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# Lessons learned from a prenatal diagnosis program for thalassemia in Thailand

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## Abstract

**Objective:** To assess the outcome of a thalassemia screening program at community hospitals by determining the proportion of at-risk couples able to obtain a prenatal diagnosis (PND) in relation to gestational age (GA).

**Methods:** We accessed records documenting prenatal screening for thalassemia in lower northern Thailand between January 2014 and December 2016. The proportion of at-risk pregnancies able to obtain a PND was determined and median GAs at the time of at-risk notification were compared. Reasons for failures to obtain PNDs were analyzed.

**Results:** Among 4633 screen-positive couples, 259 (5.6%) were identified as at-risk while 23 were excluded due to unconfirmed outcomes. Forty-one declined a PND and were excluded from the final calculations. Of the 195 remaining couples, 140 (71.8%) obtained a PND. Their median GA at the time of at-risk notification was 12.4 (5.6-29.1) weeks, which was earlier than the median GA of 17.7 (6.9-34.6) weeks for couples not undergoing PND ($P < .001$). Risks for various types of thalassemia and GA were associated with the chances of achieving a PND.

**Conclusion:** In practice, one quarter of couples identified as at-risk were unable to obtain a PND. Time-influencing factors seem to be a major determinant.

## 1 | INTRODUCTION

Thalassemia is a common genetic condition in Southeast Asian countries including Thailand. Screening and determination of at-risk couples together with prenatal diagnosis (PND) protocols for affected fetuses constitute practical strategies currently in use. For β-thalassemia, mass screening based on hemoglobin (Hb) $A_2$ fraction and mutation analysis is the preferred method to identify at-risk pregnancies.[1] For $\alpha^0$-thalassemia, screening via an osmotic fragility test (OFT) or the assessment of mean corpuscular volume (MCV) and diagnosis based on DNA methods are presently conducted.[2,3] In Thailand, the prevalence of couples at risk for Hb E (HBB:c.79G > A)/β-thalassemia, homozygous β-thalassemia, and homozygous $\alpha^0$-thalassemia is as high as 1.5% to 2.2% among all pregnancies.[3,4] Presently, carrier screening to determine at-risk couples is included in a routine package of antenatal examination for every pregnant Thai woman and her spouse. However, the efficacy of thalassemia screening programs can be difficult to estimate in practice.

Although elective abortion of affected fetuses is one outcome, this outcome index may not reflect the intended mission of the program that aims to provide parents with options. Certain at-risk couples may choose preimplantation genetic diagnosis, with the majority of at-risk pregnancies requiring PND. Finally, some couples may accept the diagnosis of an affected fetus but still wish to continue the pregnancy. Therefore, the number of at-risk couples identified or rates of PND conducted represent superior measures of the efficacy of the program. In the present study, we chose to study the rate of PND as an outcome measure. A web-based national registry tracks pregnancies participating in the thalassemia prenatal screening and diagnosis program provided by the National Health Security Office in Thailand. Based on information gathered from this database, there were 1113 PNDs (most likely invasive PND only) conducted among 2340 at-risk

  

couples (47.6%) in 2017.[5] This metric reveals that the accessibility rate is less than half of the at-risk couples identified, which is unacceptable compared with previous studies.[2,3] However, the number of at-risk couples in the database is still far below the estimated number (12 000-17 600 at-risk couples each year calculated from 800 000 annual pregnancies),[3,4,6] which stands to diminish the accuracy of the data representing the entire country. Therefore, the newly developed web-based system requires far more reliable information. To the best of our knowledge, there are two citable reports regarding the rate of PND for thalassemia specifically from Thailand. The outcomes were impressive, ranging between 78.1% (756 invasive PNDs conducted out of 968 at-risk couples) for an experienced referral center in northeastern Thailand and 97.2% (273 [31 by serial ultrasonography] PNDs conducted out of 281 at-risk couples) for a prenatal screening model implemented in six tertiary care centers.[2,3] Of note, these two studies were conducted on well-designed screening programs by experienced leading referral centers in Thailand.

Several factors were reported to have a negative impact on each step of the prenatal screening for thalassemia; among these, delayed antenatal care (ANC) stands out as a major determinant.[7-9] Given that each step in the pathway requires considerable time before the final outcome is obtained, early access to ANC is highly recommended. After at-risk notification, PND can be performed as early as 10 weeks of gestational age (GA) by chorionic villous sampling (CVS), while amniocentesis and cordocentesis are recommended as early as 16 and 18 weeks, respectively.[10] However, only half of the pregnancies in Thailand begin their ANC before 12 weeks.[11,12] Due to delayed investigation of at-risk pregnancies, the service might not provide couples with sufficient time to make an informed decision about PND[7] or it may be too late for obstetricians to offer PND at later GAs.[10] Thus, we chose to focus on the GA of pregnancies in accessing PND for analysis in our investigation.

Most Hb E/$\beta^+$-thalassemia individuals have a mild phenotype. Therefore, a certain number of couples at risk for Hb E/$\beta^+$-thalassemia do not typically require PND.[13] However, before their mutation analysis results were revealed, their initial outcomes identified them as Hb E/β-thalassemia at-risk couples. Couples at risk for Hb E/$\beta^+$-thalassemia commonly decline PND because of the milder phenotype; however, some may still choose to proceed with a PND.

The objective of this study was to determine the proportion of at-risk couples identified at community hospitals who were able to obtain a PND of their fetuses while assessing the reasons PNDs were not conducted for other couples in relation to their GAs in a prenatal diagnosis program for thalassemia carried out in lower northern Thailand.

**What is already known about this topic?**

- Given the considerable time commitment required for thalassemia prenatal screening and diagnosis programs, several studies have reported that delayed antenatal care had a negative impact on the ultimate goal. However, no studies have thoroughly demonstrated how differences in the delay affected the success and failure groups.

**What does this study add?**

- The current study determined the accessibility to prenatal diagnosis (PND) for at-risk couples in relation to gestational age (GA).
- Differences in GA were compared between the groups that obtained a PND and those that did not and among subgroups in detail.

## 2 | METHODS

The Thalassemia Research Unit at Naresuan University Hospital, Phitsanulok, was established to conduct a screening program for thalassemia in pregnant women and their spouses with the involvement of 42 community hospitals in lower northern Thailand. As part of the routine workflow, blood specimens were drawn from each couple in the initial screening for carriers (OFT or MCV) at community hospitals. A positive result in a husband or wife (single-positive) or a double-positive result (in both spouses) was sent to the Thalassemia Research Unit in paired samples for further investigation. High performance liquid chromatography (HPLC; VARIANT) was performed on all specimens to detect β-thalassemia and Hb E phenotypes using the β-thalassemia Short Program (Bio-Rad Laboratories, Hercules, CA, USA). Real-time polymerase chain reaction (PCR) with high-resolution DNA melting (HRM) analysis was conducted to confirm homozygous β-thalassemia and Hb E/β-thalassemia risk. The PCR technique to detect the $\alpha^0$-thalassemia (Southeast Asian and Thai deletions) genotype was performed only when both spouses were positive at screening. Based on the results of these investigations, at-risk and non-risk couples were determined, and their results were returned to their community care providers. After genetic counseling at their community hospitals, at-risk couples were referred to a provincial hospital for an invasive procedure and the specimens forwarded to our institute or other tertiary care centers for analysis. PNDs were conducted using chorionic villous tissue, amniotic fluid, or fetal blood. A certain number of pregnancies at risk for Hb Bart's hydrops fetalis were followed clinically with serial ultrasonography for their PND. Real-time PCR with HRM analysis to detect β-thalassemia or $\alpha^0$-thalassemia mutations was performed on chorionic villous tissue, amniotic fluid, or fetal blood specimens as previously described.[14] HPLC was conducted on fetal blood to diagnose fetal phenotype.[15]

Prenatal screening and diagnosis results recorded between January 2014 and December 2016 were reviewed. Pregnancies without documentation of GA were excluded. All at-risk pregnancies were identified. Proportions of at-risk couples able and unable to obtain a PND were determined. All at-risk pregnancies with and without PND

results were tracked back to their community care providers via e-mail or telephone to confirm their PND outcomes—possibly determined at different locations—while pregnancies without end results were excluded. Median GAs and ranges at the time of at-risk notification were analyzed and compared between the PND-able and -unable groups and among subgroups and the total number of at-risk couples (Mann–Whitney *U* test). The Chi-squared test and logistic regression models were used to determine the relationship between potential factors and the chances of obtaining a PND. A *P*-value less than .05 was set as the level of statistical significance. All data analyses were performed using SPSS (Statistical Package for the Social Sciences) software Version 17.0 (SPSS Inc., Chicago, IL). The study was approved by the institutional ethics committee of Naresuan University (approval number 0540/61).

## 3 | RESULTS

The 4633 screen-positive couples who had GA records were reviewed. Their median GA was 12.4 (2.0-41.3) weeks when their blood samples were processed for risk determination at the Thalassemia Research Unit with the return of results at 13.9 (3.1-42.1) weeks. There were 259 (5.6%) at-risk couples identified, with 154 (59.5%) at risk for Hb E/β-thalassemia, 95 (36.7%) for homozygous $\alpha^{0}$-thalassemia, and 10 (3.9%) for homozygous β-thalassemia. Twenty-three at-risk couples (8.9%) without data on their final outcomes (including six early abortions) were excluded, leaving 236 couples for evaluation. Of these, the median GA for receipt of risk results was 13.7 (5.6-34.6) weeks.

Of these 236 at-risk couples, 140 were able to obtain a PND (59.3%). Their median GA at the time of at-risk notification was 12.4 (5.6-29.1) weeks, which was significantly earlier than the 17.7 (6.9-34.6) weeks for the group unable to obtain a PND ($P < .001$). However, of the 96 that did not obtain a PND, 22 declined a PND due to a final diagnosis of Hb E/$\beta^{+}$-thalassemia risk, and 19 were offered a PND but declined. Given that these couples did not undergo a PND because of a milder diagnosis or invasive testing—inclusion of which might not truly reflect the program's efficacy—we excluded them from further analysis, leaving 140 of 195 couples who underwent PND (71.8%).

Of the 140 couples who obtained a PND, their median GA at the time of at-risk notification was earlier than that of the total number of at-risk couples ($P = .007$) (Table 1). The relationship between PND method and GA at the time of at-risk notification is shown in Table 2. Despite a late at-risk result notification at 26.4 weeks, one homozygous $\alpha^{0}$-thalassemia at-risk woman still underwent an amniocentesis. Median GAs at the time of fetal tissue sampling and PND result notification calculated from the data of 96 available cases were 18.0 (14.0-31.0) weeks and 20.1 (16.3-31.1) weeks, respectively, indicating that after the risk identification process, approximately 6 weeks remained for a couple to obtain a PND with an additional 2 weeks before receiving the outcome (Table 2). For the eight Hb E/$\beta^{+}$-thalassemia at-risk pregnancies whose mothers chose to proceed with a PND, their $\beta^{+}$ mutations in *trans* to the codon 26 G>A mutation of Hb E (HBB:c.79G>A) consisted of six nucleotide (nt) -28 A>G (HBB:c.-78A>G), one IVS-II nt 654 C>T (HBB:c.316-197C>T), and one Hb Tak (HBB:c.441_442insAC). Of the 140 pregnancies undergoing PND, there were 45 (32.1%) affected fetuses; of these, 36 women decided to terminate their pregnancies. There were 28 homozygous $\alpha^{0}$-thalassemia and eight Hb E/$\beta^{0}$-thalassemia fetuses terminated. Five couples who had a fetus affected by Hb E/$\beta^{+}$-thalassemia (four nt −28 A>G and Hb E and one IVS-II nt 654 C>T and Hb E) and four with Hb E/$\beta^{0}$-thalassemia [compound heterozygote between Hb E and: one codon 17 A > T (HBB:c.52A>T); one codon 41/42-TTCT (HBB:c.126_129delCTTT); and two unknown $\beta^{0}$-mutations] continued their pregnancies. The median GA at the time of PND result notification was 19.9 (17.3-21.3) weeks, which was the same for the 36 who decided to terminate their pregnancies at 20.4 (17.0-31.1) weeks ($P = .281$).

In this study, 96 at-risk couples (40.7%) were unable to obtain a PND. There were 41 couples who declined PND including 22 with a final diagnosis of Hb E/$\beta^{+}$-thalassemia risk (Table 1). Of the

**TABLE 1** Study outcome

| Group | Subgroup | Hb E/$\beta^{+}$ at-risk couple | Hb E/$\beta^{0}$ at-risk couple | Homozygous β at-risk couple | Homozygous $\alpha^{0}$ at-risk couple | Total | GA[a](week) | | |
|---|---|---|---|---|---|---|---|---|---|
| PND-able 140/236 (59.3%) | | 8 | 61 | 3 | 68 | 140 | 13.7 (5.6–34.6) | 12.4 (5.6–29.1) ($P = .007$*) | |
| PND-unable 96/236 (40.7%) | Late presentation and unknown reasons 55/96 (57.3%) | 0 | 39 | 2 | 14 | 55 | | 17.7 (6.9–34.6) ($P < .001$)* | 19.7 (7.9-34.6) ($P < .001$)* |
| | Declined 41/96 (42.7%) | 22 | 11 | 1 | 7 | 41 | | | 14.1 (6.9-25.1) ($P = .943$) |
| Total | | 30 | 111 | 6 | 89 | 236 | | | |

*Note*: *P*-value as compared with the median gestational age of the total at-risk couples using Mann-Whitney *U* test. *Significant difference ($P < .05$).
Abbreviations: GA, gestational age; Hb, hemoglobin; PND, prenatal diagnosis.
[a]Median gestational age and range at the time of at-risk notification.

**TABLE 2** Relationship between prenatal diagnosis method and gestational age

| PND method | Number (%) | GA[a] at the time of at-risk notification (week) | GA[a] at the time of fetal specimen sampling[b] (week) | GA[a] at the time of PND result notification[b] (week) |
|---|---|---|---|---|
| Amniocentesis | 102 (72.9) | 12.0 (5.6-26.4) | 18.0 (14.0-23.0) | 20.1 (16.3-25.0) |
| Cordocentesis | 17 (12.1) | 16.3 (11.9-29.1)* | 26.5 (17.0-31.0) | 26.9 (17.0-31.1) |
| Serial ultrasonography | 4 (2.9) | 12.1 (10.6-16.7) | — | — |
| Chorionic villous sampling | 1 (0.7) | 11.4 | — | — |
| Unknown | 16 (11.4) | 11.9 (5.7-22.1) | 17.0[c] | 18.6[c] |
| Total | 140 (100) | 12.4 (5.6–29.1) | 18.0 (14.0-31.0) | 20.1 (16.3-31.1) |

*Note*: *Significant difference ($P < .05$) as compared with the median gestational age of the total couples able to obtain a prenatal diagnosis using Mann-Whitney *U* test.
Abbreviations: GA, gestational age; PND, prenatal diagnosis.
[a]Median gestational age and range.
[b]Values calculated from 96 available cases.
[c]Raw data of one available case.

**TABLE 3** Analyses of the relationship between potential factors and a chance to obtain a prenatal diagnosis

| | Univariate analysis[a] | | | Multivariate analysis[b] | | |
|---|---|---|---|---|---|---|
| Factors | Odds ratio | 95% CI | *P* value | Odds ratio | 95% CI | *P* value |
| Gestational age | 0.85 | 0.81-0.90 | <.001[c]* | 0.80 | 0.75-0.86 | <.001* |
| Hb E/$\beta^+$-thalassemia risk | 0.18 | 0.07-0.43 | <.001* | 0.11 | 0.04-0.31 | <.001* |
| Hb E/$\beta^0$-thalassemia risk | 0.73 | 0.43-1.23 | .239 | — | — | — |
| Homozygous β-thalassemia risk | 0.68 | 0.13-3.44 | .638 | — | — | — |
| Homozygous $\alpha^0$-thalassemia risk | 3.37 | 1.88-6.06 | <.001* | 2.93 | 1.31-6.54 | .009* |
| PND decline | 0.02 | 0.01-0.03 | <.001* | — | — | — |

*Note*: *Significant difference ($P < .05$).
Abbreviation: CI, confidence interval; Hb, hemoglobin; PND, prenatal diagnosis.
[a]Chi-square test.
[b]Multivariate logistic regression analysis: Backward Stepwise - likelihood ratio method.
[c]Simple logistic regression analysis.

55 remaining couples, late gestation as judged by obstetricians was reported in 28 couples as the reason for not proceeding with PND. Their median GA at the time of at-risk notification was 23.6 (10.6-34.6) weeks, far beyond the 17.3 (7.9-31.1) weeks of the remaining 27 with unconfirmed reasons ($P = .002$). Nine couples received their at-risk results before 20 weeks; however, due to their late decisions or administrative problems, they did not see their obstetricians until after 20 weeks—too late for a PND. For the 27 remaining couples, no reasons were documented for not obtaining a PND, although their median GA of 17.3 weeks was advanced compared with all 236 at-risk couples at 13.7 weeks, but without statistical significance ($P = .059$). Therefore, of those not undergoing PND, late presentation (29.2%) and a diagnosis of Hb E/$\beta^+$-thalassemia risk (22.9%) were identified as the two most common causes of failure to obtain a PND.

Multivariate analysis showed that among all potential factors (GA, types of thalassemia risk, and PND decline), increasing GA and Hb E/$\beta^+$-thalassemia risk were associated with a decreased likelihood of obtaining a PND [odds ratio 0.80 ($P < .001$) and 0.11 ($P < .001$), respectively]. In contrast, homozygous $\alpha^0$-thalassemia risk exhibited the opposite relationship [odds ratio 2.93 ($P = .009$)] (Table 3).

Information for the 21 homozygous $\alpha^0$-thalassemia at-risk couples who were reported not to have obtained a PND (including seven who declined) was not concordant with clinical practice. In actual clinical situations wherein couples at risk for Hb Bart's hydrops fetalis decide not to proceed with an invasive PND, their obstetricians would most likely continue to monitor the fetuses by ultrasonography. The report regarding 21 pregnancies at risk for homozygous $\alpha^0$-thalassemia that were not able to obtain a PND was likely the result of miscommunication. Therefore, we performed a reanalysis after excluding the 89 homozygous $\alpha^0$-thalassemia at-risk couples. The overall outcomes were not substantially different except for the rate of PND (Table 4). Among the remaining 147 at-risk couples, 72 were able to proceed with PND (49.0%). After excluding the 34 who

**TABLE 4** Study outcome after excluding homozygous $\alpha^0$-thalassemia at-risk couples

| Group | Subgroup | Hb E/$\beta^+$ at-risk couple | Hb E/$\beta^0$ at-risk couple | Homozygous β at-risk couple | Total | GA[a](week) | | |
|---|---|---|---|---|---|---|---|---|
| PND-able 72/147 (49.0%) | | 8 | 61 | 3 | 72 | 14.0 (5.6-34.3) | 12.7 (5.6-22.1) ($P$ = .006*) | |
| PND-unable 75/147 (51.0%) | Late presentation and unknown reasons 41/75 (54.7%) | 0 | 39 | 2 | 41 | | ($P$ = .007*) 18.4 (7.0-34.3) | 21.3 (7.9-34.3) ($P$ < .001*) |
| | Declined 34/75 (45.3%) | 22 | 11 | 1 | 34 | | | 15.1 (7.0-25.1) ($P$ = .832) |
| Total | | 30 | 111 | 6 | 147 | | | |

*Note*: $P$-value as compared with the median gestational age of the total at-risk couples using Mann–Whitney $U$ test. *Significant difference ($P$ < .05).
Abbreviations: GA, gestational age; Hb, hemoglobin; PND, prenatal diagnosis.
[a]Median gestational age and range at the time of at-risk notification.

declined PND, the rate of a PND for β-thalassemia risk was 63.7% (72 of 113 couples).

## 4 | DISCUSSION

The proportion of at-risk pregnancies identified in the present study was falsely elevated due to its calculation among screen-positive couples. For our final outcome, more than half of the overall at-risk couples and half of the β-thalassemia at-risk pregnancies were able to obtain a PND, which is comparable with the national web-based registry outcome. However, given that certain factors causing failure to proceed with PND might not reflect the true efficacy of the screening program, we chose to exclude Hb E/$\beta^+$-thalassemia at-risk couples who declined a PND and non-Hb E/$\beta^+$-thalassemia at-risk couples with PND refusal from our PND rate calculation, which would raise our outcome to a more acceptable rate of three-fourths of the total at-risk pregnancies and approximately 60% of the β-thalassemia at-risk couples. Our results demonstrated a pattern of PND outcome in clinical practice at the community hospital level in lower northern Thailand. Furthermore, the possibility exists that these outcomes can be replicated in other parts of the country due to their concordance with the national database.

For the 96 couples who did not undergo PND, we excluded 41 couples who declined PND from the calculations. However, after analysis of the reasons for failure to obtain a PND, the majority of these 41 couples demonstrated a unique $\beta^+$-thalassemia mutation with a milder phenotype; furthermore, the group had a different median GA from the other 55 couples unable to obtain a PND (14.1 weeks vs 19.7 weeks, $P$ < .001), indicating their tendency toward early-onset PND refusal. Therefore, we chose to segregate these 41 couples who declined the PND into a so-called "early decline" group separate from the 55 remaining couples unable to receive a PND during the analysis. In addition, for the subgroup of 27 couples not undergoing PND without confirmed reasons within the 55 remaining couples, we conclude that there were some "late declines" in this subgroup whose GAs at the time of at-risk notification ranged between 7.9 and 31.1 weeks.[7]

Given that this study is retrospective, the reasons the couples were unable to obtain a PND are not completely known; however, the analysis suggests that late presentation and a diagnosis of Hb E/$\beta^+$-thalassemia risk are the two most common factors. Approximately one-third of the couples unable to obtain a PND were specifically reported to be too late for a PND as judged by their obstetrician. Regarding the gestational limits of PND for termination of pregnancy in thalassemia, a guideline for prenatal screening and thalassemia diagnosis addressing this issue was recommended by the Royal Thai College of Obstetricians and Gynecologists in 2009.[10] For Hb Bart's hydrops fetalis risk, there is no limitation of GA for a PND; however, for Hb E/β-thalassemia and homozygous β-thalassemia risk, the PND should be performed up until a GA of 22 weeks. Consequently, β-thalassemia at-risk pregnancies with late presentation might preclude the obstetrician from offering a PND in consideration of pregnancy termination. However, late PND may be requested by some couples for specific reasons other than termination of pregnancy. In our GA data of the groups able to obtain a PND, there were pregnancies with late presentation identified by the upper range numbers of GA for each obstetric procedure. Despite the guideline, the data may indicate that in cases of late presentation, a decision to perform a PND is partly dependent on the obstetrician based on various reasons. Some couples may need to prepare for medical or financial support in the near future; some cannot wait until their baby is born.

In the multivariate analysis, one of the reasons for failure to obtain a PND is late attendance of ANC, leading to late presentation for a PND. Among the 140 couples who underwent PND, there was only one couple who obtained a CVS. The very low rate of CVS—the method recommended for an early PND—may reflect the problem of late initiation of prenatal care within the Thai population. Only half of the pregnancies in our study (from 4633 screen-positive couples) began their thalassemia screening within a GA of 12.4 weeks, which is comparable with other studies.[11,12] Unfortunately, this proportion seems to be constant—at least for the past 5 years.[12] Therefore, to improve PND accessibility, a campaign promoting early ANC must be implemented more rigorously than ever with innovative strategies. Regarding diagnosis of thalassemia risk, which was identified by the analysis as an influential factor, presentation of the milder phenotype

of Hb E/$\beta^+$-thalassemia risk may constitute an appropriate reason to decline PND, whereas homozygous $\alpha^0$-thalassemia risk may necessitate PND to prevent serious maternal complications due to preeclampsia resulting from a deceased fetus *in utero*.

One strategy in common with the two successful models in prenatal screening for thalassemia mentioned earlier is to incorporate a late PND method—cordocentesis—in the program. In a northeastern study with a 78.1% PND rate, the proportion of cordocentesis for PND was 22.2%.[2] A high cordocentesis proportion of 44.3% was also reported in a multicenter study with a 97.2% PND accessibility.[3] According to our study results, cordocentesis offers a greater likelihood to achieve a PND as observed in couples who obtained this procedure. Their median GA at the time of at-risk notification of 16.3 (11.9-29.1) weeks was the highest among any PND method ($P$ = .005 using Kruskal–Wallis test), correlating with their median GA at the time of fetal specimen sampling of 26.5 (17.0-31.0) weeks. Therefore, if we are unable to convince mothers of the importance of early prenatal care, only a few choices remain to promote the rate of PND. The first is a strategy to expedite the process of screening and diagnosis of at-risk couples, such as a one-stop service or implementation of a universal diagnostic test without screening, and to shorten the process of referring mothers to obtain a PND after an at-risk notification.[16] Based on our findings, it takes an average of 6 weeks after an at-risk notification is sent until an at-risk couple obtains a PND. This period is critical, and the process being followed may require modifications to expedite the referral steps for obstetricians while also providing couples sufficient time to make an informed decision regarding PND. Another strategy is to improve the obstetric procedures performed in the case of late PND, especially cordocentesis. Since Hb E/β-thalassemia constitutes the majority of at-risk diagnoses (59.5% in the present study), the third strategy is a noninvasive prenatal diagnosis (NIPD) using cell-free fetal DNA from maternal plasma to detect paternal alleles for inclusion or exclusion; this could reduce the number of at-risk couples requiring an invasive procedure.[17] Additionally, some at-risk couples who decline PND may not wish to risk their pregnancies with invasive procedures; this could make NIPD even more beneficial.

As for the limitations of our study, various reasons PND was declined were not completely identified by more effective measures such as in-depth personal interviews, as the study was retrospective. Moreover, the GA of most pregnancies might not be unequivocally confirmed by obstetricians using ultrasonography. Finally, for the exclusion rate of 8.9%, most of these patients missed their follow-up appointments at community hospitals, instead attending other private healthcare facilities.

## 5 | CONCLUSION

Our study clearly demonstrated the accessibility to PND for at-risk couples in relation to GA and showed the pattern of PND outcomes in clinical practice. Despite universal thalassemia screening during pregnancy, one-fourth of at-risk couples at the community hospital level in lower northern Thailand were unable to obtain a PND. Given the considerable time requirements for thalassemia prenatal screening programs, time influencing factors seem to be a major determinant of program efficacy.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author on request.

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