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Community-Based Screening for Heterozygous α^0-Thalassemia (--SEA and --THAI Deletions) Using Multiplex Gap-PCR in Pathum Thani Province

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Abstract:

Introduction: Alpha-thalassemia is a significant public health problem in Thailand, particularly the Southeast Asian (--SEA) deletion and the THAI deletion (--THAI), which are major causes of heterozygous α^0-thalassemia and may lead to hemoglobin Bart's hydrops fetalis, a life-threatening condition in fetuses.

Objectives: This study aimed to determine the prevalence of heterozygous α^0-thalassemia due to --SEA and --THAI deletions in a community population and to evaluate its association with hematological parameters.

Materials and Method: A cross-sectional study was conducted on 95 individuals (aged ≥18 years) residing in Pathum Thani Province. Leftover EDTA blood samples from routine health check-ups were tested for α^0-thalassemia (--SEA and --THAI deletions) using multiplex gap-PCR. Hemoglobin (Hb) levels and red blood cell indices were obtained from complete blood counts. Anemia was defined by World Health Organization (WHO) criteria (Hb < 13 g/dL in males, Hb < 12 g/dL in females). Statistical analysis used Fisher's exact test to compare proportions.

Results: Of the 95 participants, 90 (94.7%) had a normal genotype ($\alpha\alpha/\alpha\alpha$), and five (5.3%) were carriers of the --SEA deletion. No --THAI deletion carriers were detected. All individuals carrying the --SEA deletion exhibited anemia with microcytosis, whereas 30% of those with normal genotype were anemic. The association between --SEA carrier status and both anemia and microcytosis was statistically significant ($p < 0.01$).

Conclusion: The heterozygous α^0-thalassemia (--SEA deletion) carrier rate was 5.3% in this community sample. Multiplex gap-PCR proved to be an effective tool for community-based thalassemia carrier screening. These findings provide essential

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baseline data for planning premarital screening programs and allocating resources for genetic counseling in Pathum Thani.

Keywords: Alpha-Thalassemia; Gene Deletion; Multiplex Polymerase Chain Reaction; Genetic Carrier Screening; Prevalence

Introduction

Thalassemia is one of the most common inherited anemias worldwide and represents a major public health challenge in many regions. The World Health Organization (WHO) estimates that approximately 7% of the global population are carriers of thalassemia genes, with around 300,000 infants born each year with severe forms of the disease.[1] Thalassemia not only affects patients' quality of life but also poses a significant barrier to achieving the United Nations Sustainable Development Goals (SDGs), particularly Goal 3: Good Health and Well-being. Prevention and control of genetic disorders such as thalassemia are directly linked to reducing infant mortality, improving maternal health, and alleviating the economic burden on families and healthcare systems.

Thailand is located within the global "thalassemia belt," resulting in a high carrier prevalence, with approximately 30–40% of the Thai population carrying at least one thalassemia gene. Combinations of these abnormal genes give rise to more than 60 thalassemia syndromes with varying clinical severity, ranging from asymptomatic conditions to fatal diseases.[2] Consequently, the Thai government has incorporated thalassemia into the National Thalassemia Prevention and Control Program, aiming to reduce the incidence of three severe forms: homozygous β-thalassemia, β-thalassemia/Hb E disease, and hemoglobin Bart's hydrops fetalis.[3] The core strategy of this program involves carrier screening in pregnant women and their partners using preliminary hematological tests such as mean corpuscular volume (MCV) and the dichlorophenolindophenol (DCIP) test for hemoglobin E (Hb E).[4]

Alpha-thalassemia is the most prevalent form of thalassemia in Thailand, with an average carrier frequency of 20.1%, one of the highest in Southeast Asia.[5] The disease results from deletions or mutations of the α-globin genes located on chromosome 16, leading to reduced or absent α-globin chain production.[6] The most severe condition, homozygous α^0-thalassemia, occurs when both parents are α^0-thalassemia carriers, resulting in a 25% chance of having a fetus with hemoglobin Bart's hydrops fetalis (––/––), which is usually fatal in utero or shortly after birth. This condition also increases the risk of serious maternal complications such as preeclampsia and postpartum hemorrhage.[7]

Reported frequencies of α^0-thalassemia deletions in Thailand vary by region and study population but consistently show that the Southeast Asian deletion (--SEA) is the predominant α^0-thalassemia determinant, whereas the Thai deletion (--THAI) is distinctly uncommon. In a 7-year screening study from Ramathibodi Hospital, Bangkok, involving 31,632 blood samples, the overall α-thal-1 carrier rate was 14.40%, comprising --SEA 14.21% and --THAI 0.18%.[6] Similarly, a large referral-center study from northeast Thailand analyzing 12,525 specimens found α^0-thalassemia alleles in 15.0% of samples, including 14.8% with the --SEA deletion and 0.2% with the --THAI deletion.[8] In northern Thailand, heterozygous --SEA was reported in 12.23% of 638 pregnant women, while a

population-based ethnic survey found --SEA in 3.5% and no --THAI carriers.[9]

Although national data on thalassemia prevalence in Thailand are well documented, particularly in northern regions with high prevalence[10], community-level data from metropolitan provinces such as Pathum Thani remain limited. Pathum Thani is a semi-urban province within the Bangkok metropolitan area, characterized by population migration from all regions of the country, resulting in high genetic diversity. Thus, this area may better reflect the genetic structure of modern Thai society compared with more genetically homogeneous rural areas. Previous community health screening in Sam Khok District, conducted by the Faculty of Allied Health Sciences, Pathum Thani University, reported an anemia prevalence of up to 20% (unpublished data, 2024), indicating the need for further investigation, particularly regarding thalassemia carrier status.

Preliminary screening using red blood cell indices is sensitive but lacks specificity and may yield false-positive results due to conditions such as iron deficiency.[4] Therefore, molecular confirmation is essential. This study employed the multiplex gap-PCR technique because of its high efficiency in simultaneously detecting --SEA and --THAI deletions in a single reaction, with high sensitivity and specificity (>95%), simple procedures, and relatively low cost compared with other molecular methods, making it particularly suitable for large-scale community screening.[11]

Objectives

1. To determine the prevalence and 95% confidence interval of heterozygous α^0-thalassemia caused by --SEA and --THAI deletions in a sample population from Pathum Thani Province.

2. To analyze the association between α^0-thalassemia carrier status and hematological parameters, particularly anemia and microcytosis.

3. To provide baseline data to support public health policy planning, population-specific genetic counseling services, and the development of effective community-based thalassemia prevention strategies in Pathum Thani Province.

Materials and Method

Study Design and Ethical Approval

This study was a cross-sectional descriptive study. The research protocol was reviewed and approved by the Human Research Ethics Committee of Pathum Thani University (Approval No. PTU-IRB-CERT-CL-2025-030; approved on 11 August 2025). Only leftover blood samples from routine health check-ups were used, and all personal identifiers were removed.

Study Population and Sample Size

The study population consisted of individuals aged 18 years and older residing in Sam Khok District, Pathum Thani Province, who participated in an annual health check-up program. Inclusion criteria were consent to use leftover EDTA blood samples and no history of blood transfusion within three months prior to testing. Exclusion criteria included hematological disorders affecting erythropoiesis (e.g., aplastic anemia, myelodysplastic syndrome) and insufficient or poor-quality DNA samples.

Sample size was calculated using a prevalence estimation formula based on a reported α-thalassemia carrier prevalence of 22.6% in Southeast Asia (Goh et al., 2020), with a 95% confidence level ($\alpha = 0.05$) and an allowable error (d) of 0.08. The minimum required sample size was approximately 90 participants. Blood samples were stored at 4°C until DNA extraction.

Study Area

Sam Khok District, Pathum Thani Province, was selected due to its semi-urban characteristics and ethnically diverse population, reflecting the overall demographic structure of the province. Previous community health screening reported a high anemia prevalence of 20% (unpublished data, 2024), underscoring the importance of genetic disease surveillance in this area.

Hematological Analysis

Hematological parameters, including hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), were obtained from routine complete blood count analysis using an automated hematology analyzer. Anemia was defined according to WHO criteria (Hb < 13 g/dL in males and < 12 g/dL in females), and microcytosis was defined as MCV < 80 fL.[12]

DNA Extraction

Genomic DNA was extracted from 300 μL of EDTA blood using a commercial DNA extraction kit (Favorgen Biotech Corp., Taipei, Taiwan). DNA concentration and purity were measured using a spectrophotometer (PerkinElmer, MA, USA) at 260 nm. DNA samples with concentrations of 25–50 ng/μL were stored at –20°C until analysis.

Detection of α^0-Thalassemia (--SEA and --THAI Deletions) by Multiplex Gap-PCR

Multiplex gap-PCR was performed using KAPA 2G Fast HotStart ReadyMix with dye (Kapa Biosystems, USA). Primer sequences were based on.[13] PCR amplification was conducted using a PCRmax thermal cycler under standard cycling conditions. PCR products were analyzed by 2% agarose gel electrophoresis and interpreted according to band size.

Quality Control and Interpretation

Each PCR run included positive controls for --SEA/αα, --THAI/αα, a normal genotype (αα/αα), and a no-template negative control. Band sizes were interpreted as follows: normal genotype (1010 bp), --SEA deletion (660 bp), and --THAI deletion (410 bp).

Statistical Analysis

Data were analyzed using statistical software. Descriptive statistics were used to summarize demographic data and carrier prevalence with 95% confidence intervals. Fisher's exact test was applied to compare categorical variables (e.g., proportions of anemia and microcytosis between groups). A p-value < 0.05 was considered statistically significant.

Results

A total of 95 participants were included in the study, comprising 73 females (76.8%) and 22 males (23.2%), with a mean age of 45.9 years. The demographic characteristics and hematological indices of the participants stratified by genotype. Carriers of the --SEA deletion had markedly lower mean hemoglobin (10.6 g/dL vs 12.9 g/dL) and hematocrit (34.6% vs 40.1%) compared to those with a normal genotype, reflecting anemia. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were also substantially lower in --SEA carriers (58.8 fL and 19.8 pg, respectively) than in individuals with normal genotype (87.5 fL and 28.1 pg), indicating pronounced microcytosis in the carrier group (Table 1). Genotypic analysis by multiplex gap-PCR identified 90 individuals (94.7%) with the normal genotype (αα/αα) and five individuals (5.3%) as heterozygous --SEA deletion carriers (--SEA/αα). No carriers of the --THAI deletion were detected in this population (Table 2). Figure 1 illustrates

the multiplex gap-PCR results: all samples from --SEA deletion carriers exhibited the expected 660 bp deletion band in addition to the normal 1010 bp band, whereas samples with the normal genotype showed only the 1010 bp band.

Table 1 Demographic and hematological characteristics of participants by α-thalassemia genotype (normal vs. --SEA deletion carrier).

Characteristics	Normal Genotype (αα/αα) (n = 90)	--SEA Deletion Carrier (--SEA/αα) (n = 5)
Sex (Male/Female)	21/69	1/4
Mean age (years)	45.8	47.6
Mean Hb (g/dL)	12.9	10.6
Mean Hct (%)	40.1	34.6
Mean MCV (fL)	87.5	58.8
Mean MCH (pg)	28.1	19.8

Table 2 Frequency distribution of α^0-thalassemia genotypes in the study population (N = 95).

Genotype	Number of individuals	Percentage (%)
αα/αα	90	94.7
--SEA/αα	5	5.3
--THAI/αα	0	0
Total	**95**	**100**

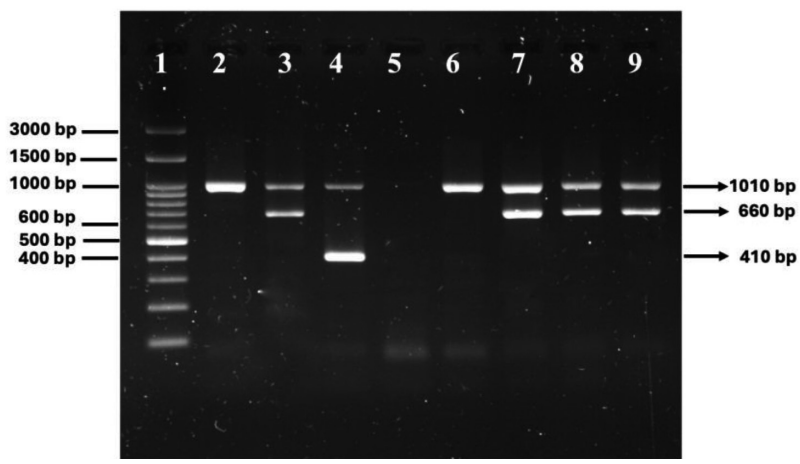

Figure 1 Multiplex gap-PCR results for detection of α^0-thalassemia (--SEA and --THAI deletions). Lane 1: 100 bp DNA ladder; Lane 2: normal genotype (αα/αα), showing a 1010 bp band; Lane 3: heterozygous --SEA deletion carrier (--SEA/αα, positive control), showing 1010 bp and 660 bp bands; Lane 4: heterozygous --THAI deletion carrier (--THAI/αα, positive control), showing 1010 bp and 410 bp bands; Lane 5: negative control (no DNA template); Lane 6: study sample with normal genotype (αα/αα); Lanes 7–9: study samples identified as heterozygous --SEA deletion carriers (--SEA/αα).

In this study, all five --SEA deletion carriers were anemic (100%), whereas among the 90 individuals with normal genotype, 27 (30.0%) had anemia and 63 (70.0%) were non-anemic (Table 3). This difference in anemia prevalence between carriers and non-carriers was statistically significant ($p = 0.0035$). Similarly, all --SEA carriers (100%) had microcytic red blood cells (MCV < 80 fL), compared to 21 (23.3%) of the normal-genotype group (Table 4). The association between --SEA carrier status and microcytosis was also significant ($p = 0.0011$). Overall, the presence of a heterozygous --SEA deletion was strongly correlated with increased risk of anemia and microcytosis in this population ($p < 0.01$).

Table 3 Anemia status of participants by α-thalassemia genotype.

Genotype	Anemic n (%)	Non-anemic n (%)	Total (N)
αα/αα	27 (30.0)	63 (70.0)	90
--SEA/αα	5 (100)	0 (0)	5
Total	**32 (33.7)**	**63 (66.3)**	**95**

Note: Anemia defined as Hb < 13 g/dL in males and Hb < 12 g/dL in females (WHO criteria). *p*-value (Fisher's exact test) = 0.0035 for difference in anemia prevalence between genotypes.

Table 4 Prevalence of microcytosis (MCV < 80 fL) among participants by α-thalassemia genotype.

Genotype	Microcytic RBC n (%)	Non-microcytic RBC n (%)	Total (N)
--SEA/αα	5 (100)	0 (0)	5
αα/αα	21 (23.3)	69 (76.7)	90
Total	**26 (27.4)**	**69 (72.6)**	**95**

Note: Microcytosis defined as MCV < 80 fL. *p*-value (Fisher's exact test) = 0.0011 for difference in microcytosis between genotypes.

Discussion

In this community-based study in Pathum Thani, the prevalence of heterozygous α^0-thalassemia (--SEA deletion) carriers was 5.3%, and no --THAI deletion carriers were identified. This finding reflects the genetic distribution in central Thailand, where the --SEA deletion is known to be the predominant α-thalassemia mutation.[11] All carriers in our study had anemia and microcytosis, highlighting the clear hematological impact of losing one pair of α-globin genes. The differences in hemoglobin level and red cell indices between carriers and non-carriers were statistically significant, consistent with reports that even a single α^0-thalassemia deletion can cause notable hematologic changes.[1,14]

The absence of --THAI deletion carriers in this sample may be explained by the limited geographic distribution of the --THAI mutation, which is restricted to certain areas of central and southern Thailand.[3,15] Additionally, the sample size and the fact that participants were drawn

from a single district could limit the detection of such a rare mutation. Regional variations in α-thalassemia prevalence have been documented; while the average α-thalas semia carrier rate in Thailand is about 20%[5], the rate can vary widely between different provinces and ethnic groups. The carrier rate of 5.3% observed in Pathum Thani is lower than the national average, potentially due to the province's diverse population and the presence of other genetic factors. Notably, all --SEA carriers in our study had only mild-to-moderate anemia. This raises the possibility of concurrent factors such as iron deficiency or co-inheritance of other hemoglobinopathies (e.g., Hb E trait), which are common in the Thai population and could influence the hematological presentation.[2,16]

The application of multiplex gap-PCR proved to be practical and effective for community screening. This technique can simultaneously detect multiple α-globin gene deletions (--SEA, --THAI, and others) in a single test, with high sensitivity and specificity (over 95%) [11]. Our findings support previous studies[11,17] that demonstrated multiplex PCR as a reliable method comparable to conventional PCR for thalassemia mutation detection, yet more efficient for screening purposes. The ability to use standard laboratory equipment without requiring advanced technology makes multiplex gap-PCR especially suitable for widespread screening in resource-limited settings.

The limitation of this study is relatively small sample size and the focus on only two deletional mutations, which may not capture all forms of α-thalassemia carriers in the community. Nevertheless, the data provide a valuable snapshot of carrier prevalence in a semi-urban Thai population and underscore the importance of proactive screening. Our results are in line with current recommendations that community-level carrier screening and genetic counseling are crucial for preventing new cases of severe thalassemia.[18] Identifying carrier couples before or during early pregnancy can effectively prevent the birth of infants with hemoglobin Bart's hydrops fetalis, a condition that is incompatible with life and poses serious risks to maternal health.[7,18] These findings reinforce the need to integrate molecular thalassemia screening into existing public health programs, such as premarital check-ups and antenatal care, as advocated by the National Thalassemia Prevention and Control Program.[2,19]

From a broader public health perspective, implementing community-based thalassemia carrier screening using multiplex PCR is a cost-effective approach to reduce the burden of severe thalassemia. Such screening can be expanded province-wide or nationally, for example, by incorporating it into routine premarital or preconception health screening programs supported by the Ministry of Public Health. Further studies on the cost-effectiveness of this strategy are warranted to confirm the long-term benefits, including reduced healthcare expenditures for thalassemia patient care and improved quality of life for at-risk populations.[4]

Conclusion

In this community sample, the carrier frequency of heterozygous α^0-thalassemia due to the --SEA deletion was 5.3%, while no --THAI carriers were detected. Multiplex gap-PCR was feasible for community-based screening in this setting. Integration of this molecular screening into public health initiatives could help prevent severe thalassemia cases and inform targeted genetic counseling strategies.

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