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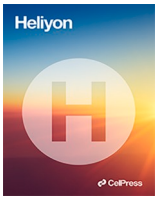

Research article

Association of thalassemia, hemoglobinopathies, and vitamin D levels with lipid profile in adults: Community-based research in southern Thai population

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ABSTRACT

This study explored the frequency of lipid-lowering drug use in the thalassemia population and investigated the association of thalassemia, hemoglobinopathies, and serum 25(OH)D levels with lipid profile and red blood cell parameters. A combination of cross-sectional and community-based studies was conducted with 615 participants from the southern Thai population. Thalassemia and hemoglobinopathies were diagnosed using hemoglobin analysis and polymerase chain reaction-based methods to genotype globin genes. Biochemical parameters such as lipid profile, fasting blood sugar (FBS), and serum 25(OH)D levels were assessed using standard enzymatic methods and electrochemiluminescence immunoassays. Differences in the means of hematological and biochemical parameters between the thalassemia and non-thalassemia groups were compared and analyzed. A significantly lower frequency of lipid-lowering drug use was observed in the thalassemia group. Thalassemia, with clearly defined abnormalities in red blood cells, is associated with a 4.72-fold decreased risk of taking lipid-lowering drugs. Among thalassemia participants, the total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels were significantly lower than those in non-thalassemia participants. The prevalence of hypovitaminosis D in carriers of thalassemia and/or hemoglobinopathies in the southern Thai population was 53 % in females and 21 % in males. The highest lipid profile was observed in samples without thalassemia and hypovitaminosis D. The genetics of thalassemia and hemoglobinopathies with obviously abnormal red blood cells could explain the variable lipid levels, in addition to lipid metabolism-related genes and environmental factors. However, the effect of thalassemia on lipid levels in each population may differ according to its prevalence. A larger sample size is required to confirm this association, especially in countries with a high prevalence of thalassemia.

1. Introduction

Lipid profiles in routine laboratory tests typically include total cholesterol (TC), triglycerides (TGs), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) [1]. Serum lipid profiles and other risk factors, including

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smoking status, alcohol consumption, physical activity, hypertension, vitamin D status, and obesity were measured, and medical histories were used to assess cardiovascular risk [2,3]. There are associations among 25(OH)D levels, lipid profiles and metabolic syndrome (MetS) in several populations. Lower 25(OH)D levels are associated with higher TC and LDL-C levels, and a higher risk of MetS [3,4]. Both genetic and environmental factors, which are multifactorial, can cause abnormal lipid profiles, namely, dyslipidemia [5]. Several genome-wide association studies (GWAS) in various populations have identified more than 250 lipid-associated genes/loci such as *LDLR*, *APOB*, *PCSK9*, *APOE*, *CETP*, *ABCG5*, and *NPC1L1* [6–9]. Interestingly, rs11549407 (A/G) of the *HBB* gene (β^0-thalassemia, stop-gain mutation, p. Gln40*) is associated with lower TC and LDL-C levels in the Sardinia region, which has a high frequency of β-thalassemia [10,11]. A genome-wide association in the Million Veteran Program (MVP) also identified the *HBB* locus (rs11549407), which is associated with TC and LDL-C [12]. Lower TC and LDL-C levels have been observed in both β-thalassemia intermedia and β-thalassemia major in several populations [13,14], and in β-thalassemia minor [15]. Furthermore, β-thalassemia mutation is a genetic modifier of familial hypercholesterolemia (FH) that lowers TC and LDL-C levels [16,17]. The relationship between lipid metabolism and red blood cell disorders is associated with increased bone marrow activity [14]. Chronic anemia can reduce cholesterol levels through cholesterol consumption to compensate for compensated erythropoiesis [18]. Cholesterol homeostasis is essential for erythroblast differentiation and terminal erythropoiesis [19].

Thalassemia and hemoglobinopathies are caused by mutations in one or more globin genes, leading to quantitative and qualitative defects, respectively. α-Thalassemia, β-thalassemia, and Hb E are the three most common thalassemia and hemoglobin variants in Southeast Asia, especially in the Thai population [20]. The prevalence of α-thalassemia, β-thalassemia, and Hb E ranges from 20 to 30 %, 3–9%, and 10–60 %, respectively [20,21]. The combination of different thalassemia and hemoglobin variants results in more than 60 different types of thalassemia, including thalassemia minor, thalassemia intermedia, and thalassemia major. Hb Bart's hydrops fetalis (−/−), homozygous β-thalassemia (β*/β*), and Hb E/β-thalassemia (β^E/β*) are three severe thalassemia syndromes for which prevention and control programs in Thailand are of concern. Previous data on thalassemia were mostly gathered from hospital-based sample collections. Community-based research has been conducted on anemia, iron deficiency, and thalassemia. Thalassemia is a major cause of anemia in the community and studied areas [22,23].

According to the recent National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines, lipid profiles are categorized according to TC, LDL-C, and HDL-C levels [24]. The prevalence of hypercholesterolemia (≥200 mg/dL) and high LDL-C (≥130 mg/dL) in the southern Thai population was 56.62 %, and 43.05 %, respectively [25]. Increasing age was associated with a higher prevalence of dyslipidemia [26,27]. Owing to the high frequency of thalassemia and HbE in the Thai population, this study aimed to explore the prevalence of thalassemia and hypovitaminosis D and to determine the association between lipid profile and thalassemia and/or hypovitaminosis D in the southern Thai population using community-based research.

2. Materials and methods

2.1. Study population

This cross-sectional study was conducted in two communities in the Nakhon Si Thammarat Province, southern Thailand, between April and August 2015. The study protocol was approved by the Institutional Review Board of Walailak University (Nakhon Si Thammarat, Thailand; protocol no.14/101). Written informed consent was obtained from all participants. We enrolled 615 participants, of whom 486 were females and 129 were males, to explore the prevalence of anemia, thalassemia, and hemoglobinopathies in the two communities. After excluding participants who had been treated with lipid-lowering drugs, antidiabetic drugs, and/or diabetic conditions ($n = 147$), 468 samples were used for the association analysis between thalassemia status, vitamin D levels, and lipid profile.

2.2. Demographic data and anthropometric measurements

Demographic data collected using a questionnaire included age, sex, religion, fish oil in take, multivitamin intake, milk consumption, sunscreen use, smoking, alcohol consumption, lipid-lowering drug use, and physical activity. Anthropometric data, such as body weight and height, systolic blood pressure, and diastolic blood pressure, were measured for each participant.

2.3. Thalassemia screening and hemoglobin analysis

Complete blood count (CBC) was performed using a Sysmex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and dichlorophenolindophenol precipitation test (DCIP) were used for thalassemia and HbE screening, respectively. Samples with MCV less than 80 fL and/or MCH less than 27 pg or positive DCIP were considered positive. All positive samples were analyzed for hemoglobin type using an automated high-performance liquid chromatography (HPLC) - Variant II thalassemia short program (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. DNA extraction, DNA concentration, and globin gene genotyping

Human genomic DNA (gDNA) was extracted from peripheral blood leukocytes using a genomic DNA extraction kit (Geneaid Biotech, Ltd., Taiwan) according to the manufacturer's instructions. The purity (OD260/OD280) and concentration (OD260) of the stock gDNA were measured using a Nanodrop ND-1000 (Thermo Fisher Scientific Inc., MA, USA). Common α-globin gene deletions ($–^{SEA}$, $–^{THAI}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$) and 3.5 *HBB* deletion were characterized by multiplex gap PCR [28,29]. Non-deletional α-globin genes (Hb

Constant Spring and Hb Pakse) and point mutations of the β-globin gene were identified by allele-specific PCR and multiplex amplification refractory mutation system (MARMS) PCR and PCR-HRM [30,31]. Uncharacterized globin gene mutations (*HBB* and *HBD* genes) were further genotyped using direct DNA sequencing [32].

2.5. Development of a new method for δ^0-thalassemia (IVS II-897, A > T) mutation detection

When an atypical pattern of the HPLC chromatogram with abnormally low level of Hb A_2 was found, the DNA sample was first subjected to α-and β-globin genotyping. Negative results for both α-globin gene deletions and β-globin gene were obtained, and direct DNA sequencing of the δ-globin gene (*HBD*) was conducted. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for secondary confirmation of *HBD* mutation, which was primarily designed by this study. The primer sequences HBD-D0-F: 5'-GGG GAT CAG TTT TGT CTA AG-3' and HBD-D0-R: 5'-ACC TTC TTA CAC ACC TGG AC-3'. The PCR mixture contained 1X PCR buffer (with 1.5 mM $MgCl_2$), 200 μM dNTPs, 0.1 μM of each primer, 1.25 U of *Taq* DNA polymerase (New England Biolabs, Ipswich, MA, USA), and 50 ng of genomic DNA, and the reaction mixture was adjusted to 25 μL with sterile deionized water. PCR was performed in a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA) as follows: 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The PCR products were analyzed on 2 % agarose gel electrophoresis, followed by digestion. The 428-bp PCR product of the *HBD* gene, including intron 2 and exon 3 junctions, was digested with the restriction enzyme *BsrB*I (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions, to discriminate between wild-type and mutant alleles. The digested PCR products (mutant alleles) and indigestible PCR products (wild-type alleles) were separated using 2 % (w/v) agarose gel electrophoresis at 100 V for 45 min and visualized using a Gel Doc™ XR + gel documentation system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) after staining with ethidium bromide.

We also developed PCR-HRM for the high-throughput genotypic screening of IVS II-897 (A > C). The primer sequences D0-HRM-F: 5'-AGA AGC CAG TCT TTA TTT CTC TG-3' and D0-HRM-R: 5'-ATT CCT TGC CAA AGT TGC-3' were designed and used to amplify the 114-bp PCR product. A total of 20 μL of PCR contained 1X HOT FIREPol® EvaGreen® HRM Mix with ROX (Solid Biodyne, Tartu, Estonia), 0.25 μM of each primer, and 20 ng of genomic DNA. After initial activation at 95 °C for 15 min, the PCR cycling conditions were 95 °C for 15 s, 56 °C for 20 s, and 72 °C for 20 s, with amplification for 40 cycles. The amplified 114-bp PCR product was denatured at 95 °C for 15 s and reannealed at 60 °C for 1 min. The melting curve was obtained from 60 °C to 95 °C with a ramp rate of 0.05 °C/s. The HRM patterns were analyzed using High-Resolution Melt Software v.3.1 (Applied Biosystems, Foster City, CA, USA).

2.6. Lipid profile, fasting blood sugar (FBS), and 25(OH)D measurements

Fasting blood samples (12-h fasted) were used for all measurements. Lipid profiles and fasting blood sugar (FBS) levels were measured using a Konelab analyzer (KONELAB 20, Tokyo; Japan). Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured using standard enzymatic methods. The Friedewald equation was used to calculate the low-density lipoprotein cholesterol (LDL-C) levels [33]. FBS was quantified using the glucose oxidase method. In addition, serum 25-hydroxyvitamin D [25(OH)D] levels were measured by using an electrochemiluminescence immunoassay reagent (VITROS® 25-OH Vitamin D Total assay) using an automated Vitros ECi (Johnson and Johnson, Rochester, NY, USA) [34].

2.7. Classification of study participants

Participants were categorized according to their thalassemia genotype, anemia status, and vitamin D status. First, thalassemia genotypes were classified into 3 groups as follows: (i) non-thalassemia - negative screening (MCV ≥80 fL, MCH ≥27 pg, and negative DCIP); (ii)thalassemia with a minor change in red blood cell parameters - heterozygous Hb CS, Hb J-Bangkok, Hb Malay with or without α-thalassemia-2, heterozygous β^+-thalassemia, heterozygous α-thalassemia-2, heterozygous HPFH, homozygous Hb CS; and (iii) thalassemia with obvious changes in red blood cell parameters - double heterozygotes for Hb E and α-thalassemia 1, heterozygous Hb E with homozygous α-thalassemia 2, heterozygous β^0-thalassemia (β^+ [severe form]-thalassemia), heterozygous α-thalassemia 1, homozygous Hb E without α-thalassemia, homozygous α-thalassemia 2, deletional Hb H disease ($-^{SEA}/-\alpha^{3.7}$) and β^0-thalassemia/Hb E. Second, vitamin D levels were categorized into 2 groups as follows: sufficiency ([25(OH)D] ≥ 75 nmol/L) and hypovitaminosis D (25 (OH)D] < 75 nmol/L) [35]. Third, the anemia status was classified using Hb levels and sex as follows: Hb less than 120 g/L was considered anemic in females, whereas Hb less than 130 g/L was considered anemic in males [36].

2.8. Data analysis

All the statistical analyses were performed using SPSS version 26 (SPSS, Chicago, IL). Prevalence data are presented as numbers and percentages according to the globin genotype. Demographic data, biochemical parameters, and red blood cell test results among the different thalassemia groups (Groups 1–3) and sexes (female and male) were compared using Student's t-test, or the nonparametric Mann -Whitney *U* test. One-way analysis of variance (ANOVA) or the Kruskal -Wallis test was used for multiple comparisons of means among the three groups. The variables of the participants are presented as mean and standard deviation (SD) for continuous data, and categorical data are described as percentages. The chi-square test was used to compare proportions between groups. Sample sizes from all analyses provided a power greater than 80 %, except for some subgroups in males, and patients with thalassemia. *P* values below 0.05 were considered statistically significant differences between two groups or among three groups were considered statistically significant.

3. Results

3.1. A community-based cross-sectional study of thalassemia and hemoglobinopathies

A total of 615 participants were enrolled in the study to explore the prevalence of thalassemia and hemoglobinopathies in the two communities of Nakhon Si Thammarat Province, including 486 females and 129 males. The mean age of all participants was 53.13 years, with the mean ages of females and males being 53.25 and 52.69 years, respectively. The overall prevalence of thalassemia and hemoglobinopathies was 29.8 % (183/615), including more than 20 globin genotypes (Table 1). Hb analysis revealed several Hb-type patterns, such as A_2A (Fig. 1A–D and E), CSA_2A Bart's (Fig. 1B), A_2A Bart's H (Fig. 1C), A_2AF (Fig. 1F and G), EA (Fig. 1H and I), EE (Fig. 1J), EF (Fig. 1K), and A_2AX (Fig. 1L). Hb E-related syndrome, including heterozygous Hb E (β^E/β^A) without or with α-thalassemia (αα/αα, $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, $-^{SEA}/\alpha\alpha$, and $-^{THAI}/\alpha\alpha$), homozygous Hb E (β^E/β^E) without α-thalassemia (αα/αα), and β-thalassemia/Hb E disease without α-thalassemia, was the most prevalent at 12.7 % (78/615). α-Thalassemia 2 heterozygotes and homozygotes ($-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, and $-\alpha^{3.7}/-\alpha^{3.7}$) were the second most prevalent at 11.9 % (73/615), whereas the prevalence of α-thalassemia 1 heterozygotes was 2.4 % (15/615). The prevalence of β-thalassemia heterozygotes, including β^0-thalassemia mutations (codons 41/42; -TTCT, IVS I-5, G > C, 3.5 kb deletion) and β^+-thalassemia mutations (−28; A > G, codon 19; A > G [Hb Malay], IVS I-1; G > T) was 3.4 % (21/615). Hb Constant Spring (Hb CS) heterozygotes and homozygotes were observed in 0.6 % (4/615) of the participants. According to the prevalence of α-thalassemia, β-thalassemia, and Hb E carriers in these communities, we found 2 thalassemia diseases, including 4 patients with deletional Hb H disease ($-^{SEA}/-\alpha^{3.7}$) and 2 patients with β^0-thalassemia/Hb E disease. Interestingly, the hereditary persistence of fetal hemoglobin (HPFH) and δ-globin gene mutations were also observed in these two communities at 1.1 % (7/615) and 0.3 % (2/615), respectively. The prevalence of anemia among males and females according to the World Health Organization's criteria is 4 % and 10 %, respectively. Thalassemia accounted for anemia in both males (98 %) and females (100 %; Table 2).

3.2. Characterization of δ^0-thalassemia (the first report in the southern Thai population)

Among 615 samples, 2 samples were characterized as δ-globin gene mutations. The first sample revealed a novel δ-globin chain variant that has already been published [32]. The second δ-globin gene mutation was the first reported case of δ^0-thalassemia in a southern Thai population. The proband was a 59-year-old woman from southern Thailand. Her complete blood count was as follows: red blood cell (RBC) count, $4.86x10^6$/μL; hemoglobin concentration (Hb), 116 g/L; hematocrit (HCT), 0.36 L/L; mean corpuscular volume (MCV), 75.3 fL; mean corpuscular hemoglobin (MCH), 23.9 pg; mean corpuscular Hb concentration (MCHC), 0.32 g/L; and RBC distribution width (RDW), 13.0 %. Hemoglobin analysis identified a normal hemoglobin type (A_2A) with a low Hb A_2 level (1.9 %) and a slightly increased Hb F level (1.5 %) (Fig. 2A). Hematological results revealed that the case was suspected to have α-thalassemia. Surprisingly, multiplex-gap PCR for 4 common α-thalassemia deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-^{SEA}$, and $-^{THAI}$) was negative. Therefore, this sample had the potential to have δ-thalassemia because of the decreased Hb A_2 level. The whole δ-globin gene (*HBD*) was subsequently amplified and sequenced. DNA sequencing revealed an A to C substitution at the 3'acceptor site of the second intron, nucleotide 1, 390th position of the δ-globin gene (IVS-II-897, A > C; AG^CT > CGCT) (Fig. 2B and C) which was classified as a δ^0-thalassemia mutation. As this mutation is the first case in the southern Thai population, we developed two new methods for genotyping this

Table 1
Study population of thalassemia and hemoglobinopathies.

Group no./Type	*HBA* genotype	*HBB* (or *HBD)* genotype	No. (%)
Group 1 (n = 432)			
Normal or non-thalassemia	αα/αα	β^A/β^A	432 (70.24)
Group 2 (n = 143)			
Heterozygous Hb E	αα/αα	β^E/β^A	54 (8.78)
Heterozygous α-thalassemia 2	$-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$	β^A/β^A	53 (8.62)
Double heterozygotes for Hb E and α-thalassemia 2	$-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$	β^E/β^A	13 (2.12)
Suspected heterozygous HPFH	αα/αα	Suspected HPFH	7 (1.14)
Heterozygous Hb Malay	αα/αα	$\beta^{Hb\ Malay}/\beta^A$	4 (0.65)
Heterozygous β^+-thalassemia	αα/αα	β^+/β^A	4 (0.65)
Double heterozygotes for Hb Malay and α-thalassemia 2	$-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$	$\beta^{Hb\ Malay}/\beta^A$	3 (0.49)
Heterozygous Hb CS	$\alpha^{CS}\alpha/\alpha\alpha$	β^A/β^A	2 (0.33)
Homozygous Hb CS	$\alpha^{CS}\alpha/\alpha^{CS}\alpha$	β^A/β^A	1 (0.16)
Double heterozygotes for Hb CS and δ^0-thalassemia	$\alpha^{CS}\alpha/\alpha\alpha$	δ^0/δ	1 (0.16)
Heterozygous Hb J-Bangkok	αα/αα	$\beta^{Hb\ J-Bangkok}/\beta^A$	1 (0.16)
Group 3 (n = 40)			
Heterozygous α-thalassemia 1	$-^{SEA}$/aa	β^A/β^A	12 (1.95)
Heterozygous β^0-thalassemia	αα/αα	β^0/β^A	10 (1.63)
Homozygous Hb E	αα/αα	β^E/β^E	5 (0.81)
Deletional Hb H disease	$-^{SEA}/-\alpha^{3.7}$	β^A/β^A	4 (0.65)
Double heterozygotes for Hb E and α-thalassemia 1	$-^{SEA}/\alpha\alpha$, $-^{THAI}/\alpha\alpha$	β^E/β^A	3 (0.49)
Homozygous α-thalassemia 2	$-\alpha^{3.7}/-\alpha^{3.7}$	β^A/β^A	2 (0.33)
β-thalassemia/Hb E disease	αα/αα	β^0/β^E	2 (0.33)
Homozygous α-thalassemia 2 with Hb A_2-Kiriwong [32]	$-\alpha^{3.7}/-\alpha^{3.7}$	$\delta_2^{HbA\ Kiriwong}/\delta$	1 (0.16)
Heterozygous Hb E with homozygous α-thalassemia 2	$-\alpha^{3.7}/-\alpha^{3.7}$	β^E/β^A	1 (0.16)

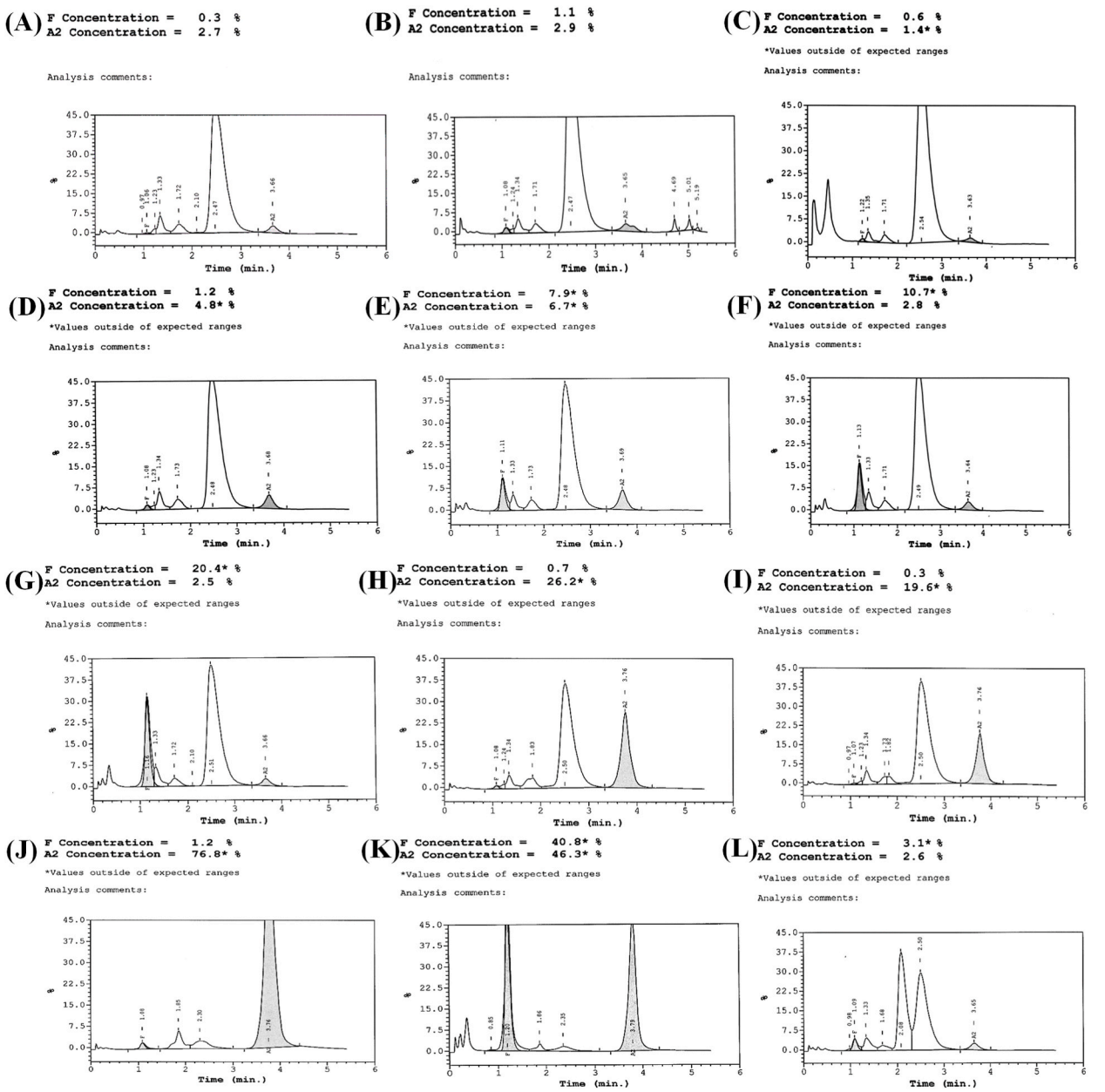

Fig. 1. Representative HPLC chromatograms from various types of thalassemia and hemoglobinopathies. (A) A_2A (normal Hb type); (B); CS A_2A Bart's (homozygous Hb CS); (C) A_2A Bart's H (deletional Hb H disease); (D) A_2A ($A_2 > 3.5$ %; heterozygous β-thalassemia); (E) A_2A ($A_2 > 3.5$ % and increased Hb F level; deletional β^0-thalassemia heterozygote); (F and G) A_2AF (HPFH, Hb F 10.7 % and 20.4 %); (H) EA (pure heterozygous Hb E, Hb A_2/E 26.2 %); (I) EA (double heterozygotes for Hb E and α-thalassemia 1; Hb A_2/E 19.6 %); (J) EE (homozygous Hb E); (K) EF (Hb E/β^0-thalassemia), and (L) A_2AX (β-globin chain variant/Hb J-Bangkok heterozygote).

δ^0-thalassemia mutation namely, PCR-RFLP and PCR-HRM, for population screening and confirmation. Primers (D0-RFLP-F and D0-RFLP-R) were used to amplify the 428-bp PCR product from different DNA templates. After *BsrB*I digestion, digestible PCR products represented the mutant allele (C allele), showing 121-bp and 307-bp fragments, whereas indigestible PCR products represented the wild-type allele (A allele), showing 428-bp fragments (Fig. 2D and E). In addition, primers (D0-HRM-F and D0-HRM-R) were used to amplify the PCR products (Fig. 2D), obtaining 114-bp, and the PCR product was subsequently used for high-resolution melting curve analysis. Very good discrimination between AA and AC genotypes was observed using the developed method (Fig. 2F).

Table 2
Anemia status in thalassemia and non-thalassemia.

Category	Females, *n* (%)			Males, *n* (%)		
	Total females	Non-anemia (Hb ≥ 120 g/L)	Anemia (Hb < 120 g/L)	Total males	Non-anemia (Hb ≥ 130 g/L)	Anemia (Hb < 130 g/L)
All samples	486 (100 %)	438 (100 %)	48 (100 %)	129 (100 %)	124 (100 %)	5 (100 %)
Non-thalassemia	337 (69 %)	336 (77 %)	1 (2 %)	95 (74 %)	95 (77 %)	0 (0 %)
Thalassemia	149 (31 %)	102 (23 %)	47 (98 %)	34 (26 %)	29 (23 %)	5 (100 %)
P value		<0.001			0.001	
OR (95%CI)		154.82 (21.10–1136.13)			NA	

P values were obtained using the chi-square test, with non-thalassemia as the reference group.

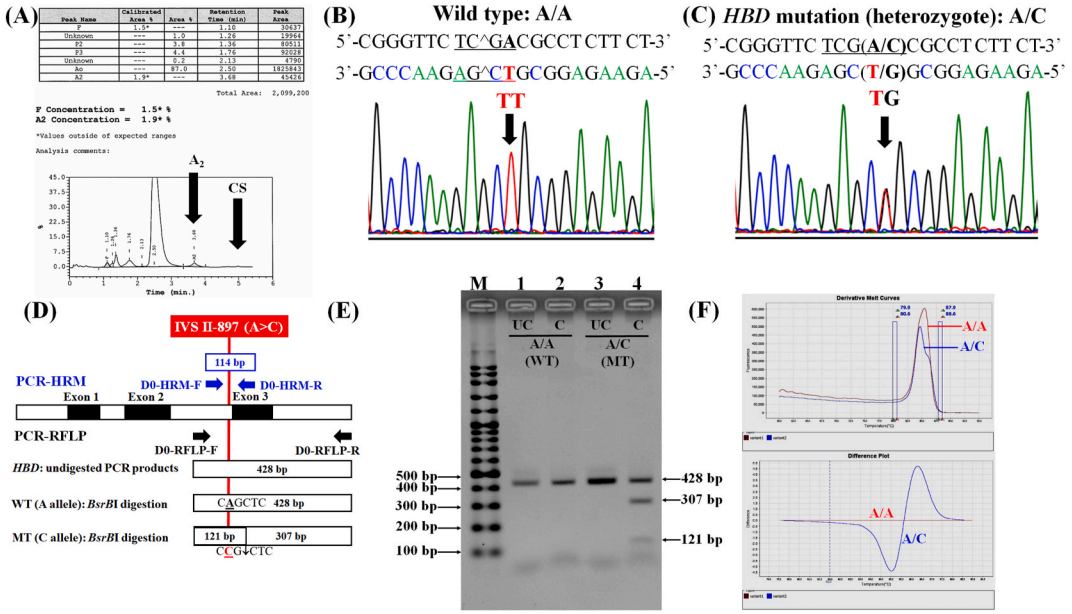

Fig. 2. Molecular characterization of double heterozygosities for δ^0-thalassemia and Hb Constant Spring in a southern Thai individual. (A) HPLC chromatogram showing decreased Hb A_2 level and a very small peak of Hb Constant Spring; (B) normal *HBD* gene sequence [wild type]; (C) *HBD* gene mutation sequence [IVS II-897, A > C; AG^CT > CGCT]; (D) schematic representation of PCR-RFLP and PCR-HRM; (E) agarose gel electrophoresis (The original image is provided in the Supplementary file); (F) HRM profile of the wild-type [red line; A/A] and mutant [blue line; A/C] genotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Frequency of the use of lipid-lowering drugs between the non-thalassemia and thalassemia groups.

Group (*n*)	Number of samples without lipid-lowering drugs	Number of samples with lipid-lowering drugs	*P* value	OR (95%CI)
Non-thalassemia (432)	346 (80 %)	86 (20 %)	Ref.	Ref.
Thalassemia gene mutation (183)	164 (90 %)	19 (10 %)	0.004	2.15 (1.26–3.65)
- Thalassemia: subgroup 1 (143)	126 (88 %)	17 (12 %)	0.030	1.84 (1.05–3.22)
- Thalassemia: subgroup 2 (40)	38 (95 %)	2 (5 %)	0.020	4.72 (1.12–20.0)
Total samples (615)	510 (83 %)	105 (17 %)		

Values are presented as numbers (%); OR, odds ratio; 95 % CI, 95 % confidence interval; Ref., reference group.
Thalassemia: subgroup 1, thalassemia with no or minimal change in RBC parameters; Thalassemia: subgroup 2, thalassemia with obvious changes in RBC parameters.
P values were obtained using the chi-square test, with non-thalassemia as the reference group.

3.3. Association of the use of lipid-lowering drugs, high total cholesterol and/or LDL-C, and thalassemia status

The frequencies of participants who had been taking lipid-lowering drugs were compared between the non-thalassemia and thalassemia groups. We found a lower frequency of lipid-lowering drug use in the thalassemia group (10 %) than in the non-thalassemia group (20 %) ($p = 0.004$). All thalassemia group members had a at 2.15-fold decreased risk of taking lipid-lowering drugs compared with those in the non-thalassemia group. Moreover, in Group 2 (thalassemia with obvious changes in red blood cell parameters), there was a 4.72-fold decreased risk of taking lipid-lowering drugs compared with that of the non-thalassemia group. Therefore, 105 patients treated with lipid-lowering drugs were excluded from further analysis (Table 3). A total of 468 samples without diabetes and without the use of lipid-lowering or antidiabetic drugs were used to analyze the association between high total cholesterol and/or LDL-C and thalassemia status. The frequency of participants who had thalassemia status in the high TC and/or LDL-C group (total cholesterol ≥5.168 mmol/L and/or LDL-C ≥ 3.359 mmol/L) was significantly lower than that in the participants who had non-thalassemia status ($p < 0.001$), and the thalassemia group was a 2.53-fold decreased risk of having high TC and/or LDL-C levels compared with those in the non-thalassemia group, as shown in Table 4.

3.4. Association of lipid profile and thalassemia status

A total of 468 samples were classified into six subgroups according to thalassemia status (three groups) and sex (female and male). As shown in Table 5, the mean age and vitamin D levels were not different between the two groups (Group 1 was set as the reference group) or among the three groups in both the male and female groups. All red blood cell parameters were significantly different between groups 1 versus Group 2 and Group 1 and 3 ($p < 0.001$), and among the three groups ($p < 0.001$) (Fig. 3A–D). Total cholesterol (TC) and LDL-C levels were significantly lower in woman in group 2 and 3. In addition, HDL-C levels were significantly lower in Group 3 females. TC and LDL-C levels decreased according to the degree of red blood cell abnormalities in both female and male groups (Fig. 4A–D).

3.5. Effect of vitamin D status and thalassemia on the lipid profile and red blood cell parameters

A higher frequency of hypovitaminosis D (54–60 %) was observed in all female groups. In contrast, a lower frequency of hypovitaminosis D (14–27 %) was observed in all male groups. There was a highly significant association between sex and hypovitaminosis D ($p < 0.001$), and the odds ratio (OR) for hypovitaminosis D was 3.82 for females versus males in all participants (Table 6). The prevalence of hypovitaminosis D in carriers of thalassemia and/or hemoglobinopathies in the southern Thai population was 53 % in females and 21 % in males. Moreover, all the patients with thalassemia (100 %) had hypovitaminosis D (Table 7). The combined effects of thalassemia and vitamin D were sub-grouped into six categories for both males and females. TC and TG levels were significantly different in the groups of females with non-thalassemia with hypovitaminosis D and vitamin D sufficiency. In Group 2, higher TGs levels were found in the hypovitaminosis D group than in the vitamin D sufficiency group (Table 8). The highest lipid profile levels were observed in the non-thalassemia group (Group 1) with hypovitaminosis D (Tables 8 and 9). In Group 1 (non-thalassemia group; male), there were highly significant differences in RBC, Hb, Hct, and RDW between the hypovitaminosis D and vitamin D sufficiency groups. Higher RBC, Hb, and Hct levels and lower RDW were observed in the vitamin D sufficiency group. In Group 2 (thalassemia with minor changes in red blood cell parameters; male), a higher BMI and lower DBP were observed in patients with hypovitaminosis D, as shown in Table 9. No significant differences were noted in the lipid profiles among the different male groups in the hypovitaminosis D and vitamin D sufficiency groups.

4. Discussion

In Thailand, Hb Bart's hydrops fetalis (homozygous α-thalassemia 1), homozygous β-thalassemia disease, and β-thalassemia/Hb E

Table 4
The frequency of high total cholesterol and/or LDL cholesterol between the non-thalassemia and thalassemia groups without diabetes.

Group (*n*)	Number of samples of non-high TC and/or LDL-C (%)	Number of samples of high TC and/or LDL-C (%)	*P* value	OR (95%CI)
Non-thalassemia (316)	100 (32 %)	216 (68 %)	Ref.	Ref.
Thalassemia gene mutation (152)	82 (54 %)	70 (46 %)	<0.001	2.53 (1.70–3.77)
- Thalassemia: subgroup 1 (117)	59 (50 %)	58 (50 %)	<0.001	2.20 (1.42–3.39)
- Thalassemia: subgroup 2 (35)	23 (66 %)	12 (34 %)	<0.001	4.14 (1.98–8.65)
Total samples (468)	182 (39 %)	286 (61 %)		

Values are presented as numbers (%); OR, odds ratio; 95 % CI, 95 % confidence interval; Ref., reference group.
The high total cholesterol and/or LDL cholesterol group was defined as a total cholesterol ≥ of 5.168 mmol/L (≥200 mg/dL) and/or LDL-C ≥ of 3.359 mmol/L (≥130 mg/dL) [24].
P values were obtained using the chi-square test, with non-thalassemia as the reference group.

Table 5
The association between thalassemia status in females and males without the use of lipid-lowering drugs and demographic data, red blood cell parameters, and biochemical tests.

Variables	Group 1 (n = 316)		Group 2 (n = 117)		Group 3 (n = 35)		P values[d]	
	Female (n = 243)	Male (n = 73)	Female (n = 95)	Male (n = 22)	Female (n = 28)	Male (n = 7)	Female	Male
Demographic data								
Age (years)	51.87 ± 13.58	51.22 ± 13.24	48.94 ± 12.16	50.14 ± 13.25	51.79 ± 16.20	52.57 ± 13.78	0.157	0.590
BMI (kg/m^2)	24.42 ± 4.02	23.04 ± 3.35	23.52 ± 4.43[b]	23.02 ± 3.50	24.77 ± 4.40	21.85 ± 5.68	0.036	0.980
SBP (mmHg)	130.46 ± 20.02	128.16 ± 15.68	126.18 ± 20.33[b]	136.59 ± 22.30[c]	133.25 ± 24.88	123.14 ± 17.29	0.102	0.090
DBP (mmHg)	80.04 ± 12.17	78.32 ± 9.02	78.71 ± 11.74	80.55 ± 12.34	76.93 ± 15.04[c]	73.29 ± 12.96	0.078	0.398
Red blood cell parameters								
RBC (10^6/μL)	4.59 ± 0.31	5.02 ± 0.37	4.90 ± 0.42[a]	5.44 ± 0.48[c]	5.53 ± 0.52[a]	5.62 ± 1.26[a]	<0.001	<0.001
Hb (g/L)	138.21 ± 8.65	155.59 ± 11.87	125.58 ± 14.50[a]	146.27 ± 12.82[b]	113.64 ± 13.74[a]	126.29 ± 24.32[a]	<0.001	<0.001
Hct (L/L)	0.41 ± 0.02	0.46 ± 0.03	0.38 ± 0.04[a]	0.44 ± 0.03[c]	0.36 ± 0.03[a]	0.41 ± 0.07[c]	<0.001	0.007
MCV (fL)	90.32 ± 3.05	92.29 ± 3.25	78.27 ± 6.18[a]	81.38 ± 3.88[a]	65.53 ± 6.02[a]	73.66 ± 8.73[a]	<0.001	<0.001
MCH (pg)	30.18 ± 1.26	31.01 ± 1.49	25.65 ± 2.52[a]	26.96 ± 1.75[a]	20.63 ± 2.38[a]	22.70 ± 1.72[a]	<0.001	<0.001
MCHC (g/L)	334.14 ± 8.56	335.85 ± 7.99	327.26 ± 11.51[a]	331.05 ± 9.75[c]	314.21 ± 12.18[a]	309.57 ± 17.30[a]	<0.001	<0.001
RDW (%)	12.38 ± 0.70	12.31 ± 0.66	13.33 ± 1.80[a]	12.61 ± 0.70	15.63 ± 3.15[a]	17.69 ± 5.02[a]	<0.001	<0.001
Biochemical tests								
FBS (mmol/L)	5.37 ± 0.62	5.48 ± 0.60	5.14 ± 0.73[b]	5.25 ± 0.51	5.40 ± 0.58	5.02 ± 0.98	0.064	0.249
Vitamin D (nmol/L)	71.47 ± 17.47	97.30 ± 29.67	74.62 ± 19.17	91.34 ± 22.37	74.54 ± 13.62	91.91 ± 28.36	0.330	0.639
TC (mmol/L)	5.66 ± 0.99	5.50 ± 0.94	5.26 ± 0.98[a]	5.21 ± 1.13	4.93 ± 1.24[a]	4.43 ± 1.20	<0.001	0.162
LDL-C (mmol/L)	3.36 ± 0.92	3.23 ± 1.00	3.09 ± 0.91[b]	3.00 ± 1.03	2.91 ± 0.99[c]	2.37 ± 0.89	0.006	0.228
HDL-C (mmol/L)	1.68 ± 0.45	1.60 ± 0.46	1.61 ± 0.43	1.55 ± 0.49	1.38 ± 0.38[b]	1.58 ± 0.66	0.002	0.486
TG (mmol/L)	1.35 ± 0.64	1.47 ± 0.74	1.23 ± 0.64	1.45 ± 0.54	1.38 ± 0.68	1.06 ± 0.27	0.153	0.248

Data are presented as the mean ± SD.
P values were calculated by the Mann− Whitney U test according to sex and participant groups and Student's t-test was used to calculate p values for age, DBP, Hb, Hct, FBS, TC, and LDL-C parameters in the male group (a comparison between two groups, Group 1 was used as the reference group in both females and males).
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] P values were calculated using the Kruskal-Wallis test (comparison among the three groups).

disease have been included in the prevention and control programs for severe thalassemia. Carrier screening, genetic counseling, and prenatal diagnosis are effective strategies to support the appropriate treatment of patients and reduce the incidence of severe thalassemia. Carrier screening in couples has helped reduce the number of severe thalassemia diseases [37–39]. Several publications have reported the prevalence of thalassemia and hemoglobinopathies in different targeted samples and regions. To explore the prevalence of thalassemia and hemoglobinopathies in Thailand, various study designs have been conducted, such as hospital-based or regional health promotion center-based sample collection, area-based research, examining a specific group with a certain condition (a parent of the thalassemia patient, blood donors), and minority ethnic groups [22,23,39–44]. This study represents the prevalence report of a community-based cross-sectional study of thalassemia and hemoglobinopathies in the southern Thai population. This study demonstrated various thalassemia syndromes (more than 25 different globin genotypes) and predicted that severe thalassemia could be observed in these two communities because of the presence of α-thalassemia 1, β-thalassemia, and HbE alleles. Therefore, this study could aid for the prevention and control of severe thalassemia through community-based awareness. In addition, this study was the first to discover double heterozygosities for δ^0-thalassemia (IVS II-897, A > C) and Hb Constant Spring in a southern Thai individual with an Hb A_2 level of 1.9 %, which is higher than in some cases (Hb A_2 levels of 1.1 and 1.7 %) from a previous report [45]. We also developed two original methods for δ^0-thalassemia genotyping: PCR-HRM and PCR-RFLP (*BsrB*I). PCR-HRM can be applied in large population screening with low cost and no post-PCR process, and positive screening samples can be subsequenced for secondary confirmation by PCR-RFLP.

Hyperlipidemia is a risk factor for cardiovascular diseases (CVDs). Several loci have been associated with lipid profiles in various population groups [6–10,12]. Lipid-lowering drugs have been used to decrease lipid levels and the risk of premature CVD [46]. In the Chinese population, 33.8 % of the population had dyslipidemia, and 14.1 % were recommended lipid-lowering medications, comparable to the 17.0 % reported in this study [47]. Genetics associated with lipid metabolism, related pathways, environmental factors, and multifactorial inheritance play important roles in this disease. In countries where thalassemia is highly prevalent, β-thalassemia major and β-thalassemia intermedia have been reported that associated with lower lipid levels in various populations [13,14]. In addition, lower TC and LDL-C levels were found in thalassemia minors and in familial hypercholesterolemia (FH) in β-thalassemia heterozygotes [16,17]. However, there are limited data on the association of the lipid levels with thalassemia minor [15,48,49]. Interestingly, this is the first report demonstrating a lower frequency of lipid-lowering drug use in patients with thalassemia. Subjects

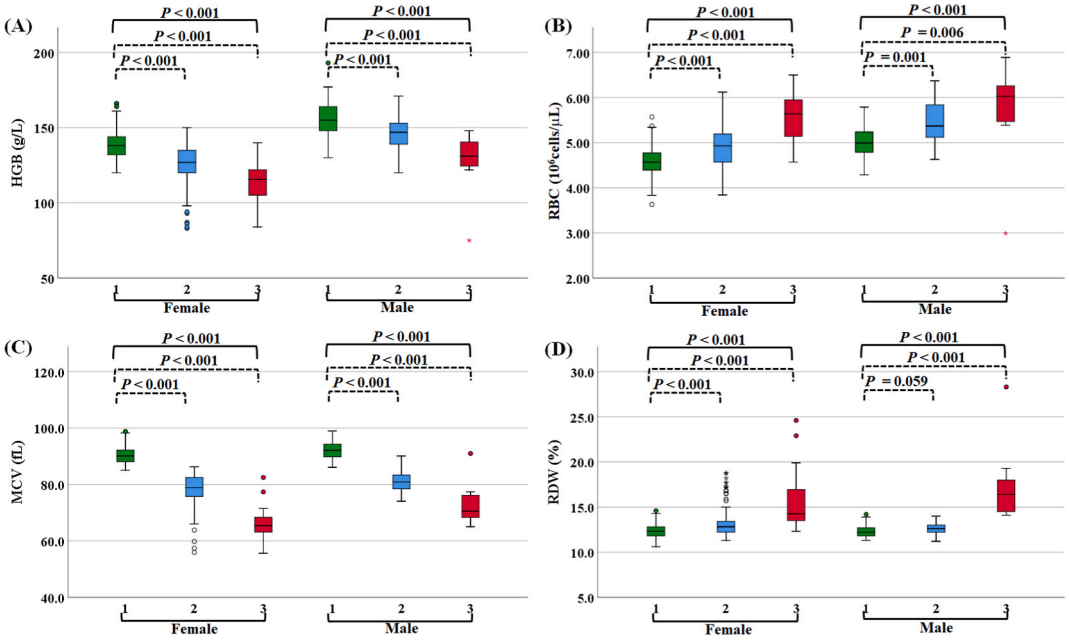

Fig. 3. Box plot of HGB (A), RBC (B), MCV (C), and RDW (D) according to sex and thalassemia status (1; non-thalassemia, 2; thalassemia with a minor change in RBC parameters, 3; thalassemia with obvious changes in RBC parameters) [dash brackets represent *p* values from two group comparisons and solid brackets represent *p* values from three group comparisons].

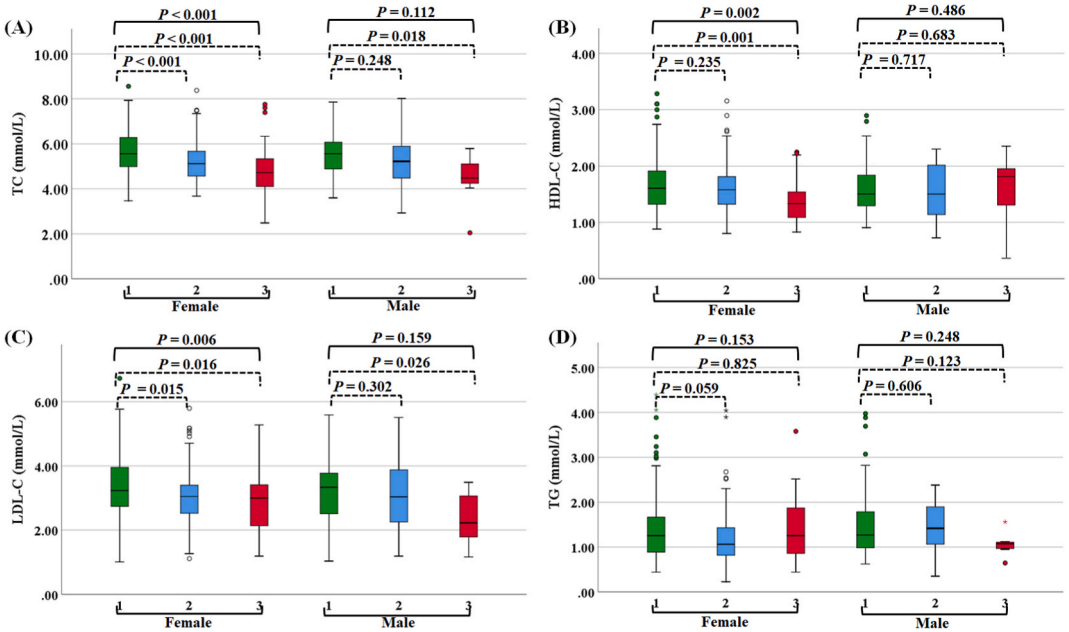

Fig. 4. Box plot of TC (A), HDL-C (B), LDL-C (C), and TG (D) according to sex and thalassemia status (1; non-thalassemia, 2; thalassemia with a minor change in RBC parameters, 3; thalassemia with obvious changes in RBC parameters) [dash brackets represent *p* values from two group comparisons and solid brackets represent *p* values from three group comparisons].

with thalassemia and obvious changes in red blood cell parameters were at a 4.72-fold reduced risk of using lipid-lowering drugs compared to those in the non-thalassemia group. In addition, among participants without the use of lipid-lowering drugs or diabetes, the thalassemia group had a significantly lower frequency of high total cholesterol and/or LDL-C levels than the non-thalassemia group, with a 2.15-fold reduced risk. This study demonstrated that lipid levels, especially TC and LDL-C, were significant lower in thalassemia group, which similar with to other populations [14–17,48–50]. The mechanism underlying the relationship between low

Table 6
The association between sex and hypovitaminosis D in Groups 1-3.

Group	All (n = 468)		Group 1 (n = 316)		Group 2 (n = 117)		Group 3 (n = 35)	
Vitamin D status/Sex	<75 nmol/L (n = 239)	≥75 nmol/L (n = 229)	<75 nmol/L (n = 165)	≥75 nmol/L (n = 151)	<75 nmol/L (n = 57)	≥75 nmol/L (n = 60)	<75 nmol/L (n = 17)	≥75 nmol/L (n = 18)
Female	212(58 %)	154(42 %)	145(60 %)	98(40 %)	51(54 %)	44(46 %)	16(57 %)	12(43 %)
Male	27(26 %)	75(74 %)	20(27 %)	53(73 %)	6(27 %)	16(73 %)	1(14 %)	6(86 %)
P value	<0.001		<0.001		0.022		0.052	
OR (95%CI)	3.82 (2.35–6.22)		3.92 (2.21–6.97)		3.09 (1.11–8.58)		8.00 (0.85–75.56)	

Values are presented as numbers (%); OR, odds ratio; 95 % CI, 95 % confidence interval.
P values were obtained by the chi-square test.

Table 7
The association between sex and hypovitaminosis D in thalassemia carriers and thalassemia patients.

Group	Thalassemia carrier (n = 146)		Thalassemia patient (n = 6)	
Vitamin D status/Sex	<75 nmol/L (n = 68)	≥75 nmol/L (n = 78)	<75 nmol/L (n = 6)	≥75 nmol/L (n = 0)
Female	62(53 %)	56(47 %)	5(100 %)	0(0 %)
Male	6(21 %)	22(79 %)	1(100 %)	0(0 %)
P value	0.002		NA	
OR (95%CI)	4.06 (1.54–10.73)		NA	

Values are presented as numbers (%); OR, odds ratio; 95 % CI, 95 % confidence interval; NA, Not applicable.
P values were obtained by the chi-square test.

Table 8
The association between thalassemia status in combination with vitamin D status and demographic data, biochemical tests, and hematological tests (females).

Thalassemia status	Group 1 (n = 244)		Group 2 (n = 95)		Group 3 (n = 28)	
Vitamin D status	<75 nmol/L (n = 146)	≥75 nmol/L (n = 98)	<75 nmol/L (n = 51)	≥75 nmol/L (n = 44)	<75 nmol/L (n = 16)	≥75 nmol/L (n = 12)
Vitamin D (nmol/L)	59.90 ± 10.01[a]	88.44 ± 11.27	61.95 ± 10.23[a]	89.31 ± 16.46	65.06 ± 5.81[a]	87.18 ± 10.23
Demographic data						
Age (years)	51.55 ± 13.47	52.12 ± 13.92	50.14 ± 11.30	47.55 ± 13.07	55.38 ± 16.95	47.00 ± 14.45
BMI (kg/m^2)	24.70 ± 4.12	23.91 ± 3.92	24.28 ± 4.75	22.65 ± 3.91	23.84 ± 4.91	26.01 ± 3.44
SBP (mmHg)	131.88 ± 20.50	128.19 ± 19.11	126.75 ± 17.23	125.52 ± 23.60	139.19 ± 30.01	125.33 ± 13.09
DBP (mmHg)	80.22 ± 11.80	79.63 ± 12.77	80.12 ± 17.76	77.07 ± 11.65	79.56 ± 18.82	73.42 ± 6.96
Biochemical tests						
FBS (mmol/L)	5.37 ± 0.69	5.34 ± 0.57	5.10 ± 0.75	5.18 ± 0.73	5.43 ± 0.65	5.35 ± 0.51
TC (mmol/L)	5.78 ± 1.02[b]	5.44 ± 0.97	5.37 ± 1.01	5.14 ± 0.93	5.10 ± 1.52	4.70 ± 0.71
LDL-C (mmol/L)	3.41 ± 0.94	3.26 ± 0.90	3.10 ± 0.99	3.07 ± 0.83	2.96 ± 1.21	2.85 ± 0.65
HDL-C (mmol/L)	1.70 ± 0.49	1.64 ± 0.40	1.63 ± 0.37	1.58 ± 0.49	1.46 ± 0.45	1.28 ± 0.26
TG (mmol/L)	1.46 ± 0.69[a]	1.18 ± 0.52	1.39 ± 0.76[c]	1.05 ± 0.40	1.49 ± 0.70	1.24 ± 0.66
Red blood cell parameters						
RBC (10^6/μL)	4.59 ± 0.32	4.57 ± 0.33	4.91 ± 0.41	4.89 ± 0.44	5.50 ± 0.58	5.57 ± 0.46
Hb (g/L)	137.68 ± 9.63	138.36 ± 9.42	126.45 ± 11.89	124.57 ± 17.12	110.81 ± 15.77	117.42 ± 9.83
Hct (L/L)	0.41 ± 0.03	0.41 ± 0.03	0.39 ± 0.03	0.38 ± 0.04	0.36 ± 0.04	0.37 ± 0.02
MCV (fL)	90.13 ± 2.14	90.60 ± 3.18	78.77 ± 4.80	77.69 ± 7.49	64.61 ± 4.98	66.76 ± 7.21
MCH (pg)	30.04 ± 1.29	30.33 ± 1.31	25.78 ± 1.88	25.51 ± 3.12	20.16 ± 2.15	21.26 ± 2.61
MCHC (g/L)	333.33 ± 9.62	334.77 ± 8.84	327.20 ± 9.45	327.34 ± 13.63	311.50 ± 13.05	317.83 ± 10.32
RDW (%)	12.44 ± 1.48	12.37 ± 0.73	13.18 ± 1.67	13.51 ± 1.95	16.33 ± 3.48	14.70 ± 2.49

Data are presented as the mean ± SD.
P values were calculated by the Mann−Whitney U test according to participant groups and vitamin D status (a comparison between the vitamin D sufficiency group and hypovitaminosis D group); vitamin D sufficiency (vitamin D ≥ 75 nmol/L) group of each group was used as the reference group.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

LDL-C levels in β^0-thalassemia and FH heterozygotes is the overexpression of low-density lipoprotein receptor (LDLR) [17] and cholesterol homeostasis during erythrocyte maturation [19,51]. The increased expression of lipid metabolism-related genes has also been observed in cultured erythroblasts [52]. In addition, increased bone marrow activity and cholesterol consumption have been purposed for reducing the cholesterol in thalassemia and chronic anemia [14,18]. Cholesteryl ester transfer protein (*CETP*) gene polymorphisms have been associated with HDL-C levels in Brazilian pediatric population with sickle cell disease (SCD) [53]. TC, HDL-C, and LDL-C levels were significantly lower in patients with hemolytic anemia-related gallstones than in those with general

Table 9
The association between thalassemia status in combination with vitamin D status and demographic data, biochemical tests, and red blood cell parameters (males).

Thalassemia status	Group 1 ($n = 73$)		Group 2 ($n = 22$)		Group 3 ($n = 6$)
Vitamin D status	<75 nmol/L ($n = 20$)	≥75 nmol/L ($n = 53$)	<75 nmol/L ($n = 6$)	≥75 nmol/L ($n = 16$)	≥75 nmol/L ($n = 6$)
Vitamin D (nmol/L)	64.47 ± 8.38[a]	108.94 ± 26.26	64.91 ± 2.74[a]	101.25 ± 17.72	99.75 ± 21.17
Demographic data					
Age (years)	53.30 ± 12.04	50.43 ± 13.69	50.33 ± 10.15	50.06 ± 14.55	56.33 ± 10.44
BMI (kg/m^2)	22.90 ± 2.38	23.10 ± 3.67	24.35 ± 3.47	22.53 ± 3.50	22.86 ± 5.50
SBP (mmHg)	129.15 ± 17.78	127.79 ± 14.98	136.50 ± 15.14	136.63 ± 24.90	124.33 ± 18.62
DBP (mmHg)	77.85 ± 8.25	78.49 ± 9.37	73.67 ± 7.66	83.13 ± 12.95	74.50 ± 13.75
Biochemical tests					
FBS (mmol/L)	5.46 ± 0.60	5.49 ± 0.62	5.11 ± 0.45	5.30 ± 0.53	5.37 ± 0.38
TC (mmol/L)	5.57 ± 0.89	5.47 ± 0.97	5.52 ± 0.85	5.09 ± 1.23	4.83 ± 0.63
LDL-C (mmol/L)	3.24 ± 0.97	3.23 ± 1.02	2.95 ± 1.05	3.01 ± 1.06	2.57 ± 0.79
HDL-C (mmol/L)	1.62 ± 0.47	1.60 ± 0.46	1.90 ± 0.36[c]	1.41 ± 0.48	1.78 ± 0.41
TG (mmol/L)	1.57 ± 0.85	1.43 ± 0.69	1.46 ± 0.40	1.44 ± 0.59	1.05 ± 0.30
Red blood cell parameters					
RBC (10^6/μL)	4.83 ± 0.35[c]	5.10 ± 0.36	5.38 ± 0.44	5.46 ± 0.50	6.06 ± 0.54
Hb (g/L)	147.45 ± 9.58[a]	158.66 ± 11.24	140.67 ± 11.31	148.38 ± 13.05	134.83 ± 9.79
Hct (L/L)	0.44 ± 0.03[b]	0.47 ± 0.03	0.44 ± 0.03	0.44 ± 0.04	0.43 ± 0.04
MCV (fL)	91.74 ± 2.98	92.50 ± 3.35	80.95 ± 2.21	81.54 ± 4.40	70.77 ± 4.60
MCH (pg)	30.55 ± 1.37	31.19 ± 1.51	26.15 ± 0.65	27.26 ± 1.94	22.30 ± 1.49
MCHC (g/L)	332.90 ± 8.90	336.96 ± 7.41	323.17 ± 6.50[b]	334.00 ± 9.22	315.17 ± 9.81
RDW (%)	12.56 ± 0.70[c]	12.22 ± 0.62	12.85 ± 0.76	12.51 ± 0.68	15.92 ± 1.98

Data are presented as the mean ± SD.
P values were calculated by the Mann–Whitney *U* test according to participant groups and vitamin D status and the Student's t-test was used to calculate *p* values for age, DBP, Hb, Hct, FBS, TC, and LDL-C parameters in male group (a comparison between the vitamin D sufficiency group and hypovitaminosis D group); the vitamin D sufficiency (vitamin D ≥ 75 nmol/L) group of each group was used as the reference group).
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

gallstones. Hypolipidemia is more common in hereditary spherocytosis (HS) than in autoimmune hemolytic anemia (AIHA) [54]. A high prevalence of hypovitaminosis D has been reported in several populations, and associated with cardiovascular risks [55–57]. Hypovitaminosis D has also been observed in thalassemia major and intermedia [58]. The prevalence of hypovitaminosis in carriers of thalassemia and/or hemoglobinopathies in this study was 54 % in females and 27 % in males in similar to that in the general population. In Thailand, a high prevalence of vitamin D deficiency was observed in elderly and postmenopausal women [59,60]. Hypovitaminosis D was associated with increased lipid levels and increased risk of having MetS, especially in postmenopausal women [3,4]. The effect of thalassemia on lipid and red blood cell parameters was more predominant than that of vitamin D levels. A recent study revealed that hypovitaminosis D in males resulted in lower RBC and Hb levels in the non-thalassemia group, similar to previous studies [61,62]. A limitation of this study is that the number of patients with thalassemia was quite low because the samples were not recruited through hospital-based collection. However, the evidence of hypocholesterolemia has been well-studied in patients with thalassemia from several populations.

5. Conclusions

Genetic heterogeneity of thalassemia and hemoglobinopathies was observed in this community-based study, and proper management of prevention and control program in the community could be implemented. The effects of several types of thalassemia and hemoglobinopathies with obvious changes in red blood cell parameters and sufficient vitamin D levels on lower lipid levels, especially TC and LDL-C levels, were noted. This phenomenon can be explained by the protective effect of red blood cell disorders against premature CVD through the reduction of cholesterol levels. Increased bone marrow activity and cholesterol consumption have been proposed as cholesterol-lowering mechanisms in thalassemia and chronic anemia. However, the combination of associated factors, such as genetic, nutritional, and environmental factors is required for the precise prediction of cardiovascular risks.

Data availability statement

The data will be made available upon request. All the related data are included in this article.

CRediT authorship contribution statement

Nutjaree Jeenduang: Writing – review & editing, Methodology, Investigation, Funding acquisition. **Dararat Horpet:** Investigation. **Thunyaluk Plyduang:** Investigation. **Manit Nuinoon:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Manit Nuinoon reports financial support was provided by The National Research Council of Thailand (NRCT). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31374.

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