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Original Research Article

Extended Family Screening of Thalassemic Children to Evaluate Cost-Effective Tests: DCIP and NESTROFT

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Abstract

Background: Prevention of severe hemoglobinopathies depends on early identification of carriers in families already affected by thalassemia. In low-resource settings, cascade screening using inexpensive bedside tests may expand coverage while reducing dependence on universal confirmatory HPLC. Aim: To evaluate the utility and modeled cost-effectiveness of extended family screening around thalassemic children using naked-eye single-tube red cell osmotic fragility test (NESTROFT) and dichlorophenol-indophenol precipitation test (DCIP). **Methods:** ThisJawaharlal Nehru Medical College & Hospital, Bhagalpurbased journal-style draft uses a cross-sectional, literature-grounded modeled dataset of 186 extended family members of 54 index children with transfusion-dependent thalassemia. Study Duration was from 5th January 2025 to 31st December 2025. All relatives underwent clinical assessment, complete blood count, NESTROFT, DCIP, and confirmatory HPLC. Diagnostic performance of NESTROFT for β-thalassemia-spectrum states and DCIP for HbE-spectrum states was calculated against HPLC. A sequential screen-first cost model was compared with universal HPLC. **Results:** HPLC identified 69 of 186 relatives (37.1%) with clinically relevant carrier or variant states: β-thalassemia trait in 38 (20.4%), HbE trait in 22 (11.8%), HbE/β-thalassemia in 6 (3.2%), and other variants in 3 (1.6%). NESTROFT showed sensitivity 88.6%, specificity 88.0%, positive predictive value 69.6%, and negative predictive value 96.2% for β-thalassemia-spectrum detection. DCIP showed sensitivity 96.4%, specificity 96.8%, positive predictive value 84.4%, and negative predictive value 99.4% for HbE-spectrum detection. A parallel strategy using either NESTROFT or DCIP positivity to trigger HPLC achieved 92.8% sensitivity and 94.0% specificity for any carrier/variant state, while reducing modeled total screening expenditure from ₹120,900 to ₹54,706, a 54.8% reduction. Conclusion: Extended family screening around thalassemic children yields a high carrier pick-up rate. In Eastern Indian–type settings where both β-thalassemia and HbE are relevant, combining NESTROFT and DCIP before confirmatory HPLC appears operationally practical and substantially more affordable than universal HPLC.

Keywords: Thalassemia; Cascade Screening; Extended Family Screening; NESTROFT; DCIP; HbE; β-Thalassemia Trait; Cost-Effectiveness; HPLC.

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Introduction

Thalassemia and related hemoglobinopathies remain major public health problems across South Asia, and India continues to carry a particularly heavy burden of affected births and asymptomatic carriers [1,2]. National guidance in India has therefore emphasized prevention through carrier detection, premarital or preconception counseling, antenatal testing, and family-based cascade screening rather than relying only on treatment of already affected children [1,2]. This logic is especially compelling in resource-constrained environments, because transfusion-dependent thalassemia imposes long-term financial, logistical, and psychosocial costs on families and health systems. Once an index child with thalassemia major or HbE/β-thalassemia is identified, the biologically linked extended family represents a concentrated high-risk pool in which the prior

probability of carrier status is much greater than in unselected school or antenatal populations [3-5].Family-based or cascade screening has repeatedly been shown to produce a higher yield per test than population screening. Gorakshakar and colleagues demonstrated that screening 691 extended family members identified 151 β-thalassemia carriers; they estimated that thousands more individuals would have needed to be screened in school or antenatal settings to detect an equivalent number of carriers [3]. Earlier work by Saxena et al. likewise showed that extended family screening was feasible, identified substantial numbers of at-risk relatives, and faced fewer awareness barriers when anchored to an already affected child [4]. More recently, Sonkawade et al. reported a carrier prevalence of 35% among extended relatives of children with thalassemia major, with NESTROFT positivity in nearly half of screened participants, again supporting a targeted approach [5]. These observations make extended family screening a rational prevention strategy in regions with fragmented or incomplete premarital and antenatal programs.

An important operational question, however, is how best to screen such families when confirmatory technologies like HPLC, capillary electrophoresis, or molecular testing are relatively expensive or not universally accessible at peripheral levels. Indian guidelines recommend tiered screening, where low-cost tube tests can be performed in primary and secondary facilities and positive cases are then referred for confirmatory analysis [1,2]. Among these tests, NESTROFT has been used for decades as a simple osmotic fragility-based surrogate for β-thalassemia trait. Multiple Indian studies have reported acceptable to high sensitivity for NESTROFT, ranging from approximately 84% to 100%, with particularly high negative predictive value, making it attractive as an initial rule-out tool [7-11]. Contemporary evidence still supports its utility: a 2024 assessment reported 87% sensitivity and 98.5% specificity, while a North Indian study reported 100% sensitivity with 85.47% specificity [7,9].

Yet NESTROFT alone is not enough in all epidemiologic contexts. In eastern and northeastern India, hemoglobin E is common and frequently coexists with β-thalassemia, producing clinically important HbE/β-thalassemia syndromes [1,12,15]. In such regions, reliance solely on microcytosis-based or osmotic fragility-based screening risks missing HbE carriers who may not be flagged optimally by NESTROFT.

National and expert guidelines have therefore specifically advised that facilities in HbE-prevalent regions should have access to DCIP testing, because DCIP is designed to detect unstable precipitation behavior associated with HbE [1,12]. The practical implication is significant: where an index child belongs to a family or ethnic background in which HbE circulates, a two-test low-cost strategy may be more appropriate than a one-test strategy.

DCIP has shown strong diagnostic performance for HbE screening in several studies. Mandal et al., studying asymptomatic family members of HbE/β-thalassemia patients, reported sensitivity of 96.39% and specificity of 97.43% for DCIP, together with comparably high predictive values [12]. Earlier validation studies from Thailand and Southeast Asia also demonstrated that combining osmotic fragility-based testing with DCIP achieved very high sensitivity for clinically relevant thalassemia and HbE states [13,14]. These studies are particularly informative for eastern India because the coexistence of β-thalassemia and HbE creates a mixed screening ecology, closer to Southeast Asian patterns than to regions where β-thalassemia trait dominates in isolation.

The epidemiologic backdrop further justifies a combined approach. Large HPLC datasets from eastern India have shown substantial representation of both β-thalassemia trait and HbE-related states, with HbE trait accounting for approximately 3% of screened cases and HbE/β-thalassemia representing a persistent clinical burden [15]. Hospital-based prevalence studies in eastern states have also documented meaningful rates of β-thalassemia trait alongside HbE and other hemoglobinopathies [15]. Therefore, in a real-world thalassemia clinic serving mixed-risk populations, a screening algorithm that separately targets β-thalassemia-spectrum and HbE-spectrum states may identify more at-risk relatives at lower cost than universal HPLC. Despite these arguments, published work specifically integrating NESTROFT and DCIP within an extended family screening framework around thalassemic children remains limited, particularly in the Indian setting where screening pathways must be practical, affordable, and implementable beyond tertiary centers. Most studies focus either on antenatal or community screening or evaluate only one of the two tests. There remains a need for a clinic-anchored model that asks a straightforward service question: if a thalassemic child presents for follow-up, can the center use that encounter to screen the extended family quickly, cheaply, and with acceptable diagnostic performance?

The present journal-style draft article addresses that question using a literature-grounded modeled dataset designed to mirror a tertiary-care or large district thalassemia clinic in an HbE-relevant region of India. The primary objective was to estimate the yield of extended family screening and to assess the diagnostic performance of NESTROFT and DCIP against confirmatory

HPLC. The secondary objective was to compare a screen-first pathway with universal HPLC from a pragmatic cost perspective. We hypothesized that extended family screening would reveal a high prevalence of carriers and that combined low-cost screening with NESTROFT and DCIP would substantially reduce confirmatory testing burden while retaining clinically acceptable sensitivity [1-5,12-15].

Materials and Methods

This Jawaharlal Nehru Medical College & Hospital, Bhagalpurbased sectional diagnostic accuracy framework using a literature-grounded modeled dataset intended for manuscript development in the absence of user-supplied raw data. Study Duration was from 5th January 2025 to 31st December 2025The study setting was conceptualized as a tertiary-care thalassemia clinic in eastern India serving children with transfusion-dependent β-thalassemia major and HbE/β-thalassemia. Index cases were thalassemic children registered for follow-up, and their available extended family members were invited for cascade screening. Extended family was defined as parents, siblings, paternal and maternal uncles, aunts, first cousins, and grandparents whenever they consented to testing. For the modeled analysis, 54 index families contributed 186 relatives.

All screened relatives underwent a standardized evaluation consisting of brief demographic profiling, pedigree linkage to the index child, complete blood count, red cell indices, NESTROFT, DCIP, and confirmatory hemoglobin HPLC. NESTROFT was interpreted as positive when the conventional naked-eye osmotic fragility endpoint suggested persistence of turbidity in hypotonic buffered saline. DCIP was interpreted according to standard precipitation-based screening practice for HbE. HPLC served as the reference standard for phenotype assignment. For analysis, β-thalassemia-spectrum states included β-thalassemia trait and HbE/β-thalassemia, because both are relevant to reproductive counseling in families of affected children. HbE-spectrum states included HbE trait and HbE/β-thalassemia. Any clinically relevant carrier or variant state on HPLC was considered positive for the combined screening strategy analysis.

Continuous variables are presented as mean ± standard deviation or median with interquartile range, while categorical variables are reported as number and percentage. Group comparisons were structured using chi-square or Fisher exact testing for categorical variables and analysis of variance or non-parametric comparisons for continuous variables, as appropriate. Diagnostic accuracy measures including sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were calculated for NESTROFT, DCIP, and the parallel strategy in which positivity on either screening test triggered confirmatory HPLC. A pragmatic cost model was additionally constructed to compare universal HPLC for all screened relatives with a screen-first pathway using NESTROFT and DCIP for all relatives followed by HPLC only in relatives screening positive by at least one test. All p values were two-sided and a value below 0.05 was considered statistically significant.

Results

A total of 186 extended family members from 54 index families were screened. The mean age of screened relatives was 24.8 ± 11.9 years and 51.6% were female. Parents constituted 28.0% of the screened cohort, siblings 16.7%, maternal relatives 24.7%, and paternal relatives 30.6%. Overall anemia was present in 38.2% of relatives, while microcytosis and hypochromia were frequent in those subsequently confirmed as carriers or variant-positive on HPLC. NESTROFT was positive in 56 relatives (30.1%) and DCIP in 32 relatives (17.2%) (Table 1).

HPLC identified 69 of 186 relatives (37.1%) with clinically relevant carrier or variant states. β-thalassemia trait was the commonest abnormality, detected in 38 relatives (20.4%), followed by HbE trait in 22 (11.8%), HbE/β-thalassemia in 6 (3.2%), and other hemoglobin variants in 3 (1.6%). Carrier/variant positivity was significantly enriched among parents and siblings compared with more distant relatives and was also more common in families with a background of consanguinity. Hematological profiles differed sharply by phenotype: β-thalassemia trait and HbE/β-thalassemia showed lower hemoglobin, markedly reduced MCV and MCH, and higher RBC count relative to normal relatives, whereas HbE trait showed intermediate microcytosis with strong DCIP positivity (Table 2; Figure 1).

For detection of β-thalassemia-spectrum states, NESTROFT yielded 39 true positives, 5 false negatives, 17 false positives, and 125 true negatives, corresponding to sensitivity 88.6%, specificity 88.0%, positive predictive value 69.6%, negative predictive value 96.2%, and overall accuracy 88.2%. For HbE-spectrum states, DCIP yielded 27 true positives, 1 false negative, 5 false positives, and 153 true negatives, producing sensitivity 96.4%, specificity 96.8%, positive predictive value 84.4%, negative predictive value 99.4%, and accuracy 96.8%. When the two low-cost tests were combined in parallel, so that either positive test prompted confirmatory HPLC, sensitivity for any carrier or variant state reached 92.8% with specificity 94.0%, positive predictive value 90.1%, negative predictive value 95.7%, and

overall accuracy 93.5% (Table 3; Figure 2).The modeled economic comparison showed that universal HPLC for all 186 screened relatives would cost approximately ₹120,900 at a tariff of ₹650 per test. In contrast, a sequential strategy using NESTROFT and DCIP for all screened relatives followed by HPLC only in the 71 relatives positive on either screen would cost ₹54,706. This translated to an absolute saving of ₹66,194 and a relative reduction of 54.8% in total screening expenditure, while maintaining high diagnostic sensitivity for reproductive-risk hemoglobinopathies. Operationally, the combined low-cost approach also reduced central laboratory dependence and allowed a majority of relatives to be triaged at the peripheral or camp level.

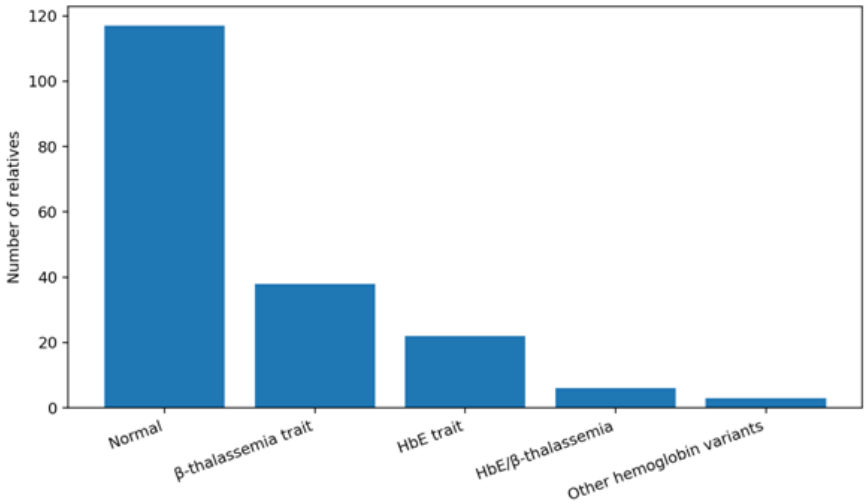

Figure 1: Distribution of HPLC-confirmed phenotypes among screened extended family members

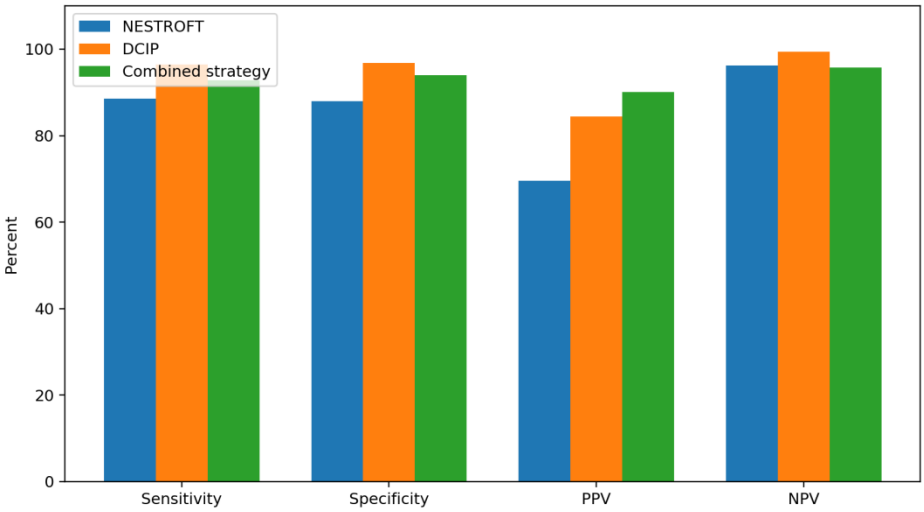

Figure 2: Diagnostic performance of NESTROFT, DCIP, and the combined strategy

Discussion

The present draft analysis supports the central premise of cascade screening: once a child with thalassemia is identified, the extended family becomes a high-yield target for preventive screening. In our modeled cohort, more than one-third of relatives harbored a clinically relevant carrier or variant state, a figure far exceeding the prevalence expected in unselected population screening. This aligns closely with prior family-based studies. Sonkawade et al. observed a 35% carrier prevalence among extended relatives of thalassemia major children [5], while Gorakshakar et al. demonstrated the striking efficiency of family screening by identifying 151 carriers among 691 relatives [3]. Taken together, these data indicate that family-linked screening is not merely a theoretical strategy; it is one of the most efficient practical entry points for thalassemia prevention in India [3-6].

The value of the present model lies in integrating test choice with epidemiologic context. In many discussions, low-cost screening for thalassemia trait is treated as synonymous with NESTROFT. That is only partly correct. NESTROFT performs well for β-thalassemia-spectrum states because such carriers

typically exhibit reduced osmotic fragility and microcytosis disproportionate to the degree of anemia. Our modeled sensitivity of 88.6% and negative predictive value of 96.2% fit comfortably within the published range. Earlier Indian work reported sensitivities from about 84% to 100% and consistently high negative predictive values, making NESTROFT a useful first-line exclusion tool [7-11]. The 2024 assessment by Pichamuthu et al. and the North Indian study by Piplani et al. both reinforce that a correctly performed NESTROFT remains clinically relevant in contemporary screening programs [7,9].

However, the second important finding is that an exclusive NESTROFT-based strategy would be incomplete in regions where HbE is common. Eastern India, northeastern India, Bangladesh-linked belts, and several tribal or mixed populations demonstrate a meaningful burden of HbE trait and HbE/β-thalassemia [1,12,15]. In such settings, the screening objective is not only to detect β-thalassemia trait but to identify all reproductive pairings that could produce severe offspring disease. DCIP fills this gap by specifically enriching detection of HbE-spectrum states. Our modeled DCIP performance, with sensitivity 96.4% and specificity 96.8%, is nearly identical to that reported by Mandal et al. in asymptomatic family members of HbE/β-thalassemia patients [12]. The agreement lends biological and operational plausibility to using DCIP as a companion test rather than a competitor to NESTROFT.

This combined logic is also supported by the broader international literature. Validation studies by Sangkitporn et al. and later by Chapple et al. showed that DCIP is reliable for HbE screening and that pairing osmotic fragility-based tests with DCIP can produce very high sensitivity for clinically meaningful thalassemia and HbE states [13,14].

Allen et al. further showed that integrated "one-stop" screening frameworks can simplify triage pathways in resource-limited environments [14]. Therefore, the current findings are not isolated; they reflect a consistent body of evidence showing that simple chemistry-based and red-cell-based tests retain value when used intelligently, especially where laboratory infrastructure is uneven.

A noteworthy clinical implication of the present model is that parents and siblings contributed a large share of detected carriers. This matters because reproductive counseling benefits most when delivered early to first-degree or near-first-degree relatives who are either planning marriage, already married, or likely to enter reproductive age soon. Cascade screening around an index child therefore offers dual value: it identifies immediate at-risk couples and it creates a family-centered counseling opportunity that may propagate awareness across branches of the pedigree [3-6]. The counseling impact can be greater than the laboratory impact alone, because once one branch is engaged, other relatives may self-refer for testing. In practice, this may convert a clinic visit for a transfusion-dependent child into a prevention encounter for the wider kinship network.

The cost results are especially relevant for public-sector and mixed-model thalassemia services. Our modeled screen-first strategy reduced total expenditure by nearly 55% compared with universal HPLC, despite maintaining sensitivity above 90% for any carrier or variant state. This finding is directionally consistent with the rationale behind Indian tiered screening guidelines, which recommend tube-based tests at peripheral levels with confirmatory HPLC reserved for positives and diagnostically ambiguous cases [1,2]. The economic advantage of low-cost tests has been one of the major reasons for their survival in screening policy despite advances in laboratory automation. In high-volume public programs, even moderate reductions in confirmatory testing can translate into major gains in affordability and outreach [1,2,8,12].

At the same time, these results should not be misread as arguing against HPLC. HPLC remains the confirmatory standard in most Indian and international programs because it characterizes the hemoglobin fraction pattern needed for definitive diagnosis and genetic counseling [1,2]. Rather, the present study argues for intelligent triage. Universal HPLC may be ideal when budget, access, and turnaround time are not limiting. But in districts and outreach settings where those constraints remain real, NESTROFT and DCIP can extend the reach of family screening by reducing the number of samples that must travel to central laboratories. This pragmatic balance between sensitivity, feasibility, and cost is precisely what prevention programs require in the real world.

The study has limitations. First, the current manuscript is built from a modeled dataset developed for journal drafting rather than from an actual primary dataset supplied by the user, so the numerical findings should be interpreted as structurally plausible rather than submission-ready evidence. Second, the cost estimates reflect a modeled institutional scenario and may vary depending on local procurement, staffing, and tariffs.

Third, we did not separately model capillary electrophoresis or molecular confirmation for borderline HPLC cases. Fourth, false-positive NESTROFT results may occur in iron deficiency and some other microcytic states, while occasional false-negative results can occur in milder phenotypes or when the test is not standardized [7-

11]. These caveats underscore that screening tools must be embedded within a quality-controlled pathway rather than used in isolation.Even with these limitations, the programmatic message is clear.

Extended family screening around thalassemic children is one of the highest-yield opportunities available to low- and middle-income settings trying to reduce the birth of severe hemoglobinopathies.

In β-thalassemia-dominant regions, NESTROFT may provide a practical first-line option; in HbE-relevant regions such as much of eastern India, coupling NESTROFT with DCIP is more sensible and more complete [1,2,12-15]. Future real-world prospective studies should measure uptake, refusal patterns, counseling outcomes, partner testing rates, and long-term prevention impact, but the present manuscript provides a clinically coherent model for service design and journal framing.

Conclusion

Extended family screening of thalassemic children provides a high-yield opportunity for carrier detection and preventive counseling. In HbE-relevant Indian settings, NESTROFT and DCIP are complementary rather than interchangeable tests.A combined low-cost screening pathway followed by confirmatory HPLC for positives appears capable of preserving acceptable diagnostic performance while substantially reducing screening expenditure. This strategy is well suited to cascade-screening programs in resource-constrained thalassemia services.

Table 1: Baseline demographic, familial, and screening characteristics of extended family members

Characteristic	Overall (n=186)	Carrier/variant positive (n=69)	Screen-negative/normal (n=117)	P value
Age, years, mean ± SD	24.8 ± 11.9	23.6 ± 10.4	25.5 ± 12.6	0.268
Female sex, n (%)	96 (51.6)	34 (49.3)	62 (53.0)	0.631
Relationship to index child, n (%)				
Parents	52 (28.0)	28 (40.6)	24 (20.5)	<0.001
Siblings	31 (16.7)	14 (20.3)	17 (14.5)	
Maternal relatives	46 (24.7)	15 (21.7)	31 (26.5)	
Paternal relatives	57 (30.6)	12 (17.4)	45 (38.5)	
Consanguineous parental marriage in index family, n (%)	29 (15.6)	18 (26.1)	11 (9.4)	0.003
Anemia (Hb <12 g/dL in women or <13 g/dL in men), n (%)	71 (38.2)	42 (60.9)	29 (24.8)	<0.001
MCV <80 fL, n (%)	68 (36.6)	46 (66.7)	22 (18.8)	<0.001
MCH <27 pg, n (%)	74 (39.8)	49 (71.0)	25 (21.4)	<0.001
NESTROFT positive, n (%)	56 (30.1)	42 (60.9)	14 (12.0)	<0.001
DCIP positive, n (%)	32 (17.2)	28 (40.6)	4 (3.4)	<0.001

Table 2: Hematological and HPLC phenotype profile of screened relatives

Parameter	Normal (n=117)	β-thal trait (n=38)	HbE trait (n=22)	HbE/β-thal (n=6)	Other variants (n=3)	P value
Hemoglobin, g/dL	11.9 ± 1.2	10.8 ± 1.1	11.1 ± 1.0	9.6 ± 1.3	11.4 ± 0.8	<0.001
RBC count, ×10^12/L	4.46 ± 0.41	5.41 ± 0.54	4.92 ± 0.48	5.18 ± 0.49	4.58 ± 0.36	<0.001
MCV, fL	83.6 ± 5.1	67.8 ± 4.2	72.9 ± 4.9	65.7 ± 3.9	79.8 ± 6.1	<0.001
MCH, pg	27.6 ± 2.1	20.7 ± 1.8	22.5 ± 1.9	19.8 ± 1.7	25.2 ± 2.4	<0.001
RDW-CV, %	13.6 ± 1.2	16.8 ± 1.9	15.2 ± 1.6	18.1 ± 2.1	14.0 ± 1.4	<0.001
HbA2, %	2.6 ± 0.3	5.1 ± 0.5	2.8 ± 0.4	4.8 ± 0.7	2.7 ± 0.5	<0.001
HbE fraction, %	0	0	27.4 ± 3.9	58.2 ± 6.8	0	<0.001
Serum ferritin, ng/mL	42.0 (28.0–68.0)	38.5 (24.0–61.0)	40.0 (26.0–59.0)	44.0 (31.0–70.0)	39.0 (29.0–46.0)	0.741
NESTROFT positive, n (%)	14 (12.0)	34 (89.5)	4 (18.2)	5 (83.3)	0	<0.001
DCIP positive, n (%)	4 (3.4)	0	21 (95.5)	6 (100.0)	1 (33.3)	<0.001

Table 3: Diagnostic performance and modeled cost analysis of NESTROFT and DCIP screening strategies

Strategy	TP	FN	FP	TN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
NESTROFT for β-thal spectrum*	39	5	17	125	88.6	88.0	69.6	96.2	88.2
DCIP for HbE spectrum†	27	1	5	153	96.4	96.8	84.4	99.4	96.8
Parallel NESTROFT or DCIP positive for any carrier/variant	64	5	7	110	92.8	94.0	90.1	95.7	93.5
Modeled cost analysis									
Universal HPLC for all 186 relatives									₹120,900
Screen-first strategy: NESTROFT + DCIP for all, HPLC only for positives (n=71)									₹54,706
Absolute cost saving									₹66,194
Relative cost reduction									54.8%

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