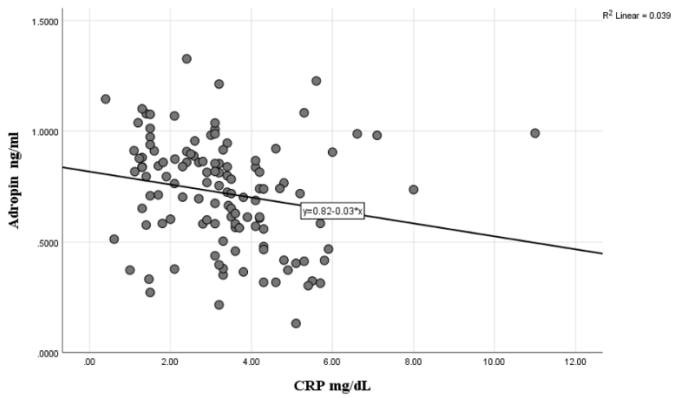
B


R² Linear = 0.178
y=1.54-0.05*x
Adropin ng/ml
BMI kg/m2

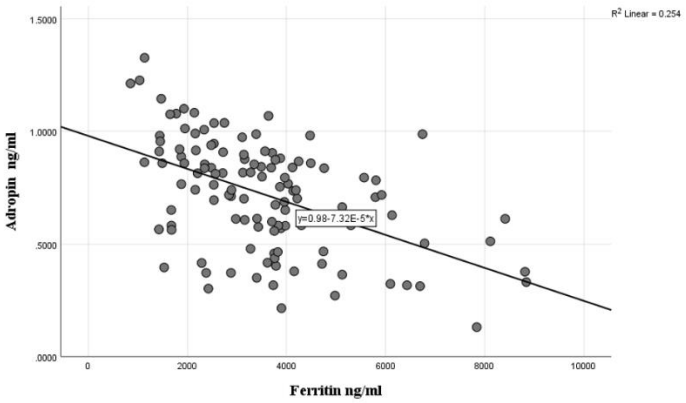
C


R² Linear = 0.039
y=0.82-0.03*x
Adropin ng/ml
CRP mg/dL

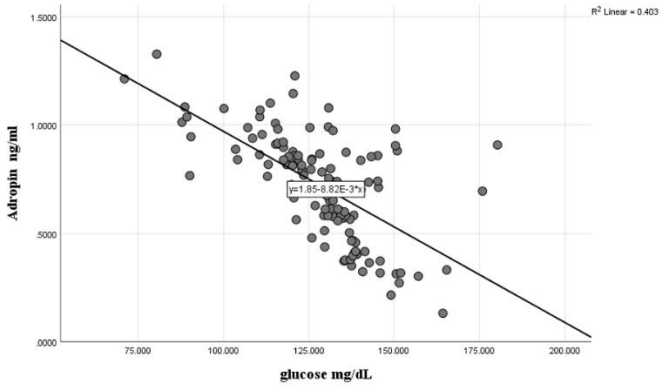
D


R² Linear = 0.039
y=0.82-0.03*x
Adropin ng/ml
CRP mg/dL

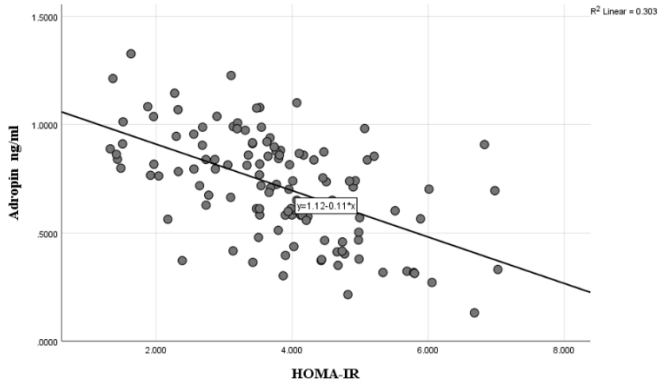
E


R² Linear = 0.254
Adropin ng/ml
y=0.99-7.32E-5*x
Ferritin ng/ml

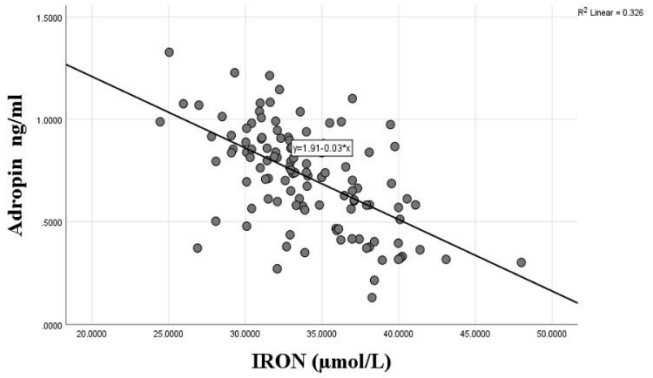
F


R² Linear = 0.403
Adropin ng/ml
y=1.85-8.82E-3*x
glucose mg/dL

G


R² Linear = 0.303
Adropin ng/ml
y=1.12-0.11*x
HOMA-IR

H


R² Linear = 0.326
Adropin ng/ml
y=1.91-0.03*x
IRON (µmol/L)

I

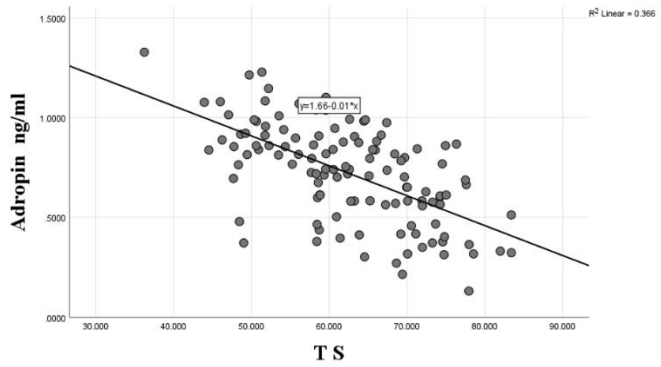
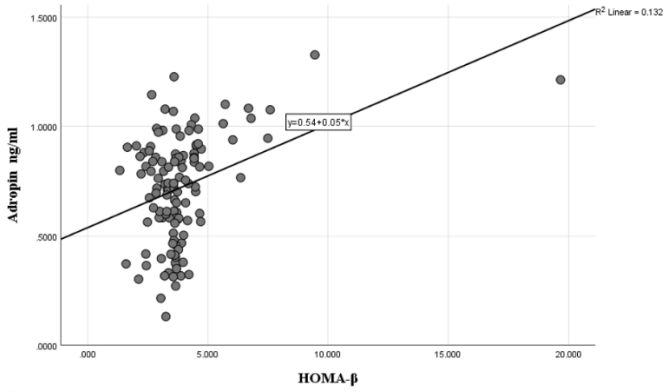
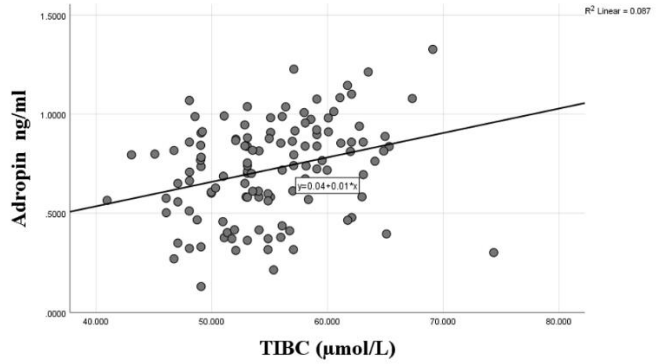


Fig.1. Negative correlations between serum level of adropin in (A) age, (B) BMI, (C) CRP, (D) ferritin, (E) glucose (F) insulin, (G) HOMA-IR (H) Iron (I) TS in patients' group.

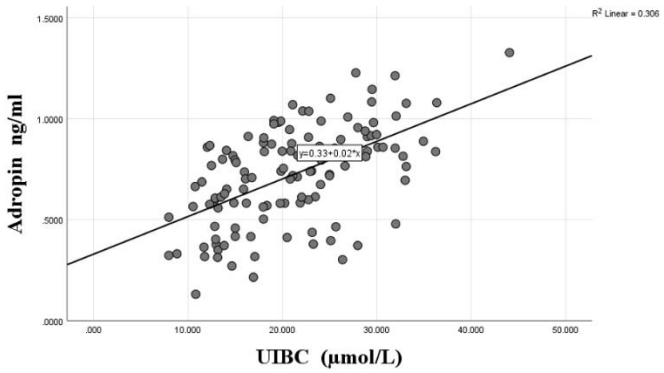
A



B



C



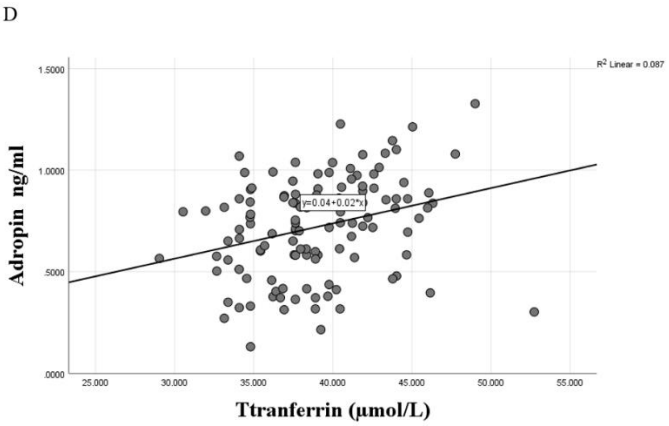


Fig.2: Positive correlation between serum levels of adropin with (A) HOMA-β, (B) TIBC, (C) UIBC, (D) Transferrin in patients' group.

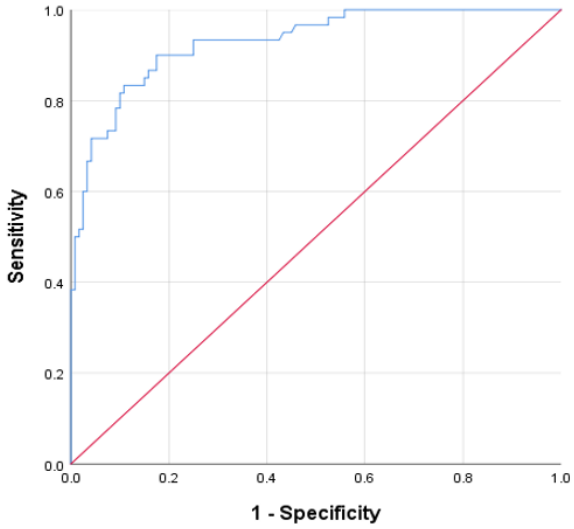


Fig.3. Receiver-operating characteristic (ROC) curves of serum adropin revealing valuable discrimination of patients with thalassemia.

DISCUSSION

The reduction or nonexistent of beta globin chain synthesis is one of the inherited blood diseases known as beta-thalassemia syndromes, which lowers the amount of hemoglobin in red blood cells (RBC) (Galanello and Origa 2010). Our research has shown that individuals with β-thalassemia major had significantly higher serum levels of CRP, ferritin, glucose, insulin, HOMA-IR than healthy volunteers, whereas adropin and HOMA-β have significantly decreased in β-TM as compared with healthy control subjects. In addition, serum adropin level revealed a significant negative correlation with CRP, ferritin, serum glucose, insulin and HOMA-IR while it exhibited a significant positive correlation with HOMA-β for β-TM.

The elevation of ferritin in the current study was found which is in the agreement with other studies (Pootrakul, Vongsmasa et al. 1981) (Dehghani, Karimzadeh et al. 2021). Iron overload, which causes an increase in ferritin in β-TM patients and leads to a buildup of iron in their important organs, is the primary cause of mortality for β-thalassemia major patients (Mishra and Tiwari 2013). Although the liver iron concentration is thought to be the most representative measure of the iron status in the body, the serum ferritin level can be used in β-TM to determine the level of iron in the human body (Angulo, Covas et al. 2008). The findings revealed that individuals with -TM had a greater likelihood of having impaired glucose (diabetes or pre-diabetes), fasting insulin levels, and

HOMA-IR than did healthy individuals. The increasing of impaired glucose and fasting insulin levels in β-TM as comparing with healthy control groups was reported in other studies as well which in matched to our findings (Soliman, Yasin et al. 2013) (Shams, Ashtiani et al. 2010). The prevalence of impaired glucose for β-TM in the current study who need frequent blood transfusions is occurred due to iron overload and the accumulation of iron in the pancreatic β cells and liver causing more inflammation of these organs (Azami, Sharifi et al. 2017) (Habib and Ayad 2021). Both insulin resistance and oxidative stress are brought on by the iron turnover brought on by the hemolysis of the microcytic erythrocytes in β-TM (Ghergherehchi and Habibzadeh 2015) (Tangvarasittichai, Pimanprom et al. 2013) (Cavallo-Perin, Pacini et al. 1995).

Adropin is a peptide hormone which mostly associated with energy homeostasis and vascular protection but it could also be linked with inflammation through its network of pathways and interactions(Brnić, Martinovic et al. 2020). Different physiological and pathophysiological conditions can contribute to changing adropin levels in the human body. Recent studies exhibited that decreased concentration of adropin level is associated with many diseases such as Rheumatoid Arthritis(Simac, Perkovic et al. 2022), type 2 diabetes mellites(Wei, Liu et al. 2022), COVID-19 (Aydın, Uzunçakmak et al. 2022), coronary artery disease(Zheng, Liu et al. 2019) and inflammatory bowel diseases(Brnić, Martinovic et al. 2020). This study reported that patients with β-thalassemia major had lower serum levels of adropin and we expect that adropin may be a risk factor or potential biomarker for predicting the development and progression of many diseases (mainly in cardiovascular disease) in patients with β-thalassemia major.

Lover F et al. and Vasquez Rey E et al. introduced the suggestion to interpret the effect of adropin on heart disease (HD). They showed the main mechanism for endothelial dysfunction. The endothelium plays an important role in the maintenance of vascular homeostasis. the decreased concentration of adropin level in the human body is contributed to endothelial dysfunction which causes the development and progression of cardiovascular disease. On other hand, enhancement of adropin level can supply a productive effect on endothelial dysfunction(Lovren, Pan et al. 2010) (Vázquez-Rey and Kaski 2003), since it is contributed to regulating endothelial cells function by upregulating endothelial nitric oxide synthase (eNOS). Lingzhe WL and colleagues assessed coronary atherosclerosis and serum adropin levels in type 2 diabetes mellitus. Researchers discovered that lower serum adropin levels and more severe angiographic coronary atherosclerosis were observed in patients with diabetes. They discovered that serum adropin has a significant association with the severity of atherosclerosis, and lower serum adropin with more severe atherosclerosis(Wu, Fang et al. 2014). In this study, our findings suggest that decreased circulating adropin levels may be caused by the risk of type 2 diabetes mellitus and atherosclerosis in patients with β-thalassemia major.

CONCLUSIONS

Patients with β-thalassemia major showed significantly lower adropin than control groups. Adropin may be more used as a biomarker for predicting the development of complications of the disorder. Decreased circulating adropin may promote diabetes mellitus, endothelial dysfunction causing cardiovascular diseases, kidney dysfunction, rheumatoid arthritis, and inflammatory bowel diseases, especially in Patients with β-thalassemia major.

Acknowledgment

The authors thank the patients for their cooperation and the medical staff in the laboratories of Thalassemia Unit" in "Al Zahra Teaching Hospital" Najaf, Iraq.

Declaration of interests

The authors declare no found conflict of interest.

Founding: None

REFERENCES

Ali, I. I., C. D'Souza, J. Singh and E. Adeghate (2022). "Adropin's role in energy homeostasis and metabolic disorders." *International journal of molecular sciences,* **23**(15): 8318.

Ali, S., S. Mumtaz, H. A. Shakir, M. Khan, H. M. Tahir, S. Mumtaz, T. A. Mughal, A. Hassan, S. A. R. Kazmi and M. Irfan (2021). "Current status of beta-thalassemia and its treatment strategies." *Molecular Genetics & Genomic Medicine,* **9**(12): e1788.

Angulo, I. L., D. T. Covas, A. A. Carneiro, O. Baffa, J. Elias Junior and G. Vilela (2008). "Determination of iron-overload in thalassemia by hepatic MRI and ferritin." *Revista Brasileira de Hematologia e Hemoterapia*, **30**: 449-452.

Aravind, K., T. Poornima, S. Sibasis and S. A. Kumar (2005). "Prevalence of insulin resistance: a prospective study in North Indian population." Indian Journal of Clinical Biochemistry, **20**: 10-17.

Aydın, P., S. K. Uzunçakmak, H. Tör İ, A. Bilen and A. Özden (2022). "Comparison of Serum Adropin Levels in Patients with Diabetes Mellitus, COVID-19, and COVID-19 with Diabetes Mellitus." Eurasian Journal of Medicine **54**(2): 197-201.

Azami, M., A. Sharifi, S. Norozi, A. Mansouri and K. Sayehmiri (2017). "Prevalence of diabetes, impaired fasting glucose and impaired glucose tolerance in patients with thalassemia major in Iran: A meta-analysis study." Caspian Journal of Internal Medicine **8**(1): 1-15.

Brnić, D., D. Martinovic, P. M. Zivkovic, D. Tokic, I. Tadin Hadjina, D. Rusic, M. Vilovic, D. Supe-Domic, A. Tonkic and J. Bozic (2020). "Serum adropin levels are reduced in patients with inflammatory bowel diseases." Scientific Reports, **10**(1): 1-9.

Cavallo-Perin, P., G. Pacini, F. Cerutti, A. Bessone, C. Condo, L. Sacchetti, A. Piga and G. Pagano (1995). "Insulin resistance and hyperinsulinemia in homozygous β-thalassemia." *Metabolism,* **44**(3): 281-286.

De Sanctis, V., A. Soliman and M. Yassin (2013). "Iron overload and glucose metabolism in subjects with β-thalassaemia major: an overview." Current Diabetes Reviews **9**(4): 332-341.

Dehghani, M., P. Karimzadeh, N. Azadeh, A. Rezvani and A. Kashkooe (2021). "Serum ferritin and hematological indices in thalassemia minor and nontransfusion dependent hemoglobinopathy." *Iraqi Journal of Hematology,* **10**(1): 17.

Farmakis, D., J. Porter, A. Taher, M. D. Cappellini, M. Angastiniotis and A. Eleftheriou (2022). "2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia." *HemaSphere,* **6**(8).

Fucharoen, S. and D. J. Weatherall (2012). "The hemoglobin E thalassemias." *Cold Spring Harbor perspectives in medicine,* **2**(8): a011734.

Galanello, R. and R. Origa (2010). "Beta-thalassemia." *Orphanet journal of rare diseases*, **5**(1): 1-15.

Galanello, R. and R. Origa (2010). "Beta-thalassemia." Orphanet Journal of Rare Dis eases **5**: 11.

Gao, S., R. P. McMillan, Q. Zhu, G. D. Lopaschuk, M. W. Hulver and A. A. Butler (2015). "Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance." *Molecular metabolism,* **4**(4): 310-324.

Ghergherehchi, R. and A. Habibzadeh (2015). "Insulin resistance and β cell function in patients with β-thalassemia major." *Hemoglobin,* **39**(1): 69-73.

Habib, R. M. and R. G. E.-S. Ayad (2021). "Assessment of hepatic and pancreatic iron overload by magnetic resonance in β-thalassemia major." *Menoufia Medical Journal,* **34**(4): 1410.

Jasaszwili, M., M. Billert, M. Z. Strowski, K. W. Nowak and M. Skrzypski (2020). "Adropin as a fat-burning hormone with multiple functions—Review of a decade of research." *Molecules,* **25**(3): 549.

Khanna, D., C. Peltzer, P. Kahar and M. S. Parmar (2022). "Body Mass Index (BMI): A Screening Tool Analysis." *Cureus,* **14**(2): e22119.

Lovren, F., Y. Pan, A. Quan, K. K. Singh, P. C. Shukla, M. Gupta, M. Al-Omran, H. Teoh and S. Verma (2010). "Adropin is a novel regulator of endothelial function." *Circulation,* **122** (11_suppl_1): S185-S192.

Luo, Y., R. Bajoria, Y. Lai, H. Pan, Q. Li, Z. Zhang, P. Yang, R. Chatterjee and Y. Liang (2019). "Prevalence of abnormal glucose homeostasis in Chinese patients with non-transfusion-dependent thalassemia." *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy,* **12**: 457.

Mishra, A. K. and A. Tiwari (2013). "Iron overload in Beta thalassaemia major and intermedia patients." *Maedica (Bucur),* **8**(4): 328-332.

Moradi, G. and E. Ghaderi (2013). "Chronic disease program in Iran: Thalassemia control program." *Chronic Diseases Journal,* **1**(2): 98-106.

Noetzli, L. J., S. D. Mittelman, R. M. Watanabe, T. D. Coates and J. C. Wood (2012). "Pancreatic iron and glucose dysregulation in thalassemia major." *American journal of hematology,* **87**(2): 155-160.

Pootrakul, P., V. Vongsmasa, P. La-ongpanich and P. Wasi (1981). "Serum ferritin levels in thalassemias and the effect of splenectomy." *Acta Haematol,* **66**(4): 244-250.

Porter, J. B. (2009). "Pathophysiology of transfusional iron overload: contrasting patterns in thalassemia major and sickle cell disease." *Hemoglobin,* **33**(sup1): S37-S45.

Sajadi Hezaveh, Z., A. Azarkeivan, L. Janani and F. Shidfar (2019). "Effect of quercetin on oxidative stress and liver function in beta-thalassemia major patients receiving desferrioxamine: A double-blind randomized clinical trial." Journal of Reserch Medical Sciences **24**: 91.

Shams, S., M. T. H. Ashtiani, M. Monajemzadeh, L. Koochakzadeh, H. Irani, F. Jafari and A. Mohseni (2010). "Evaluation of serum insulin, glucose, lipid profile, and liver function in β-thalassemia major patients and their correlation with iron overload." *Laboratory medicine,* **41**(8): 486-489.

Simac, P., D. Perkovic, I. Bozic, N. Bilopavlovic, D. Martinovic and J. Bozic (2022). "Serum Adropin Levels in Patients with Rheumatoid Arthritis." *Life,* **12**(2): 169.

Skrzypski, M., P. A. Kołodziejski, E. Pruszyńska-Oszmałek, T. Wojciechowicz, P. Janicka, M. Krążek, E. Małek, M. Z. Strowski and K. W. Nowak (2022). "Daily Treatment of Mice with Type 2 Diabetes with Adropin for Four Weeks Improves Glucolipid Profile, Reduces Hepatic Lipid Content and Restores Elevated Hepatic Enzymes in Serum." *International Journal of Molecular Sciences,* **23**(17): 9807.

Soliman, A. T., M. Yasin, A. El-Awwa and V. De Sanctis (2013). "Detection of glycemic abnormalities in adolescents with beta thalassemia using continuous glucose monitoring and oral glucose tolerance in adolescents and young adults with β-thalassemia major: Pilot study." Indian Journal of Endocrinology and Metabolism **17**(3): 490-495.

Tangvarasittichai, S., A. Pimanprom, A. Choowet and O. Tangvarasittichai (2013). "Association of iron overload and oxidative stress with insulin resistance in transfusion-dependent beta-thalassemia major and beta-thalassemia/ HbE patients." Clinical Laboratory journal **59**(7-8): 861-868.

Thein, S. L. (2018). "Molecular basis of β thalassemia and potential therapeutic targets." *Blood Cells, Molecules, and Diseases,* **70**: 54-65.

Vázquez-Rey, E. and J. C. Kaski (2003). "Cardiovascular syndrome X and endothelial dysfunction." *Revista Espanola De Cardiologia,* **56**(2): 181-192.

Wei, W., H. Liu, X. Qiu, J. Zhang, J. Huang, H. Chen, S. Qiu, R. Lin, S. Li and M. Tu (2022). "The association between serum adropin and carotid atherosclerosis in patients with type 2 diabetes mellitus: a cross-sectional study." *Diabetology & Metabolic Syndrome,* **14**(1): 27.

Wu, L., J. Fang, L. Chen, Z. Zhao, Y. Luo, C. Lin and L. Fan (2014). "Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients." *Clinical chemistry and laboratory medicine,* **52**(5): 751-758.

Ye, Z., C. Zhang and Y. Zhao (2021). "Potential effects of adropin on systemic metabolic and hormonal abnormalities in polycystic ovary syndrome." *Reproductive BioMedicine Online,* **42**(5): 1007-1014.

Zheng, J., M. Liu, L. Chen, F. Yin, X. Zhu, J. Gou, W. Zeng and Z. Lv (2019). "Association between serum adropin level and coronary artery disease: a systematic review and meta-analysis." *Cardiovascular Diagnosis and Therapy,* **9**(1): 1.