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Sickle haemoglobin: How critical are laboratory quality measures for accurate identification?

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Madam,

Adult haemoglobin (Hb) comprises of 2 alpha and 2 beta-globin chains, each having a haem molecule attached**.** In healthy individuals, around 95-98% HbA (α2β2) and 2–3.5% of Hb A2 (α2δ2) are present. The genetic defect of globin chain in which valine is replaced for glutamic acid at position 6 of β globin chain results in sickle haemoglobin (HbS). Homozygous genetic defect (βSβS) results in a symptomatic disease called 'sickle cell anaemia' whereas, heterozygous (ββS) state is asymptomatic and commonly called as "sickle cell trait' [1]. On deoxygenation and dehydration, HbS undergoes irreversible polymerization causing deleterious effects in vivo [2]. Around 3.2 million people have sickle-cell disease worldwide, with about 80% cases in Africa. About 0.5 to 1 per cent of the Pakistani population carries HbS3. Currently, high-performance liquid chromatography (HPLC) is the preferred method in which HbS elutes at retention time ranging in between 4.1 to 4.7 minutes. Several other variant haemoglobins cause interference by co-eluting at same retention time include HbA2, Hb Q-Thailand, Hb Manitoba, Hb Russ, Hb Stanlivelly-II, HbE- Saskatoon, Hb Montgomery and many more[4]. Therefore, it is difficult to identify and differentiate HbS precisely from interfering variant haemoglobins for proper

diagnosis and future genetic counselling of patients. The definitive test for this purpose is molecular detection of underlying mutation either by polymerase chain reaction (PCR) or sequencing of the beta-globin gene. Molecular tests, however, are expensive and require expertise. Therefore, these tests are not widely available in Pakistan and other resource constraint countries. World's leading quality assurance organizations such as College of American Pathologists (CAP) recommend that all cases found HbS positive in the primary screening should be confirmed by secondary testing [5], this can easily be achieved by sickling test. In the sickling test, the sample is combined with reducing agent (Sodium Met bisulfate) resulting in red cell hypoxia. Cells with HbS change to sickle shape from their normal biconcave shape, diagnosed by microscopic examination along with controls. This cost-effective and readily available technique helps to differentiate HbS from other variants. Therefore, every laboratory should confirm the presence of HbS by sickling test, and the government should ensure the availability of molecular tests at the mass level at a reduced cost supported by the efficient health insurance system. The impact of these strategies will provide accurate and timely diagnosis, and hence appropriate management and future genetic counselling of the patients could be easily achieved.

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