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ORIGINAL RESEARCH REPORT

Hematological parameters and red blood cell morphological abnormality of Glucose-6-Phosphate dehydrogenase deficiency co-inherited with thalassemia

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Abstract

Objective/Background: Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and thalassemia are genetically independent hemolytic disorders. Co-inheritance of both disorders may affect red blood cell pathology to a greater extent than normally seen in either disorder alone. This study determines the prevalence and evaluates hematological changes of G-6-PD deficiency and thalassemia co-inheritance.

Methods: G-6-PD deficiency was screened from 200 male thalassemia blood samples using a fluorescent spot test. Hematological parameters and red blood cell morphology were evaluated among G-6-PD deficiency/thalassemia co-inheritance, G-6-PD deficiency alone, thalassemia alone, and normal individuals.

Results: G-6-PD deficiency was detected together with hemoglobin (Hb) E heterozygote, Hb E homozygote, β-thalassemia trait, and β-thalassemia/Hb E, α-thalassemia-2 trait, and Hb H disease. Hb level, hematocrit, mean cell volume, and mean cell Hb of G-6-PD deficiency co-inherited with asymptomatic thalassemia carriers show significantly lower mean values compared to carriers with only the same thalassemia genotypes. Higher mean red blood cell

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distribution width was observed in G-6-PD deficiency co-inherited with Hb E heterozygote, as with numbers of hemighost cells in G-6-PD deficiency/thalassemia co-inheritance compared to those with either disorder. Apart from Hb level, hematological parameters of co-inheritance disorders were not different from individuals with a single thalassemia disease. *Conclusion:* G-6-PD deficiency co-inherited with thalassemia in males was present in 10% of the participants, resulting in worsening of red blood cell pathology compared with inheritance of thalassemia alone.

Introduction

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is an X-linked disorder that commonly causes enzymopathy in humans. In red blood cells, G-6-PD is involved in the pentose phosphate pathway required for regeneration of reduced glutathione [1,2]. Decrease in glutathione level in G-6-PD deficiency results in increased oxidative stress in red blood cells, leading to cell hemolysis and anemia; the former is triggered by several external causes, such as antibiotics, antimalarial drugs, fava beans, infections, and acute illness [2]. At present, G-6-PD deficiency affects over 400 million people worldwide, especially in Africa, Asia, the Mediterranean, and the Middle East, where thalassemia also is frequently found [1,3,4]. In Thailand, the prevalence of G-6-PD deficient individuals is in the range of 5—16% and as high as 8—20% in the male population [5—8]. The majority of G-6-PD deficiency in Thailand is due to G-6-PD variants; Canton, Kaiping, Mahidol, Union, and Viangchan [8]. Although their incidences may vary depending on the population tested as well as the detection method, all G-6-PD deficiency variants in Thailand fall into type 3, moderate/mild enzyme deficiency (10—60% normal enzyme activity).

Thalassemia is a genetic disorder caused by a defect in production of one or more globin chains of hemoglobin (Hb). Thalassemia is classified into two major groups, namely, α- and β-thalassemia according to the particular type of globin chain affected [9,10]. α-Thalassemia is mainly caused by a deletion of the α-globin gene, and can be classified into four groups depending on one, two, three, or all four of the α-globin alleles, namely, α-thalassemia 2 trait (-α/αα) or ''silent carrier", α-thalassemia 1 trait (—/αα), Hb H disease (—/-α), and Hb Bart's hydrops fetalis syndrome (—/—), respectively [9]. By contrast, β-thalassemia is generally caused by point mutations of the β-globin gene leading to a reduction (β^+) or absence (β^0) of β-globin chain production. β-Thalassemia can be classified into three clinical and hematological conditions of increasing severity, namely, β-thalassemia minor (β-thalassemia trait), β-thalassemia intermedia, and β-thalassemia major [10]. In addition, Hb variants, e.g., Hb Constant Spring (Hb CS) and Hb E, α- and β-globin variant, respectively, are commonly found in thalassemia prevalent regions of Thailand, resulting in concomitant inheritance of Hb H disease with Hb CS, β-thalassemia with Hb E (β-thal/Hb E) or Hb H disease with β-thal/Hb E (Hb EF Bart's diseases) [11,12]. The clinical manifestations of thalassemia can vary from asymptomatic individuals to those with severe anemia, depending on the degree of gene defect, with the prevalence in Thailand of 30—40% asymptomatic carriers and 1% disease cases [12].

Although G-6-PD deficiency and thalassemia are genetically independent disorders, high prevalence of both diseases in Thailand raises the possibility of the co-inheritance of G-6-PD deficiency and thalassemia, which would likely enhance oxidative stress status in red blood cells, thereby exacerbating the pathology of such affected individuals. In this communication, the prevalence of combined G-6-PD deficiency and thalassemia in representative Thai male thalassemia individuals was determined and red blood cell pathology was evaluated.

Materials and methods

Blood samples

A total of 200 residual blood samples from male thalassemia individuals, with disease and asymptomatic, were obtained immediately after routine analysis for hematological parameters and blood smear preparation from the hematology laboratory, Thalassemia Research Centre, Institute of Molecular Biosciences, Mahidol University, Thailand. Control groups, consisting of 15 genetically normal and three G-6-PD deficiency male blood samples, were collected from volunteers, at the Faculty of Medical Technology and Thalassemia Research Centre, Mahidol University. The study was approved by the Institutional Review Board of the Committee on Ethics, Mahidol University (MU-IRB 2011/147.2812).

Screening G-6-PD deficiency by fluorescent spot test

All blood samples were screened for G-6-PD deficiency within a day of blood collection using a fluorescent spot test (R&D Diagnostics Ltd., Aghia Paraskevi, Greece) according to the manufacturer's protocol. In brief, a 10 μL aliquot of whole blood was mixed with 100 μL of reaction mixture containing glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate. After a 10-minute incubation period at room temperature, the mixture was spotted onto a filter paper and fluorescence detected under long-wavelength ultraviolet (UV) light. G-6-PD deficiency blood fails to generate a fluorescent signal under UV light due to an inability to convert nicotinamide adenine dinucleotide phosphate (nonfluorescent) to reduced nicotinamide adenine dinucleotide (fluorescent).

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