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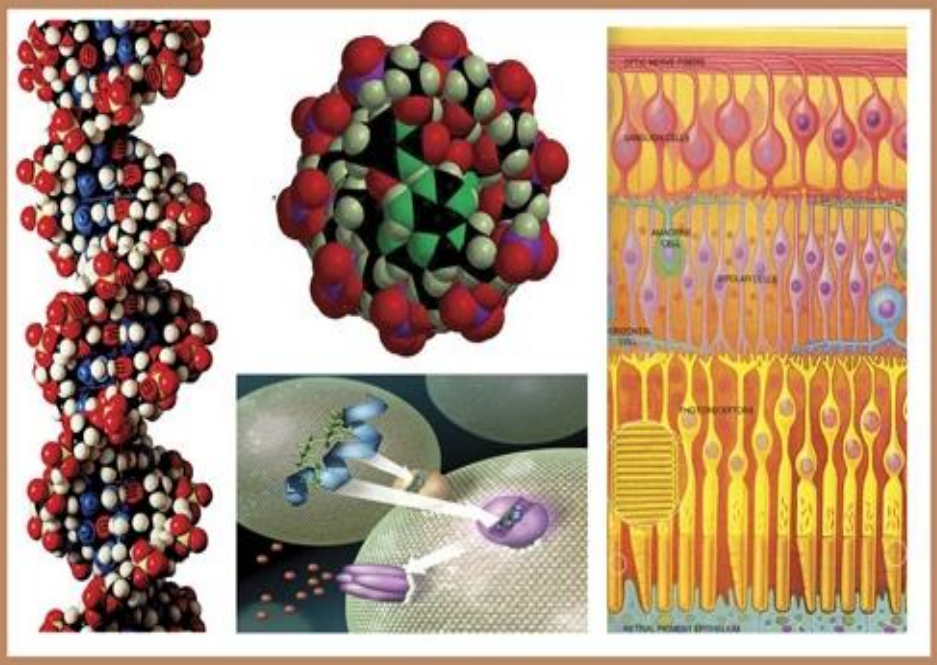

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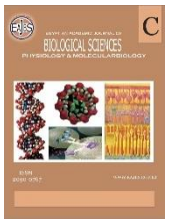

Correlation between Serum IL-33 with Iron Status and Zinc, Copper Levels in Iraqi Children with β-thalassemia Major

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ABSTRACT

Patients with β -TM exhibit a number of immunological abnormalities, the most significant of which is the impairment of neutrophil and macrophage phagocytic and killing capabilities as well as the release of certain cytokines. our study aims to assess the levels of serum IL-33, zinc, copper, and ferritin in patients with thalassemia who are dependent on transfusions and receiving iron chelation therapy. the case-control study was performed on 180 persons (120 of β -TM patients with ages ranging from 5 to 20 years and 60 of control group) of similar age and sex were also examined as a control group. By using spectrophotometry, the levels of serum iron, zinc, and copper were determined, and serum IL-33 and ferritin levels were measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method. An independent sample t-test was utilized for statistical analysis. The mean serum zinc, transferrin, TIBC and UIBC level was significantly ($p<0.01$) lower and serumIL-33, ferritin, iron and TS% level was significantly ($p<0.01$) higher in β -TM patients compared to control group. No significant difference in serum Cu between patients and control. Furthermore, a statistically significant positive connection was found between serum IL-33 levels and age, BMI, ferritin, TS%, Fe, and Cu (r = (0.701**), (0.457**), (0.649**), (0.627**), (0.488**), and (0.624**). also, serum levels IL-33 were found to be negatively correlated with TIBC, UIBC, Transferrin, and Zn, r = (-0.549**), (-0.643**), (-0.549**) and (-0.486**) respectively. In summary, we find that IL-33 is a novel inflammatory marker of the patients with β-TM.

INTRODUCTION

Beta-thalassemia major (β -TM) is an inherited blood disorder characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals (Roy 2019). Anemia in β-TM is brought on by both ineffective erythropoiesis and decreased lifespan of effete red blood cells due to the toxic mismatched -hemoglobin's' capacity to lead to the early eryptosis of the circulating thalassemic red blood cells and apoptosis of erythroid precursor cells in the bone marrow (Moustafa, Al-Hakeim *et al.*, 2023).

When the production of the globin chains that make up heterotetrametric hemoglobin is decreased or absent, it results in a variety of heterogeneous groupings of autosomal recessive anemias known as thalassemia. Two types of the syndrome can be distinguished, thalassemia caused by deletion of globin genes and -thalassemia caused by globin gene alterations (Pang, Shang *et al.,* 2019).

Additionally, thalassemic individuals have much higher amounts of IL-1 and TNF, two substances that might decrease erythropoiesis (Najafi, Ghanavat *et al.,* 2021). Interleukin-33 (IL-33) is an IL-1 family member play critical roles in controlling the activity of a variety of immunocompetent cells. Cardioprotection, innate and adaptive immune responses in mucosal membrane and the maintenance of adipose tissue cells have all been linked to essential roles of IL-33 (Erfurt, Hoffmeister *et al.,* 2021). Numerous studies conducted over the past ten years have evaluated the utility of IL-33 as a biomarker in both inflammatory and noninflammatory illnesses. (Erfurt, Hoffmeister *et al.,* 2021). Th2 cells release the cytokine interleukin (IL)-33, which activates human Th2 cells through chemoattraction. Immunoglobulin IgE causes mast cells to become activated and produce IL-33 (Gabryelska, Kuna *et al.,* 2019). According to recent research, the anemic crisis may be related to changes in cytokine levels in thalassemic patients, and elevated levels of TNF- and IL-10 may cause anemia or alter erythropoiesis. (Butthep, Wisedpanichkij *et al.,* 2015). They believed that the existence of chronic inflammation would result in a sustained systemic inflammatory response and have an impact on the cytokine levels of patients with β -TM. trace elements must be present in the body in sufficient amounts, be able to combine to form essential molecules, and be capable of taking part in a wide range of significant chemical reactions. (Shazia, Mohammad *et al.,* 2012). It has been established that a lack of trace elements contributes to the production of free radicals, which harms tissue.

Complex interactions emerge from the frequent detection of infectious diseases and trace element deficiencies. (Lukác and Massányi 2007). On the other hand, because there is limited information available regarding the trace element levels of thalassemia patients, in-depth research was necessary to identify significant variations in the clinical course of thalassemia major. The antioxidant superoxide dismutase molecule, which protects cells from free radical damage, is largely made up of copper, which also aids in the synthesis of the protein ceruloplasmin. As hormone-like molecules vital for managing blood pressure, heart rate, and healing, prostaglandins and adrenaline are produced only when copper is present. Hemoglobin, a protein in blood cells that transports oxygen, primarily consists of copper. It works in conjunction with vitamin C to produce the elastin protein, which keeps the skin, blood vessels, and lungs supple. Anemia, neutropenia, growth impairment, anomalies in the metabolism of glucose and cholesterol, and a higher incidence of infections are all symptoms of this trace element deficiency.

On the other side, a buildup of copper in the body causes Wilson's disease, which manifests as liver cirrhosis and copper accumulation (Masi, Ferrari *et al.,* 2021). Zinc is a mineral that is necessary for numerous biological activities. More than 300 enzymes rely on zinc as a cofactor, it is essential for the synthesis of proteins, the creation of DNA, cellular development, wound healing, immune system function, and metabolism. It also has antioxidant qualities (Chasapis, Ntoupa *et al.,* 2020). zinc is redistributed from plasma and bones to the bone marrow to produce new red blood cells. Inadequate zinc status (zinc deficiency or excess) could have effects on anemia (Jeng and Chen 2022). The primary objective of this study was to assess the serum levels of IL33, iron status and trace metals (zinc, and copper) in patients with β-TM and healthy controls, also to assess the relationship between the

variables under investigation and IL-33**.**

MATERIALS AND METHODS

180 individuals in this study were split into two groups. 60 healthy people make up the control group in the first group, while 120 Iraqi children with beta-thalassemia from the Department of Hematology and Transfusion Medicine at the "Thalassemia Unit" "AL-Zahra'a Teaching Hospital" in (Najaf, Iraq), from January 2022 to June 2022, make up the second group. Ages of all the study groups ranged from 5 to 20. The trial's subjects all gave venous blood samples, totaling 5 ml were collected, 24 hours before transfusion with washed cells to be sure that the circulating blood belongs to the patient and is not the transfused blood. Interleukin-33 (IL-33) was measured using the ELISA technique described in the instructions included with the kit from MELSIN.

Using a BIOLABO (France) kit, perform a photometric colorimetric test to determine the presence of iron. iron concentration has been detected by colorimetry methods(Carter 1971). Using a humane assay kit (Germany), zinc and copper levels in the serum blood were assessed. Ferritin has been determined using the enzyme-linked immunosorbent assay (ELISA) technique using the instruction as supplied with a kit from BIOLABO (France). Patients with any type of chronic illness were not allowed to participate in the study.

Exclusion Criteria: The study excluded patients and controls who had any acute illnesses or pathological wounds and tested positive for HIV and viral hepatitis.

Statistics Analysis: Using IBM's SPSS statistics program in version 26.0, the results of the current study were presented as Mean ± SD. reviewing the descriptive data Using Pearson's test of frequency The independent t-test test was used to demonstrate the difference between group variation, and correlation was utilized to assess the link between variables. P-values of 0.05 or less were considered significant for this test.

RESULTS

The age distribution of the patients and controls did not statistically differ from one another. Patient data are given in Table 1. β-TM patients had higher serum IL-33 concentrations than those of healthy controls (patients, 36.651±7.346 ng/ml; healthy controls 31.634±2.133 ng/ml) **(**Fig. 1**).** All patients with β -TM showed a substantial positive individual association between blood levels of IL-33 and age, BMI, ferritin, TS%, Fe and Cu (r = (0.701^{**}), (0.457^{**}), (0.649^{**}), (0.627^{**}), (0.488^{**}), and (0.624^{**}) respectively (Fig. 3) while there is a significant negative correlation between serum IL-33 levels and TIBC, UIBC, Transferrin and Zn (r = (-0.549^{**}), (-0.643^{**}), (-0.549^{**}) and (-0.486^{**}) respectively **(**Fig. 4**).**

Table 1: general characteristics of the patients who have signed up and the control.

	Mean ± SD (Range)		
Parameters	**patients (n=120)**	**Controls (n=60)**	***P* value**
Age (years	12.93± 4.660	12.58±4.600	N. S
BMI (kg/m2)	18.12 ± 2.146	22.60±2.418	0.00**
IL-33 (ng/ml)	36.651±7.346	31.634±2.133	0.00**
Ferritin (ng/ml)	2160.262±935.918	98.316±19.252	0.00**
IRON (µmol/L)	32.263±5.836	17.818±3.817	0.00**
TIBC (µmol/L)	49.112±14.480	54.456±12.874	0.00*
UIBC (µmol/L)	16.848±6.708	37.305±13.249	0.00**
Transferrin (g/L)	0.123±0.036	0.138 ±0.032	0.00**
TS%	71.767±23.127	34.458 ±12.404	0.00**
Cu (µg/dL)	104.733 ±8.631	102.634±2.132	0.065
Zn (µg/dL)	61.365±12.320	75.110±14.226	0.00**
Zn/Cu ratio	0.59 ±0.145	0.731 ±0.137	0.00**

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

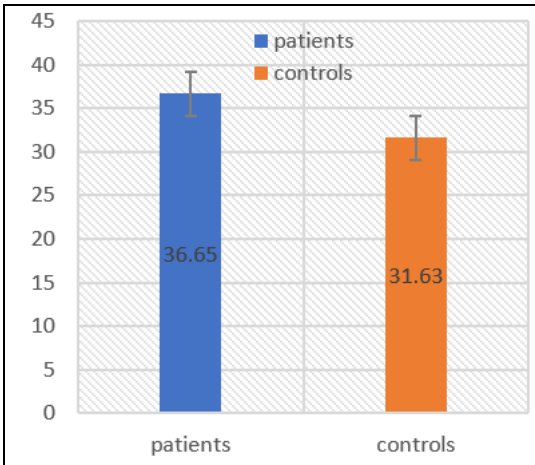

Fig. 1: Comparisons of serum IL -33 (ng/ml), levels for the patients with β-TM and control.

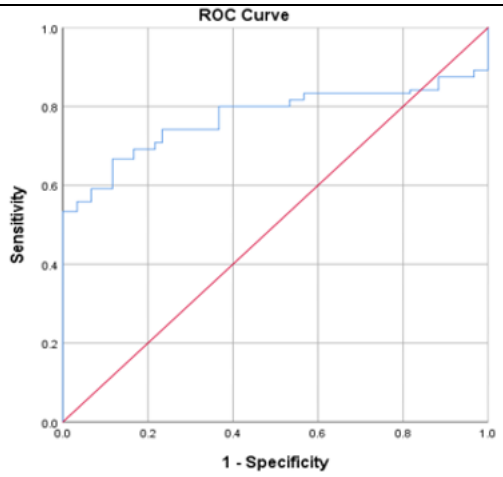

Fig. 2: Curves of the receiver-operating characteristic (ROC) for serum IL-33 levels in β-TM patients

A significant difference in Interleukin-33 levels between the patient and control groups was discovered in this investigation. The study's findings led researchers to the conclusion that a new superfamily of cytokines (IL-33) might be measured as a clinical biochemical marker measure for patients with β- TM in Iraqi children. Furthermore, this research has shown a link between elevated IL-33 levels and iron excess. Iron metabolism issues may have been a result of increased IL-33 production, which is likely the result of macrophage overstimulation. The follow-up samples of larger series of patients with β - TM should be examined before a clinical significance can be assigned to these variations in serum cytokine levels.

To determine whether any amount of serum IL-33 could be used to distinguish between patients with and without iron overload, the area under the ROC curve was calculated. With a threshold value of 34.1915 ng/mL, serum IL-33 concentrations had the highest AUCROC value (AUCROC = 0.772 [95% CI: 0.704 - 0.840; p0.001]). This variable's sensitivity and specificity were, respectively, 0.742 and 0.767**. (**Fig 2**).**

Table 2: Correlation between IL-33 ng/mL and examined variables in patients with major thalassemia.

Parameters	r	P-Value
Age (years)	0.701**	0.000
BMI (Kg/m^2)	0.457**	0.000
ferritin ng/mL	0.649**	0.000
TIBC (µmol/L)	-0.549**	0.000
UIBC (µmol/L)	-0.643**	0.000
TS %	0.627**	0.000
Transferrin (g/L)	-0.549**	0.000
Fe (µmol/L)	0.488**	0.000
Cu µg/dL	0.624**	0.000
Zn (µg/dL)	-0.486**	0.000

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

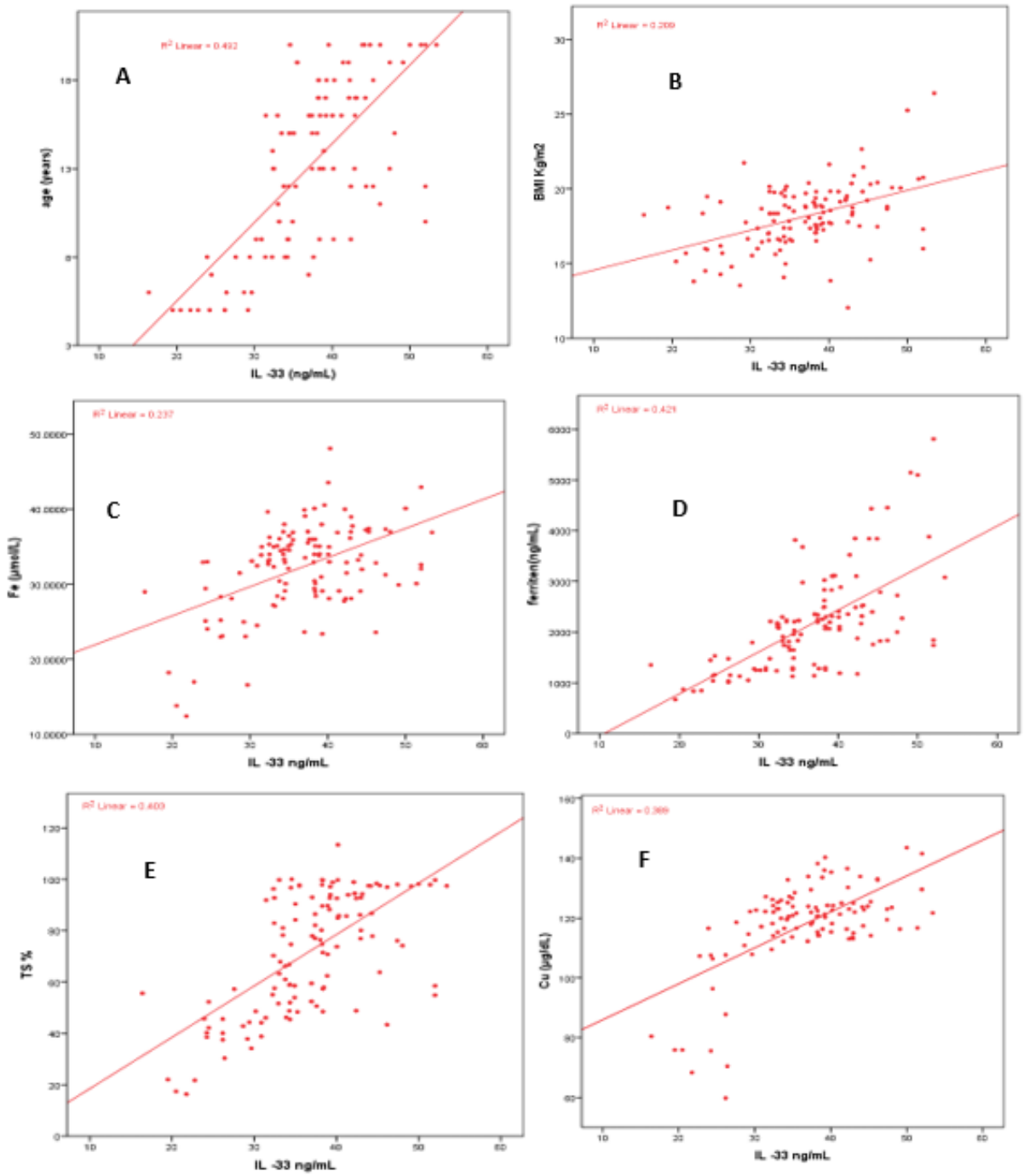

Fig. 3: positive correlation between serum levels of IL-33 (ng/mL) with (A) age, (B) BMI, (C) ferritin, (D) TS %, (E) Iron and (F) Copper in patients with β- TM

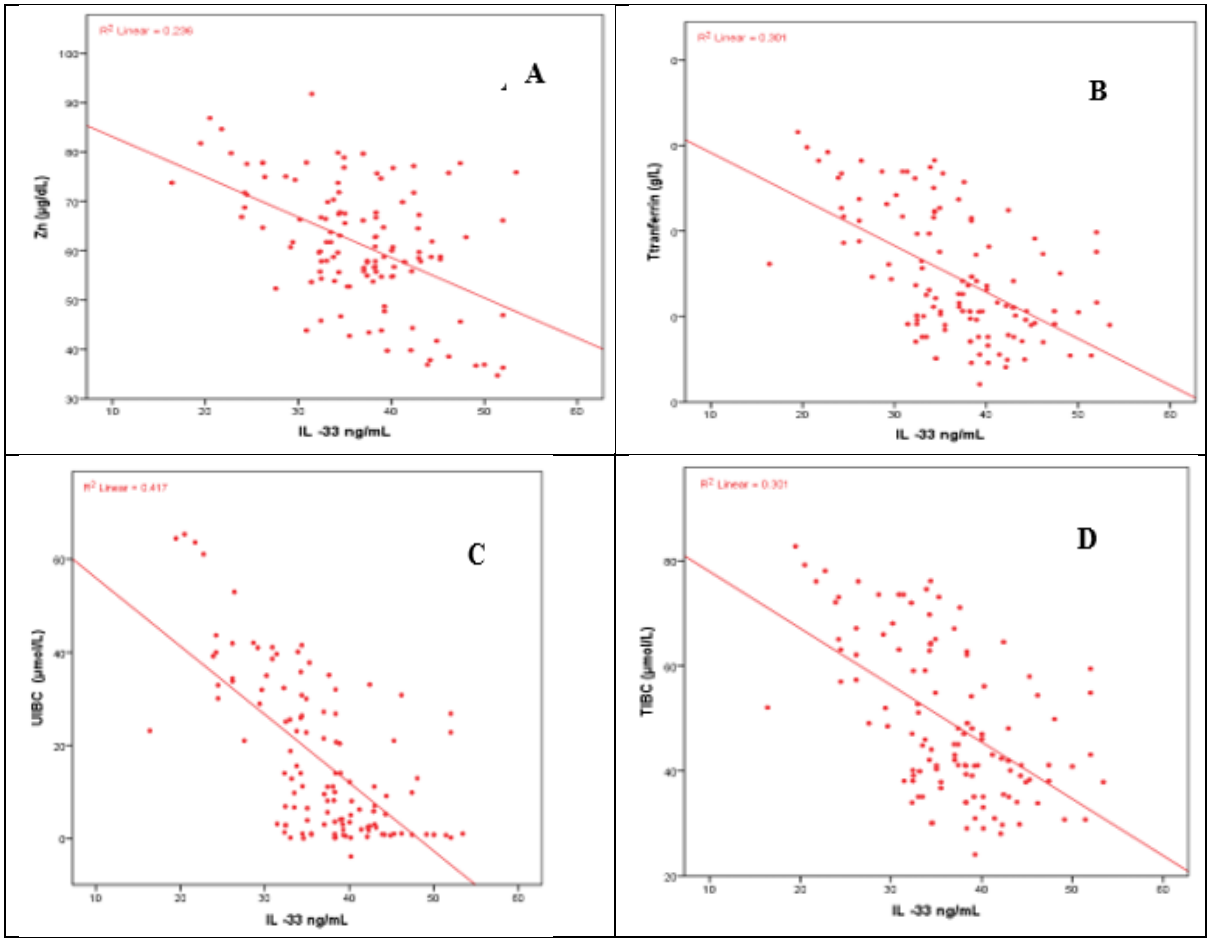

Fig. 4: Negative correlation between serum levels of IL-33 (ng/mL) with (A) Zinc, (B) Transferrin, (C) UIBC and (D) TIBC in patients with β- TM

DISCUSSION

Although Th2 (T Helper2 cell) immune responses were initially associated with IL-33, it is now recognized as a pleiotropic cytokine that can act in inflammatory diseases as an intracellular nuclear factor and as an alarmin in response to cellular injury or mechanical injury. It can also be released into extracellular space. (Liew, Girard, *et al.,* 2016). Higher lymphocyte counts are typically seen in β-TM patients, possibly as a result of the ongoing antigenic challenge from blood transfusions (Zhou and Luo 2022). β -TM patients have been found to have higher numbers of various lymphocyte subsets, such as helper T cells, suppressor T cells, NKCs, and B cells (differentiated by respective phenotypic signatures of CD3+/CD4+, CD3+/CD8+, CD3-/CD16/56+, and CD3-/CD19+) (Pourgheysari, Karimi, *et al.*, 2016).

However, in patients with β -TM, serum ferritin predictive for iron overload was 882 ng/mL (Pinto, Bacigalupo, et al. 2018), which is lower than the cut-off observed in our study was 1842.5 ng/ml. There are probably two reasons for this: firstly, serum ferritin value can increase by multiple infections and inflammatory conditions, very frequent in children. Secondly, in the Our study the threshold chosen to identify iron overload was more conservative. diseases like thalassemia, particularly thalassemia major, which frequently necessitate blood transfusions, are brought on by iron overload, may develop secondary hemochromatosis. Regular blood transfusions are necessary for β -TM patients, which, in the absence of appropriate chelation therapy, results in iron overload (Pinto and Forni 2020).

The amount of iron in thalassemia individuals might surpass ferritin's capacity for storage and purification, completely saturate transferrin, and result in the creation

of free iron, which builds up in the blood and tissues. Due to the presence of this free iron, extremely damaging substances like hydroxyl radicals will occur (OH), hydroxyl radicals attack lipids and produce lipid peroxides, which contribute to oxidative stress (Pisoschi, Pop *et al.,* 2021). Long-term blood transfusions can seriously complicate iron excess. In order to prevent early mortality from iron-induced cardiomyopathies (Saliba, Atoui *et al.* 2020).

Our findings show that compared to controls, β -TM patients had considerably higher serum IL-33 levels. one of the primary sources of serum IL-33 is hemolysis of red blood cells (RBCs), So Patients with β -TM have an increase in serum IL-33 levels, and there is a positive association between serum IL-33 levels and the degree of hemolysis. Iron metabolism issues may have been a result of increased IL-33 production, which is likely the result of macrophage overstimulation. (Wei, Zhao *et al.,* 2015). As far as we are aware, this is the initial clinical report to analyze IL-33 serum levels, as well as a favorable connection between IL-33 and age, BMI, ferritin, TS%, Fe, and Cu in β -TM. Moreover, both the positive and negative correlation, indicating that this cytokine may exert a role in β-TM patients. In our study, patients with β -TM had considerably lower zinc levels than the controls (hypozincemia). The causes of zinc deficiency in patients of β -TM could be due to inadequate intake of zinc through daily meals, irregular urinary zinc absorption, renal dysfunction, urinary zinc secretion, disturbances in zinc metabolism, and greater levels of zinc excretion in sweat (Jeng and Chen 2022).

Another critical trace element, copper, was an increase in serum level of copper in patients experiencing thalassemia major. the etiology of hypercupremia is hemochromatosis, which is a principal complication of thalassemia and also occurs in acute and chronic inflammation(Al-Samarrai, Adaay *et al.,* 2008). Significantly elevated iron levels have been observed in β -TM patients due to blood transfusions. Further research is required before recommending the use of specific antioxidants in combination with vital minerals and trace elements to minimize the degree of oxidative damage and the repercussions that follow in beta thalassemia major.

CONCLUSION

According to the study's findings, β-TM patients receiving combined iron chelator treatment may experience high serum IL-33 levels, low serum zinc and There was also a slight increase in the levels of copper in the serum, but not significant, Therefore, when patients are treated with a combined iron chelator, routine assessment of blood copper and zinc levels may be useful to prevent the detrimental consequences of changing these micronutrient concentrations in TDT.

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Declaration of Interests: The authors declare that they no conflict of interest.

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