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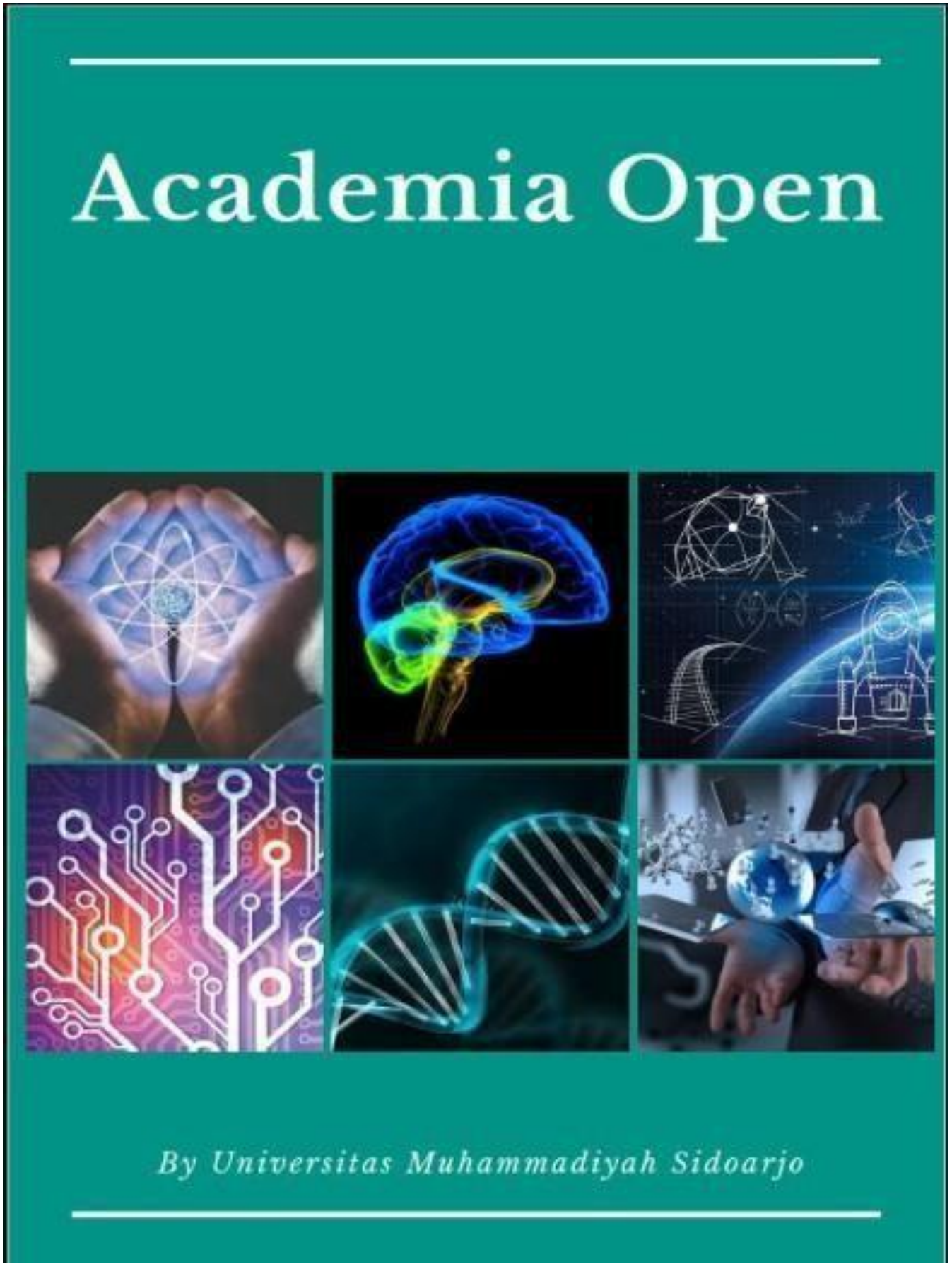

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Biochemical, Hematological, and Bone Status Parameters in Patients With Beta-Thalassaemia in Al Muthanna Province, Iraq

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Abstract

General Background: Beta-thalassaemia is one of the most prevalent monogenic disorders worldwide and is associated with multiple hematological, biochemical, and skeletal abnormalities. **SpecificBackground:** Patientswithtransfusion-dependentbeta-thalassaemiafrequentlyexperience iron overload and metabolic complications that may affect liver function and bone health.**Knowledge Gap:** Limited regional evidence is available regarding the combined assessment of hematological indices, biochemical markers, and bone status among beta-thalassaemia patients in Al Muthanna Province, Iraq. **Aims:** This study aimed to investigate differences in hematological and biochemical parameters between beta-thalassaemia patients and healthy controls and to evaluate the relationship between body mass index (BMI) and bone mineral density Z-scores. **Results:** A total of 168 participants were enrolled, including 84 beta-thalassaemia patients and 84 healthy controls. Patients demonstrated significantly lower hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and vitamin D levels. In contrast, ferritin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels were significantly elevated. Bone density assessment revealed significant differences in Z-scores across BMI categories, indicating a relationship between BMI and bone status. **Novelty:** The study provides an integrated evaluation of hematological profiles, liver-related biochemical markers, vitamin D status, ferritin levels, and bone mineral density in pediatric beta-thalassaemia patients from Al Muthanna Province. **Implications:** Routine monitoring of iron overload, liver biomarkers, vitamin D status, and bone mineral density may support early identification of complications and improve

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clinical management of beta-thalassaemia patients.

Highlights:

- Beta-thalassaemia patients exhibited reduced hematological indices and vitamin D concentrations compared with healthy controls.
- Ferritin accumulation was accompanied by increased alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels.
- Significant associations were identified between body mass index categories and bone mineral density Z-scores.

Keywords: Beta Thalassaemia, Bone Mineral Density, Ferritin, Hematological Parameters, Liver Enzymes

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Introduction

Thalassaemia is a collection of autosomal-recessive blood inheritance These are some of the world's most prevalent genetic illnesses. Several genetic defects cause the condition [1]. The first clinical diagnosis of thalassaemia was made. The occurrence of anaemia, splenomegaly, and bone deformities among Italian offspring was first documented by Thomas Cooley in 1925 [2]. With a broad range of clinical manifestations, β-thalassaemia is typically divided into three primary groups: thalassaemia major, where patients present with severe anaemia from where patients present with severe anaemia from early years and need blood transfusions on a regular basis along with life span iron chelation; thalassaemia intermedia; and thalassaemia minor, which is marked by mild, asymptomatic anaemia linked to heterozygosity (thalassaemia trait) and represents the mildest clinical symptoms [3, 4]. Thalassaemia, which is prevalent in Southeast Asia, including Iraq, the Middle East, and the Mediterranean coast, affects about 1.67% of the population [5]. (major β-thalassaemia). Severe anaemia It manifests early in life (from the 6 month until the 1st year) is the hallmark of this deadly sickness, followed by a slowdown in growth, bone marrow hyperplasia, and hypoxia [6]. The equilibrium of alpha and beta chain production determines the severity of the illness because a loss of this equilibrium causes an accumulation of alpha and beta chains inside the red blood cell, rendering haemoglobin insoluble [7]. The plasma membrane of blood cells is harmed via the accumulation of alpha chains due to the continuous production of alpha chains and the lack of beta chains. As a result, the patient requires blood transfusions and has elevated intestinal absorption of iron, which builds up in the body and causes liver, heart, and pancreas damage [8]. Since 1970, several complete blood count indices have been put out as easy and affordable ways to assess if a blood sample is more indicative of iron deficiency anaemia or β-thalassaemia [9]. Red cell indices have been identified as the initial sign of β-thalassaemia using electronic cell counters [10]. Serious organ damage and overall negative effects on essential bodily systems, such as cardiac, respiratory, endocrine, hepatic, and renal functions, are the results of iron excess and toxicity [11]. Serum ferritin levels and liver iron concentration values are excellent indicators of iron overload, which often manifests one or two years after the start of regular blood transfusions [12]. Alanine aminotransferase ALT and aspartate aminotransferase (AST) are regarded as markers of liver health. Additionally, alkaline phosphatase (ALP) is thought to be a sign of liver and bone health [13, 14]. Compared to their healthy contemporaries of the same age, patients with β-major thalassaemia have a severe vitamin D shortage [15]. No statistically significant differences related to gender variables were observed among thalassaemia patients [16]. Gender was significant; however, data from primary β-thalassaemia patients indicated a greater prevalence of males in contrast to females [17]. Patients with thalassaemia encounter several difficulties in managing their condition, including an elevated risk of osteoporosis, despite notable advancements in transfusion and iron chelation regimes [18]. Thalassaemia-related bone disease is a teenage issue that might emerge in adulthood. A crucial time for bone growth is adolescence. A key factor in this process is the thalassaemia-related development of decreased bone mass as a result of impaired bone turnover [19]. In this study, we examined the age and gender distribution of patients in Al-Muthanna province as potential factors affecting disease severity, in addition to liver function tests (certain liver enzymes), blood indicators, and bone density, enabling effective control of thalassaemia and early detection of any disparities to avoid serious, life-threatening complications.

Materials and Methods

The current study involve 168 participants, categorised into two groups: 84 a subject with β-thalassaemia (41 males and 43 females) and 84 healthy controls (41 males and 43 females). Both sick and healthy volunteers were between the ages of five and fifteen. This study was carried out in the government Women's and Children's Hospital's thalassaemia treatment facility in Al-Muthanna Province, Iraq. Before collecting any biological samples, the study began following acceptance of the approval from the ethics committee at the Women's and Children's Hospital, No. 185/IEC. We got each participant's written informed consent, and the study protocol complies with the 1975 Declaration of Helsinki's ethical standards. Haematological tests, biochemical tests, and radiographic imaging were used to acquire the data. Each patient group and the control group had five millilitres of venous blood drawn. This method allows for the investigation of blood parameters. Furthermore, the serum from the sample undergoes biochemical tests to determine iron levels and liver enzymes. The transfusion-dependent TDT patients in this study received Regularly blood transfusions and iron chelation medicine. excluded participants with splenectomy, as well as those with autoimmune illnesses. (Complete blood count, CBC) measurements for both the control and patient groups, including (haemoglobin, Hb), (haematocrit, HCT), (mean corpuscular volume, MCV), (mean corpuscular haemoglobin, MCH), (mean corpuscular haemoglobin concentration, MCHC), and (red blood cell, RBC) counts, were determined using a comprehensive set of control and calibrator reagents from the XN-350™ Automated Haematology Analyser from Sysmex (Japan). Serum concentrations and some biochemical markers, ALT, AST, and ALP, were measured using a Biorex Monarch 240 device from Biorex Diagnostics (United Kingdom). Ferritin and vitamin D3 were carried out using the Mini VIDAS device from bioMérieux (France). Anthropometric characteristics were measured for both the patient and control groups. Bone mineral density (BMD), measured in kilograms per square metre (kg/m²) [20], was evaluated using the Stratos device (dual energy X ray absorptiometry, DEXA) from DMS - Imaging (France) at the L1–L4 lumbar spine, which evaluated the results in the form of a Z-score. As advised by the Thalassaemia International Federation, patients aged ≥10 years were examined for osteoporosis every year using a DEXA scan to measure bone mineral density (BMD). Osteoporosis (< -2.5 standard deviations (SD)) and osteopenia (-2.5 to -1.0 SD) were categorised using the World Health Organization standards.

Statistical Analysis

The SPSS 2025 programme was used for statistical analysis. ANOVA was employed to assess differences between the means of independent groups. The Z-score of bone density was correlated with other variables using Pearson's correlation. If the p were less than 0.05, the statistical relationship or differences were deemed significant.

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Results

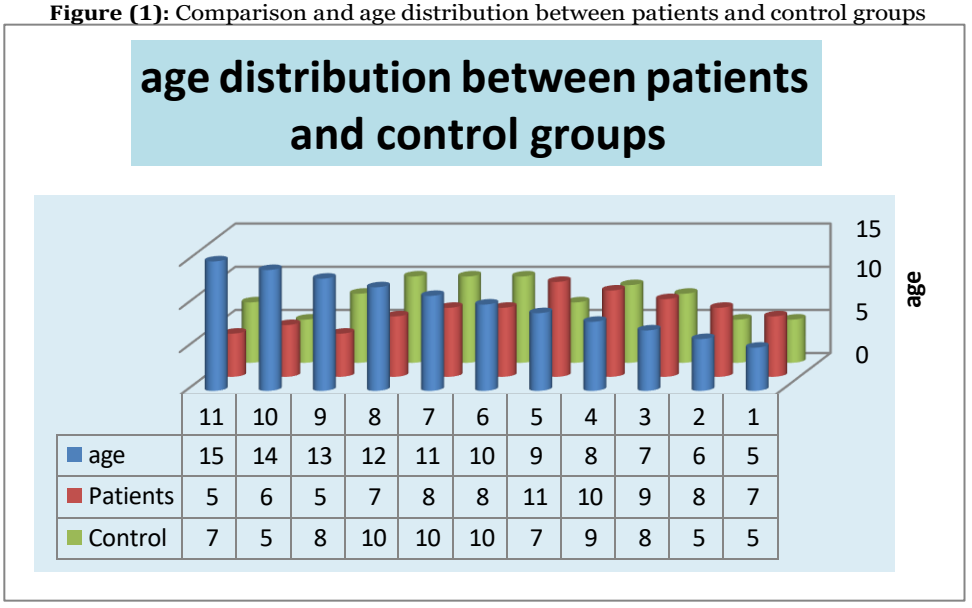
The sex characteristics of the subjects with thalassaemia group and the control healthy group were similar, lacking any statistically relevant difference ($p > 0.05$), as Table 1 displays The number of male participants was distributed as follows: 41 for each group and, for females, 43 per group.

Table 1: Sex and age data for the patient group with β-thalassaemia and the healthy control group

The characteristics	Patients group with β-thalassaemia	healthy control group	p-value
Number (N)	84	84	-
Gender Male N (%) Female N (%)	41 (48.81%) 43 (51.19%)	41 (48.81%) 43 (51.19%)	1.00
Age (year) Mean ± SD	7.63±1.91	7.636±2.01	0.5635

The descriptive data results from the current study in Table 1 indicate that there are no significant age -related β-thalassaemia patients' variations from the healthy control group (7.63±1.91 and 7.636±2.01 years, respectively). Figure 1 shows the age distribution of the two study groups.

Figure (1): Comparison and age distribution between patients and control groups



All study participants underwent blood tests to measure haematological parameters, including iron levels. It should be mentioned that the main course of treatment for each of these individuals included chelation therapy along with frequent blood transfusions. The results of a considerable statistical difference were shown by the statistical analysis ($p < 0.05$) and showed a substantial decrease in Hb ranges in the β-thalassaemia patient's group relative to the healthy control group (8.12 ± 1.45 g/dL vs 13.45 ± 0.68 g/dL). This decline was followed by a similar drop at a significance threshold of $P < 0.05$. In each of HCT (25.8 ± 4.2 vs 45.3 ± 5.1%), MCH (23.9 ± 3.1 pg vs 28.9 ± 1.5), MCV decreased to 74.4 ± 7.6 fL vs 83.2 ± 4.3 fL, and the MCHC (30.8 ± 4.2 g/dL vs 33.4 ± 1.2). On the other hand, the variations in RBC levels were not significant ($p > 0.05$). Even so, the RBC averages of the patients were lower than those of the control group of participants (3.55 ± 0.72 cells/µL vs. 4.52 ± 0.41 cells/µL). As displayed in Table 2.

Table 2: Comparing Haematological Measures Between β-thalassaemia Patients and Healthy Controls.

Parameter/ Unit	**β-** thalassaemia (n=84) Mean ± SD	Control (n=84) Mean ± SD	P-value
Hb (g / dL)	8.12 ± 1.45	13.45 ± 0.68	P-value < 0.05 *
HCT (%)	25.8 ± 4.2	45.3 ± 5.1	P-value < 0.05 *
MCH (pg)	23.9 ± 3.1	28.9 ± 1.5	P-value < 0.05 *

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MCV (fL)	74.4 ± 7.6	83.2 ± 4.3	P-value < 0.05 *
RBC count (×10⁶/µL)	3.55 ± 0.72	4.52 ± 0.41	P-value > 0.05 (NS
MCHC (g/dL)	30.8 ± 4.2	33.4 ± 1.2	P-value < 0.05 *

* P: < 0.05, NS: No significance: g/dl: Grams per decilitre, %: Per cent, ×10⁶/µL: Million per microlitre, fL: Femtolitre, pg: Picogram.

According to the current research, patients with thalassaemia had significantly greater levels of liver enzymes than the control group (p = 0.001); ALT levels (68.2 ± 12.4 IU/L vs 30.3 ± 7.7 IU/L) and ALP levels were also observed (235.6 ± 38.2 IU/L vs 140.5 ± 29.6 IU/L), respectively. Similarly, patients' AST levels significantly increased (72.8 ± 15.3 vs. 29.2 ± 8.5 IU/L). This study evaluated vitamin D, a hormone that is important for bone health. The results revealed a statistically significant decline (p = < 0.01) in the patient group as opposed to the control group (19.6 ± 8.3 vs. 31.8 ± 8.5). The ferritin test showed an increase in the patient group that was statistically significant in comparison to the control group (3120.7 ± 1280.4 vs 86.2 ± 35.7 µg/L, p -value of 0.001), as exhibited in Table 3.

Table 3: The liver's function test in 84 individuals with β-thalassaemia and 84 healthy controls.

Parameter (Unit)	**β-thalassaemia** (n=84)	Control (n=84)	P-value
ALT (IU/L)	68.2 ± 12.4	30.3 ± 7.7	P-value < 0.001***
ALP (IU/L)	201.6 ± 39.2	140.5 ± 29.6	P-value < 0.001***
AST (IU / L)	72.8 ± 15.3	29.2 ± 8.5	P-value < 0.001 ***
Vit D3 (ng / mL)	19.6 ± 8.3	31.8 ± 8.5	P-value < 0. 01 **
Ferritin (µg/L)	3120.7 ± 1280	86.2 ± 35.7	P-value < 0.001 ***

*** P; ≤ 0.001. ** P; ≤ 0.01.* P; < 0.05. IU/L; International Units per Liter. ng /mL, Nanograms per milliliter.

In individuals with β-thalassaemia, the results in Table 4 reveals a strong correlation between bone density (Z score) and body mass index (BMI). The data showed that patients with a normal BMI (16.5–22.9 kg/m²) had Z-score values of 0.11 ± 0.02 in normal cases, −1.6 ± 0.63 in cases of osteopenia, and −3.20 ± 0.25 in cases of osteoporosis. Patients with a low BMI (< 16.5 kg/m²) showed values of 0.65 ± 0.89, −1.40 ± 0.62, and −3.40 ± 0.41, respectively, while patients with a high BMI (> 16.5 kg/m²) scored 0.54 ± 0.25, −1.50 ± 0.79, and −2.90 ± 0.30, respectively, with the groups' differences being significant (P < 0.05).

Table 4: Evaluation of Z scores according to patients' BMI

Z score	**Normal BMI** **16.5 to 22.9 kg / m²**	**Low BMI** **< 16.5 kg / m²**	**High BMI** **> 16.5 kg / m²**	**P value**
	Mean ± SD	Mean ± SD	Mean ± SD	
Normal	0.11 ± 0.02	0.65 ± 0.89	0.54 ± 0.25	P-value < 0.05 *
Osteopenia	-1.6 ± 0.63	-1.40 ± 0.62	-1.50 ± 0.79	P-value < 0.05 *
Osteoporosis	-3.20 ± 0.25	-3.40 ± 0.41	-2.90 ± 0.30	P-value < 0.05 *

Discussion

In the Asian community, thalassaemia is one of the most common hereditary conditions. [21]. The high prevalence of the disorder in the Asian continent and the entire world has drawn the attention of the medical fraternity worldwide to conduct extensive research on the disorder (22, 23). β-thalassaemia is caused by the result of metabolic disorders, iron overload, hypoxia, and cellular injury, resulting in inefficient erythropoiesis, haemolysis, or anaemia, thereby requiring these patients to be sustained on blood transfusion or bone marrow transplantation to survive their lives [24]. the study samples' demographics, including their age and gender. Remarkably, our findings revealed that the participating cohorts did not significantly differ in terms of age or gender [25]. The result from the current study on haematological variables showed that the control group's levels of MCV, MCH, Hb, RBC, HCT, and MCHC were substantially more than the patients'. However, The findings align with research carried out in Iraq's Maysan Governorate [26]. β-thalassaemia patients lack the ability to synthesise beta chains of haemoglobin, resulting in fewer normal red cells and Hb in the blood [27]. Due to increased destruction of RBC and decreased production efficiency (RBC), haemoglobin concentrations are decreased in thalassaemia patients [28]. The concentration of haematocrit also decreases. Consequently, in order to properly control their illness, patients require frequent blood transfusions [29]. Additionally, The findings of the study showed that ferritin values were higher in patients than in the healthy group, which are in line with numerous other studies [30, 31, 32]. According to a previous study, iron overload has a harmful effect on hepatic cells, which raises the levels of liver enzymes [33]. The study's findings showed that patients with β-thalassaemia had greater liver enzyme levels (ALT, ALP, and AST) than the control

healthy group. These outcomes agree with findings from a study from Basra, Iraq [34]. Vitamin D3 levels in the sick group were significantly lower than those in the control group of participants, and these findings are in line with earlier research [17, 34]. The results confirmed statistically significant differences in mean Z-scores among the three BMI categories. Within each bone density category, this statistical significance indicates that BMI is a major factor influencing bone density status in β-thalassaemia major patients, which is consistent with research that considers low BMI a major risk factor in this patient group [35]. Low BMI patients had the highest mean negative Z-score for osteoporosis. This lends credence to the idea that β-thalassaemia patients with the lowest BMI have the lowest bone density since they have less muscle and body fat, which reduces bone load, and the dominance of other thalassaemia-related pathological conditions like iron overload and endocrine abnormalities [36]. Osteoporosis is a condition that weakens bones, leading to a loss in bone density [37]. All patients with thalassaemia major have osteoporosis due to poor bone density; hence, the need for annual bone mineral density measurements to prevent serious outcomes and promote optimal bone density.

Conclusion

Patients suffered from anaemia and poor bone marrow response, demanding repeated blood transfusions. This, in turn, resulted in iron overload, which caused obvious hepatotoxicity via raised enzyme levels. Iron overload, combined with vitamin D deficiency, exacerbated osteoporosis, as a low body mass index puts additional stress on the bones, making them more fragile.

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