Trace Elements and Oxidant/Antioxidant Status in Beta-Thalassemia Patients

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ABSTRACT

An genetic condition called -thalassemia major is caused by reduced or absent beta-globin chains. We attempted to measure any association between TAC and TOS with some trace elements (Zn, Cu, Mg, Co, and Se) in β-thalassemia patients as an additional tool in the diagnosis. The study included 52 Arabic Iraqi β-thalassemia primary patients and 44 aged-matched children as a control group. These patients were registered as thalassemia patients in "Miesan center for blood disease" in Maysan province-Iraq. Patients with -thalassemia showed considerably (p0.05) lower levels of Mg as well as significantly (p0.01) lower levels of serum Hb, RBC, HCT, MCV, MCH, TIBC, TAC, Zn, and Se compared to normal controls. MDA, Iron, Ferritin, MCHC, PLT, T. bilirubin, AST, ALT, and Copper levels in patients were substantially higher than in controls (p0.01) and (p0.05), respectively. The current results show that in patients with -thalassemia, trace element levels (Se, Zn, Mg, Cu, and Co) were significantly correlated with TAC and MDA levels. Our findings showed that the relationship between TAC and MDA and a few trace elements (Zn, Cu, Mg, Co, and Se) could be useful markers for predicting disease progression in -thalassemia patients.

Keywords: Beta-thalassemia, Oxidative stress, Total antioxidants capacity, Trace elements

INTRODUCTION

One of the most prevalent hereditary single gene illnesses, -thalassemia is brought on by 200 or more mutations in the beta-globin genes. Although little or limited beta-globin chain formation occurs in β-thalassemia, alpha chain production persists. Growing erythrocytes become more brittle as alpha chain synthesis increases, resulting in early damage, ineffective erythropoiesis, and anemia. Depending on the beta-globin chain deficit, there are several kinds of β-thalassemia[1]. The most severe form of the disease, beta thalassemia major, is marked by the homozygous or compound heterozygous inheritance of two beta-globin chain mutations. Due to their extreme anemia, patients with beta-thalassemia major need many blood transfusions to survive. The beta-globin chain deficiency is 50% for -thalassemia trait (minor), 100% for -thalassemia major, and 50–80% for -thalassemia intermediate[2].

Essential trace elements like iron, copper, and zinc are all impacted by a number of blood disorders, including thalassemia in affluent people. After a series of events causes the early death of red cells during hemolysis, the alteration of these components, along with high levels of hemoglobin subunits, stimulates the generation of oxygen radicals. Although serum iron is high in homologous β-thalassemia and iron-binding capacity is saturated," percent transferrin saturation and raised plasma ferritin are the primary indicators for iron overload. Because serum ferritin might be elevated as a result of inflammation or hepatocellular injury, it isn't always the most trustworthy diagnostic of iron overload[3]. Blood erythrocytes and hair zinc levels were much reduced in thalassemia major, and this was equally true in sickle cell disease and hemoglobin H illness. Serum copper levels, on the other hand, were considerably higher than in the control group[4].The goal of the current study was to use trace elements (Zn, Cu, Mg, Co, and Se) as a diagnostic tool to examine the relationship between total antioxidant capacity and oxidative stress in -thalassemia patients in the Meisan province (southern Iraq).

METHODS

Subjects: One hundred Arabic Iraqi thalassemia patients were included in the current investigation. Only 52 patients completed all biochemical analysis tests (31 men and 21 women). Their ages ranged from five to fifteen years old. Orally, the patients' parents and the people in the control group gave their approval. The "Miesan center for blood disorders" in Iraq's Maysan region identified these patients with thalassemia. The people had thalassemia major, according to their medical records, and the diagnosis was made based on "clinical symptoms, haematological, and hemoglobin HPLC analysis, hemoglobin HPLC was done utilizing the (VARIANTTM-Thalassemia Short Program) HPLC apparatus." In the ordinary range of total hemoglobin, HbA2 levels are between 1.75 and 3.25 percent; however, "heterozygous -Thalassemia circumstances give HbA2 levels between 4.0 and 9.0 percent." To be considered normal, HbF must be less than 1% of total hemoglobin. "HbF ranges 1-5 percent for heterozygous -Thalassemia and 80-100 percent for homozygous -Thalassemia, respectively." All of these individuals

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underwent blood transfusions as part of their treatment. C-reactive protein (CRP) levels in the blood in all samples are less than 6 mg/L. A normal C-reactive protein could be utilized to rule out elevated ferritin due to acute phase responses[1]. Patients with diabetes, heart disease, infection, and inflammation, as well as patients from non-Arab ethnic groups, were not included in the study. Thirty healthy children made up the control group. They were about the same age as the sufferers. There were no anemia or symptoms of a systemic illness among the individuals.

Samples Preparation: After a ten-hour fast and 30 minutes of resting in the supine posture, all samples were taken between 8:00 and 9:00 a.m. Fresh peripheral venous blood (5 mL) from people with -thalassemia and healthy volunteers was divided into two equal parts; the first (2.5 mL) was put in "EDTA-containing polypropylene tubes" and gently shaken to be used for measuring Se concentration and carrying out a complete blood count. The remaining liquid was transferred to a fresh, plain tube (without anticoagulant) and left to clot for 30 minutes at room temperature. After the blood had clotted and been centrifuged for 10 minutes at 402 x g, the serum was extracted. While the remaining serum was kept in a deep freezer at (-20oC) until needed, the serum collected was used right away to identify variables in this study.

Methods of Biochemical Estimation: Using standard methods, the blood samples of the control and -Thalassemia patients were examined for the following biochemical parameters: BMI was calculated using the formula: BMI (kg/m2) = Wt in kg / Ht in m2.[5]. The complete blood count (PLT, total leukocyte count and concentration of hemoglobin (Hb)) was assayed on a Sysmex XT-2000i haematology analyser analyzer (Japan). Level of serum Ferritin was assayed by human ELISA kit (Ealbscience- Ferritin (E-EL-H0168/USA). Also, a direct colorimetric methods were applied, and the level of some other biomarker were measured such as Iron was assayed using reagent kit (Ealbscience-Iron (E-BC-K139-M /USA), Total Iron Binding Capacity (TIBC) was assayed using reagent kit (Ealbscience-TIBC (E-BC-K071-M/USA), Total Bilirubin (TBIL) was assayed using reagent kit (Ealbscience- TBIL (E-BC-K760-M/USA), AST was assayed using reagent kit (Ealbscience- AST (E-BC-K236-M/USA), ALT was assayed using reagent kit (Ealbscience- ALT (E-BC-K235-M/USA) and total antioxidant capacity (TAC) by reagent kit (Ealbscience-TAC (E-BC-K219/USA). Flame atomic absorption spectrometry (AAS) was used to determine serum Mg and Zn concentrations (GBC 933 Plus), serum Cu and Co by flameless atomic absorption spectrometry (1TAA500-PG). The hydride generation method was used to assess the content of Se in whole blood[6].

Statistical Analysis: For the statistical analysis, "SPSS software version 21 (IBM Corporation, New York, USA)" was employed. A student t-test was used to establish the statistical significance, and Pearson correlation was used to determine relationships. The thresholds for significant differences were p0.05 and p0.01, respectively[7-13].

RESULTS

Table 1. The demographic characteristics of present study (n=96)

The characteristics		Control	β-Thalassemia Patients
Total (No.)		44	52
Age (mean ± SD)		9.42± 3.87	10.83 ± 4.21
BMI (Kg/m^2)		18.17±3.54	15.43±1.76
Gender	Male	27	31
	Female	17	21
Demographic area	Urban	28	28
	Rural	5	24
Parents consanguinity, (%)	Yes	30	23 (44.23%)
	No	3	29 (55.75%)
Similar condition in the family, (%)	Yes	-	13 (25.00%)
	No	-	39 (75.00%)
Chelation therapy, (%)	Yes	-	35 (67.30%)
	No	-	17 (32.69%)
Type of Chelation therapy, (%)	Deferoxamine	-	27 (51.92%)
	Deferiprone	-	8 (15.38%)
Splenectomy, (%)	Yes	-	11 (21.15%)
	No	-	41 (78.84%)

The results in Tables 2 showed that hemoglobin levels in patients with -thalassemia had considerably (P0.01) dropped levels to roughly 30% of the level observed in controls. This was in contrast to the healthy controls group. With MCV and MCH values of 74.88 4.71 fl and 24.12 1.32 pg in patients compared to 78.40 2.11 fl and 24.71 0.73 pg in controls, P 0.01, microcytosis without hypochromia is substantially more apparent in -thalassemia patients than controls. Additionally, patients were found to have "secondary thrombocytosis (thrombocythemia)" with PLT counts of 287.1634.87 vs. 273.17113.89 109/l, respectively, which was significantly remarkable (P0.01). Additionally, a significantly higher level of leukocytosis (P 0.01) was observed in patients compared to controls (patients: 11.90 0.86; controls: 7.30 1.02 109/l). Furthermore, in comparison to healthy controls, there is a considerable rise in serum ferritin, resulting in iron excess in patients 2541.24±57.52 ng/l vs 61.21±26.31 ng/l, p-value< 0.01. Moreover, Same table 2 reflect a significantly decreased (p<0.01) in levels of TAC, Se, Zn and Mg level and increased (p<0.01) level of MDA, Cu and Co in patients, with compared to control. The present results confirmed that TAC and MDA were highly significantly correlated with level of trace elements (Se, Zn, Mg, Cu and Co) in patients with β-thalassemia, as shown in Table 3 and Figure 1.

From the other hand, the acquired AUC data indicated that Fe, Se, Zn, Mg, Cu, Co, MDA and TAC could be potentially greater predictive biomarkers in beta thalasemia subjects (AUC = 1.000, 0.000, 0.000, 0.123, 1.000, 0.761, 1.000, and 0.000 respectively), as demonstrated in Figure 2.

Data are displayed as mean SD, SD: "Standard Deviation, SE: Standard Error, Range: is the difference between the highest and lowest values in the set, 95% CI: Confidence Intervals (Lower and Upper)", F.: Fasting, n: Number of subjects, p-value (Non-Significant [p>0.05], p0.05 indicated Significant, p0.01 indicated High Significant)" stated the significance level in relation to the corresponding control value.

DISCUSSION

Thalassemia (Mediterranean anemia) is a genetic disorder in which one or more of the hemoglobin chains are formed at a reduced rate or not at all. The lack of a regular channel to eliminate the additional iron causes iron overload, which is produced by the frequent transfusion of blood to patients. The accumulation of iron in tissues, particularly the endocrine glands, may have deleterious effects on the functions of these organs[3].

There are variations between urban and rural people with regard to pollution, the environment, society, psychology, genetics, and diet, among other things, and these discrepancies are escalating dramatically in urban regions. On the other hand, male participants' needs and desires at work have the power to change their psychology. Furthermore, the

Table 2. Total parameter levels assessed in this study for patients with -β-thalassemia and healthy controls

Parameters	β-Thalassemia Patients n=52						Control n=44	p-Value
	Mean ±SD	SE	Median	Range	95 % C.I			
					Lower	Upper	Mean ± SD	
Hb (g/dL)	7.81±0.83	0.145	7.89	5.23-8.70	7.51	8.10	11.56±1.32	p<0.01
RBC × 10^{12}/l	3.22± 0.49	0.08	3.12	2.11–4.20	3.04	3.39	4.50± 0.37	p<0.01
WBC ×10^9/l	11.90 ± 0.86	0.15	11.80	10.87–15.30	11.59	12.21	7.30 ± 1.02	p<0.01
HCT **(%)**	20.82±2.75	0.47	21.00	12.67-25.57	36.80	42.44	25.79±3.15	p<0.01
PLT×10^9/l	287.16±34.87	6.07	278.90	234.50–376.00	274.80	299.53	273.17±113.89	p<0.05
MCV (fl)	74.88±4.71	0.82	75.40	65.43– 84.70	73.21	76.56	78.40 ±2.11	p<0.01
MCH (pg)	24.12±1.32	0.23	24.56	21.34– 26.76	23.65	24.59	24.71± 0.73	p<0.01
MCHC (g /dL)	31.99± 2.57	0.44	31.23	27.98– 37.65	31.07	32.90	31.10 ± 0.71	p<0.01
Iron (mg/dL)	164.09±4.98	0.69	164	154-176.4	162.70	165.48	60.21±10.20	p<0.01
Ferritin (µg/l)	2541.24±57.52	7.97	2546	2435-2657	2525.23	2557.25	61.21±26.31	p<0.01
TIBC (µg/dL)	219.04±4.53	0.62	218	212-233	217.78	220.31	323.25±27.52	p<0.01
AST (U/l)	44.83±9.87	1.36	43.5	32.76-64	42.08	47.58	28.70±3.76	p<0.01
ALT (U/l)	36.98±7.09	0.98	34.96	26.60-69.16	35.01	38.96	29.42±2.99	p<0.01
T. bilirubin (µmol/l)	2.76±0.98	0.13	2.7	0.90-4.8	2.49	3.04	1.71±0.29	p<0.01
Se (ug/dl)	74.52±12.56	1.74	67.45	61-101.9	71.02	78.02	112.08±4.87	p<0.01
Zn (µg/dL)	41.80±3.62	0.50	42.83	34.7-46.8	40.79	42.81	72.74±9.40	p<0.01
Mg (µg/dL)	1.79±0.34	0.04	1.76	0.79-2.81	1.70	1.89	2.24±0.33	p<0.01
Cu (µg/dL)	164.65±5.45	0.75	165.3	146.21-172.56	163.13	166.17	107.98± 4.74	p<0.01
Co (µg/dL)	1.92±0.56	0.07	2.1	0.89-2.76	1.76	2.08	1.45±0.33	p<0.01
MDA(nmol/mL)	4.98±0.57	0.07	5.02	3.99-5.8	4.82	5.14	1.90±0.32	p<0.01
TAC (mmol/l)	0.95±0.14	0.02	0.96	0.59-1.17	0.91	0.99	2.44±0.39	p<0.01

Table 3. The correlation coefficient (r) among Total antioxidant capacity (TAC) and Malondialdehyde (MDA) with some trace elements in blood of β-thalassemia patients.

Variables	Total antioxidant capacity (mmol/L)		Malondialdehyde (MDA) (mmol./L)	
	r	p-Value	r	p-Value
Zinc (µg/dl)	0.847	p< 0.01	-0.866	p < 0.01
Cu (µg/dl)	-0.923	p < 0.01	0.926	p < 0.01
Mg (µg/dl)	0.473	p < 0.01	-0.588	p < 0.01
Co (µg/dl)	-0.414	p < 0.01	0.449	p < 0.01
Se (ng/ml)	0.816	p < 0.01	-0.858	p < 0.01

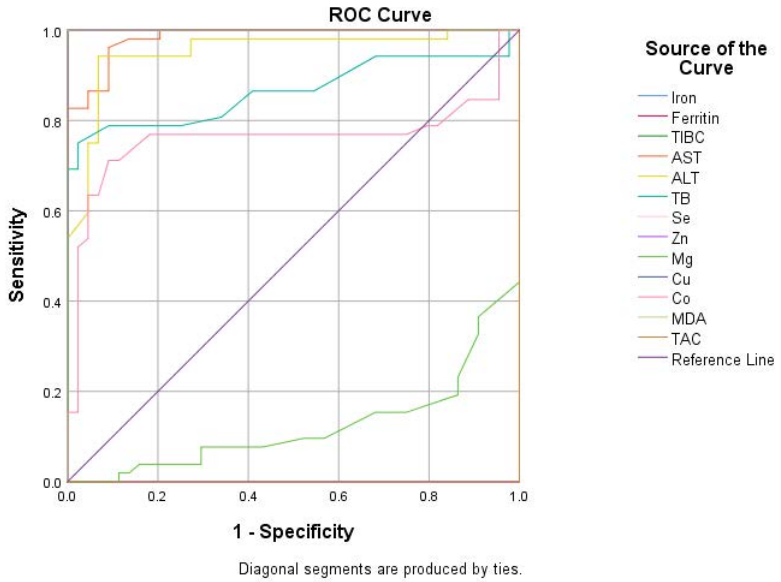

Figure 1. Correlation coefficient (r) of Total antioxidant capacity (TAC) and Malondialdehyde (MDA) with some trace elements in blood of β-thalassemia patients.

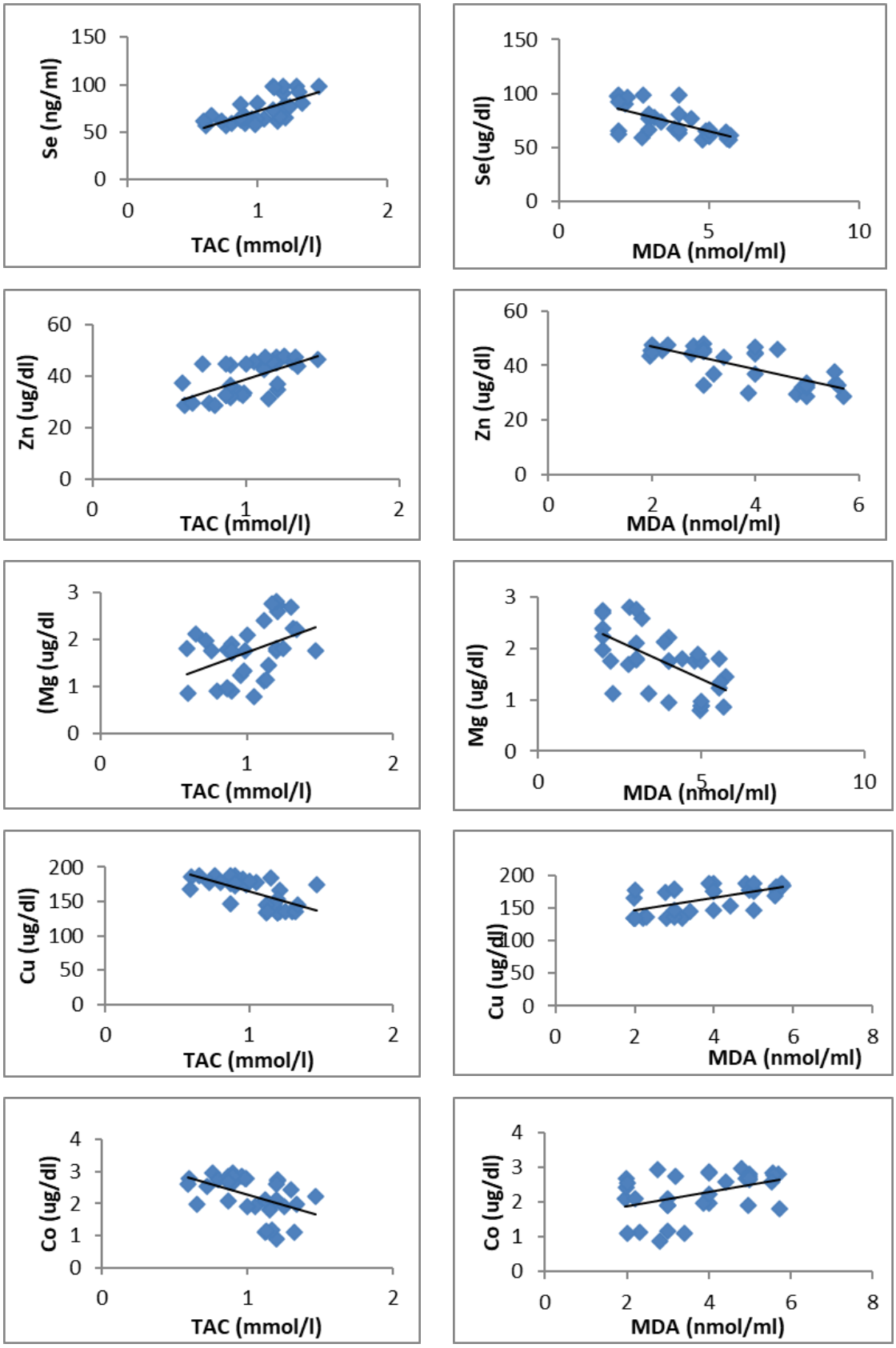

Figure 2. ROC Curve for Iron, Ferritin, TIBC, AST, ALT, TB, Se, Zn, Mg, Cu, Co, MDA and TAC for beta-thalassemia patients compared to controls

oxidant/antioxidant status issue is exacerbated by conflicts in marital and family relationships[5].

In all recent studies, the iron content of ferritin increases, which could be the most important cause of serum ferritin elevation. Congenital hemolytic anemia is caused by unstable hemoglobin variants, particularly those with -thalassemia features. Because serum ferritin is used as a diagnostic for iron overload diseases including hemochromatosis and hemosiderosis, abnormally high ferritin levels could indicate iron overload[4], our findings suggest that iron overload in the patients' group.

Because ferritin is an acute-phase reactant, it is typically elevated in infection and inflammation. Furthermore, because the thalassemic patients in this study did not have any significant inflammatory illnesses or infections, the rise in serum ferritin suggests that the repeated transfusions caused iron overload. Ferritin levels may also rise during times of acute malnutrition, which can be accompanied by anemia in thalassemic people[14]. Serum ferritin is the best single indicator of total body iron. Patients with beta-thalassemia have always suffered from disease as a result of their iron overload. The principal causes of death in thalassemia patients are congestive heart failure and catastrophic cardiac tachyarrhythmia leading to sudden cardiac death. The accessible radical-mediated pathway is the major mechanism of iron poisoning. Catastrophic cardiac ramifications occur as a result of the chain of events set in motion by iron overload[2]. Patients with thalassemia accumulate body iron over time as a result of frequent RBC transfusions, causing hepatic, endocrine, and cardiac issues. The new studies, on the other hand, showed that high levels of iron in the tissues can contribute to all of the problems that come with an iron overload[15]. Iron excess and consequent tissue iron deposition can be caused by a variety of conditions, including idiopathic "hemochromatosis" and transfusion-related siderosis. Beta-thalassemia major is a type of secondary iron overload syndrome that results in inefficient erythropoiesis and reduces red blood cell lifespan[16]. Despite the fact that the severity of the disease varies, the majority of persons who are homozygous for it become transfusion dependent. Large levels of iron from hemolyzed red blood cells, transfused erythrocytes, and excessive dietary iron accumulate in the heart and are integrated as ferritin or hemosiderin[17].

Before the use of deferoxamine-based iron chelation therapy, the cardiac symptoms of iron excess commonly ended in mortality by the end of the second decade of life. Many oxidation processes, including LDL-C oxidation, are favored by the iron released from heme and ferritin. H2O2, which is a less reactive free radical, is generally changed into the highly reactive hydroxyl radical by catalytic iron. In vitro, endothelium-dependent relaxation can be reversed by adding oxygen-derived free radical scavengers or antioxidants[18]. The current study found that thalassemic people have iron excess and are susceptible to the repercussions. End-organ damage is caused by ROS-mediated lipid peroxidation as excess iron accumulates in the liver parenchyma, endocrine organs, and cardiac myocytes[14].Our findings demonstrated that patients' red blood cells (RBC) were significantly reduced ($p<0.01$), which could be attributed to genetic abnormalities in genes essential for hemoglobin protein chain synthesis, resulting in disturbance in globin chain biosynthesis and a loss of hemoglobin manufacturing balance. However, the number, shape, and size of red blood cells as they form in the bone marrow may be affected. As a result, the red blood cells are extremely small and do not occupy the same amount of space as typical red blood cells. However, red blood cells entering the circulatory system may be phagocytosed by splenic kupffer cells, which phagocytose abnormal and aged RBCs[19].The significantly ($p<0.01$) greater WBC count in patients with -thalassemia could be related to the following factors: first, general illness conditions and immune system hyperactivation in patients who receive blood from a variety of donors on a regular basis, as demonstrated by the spread of fever soon after transfusion, The kidney's release of "erythropoietin hormone," which urges the bone marrow to increase red and white blood cell production, is boosted by the "high break percentage" of red blood cells within and outside bone marrow. Third, thalassemia causes an increase in monocytes, which break down the damaged red blood cells[20] .

PLT levels are significantly higher in thalassemia patients (p0.01), which could be associated to the proliferation and development of marrow mononuclear cells to produce colony-forming unit megakaryocytes[21]. Oxidative stress is caused by an excess of peroxides and free radicals in the body, which upsets the balance between oxidants and reductants. This imbalance will lead to oxidative stress and damage to the body's tissues and biological components. Increased levels of lipid peroxides and free-radical intermediates, as well as a reduction in overall antioxidant capacity, result in oxidative stress in beta-thalassemia major patients who frequently need blood transfusions due to severe anemia[22]. Combining iron chelators with antioxidants in patients with -thalassemia can help control the levels of antioxidants in those patients' bodies. In β-thalassemia major, the consequences of oxidative stress and alterations in antioxidant equilibrium have been extensively studied. Some studies discovered a large increase in lipid peroxide and iron levels, as well as a significant decrease in vitamin E and total antioxidant capacity. Serum zinc levels rose dramatically, whereas copper levels decreased, and erythrocyte superoxide dismutase levels increased just little. Oxidative stress and impaired antioxidant defenses may also play a role in the development of -thalassemia major [4].

Numerous biological processes, such as protein synthesis, DNA synthesis, and cellular proliferation, depend on zinc. It is virtually ubiquitous in the body and has a significant impact on both innate and acquired immunity[23] as part of the immune system. Additionally, it has strong antioxidant qualities that help shield cells from damage caused by free radicals. Additionally, it serves as the active site for metalloenzymes involved in the synthesis of nucleic acids and other host defense mechanisms, such as the production of monocytes and macrophages and granulocyte chemotaxis[17] . Additionally, the most common thalassemia complication, hemochromatosis, can also cause hypercupremia, whereas hyperzincemia, which is brought on by the release of zinc from hemolyzed red cells, can result in zinc deficiency. On the other hand, it enters the bloodstream paired with albumin after being absorbed from the small intestine. As a result, zinc deficiency is one of the variables causing growth and puberty anomalies in people with primary -thalassemia and is linked to concerns like poor development, baldness, and weight loss[18].

Hemoglobin, a protein that carries oxygen in blood cells, contains copper as one of its essential components. It creates elastin, a protein that maintains the skin, blood vessels, and lungs supple, along with vitamin C. Additionally, it is antibacterial and rich in antioxidants[24]. Ceruloplasmin, a protein that shields cells from free radical damage, is made possible by the role that copper plays in the "antioxidant superoxide dismutase molecule" and is another crucial component of copper[25]. Additionally, it is necessary for the synthesis of hormone-like molecules like prostaglandins and noradrenaline, which affect the heartbeat, blood pressure, and healing. Given chronic hemolysis's reduced rate of copper glomerular filtration and thalassaemic patients' disruption of trace element metabolism, the elevated copper level may be due to increased copper absorption from the digestive system[3].

As a byproduct of the production of sulfuric acid, selenium was

initially found. It is a well-known electro metalloid that is renowned for its capacity to combat cancer. It is a part of the enzyme glutathione peroxidase and contains a variety of important proteins, including myoglobin and hemoglobin[26]. The rare amino acid seleno-cysteine, which is required for the production of a number of vital enzymes in the body, contains se as well. It may lessen the harm done by free radicals that ferrous chloride and heme complexes generate. Because of this, a deficiency could reduce transferrin's ability to bind iron, causing greater iron buildup and tissue damage in those with -thalassemia. On the other hand, some researchers think that patients with primary -thalassemia may experience a marked increase in natural killer cell activity due to increased oxidative stress and a decrease in total antioxidant capacities, including selenium, vitamins A and E, zinc, and others[27].

Magnesium is a trace element that is necessary for the body to function properly. It is required by the body's immunological, circulatory, and musculoskeletal systems. Mg levels in the bones are double those in the intervertebral disc, despite the fact that mg is dispersed throughout the body. Therefore, a lack of this nutrient can result in age-related disorders, cardiovascular disease, osteoporosis, diabetes, hypertension, metabolic syndrome, nephropathies, and the progression of oxidative damage[17].

The sick group's Co serum level was higher than the control group's. This discovery has the potential to be big. In cell culture, the amounts of co in the serum of experimental animals and people were measured. Additionally, significant findings from investigations into the blood levels of Co in numerous organs of experimental rat models, including testicular edema and mitochondrial cardiac muscle[28,29] .

CONCLUSION

When compared to healthy persons, -thalassemia patients and controls have different levels of trace elements, oxidant/antioxidant status, liver enzymes (AST and ALT), and hematological features. Furthermore, the relationship between TAC and TOS and certain trace elements (Zn, Cu, Mg, Co, and Se) could be useful markers for predicting disease progression in -thalassemia patients. Furthermore, because stem cell culture is not possible in our country, our study demonstrates the importance of bolstering efforts for organized assessment and follow-up of thalassemia patients using iron treatment, which can be highly recommended to develop or modify the administration of the protocols, thus progressing their clinical image.

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Competing Interest: None

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