Detection of β-thalassemia (homozygous) by hemoglobin electrophoresis on agar gel and citrate agar medium: a case report

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ABSTRACT
A seven month old male baby was admitted to NICU of BPKIHS, Dharan with dyspnoea and distress. He was investigated for hemolytic anemia and suspected of β Thalassemia (Homozygous) based on the low level of Hb and high HbF%. To investigate further, Hb electrophoresis was carried out using agar gel and citrate agar gel at pH 8.6 and 6.0 respectively along with control. Electrophoretogram showed single narrow band lagging behind the healthy control moved toward anode at pH 8.6 and single narrow band moved parallel to healthy control toward cathode at pH 6.0. Thus the diagnosis of β Thalassemia (Homozygous) is favored.

Keywords: β Thalassemia, HbA, HbA2, HbF, Hb electrophoresis.

Structurally, Hemoglobin, the carrier molecule of O2, is made up of heme-iron and four globin chains arranged in a matching pairs. HbF, HbA, and HbA2 are made up of α2γ2, α2β2 and α2δ2 globin chain respectively. β Thalassemia arises as defective production of β chains and manifest as hemolytic disorders of varying degrees if it is heterozygote but traits are asymptomatic. Though DNA analysis is advocated for diagnosis in many centres, demonstration of abnormal quantities of Hb in electrophoretogram is still valuable and advocated routinely for patient care. Nepal is defined as Thalassemia zone by World Health Organization. Cases are diagnosed by electrophoresis using agar gel and citrate agar at pH 8.6 and 6.0 respectively. Among many patients diagnosed, this is a classic case of β Thalassemia (heterozygote) diagnosed by agar gel electrophoresis.

CASE REPORT
A seven month old male baby was admitted to neonatal ICU of BPKIHS, Dharan with dyspnoea and distress. On examination, he was found pale. His body weight was 5 Kg with crown rump length of 41 cm. He was referred for laboratory investigations and reported as follows:

Blood Chemistry: Total protein: 7.3 g/dl, Albumin: 4.4 g /dl, Total bilirubin: 1.5 mg/dl and Direct: 0.2 mg/dl.

Hematology: Hb: 5.9 g /dl, HbF: 45.0%

Peripheral Smear: Mild to moderate anisopoikilocytosis with mild moderate hypochromia.

Differential Count: Neutrophil-40.0% Lymphocyte-55.0% Monocyte-2.0% Eosinophil-3.0%.

He was suspected of a case of β Thalassemia (Homozygous) based on the low level of Hb and high HbF%. To investigate the cause of hemolytic anemia further, Hb electrophoresis was carried out using agar gel and citrate agar gel at pH 8.6 and 6.0 respectively along with control.

Agar gel medium at pH 8.6: single narrow band lagging behind the healthy control moved toward anode (Fig. 1.).

Citrate agar medium at pH 6.0: single narrow band moved parallel to healthy control toward cathode (Fig. 2.).

DISCUSSION
Thalassemia disorders are common in people of South East Asian Ancestry. Though Nepal lies in thalassemic zone, detection and molecular analysis of the disease by laboratory method has not been characterized. Among many diagnostic methods, identification of hemoglobins by electrophoretic techniques on cellulose acetate paper has become vital investigation and plays an important role in routine procedure. However, uses of agar gel and citrate agar as supporting medium are used with satisfactory result in this centre.
Hemolytic baby of this kind with low Hb level of 5.9 g/dl, at the age of 7 months, with muslim ethnic backgrounds, prompted the diagnosis toward $\beta$-thalassemia (Homozygous). He also had 45.0% of HbF, estimated by alkali denaturation to support the diagnosis. Again peripheral smear showed mild hypochromia and anisopoikilocytosis suggestive of hemolytic crisis.

With the above background, electrophoretic detection of hemoglobin was conducted at pH 8.6 using barbitone buffer with ionic strength of 0.05 M and constant voltage of 200-300 V. Alkaline pH favors satisfactory separation of HbA and HbA$_2$ and some common variants e.g. HbS, HbC etc based on their surface charges. At this pH, each HbA molecules acquires two negative charges more than HbS and HbA$_2$ and four more negative charges from HbC that depend on the sequence of amino acids in globin fraction. Since HbA acquires maximum negative charges at this pH, it moved farthest towards anode (+ve terminal) in electric field. (Fig. 1. control)

But patients sample lag behind confirming the absence of HbA. (Fig. 1. patient sample). This electrophoretic finding also suggests the presence of either HbA$_2$ or HbS since they bear same charges which creates diagnostic dilemma. But occurrence of HbS in people in this geographical region is rare. Additionally two hemoglobins are differentiated by electrophoretic technique using citrate agar medium at pH 6.0. At pH 6.0 which is acidic in nature, HbA and HbA$_2$ acquires positive surface charges and moved equally towards cathode (-ve terminal) (Fig. 2. control and patients sample) but HbS still it bears negative charges and moves toward anode but absent in this case. Thus presence of HbA$_2$ is confirmed and the diagnosis of $\beta$ Thalassemia (Homozygous) is favored.

REFERENCES

**Fig. 1.** Hb electrophoretogram at alkaline pH (8.6) in agar gel support medium. Hb (control) has moved toward anode and patient’s sample lagging behind confirming the absence of HbA

**Fig. 2.** Hb electrophoretogram at acidic pH (6.0) in citrate agar support medium. Hb (control) and patient’s sample have moved equally toward cathode but no movement toward anode confirming absence of HbS.