Original Research Article

Utility of hemoglobin electrophoresis to detect hemoglobinopathies in adults not presenting with hematological problems

G. J. Vani Padmaja[1*], S. S. S. Quadri[1], O. Shravan Kumar[1]

[1]Department of Pathology, Gandhi Medical College, Secunderabad, Telangana, India
[*]Corresponding author email: **drvanipadmaja@yahoo.co.in**

International Archives of Integrated Medicine, Vol. 3, Issue 7, July, 2016.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 17-06-2016 **Accepted on:** 22-06-2016
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: G. J. Vani Padmaja, S. S. S. Quadri, O. Shravan Kumar. Utility of hemoglobin electrophoresis to detect hemoglobinopathies in adults not presenting with hematological problems. IAIM, 2016; 3(7): 66-71.

Abstract

Background: Hemoglobinopathies are genetically acquired diseases. When present in new-born and in children they need to be treated and can be life threatening many a times. In the adults they can be asymptomatic and can manifest as disease during stress. They may present with other signs and symptoms not related to hemoglobinopathies.

Aim: To perform Hemoglobin (Hb) Electrophoresis to detect Hemoglobinopathies in patients not presenting with hematological problems coming to the Medical OPD at Gandhi Hospital.

Materials and methods: 3 ml Ethylene Diamine Tetra Acetic Acid (EDTA) whole blood was collected from cubital fossa from patients and Hb Electrophoresis was carried out on BIORAD - D10

Results: Hb Electrophoresis of a total of 464 patients were carried out over a period of six months (July to December 2015) and14 cases of Sickle Cell Trait, 16 cases of Thalassemia Trait, 1 case of Hb E trait , 1 case of sickle cell disease and 1 case of sickle cell disease with low Hb A2 levels were identified.

Conclusion: Hemoglobinopathies can present themselves in the adults without symptoms related to red blood cell disorders. So when Patients present themselves with other symptoms and are not being relieved of their complaints by the conventional treatment, Hb Electrophoresis can help in identifying the hemoglobinopathies, especially when the Hb is normal or near normal for that age and sex of the patient.

Key words

Hb Electrophoresis, Hemoglobinopathies, Thalassemia, HbE, Sickle cell.

Introduction

Inherited hemoglobinopathies especially thalassemia and sickle-cell disorders are now common worldwide due to migration [1, 2]. Different types of inherited hemoglobin disorders present a significant health problem all over the world accounting 71% of 229 countries [3]. About 7% of the world's population are carriers of hemoglobin disorders [4]. Data obtained from Sri Lanka as well as from two northwest states of India also show a noticeable variation in the frequency of beta thalassemia and Hb E [5, 6]. Weatherall and Clegg and added that at the younger age group inherited haemoglobin disorder are most commonly detected [7]. Hemoglobin electrophoresis is a blood test that can detect different types of haemoglobin. It uses the principles of gel electrophoresis to separate out the various types of haemoglobin and is a type of native gel electrophoresis. Hemoglobin is the protein inside red blood cells responsible for transporting oxygen. If it's abnormal in some way, it may cause too little oxygen to reach the tissues and organs. The most common types of normal haemoglobin in the Adults are:

- **Hemoglobin A:** (95 to 98 %) This is the most common type of haemoglobin found normally in adults. Some diseases, such as severe forms of Thalassemia, may cause haemoglobin A levels to be low and haemoglobin F levels to be high (**Photo – 1**).
- **Hemoglobin F (foetal haemoglobin):** (0.8 to 2%) This type is normally found in foetuses and new born babies. Haemoglobin F is replaced by haemoglobin A (adult haemoglobin) shortly after birth; only very small amounts of haemoglobin F are made after birth. Some diseases, such as sickle cell anemia, aplastic anaemia, and leukaemia, have abnormal types of haemoglobin and higher amounts of haemoglobin F (**Photo – 2**).
- **Hemoglobin A2**: (2 to 3 %) This is a normal type of haemoglobin found in small amounts in adults (**Photo – 3**).

There are more than 350 types of abnormal haemoglobin. The most common are:

- **Hemoglobin S:** (0%) This type of haemoglobin is present in sickle cell disease. Red blood cells become hard and crescent-shaped. They block small blood vessels and prevent blood from circulating properly (**Photo – 4**).
- **Hemoglobin C:** (0%) This type of haemoglobin does not carry oxygen well.
- **Hemoglobin E:** (0%) This type of haemoglobin is found in people of Southeast Asian descent.
- **Hemoglobin D:** (0%) This type of haemoglobin is present in some sickle cell disorders.

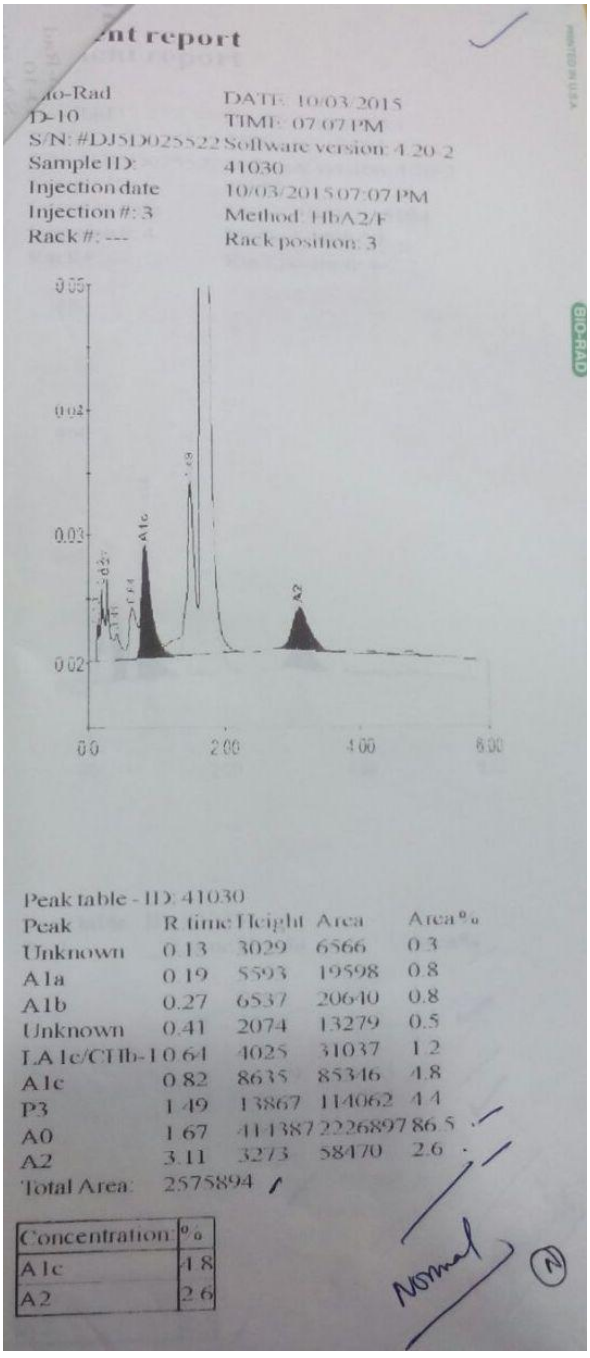
Hemoglobin S and haemoglobin C are the most common types of abnormal haemoglobin that may be found by an electrophoresis test. Electrophoresis uses an electrical current to separate normal and abnormal types of haemoglobin in the blood. Haemoglobin types have different electrical charges and move at different speeds. The amount of each haemoglobin type in the current is measured. An abnormal amount of normal haemoglobin or an abnormal type of haemoglobin in the blood may mean that a disease is present. Abnormal haemoglobin types may be present without any other symptoms, may cause mild diseases that do not have symptoms, or cause diseases that can be life-threatening. For example, haemoglobin S is found in sickle cell disease, which is a serious abnormality of the blood and causes serious problems.

Materials and methods

The blood samples of 464 patients were taken over a period of 6 months (July to December 2015). All the inclusion and exclusion criteria were met with. These patients were from the Medical OPD at Gandhi Hospital, who came with complaints of some non-specific minor

ailments and not those related to hemoglobinopathies. These patients were asked to come back for review the next week on the same day to consult the same physician.

Photo – 1: Hb electrophoresis – Normal.



Inclusion criteria

- Age: between 25 to 40 years.
- Both Males and females were included.
- The patients were not treated for and did not have symptoms related to hemoglobinopathies, like anaemia, weight loss, tiredness, fatigue, pallor, jaundice, bone aches and abdominal mass or pain. (All the above were not chronic)
- Hemoglobin values were between 10 to 12 gm%

Photo – 2: Hb electrophoresis – HbF = 5.0 = Sickle anemia.

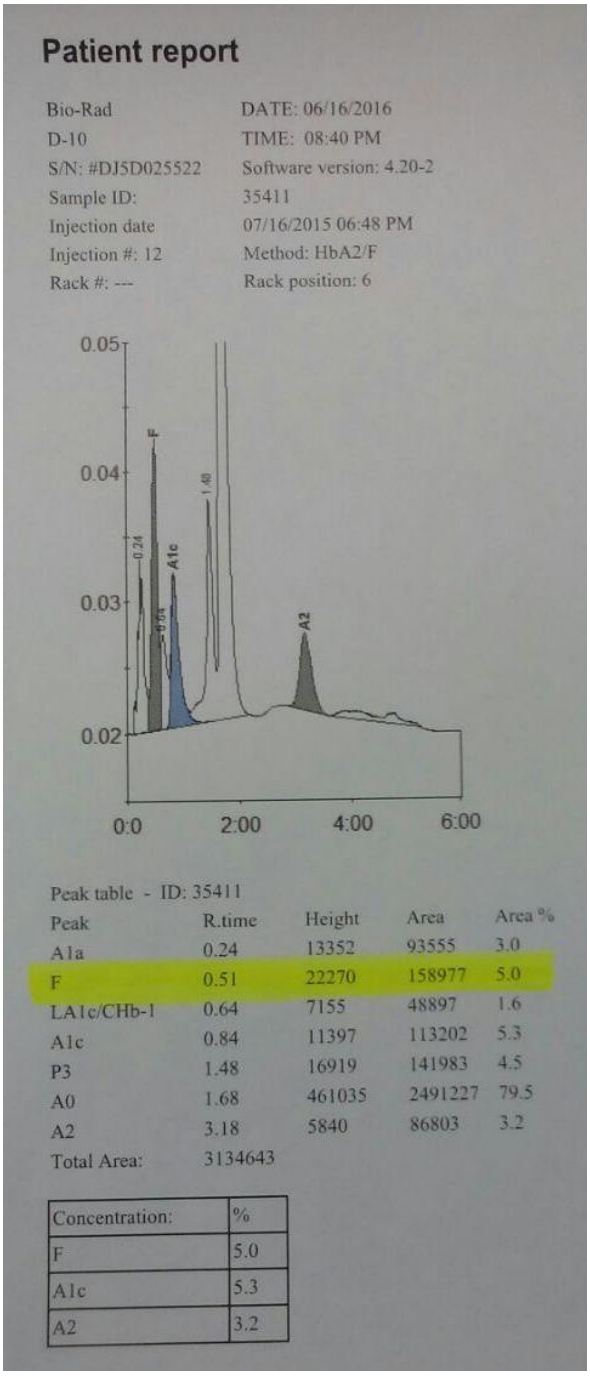
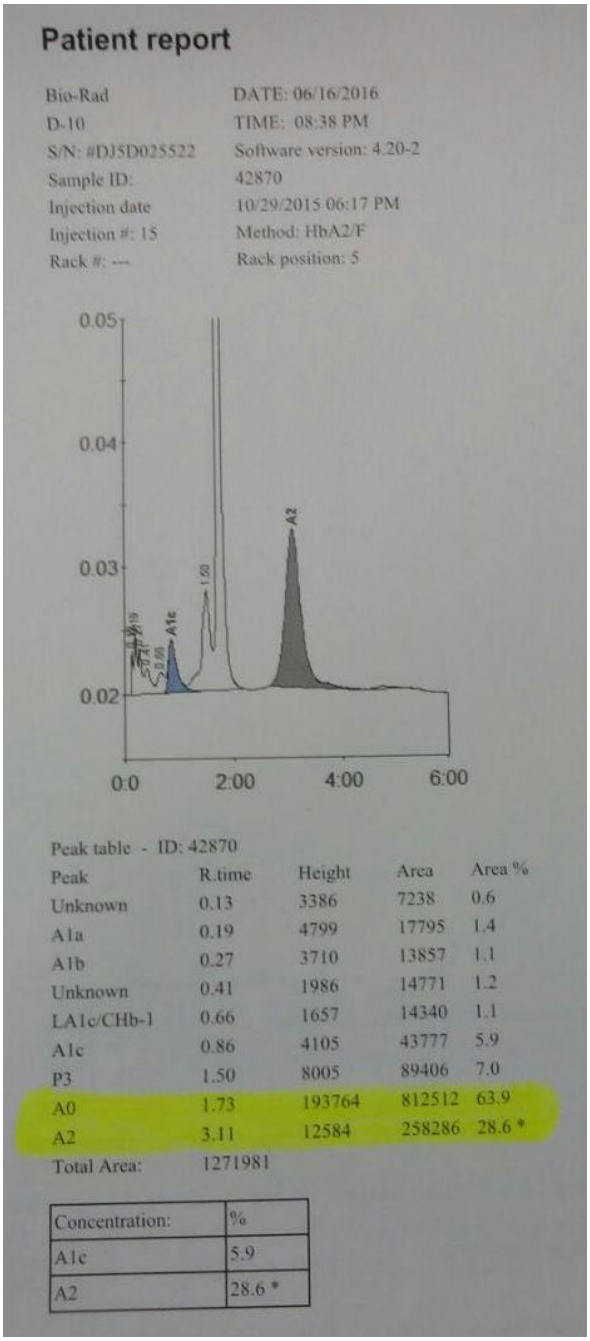


Photo – 3: Hb electrophoresis - Hb A2 = 28.6 = HbE trait.



Patient report

Bio-Rad
D-10
S/N: #DJ5D025522
Sample ID:
Injection date
Injection #: 15
Rack #: ---

DATE: 06/16/2016
TIME: 08:38 PM
Software version: 4.20-2
42870
10/29/2015 06:17 PM
Method: HbA2/F
Rack position: 5

Peak table - ID: 42870

Peak	R.time	Height	Area	Area %
Unknown	0.13	3386	7238	0.6
A1a	0.19	4799	17795	1.4
A1b	0.27	3710	13857	1.1
Unknown	0.41	1986	14771	1.2
LA1c/CHb-1	0.66	1657	14340	1.1
A1c	0.86	4105	43777	5.9
P3	1.50	8005	89406	7.0
A0	1.73	193764	812512	63.9
A2	3.11	12584	258286	28.6 *
Total Area:		1271981		

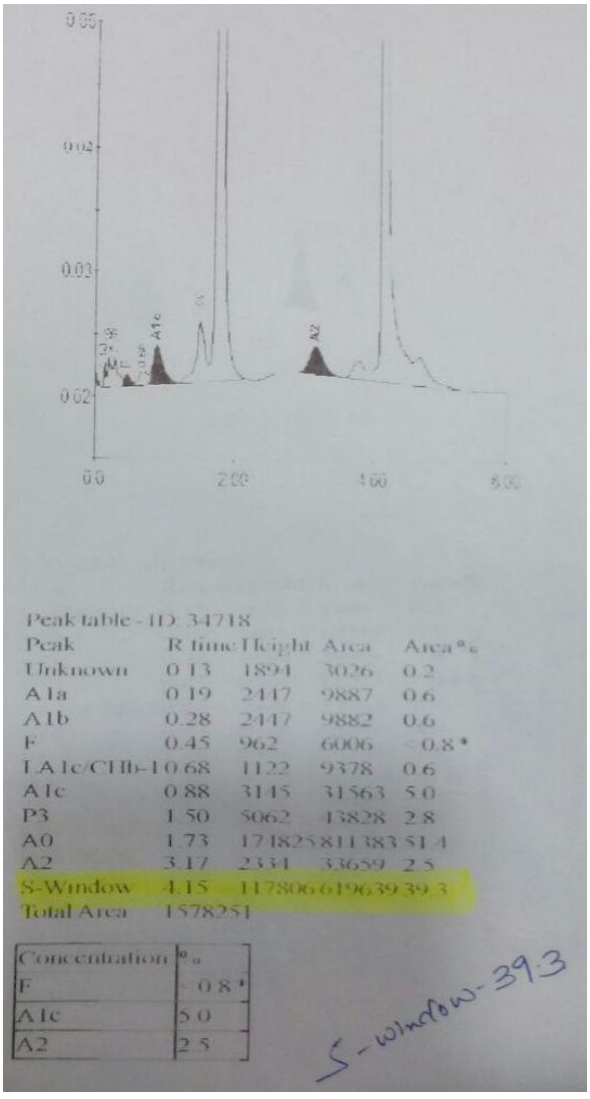
Concentration:	%
A1c	5.9
A2	28.6 *

Exclusion criteria

- Age: below 25 and above 40 years.
- Patients with specific haematological symptoms
- Hemoglobin values below 10 gm%

All aseptic precautions were taken and 3 ml EDTA whole blood was collected from cubital fossa from patients, preferably from the right side. Pressure was them applied at the site of venepuncture with cotton swab and by flexing the elbow. Once haemostasis was confirmed a small bandade was applied to further prevent bleeding from the site. Hb Electrophoresis was carried out on the whole blood collected. Hb Electrophoresis was carried out on BIORAD - D10. Complete blood count was done by automated haematology analyser. Peripheral blood film was prepared from all the samples of complete blood count and examined under microscope. The Results were then tabulated. All the Hb Electrophoresis patterns of all the patients were shown to the concerned physician. The cases where the Hb Electrophoresis showed abnormal patterns the concerned Physician advised the patient for further management.

Photo – 4: Hb electrophoresis – Hbs = 39.3 = sickle cell trait.



Peak table - ID: 34718

Peak	R.time	Height	Area	Area %
Unknown	0.13	1894	3026	0.2
A1a	0.19	2417	9887	0.6
A1b	0.28	2417	9882	0.6
F	0.45	962	6006	0.8 *
LA1c/CHb-1	0.68	1122	9378	0.6
A1c	0.88	3145	31563	5.0
P3	1.50	5062	13828	2.8
A0	1.73	171825	811383	51.4
A2	3.17	2331	33659	2.5
S-Window	4.15	117806	619639	39.3
Total Area		1578251		

Concentration	%
F	0.8 *
A1c	5.0
A2	2.5

S-Window-39.3

Results

Evaluation of the peripheral blood smear was done for all the patients. The results of the peripheral blood smear did not reveal any abnormality, except for the patient with HbSA0 (51.0) + HbS (37.7) which showed sickle cells (**Table – 1**).

Table – 1: Hb pattern.

Elevated window	Interpretation	Number
Normal Pattern		431 (204 Females)
Hb S	Sickle cell Trait	014 (09 Females)
Hb A2	Thalassemia Trait	016 (10) females
Hb A2 (28.6)	Hb E Trait	001 (01 Female)
HbF (5.0)	Sickle Thalassemia	001 (Male)
HbA0 (51.1) + HbS (37.7)	Sickle Cell Disease	001 (Female)
TOTAL		**464 (225 Females)**

Thalassemia Trait was found to be the common hemoglobinopathy, followed by Sickle cell trait, in contrary to the study carried out by Fucharoen S, et al. [8] who found that Hb E was common. There were 225 females and 139 males in our study. It was found that the incidence of hemoglobinopathies was higher if females (**Photo – 5**).

Discussion

Hemoglobinopathies can present themselves in the adults without symptoms related to red blood cell disorders. They can be a cause for constant feeling of not being well, and hence may affect the quality of life. They may manifest as other other problems which need to be addressed to. Many a times a retinue complete blood picture (CBP) may not reveal the exact morphology needed to identify the hemoglobinopathy, hence the need to perform Hb electrophoresis in such cases. Sometimes the CBP may show microcytes or crenated Red Blood cells, which may not correlate with the patients complaints. There may not be any evidence of hemolyisis to suspect a hemoglobinopathy, hence the need to do Hb electrophoresis is such patients.

Higher-than-normal amounts of both hemoglobin A2 and haemoglobin F may mean a mild form of thalessemia is present. A very low level of hemoglobin A and a high level of hemoglobin F may mean a more severe form of thalassemia. High levels of hemoglobin F may be seen in a rare condition called hereditary persistence of fetal hemoglobin.

Hemoglobin S in moderate amounts can mean that sickle cell trait is present. Hemoglobin S in high amounts means sickle cell disease.

Hemoglobin C in low amounts can mean that haemoglobin C is present. Hemoglobin C in high amounts means hemoglobin C disease, which causes anaemia and an enlaged spleen.

Hemoglobin types S and C mean hemoglobin S-C disease, which causes a mild or moderate form of sickle cell disease.

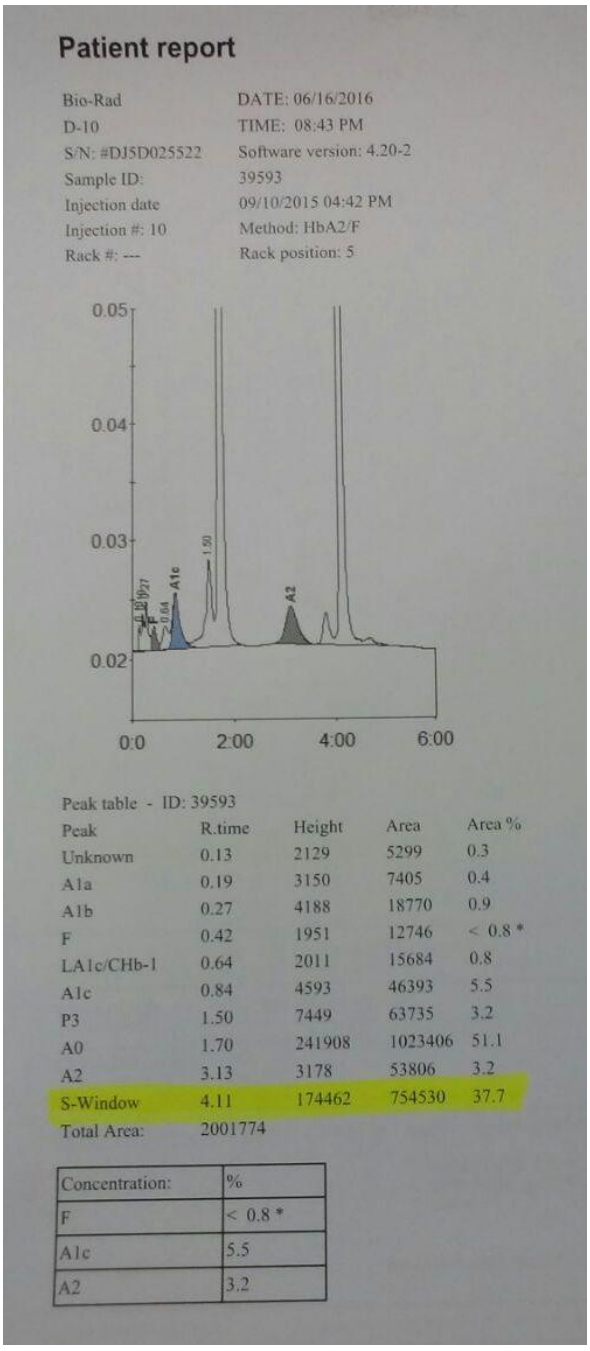
Hemoglobin E in low amounts means the presence of haemoglobin E trait. Hemoglobin E in high amounts means hemoglobin E disease, which causes anemia and smaller-than-normal red blood cells

Conclusion

In South East Asia, inherited haemoglobin disorders are one of the most common inherited disorders and put tremendous burden to the national budget. Most of the carriers remain asymptomatic and pass relatively normal life. Through population based screening programmes, it is recommended to screen all

healthy individuals to detect such asymptomatic carriers and to provide genetic counseling.

Photo – 5: Hb electrophoresis - Hb A0 = 51.1+S = 37.7 = sickle cell disease.



Patient report

Bio-Rad	DATE: 06/16/2016
D-10	TIME: 08:43 PM
S/N: #DJ5D025522	Software version: 4.20-2
Sample ID:	39593
Injection date	09/10/2015 04:42 PM
Injection #: 10	Method: HbA2/F
Rack #: ---	Rack position: 5

Peak table - ID: 39593

Peak	R.time	Height	Area	Area %
Unknown	0.13	2129	5299	0.3
A1a	0.19	3150	7405	0.4
A1b	0.27	4188	18770	0.9
F	0.42	1951	12746	< 0.8 *
LA1c/CHb-1	0.64	2011	15684	0.8
A1c	0.84	4593	46393	5.5
P3	1.50	7449	63735	3.2
A0	1.70	241908	1023406	51.1
A2	3.13	3178	53806	3.2
S-Window	4.11	174462	754530	37.7
Total Area:		2001774		

Concentration:	%
F	< 0.8 *
A1c	5.5
A2	3.2

Acknowledgement

The authors thank Mr. Srinivas for performing the tests on Biorad D-10 and the Department of Medicine Gandhi Hospital.

References

1. Salsabil MA, Islam M, Jahan D, Khan MA. Inherited Haemoglobin Disorders Among Apparently Healthy Individuals: An Analysis of 105 Cases. JAFMC Bangladesh, 2014; 10(2): 90-94.
2. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ, 2008; 6(86): 480-7.
3. World Health Organization. Thalassaemia and other haemoglobinopathies. Agenda item 5.2. 6. In: 59th World Health Assembly, 27 May 2006. EB118.R1. Avaliable from: http://www.who.int/gb/ebwha/pdf_fles/EBSS-EB118-2006-REC1/english/Res/ reseb118 _2006_rec1-en.pdf [accessed on 6 February 2008.
4. Weatherall D. J. The inherited diseases of haemoglobin are an emerging global health burden. Blood, 2010; 115(22): 4331-6.
5. de Silva S, Fisher CA, Premawardhena A. Thalassaemia in Sri Lanka: implications for the future health burden of Asian populations. Sri Lanka Thalassaemia Study Group. Lancet, 2000; 9206(355): 786-91.
6. Colah R, Gorakshakar A, Phanasgaonkar Epidemiology of beta thalassaemiain Western India: mapping the frequencies and mutations in sub-regions of Maharashtra and Gujarat [published online ahead of print March 3, 2010]. Br J Haematology.
7. Weatherall, D. J., J. B. Clegg. Inherited haemoglobin disorders: an increasing global health problem. Bulletin World Health Organization, 2001; 79: 704-12.
8. Fucharoen S, Winichagoon P. Hemoglobinopathies in South-east Asia: molecular biology and clinical medicine. Hemoglobin, 1997; 21: 299–319.