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ASSESSMENT OF SERUM CALCIUM, PHOSPHORUS AND VITAMIN D LEVELS IN MULTI-TRANSFUSED BETA THALASSEMIA MAJOR PATIENTS: A CROSS-SECTIONAL STUDY

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Abstract

Background: Beta Thalassemia Major Patients Undergoing Frequent Blood Transfusions Are At Increased Risk Of Developing Endocrinopathies Due To Iron Overload. Despite Chelation Therapy, Complications Related To Calcium-Phosphorus Metabolism And Vitamin D Deficiency Persist. Limited Data Exists On These Parameters In The Indian Thalassemic Population.

Objective: To Evaluate Serum Levels Of Calcium, Phosphorus, And Vitamin D In Beta Thalassemia Major Patients Who Had Received 20 Or More Blood Transfusions And To Assess Their Correlation With Serum Ferritin Levels.

Methods: A Cross-Sectional Study Was Conducted At The Thalassemia Ward, Department Of Paediatrics, Government Medical College, Kota, From May 2022 To March 2023. Sixty Children Aged 5-18 Years With Beta Thalassemia Major Who Had Received ≥20 Blood Transfusions Were Enrolled. Serum Calcium, Phosphorus, Vitamin D, And Ferritin Levels Were Measured And Compared With Standard Reference Values. **Results:** Mean Serum Calcium Was 6.86±1.25 Mg/Dl (Standard: 8.4-10.2 Mg/Dl), Phosphorus Was 8.1±3.5 Mg/Dl (Standard: 2.3-4.7 Mg/Dl), And Vitamin D Was 19.37±8.98 Ng/Ml (Standard: >30 Ng/Ml). Hypocalcemia Was Present In 88% Of Patients. Vitamin D Deficiency (<20 Ng/Ml) Was Observed In 75% And Insufficiency (20-29 Ng/Ml) In 18.3% Of Patients. Serum Vitamin D ($R=-0.8$, $P<0.01$) And Calcium ($R=-0.8$, $P<0.01$) Showed Significant Negative Correlation With Serum Ferritin, While Phosphorus Showed Weak Positive Correlation ($R=+0.2$, $P<0.01$). **Conclusion:** Multi-Transfused Beta Thalassemia Major Patients Demonstrate High Prevalence Of Hypocalcemia, Hyperphosphatemia, And Vitamin D Deficiency, Strongly Correlating With Iron Overload. Routine Monitoring And Appropriate Supplementation Are Recommended To Prevent Endocrine Complications And Improve Quality Of Life.

Keywords: Beta Thalassemia Major, Calcium, Ferritin, Hypoparathyroidism, Phosphorus, Vitamin D,

Introduction

Beta Thalassemia Represents One Of The Most Prevalent Inherited Hemoglobinopathies Globally, Characterized By Reduced Or Absent Synthesis Of Beta-Globin Chains, Leading To Chronic Hemolytic Anemia And Ineffective Erythropoiesis. The Mean Prevalence Of Thalassemia In India Is Approximately 3.5%, With Regional Variations Ranging From 1-17% [1]. Its Incidence Is Particularly Elevated In Specific Communities Including Sindhis, Punjabis, Khatris, Gujaratis, Mahars, And Lohanas, As Well As Certain Muslim And Christian Populations [2].

The Cornerstone Of Management For Beta Thalassemia Major Is Regular Blood Transfusion Therapy Aimed At Maintaining Hemoglobin Levels Above 9-10 G/Dl, Thereby Suppressing Ineffective Erythropoiesis And Promoting Normal Growth And Development [3]. However, This Life-Sustaining Therapy Inevitably Leads To Iron Overload, As Each Unit Of Packed Red Blood Cells Contains Approximately 200-250 Mg Of Iron, Which The Human Body Cannot Actively Excrete. Despite The Introduction Of Iron Chelation Therapy, Which Has Significantly Improved Survival And Quality Of Life, Iron-Related Complications Continue To Manifest, Particularly Affecting Endocrine Organs [4].

Owing To Limited Resources In Local Setups, Thalassemia Major Patients Undergoing Frequent Transfusions Often Receive Inadequate Monitoring, Making Them Prone To Develop Complications Likely Due To Iron Overload. Despite Chelation Therapy, Iron Overload-Related Complications Like Endocrinopathies Are Still Occurring. Common Endocrine Problems Related To Beta Thalassemia Major Are Short Stature, Hypogonadism, Hypoparathyroidism, Hypothyroidism, And Diabetes Mellitus [5,6]. Among These, Disturbances In Calcium-Phosphorus Metabolism Secondary To Hypoparathyroidism Represent A Significant But Often Underrecognized Complication.

Hypoparathyroidism In Thalassemia Major Patients Results From Iron Deposition In The Parathyroid Glands, Leading To Impaired Parathyroid Hormone (Pth) Secretion. This Manifests Clinically As Hypocalcemia And Hyperphosphatemia, Which May Present With Muscle Cramps, Paresthesias, Tetany, Seizures, Or Remain Asymptomatic Until Severe [7]. The Reported Prevalence Of Hypoparathyroidism In Multi-Transfused Thalassemia Patients Varies Widely Across Studies, Ranging From 3.6% To 40%, Depending On The Population Studied, Transfusion Practices, And Adequacy Of Chelation Therapy [8].

Vitamin D Deficiency Adds Another Dimension To The Calcium-Phosphorus Dysregulation In Thalassemia Patients. Multiple Factors Contribute To Vitamin D Deficiency In This Population, Including Inadequate Dietary Intake, Reduced Outdoor Activities Due To Chronic Illness, Skin Hyperpigmentation From Iron Deposition Reducing Cutaneous Synthesis, Hepatic Dysfunction Affecting 25-Hydroxylation, And Possible Interference With Vitamin D Metabolism By Iron Overload [9].

The Patterns Of Abnormalities Of Serum Levels Of Calcium, Phosphorus, And Vitamin D Among Patients With Beta Thalassemia Major Undergoing Repeated Blood Transfusions Remain Unexplored. Very Few Studies Have Been Undertaken Among The Indian Population. The Paucity Of Data In Our Population Prompted Us To Plan This Study Aiming To Assess Calcium, Phosphorus, And Vitamin D Status In Children With Beta Thalassemia Major Receiving Multiple Blood Transfusions. The Current Study Will Bring Forth Primary Details Which Will Guide Further Research In This Regard.

Materials And Methods

This Hospital-Based Cross-Sectional Observational Study Was Conducted At The Thalassemia Ward, Department Of Paediatrics, Government Medical College And Associated J.K. Lon Hospital, Kota, Rajasthan, India, Over A Period Of 11 Months From May 2022 To March 2023. The Study Enrolled 60 Children And Adolescents Aged 5-18 Years Who Were Diagnosed Cases Of Beta Thalassemia Major, Confirmed By Hemoglobin Electrophoresis Or High-Performance Liquid Chromatography (Hplc), And Were Registered At Our Thalassemia Ward Receiving Regular Blood Transfusions. A Consecutive Sampling Method Was Employed, Where All Patients Fulfilling The

Inclusion Criteria And Attending The Thalassemia Ward During The Study Period Were Approached For Participation.

Inclusion Criteria Comprised Children And Adolescents Aged 5-18 Years With Confirmed Diagnosis Of Beta Thalassemia Major, History Of Receiving ≥20 Packed Red Blood Cell Transfusions, Those On Regular Iron Chelation Therapy, And Patients Admitted For Blood Transfusion Between May 2022 And March 2023. Exclusion Criteria Included Age Outside The Specified Range, Other Chronic Hemolytic Anemias Apart From Beta Thalassemia Major, Presence Of Other Systemic Comorbidities Such As Chronic Kidney Disease, Celiac Disease, Or Protein-Energy Malnutrition, Current Supplementation With Calcium, Phosphorus, Or Vitamin D Preparations, And Patients On Medications Affecting Calcium Metabolism.

The Study Protocol Was Approved By The Institutional Ethics Committee Of Government Medical College, Kota. Written Informed Consent Was Obtained From Parents Or Legal Guardians Of All Participants After Explaining The Purpose And Benefits Of The Study In The Local Language. A Structured Proforma Was Used To Collect Demographic And Clinical Data Including Age, Gender, Age At Diagnosis, Total Number Of Blood Transfusions Received, And Current Chelation Therapy Regimen. Blood Samples (5 Ml) Were Collected From All Participants By Venipuncture Under Aseptic Conditions Prior To Blood Transfusion. Serum Calcium Was Estimated Using Arsenazo Iii Complexometric Method By Spectrophotometry (Normal Range: 8.4-10.2 Mg/Dl), Serum Phosphorus By Molybdate Method (Normal Range: 2.3-4.7 Mg/Dl), Serum Vitamin D (25-Hydroxyvitamin D) Using Chemiluminescent Microparticle Immunoassay (Cmia) With Results Categorized As Deficiency (<20 Ng/Ml), Insufficiency (20-29 Ng/Ml), Or Optimal (≥30 Ng/Ml), And Serum Ferritin Using Chemiluminescent Immunoassay (Normal Range: 7-140 Ng/Ml).

Data Were Entered Into Microsoft Excel And Analyzed Using Spss Version 21.0. Continuous Variables Were Expressed As Mean ± Standard Deviation. Unpaired Student's T-Test Was Used To Compare Mean Values With Standard Reference Values. Pearson's Correlation Coefficient Was Calculated To Assess Relationships Between Serum Calcium, Phosphorus, Vitamin D, And Ferritin Levels. A P-Value <0.05 Was Considered Statistically Significant.

Results

Demographic Characteristics

A Total Of 60 Children And Adolescents With Beta Thalassemia Major Were Enrolled In The Study. The Demographic Distribution Showed Male Preponderance With 34 Males (56.7%) And 26 Females (43.3%), Giving A Male-To-Female Ratio Of 1.31:1. The Mean Age Of Participants Was 12.22±4.02 Years. Age Group Distribution Revealed That 17 Patients (28.3%) Were In The 5-9 Years Group, 26 Patients (43.3%) In The 10-14 Years Group, And 17 Patients (28.3%) In The 15-18 Years Group (Table 1).

Table 1: Demographic Characteristics Of Study Participants (N=60)

Characteristic	Number (N)	Percentage (%)
Gender		
Male	34	56.7
Female	26	43.3
Age Group (Years)		
5-9	17	28.3
10-14	26	43.3
15-18	17	28.3
Mean Age ± Sd	**12.22 ± 4.02 Years**	

Serum Calcium, Phosphorus, Vitamin D, And Ferritin Levels

The Mean Values Of Serum Parameters Compared With Standard Reference Values Are Presented In Table 2. Mean Serum Calcium Was 6.86±1.25 Mg/Dl (Standard: 8.4-10.2 Mg/Dl), Serum Phosphorus Was 8.1±3.5 Mg/Dl (Standard: 2.3-4.7 Mg/Dl), Serum Vitamin D Was 19.37±8.98

Ng/Ml (Standard Optimal: ≥30 Ng/Ml), And Serum Ferritin Was 2270.6±951.9 Ng/Ml (Standard: 7-140 Ng/Ml).

Table 2: Mean Values Of Serum Calcium, Phosphorus, Vitamin D, And Ferritin

Parameter	Mean ± Sd	Standard Reference Values	Unpaired T Test
Serum Calcium (Mg/Dl)	6.86 ± 1.25	8.4-10.2	T=10.20, Df=59, P<0.01 (Highly Significant)
Serum Phosphorus (Mg/Dl)	8.1 ± 3.5	2.3-4.7	T=10.84, Df=59, P<0.01 (Highly Significant)
Serum Vitamin D (Ng/Ml)	19.37 ± 8.98	40-80 (Optimal ≥30)	T=9.16, Df=59, P<0.01 (Highly Significant)
Serum Ferritin (Ng/Ml)	2270.6 ± 951.9	7-140	-

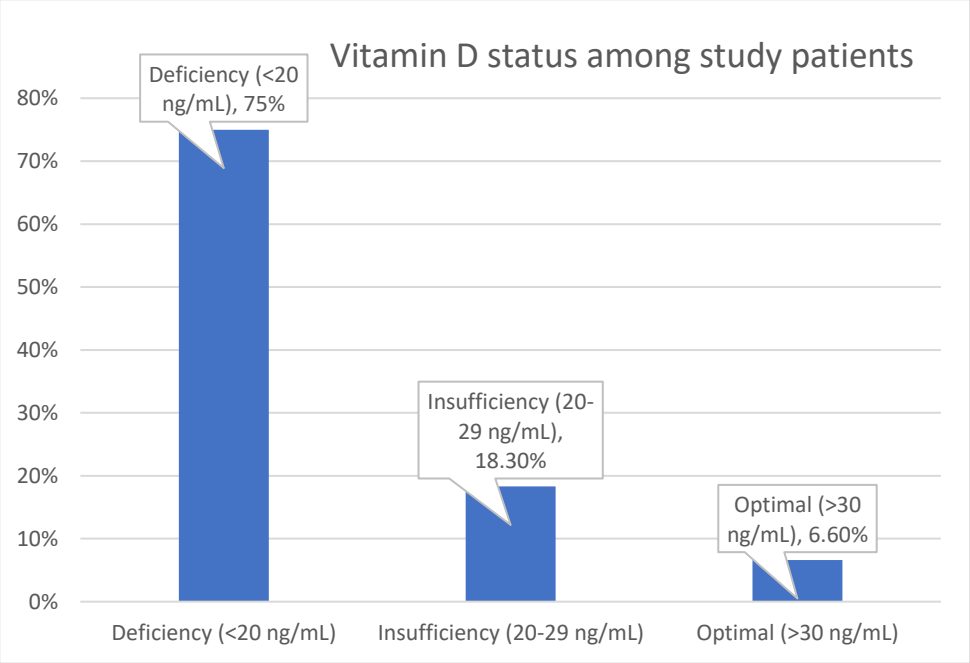
All Three Parameters (Calcium, Phosphorus, And Vitamin D) Showed Statistically Highly Significant Differences From Standard Reference Values (P<0.01).

Prevalence Of Metabolic Abnormalities

Analysis Of Individual Patient Data Revealed High Prevalence Of Metabolic Abnormalities. Hypocalcemia (Serum Calcium <8.4 Mg/Dl) Was Present In 53 Patients (88.3%). Hyperphosphatemia (Serum Phosphorus >4.7 Mg/Dl) Was Observed In 48 Patients (80%).

Vitamin D Status Assessment Showed Alarming Findings: 45 Patients (75%) Had Deficiency (<20 Ng/Ml), 11 Patients (18.3%) Had Insufficiency (20-29 Ng/Ml), And Only 4 Patients (6.7%) Had Optimal Levels (≥30 Ng/Ml). Thus, A Total Of 93.3% Of Patients Had Either Vitamin D Deficiency Or Insufficiency (Figure 1).

Figure 1: Vitamin D Status Among Study Patients



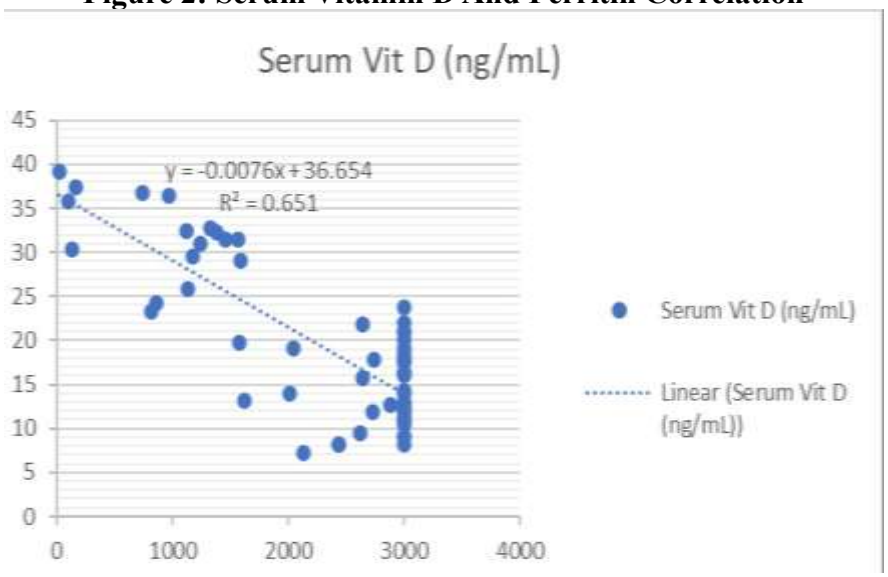
Correlation Analysis

Serum Vitamin D And Ferritin Correlation

A Strong Negative Correlation Was Observed Between Serum Vitamin D And Serum Ferritin Levels. The Pearson's Correlation Coefficient Was R=-0.8 With R^2 Value Of 0.651. The Linear Regression Equation Was Y = -0.0076x + 36.654. This Correlation Was Statistically Highly

Significant (P<0.01), Indicating That As Serum Ferritin Levels Increase (Representing Greater Iron Overload), Vitamin D Levels Significantly Decrease (Figure 2).

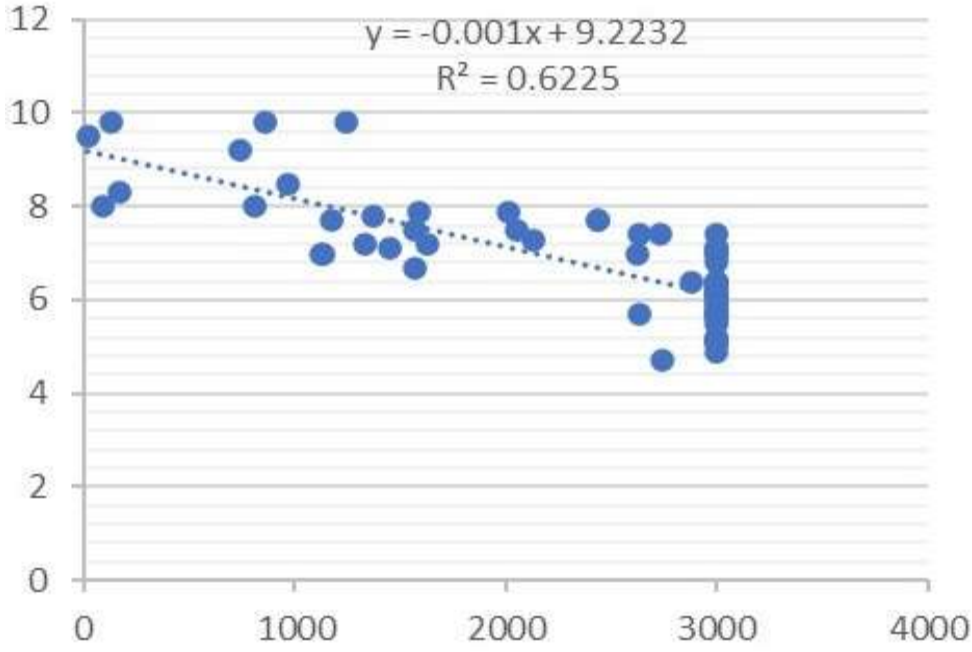
Figure 2: Serum Vitamin D And Ferritin Correlation



Serum Calcium And Ferritin Correlation

Serum Calcium Levels Showed A Significant Negative Correlation With Serum Ferritin. The Pearson's Correlation Coefficient Was R=-0.8 With R^2 Value Of 0.6225. The Linear Regression Equation Was Y = -0.001x + 9.2232 (P<0.01). Higher Iron Overload (Elevated Ferritin) Was Associated With Lower Serum Calcium Levels, Suggesting Iron-Mediated Parathyroid Dysfunction (Figure 3).

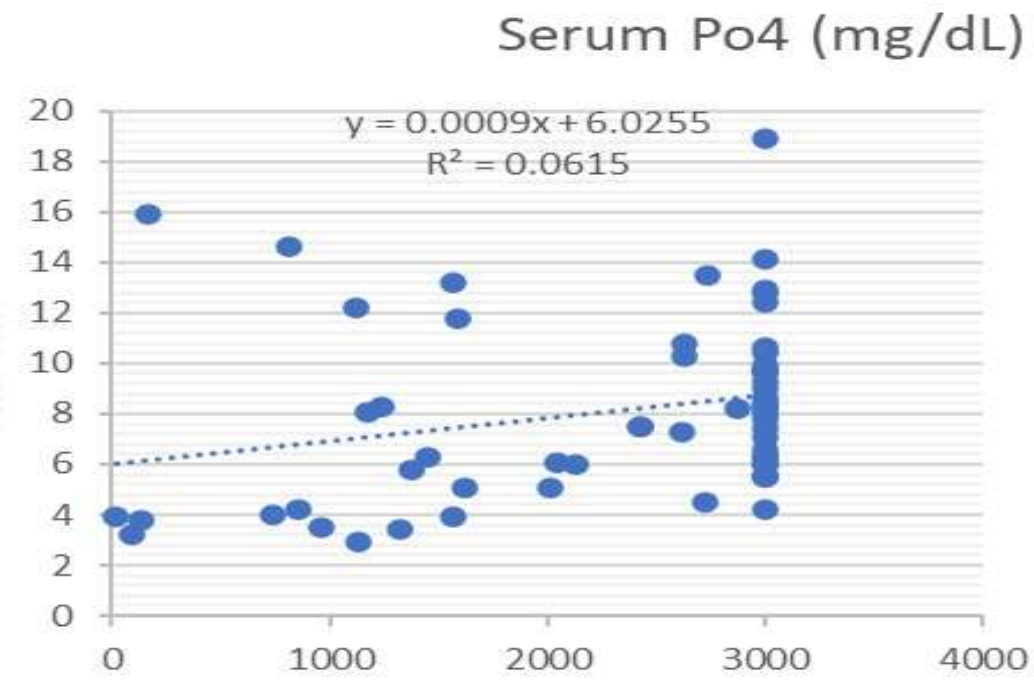
Figure 3: Serum Calcium And Ferritin Correlation



Serum Phosphorus And Ferritin Correlation

Serum Phosphorus Showed A Weak Positive Correlation With Serum Ferritin. The Pearson's Correlation Coefficient Was R=+0.2 With R^2 Value Of 0.0615. The Linear Regression Equation Was Y = 0.0009x + 6.0255 (P<0.01). Although Statistically Significant, The Correlation Was Weak, Indicating That Factors Other Than Iron Overload Also Influence Phosphorus Levels (Figure 4).

Figure 4: Serum Phosphorus And Ferritin Correlation



Discussion

The Present Study Systematically Evaluated Serum Calcium, Phosphorus, And Vitamin D Status In 60 Multi-Transfused Beta Thalassemia Major Patients Aged 5-18 Years At Our Center. Our Findings Reveal A High Prevalence Of Metabolic Bone And Mineral Abnormalities In This Population, With Strong Correlations To Iron Overload Markers. The Mean Age Of The Study Subjects Was 12.22±4.02 Years With Male To Female Ratio Of 1.31:1. The Largest Proportion Of Patients (43.3%) Belonged To The 10-14 Years Age Group, Representing The Period Of Maximum Endocrine Vulnerability Due To Puberty-Related Hormonal Changes Superimposed On Chronic Disease And Iron Overload.

The Mean Serum Calcium Level In Our Study Was 6.86±1.25 Mg/Dl, Significantly Lower Than The Normal Reference Range (8.4-10.2 Mg/Dl) With Highly Significant Statistical Difference ($T=10.20$, $P<0.01$). Low Serum Calcium Is Very Prevalent In Transfusion-Dependent Beta Thalassemia Major Patients In Our Setup, Possibly Due To Poor Chelation, As Was Confirmed By The Present Study Where 88% Of Patients Had Hypocalcemia. Our Findings Are Consistent With Several Previous Studies. Shah Et Al. Reported 49% Prevalence Of Hypocalcemia In Pakistani Thalassemia Patients, With Mean Serum Calcium Of 8.46±0.94 Mg/Dl [1]. Ahmed Z Et Al. From Ranchi, India, Reported Similar Mean Calcium Of 6.72±0.66 Mg/Dl [10]. Fahim Et Al. From Egypt Found Comparable Values (6.6±1.2 Mg/Dl) In Their Pediatric Thalassemia Cohort [6]. Meshram Rm Reported That The Mean Values Of Total Serum Calcium, Vitamin D, And Parathyroid Hormone (Pth) Showed Statistically Significant Variations Between The Case And Control Groups. In The Case Group, The Mean Serum Calcium Was 8.51 ± 0.84 Mg/Dl, Vitamin D Was 15.23 ± 10.07 Ng/Ml, And Pth Was 14.66 ± 19.86 Pg/Ml, Whereas In The Control Group, The Corresponding Values Were 9.13 ± 0.6 Mg/Dl ($P = 0.05$), 34.94 ± 9.57 Ng/Ml ($P < 0.001$), And 32.08 ± 12.42 Pg/Ml ($P < 0.001$), Respectively.[11] However, Our Prevalence Of Hypocalcemia (88%) Was Substantially Higher Than The 49% Reported By Shah, Suggesting Potentially Poorer Iron Chelation Or Longer Disease Duration In Our Population.

The Pathophysiology Of Hypocalcemia In Thalassemia Major Is Multifactorial. The Primary Mechanism Is Hypoparathyroidism Secondary To Iron Deposition In Parathyroid Glands, Leading To Impaired Parathyroid Hormone (Pth) Secretion. Iron Accumulation Causes Oxidative Stress, Lipid Peroxidation, And Cellular Damage In Parathyroid Cells, Progressively Impairing Their Function [4]. Additionally, Vitamin D Deficiency, Highly Prevalent In Our Cohort (75%), Further

Compromises Calcium Homeostasis By Reducing Intestinal Calcium Absorption. The Combination Of Inadequate Pth Secretion And Insufficient Vitamin D Creates A Dual Defect In Calcium Regulation, Explaining The Severe Hypocalcemia Observed.

Mean Serum Phosphorus In Our Study Was 8.1±3.5 Mg/Dl, Significantly Elevated Compared To The Normal Range (2.3-4.7 Mg/Dl), With 80% Of Patients Demonstrating Hyperphosphatemia. This Finding Is Characteristic Of Hypoparathyroidism, Where Reduced Pth Secretion Leads To Decreased Renal Phosphate Excretion. Our Results Align With Those Of Shah Et Al., Who Reported Mean Phosphate Levels Of 5.33±0.77 Mg/Dl And Found Hyperphosphatemia In 53% Of Hypocalcemic Patients [1]. Chahkandi Et Al. Similarly Reported Elevated Phosphorus Levels In Thalassemia Patients With Hypoparathyroidism, Confirming This As A Consistent Finding Across Different Populations [7]. The Inverse Relationship Between Calcium And Phosphorus (Hypocalcemia With Hyperphosphatemia) Is Pathognomonic Of Hypoparathyroid States And Distinguishes It From Other Causes Of Hypocalcemia. There Is Negative Correlation (R=-0.8) Between Ferritin And Serum Calcium Levels, And There Is Weak Positive Correlation (R=+0.2) Between Serum Ferritin And Phosphorus In The Present Study.

Our Study Revealed Alarmingly High Prevalence Of Vitamin D Deficiency. Mean Vitamin D Levels Were 19.37±8.98 Ng/Ml, Significantly Below The Cut-Off Of Vitamin D Deficiency Which Is 30 Ng/Ml. The Mean Vitamin D Level Was Lower Than The Cut-Off Taken, With 75% Of Patients Having Vitamin D Deficiency And 18.3% Of Patients Having Vitamin D Insufficiency In Multi-Transfused Thalassemia Major Patients. These Findings Are Consistent With Global Reports. Soliman Et Al., In Their Comprehensive Review, Documented That Vitamin D Deficiency And Insufficiency Are Highly Prevalent In Thalassemic Patients Worldwide Despite Adequate Sunshine Exposure And Routine Vitamin D Supplementation [9]. Fahim Et Al. Reported Vitamin D Levels Of 10.4±4.6 Ng/Ml In Egyptian Thalassemic Children [6]. Saffari Et Al. Found Vitamin D Deficiency In 45.5% And Insufficiency In 24.7% Of Iranian Thalassemia Patients, And Reported That Nearly 80% Of Patients Had Low Bone Mineral Density [8]. Sultan Et Al.[12] From Pakistan Observed Markedly Reduced Vitamin D Levels Compared To Standard Reference Ranges Among Children With B-Thalassemia. Similarly, Studies By Handattu Et Al., Abdelmotaleb Et Al., And Bulgurcu Et Al. Documented Vitamin D Deficiency In 60%, 49%, And 79% Of B-Thalassemia Cases, Respectively, Highlighting The High Prevalence Of Hypovitaminosis D In This Population.[13-15]

Multiple Factors Contribute To Vitamin D Deficiency In Thalassemia Major: Reduced Cutaneous Synthesis Due To Skin Hyperpigmentation From Iron Deposition; Hepatic Dysfunction Impairing 25-Hydroxylation Of Vitamin D; Reduced Outdoor Activities Due To Chronic Illness; Inadequate Dietary Intake; And Possible Direct Effect Of Iron Interfering With Vitamin D Metabolism. A Significant Negative Correlation (R=-0.8) Of Serum Vitamin D And Serum Ferritin Concentration Was Found In The Current Study, Indicating That As Iron Overload Increases, Vitamin D Levels Progressively Decline.

The Mean Serum Ferritin In Our Study Was 2270.6±951.9 Ng/Ml, Indicating Significant Iron Overload Despite Chelation Therapy. This Finding Highlights The Challenges In Achieving Adequate Iron Chelation In Resource-Limited Settings Where Compliance Issues, Drug Availability, And Cost Constraints Limit Optimal Chelation. We Observed Strong Negative Correlations Between Serum Ferritin And Both Vitamin D (R=-0.8, R^2=0.651) And Calcium (R=-0.8, R^2=0.6225), Both Highly Statistically Significant (P<0.01). These Strong Correlations Suggest That Iron Overload Is A Major Determinant Of These Deficiencies, Likely Through Iron Deposition Causing Organ Damage (Parathyroid Glands, Liver) And Functional Impairment. This Underscores The Critical Importance Of Optimal Iron Chelation Not Only For Preventing Cardiac And Hepatic Complications But Also For Preserving Endocrine Function.

The High Prevalence Of Hypocalcemia (88%), Hyperphosphatemia (80%), And Vitamin D Deficiency (93.3%) In Our Cohort Has Several Important Clinical Implications. Hypocalcemia Can Manifest As Muscle Cramps, Paresthesias, Tetany, And Seizures. The Combination Of Hypoparathyroidism, Vitamin D Deficiency, And Underlying Thalassemia-Related Bone Disease

Creates Increased Risk For Osteopenia, Osteoporosis, And Fractures. Calcium And Vitamin D Are Critical For Linear Growth And Skeletal Development During Childhood And Adolescence, And Deficiencies May Contribute To The Short Stature Commonly Seen In Thalassemia Patients.

Conclusion: It Is Evident From The Present Study That The Levels Of Calcium And Vitamin D Are Deficient Among B-Thalassemia Major Patients On Repeated Blood Transfusion, Which Is Due To Iron Overload Due To Inadequate Chelation Therapy. Multi-Transfused Beta Thalassemia Major Patients Should Be Screened For Various Endocrinopathies. Frequent Monitoring Of Serum Levels Of Calcium, Phosphorus, And Vitamin D Has To Be Done For All B-Thalassemia Major Patients Receiving Repeated Multiple Transfusions Along With Regular Supplementation Of Calcium And Vitamin D In Patients Who Are Found To Be Deficient. Monitoring Of Pth For Screening Of Hypoparathyroidism Has Also To Be Undertaken For Patients With Thalassemia Receiving Repeated Multiple Transfusions.

Limitations: Owing To Lack Of Resources, The Current Study Was Limited To A Single (Though Largest And Busiest) Transfusion Center From The Region. The Cross-Sectional Design Does Not Allow Assessment Of Temporal Relationships. Pth Levels Were Not Measured Due To Resource Constraints. Sample Size Was Modest, And Multi-Center Studies With Larger Samples Would Provide More Representative Data. High Prevalence Of Vitamin D Deficiency And Low Serum Total Calcium Levels In Our Study Emphasizes The Importance Of More Detailed Studies In Thalassemia Patients Nationwide. It Is Recommended To Monitor Serum Vitamin D And Calcium Levels Routinely And Commence Appropriate Therapy Where Necessary.

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