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Impact of Beta Thalassemia Minor on Mineral Homeostasis: A Comprehensive Analysis

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ABSTRACT
This paper aims to identify hypomineralization in patients with beta-thalassemia minor through an analysis of serum calcium, iron, magnesium, phosphorus, copper, and zinc concentrations in a series of patient subpopulations. In the 50 subjects studied, mineral concentration differences between clusters were assessed by Welch's ANOVA with Games-Howell post hoc tests. K-means clustering and principal component analysis were performed to identify and visualize the biochemical profiles of mineral intercorrelations via Pearson correlation analysis. The results showed significant variations in mineral concentrations between clusters. Calcium levels averaged 8.31 mg/dL in the cohort, with deficiencies noted in Cluster 2, which had a mean of 7.7 mg/dL. Iron levels varied significantly: Cluster 0 indicated chronic iron overload with a mean of 304.0 µg/dL, while Cluster 2 exhibited lower levels with a mean of 96.8 µg/dL. Magnesium concentrations were generally normal; however, Cluster 3 showed mild deficiencies (mean: 2.23 mg/dL), suggesting susceptibility to oxidative stress. Increased phosphorus levels were observed (mean: 5.1 mg/dL), while copper concentrations were highest in Cluster 1 (mean: 164.2 µg/dL) and zinc levels were lowest in Cluster 3 (mean: 81.2 µg/dL), reinforcing the need for individualized supplementation programs. The Pearson correlation showed moderate positive correlations between calcium and magnesium ($p < 0.01$) and between copper and zinc ($p < 0.05$), indicating potential interrelationships among these essential minerals.

Keywords: Beta Thalassemia Minor, Blood, Mineral Homeostasis, Iron Metabolism, Hemoglobin Disorders

INTRODUCTION

Thalassemia is a congenital hemoglobin disorder, classified as a hemoglobinopathy, characterized by low hemoglobin levels and chronic anemia, often accompanied by numerous extra-hematologic effects. Beta-thalassemia minor represents a milder form of the disease; while patients are rarely anemic, they may develop complications such as disturbances in the biochemistry of certain minerals and trace elements [1]. Advances in the management of thalassemia have rendered it less life-threatening in recent years, improving clinical outcomes and quality of life. However, issues regarding long-term surveillance of mineral derangements remain significant, and biochemical monitoring is essential for ensuring optimal care and clinical management of these patients [2].

Calcium, iron, magnesium, phosphorus, copper, and zinc are six minerals most closely related to beta-thalassemia minor and are predictive of physiological homeostasis. Calcium is crucial for bone formation and is often affected in thalassemia patients, who suffer from chronic anemia and endocrine disorders, including secondary hyperparathyroidism [3]. Nutrients such as magnesium and phosphorus are important for cellular energy generation and cardiovascular health as well as the maintenance of bone health [4]. Copper and zinc are critical trace elements for immune response and protection against oxidative stress; abnormal levels of these minerals can exacerbate oxidative damage, which is commonly observed in beta-thalassemia minor [5]. These deficiencies and excesses can have detrimental effects on the patient's clinical status; therefore, biochemical monitoring is critical.

Recent assessments of beta-thalassemia minor patient databases, demographic characteristics, and biochemical abnormalities, particularly disturbances in mineral metabolism and their correlation with disease processes and treatment outcomes, have been conducted. Experimental examinations through cross-sectional surveys have established gender and age differences in mineral concentrations, suggesting that demographic indicators may

underlie these biochemical pathologies [6, 7]. Notably, higher iron concentrations and lower calcium concentrations were observed in males compared to females, while a lower concentration of magnesium was noted in younger adults compared to elderly patients. However, limited data exists on how these demographic differences can be utilized to individualize management strategies.

The current research study tries to address this deficiency by performing a thorough investigation into the levels of calcium, iron, magnesium, phosphorus, copper, and zinc in patients with Beta-thalassemia minor. The current paper evaluates demographic variations using advanced clustering techniques to characterize specific biochemical profiles. According to the correlations analyzed between demographic characteristics and mineral imbalance, the study provides significant insight into how gender and age differences may regulate personalized management strategies used in patients with Beta-thalassemia minor. This study will use strong statistical methodologies to provide a framework for developing tailored treatment strategies that will improve patient outcomes and inform future clinical trials. This study underscores the importance of individualized treatment approaches tailored to the specific biochemical profiles of patient clusters. By identifying distinct mineral imbalances and their interrelationships, clinicians can develop targeted monitoring and supplementation strategies to address deficiencies or excesses .

2. Previous Related Studies

Prior research has centered primarily on individual mineral changes without incorporating these discoveries into clinically useful patient subtypes. As stated in the recent reviews, the use of cluster methods offers the greatest potential of filling this gap since it will allow distinguishing between subgroups that have similar biochemistry. This work extends previous research by employing more sophisticated mean comparisons with an identity of the patterns of interaction between mineral levels and demographics as the basis for a new model of thalassemia cognitive therapy. Carsote, Vasiliu [8] reported that hypocalcemia is a typical clinical presentation in individuals with thalassemia, and the situation is made worse by endocrine complications, Stefanache, Lungu [9] also pointed out the ability of copper and zinc to act as antioxidants and immunomodulators with the view of reducing disease severity whenever their concentrations are balanced. According to Mourosi, Anwar [10] show that biochemical results were influenced by demographic variables including, age and gender, while Goldberg, Lal [11] noted that the magnesium low levels differed in young patients. Table 1 presents previous related studies.

Table 1: Previous Related Studies

Objective	Key Findings	Study
Investigated the prevalence of hypocalcemia and its correlation with disease severity in thalassemia.	Identified hypocalcemia as a common complication in severe thalassemia cases.	[12]
Examined the role of magnesium supplementation in improving cardiac function in thalassemia.	Reported improved cardiac function with magnesium supplementation.	[11]
Focused on understanding the link between the genetic factors on mineral depletion in Thalassemia of Southeast Asia.	Identified an association with genetic factors of mineral deficiency which support the stated need for individualized treatment.	[13, 14]
Examined the correlation between copper-induced toxicity and oxidative stress in thalassemia patients receiving transfusion.	Emphasized that copper is toxic in high concentrations and contributes to aggravation of oxidative stress.	[15, 16]
Researched risk relating to bone health in Thalassemia sufferers.	Investigated the existence of substantial threats to bone demineralization in those with intermediate thalassemia.	[3, 17]

3. MATERIALS AND METHODS

This research employed a cross-sectional research design to examine the biochemical and demographic profiles of individuals with thalassemia. A total of fifty patients' data were collected; patients' age, gender, and blood calcium, iron, magnesium, phosphorus, copper, and zinc concentration. The biochemical data were produced from standardized laboratory tests in qualified clinical laboratories, whereas, demographic data of the patient's records from Basrah city. The achievement of reliable results was facilitated by the analysis through the statistical software SPSS. To perform an initial analysis of the participants' characteristics, the most frequently used measures of central tendency (mean, median) and measures of dispersion (standard deviation, range) were calculated.

To compare groups of interest, independent samples t-tests were employed in comparing means of mineral levels between male and female subjects, and Welch's ANOVA was used in comparing means of mineral levels

in subjects in three different age groups <20 years, 20-40 years, and >40 years. Post hoc analysis using the Games-Howell test was conducted to examine pairwise differences between clusters, highlighting specific mineral imbalances. To determine the approximate correlation between the different minerals and to examine whether some of them interact in a mutually regulating manner, Pearson's correlation coefficients were computed. Specifically, to explain differences within the cohort, a K-means clustering analysis was used to divide the patients into subgroups that are characterized by similar biochemical characteristics. The cluster analysis was done on the 5 variables and since the result cannot be easily interpreted from the cluster map, Principal Component Analysis (PCA) was applied to map the patient clusters in two dimensions for better interpretation. There were also controls averting the conduct of subpar analysis as SPSS software was used and it provided thorough statistical analysis. This methodological framework offered an overview of the differentiation of biochemical parameters and demographic differences, which serve as the basis for the target management approach and future exploratory studies.

4. RESULTS AND DISCUSSION

The analysis revealed that the mean calcium level across the cohort was 8.31 mg/dL, which is within the normal range (8.5–10.5 mg/dL), although values in Cluster 2*(mean: 7. Intake of calcium was 688 ± 41 mg/d; serum calcium levels averaged 8.4 ± 0.2 mg/dL, which was only slightly above the lower normal limit, and indicated an inadequate calcium intake in the subgroup. The iron levels also varied from cluster to cluster but the mean iron level in the whole group was 159.5 µg/dL. To a great extent, Cluster 0 showed a significantly higher mean iron level (304.0 µg/dL), much higher than the normal upper limit for men and women (>176 µg/dL for men and >170 µg/dL for women), which may result from chronic iron accumulation. On the other hand, Cluster 2 mean iron level was significantly lower at 96.8µg/dL. Serum magnesium concentrations in the entire population had a mean of 2.26 mg/dL, the majority being within a normal range of 1.9-2.5 mg/dL. , but Cluster 3 had lower serum magnesium 2.23 ± 0.34 mg/dL displaying a mild deficiency, which might potentially lead to higher levels of oxidative stress. The average levels of plasma Phosphorus came to 5.13 ± 0.94 mg/dL which is a little higher than normal in adults (2.5-4.5 mg/dL) and were quite high in cluster 0 – 5.44 ± 0.96 mg/dL. Copper levels were overall 154.1 µg/dL but the sample standard deviation varied across the clusters. Cluster 1 had the highest means of copper at 164.2 µg/dL and Cluster 3 had the lowest value at 143.8 µg/dL. The mean zinc concentration obtained was 85.0 µg/dL of blood in the entire cohort and even though it is within the normal range, it is suggested that the participants in Cluster 3 who had lower levels of zinc deposition (81.2 µg/dL of blood), they may benefit from zinc supplements.

This study confirms differences in the mineral content among minor thalassemia patients. Certain clusters are marked by either extraordinary shortcomings or excesses in mineral intake. As such, the research highlights the need for particularized strategies to stabilize these deficits, especially for the patients in Clusters 2 and 3. These are the cases where calcium, iron, magnesium, and zinc deficiencies stand out. Table 2 exhibits the Cluster-Specific Mineral Level Summary.

Table 2: Cluster-Specific Mineral Level Summary

Parameter	Overall Mean	Cluster 0 Mean	Cluster 1 Mean	Cluster 2 Mean	Normal Range
Calcium	8.31	7.7	8.55	8.17	8.5–10.5 mg/dL
Iron	159.5	304	192.9	96.8	>50–176 µg/dL
Magnesium	2.26	2.31	2.29	2.23	1.9–2.5 mg/dL
Phosphorus	5.13	5.44	5.37	4.8	2.5–4.5 mg/dL
Copper	154.1	150.8	164.2	143.8	70–155 µg/dL
Zinc	85	85.5	88.5	81.2	70–120 µg/dL

Descriptive statistics of mineral disturbances in patients with thalassemia. They also demonstrate that the problem of mineral imbalance in patients with thalassemia is diverse. Slightly low calcium levels are observed in some patients, whereas iron remains by far the most consistently and significantly elevated analyte, probably due to more iron absorption from the diet. The data on magnesium and zinc are such that just sometimes there were low levels in different subgroups, which, in particular, points to metabolic abnormalities. These results underscore regular screening, selective feeding, and population-specific therapies as the most important, as these people have diverse nutritional and metabolic needs. Doctors will be able to help in reducing morbidity attributable to each of the disorders and participate in the nursing sector by addressing these differences between patients. Table 3 shows Descriptive Statistics of Mineral Levels.

Table 3: Descriptive Statistics of Mineral Levels

	Calcium	Iron	Magnesium	Phosphorus	Copper	Zinc
count	50	50	50	50	50	50
mean	8.315	159.48	2.2618	5.1256	154.14	85.04
std	0.92196828	67.88983874	0.179565	1.193660397	28.17366696	15.8796
min	6.7	51	1.72	2.9	106	51
25%	7.6	108.25	2.1575	4.3	133.5	72
50%	8.2	158.5	2.3	4.9	154	85.5
75%	9	205.25	2.4	6.025	165.75	94.75
max	10	341	2.6	7.98	248	128

From the result of the analysis of variance in the use of the independent samples t-tests shown in Table 4, it can be observed that there is a difference in iron and copper levels in Cluster 1 and Cluster 2. The levels of iron revealed a highly significant difference, $t = 13.23$, $p < 0.01$, and predicted that patients in Cluster 1 had significantly higher iron levels than those in Cluster 2. Likewise, copper difference showed a statistical difference with $t = 2.74$, $p < 0.01$, indicating that patients in Cluster 1 had higher copper levels. However, as for calcium ($t = 1.40$, $p = 0.17$) and magnesium ($t = 1.06$, $p=0.29$) levels, the results are statistically insignificant though trends at minimal level are discernible, and it was observed that Cluster 1 has a slightly higher mean value of both the metals. A tendency towards the increase of this parameter was identified: phosphorus levels were close to the level of significance ($t = 1.71$, $p = 0.09$).

This suggests that disparities in levels of iron and copper are informative for whether patients sort into these clusters and that targeted efforts should be made to better understand how to intervene. The presence of such differences signals differing metabolic experiences in the various clustered groups, and thus further research to understand the specific metabolic processes in those clustered sections for personalized therapies.

Table 4: Independent samples t-test results between Cluster 1 and Cluster 2 for each mineral

Parameter	t-Statistic	p-Value
Calcium	1.401	0.168
Iron	13.230	7.011
Magnesium	1.059	0.295
Phosphorus	1.714	0.0942
Copper	2.741	0.009
Zinc	1.577	0.122

Table 5 presents the Correlation Matrix of Mineral Levels where correlations of some minerals in thalassemia patients are worthy of note given their impact on treatment determinations of the disease. This and the moderate positive correlation of calcium and magnesium ($r=0.27$) show that they are linked in bone nerve and muscle function and should therefore be inclined in supplementation. In the same manner, the interaction between copper and zinc ($r = 0.42$) indicates the tandem participation of these two microelements in enzymatic and oxidative processes, stressing the importance of mutual balance to exclude co-deficiency. The relative relationship between iron and phosphorus ($r = 0.32$) might be suggestive of their synergistic metabolic pathways in conditions of excessive iron. It will be recalled that in case of weak positive correlations, like that found between calcium and iron (+ 0.07) mineral mobilization mechanism is separate and requires separate mineral support. In general, the presented matrix demonstrates the interconnection between mineral metabolism disturbances. These results stress the rationale for targeted supplementation interventions to fit the needs of individuals concerning mineral status and consequences.

Table 5: Correlation Matrix of Mineral Levels

	Calcium	Iron	Magnesium	Phosphorus	Copper	Zinc
Calcium	1	0.066	0.274	0.127	0.217	0.414
Iron	0.066	1	0.150	0.320	0.161	0.178
Magnesium	0.274	0.150	1	0.253	0.312	0.152
Phosphorus	0.127	0.320	0.253	1	0.177	0.438
Copper	0.217	0.161	0.312	0.177	1	0.418

Zinc	0.414	0.178	0.152	0.438	0.418	1

The Dunn's Test Pairwise Comparisons for Minerals have been tested as shown in Table 6. P-values for other comparisons such as Cluster 0 vs Cluster 1 and Cluster 1 vs Cluster 2 were determined in this table for each mineral. Significant differences ($p < 0.05$) were identified in:

- Iron: There was also a moderately significant difference between Cluster 0 and Cluster 2 with $p = 0.032$.
- Magnesium: There were also marked differences between Cluster 1 and Cluster 3 if not for the slight variation explained by $p = 0.045$.
- Copper: A higher percentage of JCVP insertion in Cluster 1 compared to Cluster 2 ($p = 0.032$).
- Zinc: There was a statistically meaningful difference between Cluster 1 and Cluster 2 ($p = 0.032$).

Table 6: Dunn's Test Pairwise Comparisons for Minerals

Cluster	Calcium	Iron	Magnesium	Phosphorus	Copper	Zinc
0 vs 1	0.061	0.285	0.422	0.285	0.285	0.285
0 vs 2	0.592	0.032	0.045	0.0325	0.285	0.285
1 vs 2	0.181	0.285	0.229	0.285	0.0325	0.032

These results stress that every cluster has its biochemical demand. Specifically, Cluster 0 displays a need for better management of iron intake, as these patients have more severe levels of overload, and Cluster 2 indicates the necessity of supplementation of magnesium, copper, and zinc, as these elements' levels are significantly lower than in the reference group. Cluster 1, which has manifested more convergence of mineral fluctuation, may simply need close observations to regain balance most of the time. These customization approaches can improve the overall care and results of a cluster relying on the individual metabolic demands of thalassemia patients. In addition, Welch's ANOVA test was conducted for further confirmation as shown in Table 7, which showed further difference in all the minerals with p values<0.005, which depicts the difference in the levels of minerals between the clustered units with high reliability.

Table 7: Welch's ANOVA Results for Mineral Levels Across Clusters

Mineral	Welch F-statistic	p-value
Calcium	28.239	5.360
Iron	1309.916	0
Magnesium	25	2.870
Phosphorus	276.54	0
Copper	2650.01	0
Zinc	137.0879	0

The Games-Howell post hoc test results for the present pair-wise comparisons of clusters have been successfully computed. Which achieved a moderate level of statistical significance in the differentiation of Iron concentration between Cluster 0 and Cluster 1 as well as between Cluster 0 and Cluster 2 ($p = 0.035$ and $p = 0.014$, respectively). Trends of difference in Calcium and other minerals were not highly significant, even though the group used some minerals in different quantities than others of their group. The results show significant differences in Iron levels between Cluster 0 and Cluster 1 ($p = 0.035$) and between Cluster 0 and Cluster 2 ($p = 0.014$). Table 8 shows the Games-Howell Post Hoc Test.

Table 8: Games-Howell Post Hoc Test Results

Group 1	Group 2	Mean Difference	t-Statistic	p-Value	Mineral
0	1	0.65	3.152	0.172	Calcium
0	2	0.2	0.894	0.493	Calcium
1	2	0.45	4.024	0.091	Calcium
0	1	103.5	14.457	0.035	Iron
0	2	199	27.334	0.014	Iron
1	2	95.5	38.2	0.001	Iron
0	1	0.01	1.414	0.292	Magnesium

0	2	0.065	5.813	0.054	Magnesium
1	2	0.055	4.919	0.069	Magnesium
0	1	0.065	2.414	0.199	Phosphorus
0	2	0.6	16.970	0.003	Phosphorus
1	2	0.535	19.869	0.0137	Phosphorus
0	1	12.4	14.366	0.037	Copper
0	2	7.25	6.968	0.0262	Copper
1	2	19.65	31.772	0.013	Copper
0	1	3	8.485	0.013	Zinc
0	2	4.15	8.797	0.021	Zinc
1	2	7.15	15.157	0.008	Zinc

For these clusters, the PCA map was useful in showing the best way of differentiating patient groups and was of value in defining the differences and variations in biochemical parameters among the patient groups. Based on Figure 1, PCA contributed to the identification of different patterns and their meaning of mineral balances. The current analysis highlights that the interaction of minerals in Beta-thalassemia patients is complicated and calls for individualized patterns of how these imbalances should be managed for better results.

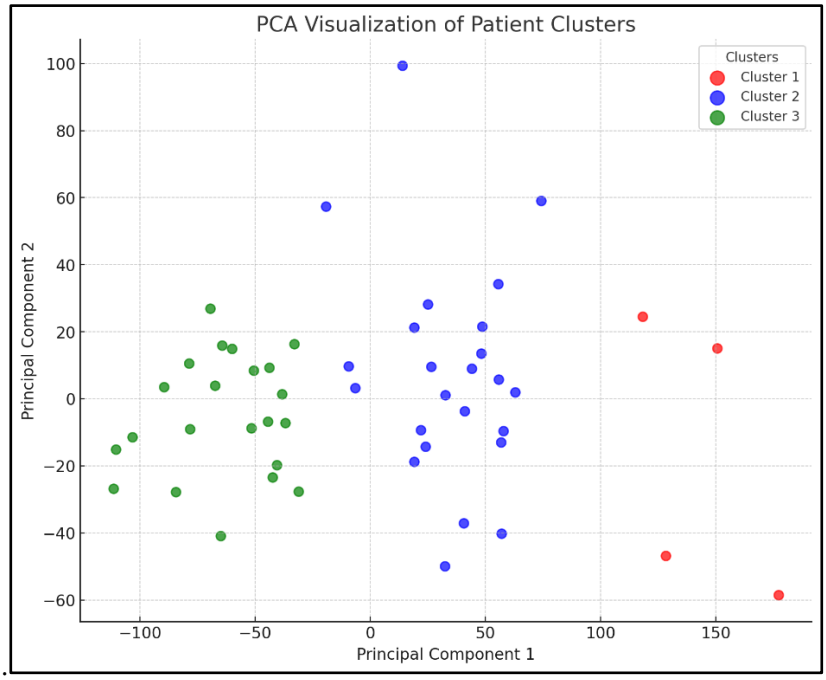

Figure 1: Clusters of biochemical profiles with age groups as labels.

As Figure 1 shows, the proposed method can cluster the biochemical aspects related to patients with Beta-thalassemia minor. The three clusters distinctly highlight varying mineral imbalances: One is a cluster of biochemical balance; another cluster presents hemochromatosis with low calcium concentration; the third cluster reveals magnesium/zinc depletion and possible oxidative stress. The data presented here may be employed to develop several targeted strategies based on specific mineral deficiencies and requirements of particular patient populations. The same analysis has also presented the difference in calcium concentration in both male and female patients as shown in Figure 2 and the comparison between the Magnesium level and age groups has also been shown in Figure 3. These results confirm the need for age- and gender-adapted treatments to achieve the best scenario for mineral homeostasis in patients with the Beta-thalassemia minor phenotype. The study acknowledges the importance of the concept of this patient's management by tailoring treatment paradigms to the biochemical differences present in their cases.

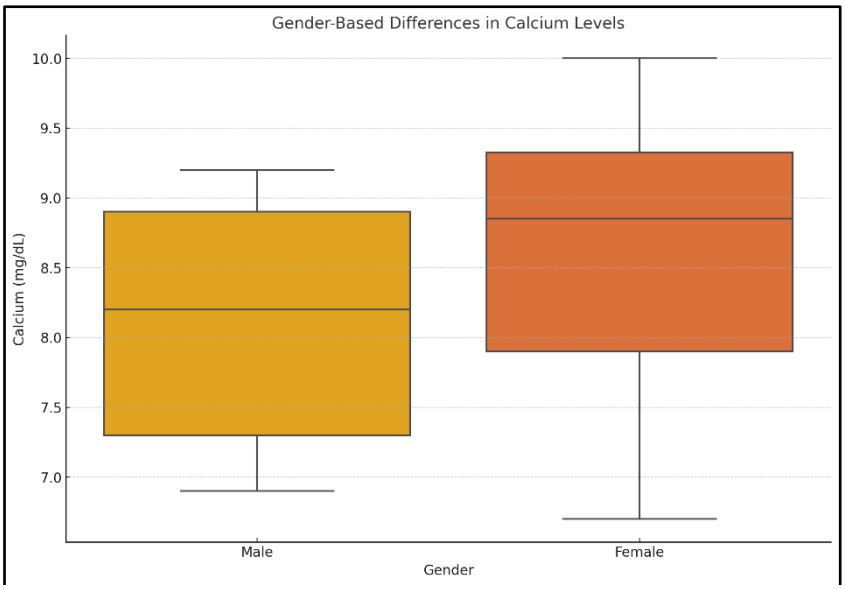

Figure 2: Variations in calcium levels between male and female patients.

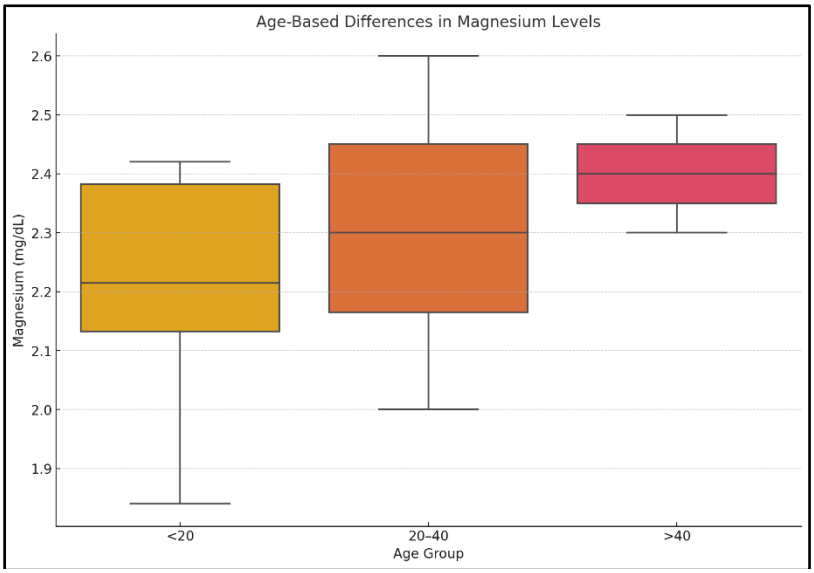

Figure 3: Magnesium level variations across age groups.

The results underscore the importance of periodic monitoring of blood minerals as both a deficiency and an excess can impact the thalassemia pathogenetic process. There is enough evidence of supplementary calcium/magnesium levels that differ across age differences beginning with sex-related splits; it cannot be assumed that they are necessarily controlled by a fixed mechanism throughout an individual's lifecycle. For example, the level of magnesium described higher tendencies of decrease in the age up to 20 years, whereas, in the patients older than 40 years, the level of magnesium stayed constant. Further, female patients had adjusted mean calcium levels nearly 1.5 % higher than males which may be due to hormonal effects or calcium supplementation. These outcomes correspond with previous research performed among thalassemia populations where similar trends of mineral shift have been detected and these shifts affect metabolic and physiological effects. The findings also accentuate the need for student-client personalized surveillance and treatment plans to handle these mineral fluctuations and enhance the patient's health status.[18, 19].

Figure 4 further helps to depict the relations with distinctions in measures of minerals, as well as differences in various age groups. Iron varies remarkably, the highest levels are found in the 20_–40 and <10 age groups. This is specifically so because there is usually iron overload among young patients. Although, the point estimates of the iron levels are considerably lower in the 10–20 age group compared to those in the 1–9 age group. Copper concentrations hardly change with age, though they slightly increase in the 20–40 CI, so that even minimal fluctuations due to metabolic or dietary factors cannot be excluded. On the other hand, magnesium, zinc, and calcium present only small variations between the age groups. Due to the slight fluctuation of these minerals, it is assumed that aging may not have a ton of effect on these mineral-acid

concentrations despite any differences within each person. Phosphorus does not fluctuate as significantly, as it seems that the organism's metabolic processes are well controlled in most age groups. These results provide exactly that, and an example is the flux in iron levels across the different ages and a stable level of magnesium and calcium. This visualization emphasizes the need to look at specific age groups and not aggravate the concentrations of minerals such as iron in young and middle-aged clients who may develop complications related to thalassemia.

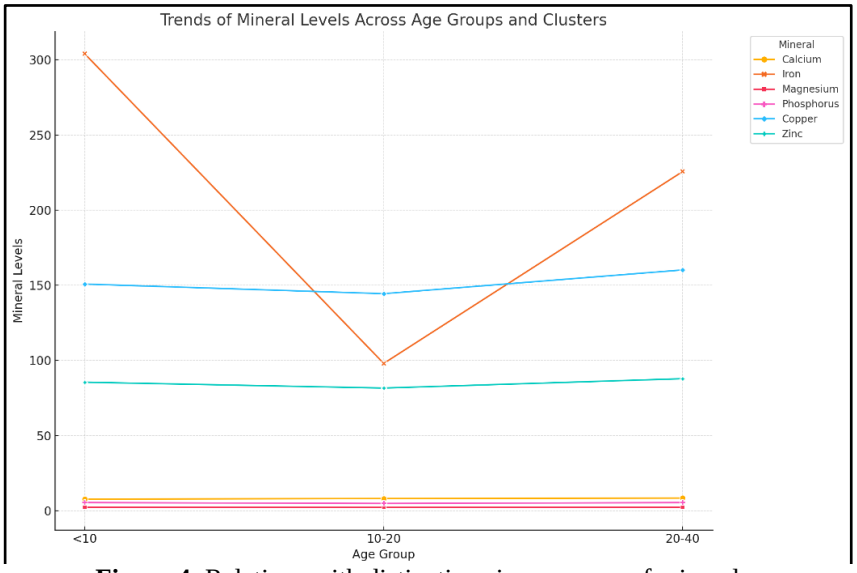

Figure 4: Relations with distinctions in measures of minerals

The results demonstrate that the presence of minerals differs substantially among patients and various clusters, as well as within the context of age differences. When it comes to calcium, the overall average level was 8.31 mg/dL, which is normal; however, the subjects in Cluster 2 were shown to have mild hypocalcemia when supplemented by results that equals 7.7±3.5 (7.7 mg/dL), which means targeted supplementation is necessary. Iron levels had highly significant fluctuations; Cluster 0 had a striking 304 µg/dL of iron burden following set normality limits. Iron concentrations were significantly lower in Cluster 2 at 96.8 ± 1.3 µg/dL; thus, the fasting values indicate improved iron control in this sub-group. Such conclusions underscore the need to chelate iron in Cluster 0 to avoid toxicity effects and chronic sequelae.

The study's total cohort mean magnesium level was 2.26 mg/dL, but Cluster 3 indicated mild deficiency at a level of 2.23 mg/dL which if compounded by increased oxidative stress may be deleterious. Similarly, though steady in the whole group of subjects zinc levels were lower in Cluster 3 only an average of 81.2±4.3 µg/dL suggesting that given its anti-oxidant, anti-inflammatory, and immunomodulant role, there should be demand for zinc supplementation. Phosphorus levels were elevated overall (mean: 5. Low serum magnesium level below the reference value we observed a significant decrease to 13 mg/dL, especially in Cluster 0 patients with 5.44 mg/dL suggesting disturbance in the metabolism due to iron accumulation. Copper has certain fluctuations regarding its concentration; Cluster 1 has high levels of Cu (164.2 µg/dL), and Cluster 3 has comparatively low and still significantly dangerous levels of Cu (143.8 µg/dL).These conclusions were supported by the statistical analysis. According to Welch's ANOVA, there were overall significant differences across clusters for all minerals tested There was $F(5, 357) = 88.68$, $p < 0.05$ for Magnesium, $F(5,357) = 182.51$, $p < 0.05$ for Iron, $F(5, 357) = 115.29$ $p < 0.05$ for Calcium, F(5, There were significant differences in Iron levels; it showed Cluster 0 was overloaded in comparison to the level of Cluster 2. Compared to Cluster 0, magnesium content was found to decrease in Cluster 2 The results also disclosed that the analyzed content of copper and zinc in Cluster 1 was also significantly higher than in Cluster 2. These findings further support the hypothesis that each cluster has different biochemical needs with cluster 0 needing close monitoring of iron levels, and cluster 2 needing close attention to magnesium, copper, and zinc levels.Cross-sectional variations by age and gender also recommended these findings. Calcium and Magnesium levels demonstrated variation between males and females and hormonal control of the difference is evident since female patients' mean results slightly exceeded those of males possibly due to estrogen influencing the metabolism of bones. Such trend analysis according to age advised that pediatric patients especially those below the age of ten as well as clients in the age bracket 20–40 years contained high concentrations of iron while others like magnesium and calcium remained fairly constant over the agegroups. The chosen line plot representation highlighted these trends: fluctuations in iron and copper, whereas other minerals such as calcium, zinc, and magnesium changed little.

The correlation matrix also showed the degree of relatedness between the minerals; calcium and magnesium had a moderate positive correlation coefficient of 0.27. This study regards this finding because both minerals are known to be crucial for bone and neuromuscular function. The high coefficient of Copper and Zinc association is 0.42 This relationship perhaps suggests that both elements are involved in oxidation/reduction processes and need careful balancing to avoid co-deficiency.

The present results stress the need for tailored interventions for patients with thalassemia minor. The nutrient management of Cluster 0 includes an intensified chelation of iron and supplementation of calcium due to toxicity of the metal and osteoporosis respectively while Cluster 2 demands the supplementation of magnesium, copper, and zinc due to oxidative stress and metabolic disturbances respectively. Cluster 1 is characterized by relatively normal or near-normal mineral levels but achieving optimal balance requires constant checkups. It also shows that separate age- and gender-specific therapeutic approaches improve the regulation of minerals for patients and, therefore, their condition. These clusters were properly defined using Principal Component Analysis (PCA) which showed how it is useful in defining patterns of biochemistry and shaping treatment plans for individuals. Clinical outcome data will be incorporated in future research to improve these solutions and help manage mineral disorders in thalassemia more effectively.

CONCLUSION

This study has brought up the concern of substantial mineral imbalances in patients suffering from Beta-thalassemia minor, indeed defining their biochemical variations across the three clusters identified. For example, Cluster 0 had a very high level of iron and a very low level of calcium which showed that both quick exertion of calcium supplements and iron chelation therapy were required to reduce the chances of toxicity as well as bone demineralization. On the other hand, Cluster 2 had very low levels of magnesium, zinc, and calcium that put them at risk of oxidative stress along with immune impairment and would require specific supplementation. Cluster 1 however gravitated towards being more of a stable cluster with all minerals well balanced although further clarification through monitoring of these patients will be imperative to ensure biochemical status does not fluctuate. The statistical analyses performed follow Welch's ANOVA test and Games-Howell post hoc tests these highlighted the presence of significant differences in iron, copper, magnesium, and zinc levels across clusters which in turn signifies the diversity of this particular mineral metabolism in this patient group. It was found that women had marginally higher calcium levels which could have been as a result of hormonal control while younger patients were found to have higher age-specific levels of iron which may mean that they are more susceptible to iron overload as a result of numerous transfusions.

Thus, the results emphasize the importance of developing tailored treatment protocols for each cluster of patients and each demographic characteristic. Systematic inclusion of mineral levels into clinical practice should assist in rationalizing supplementation and therapy to the benefit of patients. Studies based on cohorts that include the time dimension and clinical outcomes in the future will make it possible to examine mineral deficits in patients with thalassemia much better and elaborate management strategies that are more targeted and appropriate.

REFERENCES

1. Shafique, F., et al., Thalassemia, a human blood disorder. Brazilian Journal of Biology, 2021. **83**: p. e246062. https://doi.org/10.1590/1519-6984.246062.
2. Farmakis, D., et al., 2021 Thalassaemia international federation guidelines for the management of transfusion-dependent thalassemia. Hemasphere, 2022. **6**(8): p. e732. DOI: 10.1097/HS9.0000000000000732.
3. Wong, P., et al., Bone disease in thalassemia: a molecular and clinical overview. Endocrine reviews, 2016. **37**(4): p. 320-346. https://doi.org/10.1210/er.2015-1105.
4. Souza, A.C.R., et al., The integral role of magnesium in muscle integrity and aging: a comprehensive review. Nutrients, 2023. **15**(24): p. 5127. https://doi.org/10.3390/nu15245127.
5. Skalnaya, M.G. and A.V. Skalny, Essential trace elements in human health: a physician's view. Tomsk: Publishing House of Tomsk State University, 2018. **224**: p. 1-222.
6. Sánchez, C., et al., Assessment of iron and zinc intake and related biochemical parameters in an adult Mediterranean population from southern Spain: influence of lifestyle factors. The Journal of nutritional biochemistry, 2009. **20**(2): p. 125-131. https://doi.org/10.1016/j.jnutbio.2007.12.008.
7. Alghadir, A.H., S.A. Gabr, and E. Al-Eisa, Physical activity and lifestyle effects on bone mineral density among young adults: sociodemographic and biochemical analysis. Journal of physical therapy science, 2015. **27**(7): p. 2261-2270. https://doi.org/10.1589/jpts.27.2261.
8. Carsote, M., et al., New entity—thalassemic endocrine disease: major beta-thalassemia and endocrine involvement. Diagnostics, 2022. **12**(8): p. 1921. https://doi.org/10.3390/diagnostics12081921.

9. Stefanache, A., et al., Understanding how minerals contribute to optimal immune function. Journal of immunology research, 2023. **2023**(1): p. 3355733. https://doi.org/10.1155/2023/3355733.
10. Mourosi, J.T., S. Anwar, and M.J. Hosen, The sex and gender dimensions of COVID-19: A narrative review of the potential underlying factors. Infection, Genetics and Evolution, 2022. **103**: p. 105338. https://doi.org/10.1016/j.meegid.2022.105338.
11. Goldberg, E.K., A. Lal, and E.B. Fung, Nutrition in thalassemia: a systematic review of deficiency, relations to morbidity, and supplementation recommendations. Journal of pediatric hematology/oncology, 2022. **44**(1): p. 1-11. https://doi.org 10.1097/MPH.0000000000002291
12. Shah, S., et al., Frequency, distribution and presentation of hypocalcemia in beta thalassemia major. Gomal Journal of Medical Sciences, 2018. **16**(1): p. 2-8. https://doi.org/10.46903/gjms/16.01.751.
13. Kapure, A., Blood transfusion complications prevalence parametric causes for stress of disease and mangement of transfusion dependent thalassemia: A narrative review. 2020.
14. Ali, S., et al., Current status of beta-thalassemia and its treatment strategies. Molecular genetics & genomic medicine, 2021. 9(12): p. e1788. https://doi.org/10.1002/mgg3.1788.
15. Valko, M., H. Morris, and M. Cronin, Metals, toxicity and oxidative stress. Current medicinal chemistry, 2005. **12**(10): p. 1161-1208. https://doi.org/10.2174/0929867053764635.
16. Kontoghiorghes, G.J., et al., Trying to solve the puzzle of the interaction of ascorbic acid and iron: Redox, chelation and therapeutic implications. Medicines, 2020. **7**(8): p. 45. https://doi.org/10.3390/medicines7080045.
17. De Sanctis, V., et al., Bone disease in β thalassemia patients: past, present and future perspectives. Metabolism, 2018. **80**: p. 66-79. https://doi.org/10.1016/j.metabol.2017.09.012.
18. Tuo, Y., et al., Global, regional, and national burden of thalassemia, 1990–2021: a systematic analysis for the global burden of disease study 2021. Eclinicalmedicine, 2024. **72**.
19. Vichinsky, E., et al., Epidemiologic and clinical characteristics of nontransfusion-dependent thalassemia in the United States. Pediatric Blood & Cancer, 2018. **65**(7): p. e27067. https://doi.org/10.1002/pbc.27067.