

# Human hemoglobin G-Makassar variant masquerading as sickle cell anemia

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## Abstract

Human hemoglobin of G-Makassar variant has been reported very rarely with Beta Thalassemia. In year 1969 Hb G-Makassar was first identified in Makassar, Sulawesi (Celebes), Republic of Indonesia. The disease was first published in 1969 and 33 years later it has been reported at a family of Thailand origin. We report a 45-year-old Malay man who was investigated for anemia and thrombocytopenia then diagnosed with Hb G-Makassar. This finding describes as a new Hemoglobin G-Makassar discovered in Malaysia after 14 years diagnosed in Thailand.

## Introduction

Hemoglobin (Hb) G-Makassar mutation was first identified in Indonesia, which are bound Malaysia and the Southeast Asia. Blackwell et al. were described the patient was living and origin of Thailand.<sup>1</sup> The Hb G-Makassar electrophoretic mobility slower-moving  $\beta$  hemoglobin variant exhibited to have its anatomical abnormality at the  $\beta$ -6 or A3 location where the glutamyl residue typically is replaced by an alanine residue.<sup>2,3</sup> Meanwhile the substitution of single amino acid in the gene encoding  $\beta$ -globin subunit  $\beta$ -6 glutamyl to valine will result as sickle cell disease.<sup>4</sup> Both routine hemoglobin electrophoresis separation by cation-exchange High Performance Liquid Chromatography (HPLC) and cellulose acetate electrophoresis were unable to separate Hb G-Makassar and Hb S where they were found to share the same identical properties. As a result, Hb G-Makassar and Hb S could be incorrectly identified for each other and therefore can be misdiagnosed as Sickle Cell Disease (SCD).<sup>5</sup>

## Case Report

A 45-year-old man was previously investigated at Malaysian private hospital for anemia and thrombocytopenia for a year. His platelet was normalized while anemia remained. He then presented to Malaysian government hospital of Haematology Centre with symptomatic anemia and bleeding haemorrhoids. He had frequent bleeding haemorrhoids in the past that required intermittent blood transfusions management. He has no hepatplenomegaly.

Counts on presentation at our centre was; Hb: 5.7 g/dL, MCV: 68.9 fL MCH: 20.2 pg MCHC: 29.3 g/dL, platelet: 407 and WBC:  $8.5 \times 10^3/\mu\text{L}$ . The initial iron (Ferum) was 2.4  $\mu\text{mol/L}$  and ferritin was 5.5  $\mu\text{g/L}$  before started iron therapy. Both iron and ferritin level were improved after iron therapy. The latest ferritin level was 53.4  $\mu\text{g/L}$  in 2017.

Peripheral blood film showed thalassemia pictures but did not reveal any sickle cells. Hb analysis was initially suggestive of compound heterozygous state of Hb S/E. However,  $\beta$  DNA analysis revealed compound Heterozygous codon 26 [GAG>AAG] HbE ( $\beta^E$ ) and codon 6 (GAG->GCG) Hb G-Makassar mutations while  $\alpha$  DNA analysis showed negative result of mutations. Apart of haemorrhoids, the patient is otherwise asymptomatic. After he had surgery for haemorrhoids, his Hb was stable ranging from 9 g/dL to 12 g/dL, MCH 19 pg to 25 pg, MCV 63 fL to 74 fL. He was transfusion independent and only treated with iron therapies. He also does not need regular follow up. During follow up, he never has jaundice. His total bilirubin was 8  $\mu\text{mol/L}$  and did not raised. The reticulocyte percentage was normal but the absolute reticulocyte count was low with 0.67% and  $0.029 \times 10^6/\mu\text{L}$  respectively.

## Results

Figure 1 reveals that the morphology of red blood cell on peripheral blood smear of this patient was microcytic hypochromic anemia with presence of occasional target cells (stain was purchased from Sysmex Malaysia Sdn. Bhd.). This finding could be related to heterozygous  $\beta$  variant (Hb E) detected in this patient as in Hb G-Makassar, it was reported as normal morphology. The typical feature of sickle cell anemia such as boat-shaped cell or sickle cell was not seen.

Figure 2 demonstrates that Hb Makassar variant moved to the same location as Hb S, which was detected at zone

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Key words: Hemoglobin G-Makassar; electrophoresis; beta thalassemia; DNA analysis; sickle cell disease.

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five (Z5) from the routine hemoglobin capillary electrophoresis. Moreover, the other  $\beta$  hemoglobin variants on the same Z5 are Hb Dhofar, Hb S-Antilles, Hb Hamadan, while  $\alpha$  hemoglobin variants on the same Z5 are Hb Arya, Hb Hasharon, Hb Handsworth, Hb Ottawa, Hb Fort de France, Hb Montgomery, Lombard-Hb A2 variant, Cemenelum-Hb A2 variant and Jackson-Hb A2 variant. Denatured Hb O-Arab also observed at the Z5 peak.<sup>5</sup>

In this particular case, Hb S fraction was 63% with the presence of Hb E 25.7%, while minimal Hb A and Hb F was identified. Hb A2 was minimally elevated. This can be mistakenly identified as Compound Heterozygous state of Hb S/E thalassemia.

Figure 3 demonstrates that the mobility of the hemoglobin variant Hb G-Makassar in cellulose acetate electrophoresis was identical to Hb S. Therefore, the effect of alkaline pH was not accomplished to separating Hb S, Hb Punjab and Hb Tak through cellulose acetate hemoglobin electrophoresis. Hence, they appear to migrate to the same position as the Hb F. Hb G-Makassar also retained at the same location as Hb S at acidic pH on cellulose acetate hemoglobin electrophoresis. Because of this similarity, the differential diagnosis of the variants are periodically problematic.<sup>6-8</sup>

Figure 4 describes a DNA sequence on  $\beta$  DNA analysis using PCR amplification that confirmed Hb G-Makassar mutation (GAG  $\rightarrow$  GCG). DNA molecular analysis of the  $\beta$ -globin genes identified the GAG  $\rightarrow$  GCG mutation in codon 6 where the mutation bound for Hb G-Makassar [ $\beta$ 6(A3) Glu  $\rightarrow$  Ala]. In addition, he also had a single point mutation in the codon 26 causing  $\beta^E$ -thalassemia, [GAG  $\rightarrow$  AAG]; he is aggregate heterozygote for Hb G-Makassar/  $\beta^E$ -thalassemia. The Hb G-Makassar mutation was first diagnosed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. It was detected by *Acil* restriction site in the 886 bp fragment, and this fragment was further absorbed on 645 and 241 bp fragments, correspondingly in the Hb G-Makassar allele, variant of the second nucleotide of the codon 6, A  $\rightarrow$  C.

## Discussion

Hemoglobinopathies are inherited abnormalities of globin chain synthesis and Sickle cell anemia is the commonest monogenic diseases as described by Thom *et al.*<sup>9</sup> Piel and David recently reported more than 1000 natural mutation in the human hemoglobin variants. These hemoglobin variants were found to be the effect of single amino acid substitutions throughout the gene molecule. The clinical effects of the hemoglobin variants are vary ranging from clinically insignificant to severe forms of hemoglobin disorder.<sup>10</sup> Nevertheless, over 300,000 babies are estimated to be delivered every year with one of these abnormalities.<sup>11</sup> Sickle-cell disease is most commonly found in sub-Saharan Africa, and parts of the Mediterranean region, the Middle East and the Indian subcontinent. Meanwhile Beta-thalassemia is most common among populations of Mediterranean, African and South Asian ancestry. The prevalence for Southeast Asia is 0-11% of population. However, the incidence of Hb G-Makassar remains less informative as reported by Weatherall and Piel.<sup>12-14</sup>

Many variants of the  $\beta$ - and  $\alpha$ -globin chain will migrate like Hb S under the conditions of alkaline electrophoresis though some variants such Hb D or Hb G can be separated by acid electrophoresis but not Hb G-Makassar. Hb G-Makassar cannot be distinguished from Hb S by isoelectric focusing, HPLC, globin chain electrophoresis or hemoglobin electrophoresis.<sup>15</sup> Table 1 shows the identification of Hb G-Makassar in this case was obtained by  $\beta$  DNA sequence analysis, which revealed a single nucleotide substitution GAG $\rightarrow$ GCG of  $\beta$ -

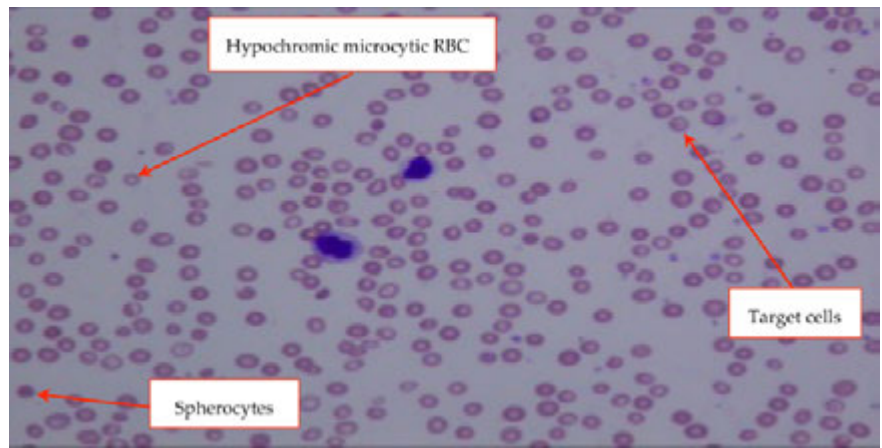


Figure 1. Peripheral blood smears film using May-Grünwald-Giemsa staining shows negative sickle cell of Hb G-Makassar.

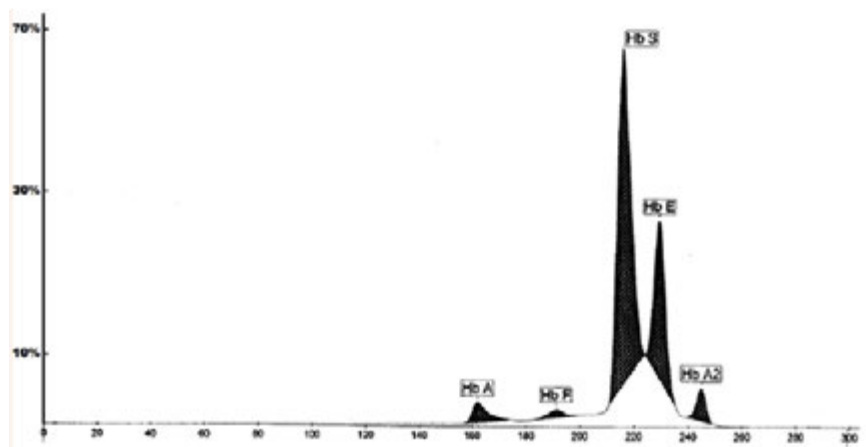


Figure 2. Hemoglobin capillary electrophoresis analysis showing presence of Hb G-Makassar which has properties identical to those Hb S. Where Hb A = 4.6%, Hb F = 2.1%, Hb S = 63.0%, Hb E = 25.7%, Hb A2 = 4.6%.

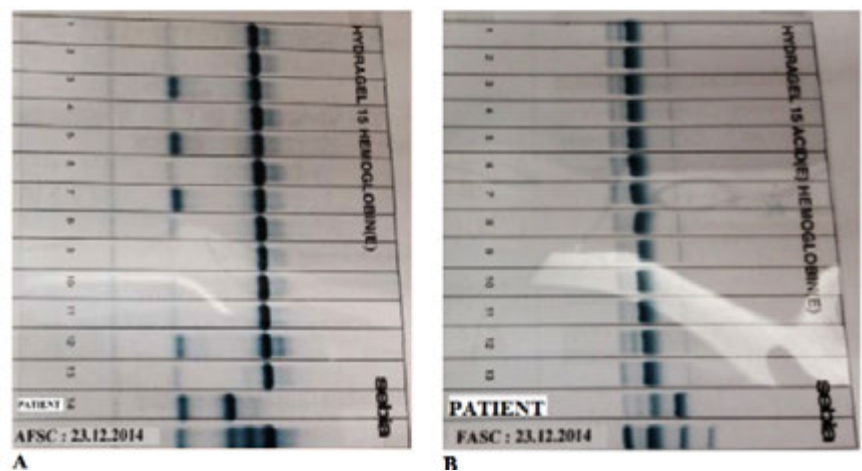


Figure 3. Cellulose acetate hemoglobin electrophoresis at (A) alkaline pH (B) acid pH show presence of Hb S.

globin gene at codon 6 [ $\beta$ :Glu $\rightarrow$ Ala] and Heterozygous codon 26 [GAG>AAG] HbE ( $\beta^E$ ). As there was no sickle cell noted on peripheral blood film, sickling test can be performed to identify the presence of Hb S.<sup>16,17</sup>

Hemoglobin electrophoresis of this patient showed predominantly Hb G-Makassar (63.0%), presence of Hb E (25.7%), minimal Hb A (4.6%) and Hb F (2.1%). After reviewed his DNA analysis, he was diagnosed as compound heterozygote Hb G-Makassar/ $\beta^E$ -thalassemia. The clinical expression of this was a  $\beta$ -thalassemia minor phenotype. In correlation to his clinical features, he has no splenomegaly and no signs of hemolysis. Hence, the presence of Hb G-Makassar was appeared to be functionally proportionate to Hb A. The level of Hb A2 in Hb G-

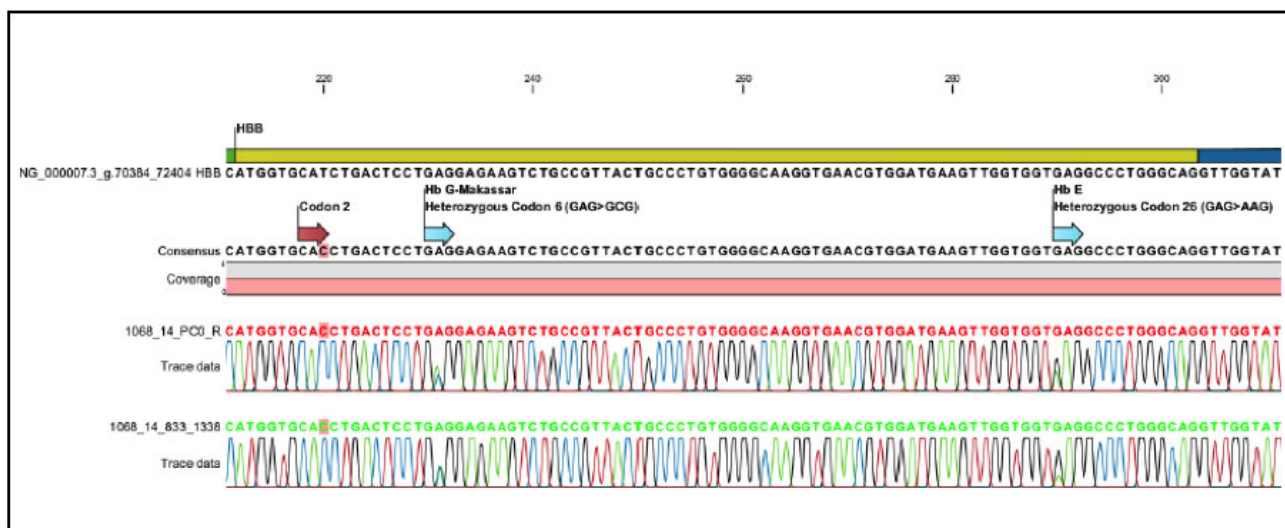
Makassar trait (ranging from 3.7% to 4.7%) and compound heterozygosity with  $\beta^0$ -thalassemia (9.1%) were noted to be higher than typically present in normal controls (ranging from 2.3% to 3.2%) and  $\beta^0$ -thalassemia traits (ranging from 4.0% to 6.0%). This is similar to Hb S trait and Hb S/ $\beta$ -thalassemia. Increased of Hb A2 might reflect of an elevation of  $\alpha$ -globin chains available for tetramer formation with  $\delta$ -globin chains, indicating a minimally reduced affinity of the  $\beta$ S with the  $\alpha$  chain. An analogous explanation may employ to Hb G-Makassar variant.

Position of the structural change of Hb G-Makassar occurs at the same as in Hb S, however their clinical manifestations are absolutely different. Homozygous expression of Hb S concludes sickle cell disease, which is a vaso-occlusive condition and

chronic hemolytic anemia that can sometimes be fatal.<sup>15</sup> Hb G-Makassar heterozygotes are haematologically normal and clinically asymptomatic but Hb G-Makassar/ $\beta^0$ -thalassemia compound heterozygote has attribute to thalassemia minor. On the other hand, homozygous Hb G-Makassar is almost normal and did not have any abnormal clinical feature. Nevertheless, the Hb G-Makassar was viewed and become less informative on their clinical phenotype and haematological disorder. The difference in clinical manifestations of these two hemoglobin variants might be due to the alteration of the Glu  $\rightarrow$  Ala side chain which is not produced enough for polymerization and generation of red cell sickling.

**Table 1. DNA analysis of  $\beta$ -globin gene/cluster and test methods conducted at Institute for Medical Research (Malaysia) Laboratory.**

Method 1: Multiplex application refractory mutation system (ARMS) of polymerase chain reaction (PCR)	Method 2: Big dye cycle sequencing kit and analysed on the ABI 3730XL DNA analyzer
-88 [C>T] ( $\beta^+$ ), -29 [A>G] ( $\beta^+$ ), -29 [A>G] ( $\beta^+$ ), cap +1 [A>G] ( $\beta^+$ ), initiation codon [A TG>AGG] ( $\beta^0$ ), codon 6[GAG>GTG] Hb S ( $\beta^S$ ), codon 17 [AAG>TAG] ( $\beta^0$ ), codon 19 [AAC>AGC] Malay ( $\beta^+$ ), codon 26 [GAG>AAG] Hb E ( $\beta^E$ ), IVS 1-1[G>T] ( $\beta^0$ ), IVS 1-1 [G>A] ( $\beta^0$ ), IVS 1-5[G>C] ( $\beta^0$ ), codon 41/42 [-TTCT] ( $\beta^0$ ), codon 43 [GAG>TAG] ( $\beta^0$ ), codon 71/72 [+A] ( $\beta^0$ ), IVS 2-654 [C>T] ( $\beta^0$ ), Poly A[AATAAA>AATAGA] ( $\beta^+$ )	B-Thalassemia from -100 of the 5' untranslated region to +320 3' unstranlated region to +320 of the 3' untranlated region



**Figure 4. Direct genomic sequencing results of  $\beta$  DNA analysis showing the Compound heterozygous codon 6 (GAG  $\rightarrow$  GCG) Hb G-Makassar and codon 26 [GAG  $\rightarrow$  AAG] Hb E ( $\beta^E$ ) mutations.**

## Conclusions

There are high possibilities of Hb S and Hb G-Makassar presences in ethnic group such multiracial groups as a result of population migration. As Hb G-Makassar cannot be differentiated from Hb S on both acid and alkaline electrophoresis, DNA analysis would be the definitive for the diagnosis of Hb G-Makassar.

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