Co-inheritance of Southeast Asian Ovalocytosis and Hb E: Does it affect the red blood cells indices?

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Complete blood count (CBC) is used broadly to screen individual's general health status. Some inherited red blood cell (RBC) disorders influence the RBC parameters. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) are amongst the important RBC parameters used in thalassaemia-haemoglobinopathy screening [1-2]. Globin chain disorders and Southeast Asian Ovalocytosis (SAO) are common RBC disorders in Southeast Asian countries [3]. We evaluated the RBC parameters in patients with Hb E and those with SAO co-inheritance.

A total of 33 from 1500 Malay patient's samples that were sent for thalassaemia-haemoglobinopathies screening in Hospital Kuala Lumpur (HKL) were identified and consented (30 cases with Hb E and 3 cases with co-inheritance of Hb E and SAO). The inclusion criteria were Malay patients with MCV and MCH levels less than 78 fL and 27 pg respectively with presence of oval and stomatocytic RBCs in the peripheral blood film. DNA extraction was performed in samples suspected of having co-inheritance of SAO and Hb E. Primers 198 and 199 (AIT biotech Pte Ltd. Singapore) were designed for SAO detection [4], [5]. Hb E mutation was detected using ARMS PCR [6].

SAO was characterised by presence of an in frame 27bp deletion in exon 11 of the band 3 gene. A band of 175bp was observed in normal subjects and two bands, 175bp and 148bp were observed in heterozygous SAO subjects (Fig. 1).

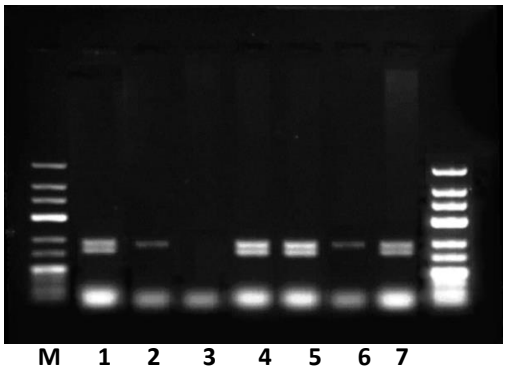

Fig. 1: PCR products of genomic DNA with primer of 198 and primer 199. Lane M is a 100-300bp DNA ladder. Lane 1 shows a positive control of SAO. Lane 2 shows a negative control of SAO. Lane 3 is the no template control (NTC). Lane 4, 5 and 7 show the samples with heterozygous SAO. Lane 6 is a normal sample.

There were only three Hb E-SAO cases identified. All of them were female cases with an average age of 35 years (19 to 59 years old). Their mean RBC count, Hb, MCV, MCH, mean corpuscular haemoglobin concentrate (MCHC) and red cell distribution width (RDW) were 4.5 x $l0^6$/µL, 10.33 g/dL, 66.33 fL, 22.5 pg, 33.96 g/dL, and 18.3%, respectively. One-way ANOVA was used to test the differences between the co-inheritance group and Hb E trait group. Statistical analysis showed that the co-inheritance SAO and Hb E trait significantly affect the MCV and RDW parameters of FBC (p<0.05). Other RBC parameters including Hb, RBC count, MCH and MCHC between co-inheritance group and Hb E group were not statistically significant (p>0.05).

SAO and Hb E trait are both common genetic defects in Malaysia [3]. Prevalence of SAO and Hb E among the Malay population are 5.1% and 4% respectively [3,5]. Therefore, the chances of both disorders being co-inherited may be high and could have some effects on RBC parameters. However, the data and research posed in patients with association of these two disorders are limited. Co-inheritance of SAO and Hb E did not demonstrate any differences in RBC indices when compared to Hb E alone [7]. In this study, we found only the MCV and RDW were different between the co-inheritance group and Hb E trait group.

MCH is the most important and preferred parameter compared to MCV for thalassaemia and Hb variants screening. The MCH cut-off value of <27 pg is suggestive for thalassaemia-haemoglobinopathies [1-2], The cut-off value of MCV in some individuals with Hb E trait is considered as less than 80 fL, while some Hb E trait patients have MCV levels of more than 80fL [5-6]. Therefore, the level of MCV alone might lead to missed to be detected and is not a reliable parameter for Hb E trait detection. However, it is clear that MCV is usually decreased in Hb E and when in combination with SAO, the MCV is significantly lowered. Therefore, in co-inheritance cases, the MCV can be as useful as MCH for screening purposes. A low MCV with high RDW is not only indicative of iron deficiency anaemia but also for Hb E-SAO as shown in this study.

Co-inheritance of SAO and Hb E in the Malay population does indicate reduced MCV values, however it does not significantly affect the cut-off values of other parameters that are fundamental for haemoglobinopathy screening.

Keywords: Hb E, SAO, Co-inheritance of SAO and Hb E, RBC parameters

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References:

1. Hartwell, S. K., et. al., Review on screening and analysis techniques for hemoglobin variants and thalassemia. *Talanta*, 2005. **65**(5): p. 1149-61.
2. Ryan, K., et. al., Significant haemoglobinopathies: guidelines for screening and diagnosis. *British journal of haematology*, 2010. **149**(1): p. 35-49.
3. Fucharoen, G., et. al., Coexistence of Southeast Asian ovalocytosis and beta-thalassemia: a molecular and hematological analysis. *American journal of hematology, 2007*. **82**(5): pp. 381-385.
4. McMullin, M. F., The molecular basis of disorders of the red cell membrane. *Journal of clinical pathology*, 1999. **52**(4): p. 245-248.
5. An, X., Mohandas, N., Disorders of red cell membrane. *British journal of haematology*, 2008. **141**(3): p. 367-375.
6. Clark, B. E., Thein, S. L., Molecular diagnosis of haemoglobin disorders. *Clinical and laboratory haematology*, 2004. **26**(3): p. 159-176.
7. George, E., Kudva, M. V., Homozygous haemoglobin E in association with hereditary ovalocytosis. *The Medical journal of Malaysia*, 1989. **44**(3): p. 255-258.