Hematological Parameters: Manual vs Automated Method; Among the β-Thalassemia and other Haemoglobinopathies in a Tertiary Care Hospital in Kolkata

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Abstract

Background: According to a report of WHO in 2007, 7% of world populations are carrier for Haemoglobin disorder and accurate and timely detection of various Hb variants including beta thalassemia trait can prevent occurrence of more serious disorders like thalassemia major in new-borns. But in a developing country like us use of high pressure liquid chromatography (HPLC) is limited, manual testing is done and accurate assessment is quite impossible. So, an observational cross-sectional study was done among 117 β-thalassemia and other Hemoglobinopathies cases and carriers.

Objective: To find out the variation between the results of haematological tests obtained by automated counting chamber and manual method.

Materials & Methods: This Observational, cross-sectional study was done at Thalassemia control unit and Physiology department of R G Kar Medical College and Hospital, Kolkata. β-thalassemia and other Hemoglobinopathies cases and carriers detected by complete blood count with HPLC from Thalassemia Outpatient Department (OPD) and antenatal mothers from antenatal clinic (ANC) were the population of this study. Findings and variations of the features of different hematological parameters amongst the β-thalassemia and other hemoglobinopathies were reviewed. A finding by HPLC was HbA, HbA2, HbF, HbD, and HbE.

Results: Result of only Hb% obtained from HPLC and manual method was significantly different in β-THALASSEMA Trait (p=0.0001), HbE Trait (p=0.0001), HbS trait (p=0.0001) and not significant in HbE disease and no other Red cell indices were significantly different in two methods. Conclusion: As hematological features like TC of RBC or PCV results by manual method shows no significant differences with HPLC/ Automated counter

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assessment, such methods can be utilized even at primary level cost effectively for assessment of hematological disorders. But significant difference is seen in estimating Hemoglobin percentage in between Shalli’s method and HPLC/Automated counter assessment. So some alternate method may introduce at lower level of health system other than Shalli’s method.

**Keywords:** Anaemia, haemoglobinopathies, thalassemia, automated counting chamber, manual method, Kolkata

**Introduction**

Anaemia is a major public health problem among the children, pregnant women and non-pregnant women in South East Asia, of which India is a part. Thalassemia and Hemoglobinopathies are important amongst the causes of anemia. Abnormalities of haemoglobin (Hb) synthesis are extremely common inherited disorder worldwide, ranging from almost functionally normal Hb to severe transfusion dependent disorder and are quantitative (Thalassemia Syndrome) or qualitative (Hb variant) or a combination of both. Such diseases are prevalent in India also.

Hemoglobinopathies (Hb variant) and β-Thalassemia carrier constitutes 4.5% of world population-as per a report of World health organization (WHO) in 1989. It shows an increasing trend and according to a report of WHO in 2007, 7% of world populations are carrier for Haemoglobin disorder. Each year about 30,000 infants is born with major Hb disorders. Around 85% of sickle-cell disorders and over 70% of all affected births occur in Africa.

Accurate and timely detection of various Hb variants including beta thalassemia trait can prevent occurrence of more serious disorders like thalassemia major in new-borns. Revolutionary changes have taken place in the last decade in the field of detection of β-Thalassemia and other Hemoglobinopathies. Automated high performance or high pressure liquid chromatography (HPLC) plays an important role to detect those disorders. Cation exchange HPLC offers a reliable tool for early, accurate detection thereby aiding in prevention and management of various Hemoglobinopathies. This is especially important in view of high incidence of β-thalassemia trait in the Indian subcontinent. Early detection of traits will prevent occurrence of thalassemia major in offspring. Detection of other variants becomes important due to complex interactions in cases with double heterozygous and homozygous states, which may lead to severe hematological abnormalities. Findings must be supplemented by hemogram findings, family/sibling studies, Hb electrophoresis, other confirmatory techniques and molecular studies based on HPLC findings and on a case-to-case basis. But in a developing country like us use of HPLC is limited. In most of the health centres HPLC is not available and blood is tested manually. There are very few studies in West Bengal regarding the review of hematological features of HPLC study and comparison between the laboratory findings among manual and automated counter examination of blood, in the patients of Haemoglobin abnormalities. With this perspective this study aims to explore and compare the results obtained by HPLC and manual testing of some required haematological parameters and serves as a guideline for screening of Hb disorders in remote areas.

**Aims & Objectives**

- To explore and compare the results obtained by HPLC and manual testing of some required haematological parameters which may serve as a guideline for screening of Hb disorders in remote areas.
- To find out the variation between the results of haematological tests obtained by automated counting chamber and manual method

**Materials and Methods**

An Observational, cross-sectional study was conducted over a period of 1 year at thalassemia control unit and Physiology department of R G Kar Medical College and Hospital, Kolkata. Beta-thalassemia and other hemoglobinopathies cases and carriers detected by the thalassemia control unit by complete blood count with HPLC from thalassemia out patient department (OPD) and antenatal mothers from antenatal clinic (ANC) were the population
of this study. From the review of the records of last few years total number of ß-thalassemia and other Haemoglobinopathies detected per month on an average was around 14 (including special OPD, ANC and camp/camps). Thus, the expected number of patients of ß-thalassemia and other Haemoglobinopathies in the stipulated nine-month period of data collection for this study came to be 126. So in our study sample size was 120. In this study 127 patients were reviewed. Out of them 10 patients did not give their consent for giving blood for 2nd time. In this study multi phase sampling was done. In the first stage systemic random sampling was done to select 50% of the targeted collection centre, while in the second stage purposive sampling was done to select the cases and carriers of β-thalassemia and other Hemoglobinopathies after considering exclusion and inclusion criteria.

**Inclusion criteria:**

1. Diagnosed case of β-thalassemia and other hemoglobinopathies by complete blood count (CBC) and high-pressure liquid chromatography (HPLC)
2. Both sexes

**Exclusion criteria:**

1. History of having recent transfusion (within 3 months)
2. History of having repeated transfusion
3. Patient on haematinics
4. Hospitalized patients
5. Patient aged over 60 years and below 6 months
6. Patient whose records are not filled up properly

**Study technique:** After getting ethical clearance, the descriptive cross-sectional study was done among the β-thalassemia and Hemoglobinopathies (both cases and carriers) detected at Thalassemia control Unit and Physiology department of R.G. Kar Medical College, Kolkata by HPLC and hemogram by automated counter. Thalassemia control Unit collected blood from Antenatal OPD, Thalassemia OPD. About 50% of such points were selected by simple random sampling within the period of study. All the cases and carriers detected at those points were approached for the study. Findings and variations of the features of different hematological parameters amongst the β-thalassemia and other Hemoglobinopathies were reviewed. During the time of collection of blood from new visitors (referred for diagnosis) for Complete Blood Count by automated chamber and HPLC, blood was also collected in EDTA vial from diagnosed cases revisiting for collection of their reports and haemoglobin (Hb%), Packed cell volume (PCV) and Total count (TC) of red blood cell (RBC) were manually tested at Physiology department and this manual examination was done after getting proper consent and after explaining the whole procedure to the patients. Reports of automated counter and manual procedure were compared. Hemoglobin estimations were done by Sahli’s acid hematin method; TC of RBC were done by improved Neubauer counting chamber using normal saline as diluting fluid and Packed cell volume by using Wintrobes haematocrit tube and centrifuge machine (at 3000RPM for twenty minutes).

Parameter studied was medical history of study participants; and hematological findings – Hb%, TC of RBC, TC white blood cell (WBC), platelet count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell width (RDW). A finding by HPLC was HbA, HbA2, HbF, HbD, and HbE.

**Special investigations:** In this study for measurement of different haematological parameters and variant haemoglobin concentration two important & advanced procedure were used, one was HPLC technique for assessing type of Hb and another, automated blood cell counter (Sysmex Kx-21 type). Parameters measured by automated cell counter was complete blood count (CBC), TC of RBC, Hb%, PCV, RBC indices, and RDW.

**Data analysis:** Data were collected in a pre-tested and pre designed schedule from registers and record books of the control unit and screening sheet of the camps and the hematological tests performed manually. Data were put in a master chart and analysed by using SPSS Version 20.
Results

Table 1: Distribution of different hemoglobin disorders (n=117)

<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>β- Thalassemia Trait (BTT)</td>
<td>45</td>
<td>38.50</td>
</tr>
<tr>
<td>β- Thalassemia Major (BTM)</td>
<td>2</td>
<td>1.72</td>
</tr>
<tr>
<td>E- β- Thalassemia (E βT)</td>
<td>2</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Table 1 shows the spectrum of different haemoglobin disorders in the present study. Maximum persons had HbE trait (51.3%) next to which β- Thalassemia trait (38.5%), HbS trait (4.3%), HbE Disease (2.6%), β- Thalassemia Major (1.4%) and E βThalassemia (1.4%).

Table 2: Findings of different hematological parameters measured by HPLC/ Automated Counter of different haemoglobin disorders

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Type of Hb Disorders</th>
<th>TC of RBC (millions/cmm) Mean ± SD</th>
<th>Hb % (gm/ccmm ) Mean ± SD</th>
<th>PCV (%) Mean ± SD</th>
<th>MCV (fl ) Mean ± SD</th>
<th>MCH (pg ) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β- Thalassemia Trait</td>
<td>5.1604 ± 0.9124 2</td>
<td>10.404 ± 1.4652</td>
<td>33.993 ± 5.9717</td>
<td>68.060 ± 5.9240</td>
<td>20.160 ± 2.1201</td>
</tr>
<tr>
<td>2</td>
<td>β- Thalassemia Major</td>
<td>2.4 ± 0.6222 3</td>
<td>4.950 ± 1.0607</td>
<td>17.250 ± 2.7577</td>
<td>72.850 ± 7.4246</td>
<td>20.750 ± 0.9192</td>
</tr>
<tr>
<td>3</td>
<td>E- β- Thalassemia</td>
<td>3.72 ± 1.08894</td>
<td>6.250 ± 0.9192</td>
<td>23.150 ± 2.7577</td>
<td>63.900 ± 11.3137</td>
<td>17.200 ± 2.5456</td>
</tr>
<tr>
<td>4</td>
<td>HbE disease</td>
<td>4.6333 ± 0.57073</td>
<td>9.233 ± 0.3786</td>
<td>29.900 ± 1.9925</td>
<td>64.867 ± 4.7343</td>
<td>20.067 ± 1.7243</td>
</tr>
<tr>
<td>5</td>
<td>HbE Trait</td>
<td>4.5727 ± 0.65601</td>
<td>11.185 ± 1.4537</td>
<td>35.552 ± 4.6992</td>
<td>77.997 ± 4.1348</td>
<td>24.675 ± 1.5050</td>
</tr>
<tr>
<td>6</td>
<td>HbS Trait</td>
<td>4.1380 ± 0.23805</td>
<td>10.684 ± 0.2971</td>
<td>33.420 ± 2.0474</td>
<td>80.760 ± 2.4583</td>
<td>25.560 ± 0.8142</td>
</tr>
</tbody>
</table>

Table 3: Shows result of HPLC/automated counter vs manual method of Hemoglobin % among the persons having different Hemoglobin disorders

<table>
<thead>
<tr>
<th>Hematological disorders Mean ±SD</th>
<th>Automated Counter/HPLC n=45</th>
<th>Manual Method n=45</th>
<th>t and p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β- Thalassemia Trait (n=45)</td>
<td>10.404 ± 1.4652</td>
<td>12.27 ± 2.137</td>
<td>t (88) = 4.8310 p=0.0001 Significant</td>
</tr>
<tr>
<td>Hemoglobin E Trait (n=60)</td>
<td>11.185 ± 1.4537</td>
<td>13.28 ± 2.248</td>
<td>t (118)=6.0618 p=0.0001 Significant</td>
</tr>
<tr>
<td>Hemoglobin E Disease (n=3)</td>
<td>9.233 ± 0.3786</td>
<td>11.17 ± 1.258</td>
<td>t (4)=2.5538 p=0.0631 Not Significant</td>
</tr>
<tr>
<td>Hemoglobin S Trait (n=5)</td>
<td>10.684 ± 0.2971</td>
<td>13.16 ± 0.329</td>
<td>t (8)=12.4895 p=0.0001 Significant</td>
</tr>
</tbody>
</table>
The above table 3 shows that result of Hb% is significantly different in β- Thalassemia Trait (p=0.0001), HbE Trait (p=0.0001), HbS trait (p=0.0001) and not significant in HbE disease.

Moreover two patients with β- Thalassemia Major and two patients with E β- Thalassemia have not been included as they were severely ill and could not come to Physiology department.

**Discussion**

A descriptive cross-sectional study was done among 117 persons having β-thalassemia and Haemoglobinopathies (both cases and carriers) detected at Thalassemia control Unit and subsequently their 2nd sample of blood was collected for manual examination of Hb%, PCV and TC of RBC at Physiology department of R G Kar Medical College, Kolkata. Three of them refused to give 2nd sample of blood.

In the present study out of total 117 persons having different Hb disorders, 60 were HbE trait (51.3 %), 45 were β-Thalassemia trait (38.5 %), 5 were HbS trait (4.3 %), 3 were HbE disease (2.6%), 2 were β-Thalassemia major (1.7%) and 2 had E-β Thalassemia (1.7%). In most of the studies Though β-Thalassemia trait is the commonest form of disorder, in our study Hemoglobin E trait took the first place and our findings are almost similar to the findings of Ghosh N et al – a study done among the antenatal mothers in Darjeeling district in West Bengal.10 In a study in Bangladesh by M. Mesbah Uddin, et al in 2012 among 600 cases of anaemic patients referred from various parts of the country for diagnosis and counselling during 3 months (2011) of time the most common form of Hb formation disorder observed was β-thalassemia minor (21.3%) followed by E-β-Thalassemia (13.5), HbE trait (12.1%), HbE disease (9.2%), Hb D/S trait (0.7%), β-thalassemia major (0.5%), and δ-β-thalassemia (0.5%).10 In the current study 37 (31.6%) were male and 80 (68.4%) were female. Most of our cases were seen within 39 years of age (98.3%).

Different haematological parameters in the different Hb disorders were examined in this study. Patients of Thalassemia major (n=2) had severe symptoms, so they and E- β- Thalassemia (n=2) who were also admitted, were unable to attend Physiology department and manual testing was not done. Among other cases, it was found that except Hemoglobin E disorder, significant difference was persistent regarding the result of hemoglobin percentage between manual examination and HPLC/ Automated counter examination. Regarding manual methods no significant differences were found with the results of HPLC/ Automated chamber while measuring TC of RBC and PCV. These findings are similar to several other studies.11-13 A study in West Bengal by I. Chakraborty et al all found 3.4% beta thalassemia trait, 0.6% E-beta thalassemia. Various RBC indices were significantly low in them with concurrent iron deficiency.14 An accurate RBC counting enables the MCV and MCH to be correctly calculated. In well-equipped laboratories, where these indices are provided by an automated system, they are of considerable clinical importance and are widely used in the classification of anemia. Where automated analyzers are not used, manual RBCs counting (and consequently, calculations of these red cell indices) are inaccurate and time-consuming.15

For assessing the hematological features like TC of RBC or PCV manual methods shows no significant differences with HPLC/Automated counter assessment. So, such methods can be utilized even at primary level cost effectively for assessment of such cases. But significant difference is seen in estimating Hemoglobin percentage in between Shalli’s method and HPLC/Automated counter assessment. So some alternate method may introduce at lower level of health system other than Shalli’s method. PCV and TC of RBC have no variation in results obtained by automated counting chamber and manual method in different haemoglobin disorders. So MCV can be done manually to differentiate between Iron deficiency anaemia where MCV is low and haemoglobinopathies where MCV is normal both of which are prevalent in rural Bengal. So at Primary health centre level iron can be prescribed only after performing such a minor test with a few instruments. So, if PHCs are provided with colorimeter for Hb estimation and assessment of MCHC can be done, preliminary treatment of anaemia can be started and possibility of Iron overload in haemoglobinopathies can be avoided at the root level.

**Conclusion**

From this study it can be said that the hematological features like TC of RBC or PCV results by manual method shows no significant differences with HPLC/Automated counter assessment. So, such methods can be utilized even at primary
level cost effectively for assessment of such cases. But significant difference is seen in estimating Hemoglobin percentage in between Shalli’s method and HPLC/Automated counter assessment. So some alternate method may introduced at lower level of health system other than Shalli’s method.

Recommendations

MCHC not MCH is more reliable for diagnosis of Iron deficiency anaemia and Haemoglobinopathies and haemoglobin estimation is also a must for this diagnosis. Manual method is not suitable for this purpose according to our study report. So, if PHCs are provided with colorimeter for Hb estimation and assessment of MCHC can be done, preliminary treatment of anaemia can be started and possibility of Iron overload in haemoglobinopathies can be avoided at the root level.

Limitation

Sample size was small due to time and manpower constrains.

Conflict of Interest: None

Ethical clearance: Approved by Institutional Ethics Committee, R G Kar Medical College and Hospital, Kolkata

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References