Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

TO STUDY IMPORTANCE OF ALKALINE PAPER ELECTROPHORESIS TECHNIQUE FOR THE DIAGNOSIS OF SICKLE CELL ANEMIA.

Dr.R. Salma Mahaboob[1], Dr. G. Obulesu [2*], Dr. Ramanesh Murthy [3], Dr. Madhumitha Murthy[4], Dr. Rajesh kumar Srivastava [5].

1. Associate Professor in Biochemistry at **RSDKS** Government Medical college and Hospital, Ambikapur, Chhattisgarh. India.
2. Associate Professor in Microbiology at **RSDKS** Government Medical college and Hospital, Ambikapur, Chhattisgarh. India.
3. DEAN, Professor and HOD Department of Microbiology at **RSDKS** Government Medical college and Hospital, Ambikapur, Chhattisgarh. India.
4. Professor and HOD in Anesthesia at **RSDKS** Government Medical college and Hospital, Ambikapur, Chhattisgarh. India.
5. Professor and HOD in Biochemistry at **RSDKS** Government Medical college and Hospital, Ambikapur, Chhattisgarh. India.

***Corresponding Author: Dr. G.OBULESU**

Associate Professor in Microbiology. **RSDKS** Government Medical college and Hospital. Ambikapur, Pin- 497001. Chhattisgarh. India.

Keywords: Sickle cell anemia, Alkaline Paper Electrophoresis, Alkaline buffer solution.

ABSTRACT.

INTRODUCTION: Sickle cell disease is caused by a hereditary defect in the hemoglobin molecule. The two beta chains in normal hemoglobin contain a glutamic acid residue at position 6.In people with sickle cell disease a valine residue occurs in this position due to an A to T transverse mutation in the glutamate codon GAG to give the valine codon GTG.(1) This residue is on the outer surface of the molecule and this single difference in the sequence of the 146 amino acids of the beta chain is enough to produce a sticky hydrophobic spot on the surface that results in the abnormal quaternary association of the α and β chains of the abnormal hemoglobin. Both deoxygenated HBA and HBS have a normal complementary stick patch on the surface of the chains to which the abnormal hydrophobic spot on deoxygenated HBS will bind. (2) This normal patch is masked when the hemoglobin is oxygenated. Thus when oxygen concentrations fall below a critical level the HbS polymerises into linear, insoluble arrays of fibers within the erythrocyte which become deformed and function abnormally. This only happens in sickle cell homozygotes, since the presence of deoxyHbA produced by the normal allele in heterozygotes will terminate the polymerization. Heterozygotes are phenotypically normal but are said to carry the sickle cell trait.(2-4)

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

Glu to Valine substitution also causes a charge difference between HbA and HbS that affect the mobility of the molecule in an electric field. thus electrophoresis of hemoglobin can be used as a diagnostic aid and can readily distinguish between normal sickle cell homozygote and sickle cell heterozygote individuals.(5-6)

ELECTROPHORESIS:

Electrophoresis separation encompasses a variety of methods in which solutes (usually charged macromolecules such as proteins, nucleic acids, etc) are resolved on the basis of their mobility in an electric field. In general an electric field is applied through some supporting medium and the solutes migrate in that field according to:

1. Their net charge
2. The friction between solute and support (which is dependent upon size)
3. The magnitude of the potential difference (The electric field).

Alkaline Paper Electrophoresis:

Electrophoresis is used to separate and identify the different hemoglobin by their migration within an electric field. Hemoglobin variants separate at different rates due to differences in their surface electric charges as determined by their amino acids structure.

Figure 01:Electrophoresis machine.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

Figure 02: Electrophoresis run

METHODS AND MATERIALS:

Total 116 patients were taken into this study. All the Patients samples were collected in Sickle cell unit which is under the department of Biochemistry at RSDKS Govt. medical college, Ambikapur, Chhattisgarh, India. from 25 November 2021 to 31 Jan 2022.

The exclusion criteria were patients with no clinical or laboratory evidence of diabetes mellitus, liver diseases, lupus nephritis, acute illness, respiratory diseases. None of the patients had history of antioxidant drugs supplementation.

PRICIPLE OF ALKALINE PAPER ELECTROPHORESIS:

At alkaline P^H hemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate towards the anode. Structural variants that have a change in the charge on the surface of the molecule at alkaline P^H will separate from Hb A. Hemoglobin variant that have an amino acid substitution that is internally sited may not separate, and those that have an amino acid substitution that has no effect on overall charge will not separate by electrophoresis.

REAGENTS:

1. Cellulose acetate strips.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

2. 10X Alkaline buffer solution.
3. 10X Washing solution.
4. Hemolysing reagent 1.
5. Hemolysing reagent 2.
6. Staining solution.
7. Distaining solution.
8. Distilled Water.

GENERAL PREPARATION INSTRUCTIONS:

1. **Dilute 10X Alkaline buffer solution as follows**

10 X Alkaline buffer	Distilled Water	Total Volume
50ml	450ml	500ml

2. Dilute 10X washing solution as follows

10 X Washing Solution	Distilled Water	Total Volume
10ml	90ml	100ml

Method:

Sample Preparation:

1. Use freshly collected blood sample

2. Red blood cells are to be separated from whole blood sample by centrifugation

3. Centrifuge 300 micro liters of fresh blood sample at 10000 RPM for 10 minutes.

4. Discard supernatant carefully without disturbing the pellet.

5. Wash the red blood cells with 1ml of diluted wash solution by re-suspending pellets with pipette.

6. Centrifuge the pellet at 10000 RPM for 10 minutes again discard the supernatant and wash the pellet with another 1ml of diluted wash solution.

7. Wash the cells again one more time with diluted wash solution.

8. Add 300microliter of haemolysing reagent 1 to the red blood cells pellets .Mix gently and leave for 5 minutes.

9. Add 150 micro liters of hemolysing reagent 2 to the above hemolysate and vortex for 2 minutes.

10. Centrifuge the hemolyate at 10000 RPM for 10 minutes

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

11. Transfer the supernant to clean sample container without disturbing the middle pellet layer and lowermost clear phase.

12. Decent the staining solution and remove the excess stain using the destining solution.

13. Repeat the procedure for 2-3 times.

14. Decant the de staining solution and blot the strip using clean blotting paper.

15. Observe the hemoglobin bands on the cellulose acetate strip and store in a protective plastic envelope.

Results:

Total 116 patients were taken into this study. All the Patients samples were collected in Sickle cell unit which is under the department of Biochemistry at RSDKS Govt. medical college, Ambikapur, Chhattisgarh. India, from 25 November 2021 to 31 Jan 2022.

Table 01 : Shows November and December 2021 Patients Results.

SN	**Month**		Solubility Test				Hb Electrophoresis					**Grand Total Tes**
			Positive	Negative	Not Conclusive	Total	SS	AS	AA	Not Conclusive	Total	
SN	November		7	7	0	7	2	1	3	1	7	35
	December	OPD	22	18	0	40	6	15	1	1	23	126
		IPD	5	12	0	17	3	0	0	1	21	59
Total			34	37	0	64	11	16	4	3	51	220

Table 02 : Shows January 2022 Patients Results.

S.No.	Mon.2022 Jan.	Hb Electrophoresis				Total
		SS	AS	AA	Not Conclusive	
1	OPD	3	7	3	0	13
2	IPD	4	2	0	2	8
Total		7	9	3	2	21

S.No.	Mon.2022 Jan.	Solubility Test Positive	Solubility Test Negative	Total
1	OPD	10	17	27
2	IPD	9	16	25
Total		19	33	52

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

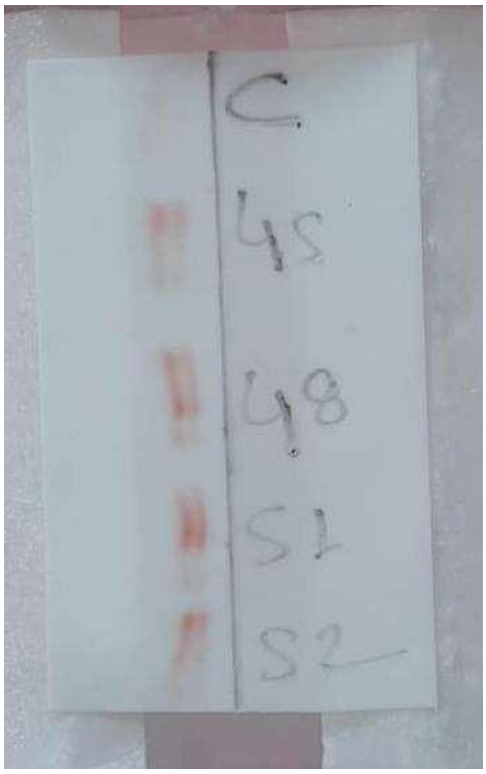

Figure 03:Electrophoresis run results.

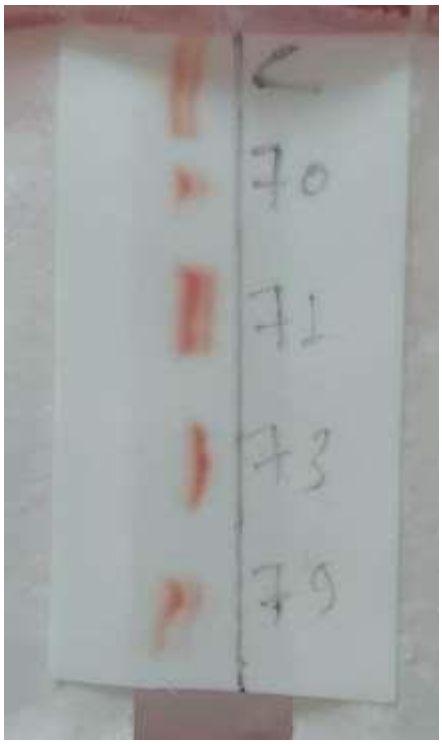

Figure 04: Electrophoresis run results.

DISCUSSION:

Paper electrophoresis of abnormal hemoglobin's is a simple and convenient technique for the study of hereditary hemoglobinopathies. A semi quantitative Paper electrophoresis technique is described which allows rather accurate quantification of the various hemoglobin components by inspection alone.

CONCLUSION :

HPLC like technology have an advantage in diagnosis of haemoglobinopathies at a molecular level. These technology present only at advanced laboratories. Alkaline paper electrophoresis is a simple technique which can be arranged even at primary centers and thus aids in the diagnosis of sickle cell anemia.

Ethical committee Clearance Certificate: IEC/14/GMS.24/07/2023.

REFERENCES:

1. World Health Organization. Sickle cell anaemia: report by the secretariat, 59th World Health Assembly. Provisional agenda item 11.4; A59/9. 2006.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

2. Omisakin CT, Esan AJ, Ogunleye AA, Ojo-Bola O, Owoseni MF, Omoniyi DP. Glucose-6-phosphate dehydrogenase (G6PD) deficiency and sickle cell trait among blood donors in Nigeria. Am J Public Health Res.2014;2(2):51–5.
3. 3. Carson JL, Grossman BJ, Kleinman S, Tinmouth AT, Marques MB, Fung MK, et al. Red blood cell transfusion: a clinical practice guideline from the AABB. Ann Intern Med. 2012;157(1):49–58.
4. 4. Wang WC. Sickle cell anaemia and other sickling syndromes'. In: Wintrobes clinical haematology, 12th edn. Philadelphia: Lippincott Williams and Wilkins. 2009. pp. 1038–1082.
5. 5. Thurston GB, Henderson NM, Jeng M. Effects of erythrocytapheresis transfusion on the viscosity of sickle cell blood. Clin Hemorheol Microcirc.2004;30(1):61–75.
6. Tsaras G, Owusu-Ansah A, Boateng FO, Amoateng-Adjepong Y. Complications associated with sickle cell trait: a brief narrative review. Am J Med.2009;122(6):507–12.