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ORIGINAL ARTICLE

FREQUENCY, DISTRIBUTION AND PRESENTATION OF HYPOCALCEMIA IN BETA THALASSEMIA MAJOR

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ABSTRACT

Background: Disturbance of calcium hemostasis is common in patients of ß-thalassemia major. The objectives of this study were to determine the frequency, distribution and presentation of hypocalcemia in ß-thalassemia major children.
Materials and Methods: This cross-sectional study was conducted in Fatimid Foundation Peshawar, Pakistan from to June 2015 to August 2015. 100 patients of ß-thalassemia major, aged 5-20 years were enrolled. After history and examination, serum calcium levels were determined through semi-automated analyzer Microlab 200. Those having levels less than 8.6 mg/dl were labelled as hypocalcemics. Sex, hypocalcemia in ß-thalassemia major, symptomatic hypocalcemia, presence of paresthesias & numbness, myalgias, hyperpigmentation and carpopedal spasm were variables. Frequency and percentage were calculated for all variables. Observed to expected frequencies of all variables were analyzed by chi-square goodness-of-fit test.
Results: The frequency of hypocalcemia in ß-thalassemia major was 49/100 (49%), including 25 boys and 24 girls. The frequency of symptomatic hypocalcemia in ß-thalassemia major was 15/49 (30.61%). Paresthesias & numbness was present in 7/49 (14.29%) cases, hyperpigmentation in 6/49 (12.24%), myalgias in 3/49 (6.12%) and carpopedal spasm in 2/49 (4.08%) cases. The frequency of hypocalcemia in ß-thalassemia major and frequency of symptomatic hypocalcemia were higher in our sample than expected for the population. The frequency of paresthesia & numbness and of carpopedal spasm were similar while that of hyperpigmentation and myalgias were lower than expected.

Conclusion: Hypocalcemia in ß-thalassemia major is very prevalent and mostly asymptomatic and chronic, therefore calcium levels of such patients should be periodically assessed and calcium supplementation advised where necessary.
KEY WORDS: ß-thalassemia major; Hypocalcemia; Paresthesias; Hyperpigmentation; Myalgias; Carpopedal spasm.

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INTRODUCTION

Background: Calcium is one of the abundant trace metals of the body and most prevalent cation in human body. It plays important roles in the body like skeletal mineralization, blood coagulation, neuromuscular conduction, maintenance of normal vascular tone, excitability of skeletal and cardiac muscles, stimulating secretions by exocrine glands and preserving cell membrane integrity and permeability particularly in terms of sodium and potassium exchange.[1]

Hypocalcemia is a common biochemical abnormality. It arises mostly as a consequence of hypoparathyroidism or vitamin D deficiency or as a result of resistance to these hormones. However it may be a result of renal disease, end stage liver disease or drugs.[2-4]

Hypocalcemia varies in presentation from being asymptomatic to life threatening medical emergency. Common symptoms of hypocalcemia are paresthesias & numbness, muscle spasms and myalgias, cramps, tetany, convulsions and dermatitis. Hypocalcaemia can also present as neuromuscular irritability, cognitive impairment

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and laryngospasm. Complications of chronic hypocalcemia are mainly that of bone disease. Severe hypocalcemia can however result in cardiovascular collapse, hypotension not responding to fluids and dysrhythmias. Neurological complications of hypocalcemia are acute convulsions, tetany, basal ganglia calcification, parkinsonism, choreoathetosis and hemiballismus.[5,6]

Hypoparathyroidism leading to hypocalcemia resulting from iron overload in thalassemics was first elaborated by Gabriele in 1971. Later many more researchers detected it in their surveys too. The reason for this complication is attributed to iron deposition in the parathyroid glands consequent upon delay in chelation therapy.[7]

Parathyroid gland damage is mediated through many mechanisms like sensitivity to iron damage, increased collagen deposition secondary to increased activity of iron-dependent protocollagen prolyl hydroxylase enzyme with subsequent disturbed microcirculation in patient. Low parathyroid hormone levels lead to excessive calcium loss in urine, decrease bone remodeling, and decreased intestinal absorption of calcium leading to hypocalcemia.[8]

Knowledge Gaps/ Research Problems: Fatimid Foundation is the largest organization in Khyber Pakhtunkhwa Province and also in Pakistan, proving blood, blood components and other related services to thalassemia patients. Many studies have been done so far on hypocalcemia in thalassemia major patients and frequency reported varied from center to center.[7-10] Most of the researchers found that hypocalcemia was mostly asymptomatic in these patients. No recent studies are there showing frequency, distribution by sex and presentation of hypocalcemia in ß-thalassemia major patients in our population. These knowledge gaps were our research problems.

Research Questions (RQs):

1. What is the overall and sex wise frequency of hypocalcemia in ß-thalassemia major in sample and population?
2. What is the frequency of symptomatic hypocalcemia in ß-thalassemia major in sample and population?
3. What is the frequency of paresthesias & numbness, hyperpigmentation, myalgias and carpopedal spasm in symptomatic hypocalcemia in ß-thalassemia major in sample and population?
4. Is there any difference between the sample and population in the distribution of hypocalcemia in ß-thalassemia major?
5. Is there any difference between the sample and population in the distribution of symptomatic hypocalcemia in ß-thalassemia major?
6. Is there any difference between the sample and population in the distribution of paresthesias & numbness, hyperpigmentation, myalgias and carpopedal spasm in symptomatic hypocalcemia in ß-thalassemia major population?

Objectives:

The objectives of our study were to determine the overall and sex wise frequency, distribution and presentation of hypocalcemia in ß-thalassemia major children in our population.

Null Hypotheses:

H_{01}: There is no statistically significant difference between the sample and population in the distribution of hypocalcemia in ß-thalassemia major. (RQ 4)

H_{02}: There is no statistically significant difference between the sample and population in the distribution of symptomatic hypocalcemia in ß-thalassemia major. (RQ 5)

H_{03}: There is no statistically significant difference between the sample and population in the distribution of paresthesias & numbness in symptomatic hypocalcemia in ß-thalassemia major. (RQ 6)

H_{04}: There is no statistically significant difference between the sample and population in the distribution of hyperpigmentation in symptomatic hypocalcemia in ß-thalassemia major. (RQ 6)

H_{05}: There is no statistically significant difference between the sample and population in the distribution of myalgias in symptomatic hypocalcemia in ß-thalassemia major. (RQ 6)

H_{06}: There is no statistically significant difference between the sample and population in the distribution of carpopedal spasm in symptomatic hypocalcemia in ß-thalassemia major. (RQ 6)

MATERIALS & METHODS

Design, Duration & Setting: This cross-sectional study was conducted in Fatimid Foundation Center, Hayatabad, Peshawar, Pakistan from to June 2015 to August 2015. Fatimid Foundation, a non-profit organization is the pioneer of voluntary blood transfusion services in Pakistan.

Population, Sample size, Technique & Selection: The population for the study comprised 1500 patients of ß-thalassemia major registered at Fatimid Foundation, Peshawar at this time. Sample size was calculated to be 100 by Raosoft® on line sample size calculator with 7.95% margin of error, 90% confidence interval and 50% response distribution. 100 patients of ß-thalassemia major, aged 5-20 years, whose diagnosis was confirmed by hemoglobin electrophoresis and were receiving regular transfusion and chelation therapy and coming for

their follow up were enrolled in this study through consecutive non-probability sampling technique. Patients who were very ill, receiving calcium and vitamin D supplementation, or drugs affecting calcium levels like bisphosphonates, proton pump inhibitors, diuretics, antiepileptics and cisplatin were excluded from the study.

Equipment & Procedure of Conduct: Detailed history and general physical and systemic examination of the patients were conducted. Serum calcium levels were analyzed on semi-automated analyzer Microlab 200 (Merck Laboratories, Kenilworth, New Jersey) using kits from Weiner Laboratories (Rosario, Argentina). Three ml of blood was taken from patients in plain tubes with gel through venipuncture. Blood was allowed to clot and then centrifuged for separating the serum. Sera obtained were then stored at -20 ^{0}C. Calcium levels were then determined in the obtained sera through photocolorimetric method. Plain test tubes were taken and 750μl buffer, 250μl cresolphtalein (CPX) solution, and 10μl of patients' serum were added in each. The contents were mixed well and readings were taken after 10 minutes at 570 nm. Patients having serum calcium levels less than 8.6 mg/dl were labelled as hypocalcemics.

Data Collection Plan: Sex (boys & girls) was a demographic variable while presence of hypocalcemia in ß-thalassemia major, presence of symptomatic hypocalcemia in ß-thalassemia major, presence of paresthesias & numbness, presence of hyperpigmentation, presence of myalgias and presence of carpopedal spasm were six research variables. All these six variables were measured on nominal and binary scale, each having two attributes of present and absent.

Data Analysis Plan: Descriptively all the seven variables were analyzed by frequency and percentage. Estimation of parameter for proportion for population was given as confidence interval at confidence level of 80%. The distribution of hypocalcemia in ß-thalassemia major, distribution of symptomatic hypocalcemia in ß-thalassemia major and distribution of paresthesias & numbness, hyperpigmentation, myalgias and carpopedal spasm were all analyzed separately by chi-square goodness-of-fit test.[11-12] Observed counts, expected counts, their difference, chi-square statistic, degree of freedom and level of significance were given at alpha 0.5. Data was analyzed by an online calculator.[13]

RESULTS

Descriptive Analysis & Estimation of Parameter: Out of 100 children with ß-thalassemia major, 53 (53%) were boys and 47 (47%) were girls. The frequency of hypocalcemia in ß-thalassemia major was 49 in 100 children (49%, 80% CI 42.59-55.41%), including 25 (25%, 80% CI 19.45-30.55%) boys and 24 (24%, 80% CI 18.53-29.47%) girls.

The frequency of symptomatic hypocalcemia in ß-thalassemia major was 15 in 49 (30.61%, 80% CI 22.17-39.05%) while 34 in 49 (69.39%, 80% CI 60.95-77.83%) were asymptomatic. Thirteen out of 15 presented with one feature each, one with two features of paresthesias & numbness and myalgias and one with three features of paresthesias & numbness, myalgias and hyperpigmentation.

The frequencies with 80% CI of four presentations of symptomatic hypocalcemia in ß-thalassemia major are given in Table 1. Most of the patients presented with paresthesias & numbness.

Testing of Hypotheses:

RQ 4, H_{01}: The observed distribution of hypocalcemia in ß-thalassemia major of the sample was compared to the expected distribution of the population by chi-square goodness-of-fit test at alpha 0.05. Table 2

H_{01} proved to be false, hence rejected, showing that the observed counts of hypocalcemia have not a good fit to the expected counts. In other words the difference between the observed and expected counts for hypocalcemia in ß-thalassemia major was statistically significant. More simply, the frequency of hypocalcemia in our sample was significantly higher than expected for the population. The expected counts were taken from a study by Adil A, et al from Karachi, Pakistan.[13]

RQ 5, H_{02}: The observed distribution of symptomatic hypocalcemia in ß-thalassemia major of the sample was compared to the expected distribution of the population by chi-square goodness-of-fit test at alpha 0.05. Table 3

H_{02} proved to be false, hence rejected, showing that the observed counts of symptomatic hypocalcemia has not a good fit to the expected counts. In simple words, the frequency of symptomatic hypocalcemia in ß-thalassemia major in our sample was significantly higher than expected. The expected counts were taken from a study by De Sanctis et al.[16]

RQ 6, H_{03}: The observed distribution of paresthesias & numbness in symptomatic hypocalcemia in ß-thalassemia major of the sample was compared to the expected distribution of the population by chi-square goodness-of-fit test at alpha 0.05. Table 4

H_{03} proved to be true, hence accepted, showing that the observed counts of paresthesias & numbness in symptomatic hypocalcemia has a good fit to the expected counts. In simple words the frequency of paresthesias & numbness in symptomatic hypocalcemia in ß-thalassemia major in our sample was same as expected. The expected counts are from a study by Aleem, et al from Saudi Arabia.[8]

RQ 6, H_{04}: The observed distribution of hyperpigmentation in symptomatic hypocalcemia in ß-thalassemia major of the sample was compared to the expected distribution of the population by chi-square good-

ness-of-fit test at alpha 0.05. Table 5

H_{04} proved to be false, hence rejected, showing that the observed counts of hyperpigmentation in symptomatic hypocalcemia has not a good fit to the expected counts. In simple words the frequency of hyperpigmentation in symptomatic hypocalcemia in ß-thalassemia major was significantly less in our sample than expected. The expected counts are from a study by Fahmey SS, et al.[16]

RQ 6, H_{05}: The observed distribution of myalgias in symptomatic hypocalcemia in ß-thalassemia major of the sample was compared to the expected distribution of the population by chi-square goodness-of-fit test at alpha 0.05. Table 6

H_{05} proved to be false, hence rejected, showing that the observed counts of myalgias in symptomatic hypocalcemia has not a good fit to the expected counts. In simple words the frequency of myalgias in symptomatic hypocalcemia in ß-thalassemia major was significantly less in our sample than expected. The expected counts are from a study by Shapira, et al.[19]

RQ 6, H_{06}: The observed distribution of carpopedal spasm in symptomatic hypocalcemia in ß-thalassemia major of the sample was compared to the expected distribution of the population by chi-square goodness-of-fit test at alpha 0.05.

H_{06} proved to be true, hence accepted, showing that the observed counts of carpopedal spasm in symptomatic hypocalcemia has a good fit to the expected counts. In simple words the frequency of carpopedal spasm in symptomatic hypocalcemia in ß-thalassemia major in our sample was same as

Table 1: Presentation of symptomatic hypocalcemia (n=49) in in ß-thalassemia major.

S.No.	Variables	Present	80% CI	Absent	Total
		Count (%)	Lower-Upper	Count (%)	Count (%)
1	Paresthesias & numbness	7 (14.29%)	7.88-20.7%	42 (85.71%)	49 (100%)
3	Hyperpigmentation	6 (12.24%)	6.24-18.24%	43 (87.76%)	49 (100%)
2	Myalgias	3 (06.12%)	1.73-10.51%	46 (93.88%)	49 (100%)
4	Carpopedal spasm	2 (04.08%)	0.46-07.70%	47 (95.92%)	49 (100%)

Table 2: Comparison of observed (sample) to expected (population) distribution of 18:33 of hypocalcemia in ß-thalassemia major (n=100).

Hypocalcemia	O	E	O-E	(O-E)2	$\frac{(O-E)2}{E}$	χ2	d.f.	P-value
Yes	49	35.3	13.70	187.69	5.32	8.218	1	.00415
No	51	64.7	-13.70	187.69	2.90			

O = Observed Counts, E = Expected Counts, χ^2 = Chi-square value, d.f. = degree of freedom

Table 3: Comparison of observed (sample) to expected (population) distribution of 3:21 of symptomatic hypocalcemia (n=49) in ß-thalassemia major.

Symptomatic Hypocalcemia	O	E	O-E	(O-E)2	$\frac{(O-E)2}{E}$	χ2	d.f.	P-value
Yes	15	06.22	8.88	78.77	12.86	14.70	1	0.00013
No	34	42.88	-8.88	78.77	1.84			

O = Observed Counts, E = Expected Counts, χ^2 = Chi-square value, d.f. = degree of freedom

Table 4: Comparison of observed (sample) to expected (population) distribution of 2:6 of paresthesias & numbness in symptomatic hypocalcemia (n=49) in ß-thalassemia major.

Paresthesias & numbness	O	E	O-E	(O-E)2	$\frac{(O-E)2}{E}$	χ2	d.f.	P-value
Yes	7	12.25	-5.25	27.56	2.25	3.00	1	0.0832
No	42	36.75	5.25	27.56	0.75			

O = Observed Counts, E = Expected Counts, χ2 = Chi-square value, d.f. = degree of freedom

Table 5: Comparison of observed (sample) to expected (population) distribution of 17:37 of hyperpigmentation in symptomatic hypocalcemia (n=49) in ß-thalassemia major.

Hyperpigmentation	O	E	O-E	(O-E)2	$\frac{(O-E)2}{E}$	χ2	d.f.	P-value
Yes	6	15.43	-9.43	89.02	5.77	8.42	1	0.00371
No	43	33.57	9.43	89.02	2.65			

O = Observed Counts, E = Expected Counts, χ2 = Chi-square value, d.f. = degree of freedom

Table 6: Comparison of observed (sample) to expected (population) distribution of 2:4 of myalgias in hypocalcemia (n=49) in ß-thalassemia major.

Myalgias	O	E	O-E	(O-E)2	$\frac{(O-E)2}{E}$	χ2	d.f.	P-value
Yes	3	16.17	-13.17	173.45	10.73	16.01	1	0.00006
No	46	32.83	13.17	173.45	05.28			

O = Observed Counts, E = Expected Counts, χ2 = Chi-square value, d.f. = degree of freedom

Table 7: Comparison of observed (sample) to expected (population) distribution of 2:58 of carpopedal spasm in hypocalcemia (n=49) in ß-thalassemia major.

Carpopedal spasm	O	E	O-E	(O-E)2	$\frac{(O-E)2}{E}$	χ2	d.f.	P-value
Yes	2	01.62	0.38	0.15	0.090	0.093	1	0.759
No	47	47.38	-0.38	0.15	0.003			

O = Observed Counts, E = Expected Counts, χ2 = Chi-square value, d.f. = degree of freedom

expected. The expected counts are from a study by Hagag et al in 2015.[20]

DISCUSSION

Beta thalassemias are hereditary blood disorders caused by a defect in synthesis of beta globin gene. They are treated by blood transfusions along with chelation therapy. Disturbance of calcium hemostasis in ß-thalassemia major can be due to hypoparathyroidism, vitamin D deficiency, bone marrow expansion, chronic liver impairment or chelation therapy or any combination of these factors.[14,15]

RQ 4, H_{01}: In our study 49 out of 100 (49%) patients came out to be hypocalcemics. Adil A, et al from Karachi, Pakistan reported hypocalcemia in thalassemia major patients of 18 out of 51 (35.3%) cases.[16] Shemran A, et al from Babylon, Iraq reported hypocalcemia in thalassemia patients of 28 out of 129 (21.7%).[17] Sleem GA, et al from Royal Hospital Oman, Jordan reported 1 out of 28 adult cases of hypocalcemia in beta-thalassemia major (3.57%).[7] Hypocalcemia due to hypoparathyroidism was positive in 8 out of 40 (20%) cases of thalassemia major patients in Saudi Arabia.[8] In our study the frequency of hypocalcemia came to be high than all the studies given above (Table 2). The reason for this might be the delay in starting chelation and poor compliance with the therapy.

RQ 5, H_{02}: In our study, the frequency of symptomatic hypocalcemia was 15/49 (30.61%). The rest of the patients 34/49 (69.39%) were asymptomatic. A study by De Sanctis et al[18] showed that 3 out 24 (12.5%) of hypocalcemics had symptoms. The frequency of symptomatic hypocalcemia in ß-thalassemia major in our sample was higher than this study. (Table 3)

RQ 6, H_{03}: Paresthesias & numbness was the most common presentation of hypocalcemia in ß-thalassemia major in our study where 7/49 (14.29%) of patients presented with these complaints. A previous study done by Aleem A, et al[8] showed that majority of hypocalcemics in thalassemia were asymptomatic and only 2/8 (25%) showed paresthesias of hands. This figure is similar to ours' finding as in Table 4.

RQ 6, H_{04}: Hyperpigmentation were noted in 6/49 (12.24%) patients of hypocalcemia in our study. A recent study by Fahmey SS, et al[19] at the Hematology Clinic of Beni-Suef University Hospital, Egypt showed hyperpigmentation in 17 out of 54 (31.48%) children having ß-thalassemia major. Hence the frequency of hyperpigmentation in symptomatic hypocalcemia in ß-thalassemia major was significantly less in our sample than the study by Fahmey SS, et al. (Table 5)

RQ 6, H_{05}: The frequency of myalgias in hypocalcemics have been reported to be 16 to 30% in different

studies[20,21], whereas in our study only 3/49 (6.12%) of patients suffering from hypocalcemia complained of myalgias. A study by Shapira et al[22] from Israel showed that myalgias were present in 2 out of 6 (33.33%) patients of hypocalcemia in thalassemia major. Hence the frequency of myalgias in symptomatic hypocalcemia in ß-thalassemia major was less in our sample than the study from Israel. (Table 6)

RQ 6, H_{06}: Carpopedal spasm was a presentation in 2/49 (4.08%) of hypocalcemia patients in ß-thalassemia major in our study. A study done by Hagag et al[23] in 2015 showed that 2/60 (3.33%) of hypocalcemia in ß-thalassemia major cases had developed carpopedal spasm. The frequency of carpopedal spasm in symptomatic hypocalcemia in ß-thalassemia major in our sample was similar to the study by Hagag et al. (Table 7)

Limitations and Recommendations: Due to lack of resources the current study was limited to a single center and serum calcium measurements were done once. More detailed studies are recommended involving multiple centers and periodic assessments of calcium levels in these patients.

CONCLUSIONS

It can be concluded that hypocalcemia in ß-thalassemia major is very prevalent and mostly asymptomatic and chronic, therefore calcium levels of such patients should be periodically assessed and calcium supplementation advised where necessary.

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Important note by the author (Muhammad Marwat):

A novel presentation has been done in this research document by addressing the sample (descriptive statistics), the population (inferential statistics: estimation of parameters) and comparing the observed data of the sample to the expected/ published data of the population (inferential statistics: testing of hypotheses) as demanded by the title. There is clear and categorical placement of the research problems, knowledge gaps, research questions, research objectives and research hypotheses (tentative answers to the research questions). Data are collected for relevant variables, analyzed and interpreted to testify the null hypotheses, so that the research problems are solved, knowledge gaps are filled, research questions are answered in a logical way and thence objectives are met with.

I have proposed this unpublished eight steps flow for any type of research activity in any discipline, including but not limited to medical, biomedical, biological, behavioral and social sciences journals for similar research problems/ designs.

It is termed as "Marwat's Logical Trajectory for Research Process". Individual authors are allowed, rather facilitated to adopt it for their research projects but the organizations and institutions have to seek prior permission for its use/ incorporation to their research guidelines.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	SS, SM
Acquisition, Analysis or Interpretation of Data:	SS, MM, AB, MB
Manuscript Writing & Approval:	SS, MM, MS, AB, SM

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.