

Assessment of Biochemical Parameters and Study its Correlation in β -Thalassemia Major Patients and Healthy Controls in Kirkuk City, Iraq

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Abstract

Background: Thalassemia is the name of a group of genetically (acquired) blood diseases, which include defects on the production of hemeprotein, and fractional or complete damage to the combination of a particular sort of simple protein chain. The defect might have an effect on the “ α , γ and δ ” chains or might have an effect on few combination of the “ β , γ and δ ” chains within the same patient. **Objective:** The aim of the study was to evaluate the various protein parts in the β -thalassemia major (BTM) patients in the city of Kirkuk compared to the healthy control group. **Materials and Methods:** Seventy blood samples were divided into two groups – 45 patients with BTM samples; they visited Azadi Hospital/Kirkuk city and 25 samples of healthy controls as a control group. **Results:** The results of the correlation coefficient showed that there were significant positive correlation between total protein (TP) and albumin and albumin and albumin/globulin ratio and significant negative correlation between globulin and albumin/globulin ratio. Finally, nonsignificant positive correlation between TP and thiol, TP and globulin, TP and albumin-globulin ratio, albumin/thiol, globulin and free amino and globulin and thiol and non-significant negative correlation between TP and free amino, thiol and carbonyl, thiol and free amino, carbonyl and free amino, albumin and carbonyl, albumin and free amino, albumin and globulin, and globulin and carbonyl. **Conclusion:** The correlation studies between the above parameters indicated a negative relationship between TP and free amine in β -thalassemia patients. These results reinforce the importance of measuring both TP and free amine concentration to investigate their development of β -thalassemia complications. Also, measuring both thiol and free amine yields is also important for the same reason.

Keywords: Carbonyl and free amino, thiol total protein, β -thalassemia major

INTRODUCTION

Thalassemia is the name of a group of genetically (acquired) blood diseases, which include defects on the production of hemeproteins, and fractional or complete damage to the combination of a particular sort of simple protein chain. The defect might have an effect on the “ α , γ and δ ” chains or might have an effect on few combinations of the “ β , γ and δ ” chains within the same patient. However, this defect does not effect on α and β chain along.^[1] β -thalassemia major (BTM) related with extreme iron deficiency typically hemoglobin level lower than 7 g/dl, in this manner, blood transfusion is required every “2–5” weeks to keep up a pre-transfusion hemoglobin level over 10 g/dl to keep up typical life.^[2] Thalassemia was first reported by Cooley and Lee,^[3] diagnosed by the “complete blood count, special hemoglobin and genetic” tests. Before blood transfer, the

analysis may make complete prenatal, difficult.^[4] The types of β -thalassemia mutations in Iraqi population were geographically distributed around the Iraqi regions and were centralized within southern and northern parts may be due to the history of malaria which predispose to a selective advantage against β -thalassemia prevalence.^[5] Recently, it was found that severe β -thalassemia accounts for 50,000–100,000 deaths/year of all deaths of children under 5 years in low- or middle-income countries and in Iraq it was found that there were over 2,000 cases of thalassemia

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in the Kurdistan Region and Kirkuk with a round 30,000 people are carries of β -thalassemia disorder.^[6]

Many studies have demonstrated increased oxidative stress in β -TM patients and biomarkers of oxidative stress such as plasma melon dialdehyde^[7] and thiobarbituric acid reactive substances are typically elevated in these patients.^[8] BTM patients simply have a lower overall capacity of various enzymes and other endogenous compounds (e.g., vitamins) to exert their antioxidant effects.^[9] Carbonated proteins are involved in various biological phenomena^[10] like “Age-associated disorders, cytotoxicity, Parkinson’s and Alzheimer’s disease, cancer, chronic lung disease, renal failure, diabetes, sepsis, arthritis, skeletal muscle dysfunctions, chronic arterial occlusion, acute pancreatitis, thalassemia major ... etc.”^[11]

Thiols are the most important reluctant molecules in the body. Reactive oxygen species (ROS) formed in the organism transfer excessive electrons to the thiols and oxidize them, thereby forming disulfide bonds. However, these disulfide bonds are reversible. They can turn back into thiols depending on the organism’s antioxidant–oxidant balance. Thus, they can refer to a dynamic thiol–disulfide homeostasis condition.^[12] There has been an increasing interest in the use of antioxidants, or naturally occurring products that possess antioxidant properties, in thalassemia patients.^[13]

This study aimed to evaluate the levels of some biochemical parameters including total protein (TP), albumin, globulin, albumin/globulin ratio, free amino, carbonyl and thiol” inpatients with BTM compared to healthy controls and find different phenotype links between these biochemical parameters in the patients group.

MATERIALS AND METHODS

Study design and subjects

A total of 70 blood samples comprising of 25 participants (15 males and 10 females) healthy controls as a control group with age ranges between 10 and 40 years and 45 samples (16 males and 29 females) as BTM patients with the same age range as healthy controls. Those patients visited “Azadi teaching hospital”/Kirkuk city/Iraq, from “April to October” 2019 and diagnosed by specialist doctors. Any cases that may interfere with this study such as diabetes mellitus, hypertension, anemia and liver diseases were excluded from the study.

Blood collection

Using a disposable syringe, 2–3 ml of blood was collected by venipuncture in glass tubes within gel tube for separated. The tubes were centrifuged for 10 min with 1500×g. The serum was separated from the cells and buffy coat removed. Blood samples were collected for testing some biochemical parameters, as discussed in the following sections.

Biochemical assays

Quantitative TP determination was achieved by absorbance measurements at 660 nm according to Lowry method^[14] using

bovine serum albumin as a standard. Albumin concentration was determined using bromo cresol green reagent method.^[15] The spectrophotometric estimation of free amino groups was performed according to the Zaia *et al.* method.^[16] Thiol groups were assayed according to the method of Ellman.^[17] The carbonyl group in the protein was estimated using the method of Levine *et al.*^[18]

Statistical analysis

Statistical analysis was done using GraphPad prism version 6 software (GraphPad Software, San Diego, CA, USA). Values were expressed as mean $\pm$ standard deviation (SD) and $P \leq 0.05$. The comparison of (mean $\pm$ SD) was performed using the Student’s *t*-test. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Table 1 represents the levels of some biochemical parameters as (mean $\pm$ SD) for all the studied groups:

The results Table 1 indicated that there was significant ($P \leq 0.05$) decrease in TP, albumin, globulin, and albumin/globulin ratio levels and a significant ($P \leq 0.05$) increase was observed in thiol, thiol/protein, free amino, free amino/protein, carbonyl and carbonyl/protein levels in the patients group compared to healthy controls.

Various phenotype correlations were established between the studied biochemical parameters in the patients group. The results showed that there was statistically significant ($P \leq 0.05$) positive correlation between TP and albumin and albumin and albumin/globulin ratio and significant ($P \leq 0.05$) negative correlation between the globulin and albumin/globulin ratio.

Finally, nonsignificant ($P \geq 0.05$) positive correlation was found between TP and thiol, TP and globulin, TP and albumin-globulin ratio, albumin/thiol, globulin and free amino and globulin and thiol and nonsignificant ($P \geq 0.05$) negative correlation was found between TP and free amino, thiol and carbonyl, thiol and free amino, carbonyl and free amino, albumin and carbonyl, albumin and free amino, albumin and globulin and globulin and carbonyl, as shown in Table 2.

DISCUSSION

Serum levels of albumin and globulin and albumin/globulin ratio are easily measurable biomarkers which can be used in combination to predict the survival of patients with many diseases. TP is considered as the most abundant compounds in serum. The proteins involved include enzymes, hormones and antibodies as well as regulators to osmotic pressure balance. The possible cause of decreased serum TP secondarily may be due to decrease in the synthesis of protein by the liver. Albumin is the major constituent of serum protein (usually over 50%). It is manufactured by the liver and it helps in “osmotic pressure regulation, nutrient transport and waste removal.”^[17]

Table 1: Levels of some biochemical parameters as (mean $\pm$ standard deviation) in all studied groups

Parameters	Mean $\pm$ SD		P
	Healthy controls (n=25)	β -thalassemia major (n=45)	
TP (g/dl)	5.803 $\pm$ 1.07	2.49 $\pm$ 0.64	0.0028*
Albumin (g/dl)	4.395 $\pm$ 0.86	1.931 $\pm$ 0.57	0.015*
Globulin (g/dl)	1.019 $\pm$ 1.0	0.8224 $\pm$ 0.56	0.0001*
Albumin/globulin (g\dl)	8.230 $\pm$ 6.8	3.576 $\pm$ 2.76	0.0001*
Thiol (μ mole/L)	18.80 $\pm$ 3.088	31.24 $\pm$ 8.732	0.0001*
Thiol (μ mole/mg protein)	3.366 $\pm$ 1.161	14.49 $\pm$ 5.744	0.0001*
Free amino (m mole/L)	2.579 $\pm$ 0.963	6.327 $\pm$ 2.954	0.0001*
Free amino (m mole/mg protein)	0.4209 $\pm$ 0.1966	3.110 $\pm$ 1.737	0.0001*
Carbonyl (n mole/ml)	57.95 $\pm$ 28.25	71.22 $\pm$ 23.86	0.0001*
Carbonyl (n mole/mg protein)	9.589 $\pm$ 5.104	31.39 $\pm$ 12.30	0.0001*

*Significant ($P\leq 0.05$). SD: Standard deviation, TP: Total protein**Table 2: Correlation between the studied serum parameters in patients group**

Parameters	r/P
TP/thiol	0.0776/0.6124
TP/carbonyl	0.2967/0.0478*
TP/free amino	-0.1348/0.3772
Thiol/carbonyl	-0.03817/0.8034
Thiol/free amino	-0.007353/0.9618
Carbonyl/free amino	-0.08/0.5629
TP/albumin	0.5631/0.0001*
TP/globulin	0.2441/0.1052
TP/albumin-globulin ratio	0.1494/0.3272
Albumin/carbonyl	-0.1685/0.2684
Albumin/free amino	-0.00244/0.9872
Albumin/thiol	0.1753/0.2493
Albumin/globulin	-0.2198/0.1469
Albumin/albumin-globulin ratio	0.5347/0.0002*
Globulin/carbonyl	-0.1816/0.2325
Globulin/free amino	0.07648/0.6176
Globulin/thiol	0.009161/0.9524
Globulin/albumin-globulin ratio	-0.7071/0.0001*
Albumin-globulin ratio/carbonyl	0.02758/0.8573
Albumin-globulin ratio/free amino	-0.1180/0.4403
Albumin-globulin ratio/thiol	0.1510/0.3222

Coefficient of correlation/significance r/P. *Significant ($P\geq 0.05$).

TP: Total protein

The physiological function of albumin is to regulate “osmotic pressure and the transport of fatty acids, bilirubin, cholesterol and drugs.” Albumin is the main fatty acid-binding protein in plasma and possesses seven binding sites for fatty acids with moderate and high affinity.^[19] It has been shown that albumin plays a key role in the anti-oxidative capacity of blood plasma against ROS.^[20] Globulins are the extra main constituent of full serum proteins, and role as carters of sex hormones and play a major role in immunity and inflammation.^[21]

The AGR, which syndicates albumin and globulin, is too one of the representative parameters used to measure systemic inflammation rank. In addition, this parameter can be measured easily and at a low cost. In general, albumin and globulin

are mainly produced by hepatic cells. Hepatic dysfunction, malnutrition, or systemic inflammation can lead to decreased serum albumin levels. In contrast, serum globulin levels tend to increase in infectious disease and/or systemic inflammation. Thus, the inverse correlation of these two parameters in systemic inflammation status can strongly affect the AGR in patients with malignant disease.^[22]

The results of albumin concentration in this study were inconsistent with the results obtained by Livrea *et al.*^[23] and Al-Rubaei *et al.*,^[24] who studied the BTM patients. They suggested that the decrease in TP in the serum was due to the reduction of secondary protein synthesis by the liver. The (A/G) ratio of β -thalassemia major patients is much less than one can give clues about problems in the body.^[24] Furthermore, the results of TP and albumin were not in accordance with the study conducted by Azad *et al.*,^[25] Guzalcicek *et al.*,^[26] Kawahara *et al.*^[27] and of Pipaliya *et al.*^[28] who have studied the BTM patients. TP concentration is not consistent with the results of Sherief *et al.*^[29] on BTM patients and Karar *et al.*'s^[30] study on breast cancer patients. The results of albumin concentration were not in accordance with the results of Yassin *et al.*^[31]

The results in Table 1 also indicated that there was a significant increase ($P\leq 0.05$) in the thiol, thiol/protein, free amino, free amino/protein, carbonyl, and carbonyl/protein levels of the BTM patients compared to healthy subjects. Serum total thiols as a marker of oxidative stress have been studied in various systemic diseases such as ischemic heart disease, diabetes, pulmonary diseases, Alzheimer's disease, preeclampsia, kidney diseases, and alcoholism.^[32]

The results of both thiol and carbonyl levels are consistent with Livrea *et al.*'s^[23] study on BTM and Trombetta *et al.*'s^[33] study. Previous studies have shown that oxidative stress may play a role in the pathogenesis of many diseases, and thus thiol chemistry has become more important. Thiols are very important molecules in the antioxidation process that contain -SH groups. Sulfhydryl groups can reduce electrons so that the organism can be protected from oxidative damage caused by ROS.

Thiols are converted to disulfides by this reaction; this double-sided balance had been measured.^[12] The increase in the level of thiol was consistent with the results obtained by Guzelcicek, *et al.*^[34] who studied the level of thiol in β -thalassemia major. Our result was also consistent with the results obtained by Karar *et al.*^[30] who studied the concentrations of carbonyl, thiol, and free amino in breast cancer. Amino, carbonyl, and thiol groups present in the surfaces of proteins participate in protein modification with glucose.^[34] The products created in the first phase of the reaction change in a series of subsequent reactions.^[35] Thiol group is a strong nucleophile, at physiological pH values is stronger than that amine and carbonyl groups with Lys and Arg side chains.^[36] The potential importance of SH and D-glucose reaction in protein cross-linking has only been reported by Zeng and Davies^[36] and Zeng and Davies;^[37] they concluded that the product of the initial reaction between a thiol group and D-glucose can be a target for the next reaction with an amine group or, vice versa, the initial product of a reaction between an amine group and D-glucose can be a target for this group.^[35]

CONCLUSION

Decrease in TP and decrease in albumin and globulins with no increased concentration of thiol, free amino and carbonyl in sera for β -thalassemia patients compared to health materials will continue to play a key role in the evaluation and treatment of β -thalassemia patients. The correlation studies between the above parameters indicated a negative relationship between TP and free amine in β -thalassemia patients, and these results reinforce the importance of measuring both TP and free amine concentration to investigate their development of β -thalassemia complications. Also, measuring both thiol and free amine yields is also important for the same reason.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with a patient's verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a Local Ethics Committee Azadi teaching hospital/Kirkuk city/Iraq.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Ji L, Yu J, Zhou Y, Xia Y, Xu A, Li W, *et al.* Erroneous HbA1c measurements in the presence of β -thalassemia and common Chinese hemoglobin variants. *Clin Chem Lab Med* 2015;53:1451-8.
- Kadhum SJ. The prevalence of hypothyroidism among patients with beta-thalassemia major, Western Iraq. *Iraqi Acad Sci J* 2018;17:170-4.
- Awjagh IS. Study of zinc and copper in patients with Beta Thalassemia major and splenotomized in Kirkuk city. *Kirkuk Univ J Sci Stud* 2018;13:239-48.
- Abdul-Hussian MA, Kareem AZ, Jameel YM. Evaluation of Vitamin-D3 Concentration in patients with thalassemia in Baghdad city. *Diyala J Med* 2018;14:10-9.
- Saud AM. Molecular and biochemical study on β -thalassemia patients in Iraq. *Current Res Microbiol Biotechnol* 2013;1:160-5.
- Tawfeeq AA. A prospective study for the outcomes of thalassemia in Kirkuk 2016. *Kirkuk Univ J Sci Stud* 2017;12:140-56.
- Walter PB, Macklin EA, Porter J, Evans P, Kwiatkowski JL, Neufeld EJ, *et al.* Inflammation and oxidant-stress in beta-thalassemia patients treated with iron chelators deferasirox (ICL670) or deferoxamine: An ancillary study of the Novartis CICL670A0107 trial. *Haematologica* 2008;93:817-25.
- Awadallah S. Protein antioxidants in thalassemia. *Adv Clin Chem* 2013;60:85-128.
- Tsamesidis I, Fozza C, Vagdatli E, Kalpaka A, Ciroto C, Pau MC, *et al.* Total antioxidant capacity in Mediterranean β -thalassemic patients. *Adv Clin Exp Med* 2017;26:789-93.
- Liggins J, Furth AJ. Role of protein-bound carbonyl groups in the formation of advanced glycation endproducts. *Biochim Biophys Acta* 1997;1361:123-30.
- Suzuki YJ, Carini M, Butterfield DA. Protein carbonylation. *Antioxid Redox Signal* 2010;12:323-5.
- Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014;47:326-32.
- Ozdemir ZC, Koc A, Aycicek A, Kocyigit A. N-Acetylcysteine supplementation reduces oxidative stress and DNA damage in children with β -thalassemia. *Hemoglobin* 2014;38:359-64.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
- Müller K, Brunnberg L. Determination of plasma albumin concentration in healthy and diseased turtles: A comparison of protein electrophoresis and the bromocresol green dye-binding method. *Vet Clin Pathol* 2010;39:79-82.
- Zaia DA, Barreto WJ, Santos NJ, Endo AS. Spectrophotometric method for the simultaneous determination of proteins and amino acids with p-benzoquinone. *Anal Chim Acta* 1993;277:89-95.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
- Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, *et al.* Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;186:464-78.
- Pieniazek A, Gwozdziński L, Zbrog Z, Gwozdziński K. Alterations in conformational state of albumin in plasma in chronic hemodialyzed patients. *PLoS One* 2018;13:e0192268.
- Taverna M, Marie AL, Mira JP, Guidet B. Specific antioxidant properties of human serum albumin. *Ann Intensive Care* 2013;3:4.
- Toiyama Y, Yasuda H, Ohi M, Yoshiyama S, Araki T, Tanaka K, *et al.* Clinical impact of preoperative albumin to globulin ratio in gastric cancer patients with curative intent. *Am J Surg* 2017;213:120-6.
- Oki S, Toiyama Y, Okugawa Y, Shimura T, Okigami M, Yasuda H, *et al.* Clinical burden of preoperative albumin-globulin ratio in esophageal cancer patients. *Am J Surg* 2017;214:891-8.
- Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, *et al.* Oxidative stress and antioxidant status in beta-thalassemia major: Iron overload and depletion of lipid-soluble antioxidants. *Blood* 1996;88:3608-14.
- Al-Rubaei ZM, Al-Shammaa NM, Malik EM, Malik AM. A comparative biochemical study of proteins profile in Iraqi children and adolescent with β -Thalassemia. *Iraq J Pharmaceut Sci* 2010;19:19-23.
- Azad SM, Mukherjee A, Kapoor R, Bannerji R, Ray J. Superior sagittal sinus thrombosis in a case of beta thalassaemia major. *Int J Adv Med* 2017;4:585.
- Guzelcicek A, Cakirca G, Erel O, Solmaz A. Assessment of thiol/disulfide balance as an oxidative stress marker in children with β -thalassemia major. *Pak J Med Sci* 2019;35:161-5.
- Kawahara A, Morioka T, Otani Y, Kanno K, Edahiro T, Fukushima N, *et al.* Successful treatment of acute chest syndrome with manual exchange transfusion in a patient with sickle beta+-thalassemia. *Intern Med* 2019;58:1629-34.

28. Pipaliya N, Solanke D, Parikh P, Ingle M, Sharma R, Sharma S, *et al.* Comparison of tissue elastography with magnetic resonance imaging T2* and serum ferritin quantification in detecting liver iron overload in patients with thalassemia major. *Clin Gastroenterol Hepatol* 2017;15:292-80.
29. Sherief LM, Dawood O, Ali A, Sherbiny HS, Kamal NM, Elshanshory M, *et al.* Premature atherosclerosis in children with beta-thalassemia major: New diagnostic marker. *BMC Pediatr* 2017;17:69.
30. Karar F, Zainal IG, Mohammed MO. Effects of ascorbic and cinnamic acids on the albumin glycation level in breast cancer patients. *J Glob Pharm Technol* 2018;10:28-35.
31. Yassin MA, Soliman AT, De Sanctis V, Hussein RM, Al-Okka R, Kassem N, *et al.* adenu® substituting Exjade® in iron overloaded β -thalassemia major (BTM) patients: A preliminary report of the effects on the tolerability, serum ferritin level, liver iron concentration and biochemical profiles. *Mediterr J Hematol Infect Dis* 2018;10:e2018064.
32. Kundi H, Ates I, Kiziltunc E, Cetin M, Cicekcioglu H, Neselioglu S, *et al.* A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. *Am J Emerg Med* 2015;33:1567-71.
33. Trombetta D, Gangemi S, Saija A, Minciullo PL, Cimino F, Cristani M, *et al.* Increased protein carbonyl groups in the serum of patients affected by thalassemia major. *Ann Hematol* 2006;85:520-2.
34. Baynes JW, Thorpe SR. Glycoxidation and lipoxidation in atherogenesis. *Free Radic Biol Med* 2000;28:1708-16.
35. Westwood ME, Thornalley PJ. Molecular characteristics of methylglyoxal-modified bovine and human serum albumins. Comparison with glucose-derived advanced glycation endproduct-modified serum albumins. *J Protein Chem* 1995;14:359-72.
36. Zeng J, Davies MJ. Evidence for the formation of adducts and S-(carboxymethyl) cysteine on reaction of α -dicarbonyl compounds with thiol groups on amino acids, peptides, and proteins. *Chem Res Toxicol* 2005;18:1232-41.
37. Zeng J, Davies MJ. Protein and low molecular mass thiols as targets and inhibitors of glycation reactions. *Chem Res Toxicol* 2006;19:1668-76.