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Detection of BCL11A, HMIP, and XmnI Polymorphisms among Anemic Pregnant Women in Hospital Universiti Sains Malaysia

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Abstract:

BACKGROUND: Anemia is one of the most common conditions in women during pregnancy. Fetal hemoglobin (HbF) levels are usually <1.0%. There are several genetic loci that have a significant influence on HbF levels.

AIMS AND OBJECTIVES: The aim of this study is to determine the association of HbF level and DNA polymorphism at *BCL11A* rs1186868, *HMIP* rs9376090, and *XmnI* rs7482144 in anemic pregnant women.

MATERIALS AND METHODS: Blood samples were collected from 164 anemic pregnant women at Obstetrics and Gynecology Clinic, Hospital Universiti Sains Malaysia. High-performance liquid chromatography was used to determine the HbF and HbA2 levels.

RESULTS: Multiplex amplification-refractory mutation system-polymerase chain reaction (PCR) and gap-PCR were performed for 44 samples with a high HbA2 level (>3.2%) and normal HbA2 level (≤3.2%) to detect mutation at β-globin gene cluster. Allelic discrimination for rs1186868, rs9376090, and rs7482144 was performed using the real-time PCR technique for samples with no mutation detected. The mean age of patients was 31 ± 5.9 years. The HbF and Hb levels showed a strong positive correlation (r = 0.61). Out of 22 samples, 15 mutations at the β-globin gene and no mutation at the δβ-globin gene were detected. The mean difference of HbF level of patients with and without β-globin gene cluster was 1.07 ± 0.6 and 1.79 ± 3.5, respectively. The minor allele frequency (MAF) for the studied single nucleotide polymorphisms as follows: rs1186868 (MAF = 0%), rs9376090 (MAF = 19%), and rs7482144 (MAF = 32.8%).

CONCLUSION: In conclusion, the baseline HbF levels were unaltered by *BCL11A*, *HMIP*, and *XmnI* genetic polymorphisms.

Keywords:

Anemia, fetal hemoglobin, heterozygous, homozygous, pregnancy, single nucleotide polymorphism

Introduction

Severe anemia in pregnancy could lead to serious medical complications. According to the United Nations, up to 56% of women in low-income countries are anemic.[1] The US Centers for Disease Control and Prevention defines pregnancy anemia as a hemoglobin level below 11 g/dL during the first or third trimesters.[2] Severe anemia during pregnancy can lead to neonatal complications such as early birth, low birth weight, and increased risk of maternal and perinatal death.[3] Therefore, appropriate measures for the diagnosis of early anemia should be taken, especially during the first trimester which can lead to a good perinatal outcome.[3]

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The primary oxygen carrier in fetal erythrocytes is fetal hemoglobin (HbF) for fetuses and newborns aged up to 6 months.[4] In adults, adult hemoglobin HbA and HbA2 are the predominant Hb, while HbF levels in normal adults are <1%.[5] HbF differs from HbA by binding to oxygen with higher affinity.[6]

β-thalassemia is a condition of impaired production of β-globin chains, leading to a relative excess of α-globin chains.[7] However, normal red blood cells (RBCs) each contain about 300 million molecules of Hb.[8] In β-thalassemia, there is usually a point mutation in a β-globin promoter region, leading to a decrease in β-chain production and a relative excess of alpha-chains.[8] Additionally, the level of HbF is influenced by several genetic loci. HbF levels are affected by the *XmnI* polymorphism, the *HMIP* region, and the *BCL11A* gene.[9] Variants in the *BCL11A*, *HBS1L-MYB*, and HBB loci have been linked to HbF levels and have been proven to reduce the severity of β-thalassemia.[9] Deletions affecting the β-globin gene cluster, on the other hand, result in the loss of the δ- or β-genes while leaving one or both γ-genes intact.[10] As a result, β-thalassemia, Hereditary persistence of fetal hemoglobin type-6 (HPFH), δβ-thalassemia, Hb Lepore, and γδβ-thalassemia can develop. β-thalassemia deletions are defined as deletions that delete all or part of the β-globin gene.[10] However, δβ-thalassemia is caused by deletions of the δ- and β-globin genes, and it is commonly linked with a slight increase in HbF.[11] To compensate for the lack of β-globin production, this deletion causes high expression of both γ-globin genes.[11]

During fetal life, HbF is expressed during gestational week 10 to birth[12] followed by gradual replacement with HbA after birth because of the change from γ- to β-globin gene expression.[13] Single nucleotide polymorphism (SNP) is a variation at a single position in a DNA sequence among individuals.[14] If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then the variation can be classified as SNP. A previous study has shown that SNP in the *HMIP* on chromosome 6q, *XmnI* on chromosome 8q, and the *BCL11A* locus on chromosome 2p are associated with HbF levels.[15] Thus, analyzing SNPs that affect HbF levels can provide valuable genetic information for patient stratification and can aid in the prediction of disease severity in newborns with β-thalassemia and sickle cell disease.[16] Furthermore, such information could in future assist clinicians in providing more personalized patient therapy based on the predicted phenotype.[16] Finally, determining these SNPs will aid in a better understanding of the molecular mechanisms of action of modifying agents that influence hemoglobin synthesis.[16] Pregnancy, aplastic anemia, thyrotoxicosis, hepatoma, myeloproliferative diseases, and hypoplastic myelodysplastic syndrome all cause a rise in HbF levels.[17] The aim of this study was to assess SNPs (*HMIP*, rs9376090; *XmnI*, rs7482144; and *BCL11A*, rs1186868) in anemic pregnant women in Hospital Universiti Sains Malaysia (HUSM). In this study, we assessed the significance of high HbF among anemic pregnant women and DNA polymorphism at *BCL11A*, *HMIP*, and *XmnI* site loci among anemic pregnant women in Hospital USM.

Materials and Methods

Study design

As there is no a prior information available for our population, we adopted prevalence data of maternal anemia during pregnancy from other populations in the following equation. For this study, we calculated that the minimum use of 164 blood samples was enough to detect 85% study power at a significance value of 0.05%.

$$n = \frac{Z^2 \times p(1-p)}{0.05^2}$$

where

n = sample size

Z = Z statistic for a level of confidence = 1.96 (95% confidence interval)

P = expected prevalence or proportion (in proportion of one; if 100%, P = 1)

= precision (in proportion of one; if 5%, = 0.05).

Patient recruitment and blood collections

A total of 164 anemic pregnant women were recruited at Obstetrics and Gynecology Clinic, Hospital USM, with Hb <11 g/dl. Briefly, 3 ml of blood was collected from each donor with appropriate informed consent as approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/16090283). Routine full blood counts (FBCs) and Hb analysis were carried out on all samples. The FBCs were carried out using the automated hematology analyzer (Sysmex XN-1000™, Japan).

High-performance liquid chromatography analysis

The Hb analysis was performed to determine HbF and HbA2 levels using cation-exchange high-performance liquid chromatography (HPLC) (Bio-Rad Variant II System, USA).

Multiplex amplification-refractory mutation system-polymerase chain reaction and gap-polymerase chain reaction

Genomic DNA was isolated using NucleoSpin® Blood L kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. Multiplex amplification-refractory mutation system (ARMS)-polymerase chain reaction (PCR) and multiplex gap-PCR were performed for 44 samples with high HbA2 level (>3.2%) and normal HbA2 level (≤3.2%) to detect mutation and deletion at β-globin gene cluster, respectively.

To detect β-globin gene mutation, multiplex ARMS-PCR primers (1st Base, Singapore) were used to screen for IVS 1–5 (0.300 μM), Cd 41/42 (0.040 μM), Cd 17 (0.050 μM), and Cd 26 (0.040 μM) HbE mutations. Cd 121 primer was used as an internal control to amplify 861 bp. DNA amplification was carried out using HotStarTaq Plus Master Mix kit (Qiagen GmbH, Hilden, Germany), and 100 ng/ml of genomic DNA was mixed with 9-μl primers. The primers used in this study and their sequences are listed in Table 1.

Amplifications were carried out in a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA). The thermal cycling consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles at 94°C for 25 s, annealing at 64°C for 45 s, at 72°C for 1 min 30 s, and 1 cycle with final extension at 72°C for 7 min. Electrophoresis of PCR products was carried out on agarose gel prestained with FloroSafe DNA Stain (Apical Scientific), and the results were observed under gel analyzing system (Bio-Rad, United States).

Gap-PCR primers were used to screen for Siriraj, HPFH-6, Thai, and Lepore deletions. Briefly, 100 ng/ml DNA was added into a 19-μl master mix together with four different types of primers. Siriraj (0.10 uM), Thai (0.20 uM), and HPFH-6 (0.50 uM) primers were used as positive controls. The primers were designed to detect point mutation of the β-globin gene which causes δβ-thalassemia [Table 2].

The gap-PCR was performed on a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA). The thermal cycling consisted of an initial denaturation at 94°C for

Table 1: List of primers and sequences

Mutation	Primers	Sequences	Product size (bp)
Siriraj J Gγ (Aγδβ)-thalassemia (~118 kb deletion)	Siriraj Forward	5'-GGT CAC GAA TTT GCT TGG TC -3'	620
	Siriraj Reverse	5'-GCG CTG AAA CTG TGG CTT TA –3'	
Thai (δβ)-thalassemia (~12.5 kb deletion)	Thai-Forward	5'-TTC TCC CCA TCA CTT TCA GC -3'	1447
	Lepore Reverse	5'-GGA GGA CAG GAC CAG CAT AA-3'	
HPFH-6	HPFH-6 Forward	5'-CAG GAT GGG GCT CAG AAA TA -3'	974
	HPFH-6 Reverse	5'-AAG GAA ATG AGC CAG CAG AA -3'	
Hb Lepore	Lepore Forward	5'-TGG TGC AAA GAG GCA TGA TA-3'	1159
	Lepore Reverse	5'-GGA GGA CAG GAC CAG CAT AA-3'	
Internal control	Lepore Forward	5'-TGG TGC AAA GAG GCA TGA TA-3'	304
	619 bp - Reverse	5'-CAC AGT CTG CCT AGT ACA T-3'	
ARMS A	IVS 1-5 (G–C)	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG	319
	Cd 41/42 (–TTCT)	GAG TGG ACA GAT CCC CAA AGG ACT CAA CCT	476
	Cd 17 (A–T)	CTC ACC ACC AAC TTC ATC CAC GTT CAG CTA	275
	Cd 26 (G–A)	TAA CCT TGA TAC CAA CCT GCC CAG GGC GTT	301
ARMS B	IVS 1-1 (G–T)	TTA AAC CTG TCT TGT AAC CTT GAT ACG AAA	315
	Cd 8/9 (+G)	CCT TGC CCC ACA CGG CAG TAA CGG CAC ACC	250
	–28 (A–G)	TAA GCA ATA GAT GGC TCT GCC CTG AGT TC	145
	Cd 71/72 (+A)	GGT TGT CCA GGT GAG CCA GGC CAT CAG TT	569
	Control A	CAA TGT ATC ATG CCT CTT TGC ACC	
	Control B	GAG TCA AGG CTG AGA GAT GCA GGA	
	Common E	TGA AGT CCA ACT CCT AAG CCA GTG	

bp=Base pair, del=Deletion; thal=Thalassemia; Cd=Codon

Table 2: List of QTLs that are associated with fetal hemoglobin level

Gene	Location	Sequences
BCL11A rs1186868 (TaqMan MGB probes, FAM and VIC dye-labeled)	2p16	GTGGGCGGGGTGACGGTTTTCTCTT[A/G] GAAATGAAAAACAGCCTTTCCTGCT
HMIP-2 rs9376090 (TaqMan MGB probes, FAM and VIC dye-labeled)	6q23	AGCTAAGTCTAGCTGAGTGTTAGCC[C/T] GGGGGATACTGCCAGGAACAAATGA
XmnI rs7482144 (TaqMan MGB probes, FAM and VIC dye-labeled)	11p15	AACTGTTG CTTTATAGGA TTTT

BCL11A, HMIP, and XmnI - intergenic region. QTLs=Quantitative trait locus

1 min, followed by 1 cycle with template denaturation at 94°C for 30 s, 35 cycles at 62°C for 2 min, annealing at 59°C for 1 min, at 72°C for 1 min, and 1 cycle with final extension at 72°C for 10 min.

Single nucleotide polymorphisms and genotyping

A total of 29 samples, those who were found to be mutation-free through multiplex ARMS PCR and gap-PCR, were subjected to genotyping analysis in the following SNPs: *BCL11A* rs1186868 and *HMIP-2* rs9376090 and *XmnI* rs7482144. The SNPs used in this study and their sequences are listed in Table 2. SNPs of known functional significance were selected based on previous findings and in accordance with the following criteria: (a) the minor allele frequency (MAF) ≥10% in the East Asian population (SNP information was retrieved from the National Center for Biotechnology Information, dbSNP, http://www.ncbi.nlm.nih.gov/SNP/). To detect allelic discrimination of SNP genotype, the DNA (20ng/ml) was mixed with 20x Taqman SNPs genotyping assay mix, 2X TaqPath™ ProAmp Master Mix and nuclease free water according to the manufacturer's instructions. Briefly, ready-made TaqMan SNP Genotyping Assay (TaqMan MGB probes, FAM and VIC dye-labeled) (Thermo Fisher Scientific, USA) was used. Subjects were genotyped for rs1186868 polymorphism (G > A), rs7482144 polymorphism (C > T), and rs9376090 polymorphism (T > C) by using CFX96 real-time PCR (Bio-Rad Laboratories, US). Allelic discrimination analysis was performed using CFX96 Manager software (USA).

Statistical analysis

All statistical analyses were performed using an independent *t*-test by Statistical Packages for the Social Sciences (SPSS) version25 (IBM Corp.®., Armonk, N. Y., USA). Qualitative data were expressed as frequency and percentage and quantitative data were expressed as mean ± standard error of the mean and median. $P < 0.05$ was considered statistically significant. Genetic association analysis was performed by multiple regression. Chi-square test was used to evaluate Hardy–Weinberg equilibrium (HWE).

Table 3: Demographic and hematological profiles of patients

Variable	Mean±SEM	Median
Age	31±0.46	32
Hb (g/dl)	11.04±1.15	11
RBC (×10^{12}/L)	4.3±0.61	4.2
MCV (fL)	81.9±8.50	82.6
MCH (pg)	26.2±3.38	26.3
MCHC (g/dl)	32±1.31	32
HbF (%)	0.7±1.58	4
HbA2 (%)	5.5±7.72	2.8

The demographic and hematological values are indicated as mean±SEM. Hb=Hemoglobin; RBC=Red blood cell; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=MCH concentration; HbF=Fetal Hb; HbA2=Adult hemoglobin; SEM=Standard error of mean

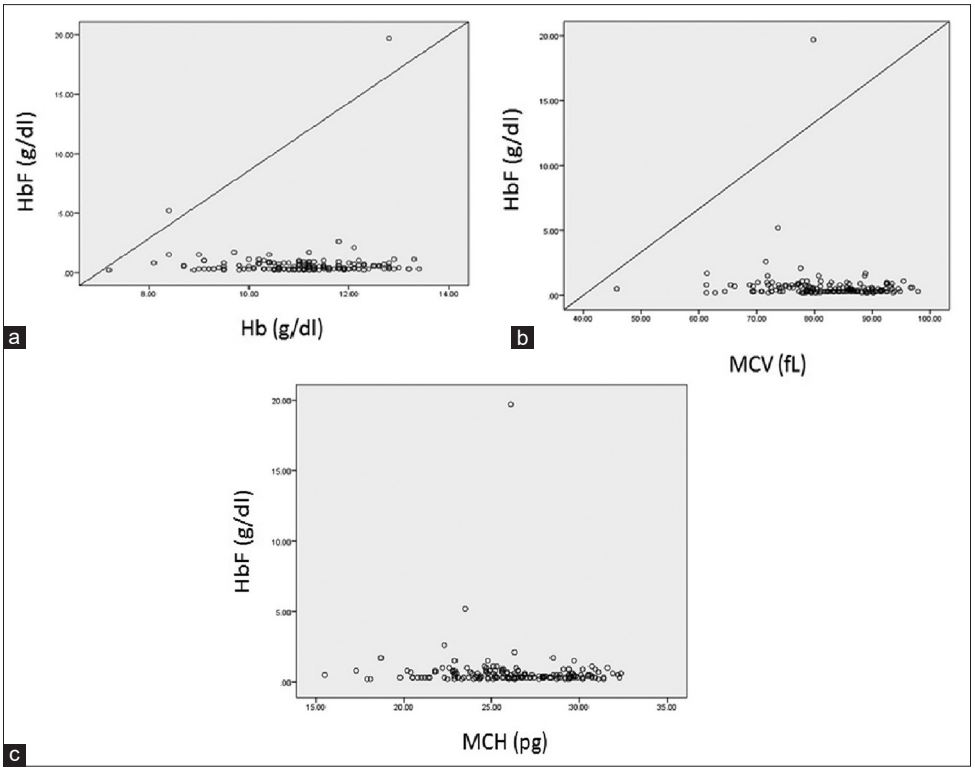

Figure 1: The correlation between HbF and Hb level (a), The correlation between HbF and MCV (b), and the correlation between HbF and MCH (c). HbF: Fetal hemoglobin, MCV: Mean corpuscular volume

Results

Laboratory parameters of recruited patients

A total of 164 pregnant women with Hb <11 g/dl have participated in this study and met our study criteria. The demographic and hematological parameters of patients were summarized, as shown in Table 3.

The correlation between HbF and Hb [Figure 1a], HbF and mean corpuscular volume (MCV) [Figure 1b], and HbF and mean corpuscular hemoglobin (MCH) [Figure 1c] was compared. The HbF and Hb level showed a strong positive correlation ($r = 0.61$). However, the correlation between HbF and MCV was very weak negative ($r = -0.073$), while HbF and MCH showed a very weak negative correlation ($r = -0.045$).

Detection of hemoglobin variants by high-performance liquid chromatography

As illustrated in Figure 2, the concentrations of HbF and HbA2 were measured using a calibration curve. Peak area, which shows the amount of compound that has passed through the detector, was used in the study. HPLC analysis was carried out for 164 samples to determine HbF and HbA2 levels. The HPLC data have shown that 19 of the patients have high HbF and 53 have high HbA2.

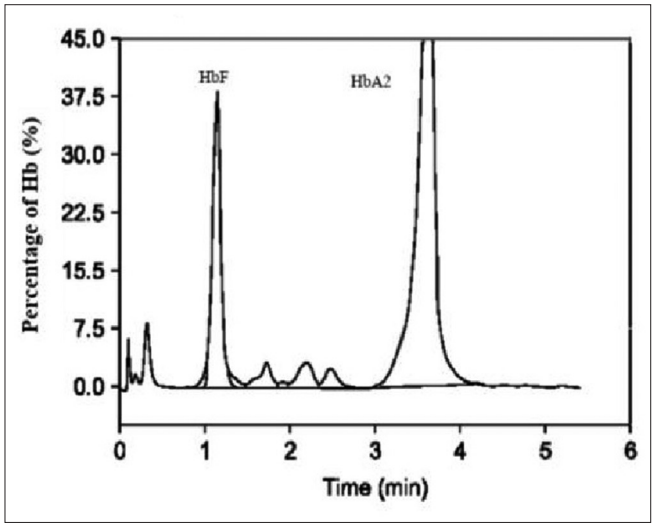

Figure 2: Representative plot of CE-HPLC output. The X-axis indicates the retention time which is the time taken for Hb variants to pass through a chromatography column while the Y-axis indicates the percentage of Hb variants. CE-HPLC: Cation-exchange high-performance liquid chromatography

Gap-polymerase chain reaction

The results indicated that the Internal controls band of a 304-bp control band has been successfully amplified. All samples were analyzed simultaneously with positive controls (Siriraj, HPFH-6, and Thai) for a deletion [Figure 3].

Multiplex amplification refractory mutation system-polymerase chain reaction

The results indicated that the Internal controls band of a 861-bp control band has been successfully amplified. All samples were analyzed simultaneously with positive controls (Cd 26 and Cd 41/42) for a mutation [Figures 4 and 5].

Single nucleotide polymorphism genotyping

SNPs act as modifiers of the HBB gene, causing the elevation of HbF level. The three SNPs rs1186868, rs9376090, and rs7482144 were investigated for their association with HbF levels. Multiple linear regression was used to test

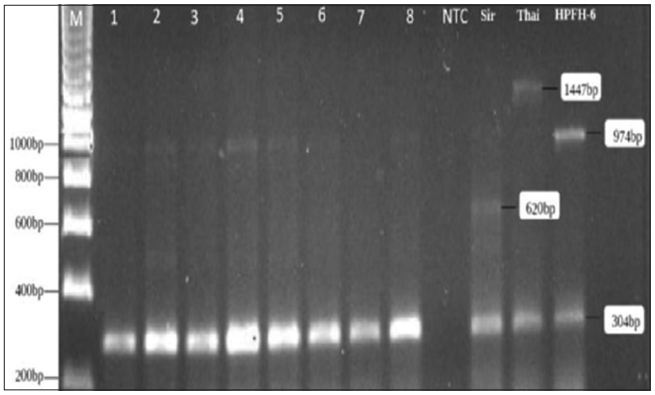

Figure 3: Gap-PCR was performed using 50 ng/μL of DNA samples and was run on 1.2% agarose gels. The 974 bp represents deletion fragment HPFH-6, the 1447 bp represents deletion fragment Thai, the 620 bp represents deletion fragment Siriraj, and 304 bp represents internal control. M represents the 200 bp DNA ladder. bp: Base pair, NTC: Nontemplate control, HPFH-6: Hereditary persistence of fetal hemoglobin type-6. PCR: Polymerase chain reaction

Table 4: Effect of fetal hemoglobin and the genetic fetal hemoglobin modifier variants on hematological outcome variables

Variables	HbF (ln HbF%)	rs1186868			rs9376090			rs7482144		
		GG	GA	AA	TT	TC	CC	CC	CT	TT
HbF	1.000 (0.000)	1.000 (0.000)	-	-	−053 (0.423)	0.981 (0.000)	-	0.428 (0.095)	−0.090 (0.396)	1.000 (0.000)
Hb	0.139 (0.263)	0.139 (0.263)	-	-	0.170 (0.264)	0.349 (0.249)	-	0.434 (0.091)	−0.172 (0.307)	1.000 (0.000)
RBC	0.203 (0.177)	0.203 (0.177)	-	-	−106 (0.348)	0.485 (0.165)	-	0.238 (0.240)	−0.130 (0.352)	−1.000 (0.000)
MCV	−0.148 (0.250)	−0.148 (0.250)	-	-	0.303 (0.127)	−0.467 (0.175)	-	0.178 (0.300)	0.099 (0.387)	1.000 (0.000)
MCH	−0.111 (0.306)	−0.111 (0.306)	-	-	0.418 (0.053)	−0.279 (0.296)	-	0.196 (0.281)	−0.060 (0.430)	1.000 (0.000)
MCHC	0.052 (0.407)	0.052 (0.407)	-	-	0.478 (0.031)	0.132 (0.402)	-	0.144 (0.336)	−0.353 (0.143)	-

Linear multiple regression included HbF, Hb, RBC, MCV, MCH, and MCHC. Allelic effects are presented as the regression coefficient (*P*-values in brackets). Hb=Hemoglobin; RBC=Red blood cell; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC=MCH concentration; HbF=Fetal Hb; HbA2=Adult hemoglobin

Table 5: Comparison of fetal hemoglobin levels between single nucleotide polymorphism genotypes

Locus	SNP/genotypes	*n*	Mean±SD HbF level (%)	MAF (%)	*P*
BCL11A	rs1186868				
	GG	29	1.86	0	0.00
	GA + AA	0			
HMIP	rs9376090				
	TT	22	2	19.6	0.287
	TC + CC	7	1.26		
XmnI	rs7482144				
	CC	13	1.02	33.9	0.224
	CT + TT	16	2.4		

BCL11A, HMIP, and *Xmnl*: Intergenic region. MAF=Minor allele frequency, Hb=Hemoglobin; HbF=Fetal Hb; SD=Standard deviation; MAF=Minor allele frequency; SNP=Single nucleotide polymorphism

for the association of genetic markers with hematological parameters and HbF, as well as for the influence of HbF on blood cell parameters [Table 4]. The MAF for *BCL11A* rs1186868, *HMIP-2* rs9376090, and *Xmn1* rs7482144 was 0.00%, 19%, and 32.8%, respectively [Table 5]. The genotype frequencies of all tested SNPs were checked for their consistency with HWE [Table 6].

Genotyping of *BCL11A* (rs1186868)

Figure 6 shows that allele 1 labeled with FAM dye while allele 2 labeled with VIC dye. Homozygous GG genotypes were auto-assigned to samples containing allele 1. Samples that involve allele 2 were genotyped as homozygous AA. Heterozygous genotypes were assigned to samples containing both allele 1 and allele 2. In this study, all samples displayed homozygous GG which is a wild-type allele. There is no mutant allele which means no presence of SNPs genotyping for *BCL11A* gene (rs1186868) in all our tested samples.

Genotyping of *HMIP-2* (rs9376090)

In this study, 69% of the samples are homozygous TT, which is wild type allele, 24.1% are heterozygous TC, and 6.9% are homozygous CC which is mutant allele [Figure 7].

Genotyping of *XmnI* (rs7482144)

Homozygous CC for 13 samples accounts for 44.8% of the total samples, 13 heterozygous CT samples account for 44.8% of the total samples, and the remaining three homozygous mutant TT samples account for 10.3% of the total samples.

MAF measures the relative frequency of minor alleles of a SNP such as the A allele for *BCL11A* rs1186868, the C allele for *HMIP-2* rs9376090, and the T allele for *XmnI* rs7482144. Our study suggests that there was no significant difference in HbF level between genotypes containing HbF-promoting alleles of rs9376090 and rs7482144 ($P > 0.05$). The results indicated that MAF for rs1186868 was 0%, rs9376090 was 19.6%, and rs7482144 was 33.9%.

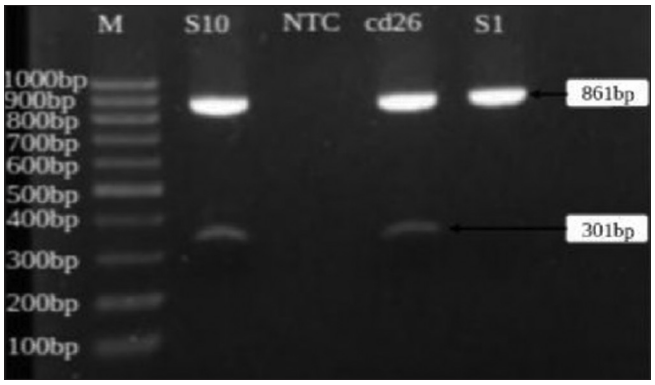

Figure 4: Multiplex ARMS-PCR was performed using 40 ng/μL of DNA samples and was run on 1.0% agarose gel. The 301 bp represents mutation fragment Cd26 and 861 bp represents internal control. M represents the 100 bp DNA ladder. bp: Base pair, NTC: Nontemplate control, ARMS: Amplification-refractory mutation system, PCR: Polymerase chain reaction

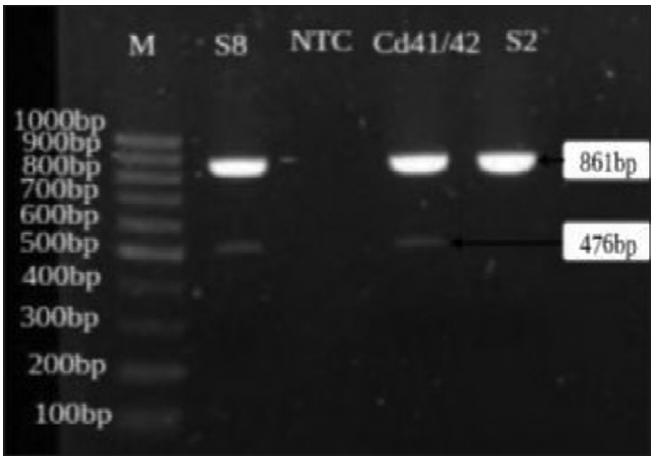

Figure 5: Multiplex ARMS-PCR was performed using 40 ng/μL of DNA samples and was run on 1.0% agarose gel. The 476 bp represents mutation fragment Cd41/42 and 861 bp represents internal control. M represents the 100 bp DNA ladder. bp: Base pair, NTC: Nontemplate control, ARMS: Amplification-refractory mutation system, PCR: Polymerase chain reaction

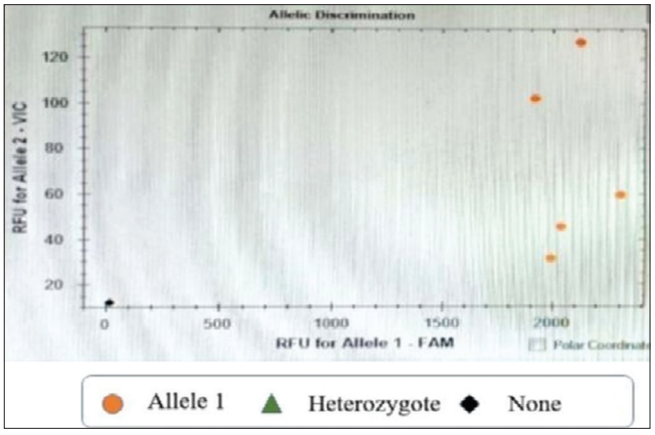

Figure 6: Allelic discrimination real-time PCR genotype of rs1186868. Genotype clusters GG (circle), GA (triangle), and NTC (diamond) are distant from each cluster. PCR: Polymerase chain reaction, NTC: Nontemplate control

Table 6: Genotype frequencies of all single nucleotide polymorphisms

SNPs	Position (chromosome)	Alleles	Genotype frequency (%)			Observed Allele Frequency	HWE	MAF (EAS)
rs1186868	2p	G/A	100.0 (GG)	0.00 (GA)	0.00 (AA)	1.00/0		0.00
rs9376090	6q	T/C	69.0 (TT)	24.1 (TC)	6.9 (CC)	0.69/0.31	1.497	0.353
rs7482144	8q	C/T	44.8 (CC)	44.8 (CT)	10.4 (TT)	0.448/0.552	0.007	0.102

Data were obtained from Ensemble database. EAS=East Asian; MAF=Minor allele frequency; HWE=Hardy–Weinberg equilibrium; SNPs=Single nucleotide polymorphisms

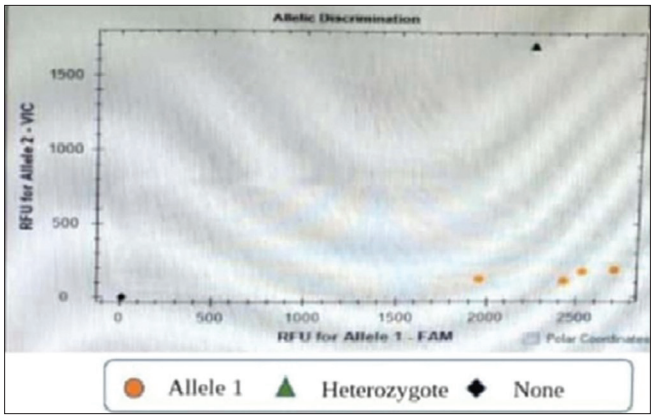

Figure 7: Allelic discrimination real-time PCR genotype of rs9376090. Genotype clusters TT (circle), TC (triangle), and NTC (diamond) are distant from each cluster. PCR: Polymerase chain reaction. PCR: Polymerase chain reaction, NTC: Nontemplate control

Discussion

Anemia is a disorder in which the blood's capacity to carry oxygen to tissues is diminished, evidenced by decreased Hb, RBC, and hematocrit counts.[18] Easy weariness and loss of energy, unusually rapid heartbeat, especially with activity, shortness of breath and headache, especially with exercise, difficulty concentrating, dizziness, pale complexion, leg cramps, and insomnia are all common symptoms of anemia.[19]

Elevated HbF in adults is caused by both inherited and acquired conditions. Examples of inherited disorders are HPFH, hereditary spherocytosis, sickle cell anemia, and thalassemia. Furthermore, some genetic loci may have a major impact on HbF levels.[20] The *HMIP-2* locus on chromosome 6, the *BCL11A* locus on chromosome 2, and the *XmnI* polymorphism on chromosome 11 are all known to affect γ-globin gene expression in adults.[21] Hence, this research investigated the relationship between high HbF and hematological parameters, as well as the presence of rs1186868, rs9376090, and rs7482144 in anemic pregnant women with acquired causes.

The expression of γ-globin genes in adults results in increased HbF level.[22] A previous study has shown that β-thalassemia carriers are expected to have lower MCV and MCH levels, higher HbA2 levels, and roughly 50% have higher HbF levels; on the other hand, α-thalassemia carriers will have reduced RBCs and lower HbA2 levels.[23] This study demonstrates that the mean MCV was 81.9 ± 8.50 fL and MCH was 26.2 ± 3.38 pg. Hypochromic microcytic anemia is described as a low MCV and MCH and high HbF.[24] That means the results suggest hypochromic microcytic anemia because MCV and MCH were low.

The characterization of undiagnosed β-globin gene mutations in pregnant women with β-thalassemia and δβ-thalassemia was performed using various DNA-based diagnostic techniques. Multiplex ARMS technique was used to identify the most common mutations specific primers for amplification of IVS 1–5 (G-C), Cd 41/42 (–TTCT), Cd 17 (A-T), and Cd 26 (G-A) known in the Malaysian population followed by other techniques such as PCR analysis to detect uncommon or unknown mutations.[25] We found a high frequency of carriers with Cd 26 (G–A) and Cd 41/42 (–TTCT) among the patients. δβ-thalassemia results from the deletion of both δ- and β-genes but with preservation of the γ-genes. Homozygous δβ-thalassemia cannot synthesize HbA2 or HbA as they have no δ- and β-genes but comprise 100% of HbF concentration.[23] Multiplex gap-PCR was used to detect globin cluster deletions in the Malaysian population.[26] We found no deletion among the patients. A previous study has shown that multiplex gap-PCR can be used for detection of β-globin gene cluster deletions. In this multiplex PCR system, the 304 bp is internal control of the PCR reaction. The 620 bp, 974 bp, and 1447 bp indicate the presence of the Siriraj, HPFH-6, and Thai, respectively.[27]

Detailed analysis of the association of HbF QTL– *BCL11A* region, *HMIP-2*, and *XmnI* region genes in Malaysia revealed that SNP rs1186868 was significantly associated with borderline and higher HbF levels in the present, but not rs9376090 and rs7482144.[28] *BCL11A* polymorphisms have been strongly related to HbF concentrations in normal people and numerous distinct populations of sickle cell anemia or thalassemia patients, with the *BCL11A* QTL having the largest effect on HbF/F-cell levels so far.[28]

In sickle cell patients, the presence of *XmnI* sites is linked to an increase in HbF production.[29] By comparing MAF in rs1186868, rs9376090, and rs7482144, there was no significant association between all studied SNPs and the HbF levels ($P > 0.05$). This study investigated the rs1186868 polymorphism and discovered that none of the participants were carriers. The SNP rs1186868 was monomorphic, and all the patients were homozygous for G/G. With a

frequency of 1.00, allele G was found to be dominant, while allele A was not found. The rs1186868 allele distribution is similar to the Ensembl database, which shows that the East Asia Summit (EAS) population lacks allele A. The SNP (rs9376090) was homozygous for T/T with a frequency of 69.0, while the T/C allele had a frequency of 24.1, and the C/C allele with a frequency of 6.9. Finally, SNP rs7482144 was homozygous for C/C, with a frequency of 44.8, while the C/T allele had a frequency of 44.8, and the T/T allele with a frequency of 10.4.

Conclusion

Allele G at SNPs *BCL11A* (rs1186868) may act as HbF-promoting alleles. Furthermore, the HbF levels are not influenced by SNPs *HMIP-2* (rs9376090) and *XmnI* (rs7482144).

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Conflicts of interest

There are no conflicts of interest.

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