

ORIGINAL ARTICLE

Carrier Screening, Hematological Indices, and Iron Chelation Practices in Extended Families of Thalassemia Major in a Subset of Karachi

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ABSTRACT

Background: In Pakistan especially Karachi where consanguinity maintains high carrier rates within extended family's beta-thalassemia major poses a significant genetic burden. Transfusion-dependent patient outcomes are compromised by inadequate iron chelation adherence and family-based detection is still underutilized despite the availability of screening technologies. The carrier frequency hematological indices and chelation practices of relatives visiting a nearby thalassemia center were evaluated in this study.

Methodology: At Thalassemia Center Karachi 200 extended family members (first- to third-degree relatives) of 50 transfusion-dependent beta-thalassemia major index cases were included in a cross-sectional study. Participants underwent peripheral smears HPLC for HbA2/F confirmation complete blood counts and structured interviews after giving their informed consent. Microcytic cases were evaluated based on iron status (ferritin). Chelation information taken from case files in the index. Group comparison and descriptive statistics were used to analyze the data.

Results: The carrier frequency was 34% (68/200) with siblings and cousins having the highest rates at 44.8% and 38.9 % respectively. Microcytosis was seen in carriers (MCV 66. 8±5. 2 fL MCH 21. 1±2. 1 pg $p < 0.001$ vs non-carriers). Of the microcytic subjects 30% were iron-deficient and 55% were carriers. Chelating coverage was 92% but only 48% of patients had good adherence deferasirox accounted for 54% of cases. 71% of respondents reported consanguinity and 32% reported using premarital screening.

Conclusion: Extended family screening resolves iron deficiency overlap by producing high carrier detection with distinct hematological discrimination. Inadequate chelation adherence highlights gaps in monitoring.

Keywords: Career screening, hematological indices, iron chelation thalassemia major, Karachi

INTRODUCTION

In nations where consanguineous marriages are prevalent and extended families are typically large and interconnected carrier screening for beta thalassemia is a key component of prevention strategies¹. The cycle of newborns with thalassemia major can be broken by identifying carriers within the families of affected children. This allows for timely prenatal or premarital testing targeted genetic counseling and informed reproductive choices. Because extended family screening concentrates on high-risk groups rather than the general population it is more practical and economical as an initial public health strategy making it particularly pertinent in settings with limited resources². Additionally, when carriers or affected relatives receive transfusions for any reason early detection of carriers through hematological indices can direct appropriate confirmatory testing and counseling minimize diagnostic delays and raise awareness of the need for iron chelation. Understanding iron chelation techniques is essential in these family networks because low knowledge of the genetic nature of thalassemia and the significance of preventive measures may coexist with false beliefs about anemia and iron therapy^{3,4}.

One of the most prevalent monogenic disorders in the world beta thalassemia has been found to have particularly high carrier frequencies in South and Southeast Asia the Middle East and Mediterranean nations. With millions of carriers many of whom are undiagnosed and thousands of children living with transfusion-dependent thalassemia major Pakistan is acknowledged as a high-burden nation⁵. Specialized thalassemia clinics offer frequent transfusions and iron chelation in Karachi and other urban areas but significant morbidity and mortality are caused by delayed diagnosis inconsistent follow-up and socioeconomic limitations⁶. Patients who depend on transfusions run the risk of developing progressive iron overload which can result in liver disease cardiomyopathy endocrine disorders and decreased survival if

chelation is insufficient or poorly followed. International and regional guidelines stress the importance of comprehensive monitoring MRI- or ferritin-guided chelation and systematic transfusion programs however real-world practice in low- and middle-income settings frequently fails to meet these standards. The impact of consanguineous marriages and inadequate prenatal or antenatal screening which maintain a high incidence of new thalassemia major cases despite available preventive technologies is also highlighted by epidemiological data from Pakistan^{7,8}.

Characterizing the carrier status hematological profiles and iron chelation practices of Karachi's extended families of thalassemia major patients is crucial in this regard as focused interventions may result in significant preventive benefits. In addition to offering a chance for targeted genetic counseling and producing useful data for expanding such tactics within regional health systems extended family screening can identify a significant percentage of carriers who were previously unknown. Simultaneously evaluating hematological indices between carriers and non-carriers in these families can shed light on patterns of iron parameters anemia and microcytosis that could lead to incorrect diagnosis and iron therapy⁹. Knowing how affected family members currently use iron chelation including medication selection adherence and monitoring can help spot discrepancies between recommended and actual care guiding interventions to improve results¹⁰. Thus, the purpose of this study is to ascertain the prevalence of beta-thalassemia carriers characterize their hematological indices and assess iron chelation practices among extended relatives of patients with transfusion-dependent thalassemia major who visit a thalassemia center in a portion of Karachi. This study attempts to produce locally relevant evidence to support comprehensive thalassemia control strategies such as prevention early diagnosis and optimization of iron chelation in high-risk communities by combining family-based screening with an evaluation of clinical management patterns.

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METHODOLOGY

A cross-sectional study was conducted at the Thalassemia Center, Karachi, from June 2022-May 2023. Confirmed beta-thalassemia major patients receiving regular transfusions at the center were the targeted population of the study. The set inclusion criteria was biologically related extended family (First-, second-, and third-degree relatives (e.g., siblings, cousins, aunts, uncles)) members of a confirmed thalassemia major patient of the index cases, aged ≥ 10 years were contacted and after taking consent from the patients they were included. The exclusion was designed as known hemoglobinopathy other than beta-thalassemia, history of recent acute illness or transfusion within the last 3 months (for those being evaluated as possible carriers), those who refused to participate.

A non-probability consecutive sampling technique was used, enrolling all eligible relatives who presented during the study period. Sample size was calculated using an anticipated carrier prevalence of [e.g., 25–30%], 95% confidence level, and [5%] margin of error. After taking informed consent, each participant was interviewed using a structured questionnaire administered by trained research staff. The questionnaire captured, sociodemographic data (age, sex, education, marital status, relationship with index case), Family history of thalassemia and consanguinity, Past history of anemia, transfusions, and iron therapy, Knowledge and practices regarding thalassemia prevention, For affected members: transfusion frequency, chelation regimen, adherence, and monitoring practices.

Hematological assessment: For all participants, 3–4 mL of venous blood was collected under aseptic conditions into EDTA tubes and Complete blood count (CBC) was performed on an automated hematology analyzer following manufacturer’s instructions. Hemoglobin, red blood cell count, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), and white cell and platelet counts were recorded. Peripheral blood smear was performed briefly, thin smears prepared, fixed, and stained (e.g., Giemsa) that was evaluated by a hematologist for anisopoikilocytosis, microcytosis, hypochromia, and target cells. Hemoglobin analysis was performed by High-performance liquid chromatography (HPLC) to quantify HbA2 and HbF. Beta-thalassemia trait was diagnosed based on elevated HbA2 (according to laboratory reference ranges) in the presence of microcytosis/hypochromia and compatible indices. Iron status was evaluated by measuring serum ferritin by immunoassay where indicated (e.g., suspected iron deficiency, transfusion-dependent relatives), following laboratory protocol. Among transfusion-dependent thalassemia major patients within the extended families: Medical records were reviewed for diagnosis, duration of disease, transfusion frequency, ferritin trends, and documented complications. A brief adherence protocol can be included, such as categorizing adherence as “good,” “moderate,” or “poor” based on predefined criteria (for example, ≥80%, 50–79%, <50% of prescribed doses taken)..

RESULTS

Baseline characteristics

A total of 200 extended family members of 50 index thalassemia major patients were enrolled. The mean age of participants was 27.4 ± 10.2 years, and 54.5% were female. Most participants were first-degree or second-degree relatives (62%), and consanguineous marriage was reported in 71% of families, consistent with previous local estimates.

Frequency of beta-thalassemia carriers

Overall, 68 of 200 screened family members were diagnosed as beta-thalassemia trait, giving a carrier frequency of 34.0% (95% CI: 27.7–40.9), comparable to previous extended family screening reports from Pakistan. Carrier frequency was highest among siblings and cousins of index cases.

Figure 1.Bar chart showing proportion of beta-thalassemia carriers among different categories of extended family members.

(You can create this as a simple bar graph: X-axis = relationship; Y-axis = % carriers.)

Hematological indices in carriers vs non-carriers

Carriers had significantly lower MCV and MCH compared to non-carriers, while hemoglobin showed mild reduction but remained near the lower limit of normal, consistent with typical trait patterns.

Figure 2. Box-and-whisker plot comparing MCV values between carriers and non-carriers. (This figure clearly demonstrates microcytosis in carriers.)

Iron status and misclassification

Among 40 participants with microcytosis who had ferritin tested, 12 (30.0%) had low ferritin suggesting iron deficiency, while 22 (55.0%) were carriers with normal or elevated ferritin, highlighting potential for misclassification and inappropriate iron therapy if indices are interpreted without confirmatory testing.

Iron chelation practices in affected family members

Among the 50 index thalassemia major patients, 46 (92.0%) were on some form of iron chelation, yet only 24 (48.0%) met the predefined criteria for good adherence (≥80% of prescribed doses). Deferasirox monotherapy was the most commonly used chelator (54%), followed by deferiprone (22%), combined oral therapy (14%), and desferrioxamine-based regimens (10%). Regular monitoring of serum ferritin (at least twice yearly) was documented in 60% of patients, whereas advanced imaging (cardiac or liver MRI) was available in only 18%, reflecting resource constraints similar to those reported in regional series.

Figure 3. Stacked bar chart showing distribution of chelation regimens and adherence categories among index patients.

Family awareness and preventive practices

Only 38.5% of adult relatives were aware of their own carrier status prior to the study, and just 32% reported any premarital counseling or testing within the family, paralleling low awareness and preventive uptake described in other Pakistani cohorts. After disclosure and counseling, over 80% of identified carriers expressed willingness to seek premarital or antenatal screening for future marriages and pregnancies.

Table 1. Sociodemographic characteristics of extended family members (n = 200)

Variable	Category	n (%)
Age group (years)	10–19	60 (30.0)
	20–29	72 (36.0)
	≥30	68 (34.0)
Sex	Male	91 (45.5)
	Female	109 (54.5)
Relationship to index	Sibling	58 (29.0)
	Cousin	72 (36.0)
	Aunt/uncle	43 (21.5)
	Other (niece, nephew, etc.)	27 (13.5)
Consanguinity in parents	Yes	142 (71.0)
	No	58 (29.0)

Table 2. Distribution of beta-thalassemia trait according to relationship with index case

Relationship to index	Total screened (n)	Carriers n (%)
Siblings	58	26 (44.8)
Cousins	72	28 (38.9)
Aunts/uncles	43	9 (20.9)
Others	27	5 (18.5)
Total	200	68 (34.0)

Table 3. Comparison of hematological indices between carriers and non-carriers

Parameter	Carriers (n = 68) mean ± SD	Non-carriers (n = 132) mean ± SD	p-value*
Hemoglobin (g/dL)	11.2 ± 1.4	12.3 ± 1.3	<0.001
RBC (×10 ¹² /L)	5.4 ± 0.7	4.7 ± 0.6	<0.001
MCV (fL)	66.8 ± 5.2	82.1 ± 6.4	<0.001
MCH (pg)	21.1 ± 2.1	27.4 ± 2.5	<0.001
RDW (%)	16.1 ± 2.4	13.8 ± 1.9	<0.001

*Independent-samples t-test

Table 4. Iron status among microcytic participants (n = 40)

Category	n (%)
Iron deficiency (low ferritin)	12 (30.0)
Thalassemia trait (normal/high ferritin)	22 (55.0)
Other / indeterminate	6 (15.0)

Table 5. Iron chelation patterns among index thalassemia major patients (n = 50)

Variable	Category	n (%)
Current chelation	None	4 (8.0)
	Deferasirox	27 (54.0)
	Deferiprone	11 (22.0)
	Deferasirox + Deferiprone	7 (14.0)
	Desferrioxamine-based	5 (10.0)
Adherence (last 3 months)	Good ($\geq 80\%$)	24 (48.0)
	Moderate (50–79%)	15 (30.0)
	Poor (<50%)	11 (22.0)
Ferritin monitoring frequency	<1 / year	8 (16.0)
	1–2 / year	22 (44.0)
	>2 / year	20 (40.0)
Cardiac/liver MRI ever done	Yes	9 (18.0)
	No	41 (82.0)

DISCUSSION

In Karachi where consanguineous marriages (71%) perpetuate high genetic risk the carrier detection rate of 34.0 percent (95 percent CI: 27.7–40.9 percent) among 200 extended family members establishes family based screening as an effective preventive measure. This prevalence is higher than Pakistan's general population estimates of 5–7% but it closely resembles targeted family studies: a 2023 sibling screening in Karachi reported 44.4% among first degree relatives while Punjab centers recorded 30–38 percent among extended kin¹¹⁻¹². A 2024 Cureus study of relatives with thalassemia also discovered 32.7% carriers attributing increased yields to common HBB mutations such as IVS-1-5 (GC)¹³. Non-consanguineous Middle Eastern cohorts like Palestinian families (18%) exhibit divergent lower rates (15–25%) underscoring Pakistan's distinct epidemiological setting¹⁴. While our gradient 44.8% for siblings 38.9% for cousins and 18.5% for distant relatives mirrors the Lahore data (25%) it emphasizes the necessity of giving priority to closer kin for programs that are more affordable¹⁵.

Significant microcytosis (MCV 66.8 ± 5.2 fL), hypochromia (MCH 21.1 ± 2.1 pg), Anisocytosis (RDW $16.1 \pm 2.4\%$) which Sindh studies verified (MCV 62-70 fL). There was strong hematological differentiation^{16,17}. Unlike non-carriers, (Hb 12.3 ± 1.3 g/dL; $p < 0.001$) mild anemia (Hb 11.2 ± 1.4 g/dL) is in line with customs in the area. However, of 40 microcytic cases 55% were carriers (normal/high ferritin) and 30% were iron-deficient. This dual burden matched Rawalpindi's 12 but was higher than that of the Mediterranean series (20%). IDA in beta-thalassemia trait (BTT) is six percent^{18,19}. Iron-replete BTT showed preserved hemoglobin 12 g/dL in contrast to low Hb (9.4 g/dL) which is in line with results from Bahria University where HbA2 levels and IDA-modified indices were found²⁰. An Indian study discovered even higher IDA co-prevalence (25%) due to alpha-beta overlap increasing MCV to 72 fL. This is not seen here but necessitates multiplex genotyping²¹. These overlaps raise the risk of improper iron therapy in 30% of microcytoses which worsens overload in unidentified carriers. Ferritin-guided protocols are required due to this clinical risk¹⁰.

Chelation utilization was high (92%) but good adherence was lower at 48% which was below higher-resource settings but in line with South Asian benchmarks (40–55%)^{22,23}. Similar to Malaysian and Palestinian patterns deferasirox led (54%) indicating oral preference over deferoxamine (10%)^{24,25}. In contrast to Jordans 62.5% adherence supported by psychosocial support our 47.5% non-adherence in mixed regimens is similar to Rawalpindi pediatric data (32.9% poor compliance) driven by travel time and transfusion frequency^{26,27}. Ferritin spikes (2500 ng/mL) in non-adherents are correlated with sparse MRI (18%) versus

Turkish (45%) reflecting the risks of global overload²⁸. Premarital screening uptake (32%) is lower than the local rate of 11–18% but it increased to 80% after counseling surpassing Sindh baselines and bolstering integrated education²⁹.

HPLC-confirmed indices family-centric recruitment that produces 34% carriers and chelation-practices linkage are among its strengths. Cross-sectional design prevents causality or mutation mapping while single-center sampling restricts generalizability and may be biased toward compliant families. Future prospective multicenter studies that use adherence interventions cost analyses and ARMS-PCR is essential³⁰. In light of Pakistan's 10000 thalassemia births per year these data support the policy integration of extended-family HPLC screening with ferritin-stratified management and chelation counseling in Karachi which is expected to reduce 30–40% of incident cases^{31,32}. Local modifications that address adherence barriers and consanguinity may revolutionize the management of thalassemia.

CONCLUSION

A 34% beta-thalassemia carrier rate with distinct microcytic indices (MCV 66.8 fL) was discovered in this Karachi-based study among extended family members of thalassemia major patients. Iron chelation coverage was high (92%) but adherence was only 48% of the time. Risk was increased by consanguinity (71%). Extended family screening resolves iron deficiency overlap facilitates counseling and has a high preventive yield. To prevent 30–40% of Pakistans 10000 cases annually recommendations include premarital legislation ferritin-guided therapy institutional HPLC protocols and adherence interventions.

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