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RESEARCH ARTICLE

Alpha Thalassemia Carrier Detection a Diagnostic Approach

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Abstract: Alpha-thalassemia is one of the most common single-gene disorders, and its carrier state often remains undetected due to nonspecific hematological findings. This study aimed to identify alpha-thalassemia carrier status among people being investigated for hemoglobinopathy in a tertiary care setting. A cross-sectional laboratory-based study was conducted from January 2023 to January 2024. Hemoglobin variant analysis reports by High Performance Liquid Chromatography (n = 384) were categorized as hemoglobinopathy (n = 129), inconclusive (n = 44), and normal (n = 211). Samples from the inconclusive and normal groups (n = 255) were further evaluated using complete blood count, peripheral smear, iron profile, and Sehgal Index (SI). Based on sample selection criteria, only ten selected samples were subjected to genetic analysis using the alpha globin strip assay. The frequency of hemoglobinopathies based on routine lab investigations was as follows: Beta thalassemia trait-23.4% (n = 90); HbS-5.72% (n = 22); HbD-2.08% (n = 8); HbE-1.56% (n = 6), and Beta thalassemia major-0.78% (n = 3). Only ten samples (2.6%) were subjected to genetic analysis, among which $\alpha^{-3.7}$ deletional mutation (n = 3) and α2 Codon 19-point mutation (n = 2) were detected. These findings correspond to a carrier frequency of 3.3% in the selected subcohort. The present exploratory pilot study findings highlight the potential utility of targeted molecular testing for detection of alpha-thalassemia carriers, particularly in cases with inconclusive hematological findings, and require further exploration.

Keywords: Alpha Thalassemia, Anemia, Genetics, Hemoglobinopathy, Iron Deficiency, Mutation

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Introduction

Haemoglobinopathies are a major cause of morbidity and mortality worldwide, imposing a significant healthcare burden [1]. Among these, thalassemia is one of the most common inherited disorders of red blood cells [2]. In India, approximately 10,000–15,000 infants are born annually with thalassemia major, and nearly 42 million individuals are β-thalassemia carriers [1].

Alpha-thalassemia is a single-gene disorder caused by defects in HBA1 and HBA2 genes, with clinical manifestations ranging from silent carrier state to Bart's hydrops fetalis [3]. Globally, its prevalence is estimated at approximately 5% [4]. In

India, alpha-thalassemia prevalence varies widely, from low levels in some populations to very high frequencies in malaria-endemic and tribal regions [5]. In our district, the overall prevalence of hemoglobinopathies has been reported to be 18.2% [6]; however, data specifically addressing alpha-thalassemia in this region remain scarce.

Growing evidence indicates that genetic disorders such as Alpha-thalassemia also contribute substantially to the anemia burden in India. Alpha-thalassemia carriers often present as microcytic anemia, closely mimicking iron deficiency, leading to repeated investigations and remaining undiagnosed because routine hematological parameters and High-Performance Liquid Chromatography (HPLC) findings are inconclusive. The Sehgal Index (SI) is a useful preliminary screening tool for differentiating Iron Deficiency Anemia (IDA) from thalassemia traits, demonstrating good sensitivity and specificity [7]. However, SI alone cannot reliably distinguish between alpha and beta thalassemia carrier states, and its diagnostic accuracy is reduced when iron deficiency anemia coexists with thalassemia [2]. Molecular diagnostic methods are considered the gold standard for definitive diagnosis; however, their availability is limited in many resource-constrained settings due to cost and infrastructure requirements. [8].

The above findings highlight the need for improved diagnostic strategies, which led to the present exploratory study.

Pilot Laboratory Based Study

Materials and Methods

Study design: Cross-sectional laboratory-based study.

Participants

The collated lab data consisted of all the samples received for haemoglobin variant analysis by HPLC (n = 384) from patients aged one month to 95 years. Data pertaining to RBC indices, peripheral smear, and iron were compiled.

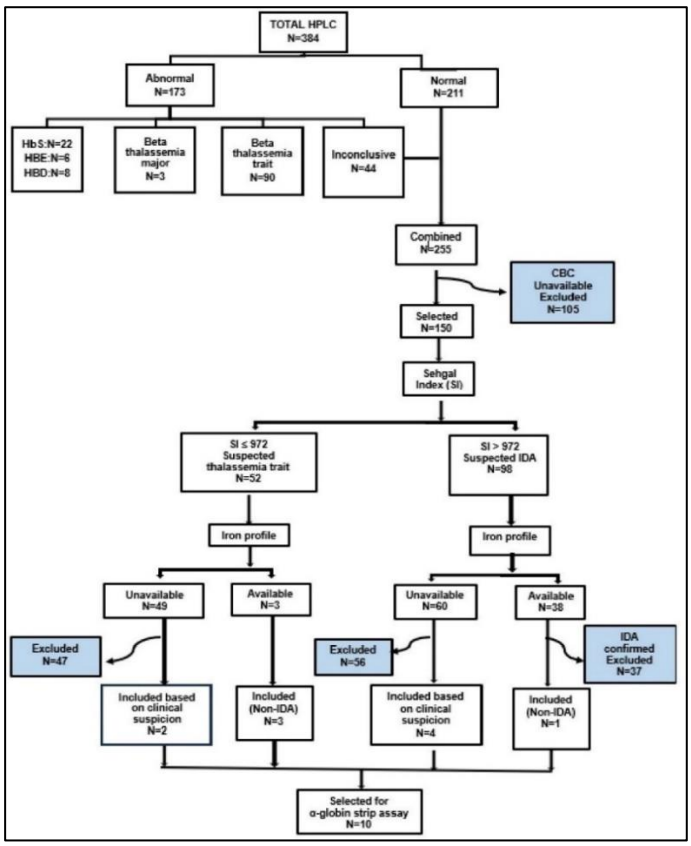

Fig. 1: Algorithm for selection of suspected alpha-thalassemia carriers

Inclusion criteria: Patients with HbA2 (<3%), MCV (Mean corpuscular volume) (<80 fL), and MCH (Mean Corpuscular Hemoglobin) (<27 pg) were considered.

Exclusion criteria: Those diagnosed with definitive haemoglobinopathy based on HPLC, HbA2 >3.5%, confirmed iron deficiency anemia, and incomplete hematological or iron profile data were excluded.

Type of sampling and reason for selection: Convenience sampling for data collection. Sample selection for molecular analysis was based on inclusion criteria, inputs from the referring clinician, and the pack size of the alpha globin strip assay (n = 10); only ten participants (Age range: 10 months 51 years) were selected as a targeted sub-cohort (Fig. 1).

Sample Analysis

BIORAD Dual D-10 HPLC system based on the principle of cation exchange chromatography was used to quantify haemoglobin A2, HbF and HbS. Red cell indices and retics were measured using the Sysmex XN-10 automated haematology analyser, which utilizes a combination of impedance, photometry, and fluorescence/optical methods for accurate analysis. Ferritin levels were estimated by Electrochemiluminescence Immunoassay (ECLIA), and serum iron levels were determined using the Ferrozine method on the ROCHE Modular Analytics platform. Peripheral smear reports were documented. Sehgal index (SI) = [MCV^2/RBC] was calculated.

The α-Globin Strip Assay Kit (cat 4-160/4-161) from Vienna Lab (Labor Diagnostika GmbH) was used exclusively for the detection of alpha-thalassemia mutations. Figure (2) and interpreted using the Online Calculator v2.19 of Vienna Lab.

The pre-procedure consisted of three key steps:

1) DNA isolation using the Spin Micro DNA Extraction Kit
2) PCR (polymerase chain reaction) amplification with biotinylated primers to amplify the target DNA
3) Agarose gel electrophoresis of the amplicons to confirm the quality of PCR

Fig. 3 DNA Ladder (L) (100bp–1000bp) served as a standard for accurate determination of amplicon sizes. Empty (E) control lanes were included to monitor for potential contamination. Gels were subsequently stained with ethidium bromide and visualized under ultraviolet (UV) light, and images were captured on a GelDoc for documentation and analysis (Fig. 3).

Statistical Analysis

Microsoft Excel was used to collate data and calculate the proportion of β-thalassemia cases and carriers. Molecular analysis results are presented as obtained. The statistical evaluation could not be performed on the lab parameters of the sub-cohort as the values are age-specific and cannot be compared between the diverse age ranges.

Results

Based on Hb electrophoresis, 33.5% were diagnosed as haemoglobin variants consisting of beta thalassemia trait (23.4%; n = 90), HbS (72%; n = 22), HbE (1.56%; n = 6), HbD (2.08%; n = 8), and beta thalassemia major (0.78%; n = 3). Only 10 out of 384 samples (2.6%) were subjected to molecular analysis based on combined hematological, biochemical, and clinical criteria and the kit size (Fig. 1). The selected samples had haemoglobin (Hb) levels ranging from 5.4–17.1 g/dL, Mean Corpuscular Volume (MCV) from 51.9– 88.3 fL, mean corpuscular haemoglobin (MCH) from 12.1– 28.5 pg, Mean Corpuscular Haemoglobin Concentration (MCHC) from 22.4– 34.2 g/dL, and Red Blood Cell (RBC) count from 4.1–6.2 million/cumm. Peripheral smear examination ranged from borderline normocytic normochromic anaemia to microcytic hypochromic anaemia, the latter being the most prevalent. (Table 1) Iron profile was available for only four and reticulocyte data for six samples.

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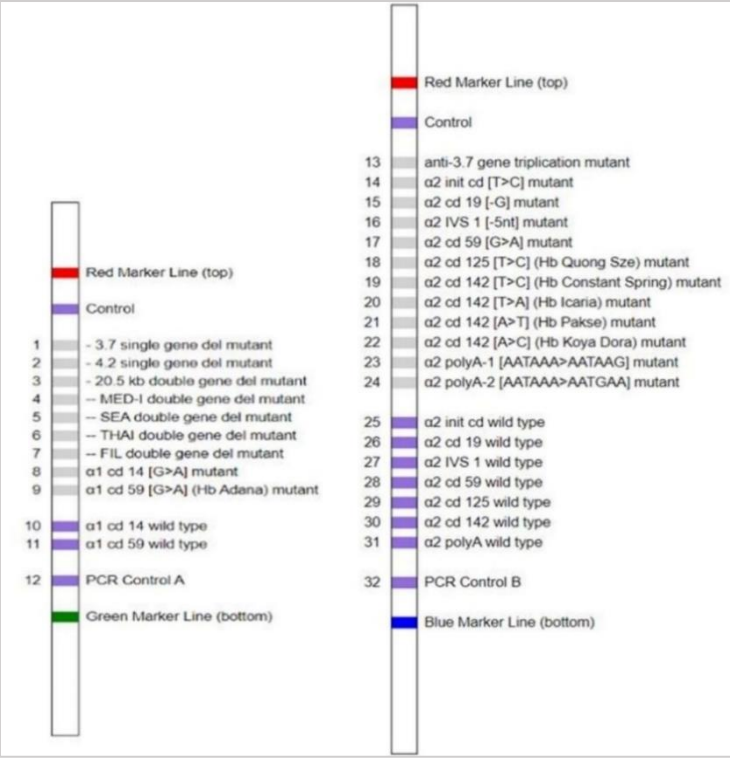

Fig. 2: Alpha globin hybridization strips with list of mutations detected

Table 1: Complete blood count with peripheral smear

Mutation detected	Age	Sex	Hb (gm/dl)	MCV (fL)	MCH (pg)	MCHC (gm/dl)	RDW (%)	RBC count (millions/cum)	Retics (%)	SI	Peripheral smear
Δ3.7 hetero zygous	14	M	14.3	76	23.2	30.6	14.3	6.2†	-	937.7*	Microcytic hypochromic anemia
	10m	M	5.1	51.9	12.1	23.4	25.7	4.2	1.6	641.3*	Microcytic hypochromic anemia
	26	F	12.1	78.1	23.3	29.8	15.3	5.2	-	1173.0	Normocytic normochromic blood picture
α2 cd19 hetero zygous	51	M	17.1	88.3	27.4	31	15.2	6.3†	0.5	1247.5	Normocytic normochromic blood picture
α2 cd19 homo zygous	20	M	10	66.2	21	31.7	17.1	4.8	0.5	916.8*	Microcytic hypochromic anemia
No mutation	42	F	9.5	66.7	18.8	28.2	20.3	5.1	0.2	880.9*	Microcytic hypochromic anemia
	41	F	12.9	83.3	28.5	34.2	13.4	4.5	0.4	1535.2	Normocytic normochromic blood picture
	30	F	10.8	77.4	23.7	30.6	17	4.6	-	1313.8	Microcytic hypochromic anemia
	33	F	10.5	81.7	24.4	29.8	15.5	4.3	-	1548.7	Normocytic normochromic blood picture
	10m	M	5.4	56	12.6	22.4	24.9	4.3	1.4	729.3*	Microcytic hypochromic anemia

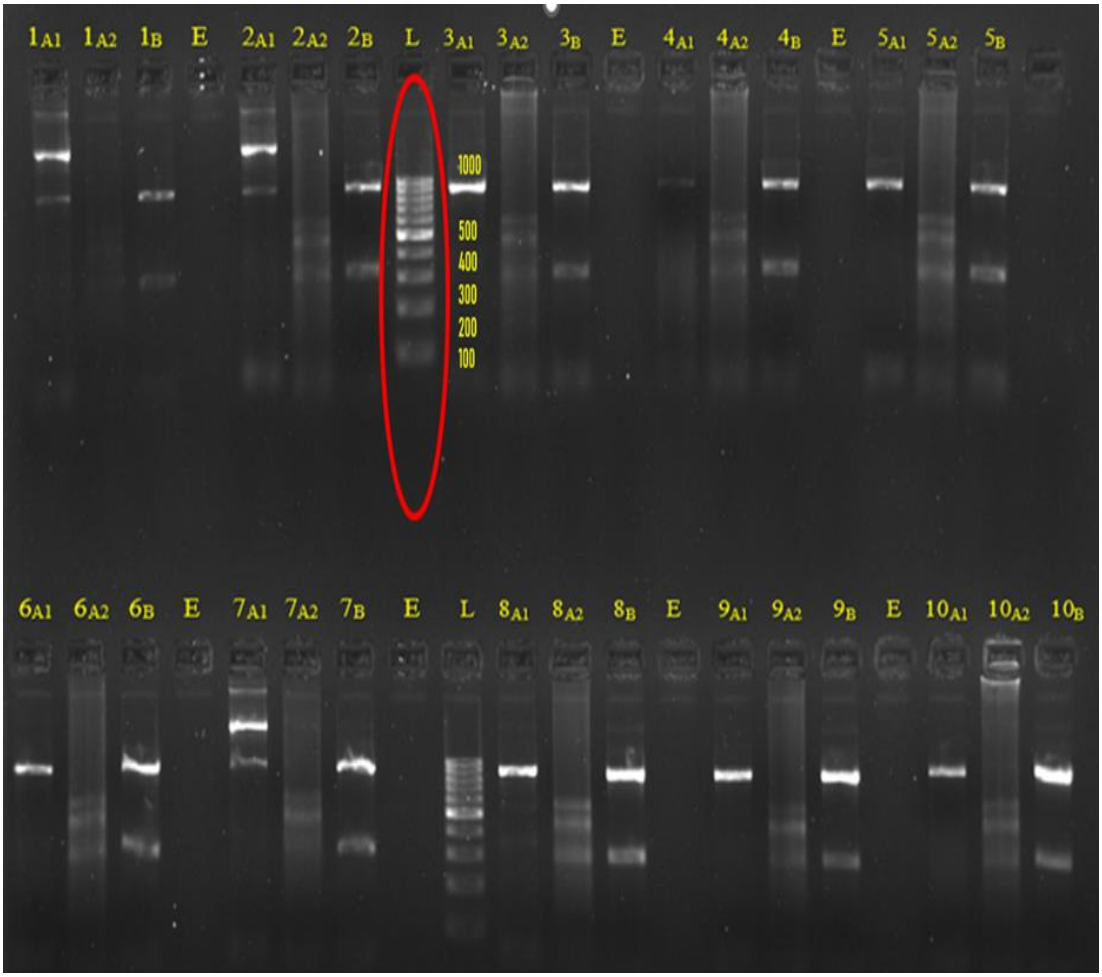

Fig. 3: Amplification products analysed by gel electrophoresis of study samples

Five patients tested positive for alpha-thalassemia mutations based on alpha globin strip assay (Fig. 4). Three patients (Samples 1, 2, and 7) were diagnosed as heterozygous for the -$\alpha^{3.7}$ deletion, supported by the presence of a distinct hybridization band at probe position 1 on their respective strips. Two patients (Samples 3 and 4) showed an α2 cd19 mutation, a non-deletional mutation, of which Sample 3 was a heterozygous carrier displaying a positive band at probe position 15 while Sample 4 was homozygous, indicated by a prominent and distinct hybridization band at the same location and a missing band at position 26 (Fig. 4). The findings demonstrate that the strip assay can accurately detect both deletional (-$\alpha^{3.7}$) and non-deletional (α2 cd19) alpha-thalassemia mutations, as mentioned in an earlier study. [10] this also reveals the presence of a diverse range of genotypes within the subcohort.

Overall ranges of the hematological parameters of the patients with and without mutations revealed no distinct differences between them. (Table 2) HPLC findings showed markedly reduced HbA2 (0.7%) and total HbA (80.1%) and a prominent unknown peak (18.5%) at RT (retention time) of 1.8 minutes only in the homozygous patient. In all other patients, HbA2 levels ranged from 1.8–2.9% with age-specific normal HbF concentrations and no abnormal peaks (Table 3).

Table 2: Haematological findings in the selected sub-cohort

Mutation detected	Hb (gm/dl)	MCV (fL)	MCH (pg)	MCHC (gm/dl)	RDW (%)	RBC count (millions/cum)	Retics (%)
Yes (n = 5)	5.1-14.3	51.9-88.3	12.1-27.4	23.4-31.7	14.3-25.7	4.2-6.16	0.5-1.6
No (n = 5)	5.4-12.9	56.0-83.3	12.6-28.5	22.4-34.2	13.4-24.9	4.3-5.05	0.2-1.4

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Table 3: HPLC findings of selected sub-cohort

Serial No.	Age	Gender	F	A2	Unknown peak	Total HbA	Mutation detected
1	14	M	0	2.9	0	93.3	Δ3.7 heterozygous
2	10m	M	0.8	2.5	0	93.2	
3	26	F	0	2.4*	0	94.7	
4	51	M	0.8	2.5	0	94.3	α2 cd19 heterozygous
5	20	M	0	0.7*	18.5 (RT 1.8 min)	80.1	α2 cd19 homozygous
6	42	F	0.8	2.6	0	94.3	No mutation
7	41	F	0.8	2.8	0	94.2	
8	30	F	0	1.8*	0	68.1	
9	33	F	0.8	2.8	0	93.4	
10	10m	M	1.4	1.9	1.3	86	

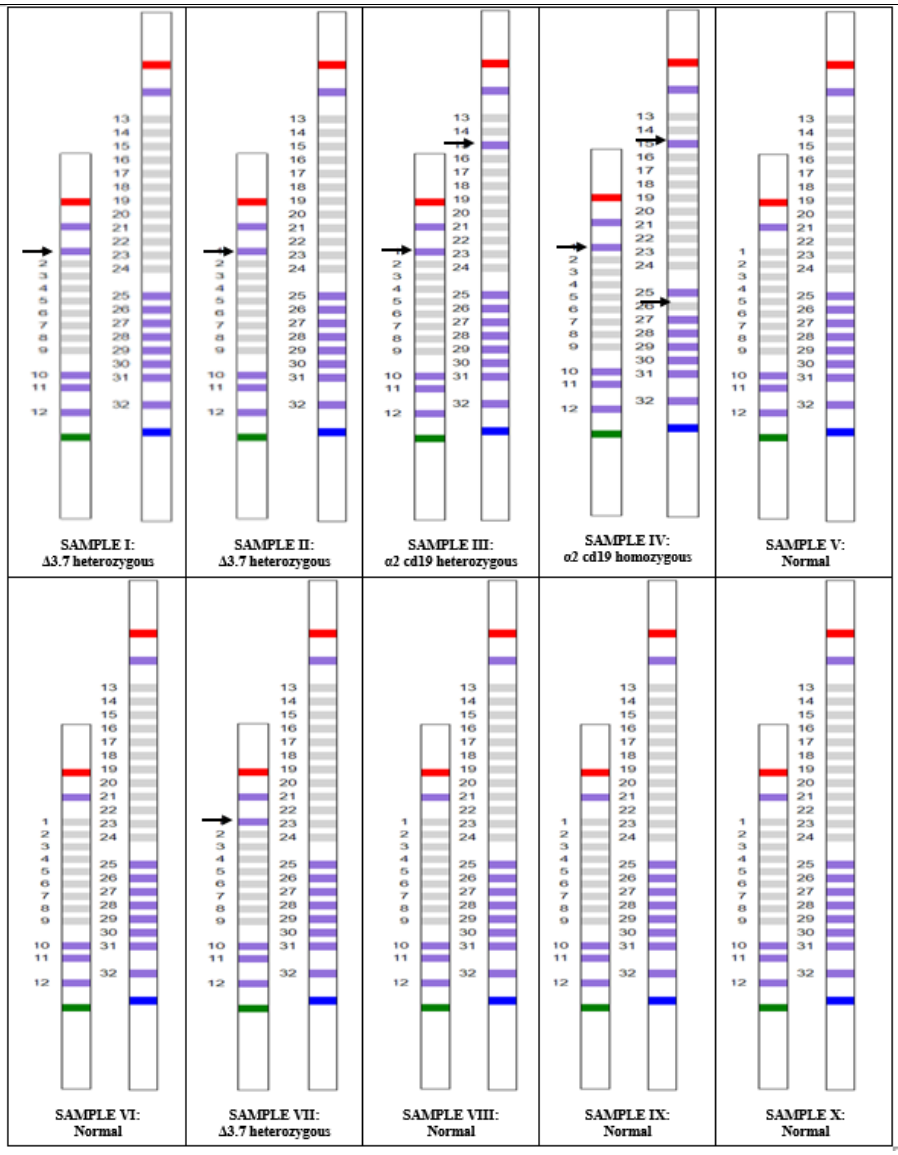

Fig. 4: Alpha globin strip assay of selected sub-cohort

Discussion

In this laboratory-based study, the prevalence of hemoglobinopathies was 33.5%, with a substantial proportion contributed by Beta Thalassemia Trait (BTT) (23.4%). In India, beta thalassemia is the most frequently diagnosed hemoglobinopathy, with approximately 42 million carriers reported nationwide [1].

Based on the selection criteria, the prevalence of the alpha-thalassemia carrier state in the present study was 3.3%. (Fig. 1). The prevalence findings apply only to the selected study cohort and are not representative of the general population. The reported prevalence of alpha-thalassemia trait in India is highly variable, ranging from 11% to more than 71% across different regions and ethnic groups [5]. Globally, the most common alpha-thalassemia mutation is the α-3.7 deletion [3]. This mutation is believed to confer partial protection against Plasmodium falciparum malaria, which explains its high frequency in malaria-endemic regions [3, 5]. Among the samples subjected to molecular analysis, an α-3.7 heterozygous deletion mutation was detected in one iron-sufficient and one iron-deficient patient. Figs. (1, 4). Our district is a malaria-endemic region, and a relatively higher prevalence of alpha-thalassemia traits may be expected, underscoring the need for vigorous screening and detection strategies.

No distinct differences in hematological parameters or HbA2 levels were seen among those with and without alpha globin gene mutations (Tables 2 and 3), attributed primarily to the small sample size (n = 10) and diverse age range. These factors are limitations for conducting a statistical analysis and prompt larger explorations of the study.

SI identified 34.6% of cases as suspected thalassemia carriers, and 65.6% as IDA (Fig. 1), but an incomplete iron profile limited definitive interpretation of these findings. A greater number of deletions in the alpha globin gene are associated with progressively lower Mean Corpuscular Volume (MCV) and HbA_2 levels, along with potentially higher ferritin levels, which may mask the presence of underlying iron deficiency anemia [2, 9] and also lead to incorrect SI values. To enhance the accuracy of screening, SI should be used in combination with iron studies and HPLC in the interpretation of diagnostically equivocal cases [9].

It was observed that patients with mutations had microcytic hypochromic to normocytic normochromic blood picture. Low MCV and MCH with a normal or high RBC count are useful for differentiating alpha-thalassemia carriers from iron deficiency anemia in clinical practice. In the present study, four of them satisfied these findings while one did not. The Red Blood Cell (RBC) count typically remains normal or may be elevated in individuals with alpha-thalassemia, in contrast to iron deficiency anemia, where the RBC count is usually reduced [3]. This relative preservation of the RBC count reflects a compensatory erythropoietic response of the bone marrow aimed at maintaining adequate oxygen delivery despite ineffective hemoglobin synthesis [2].

It has been said that no single laboratory investigation is definitively diagnostic for alpha-thalassemia [3], as alpha-thalassemia carrier patients have varied presentations with near-normal laboratory findings. Though hematological and biochemical investigations are available, molecular testing for conclusive diagnosis is still the gold standard. Several molecular techniques available for the diagnosis of genetic defects in thalassemia include PCR-based methods such as Gap-PCR and multiplex PCR for the detection of common deletional mutations, as they are relatively cost-effective, rapid, and suitable for routine diagnostic laboratories. Complementary techniques such as Multiplex Ligation-dependent Probe Amplification (MLPA) and reverse dot blot hybridization facilitate the detection of larger deletions and multiple known mutations simultaneously. Advanced approaches, including Sanger sequencing and Next-Generation Sequencing (NGS), provide comprehensive characterization by identifying rare, novel, and non-deletional variants that may be missed by targeted assays [8]. However, these techniques require substantial financial investment, specialized equipment, and trained personnel, which may limit their availability in resource-constrained settings. The strip assay evaluated in the present study offers a user-friendly workflow, standardized interpretation, and the ability to detect multiple common mutations in a single assay. Nevertheless, it remains relatively expensive on a per-test basis and is restricted to a predefined mutation panel, limiting its ability to identify rare or novel variants. Therefore, while the strip assay is clinically feasible for targeted screening and confirmatory testing in populations with well-characterized mutation spectra, more comprehensive methods such as sequencing may be necessary when clinical suspicion persists despite negative strip assay results. A limitation of the present study is the absence of complete iron profile data for all participants. Iron deficiency can influence red cell indices and, in some cases, lower HbA_2 levels, potentially masking the β-thalassemia carrier state and may have resulted in misclassification of certain individuals, particularly those with borderline hematological parameters. Findings from this pilot study suggest that the strip assay may be a useful adjunct tool for preliminary thalassemia mutation screening.

Rationale of the Study

Alpha-thalassemia carriers go undetected as the routine hematological tests do not definitely identify the condition. People with microcytic hypochromic anemia with normal or inconclusive HPLC results may be categorized as IDA and treated. This raises the frequency of unidentified carrier couples having miscarriages, children with severe anemia, or carriers of alpha-thalassemia.

Strengths

Highlights the value of genetic testing with routine hematological investigations in a malaria-endemic area for capturing the prevalence of alpha-thalassemia carriers.

Limitation

This is a single center study with a small cohort subjected to molecular analysis (n = 10), which limits generalizability. The prevalence findings apply only to the selected study cohort and are not representative of the general population. Additionally, incomplete iron profile data may have influenced patient classification and mutation detection.

Conclusion

This study underscores the need for integrating molecular diagnostics with routine laboratory investigations in malaria-endemic regions. Public health initiatives such as premarital and prenatal screening, genetic counseling, and community awareness programs are essential to reduce the burden of thalassemia.

Acknowledgment

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Author's Contributions

H. R. Samruddhi: Data acquisition, analysis, and drafting the primary manuscript.
Anupama Hegde: Design, supervision patient selection and data analysis.
Harsha Prasada Lashkari: Design, supervision and patient recruitment.
Sridevi HB: Design and interpretation of hematology investigations.
Suchitra Shenoy M: Supervision and interpretation of molecular diagnostics.
Rukmini M. S: Supervision analysis and proof readed.

Ethics

A cross-sectional laboratory-based study was conducted at the clinical laboratory of a tertiary care hospital for a period of one year. The study was conducted as per the Declaration of Helsinki and after Institutional Ethics Committee (IEC) approval was obtained. (IEC/KMCMLR/09/2023/379).

Conflict of Interest

The authors declare no conflict of interest.

Consent to Participate Statement

Written informed consent was obtained from the participants (or their parent/legal guardian/next of kin) selected for genetic analysis on the basis of selection criteria.

References

1. Sinha, S., Seth, T., Colah, R. B., & Bittles, A. H. (2020). Haemoglobinopathies in India: estimates of blood requirements and treatment costs for the decade 2017–2026. Journal of Community Genetics, 11(1), 39–45. https://doi.org/10.1007/s12687-019-00410-1
2. Sadiq, I. Z., Abubakar, F. S., Usman, H. S., Abdullahi, A. D., Ibrahim, B., Kastayal, B. S., Ibrahim, M., & Hassan, H. A. (2024). Thalassemia: Pathophysiology, Diagnosis, and Advances in Treatment. Thalassemia Reports, 14(4), 81–102. https://doi.org/10.3390/thalassrep14040010
3. Sankaran, V. G., & Nathan, D. G. (2023). Alpha-thalassemia: A practical overview. Blood Reviews, 67, 100772. https://doi.org/10.1016/j.blre.2023.101165
4. Musallam, K. M., Viprakasit, V., Lombard, L., Gilroy, K., Rane, A., Vinals, L., Tam, C., Rizzo, M., & Coates, T. D. (2024). Systematic review and evidence gap assessment of the clinical, quality of life, and economic burden of alpha-thalassemia. EJHaem, 5(3), 541–547. https://doi.org/10.1002/jha2.882
5. Thaker, P., Mahajan, N., Mukherjee, M. B., & Colah, R. B. (2022). Molecular Heterogeneity of Hb H Disease in India. Thalassemia Reports, 12(3), 73–84. https://doi.org/10.3390/thalassrep12030012
6. Rastogi, N., & Bhake, A. S. (2020). Sehgal index and its comparison with Mentzer's index and Green and King index in assessment of peripheral blood smear with marked anisopoikilocytosis. International Journal of Research in Medical Sciences, 8(8), 2972. https://doi.org/10.18203/2320-6012.ijrms20203449
7. Putchen, D. D., Kulkarni, S., Nanjundarao, S. S., Bhat, D. G., Venkatachala, P. K., & Prasad, S. R. (2024). Retrospective study on the distribution of hemoglobinopathies in Karnataka—A laboratory experience. Indian Journal of Pathology and Microbiology, 67(3), 585–591. https://doi.org/10.4103/ijpm.ijpm_893_22
8. Vijian, D., Rahman, W. S. W. A., Ponnuraj, K. T., Zulkafli, Z., & Noor, N. H. M. (2021). Molecular Detection of alpha Thalassemia: A Review of Prevalent Techniques. Medeniyet Medical Journal, 36(3), 257–269. https://doi.org/10.5222/mmj.2021.14603
9. Keikhaei, B., Bitaraf, S., Mafakher, L., Galehdari, H., Saki-Malehi, A., & Bahadoram, M. (2024). Hematologic parameters cut-off assessment of adult alpha-thalassemia patients in Iran. International Journal of Hematology-Oncology and Stem Cell Research, 18, 2148–2152.
10. Leixner, G., A. Voill-Glaninger, J. Gaugeler-Kurzweil, T. Kusstatscher and T. Panic-Jankovic. 2021. Molecular detection of alpha-thalassemia: Evaluation of a reverse hybridization strip assay for common α-globin gene mutations. Medeni Medical Journal, 36(3): 257–269. https://doi.org/10.5222/mmj.2021.14603