

Original Research Article

Evaluation of efficacy of BIORAD D10TM testing system in detection of beta thalassaemia carrier

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ABSTRACT

Background: Hemoglobinopathies especially thalassaemia and its interaction with HbE and HbS are significant cause of morbidity and mortality in our country. With no feasible treatment, prevention of cases by carrier detection is the only option for successful reduction of the disease burden. VARIANT hemoglobin testing system BIORAD using principle of cation exchange chromatography has been in use and considered as gold standard for carrier detection. The purpose of this study is to compare the efficacy of a different, cheaper instrument; D10 by the same manufacturer BIORAD for carrier detection in beta thalassaemia.

Methods: Patients diagnosed as beta thalassaemia carrier by VARIANT hemoglobin testing system (HbA2 value between 4.0-9.0) were retested using D10 instrument and checked for agreement.

Results: There was good correlation between VARIANT and D-10 methods with Intraclass correlation coefficient 0.832 (95% Confidence Interval 0.756-0.884). Bland-Altman analysis showed mean bias of +0.3526 (95% CI -0.3958 to +1.101).

Conclusions: Although further study is needed with larger sample size for assessment of sensitivity and specificity of D10 instrument, it is evident from this study that this instrument can be an effective and cheaper alternative of VARIANT hemoglobin testing system.

Keywords: Beta thalassaemia, BIORAD D10, BIORAD variant, Carrier, Detection

INTRODUCTION

Hemoglobinopathies are the commonest hereditary disorders in India. Inherited disorders of hemoglobin, especially beta thalassaemia and associated hemoglobin E and haemoglobin S are significant cause of morbidity and mortality in our country. According to previous studies the overall prevalence of β -thalassaemia is 3-4% with an estimated 8,000 to 10,000 new births reported each year.^{1,2} Most of these children have severe clinical

features and require regular blood transfusion and supportive care. Thus, they cause significant burden on the health care system. The only curative treatment for these diseases is stem cell transplantation. However, most of our patients cannot afford this form of treatment. Thus, preventing the birth of affected children is the only way to reduce the burden of this problem. This is only possible through carrier (heterozygous state) detection. Beta thalassaemia and related hemoglobinopathies are diagnosed by a number of techniques including

hemoglobin electrophoresis, high performance liquid chromatography (HPLC), iso-electric focusing, capillary gel electrophoresis and molecular analysis.³ High performance liquid chromatography (HPLC) is universally accepted as the most useful initial screening method for thalassemia and other hemoglobinopathy detection.⁴ The conventional HPLC instrument (VARIANT HEMOGLOBIN TESTING SYSTEM, BIO-RAD) although well standardized, is very costly (more than Rs. 2.5 million). The “D-10 HEMOGLOBIN TESTING SYSTEM” (BIO-RAD) on the other hand uses the same principle (HPLC) as that of Variant instrument but it is comparatively less expensive (about Rs.1 million). Thus “D-10 HEMOGLOBIN TESTING SYSTEM” is used for thalassemia detection in many of the laboratories. However, a comparative study with conventional system (VARIANT, BIO-RAD) is yet to be done.

The present study analyses the utility of “D-10 HEMOGLOBIN TESTING SYSTEM” for the diagnosis of Beta thalassaemia carrier state.

METHODS

Patients attending out- patient and in- patient departments of Ramakrishna Mission Seva Pratishthan and Vivekanada Institute of Medical Sciences, who were advised for haemoglobin HPLC during the period of January 2012 to December 2012.

Definition of population

Samples of patients who are diagnosed as β -thalassaemia trait, are included in the study.

Inclusion criteria

Cases diagnosed as beta thalassaemia carrier by VARIANT™ II Hemoglobin Testing System.

Exclusion criteria

- Children below 6 months of age.
- Patients who have received prior blood transfusion.
- Patients with borderline HbA2 values (HbA2 value between 3.5-4.0).

Study variables

Coexistent hematinic deficiency can affect results of high-performance liquid chromatography. This is most significant in cases with borderline HbA2 values. So co-existent hematinic deficiency needs to be excluded in cases with borderline HbA2 values.

Methods of data collection

A copy of chromatogram and hemogram are preserved. The red blood cell indices, retention times and relative

percentages of all the hemoglobin fractions in the chromatogram are noted.

Author obtained the RBC indices from KX-21, Sysmex Automated Cell Counter at our institute. Due to financial constraint we ran the samples first at Variant instrument and KX-21 and correlated the chromatogram findings with the complete hemogram results. Cases diagnosed as Beta thalassaemia carrier by VARIANT™ II Hemoglobin Testing System were re-tested by D10. This study is solely based on the findings obtained from Variant and D-10. Author have not done any gene mutation study for confirmation of cases.

Statistical analysis

All statistical analyses were carried out by R version 3.3.3 R Studio version 1.0.136 (R foundation) statistical software (Language). $P < 0.05$ was considered as statistically significant. Logistic regression was carried out to evaluate the association of different predictor variables. Evaluation of D10 was done as described by Bland and Altman. HbA2 value as measured by Biorad Variant machine was considered as the reference value.

Therefore, mean value for HbA2 with both the methods is plotted against the difference between the HbA2 measured by Variant and D10 machine. The 95% limits of agreement were calculated as $d \pm 1.96SD$ where d = mean difference between the two measurements; and SD = standard deviation of differences.

RESULTS

Author collected samples of 394 suspected Beta Thalassaemia carrier patients. 114 out of them were positive in VARIANT™ II Hemoglobin Testing System and tested again with D-10 Hemoglobin Testing System. Most of the patients were female (69 out of 114, 60.5%) and mean age was 30.94 years (SD = 18.54 years). Baseline profiles of the subjects are summarized in Table 1. Mean HbA2 for the entire population was 5.173 (SD = 0.466) by VARIANT method and 5.525 (SD = 0.518) by D-10 method.

There was good correlation between VARIANT and D-10 methods as depicted in Figure 1. Intraclass correlation coefficient was 0.832 (95% confidence interval 0.756-0.884).

Agreement between these two methods by Bland-Altman Analysis is shown in Figure 2. The mean difference was found to be +0.3526 (95% CI-0.3958 to +1.101). Only 3 out of 114 (2.63%) sample was outside the 95% CI limits of bias which denotes a good agreement between them.⁵

However, the pattern of distribution of differences was similar throughout the range of values. It indicates the bias did not change with higher values of HbA2 within the study range.

Table 1: Baseline profiles of the study subjects.

	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	114	1	85	30.94	18.543
Hemoglobin (gm/dl)	114	5.7	14.5	10.439	1.6424
RBC Count (millions/dl)	114	2.81	7.22	5.1332	0.81880
MCV (fl)	114	58.4	87.0	68.104	5.4669
MCH (pg)	114	15.9	30.3	20.452	1.9890
RDW (CV)	114	13.6	27.0	17.597	2.4536
HbF (%)	113	.0	5.8	1.050	1.1196
HbA0 (%)	114	35.4	87.5	84.108	4.8794
HbA2 by VARIANT (%)	114	4.1	6.2	5.173	0.4668
HbA2 by D10 (%)	114	4.3	7.0	5.525	0.5180

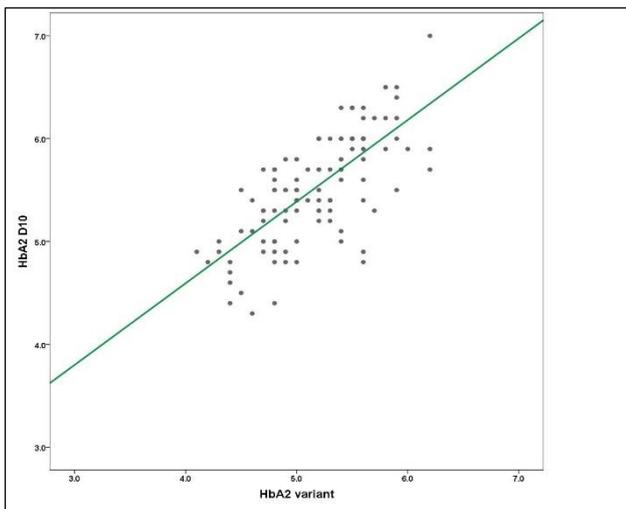
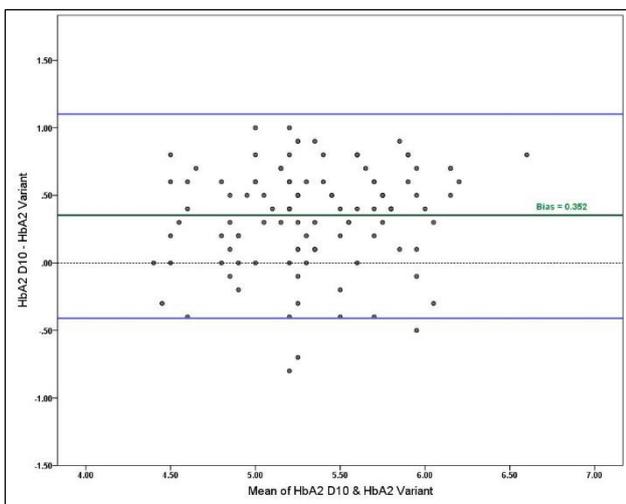


Figure 1: Distributions of HbA2 values detected by variant and D10 hemoglobin testing system.



The mean bias was found to be +0.3526 (95% CI-0.3958 to +1.101).

Figure 2: Bland-Altman plot showing agreement between variant and d10 hemoglobin testing system.

DISCUSSION

Beta thalassaemia is an important disease burden in our country India.¹ Each year a huge number of children are born that add to the already existing disease burden. The only curative treatment is bone marrow transplantation which demands not only a huge financial support but also intensive monitoring as it is full of complications and their negative influence on the outcome of the treatment.⁶ So as of the now carrier detection and prevention of birth of affected children is the only feasible option for prevention of thalassaemia and to reduce humongous burden of the disease.

For this very purpose we need an instrument which estimates different types of normal and abnormal hemoglobin accurately and at the same time which is cheap and very easy to use. Cation exchange high performance liquid chromatography (HPLC) is the method of choice for carrier (heterozygous state) detection and screening for different haemoglobin disorders (homozygous state) and Variant Hemoglobin Testing System by BIO-RAD Company has emerged as the instrument of choice for this purpose.⁷⁻¹⁰ It is a well standardized instrument. Retention times of most of the hemoglobin are known. Calibration facilities for quantification of HbA2 and HbF are available. But this instrument is very costly. D-10 Hemoglobin Testing System that was earlier launched for HbA_{1c} estimation in blood by HPLC is relatively cheaper than Variant. Its price is less than half of that of the Variant. By an extended programme it can detect heterozygous as well as homozygous states for β -thalassemia and other hemoglobinopathies like HbE, HbS, HbC, HPFH and also double heterozygous states for different haemoglobin disorders.^{11,12}

To our best of knowledge, no large-scale comparative study is available comparing the efficacy of Biorad D-10 Hemoglobin Testing System with Variant system. We could not run all the samples in both the machines due to resource constraints, thus sensitivity and specificity of the

D10 machine could not be assessed. But our study showed significantly high correlation between HbA2 measured by these two instruments. According to our study there is a mild positive bias for the HbA2 value measured by D10 instrument, but no cases were missed as thalassaemia carrier by D10.

CONCLUSION

Thalassaemia is major disease burden in our country. Only effective way to prevent the disease burden is prevention of the disease by carrier detection. Biorad D10 can be an effective cost-effective alternative of standard Variant instrument for carrier detection.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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