

Agar Gel Alkaline Electrophoresis As A Cost Effective Screening Modality For Abnormal Hemoglobins

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Abstract: Alkaline agar gel electrophoresis is a basic manual laborious technique for identifying Hemoglobin variants in the screening for Hemoglobinopathies. It's a cheaper alternative to the now preferred method of detection of Hb variants, i.e., HPLC.

In this study, at a government hospital clinical biochemistry laboratory setup, we try to compare and emphasize the importance of this manual procedure. We have done this observation from the archives of lab and case sheet data for the past four and half years; that 1891 electrophoresis were done and 65% were normal, 20% shown Thalassemia trait, and 6% were Thalassemia major and 8% were shown sickle Hb pattern. Majority correlated clinically with the electrophoresis report and in doubtful cases (1.32%), HPLC confirmed the electrophoresis report.

Hence wherever feasible, we recommend this manual alkaline agar gel electrophoresis where cost is a limiting factor.

Keywords: Agar gel alkaline electrophoresis; HPLC, High Performance Liquid Chromatography; SCD, Sickle cell disease; Thalassemia.

I. Introduction

Abnormalities of hemoglobin (Hb) synthesis are among the most common inherited disorders of either quantitative (thalassemia syndrome) or qualitative (variant Hbs) (1). The laboratory diagnosis of hemoglobinopathies and thalassemias is of growing importance, particularly because of an increasing requirement for antenatal diagnosis of significant disorders of globin chain synthesis (2). The identity of a hemoglobin variant is generally inferred from its electrophoretic mobility, its quantity, and the patient's ethnic background (3-5). Alkaline and acid hemoglobin electrophoresis is the most widely used methods for investigating hemoglobin variants and hemoglobinopathy. Alkaline electrophoresis is a reproducible method which is capable of separating common hemoglobin variants, such as hemoglobin A (Hb A), Hb F, Hb S and Hb C but Hb S, Hb D, Hb G and Hb Lepore are unresolved from each other, as are Hb C, Hb A2, Hb O-Arab, and Hb E. In addition, there are other variants with electrophoretic motilities identical or similar to those of Hb S and Hb C. Consequently, acid electrophoresis is needed for the identification of the aforementioned variants (4). Hemoglobin fraction analysis by cation-exchange HPLC has become the preferred method for quantifying Hb F and Hb A2 along with hemoglobin variant screening as a routine in the recent years.

In our hospital, Agar gel alkaline electrophoresis is the technique still in use for detection of thalassemias and hemoglobin variants, since long time. All the doubtful results of this screening were subjected to HPLC for further confirmation as a routine practice. Hence our study is designed to compare the results of electrophoresis with clinical condition and or reports of HPLC in diagnosing hemoglobin disorders.

II. Materials & Methods

This is an observational study from the archives of laboratory records and case sheets at Niloufer hospital for women& children, a Government hospital. A total of 1902 patients samples were received for hemoglobin electrophoresis at Clinical Biochemistry Laboratory, from January 2012 to august 2016 (duration of four and half years), were included in this study. Out of those 1902 samples, 6 were severely hemolysed, one has severe iron deficiency anemia and 4 samples were insufficient for the analysis hence those 11 samples were discarded. Hb electrophoresis was done for the remaining 1891 samples and electrophoretic patterns observed for abnormalities. These samples mainly constitute of pediatric age group or parents of those children in which carrier testing has to be done. Full case history including family history, ethnicity was noted. Complete blood picture, Sickling test results were verified from case records wherever necessary, in aiding the diagnosis.

Electrophoresis was performed by GOLDBERG method, on Biotech® electrophoretic chamber with manually prepared agarose gel slide (6). For all the samples, hemosylate was prepared and HbF% calculated by JONXIS method (7). Electrophoresis was performed for all the samples and patterns observed. Those samples whose diagnosis were ‘doubtful’ or need further identification like Hb E trait or Sickle β Thalassemia were only sent for analysis on automated HPLC (VARIANT TM, Bio-Rad) system (with the Beta-thalassemia Short Program was used) at outside resources and the reports were obtained by the clinical staff and were filed in the patient case records.

III. Results

Distribution of the samples was shown in figure-1, along with their diagnosis, and the number of cases was given in the table-1.

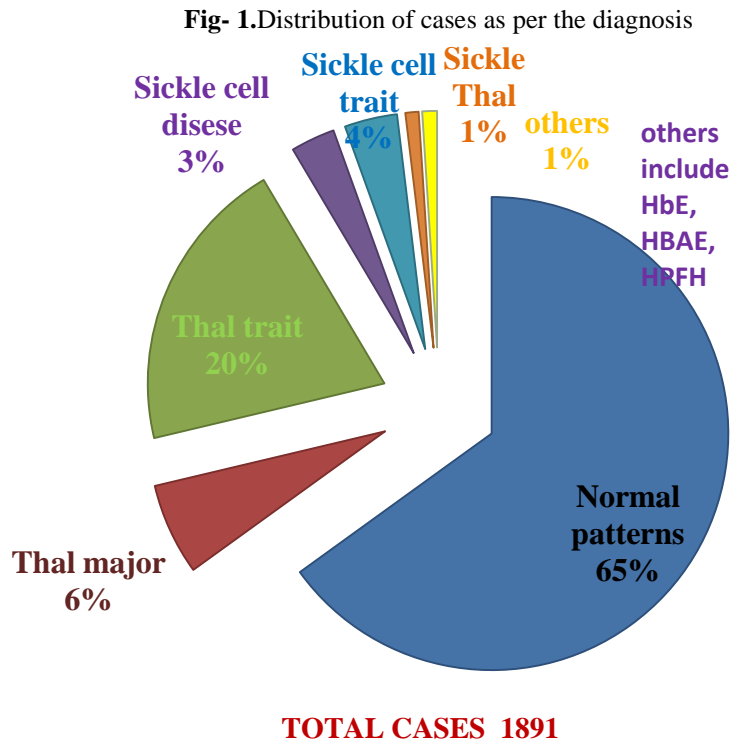


Table-1a. Distribution of cases by Diagnosis

S. NO	DIAGNOSIS	No. of cases
1	Normal patterns	1229
2	Thalassemia major	118
3	Thalassemia trait	381
4	Sickle cell disease	59
5	Sickle cell trait	68
6	Sickle β Thalassemia	17
7	Hb E disease	8
8	Hb AE	5
9	HPFH	6
	total	1891

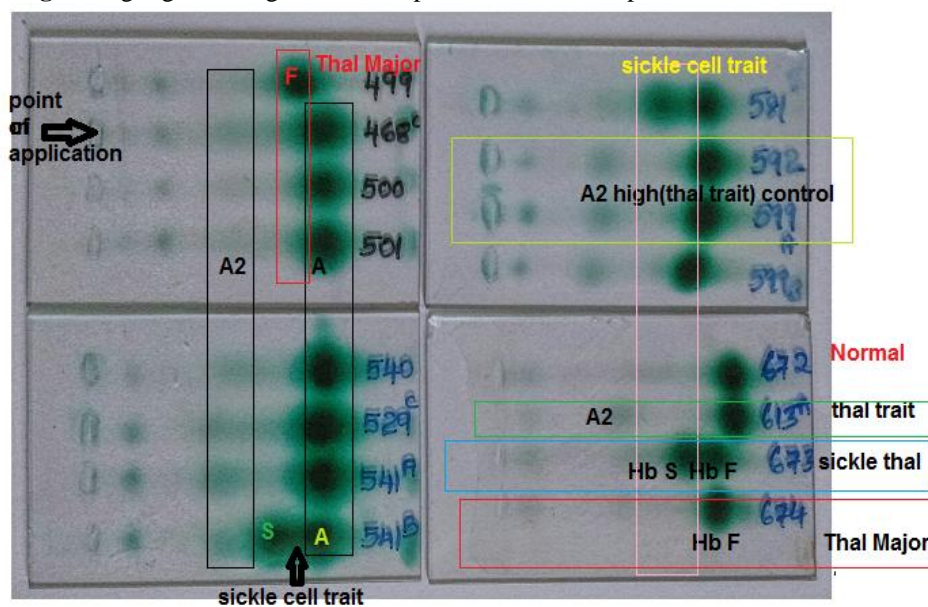
Table-1b. Distribution of Thalassemia

	Thalassemia major	Thalassemia trait	
		Pediatric patients	parents of Thalassemia major patients
No. of cases	118	61	320
		total 381	

Out of the 1891 samples run, 1229 samples were shown normal. Thalassemia major and trait were 118 and 381 respectively. Out of 118 Thalassemia major cases, 6 were known cases that are already on treatment and remaining all were correlating clinically with the clinical diagnosis. Out of 381 thalassemia traits (high A2 and Hb A present), 61 were from pediatric patients and 320 were from the parents of thalassemia major cases. Only one pediatric patient sample has to be differentiated from HbE trait and further analysis by HPLC was negative for Hb E trait (i.e., Hb AE). There were 13 cases with high density A2 zone bands with a high index of suspicion were sent for further analysis were confirmed the presence of Hb E and were diagnosed as HbEE: 8 cases and HbAE: 5 cases.

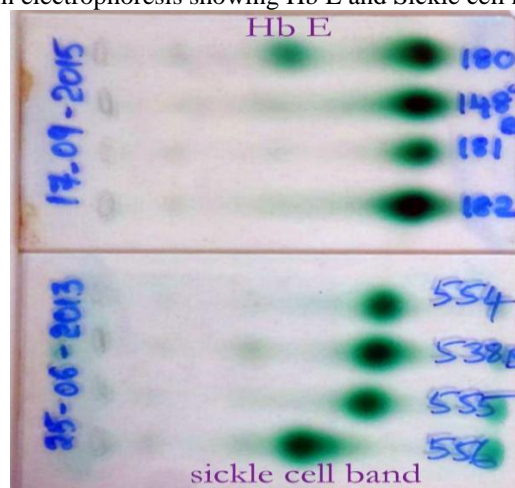
The 6 cases with increased (>20%) HbF and with Hb A band and normal A2 were given a diagnosis of syndrome of Hereditary Persistence of Fetal Hemoglobin, i.e., HPFH and were well correlated with clinical features and mild degree of anemia, were again confirmed with HPLC. Sickle cell disease (SCD) and trait (SCT) were found to be 59 and 68 respectively. All SCD samples were correlating with clinical and pathological diagnosis. Out of 68 SCTs, one has to be differentiated from Sickle beta Thalassemia with doubtful Hb A₂ and A concentrations, needed confirmation by HPLC. There were 17 cases of Sickle beta Thalassemia in which 4 were doubtful and further needed confirmation by HPLC. These variants are shown in Fig 2a & 2b.

Fig-2a. Agar gel Hemoglobin electrophoresis in alkaline pH. Hb variants are shown.



All the thalassemia major patients' parents were screened for hemoglobinopathies and were found to have thalassemia trait by alkaline gel electrophoresis. These samples were not sent for further confirmation by the HPLC analysis as they were found to have only a mild degree of anemia. All the 25 cases (1.32%) sent for HPLC out of 1891 were only further confirming the electrophoresis pattern and none differed from what was expected.

Fig 2b. Hemoglobin electrophoresis showing Hb E and Sickle cell homozygous pattern.



IV. Discussion

Hemoglobin abnormalities are the most frequent genetic disease, affecting approximately 7% of the world population. Abnormal Hbs are generally discovered during a systematic study performed within programmes for prevention of thalassemias or sickle cell disease. In several regions (India, Turkey, Iraq, Iran, Gaza strip, Saudi Arabia, Cyprus, *etc.*), these are found during a premarital screening. The investigation of a patient presenting with a hematological disorder such as a chronic hemolytic anemia, an unexplained polycythaemia or a permanent cyanosis is another, completely different condition, leading to the discovery of an abnormal Hb (8, 9). Most of the samples received in our laboratory were mainly of this type rather than for premarital screening. Electrophoresis, a test based on the migration of electrically charged molecules under an applied electric field, occupies one of the most important places in the history of abnormal Hb detection (9). The identification of hemoglobin variants is often presumptive, based on characteristic electrophoretic mobility, quantity, and/or ethnic origin. Fig 2a, 2b&3 shows various electrophoretic patterns and the apparatus along with our staff, respectively.

In our study we tried to compare the results obtained by basic gel electrophoretic technique with the gold standard HPLC method, which had replaced electrophoretic techniques in most labs of the India. Our results by Alkaline gel electrophoresis and HPLC technique which were similar to a study conducted by the *Monica et al* from Canada (19); with the suggestion that, both the techniques must be repeated after correcting iron deficiency anemia. We found no differences between alkaline gel electrophoresis and HPLC for screening hemoglobinopathies. Only 1.32% of all the cases required a further clarification by HPLC. From this, we can suggest that, in a developing country like India especially in remote places where HPLC cannot be affordable, alkaline gel electrophoresis still holds its place in screening hemoglobinopathies, with commitment for a laborious procedure.



Fig 3. Hb Electrophoresis apparatus and reagents (and due respects to the committed technical staff Mrs.Sameena, Ms.Suneetha, and Mrs.Ajitha & Mrs.Hemalatha).

Definite identification usually requires DNA analysis or amino acid sequencing (2). HPLC has been shown to be a sensitive, specific, and reproducible alternative to electrophoresis. Its use has been dramatically expanded, especially with the development of rapid, well-resolving, and fully automated analyzers. In the past decade HPLC, with its automation and its quantitative power, has appeared to be an appropriate candidate for direct identification and sensitive quantification of major and minor, normal and abnormal, hemoglobin fractions (10, 11, 12–18). Though Alkaline electrophoresis (pH 9.2) is the most common initial screening method for the detection and preliminary identification of hemoglobinopathies, Isoelectric focusing electrophoresis (IEF) has greater resolving power than conventional electrophoresis, but it is more expensive, time consuming; hence not adopted in many laboratories. HPLC technique is precise for the quantification of both Hb F and Hb A₂, which made HPLC the preferred method for hemoglobinopathy and thalassemia screening for many laboratories. Capillary electrophoresis is more recent method and has advantage of quantification of Hb H, and identification of Hb Lepore. Electrospray mass spectrometry is becoming the method of choice for the complete characterization of newly discovered Hb variants. DNA analysis is used in

the investigation of thalassemias and hemoglobinopathies to identify, in populations with a known high incidence of disease (20).

Limitations of the study

Electrophoresis in Acidic medium was not practiced in our lab, for; HPLC was the next investigation of choice.

V. Conclusion

Advances in technology like DNA sequencing, capillary electrophoresis and HPLC; has made the diagnosis of hemoglobinopathy easily detectable and aided in 100% confirmation of disease though they will not change the treatment options. In our study we attempted to compare the basic electrophoretic technique with HPLC and found 100% agreement between two for screening of hemoglobinopathy. Many laboratories are now using HPLC or capillary methods for screening hemoglobinopathies, but alkaline electrophoresis still holds good for the same especially in government sector or rural areas where cost is the major issue.

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Conflict of Interest: None to declare.

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