

FALSE POSITIVE RATES OF THALASSEMIA SCREENING IN RURAL CLINICAL SETTING: 10-YEAR EXPERIENCE IN THAILAND

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Abstract. This retrospective study aimed to describe the magnitude of false positive screening for thalassemia in a primary care setting of Thailand. The study was conducted from 1999 to 2008 and analyzed 13,745 positive cases. It involved a combination of one tube osmotic fragility (OF) and dichlorophenol indophenol (DCIP) precipitation tests. The number of cases increased over the ten-year period, corresponding well to an exponential model. Based on hemoglobin and DNA analysis, cases with α -thalassemia 1, β -thalassemia, and hemoglobin E were defined as true positive cases, and the remaining were considered as false positives. The false positive rate was in the range of 20.1-36.1%. The proportion of false positive cases of thalassemia from the screening tests was associated with a trend which was statistically significant ($p < 0.001$). The estimated cost of further hemoglobin analysis resulting from false positive cases was approximately 40.2-72.2 million THB/year for an estimated 800,000 annual births. The combination of the OF and DCIP test, which has been the strategy for screening of thalassemia and HbE in pregnant women throughout this country, resulted in a large economic burden in terms of high cost and workload associated with further hemoglobin and DNA analyses of false positive samples. Measures to reduce false positive should be developed and implemented.

INTRODUCTION

Thalassemias and hemoglobinopathies are common genetic disorders and a serious economic problem in Thailand and other Southeast Asian countries (Weatherall and Clegg, 2001). The prevalence rates of abnormal genes in Thailand are estimated as follows: 30-40% for α -thalassemia, 1-9% for β -thalassemia, and 8-60% for HbE (Fucharoen *et al*, 1992). It is estimated that about 30-40%

of Thai people carry abnormal hemoglobin genes.

The Thai Ministry of Public Health conducts a program for prevention and control of thalassemia through screening of pregnant women (Wuttipong, 1997). The aim is to decrease the number of newborns with severe thalassemia diseases: homozygous α -thalassemia 1 causing hemoglobin Bart's hydrops fetalis syndrome, homozygous β -thalassemia, and β -thalassemia/HbE disease. Thus, identification of carriers of α -thalassemia 1, β -thalassemia and HbE at antenatal clinics is an important step in this prevention and control program. The combination of a one tube osmotic fragility (OF) test

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and a dichlorophenol indophenol (DCIP) precipitation test is widely performed for mass screening as it is a simple, reliable, cost-effective and practical method (Winichagoon *et al*, 2002). However, the validity of these tests for detecting abnormal genes effectively has been questioned as a high rate of false positive results have been reported (Fucharoen *et al*, 2004; Sangkitporn *et al*, 2005). Although false positive results are not a major concern in a screening program, it can have adverse effects for pregnant women, who may suffer from anxiety and embarrassment when informed about being possible carriers of severe thalassemia (Lerman *et al*, 2002). Furthermore, the financial cost associated with the need to conduct further hemoglobin and polymerase chain reaction (PCR) analyses should also be considered.

None of previous studies estimating the false positive result rate from the screening program for thalassemia conducted in the primary health care setting. In this study, we calculated the true positive and false positive rates in the thalassemia screening program over a ten-year period (1999-2008) at rural hospitals in Thailand.

MATERIALS AND METHODS

Samples

The retrospective dataset was drawn from the thalassemia screening program of Health Promotion Center 5 (HPC-5), Nakhon Ratchasima, Thailand from 1999 to 2008. The study sample consisted of 13,747 cases with positive results from the screening test involving a combination of the one tube osmotic fragility (OF) test and dichlorophenol indophenol (DCIP) precipitation test, conducted in public general and community hospitals in four provinces of Thailand namely, Nakhon Ratchasima, Chaiyaphum, Surin, and Buri Ram Provinces. The data were compared with hemo-

globin typing and DNA analyses. The number of cases, which were sent to HPC-5, covered more than 70% of the total cases from the four provinces.

Screening test and definition of false positive result

With the OF test, a sample of 20 μ l of fresh whole blood was mixed with 2 ml of 0.34% phosphate buffered saline solution in a test tube and left at room temperature for 15 minutes before being interpreted (Fucharoen *et al*, 2004). With the DCIP precipitation test, 20 μ l of fresh whole blood was added to 2 ml of DCIP reagent and the mixture was incubated at 37°C for 15 minutes before the addition of 20 μ l of stopping reagent to eliminate and decolorize excess DCIP dye (Fucharoen *et al*, 2004). Both tests were interpreted by visualization as being negative or positive. Negative samples are characterized by a clear solution and positive samples by a cloudy appearance.

Positive samples from both tests, either positive on the OF and negative on the DCIP test (+/-), negative on the OF and positive on the DCIP test (-/+) or positive on both the OF and DCIP tests (+/+), were transferred to HPC-5 where confirmation tests were performed. Based on confirmation tests, cases with α -thalassemia 1, β -thalassemia and HbE were defined as true positive results. The remaining samples were considered as false positives.

Confirmation tests

Hemoglobin analysis and levels were determined using automated high performance liquid chromatography (VARIANT Classic, Bio-Rad Laboratories, Hercules, CA). For DNA analyses, identification of the α -thalassemia 1 (SEA deletion) gene was performed using an allele specific polymerase chain reaction (PCR) (Panyasai *et al*, 2002).

Statistical analysis

Data were entered into Microsoft Excel

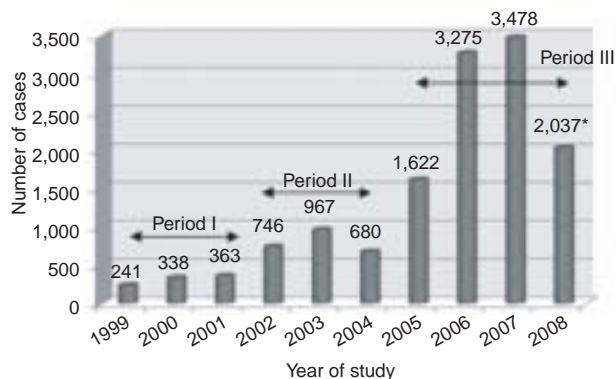


Fig 1—Number of thalassemia screening positive cases sent to HPC-5 for further analysis from 1999 to 2008. *Cases for 2008 were collected from January to June only.

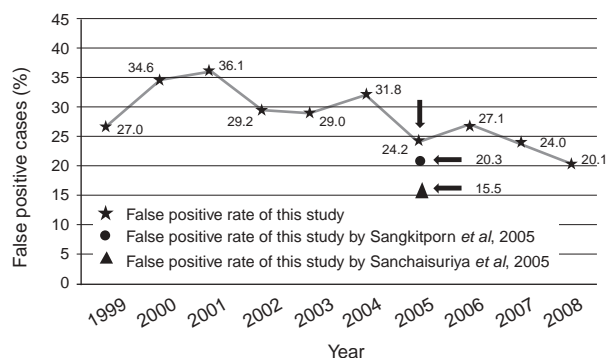


Fig 2—Percent of false positive cases from the thalassemia screening test in rural hospitals from 1999 to 2008.

(version 2007). Descriptive statistics, including mean, standard deviation (SD), and proportion, were used to describe the rate of false positive cases. Chi-square tests using STATA version 10 were employed to test the annual trend of the proportion of cases.

RESULTS

From 1999 to 2007, the number of positive cases sent to HPC-5 increased from 241 to 3,478 cases per year. The study duration can be divided into 3 periods (due to policy

changes as detailed in Discussion) (Fig 1). From 1999 to 2001, the number of positive cases were 241 and 363 cases, respectively, with a mean of 314 ± 64 . From 2002 to 2005, the number of positive cases increased from 746 to 1,622 cases, with a mean of $1,004 \pm 430$. During the third period from 2006 to 2007, the number of positive cases increased from 3,275 to 3,478, with a mean of $3,376 \pm 144$. In 2008 the cases were collected only from January to June. The data showed an exponential increase in goodness-of-fit (r^2) of 0.94.

Analysis of the data of the proportion of false positive cases from the thalassemia screening tests over a ten year period showed that the associated trend is statistically significant ($p < 0.001$). Results from the confirmatory tests indicated a false positive rate in the range of 20.1-36.1% during the 10 year period of study. False positive results with these two screening tests were higher than those at previous studied reference laboratories of 15.5% (Sanchaisuriya *et al*, 2005) and 20.3% (Sangkitporn *et al*, 2005) (Fig 2).

Table 1 shows the estimated cost resulting from false positive cases from 1999 to 2008 from estimated 800,000 annual births, which would need further hemoglobin analysis. The cost of hemoglobin analysis is 250 THB/case.

DISCUSSION

The number of positive cases from thalassemia screening of pregnant women increased exponentially from 1999 to 2007, during the 3 study periods. During the first period, the number of positive cases was small as subjects had to undertake the laboratory diagnosis at their own cost. During the second period, the number of positive cases increased two-fold due to the introduction in 2002 by the Ministry of Public Health of Thailand of a Universal Health Care Policy (30 THB scheme). However, this pro-

Table 1
Estimated budget of hemoglobin analysis for false positive thalassemia cases from 1999 to 2008.

	Number	Unit cost of hemoglobin analysis	Budget (THB/year)
Annual birth	800,000		
Pregnant women requiring hemoglobin analysis (20.1- 36.1%)	160,800 - 288,800	250 THB/case	40,200,000- 72,200,000

gram was not successful because there was a lack of an organization responsible for the overall policy implementation. Furthermore, implementation of this policy relied on administrators or policy makers, and if there was no interest, the policy was disregarded (Putthasri *et al*, 2004). However, in 2005 the National Health Security Office allocated a budget for the thalassemia prevention and control program with the Department of Health acting as an organization focal point. As a result, the program provided better care for thalassemia patients and promoted the prevention and control measures, resulting in an increasing number of screening tests.

The screening protocol for the thalassemia prevention and control program relied on OF and DCIP tests. Couples with positive results on both tests were subjected to further investigation using HPLC or DNA analyses to confirm for the presence of α -thalassemia 1 trait, β -thalassemia trait or HbE carrier status. There was higher false positive rates (20.1-36.1%) from the two screening tests conducted in this study compared with previous cross-sectional studies [20.3% (Sangkitporn *et al*, 2005) and 15.5% (Sanchaisuriya *et al*, 2005)] conducted in reference laboratories. This study supports previous findings of false positive rates of 11.6-34.6% in three community hospitals (Fucharoen *et al*, 2004). Additionally, the results varied according to the laboratory. It indicates quality control measure is needed

to improve performance in all laboratories. However, during 2006-2008 the false positive rates decreased continuously (Fig 2), because the committee on the thalassemia prevention and control program has provided training of staff who are responsible for screening of thalassemia.

The results of the present study indicate that thalassemia screening tests are limited in routine application due to their ineffectiveness in identifying abnormal genes. The false positive results may occur due to the presence of a mild form of thalassemia or hemoglobinopathy, such as Hb Constant Spring, homozygous α -thalassemia 2 or iron deficiency anemia (Fucharoen *et al*, 1999, Jopang *et al*, 2004; Sanchaisuriya *et al*, 2005).

Further study needs to be carried out to look for ways of reducing false positive results in thalassemia screening, since the results can lead to anxiety and embarrassment (Lerman *et al*, 2002) and impose an economic burden on the country's health budget.

In summary, the combination of OF and DCIP precipitation tests has problems with routine application but still remains effective for mass screening in the thalassemia prevention and control program due to their cost-effectiveness and practicality. However, it is necessary to improve testing techniques to achieve better test results to reduce the economic burden in terms of higher cost and associated workload.

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