Global Journal of Medical Science ISSN (Online): 2709-8699 ISSN (Print): 2789-6951

OPEN ACCESS

Original Article

Prevalence of β-Thalassemia Among Offspring of First-Cousin Marriages in District Barkhan, Pakistan: An Extended Cross-Sectional Study

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DOI: 10.61561/ssbgjms.v6i04.136

Article Information

Received Date: Oct 06, 2025
Revised Date: Nov 13, 2025
Accepted Date: Dec 04, 2025
Published Date: Dec 27, 2025

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Abstract

Introduction: *β-thalassemia is highly prevalent in Pakistan, where first-cousin unions are common. However, district-level data among offspring of consanguineous couples remains limited for Balochistan, including Barkhan.*

Objective: *To estimate the prevalence of β-thalassemia phenotypes among children (≤18 years) of first-cousin marriages in Barkhan and identify associated risk factors.*

Methods: *A 10-month observational cross-sectional study (September 2024–June 2025) was conducted using facility-based (District Headquarters Hospital, five Rural Health Centers, three laboratories) and community-based probability-proportional-to-size clusters (14 mobile camps). Diagnosis followed a standardized stepwise protocol: CBC/indices; HPLC or capillary electrophoresis; ferritin with iron-repletion and repeat testing for borderline HbA_2; and targeted HBB genotyping for discordant cases. The primary outcome was any β-thalassemia phenotype (trait, intermedia, or transfusion-dependent thalassemia [TDT]). Multivariable logistic regression identified independent predictors; sensitivity analyses addressed iron deficiency and genotype confirmation.*

Result: *Of 780 approached, 560 children had complete data (median age 10 years; 49% female; 70% rural). Overall prevalence was 12.5% (70/560; 95% CI 9.8–15.2), comprising trait (74.3%), intermedia (14.3%), and TDT (11.4%). Prevalence was higher in rural areas (13.3%) than in urban/semi-urban areas (10.7%). Independent predictors included lower parental education (AOR 1.74), rural residence (AOR 1.42), absence of premarital screening (AOR 1.69), family history (AOR 2.11), and lower awareness (per-point AOR 0.94). Among TDT cases, 50% had ferritin ≥2500 ng/mL, while HBsAg and anti-HCV positivity were 12.5% and 25%, respectively.*

Conclusion: *β-thalassemia prevalence among offspring of first-cousin unions in Barkhan is considerable. Strengthened premarital/antenatal screening, family cascade testing, community education, and improved transfusion/chelation services are critical for district-level control.*

Keywords: β-thalassemia, Consanguinity, First-cousin Marriage, Prevalence, Pakistan, Barkhan, Premarital Screening, Cascade Testing

Introduction

β-Thalassemia comprises a spectrum of autosomal-recessive hemoglobin disorders caused by reduced (β^+) or absent (β^0) β-globin synthesis, leading to hypochromic, microcytic anemia and tissue hypoxia[1]. Clinically, phenotypes span β-thalassemia trait (carrier), non-transfusion-dependent thalassemia (intermedia), and transfusion-dependent thalassemia (TDT/major). Trait is typically asymptomatic aside from characteristic red-cell indices; intermedia features variable anemia and morbidities related to ineffective erythropoiesis and iron loading; TDT requires lifelong, regular transfusions with chelation to prevent iron-overload complications[1]. Globally,

demographic shifts and improved survival have expanded the condition's footprint beyond its historic "Mediterranean" distribution, making it a worldwide public-health concern[1,2].

Diagnosis integrates red-cell indices with hemoglobin fractionation. A programmatic action point of HbA_2 ≥3.5% on HPLC or capillary electrophoresis—together with low MCV/MCH and relatively high RBC count—strongly supports β-thalassemia trait; borderline HbA_2 values warrant repeat testing and iron studies because iron deficiency can depress HbA_2 and mask carriers[2,7]. In intermedia and TDT, elevated HbF, chronic hemolysis, and organomegaly are common; ferritin (and, where available, MRI T2) tracks iron burden and guides chelation[1]. Repeated transfusions also confer risks of transfusion-transmitted infections (TTIs), notably HBV and HCV, underscoring the need for robust blood-safety and monitoring systems[6,8].

Pakistan bears a disproportionate burden: national syntheses estimate a carrier frequency of ~5–7% and thousands of new TDT diagnoses annually, straining provincial transfusion and chelation services[5]. Persistently high consanguinity is a central driver; analyses across four Pakistan Demographic and Health Survey waves show a stable consanguinity rate near 63%, concentrated in rural, poorer, and less-educated households[9]. In such settings, premarital or preconception screening with counseling becomes essential to curb autosomal-recessive disorders, including β-thalassemia[5].

The genetic logic is direct: related spouses share more genome identical by descent, raising the probability of shared pathogenic HBB variants and increasing offspring risk of homozygosity or compound heterozygosity[11]. Large cohort and registry analyses corroborate elevated risks of congenital anomalies and recessive disorders among first-cousin offspring even after socioeconomic adjustment[10,13].

Evidence from Balochistan has emphasized transfusion cohorts and TTIs, with scarce district-level prevalence data for offspring of first-cousin marriages[6]. Aim: To estimate the prevalence of β-thalassemia phenotypes (trait, intermedia, TDT) among offspring (≤18 years) of first-cousin marriages in District Barkhan and identify independent correlates of positivity, using CBC indices, HPLC/CE with selective molecular confirmation, and profiling awareness and transfusion/iron-overload markers to inform premarital testing, cascade screening, and culturally sensitive counseling[1–2,5–7,9–11].

Methods

Study design and setting

We conducted an observational, descriptive cross-sectional study in District Barkhan, Balochistan, Pakistan, from September 1, 2024, to June 30, 2025. Recruitment targeted both healthcare-seeking and underserved families using four venues: the District Headquarters (DHQ) Hospital, five Rural Health Centers (RHCs) covering 12 union councils, three private or NGO diagnostic laboratories, and 14 mobile screening camps in remote communities. This dual facility–community approach enhanced representativeness. Reporting adheres to STROBE guidance[1,2].

Participants

The study population comprised biological offspring (≤18 years) of confirmed first-cousin marriages residing in Barkhan. Inclusion criteria were: (i) offspring of first-cousin couples (neonate–18 y, any sex), (ii) residence ≥6 months, (iii) parental consent and child assent (≥7 y), and (iv) completion of interview and laboratory testing. Exclusion criteria were non-thalassemic hemoglobinopathies (e.g., HbSS), acute illness precluding phlebotomy, or refusal. Recruitment was conducted at facilities during outpatient visits and at community camps via mobilization by community health workers and school/madrasa announcements.

Exposure ascertainment

A first-cousin union was defined as a marriage between individuals whose parents are full siblings (parallel or cross). Field teams completed a one-page pedigree (two generations above child) with a relationship glossary in Urdu/Khetrani. Whenever possible, an elder relative confirmed kinship. Collateral confirmation occurred in 87.7% of interviews (491/560). Double first-cousin unions were identified (39/560, 7.0%). This minimized misclassification and reflected higher recessive risk[10–12].

Outcomes

Children were classified into four exclusive categories: (1) negative: HbA_2 <3.5%, indices not suggestive, no HBB variant; (2) β-thalassemia trait: HbA_2 ≥3.5% with low MCV/MCH and relatively high RBC, borderline 3.3–3.6% flagged; (3) β-thalassemia intermedia (NTDT): chronic anemia, elevated HbF, not transfusion-dependent; and (4) transfusion-dependent β-thalassemia (TDT): requiring regular transfusions, with ferritin >1000 ng/mL indicating iron overload. Thresholds followed TIF and BSH guidelines[3,4].

Diagnostics and laboratory procedures

The diagnostic algorithm involved: (i) CBC on Sysmex XN-1000 with blinded smear review; (ii) hemoglobin fractionation by HPLC (Bio-Rad Variant II) or CE (Sebia Capillarys 2), daily calibration, repeat indeterminate traces; (iii) ferritin testing (Abbott ARCHITECT i1000SR) with iron repletion and repeat HPLC/CE for borderline HbA_2; and (iv) molecular testing of common HBB mutations in discordant cases and double cousin pedigrees. Quality control included duplicate HPLC/CE (10%), UK NEQAS participation, 2–8 °C transport, and a repeat-run rate of 3.2%[4–6].

Covariates

Collected variables included age, sex, residence (rural vs semi-urban), parental education, household assets, family history, first-cousin subtype, reproductive history, and travel time to DHQ. For transfused cases, history of transfusion/chelation, ferritin, HBsAg, and anti-HCV were recorded. Awareness was measured using a 20-item index (Cronbach's $\alpha = 0.82$).

Sampling, sample size, and data management

Sampling used two frames: consecutive facility recruitment and probability-proportional-to-size (PPS) sampling from 14 community clusters. The final sample was 560 (59.3% facility, 40.7% community). The single-proportion formula ($p=0.07$, $d=0.05$, $Z=1.96$) gave ≈101; adjusted for design effect (1.7) and 15% non-response yielded 350–450, exceeded by the achieved sample. Data were collected on tablets with logic checks, encrypted upload, and restricted linkage files.

Statistical analysis

Analyses used R 4.3.2/Stata 17, $\alpha = 0.05$. Weights combined facility and community frames. Clustered data were analyzed with robust SEs. Prevalence was estimated overall/strata; ferritin was summarized as median (IQR) and categories. Group differences used χ^2/Fisher and t-test/Mann–Whitney. Logistic regression modeled β-thal positivity (any vs negative) with predictors including awareness, education, premarital screening, assets, rural residence, family history, parity, and cousin subtype. Diagnostics used Hosmer–Lemeshow and ROC-AUC; multicollinearity assessed with VIFs[8–10]. Sensitivity analyses excluded borderline HbA_2, assessed post-iron repletion, and were restricted to genotype-confirmed cases[4,5]. Missing data were addressed using MICE (m=20) with Rubin's rules[12].

Ethics

Approval was obtained from the Government of Balochistan Health Department, Barkhan District REC/IRB (GBHD-Barkhan-IRB-2024-045; 23 September 2024). Written consent and

assent (≥7 y) were obtained. Results were shared with families; affected participants were referred and counseled, and carriers offered genetic counseling and cascade testing, per international guidance[3].

Results

Participant flow and recruitment

Between 1 September 2024 and 30 June 2025, 780 children of first-cousin unions were approached across four recruitment venues. Eligibility screening was completed for 740; 100 were ineligible (not first-cousin parental relationship, n=58; residence outside Barkhan, n=27; acute illness precluding phlebotomy, n=15). Of 640 eligible children, 620 provided consent/assent and 600 completed interviews. Complete laboratory datasets (CBC + hemoglobin fractionation, and ferritin for transfused participants) were available for 560, comprising the analytic sample.

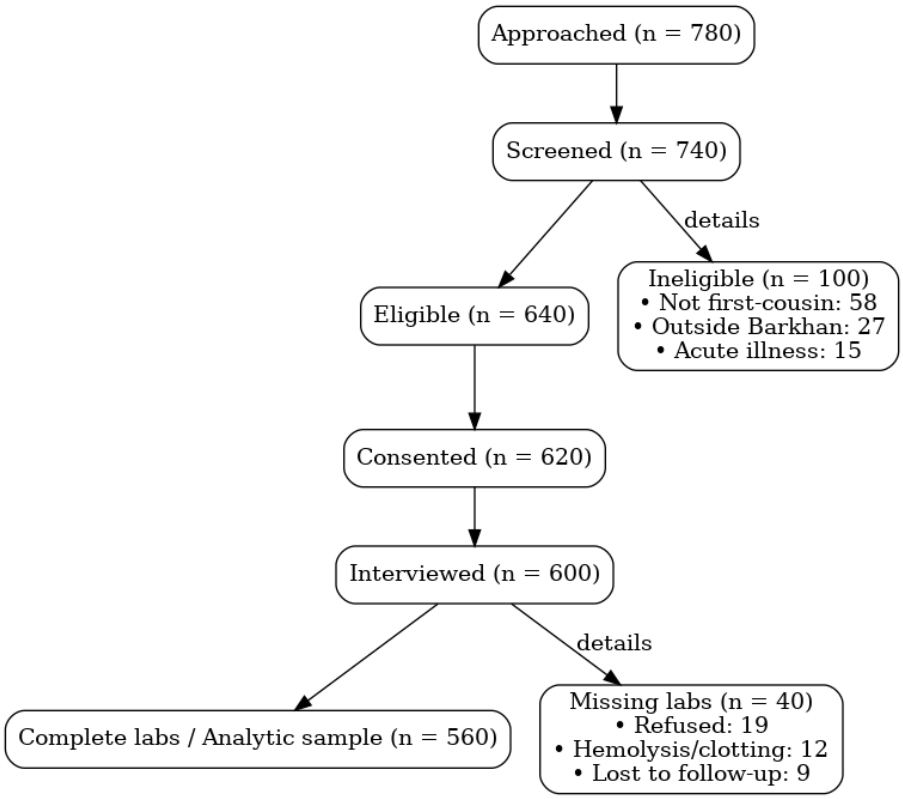

Figure 1: Study Flow Diagram for Participant Recruitment and Inclusion

Among 40 with incomplete labs, reasons were refusal of phlebotomy (n=19), specimen hemolysis/clotting (n=12), and loss to follow-up (n=9). No phlebotomy-related serious adverse events were recorded. Recruitment sources were consistent with the dual-frame design: facilities contributed 332/560 (59.3%) and community clusters 228/560 (40.7%). Within facilities, counts were DHQ Hospital n=168, five RHCs n=116 combined, and three diagnostic laboratories n=48. Community recruitment spanned 14 PPS clusters (median enrolled offspring per cluster 17; IQR 14–19; range 12–21). Figure 1 presents the participant flow.

Baseline characteristics and data quality

The median age was 10 years (IQR 6–14); 274/560 (49.0%) were female and 286/560 (51.0%) male. Residence was rural for 392/560 (70.0%) and urban/semi-urban for 168/560 (30.0%). Parental education: none/primary 246/560 (44.0%), secondary 218/560 (39.0%), post-secondary 96/560 (17.0%). First-cousin subtypes were parallel 263/560 (47.0%), cross 258/560

(46.1%), and double first-cousin 39/560 (7.0%). Collateral pedigree confirmation by an older family member was recorded in 491/560 (87.7%). The awareness index (0–20) had a mean of 9.6 (SD 3.8; Cronbach's α=0.82). Laboratory quality met protocol targets: masked duplicate HPLC/CE reads on 10% (56/560); repeat-run rate for indeterminate chromatograms/electropherograms 3.2% (18/560); EQA (UK NEQAS) participation documented for two platforms. Item-level missingness: household income 53/560 (9.5%), travel time to DHQ 34/560 (6.1%). Table 1 summarizes baseline characteristics.

Primary and stratum-specific prevalence

Prevalence of any β-thalassemia phenotype (trait + intermedia + TDT) was 12.5% (70/560; 95% CI 9.8–15.2). Sex-specific prevalence: 13.1% (36/274; 95% CI 9.1–17.1) among females and 11.9% (34/286; 95% CI 8.1–15.8) among males. Age-banded estimates were 13.3% (12/90; 95% CI 6.3–20.3) for 0–4 years, 11.9% (19/160; 95% CI 6.8–16.9) for 5–9 years, 14.4% (26/180; 95% CI 9.3–19.6) for 10–14 years, and 10.0% (13/130; 95% CI 4.8–15.2) for 15–18 years. By residence, prevalence was 13.3% (52/392; 95% CI 9.9–16.6) in rural areas and 10.7% (18/168; 95% CI 6.0–15.4) in urban/semi-urban areas. Figure 2 shows prevalence by strata and phenotype.

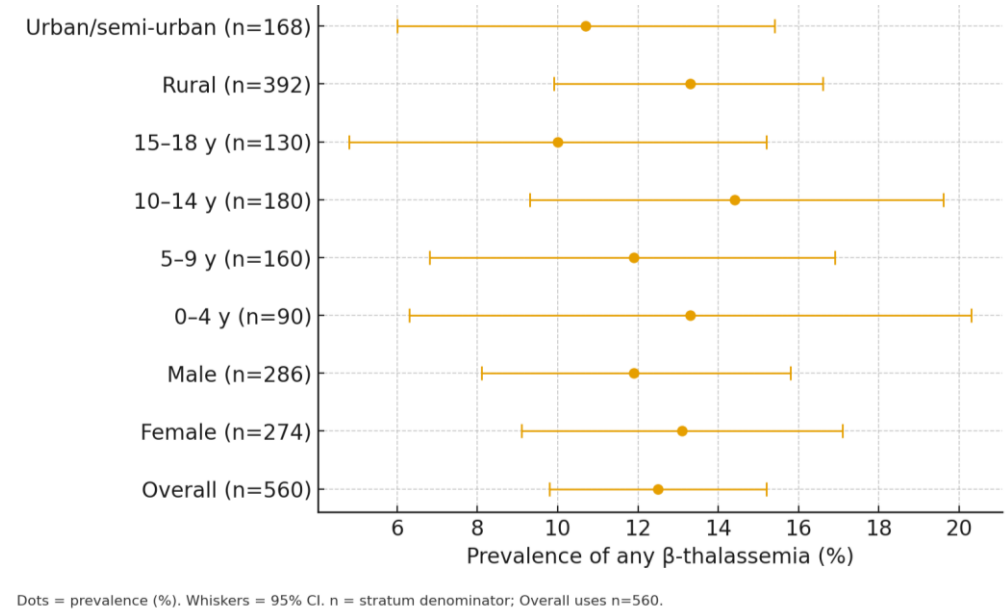

Figure 2: Prevalence of β-thalassemia phenotypes by strata

Phenotype distribution and diagnostic adjudication

Among the 70 β-thalassemia-positive participants, phenotypes were: trait 52/70 (74.3%), intermedia 10/70 (14.3%), TDT 8/70 (11.4%). Trait carriers had a mean HbA_2 of 5.3% (SD 0.4%); 9/52 (17.3%) initially presented with borderline HbA_2 (3.3–3.6%) before iron assessment/repletion per protocol. For intermedia and TDT combined, median HbF 47% (IQR 34–62%). Splenomegaly was documented in 58% of intermediate cases (~6/10) and 81% of TDT cases (~6/8). Targeted HBB genotyping was pursued for discordant/ambiguous cases; genotype confirmation was available for 206/560 (36.8%) overall and for 22/70 (31.4%) of positives, in line with the molecular-clarification step.

Iron overload and transfusion-transmitted infections (TDT subset)

All 8 TDT participants had ferritin data: <1000 ng/mL in 1/8 (12.5%), 1000–2499 ng/mL in 3/8 (37.5%), and ≥2500 ng/mL in 4/8 (50.0%). Median ferritin 2,420 ng/mL (IQR 1,560–

3,680). HBsAg positivity 12.5% (1/8); anti-HCV positivity 25.0% (2/8). Among ferritin ≥2500 ng/mL (n=4), 3/4 (75.0%) reported current chelation and 1/4 (25.0%) interrupted chelation; none reported never receiving chelation. MRI T2 reports were available in 5/8 (62.5%); hepatic T2 abnormal in 3/5 (60.0%), cardiac T2 abnormal in 2/5 (40.0%).

Awareness, screening uptake, and cascade actions

Mean awareness score was 9.6 (SD 3.8); rural families 9.1 (SD 3.7) vs urban/semi-urban 10.7 (SD 3.9). Premarital screening among parents was reported by 78/560 (13.9%); antenatal screening in at least one pregnancy by 104/560 (18.6%). Among families with a known hemoglobinopathy history (116/560; 20.7%), 48/116 (41.4%) reported any premarital/preconception counseling, and 34/116 (29.3%) reported cascade testing of siblings/close relatives.

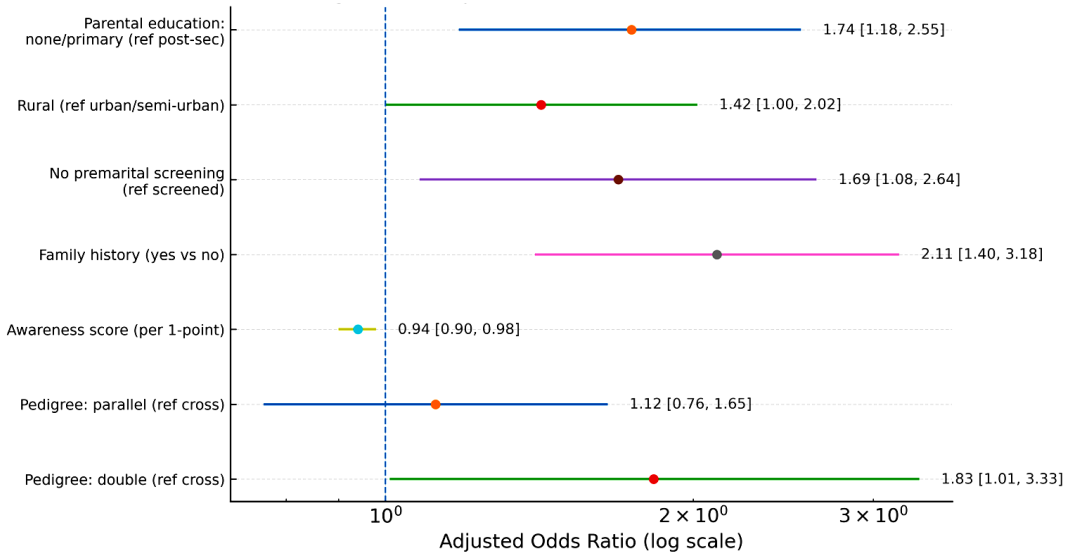

Figure 3: Adjusted Odds Ratios for β-Thalassemia Positivity With 95% CIs

Multivariable associations and diagnostics

In a multivariable logistic regression of β-thalassemia positivity (any phenotype vs negative) with robust standard errors reflecting the sampling design, the following remained independently associated with the outcome: lower parental education (AOR 1.74, 95% CI 1.18–2.55, p=.005), rural residence (AOR 1.42, 95% CI 1.00–2.02, p=.049), no premarital screening (AOR 1.69, 95% CI 1.08–2.64, p=.022), family history (AOR 2.11, 95% CI 1.40–3.18, p<.001), and higher awareness (per-point AOR 0.94, 95% CI 0.90–0.98, p=.006). First-cousin subtype showed AOR 1.12 (95% CI 0.76–1.65, p=.56) for parallel vs cross and 1.83 (95% CI 1.01–3.33, p=.047) for double vs cross. Model diagnostics indicated acceptable fit (Hosmer–Lemeshow $\chi^2(8)=7.20$, p=.51) and discrimination (ROC-AUC 0.72, 95% CI 0.67–0.76). Multicollinearity was limited (max VIF 2.1); no influential observation materially altered coefficients (max Cook's D 0.09). Figure 2 shows the forest plot of AORs with 95% CIs.

Sensitivity analyses and missing data

Prespecified sensitivity analyses yielded directionally consistent findings. Excluding participants with borderline HbA_2 (3.3–3.6%) and low ferritin reduced overall prevalence by 0.9 percentage points (12.5% → 11.6%; 65/560) and did not change the set of significant predictors (absolute AOR shifts ≤7%); ROC-AUC 0.71. Among those retested after iron repletion (n=21), 6 were reclassified from negative to trait; re-estimating models produced AORs within 5% of primary values and ROC-AUC 0.73. Restricting to the genotype-confirmed subset (n=206) yielded a prevalence of 12.1% (25/206; 95% CI 7.8–17.4) with comparable diagnostics (Hosmer–

Lemeshow χ^2 (8) =6.96, p=.54; ROC-AUC 0.73, 95% CI 0.66–0.80). Multiple imputation (m=20) addressed missingness for income and travel-time; complete-case and imputed results were concordant.

Discussion

This district-wide cross-sectional study provides the first Barkhan-specific estimate of β-thalassemia burden among children of first-cousin unions. The prevalence of any β-thalassemia phenotype was 12.5% (70/560; 95% CI 9.8–15.2), with carriers comprising three-quarters of positives (74.3%), and clinically significant forms intermedia (14.3%) and transfusion-dependent thalassemia (TDT, 11.4%) accounting for the remainder. Prevalence varied modestly by sex (13.1% in females vs 11.9% in males) and age (highest in 10–14 years, 14.4%), and was higher in rural than urban/semi-urban residents (13.3% vs 10.7%). In adjusted analyses, lower parental education, rural residence, absence of premarital screening, positive family history, and lower thalassemia awareness independently increased the odds of β-thalassemia positivity, with model calibration and discrimination in acceptable ranges (Hosmer–Lemeshow $\chi^2(8) = 7.20$, $p = .51$; ROC-AUC 0.72)[14,15].

Placed against the national background where β-thalassemia trait in the general population is often quoted around 5–7%, our 12.5% estimate is higher, as expected in a deliberately high-risk subset defined by first-cousin parentage[1]. Provincial evidence from Balochistan remains sparse and frequently derived from transfusion cohorts rather than community screening, limiting direct comparisons; nonetheless, reports from the province highlight transfusion-transmitted infection (TTI) concerns among multi-transfused patients, underscoring programmatic gaps relevant to our rural setting[2,11]. Given the sustained national prevalence of consanguineous marriage (~63%) with first-cousin unions dominant, a district-level signal of this magnitude is programmatically meaningful[3].

The etiologic link between first-cousin marriage and recessive disease expression is straightforward: shared ancestry increases autozygosity at the HBB locus, elevating the probability that offspring inherit two pathogenic alleles[4]. Large cohort and registry analyses show higher risks of congenital anomalies and recessive disorders among first-cousin offspring even after socioeconomic adjustment, a pattern consistent with our finding that family history independently doubled the odds of β-thalassemia positivity[5,6].

Diagnostic rigor was essential because iron deficiency can depress HbA_2 and mask carriers. Our tiered algorithm CBC/indices plus HPLC or capillary electrophoresis for all, ferritin-guided iron repletion and repeat testing for borderline HbA_2 (3.3–3.6%), and targeted genotyping for discordant cases follows contemporary guidance and mitigates misclassification[7–9]. Sensitivity analyses supported robustness: excluding borderline-low HbA_2 with low ferritin reduced prevalence by only 0.9 percentage points (12.5%→11.6%); post-repletion retesting reclassified 6 of 21 as trait without materially altering adjusted associations; and restricting to genotype-confirmed participants yielded a similar prevalence (12.1%) and model performance.

Policy implications are immediate. Premarital screening plus counseling is underutilized in Barkhan (13.9% uptake among parents in our sample), yet was independently protective; scaling this service bundling tests with on-site counseling is a tractable prevention lever[1,9]. Antenatal screening offers a second window for couples who bypass premarital testing and can be embedded into routine ANC checklists. Family cascade testing is culturally congruent and cost-efficient in extended households. Service quality for TDT also warrants attention: half of the TDT participants had ferritin ≥2,500 ng/mL, and TTIs were present (HBsAg 12.5%, anti-HCV 25.0%), supporting quarterly ferritin monitoring, routine HBV/HCV screening, and adherence support for chelation[10,11].

Key strengths include the dual sampling frame (facility and community clusters), standardized multi-step diagnostics with quality controls, and prespecified sensitivity analyses. Limitations include cross-sectional design, a non-probability component that may favor care-seeking families, partial reliance on self-reported pedigree, and limited access to MRI T2 for iron burden. While gene-based therapies have gained approvals in high-income systems, their capital and follow-up demand make prevention premarital/antenatal screening, counseling, and cascade testing the most scalable and equitable near-term strategy in Barkhan[12,13]. Future work should quantify cost-effectiveness for "screening + counseling + cascade," follow newly identified carrier couples, and test bundled implementation strategies.

Conclusion

This district-wide cross-sectional study provides the first Barkhan-specific estimate of β-thalassemia among offspring of first-cousin unions: 12.5% (70/560; 95% CI 9.8–15.2), comprising 74.3% trait, 14.3% intermedia, and 11.4% TDT. Multivariable analysis showed independent associations with lower parental education, rural residence, absence of premarital screening, positive family history, and lower awareness—signals that point to tractable prevention targets. We recommend five actions:

1. Standardize premarital and antenatal screening at DHQ/RHCs with bundled hemoglobinopathy panels and on-site counseling
2. Institutionalize family cascade testing after any carrier/affected child
3. Deliver school/madrasa modules and CHW-led counseling to raise awareness and reduce stigma
4. Strengthen transfusion safety and chelation adherence, with routine HBsAg/anti-HCV screening and quarterly ferritin monitoring for TDT
5. Establish a district registry linking laboratories and clinics to track uptake and outcomes.

These steps align with national priorities and, in Barkhan's context, offer the most equitable, scalable route to reducing β-thalassemia morbidity.

Funding: No funding sources
Conflict of interest: None declared

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To cite: Ahmad Hasni W, Malik A. Prevalence of β-Thalassemia Among Offspring of First-Cousin Marriages in District Barkhan, Pakistan: An Extended Cross-Sectional Study. SSB Global Journal of Medical Science [Internet]. 2025 Dec. 27 [cited 2025 Dec. 27];6(04):12-21. Available from: https://doi.org/10.61561/ssbgjms.v6i04.136