



EFFICACY OF AUTOMATED CELL COUNTER WITH PERIPHERAL BLOOD FILM FOR SCREENING OF HEMOGLOBINOPATHIES.

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Abstract:

Background: Objective: Methods: The present study was conducted in the department of Pathology and Gynaecology, in a tertiary health care center of North India and comprised of 361 anaemic antenatal women attending outdoor and admitted in indoor department of Gynaecology willing to enroll for the study were randomly selected and subjected to screening procedure for hemoglobinopathies. **Result:** Antenatal group included total 361 cases, most of them were in the second trimester (60.1%). There was found negative correlation between number of pregnancies and mean haemoglobin value. Out of 361 cases; 27 were positive for various haemoglobinopathies giving a prevalence of 7.5 % in anemic mothers. The positive cases included 22 cases of β -thalassemia trait, 2 cases of HbS trait, 1 case of HbD trait, 1 case of HbE trait and 1 case of HbE homozygous.

Conclusion: Automated cell counter based parameters and formulae are good, rapid, cheaper and easily available methods for screening of haemoglobinopathies especially for thalassemia trait detection.

Keywords: Haemoglobin, anaemia, pregnancy, thalassemia

INTRODUCTION: Anaemia is defined as reduction in circulating haemoglobin mass below the critical level. The normal haemoglobin (Hb) concentration in a person is between 12-14 gm%. WHO has accepted up to 11gm% as the normal haemoglobin level in pregnancy. Therefore any haemoglobin level below 11gm% in pregnancy should be considered as anaemia. However in India and most of the other developing countries the lower limit is often accepted as 10 gm %. WHO has estimated that prevalence of anaemia in developed and developing countries in pregnant women is 14% and 51% respectively and in India it is 65-75 %.¹

Anaemia in pregnancy is a special issue as it has deleterious effects both on mother as well as fetus. In India, anaemia is the second most common cause of maternal deaths, accounting for 20% of total maternal deaths.²

Clinically the thalassemias are classified according to their severity into major, intermediate and minor forms. Thalassemia major is a severe, transfusion-dependent disorder. Thalassemia intermedia is characterized by anaemia and splenomegaly, though not of such severity as to require regular transfusion. Thalassemia minor is the symptomless carrier state.³

An accurate diagnosis of beta thalassemia carriers as well as homozygous patients and various haemoglobinopathies is important for epidemiological studies as well as for management and prevention of major haemoglobin disorders.

The α -thalassaemias are divided into four clinical subsets that reflect the extent of impairment in α -globin chain production: silent carrier, α -thalassemia trait, Hb H disease and hydrops fetalis.⁴ Adult individuals with three functional α -genes may have a completely silent phenotype or may have a reduced MCV and MCH and very mild anaemia.

The β thalassemias and their interactions with certain structural Hb variants like HbE, HbD and HbS also produce thalassemic manifestations and are a major health problem in India.⁵

The sickling disorders are by far the most common, followed by the compound heterozygote condition HbE β -thalassemia. The diagnosis of sickle cell anaemia rests on electrophoretic or chromatographic separation of haemoglobins in hemolysates prepared from peripheral blood. The sickling phenomenon can be induced by 2% sodium metabisulphite.⁶

HbE, in interaction with β -thalassaemia, results in a thalassaemia syndrome of intermediate severity, although the clinical spectrum is very heterogenous, ranging from a mild phenotype to severe transfusion dependent anaemia. HbE heterozygotes are clinically normal with only minimal hematologic changes. Homozygotes for HbE are usually asymptomatic and have normal Hb levels, but in some cases a mild anaemia may be present.⁷

HbD occurs in four forms : heterozygous Hb D trait, Hb D-thalassaemia, HbS-D disease and the rare homozygous Hb D disease, which is usually associated with mild hemolytic anaemia and mild to moderate splenomegaly.⁸

The aim of screening is to identify carriers of β -thalassaemia, as well as structural variants like Hb S and Hb E to identify couples at risk of having a child with β -thalassaemia major, sickle cell disease, Hb S- β thalassaemia and Hb E - β thalassaemia. The compound heterozygous disorders (HbSD-punjab, HbSE, HbS/ β thalassemia) or unusual variants (HbD Iran, HbJ) are all clinically significant with varying degree of severity, making precise identification important.

High-performance liquid chromatography (HPLC) is a new technique introduced for the accurate diagnosis of haemoglobinopathies and thalassemias. It has been shown to be highly sensitive and specific method of diagnosis. HPLC offers the distinct advantage over classic haemoglobin electrophoresis as it can accurately identify and quantitate abnormal haemoglobins. Specific elution windows are defined for HbA₂, Hb F and other variant haemoglobins like Hb S, D and C. This method has helped in identification of rare Hbs like HbQ India, HbJ Meerut, HbD Iran, and other unstable haemoglobins.⁹

Material and Methods: The Present study was conducted in department of Pathology, in a tertiary health care center of North India. The study included 361 Antenatal anaemic mothers who belonged to different communities of Haryana and its nearby states and having haemoglobin less than 10 gm%.

Venous blood samples were collected in EDTA vacutainer. All samples were subjected to complete blood count by automated blood cell counter, 5-Part Differential (BC-5800). Five part differential showed the various indices including white and red blood cell count, differential leucocyte count, haemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelet count, platelet distribution width, mean platelet volume. The normal reference ranges taken as laboratory control were as follow:¹⁰

- Hb: 13.5 ± 1.5 gm%

- RBC Count: $4.3 \pm 0.5 \times 10^{12}/l$
- Hct: 0.41 ± 0.05 (l/l)
- MCV: 92 ± 9 fl
- MCH: 29.5 ± 2.5 pg
- MCHC: 33.0 ± 1.5 gm/dl
- RDW : $12.8 \pm 1.2\%$

Peripheral blood smear were prepared and stained with Leishman stain examined for red cell morphology, anisopoikilocytosis, presence of target cells, tear drop cells, fragmented red cells, nucleated cells, haemoglobin distribution and presence of any inclusion. Reticulocyte preparation was made and reticulocyte count was carried out.¹¹

Samples were subjected to HPLC Biorad Varaint-II system for further screening if any of the following features found including deranged red cell indices:

- Hb below $10\text{gm}\%$ ¹ and Red cell distribution width normal (11-14)
- $\text{MCV} \leq 75$ fl or $\text{MCH} \leq 27$ pg¹²
- RBC Count ≥ 4.5 million
- Peripheral blood film was microcytic hypochromic, target cells present and with or without normal to high reticulocyte count

High Performance Liquid Chromatography (HPLC) Biorad Varaint-II system was used for variant analysis of the samples for various haemoglobinopathies. Samples were stored between $2-8^{\circ}\text{C}$ maximum for a period of 7 days and subjected to HPLC analysis in batches. Samples were run together with two levels of A2/F controls, also supplied by the manufacturer, in each run.

Data Analysis:

Descriptive statistics (mean, standard deviation, range, percentages) was applied wherever appropriate. Statistical analysis performed using Pearson correlation test, independent t test, ANOVA test and ROC curve. Sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of various red cell indices and cell counter based formulae were calculated.

Results: Out of total 361 anaemic antenatal cases 27 were positive for various hemoglobinopathies giving a prevalence of 7.5 %. Maximum number of subjects were in second trimester of pregnancy. Negative correlation between number of pregnancies and mean haemoglobin value with a P value < 0.05 which was statistically significant.

TABLE 1: MCV Wise Distribution Of Thalassaemic Cases And Their Correlation With Other Parameters

MCV (fl) Range	Positive cases of Thal. Syndrome No %	HB (gm%) MEAN $\pm$ SD RANGE	MCH (pg) MEAN $\pm$ SD RANGE	MCHC (gm/dl) MEAN $\pm$ SD RANGE	RDWCV (%) MEAN $\pm$ SD RANGE	RBC COUNT MEAN $\pm$ SD RANGE	RETIC COUN T MEAN $\pm$ SD RANGE
50-60 n = 16	6 * 37.5	8.3 ± 1.4 5.3 – 10.0	19.7 ± 3.5 16.0 – 28.0	31.2 ± 2.3 26.0 – 35.0	15.8 ± 2.8 8.0 – 28.0	4.3 ± 0.8 3.2 – 6.0	3.2 ± 3.0 0.9 – 6.0
61-70 n = 50	11 ** 22.0	8.6 ± 1.3 5.7 – 10.0	21.4 ± 2.7 18.0 – 30.0	30.9 ± 2.5 21.0 – 36.0	16.6 ± 2.4 12.8 – 27.0	4.1 ± 0.7 2.4 – 6.6	3.1 ± 1.0 1.0 – 5.0
71-80 n = 201	10 *** 5.0	9.5 ± 1.0 3.0 – 10.0	26.3 ± 2.2 20.0 – 33.0	32.3 ± 1.7 23.0 – 37.0	14.9 ± 2.3 11.0 – 29.0	3.8 ± 0.7 1.2 – 7.0	1.9 ± 1.0 0.2 – 6.0

81-90 n = 60	-	-	9.5 ± 1.1 3.5 – 10.0	28.8 ± 1.4 26.0 – 34.0	33.0 ± 1.3 30.0 – 39.0	14.4 ± 2.0 11.2 – 24.0	3.6 ± 0.4 2.5 – 4.5	0.8 ± 0.9 0.2 – 5.0
91-100 n = 12	-	-	9.3 ± 1.8 3.4 – 10	31.1 ± 3.2 27.0 – 38.0	34.1 ± 1.4 30.0 – 36.0	14.3 ± 1.6 11.6 – 17.5	3.4 ± 0.4 2.8 – 4.1	0.9 ± 0.6 0.2 – 2.3
>101 n = 22	-	-	9.1 ± 0.9 7.1 – 10.0	30.8 ± 4.1 25.0 – 38.0	33.8 ± 1.0 30.0 – 36.0	14.8 ± 1.2 12.4 – 17.0	3.5 ± 0.6 1.8 – 4.9	0.9 ± 0.7 0.2 – 2.5
TOTAL L n = 361	27	7.5	9.3 ± 1.1 3.0 – 10.0	26.2 ± 3.7 16.0 – 38.0	32.3 ± 1.9 21.0 – 39.0	15.1 ± 2.3 8.0 – 29.0	3.8 ± 0.6 1.2 – 7.0	1.8 ± 1.2 0.2 – 6.0

*include 1 case of HbE **include 1 case of HbD ***include 2 cases of HbS and 1 case of HbE

➤ In Thal. Synd. Cases : Minimum MCV : 54.0 fl

Maximum MCV : 77.0 fl

➤ In total 361 cases range of MCV was 54.0 fl (minimum) to 114.0 fl (maximum)

➤ 27 cases were positive for thalassemia syndrome.

Table 4: MCH Wise Distribution Of Thalassemic Cases And Their Correlation With Other Parameters

MCH (pg)	Positive cases of Thal. Synd. No %	HB MEAN± SD RANGE	MCV MEAN± SD RANGE	MCHC MEAN± SD RANGE	RDWCV MEAN± SD RANGE	RBC COUNT MEAN±SD RANGE	RETIC COUNT MEAN± SD RANGE
15 – 24 n = 92	17* 18.5	8.6 ± 1.3 3.0 – 10.0	67.8 ± 5.9 54.0 – 80.0	30.9 ± 2.1 23.0 – 36.0	16.3 ± 2.2 13.0 – 27.0	4.0 ± 0.7 1.2 – 6.6	2.8 ± 1.1 0.5 – 6.0
25 – 34 n = 263	10** 3.8	9.5 ± 0.9 3.5 – 10.0	81.3 ± 10.3 56.0 – 114.0	32.8 ± 1.5 21.0 – 39.0	14.7 ± 2.2 11.0 – 29.0	3.7 ± 0.6 2.0 – 7.0	1.5 ± 1.0 0.2 – 6.0
35 – 44 n = 6	- -	7.9 ± 2.6 3.4 – 10.0	105.8 ± 6.1 98.0 – 113.0	34.8 ± 1.1 33.0 – 36.0	15.2 ± 1.9 12.6 – 17.0	2.7 ± 0.5 1.8 – 3.6	1.0 ± 0.9 0.2 – 2.5
TOTAL n = 361	27 7.5	9.3 ± 1.1 3.0 – 10.0	78.2 ± 11.6 54.0 – 114.0	32.3 ± 1.9 21.0 – 39.0	15.1 ± 2.3 11.0 – 29.0	3.8 ± 0.6 1.2 – 7.0	1.8 ± 1.2 0.2 – 6.0

*include 1 case of HbD (22 pg) and 2 cases of HbE (20 pg, 24pg)

**include 2 cases of HbS (25 pg , 26 pg)

➤ In Thal. Synd. Cases : Minimum MCH : 16.4 pg and Maximum MCH : 27.0 pg

➤ In total 361 cases range of MCH was 16.0 pg (minimum) to 38.0 pg (maximum)

Table 5: MCHC Wise Distribution Of Thalassemic Cases And Their Correlation With Other Parameters

MCHC	Positive cases of Thal. Synd. No %	HB MEAN± SD RANGE	MCH MEAN± SD RANGE	MCV MEAN± SD RANGE	RDWCV MEAN± SD RANGE	RBC COUNT MEAN± SD RANGE	RETIC COUNT MEAN± SD RANGE
15 - 24 n = 3	-	6.5 ± 3.2 3.0 – 9.3	23.0 ± 3.6 20.0 – 27.0	75.0 ± 6.2 68.0 – 80.0	18.3 ± 4.0 14.0 – 22.0	3.5 ± 2.0 1.2 – 4.9	2.9 ± 2.5 1.0 – 5.8

25 – 34 n = 331	23* 6.9	9.3 ± 1.1 3.5 – 10.0	26.0 ± 3.5 16.0 – 38.0	77.9 ± 11.4 54.0 – 114.0	15.1 ± 2.3 11.0 – 29.0	3.8 ± 0.6 1.8 – 6.6	1.8 ± 1.2 0.2 – 6.0
35 -44 n = 27	4 14.8	9.4 ± 1.4 3.4 – 10.0	29.0 ± 4.8 19.0 – 38.0	83.2 ± 13.8 54.0 – 110.0	14.2 ± 1.8 11.0 – 18.0	3.7 ± 0.9 2.5 – 7.0	1.7 ± 1.0 0.2 – 3.5
TOTAL n = 361	27 7.5	9.3 ± 1.1 3.0 – 10.0	26.2 ± 3.7 16.0 – 38.0	78.2 ± 11.6 54.0 – 114.0	15.1 ± 2.3 11.0 – 29.0	3.8 ± 0.6 1.2 – 7.0	1.8 ± 1.2 0.2 – 6.0

*include 2 cases of HbE (32 gm/dl, 33 gm/dl), 1 case of HbD (32 gm/dl) and 2 cases of HbS (32 gm/dl, 33 gm/dl)

➤ In Thal. Synd. Cases : Minimum MCHC : 28.0 gm/dl and Maximum MCHC : 35.0 gm/dl

➤ In total 361 cases range of MCHC was 21.0 gm/dl (minimum) to 39.0 gm/dl (maximum)

Table 6: RDW-CV Wise Distribution Of Thalassaemic Cases And Their Correlation With Other Parameters

RDWC V (%)	Positive cases of Thal. Synd. No %	Hb MEAN± SD RANGE	MCH MEAN± SD RANGE	MCHC MEAN± SD RANGE	MCV MEAN± SD RANGE	RBC COUNT MEAN± SD RANGE	RETIC COUNT MEAN± SD RANGE
10 -12 n = 15	1 6.7	10.0 ± 0.0 10.0 – 10.0	28.4 ± 2.6 25.0 – 34.0	33.6 ± 2.1 30.0 – 39.0	80.1 ± 7.7 60.0 – 91.0	4.2 ± 0.7 3.4 – 6.4	1.5 ± 0.9 0.3 – 3.4
12.1 - 14 n = 110	6* 5.4	9.6 ± 0.9 3.0 – 10.0	27.3 ± 2.5 20.0 – 38.0	32.9 ± 1.6 23.0 – 37.0	80.6 ± 8.3 63.0 – 110.0	3.7 ± 0.6 1.2 – 5.3	1.6 ± 1.2 0.2 – 6.0
14.1 - 16 n = 154	15** 9.7	9.4 ± 0.9 3.4 – 10.0	26.2 ± 3.6 17.0 – 38.0	32.5 ± 1.4 29.0 – 36.0	78.7 ± 13.5 56.0 – 113.0	3.9 ± 0.7 2.7 – 7.0	1.7 ± 1.1 0.2 – 6.0
16.1 - 18 n = 56	3 5.3	8.7 ± 1.1 6.0 – 10.0	23.9 ± 4.8 16.0 – 38.0	31.5 ± 1.8 28.0 – 35.0	73.1 ± 12.6 56.0 – 114.0	3.8 ± 0.6 1.8 – 5.2	2.3 ± 1.2 0.3 – 6.0
>18.1 n = 26	2*** 7.7	7.7 ± 1.9 3.5 – 10	25.0 ± 4.0 16.0 – 29.0	30.3 ± 3.2 21.0 – 34.0	75.2 ± 6.8 60.0 – 87.0	3.3 ± 0.7 2.0 – 4.9	2.6 ± 1.4 0.3 – 5.0
TOTAL n = 361	27 7.5	9.3 ± 1.1 3.0 – 10.0	26.2 ± 3.7 16.0 – 38.0	32.3 ± 1.9 21.0 – 39.0	78.2 ± 11.6 56.0 – 114.0	3.8 ± 0.6 1.2 – 7.0	1.8 ± 1.2 0.2 – 6.0

*include 1 case of HbS (12.3 %)

** include 2 cases of HbE (14.8 %, 15.0 %) and 1 case of HbS (14.8 %)

***include 1 case of HbD (19.0 %)

➤ In Thal. Synd. Cases : Minimum RDW-CV : 12.0 %

Maximum RDW-CV : 19.0 %

➤ In total 361 cases range of RDW-CV was 11.0 % (minimum) to 29.0 % (maximum)

Table 7: RBC Count Wise Distribution Of Thalassaemic Cases And Their Correlation With Other Parameters

RBC Count (million/μl)	Positive cases of Thal. Synd. No %	HB MEAN± SD RANGE	MCH MEAN± SD RANGE	MCHC MEAN± SD RANGE	RDWCV MEAN± SD RANGE	MCV MEAN± SD RANGE	RETIC COUNT MEAN± SD RANGE
≤ 2	1 25.0	5.9 ± 3.2	27.7 ± 7.1	31.0 ± 5.3	18.2 ± 7.3	83.7 ±	3.2 ± 2.2

n = 4		3.0 – 10.0	22.0 – 38.0	23.0 – 34.0	13.1 – 29.0	16.9 – 72.0 – 109.0	0.4 – 5.8
2.1 - 4 n = 244	5* 2.0	9.2 ± 1.2 3.4 – 10.0	27.0 ± 3.5 16.0 – 38.0	32.5 ± 1.7 26.0 – 39.0	15.1 ± 2.3 11.0 – 27.0	80.2 ± 11.6 – 54.0 – 114.0	1.6 ± 1.1 0.2 – 6.0
>4.1 n = 113	21**18.6	9.6 ± 0.7 7.2 – 10.0	24.4 ± 3.5 16.4 – 33.0	32.0 ± 2.1 21.0 – 36.0	14.9 ± 2.0 11.2 – 22.0	73.7 ± 10.2 – 54.0 – 110.0	2.2 ± 1.2 0.2 – 6.0
TOTAL n = 361	27 7.5	9.3 ± 1.1 3.0 – 10.0	26.2 ± 3.7 16.0 – 38.0	32.3 ± 1.9 21.0 – 39.0	15.1 ± 2.3 11.0 – 29.0	78.2 ± 11.6 – 54.0 – 114.0	1.8 ± 1.2 0.2 – 6.0

*include 1 case of HbS (3.7 million/ μ l)

**include 1 case of HbD (4.6), 2 cases of HbE (5.0, 5.1) and 1 case of HbS (4.9)

➤ In Thal. Synd. Cases : Minimum RBC Count : 2.0 million/ μ l

Maximum RBC Count : 7.0 million/ μ l

➤ In total 361 cases range of RBC Count was 1.2 million/ μ l (minimum) to 7.0 million/ μ l (maximum)

Table 8: Retic Count Wise Distribution Of Thalassaemic Cases And Their Correlation With Other Parameters

RETI C Count (%)	Positive cases of Thal. Synd. No %	HB MEAN± SD RANGE	MCH MEAN± SD RANGE	MCHC MEAN± SD RANGE	RDWCV MEAN± SD RANGE	RBC COUNT MEAN± SD RANGE	MCV MEAN± SD RANGE
0.2 - 2 n = 235	8* 3.4	9.5 ± 0.8 3.4 – 10.0	27.4 ± 3.0 18.0 – 38.0	32.6 ± 1.8 – 21.0 – 39.0	14.7 ± 1.8 11.0 – 22.0	3.7 ± 0.5 1.8 – 6.4	81.7 ± 11.4 54.0 – 113.0
2.1 - 4 n = 109	13**119	9.1 ± 1.3 3.5 – 10.0	24.3 ± 3.7 16.0 – 38.0	32.2 ± 1.9 – 26.0 – 37.0	15.5 ± 2.7 11.0 – 29.0	3.9 ± 0.7 2.0 – 7.0	72.3 ± 8.8 54.0 – 114.0
>4.1 n = 17	6 35.3	7.4 ± 2.1 3.0 – 10.0	21.0 ± 3.5 16.4 – 28.0	30.1 ± 2.4 – 23.0 – 34.0	17.0 ± 3.8 12.4 – 27.0	3.8 ± 1.4 1.2 – 6.6	68.5 ± 8.1 56.0 – 87.0
Total n = 361	27 7.5	9.3 ± 1.1 3.0 – 10.0	26.2 ± 3.7 16.0 – 38.0	32.3 ± 1.9 – 21.0 – 39.0	15.1 ± 2.3 11.0 – 29.0	3.8 ± 0.6 1.2 – 7.0	78.2 ± 11.6 54.0 – 114.0

*include 2 case of HbS (1.0 %, 2.0 %) and 1 case of HbE (1.5 %)

**include 1 case of HbE (3.5 %) and 1 case of HbD (3.0 %)

➤ In Thal. Synd. Cases : Minimum Retic Count : 1.0 %

Maximum Retic Count : 6.0 %

- In total 361 cases range of Retic Count was 0.2 % (minimum) to 6.0 % (maximum)

Table 9: Microcytic Hypochromic Blood Picture – Distribution of Indices

Hb (gm %)	Positive cases of Thal. Synd. No. %	MCV MEAN ±SD Range	MCH MEAN ±SD Range	MCHC MEAN ±SD Range	RDWCV MEAN ±SD Range	RBC COUNT MEAN ±SD Range	RETIC COUNT MEAN ±SD Range	AGE MEAN Range
2 - 4 n = 3	-----	76.3±1.1 75.0 – 77.0	24.6 ±2.5 22.0 – 27.0	29.6 ±5.7 23.0 – 33.0	18.3±9.2 12.0-29.0	1.93±0.7 1.2 –2.6	5.3±1.1 4.0 – 6.0	23.6 20 – 27.0
4.1 – 6 n = 4	-----	71.5±9.9 60.0 – 84.0	22.5 ±4.7 16.0 – 27.0	31.0 ±3.4 26.0 – 34.0	20.2±4.3 15.0 – 25.0	2.6 ±0.5 2.1 –3.3	3.3 ±1.6 1.0 – 5.0	21.7 20 – 23.0
6.1 – 8 n = 31	7 22.6	67.4±9.9 54.0 – 78.0	21.0 ±3.5 16.0 – 29.0	30.6 ±2.6 21.0 – 35.0	16.8 ±1.3 14.0 – 20.0	3.8 ±0.5 3.1 –5.2	3.0 ±1.3 0.9 – 6.0	24.8 20 – 35.0
8.1 – 10 n = 206	20* 9.7	74.5±5.8 56.0 – 93.0	25.4 ±2.8 17.0 – 33.0	32.3 ±1.7 24.0 – 37.0	14.8±1.9 18.0 – 24.0	4.0 ±0.7 2.0 –7.0	2.2 ±0.8 0.5 – 6.0	24.0 19 – 35.0
TOTAL n = 244	27 11.1	73.6±6.9 54.0 – 93.0	24.8 ±3.3 16.0 – 33.0	32.0 ±2.0 21.0 – 37.0	15.2±2.3 12.0 – 29.0	3.9 ±0.7 1.2 –7.0	2.3 ±1.0 0.5 – 6.0	24.0 19.0– 35.0

*include 2 cases of HbS (9.0 gm%, 10.0 gm%) and HbE (10.0 gm% each), and 1 case of HbD (10.0gm%)

- In Thal. Synd. Cases : Minimum Hb : 6.5 gm%

Maximum Hb : 10.0 gm%

- In total 489 cases range of Hb was 3.0 gm % (minimum) to 10.0 gm % (maximum)

Table 10: Dimorphic Blood Picture – Distribution Of Indices

Hb (gm%)	MCV MEAN ±SD RANGE	MCH MEAN ±SD RANGE	MCHC MEAN ±SD RANGE	RDWCV MEAN ±SD RANGE	RBC COUNT MEAN ±SD RANGE	RETIC COUNT MEAN ±SD RANGE	AGE MEAN RANGE
2 - 4 n = 1	85.0 85.0	28.0 28.0	32.0 32.0	21.0 21.0	2.5 2.5 – 2.5	4.0 4.0 – 4.0	22.0 22.0
4.1 – 6 n = 0	-----	-----	-----	-----	-----	-----	-----
6.1 – 8 n = 5	76.8 ±7.7 68.0 – 87.0	25.0 ±5.0 19.0 – 29.0	30.6 ±1.5 29.0 – 32.0	22.2 ± 3.4 19.0 – 27.0	3.1 ± 0.2 2.7 – 3.4	3.2 ± 2.3 0.6 – 5.0	25.8 ±1.9 23.0 – 28.0
8.1 – 10 n = 3	79.3 ±1.1 78.0 –	28.3 ±0.5 28.0 –	33.0 ±1.0 32.0 –	20.2 ± 0.7 19.5 –	3.3 ± 0.1 3.2 – 3.5	1.5 ± 0.4 1.2 – 2.0	24.3 ±2.0 22.0 –

	80.0	29.0	34.0	21.0			26.0
TOTAL n = 9	78.5 ±6.1 68.0 – 87.0	26.4 ±3.9 19.0 – 29.0	31.5 ±1.6 29.0 – 34.0	21.4 ± 2.6 19.0 – 27.0	3.1 ± 0.3 2.5 – 3.5	2.7 ± 1.9 0.6 – 5.0	24.8 ±2.1 22.0 – 28.0

- Out of total 9 cases none was found positive for thalassaemia syndrome.
- Minimum Hb : 3.5 gm%
- Maximum Hb : 10.0 gm%

Table 11: Macrocytic Blood Picture – Distribution of Indices

Hb (gm%)	MCV MEAN± SD RANGE	MCH MEAN± SD RANGE	MCHC MEAN± SD RANGE	RDWCV MEAN± SD RANGE	RBC COUNT MEAN±SD RANGE	RETIC COUNT MEAN±SD RANGE	AGE MEAN RANGE
2 - 4 n = 1	99.0 99.0 – 99.0	38.0 38.0 – 38.0	36.0 36.0 – 36.0	16.0 16.0 – 16.0	2.8 2.8 – 2.8	1.6 1.6 – 1.6	25.0 25.0 – 25.0
4.1 – 6 n = 0	-----	-----	-----	-----	-----	-----	-----
6.1 – 8 n = 4	108.0 ± 1.8 106.0 - 110.0	32.5 ± 6.3 27.0 – 38.0	34.0 ± 0.8 33.0 – 35.0	15.9 ± 1.2 14.8 – 17.0	2.9 ± 0.8 1.8 – 3.6	1.0 ± 0.9 0.4 – 2.5	23.2 ± 1.2 22.0 – 25.0
8.1 – 10 n = 22	101.4 ± 22.7 106.0 – 114.0	30.0 ± 3.3 25.0 – 38.0	33.9 ± 1.0 30.0 – 36.0	14.5 ± 1.0 12.4 – 16.6	3.6 ± 0.5 2.7 – 4.9	0.9 ± 0.6 0.2 – 2.5	24.2 ± 2.8 20.0 – 32.0
TOTAL n = 27	102.3 ± 20.5 99.0 – 114.0	31.7 ± 4.1 25.0 – 38.0	34.0 ± 1.0 30.0 – 36.0	14.8 ± 1.1 12.4 – 17.0	3.5 ± 0.6 1.8 – 4.9	1.0 ± 0.6 0.2 – 2.5	24.1 ± 2.6 21.0 – 32.0

- Minimum Hb : 3.4 gm%
- Maximum Hb : 10.0 gm%

Table 12: Normocytic Normochromic Blood Picture- Distribution of Indices

Hb (gm%)	MCV MEAN± SD RANGE	MCH MEAN± SD RANGE	MCHC MEAN± SD RANGE	RDWCV MEAN± SD RANGE	RBC COUNT MEAN±SD RANGE	RETIC COUNT MEAN±SD RANGE	AGE MEAN RANGE
2 - 4 n = 0	-----	-----	-----	-----	-----	-----	-----
4.1 – 6 n = 0	-----	-----	-----	-----	-----	-----	-----
6.1 – 8 n = 0	-----	-----	-----	-----	-----	-----	-----
8.1 – 10 n = 79	84.4 ±4.7 78.0 – 98.0	29.1 ±1.6 27.0 – 35.0	32.9 ±1.4 28.0 – 39.0	14.2 ± 1.5 11.2 – 21.0	3.7 ± 0.3 2.9 – 4.6	0.4 ± 0.3 0.2 – 2.0	23.3 ±2.2 20.0 – 35.0

TOTAL	84.4 ±4.7	29.1 ±1.6	32.9 ±1.4	14.2 ± 1.5	3.7 ± 0.3	0.4 ± 0.3	23.3 ±2.2
n = 79	78.0 –	27.0 –	28.0 –	11.2 –	2.9 – 4.6	0.2 – 2.0	20.0 –
	98.0	35.0	39.0	21.0			35.0

- Minimum Hb : 8.2 gm%
- Maximum Hb : 10.0 gm%

Discussion: In antenatal group of 19 to 35 years , women with haemoglobin upto 10 gm% were included. Out of 361 cases 27 (7.5 %) were found positive for haemoglobinopathies. These included 22 (6.1%) cases of beta thalassemia trait, 2 (0.6%) cases of HbS trait, 1 (0.3%) case of HbD Punjab, 2 (0.6%) cases of HbE (1heterozygous and 1 homozygous).

Thalassemia Intermedia:

In Present study we found 2 cases of thalassemia intermedia having age 22 years and 30 years. Mean age of presentation was 13.5 years in a study done by Tyagi et al¹³ and 11.8 years in the series of Phadke et al.¹⁴

The Hb levels in 93 patients of thalassemia intermedia in the study by Tyagi et al¹³ ranged from 2.2 gm% to 11.3 gm% with mean of 7.4 gm%. Mean Hb at diagnosis was 7.5 gm% by Phadke et al¹⁴ study. Our cases had Hb 2.6 gm% and 10.8 gm %. Such low Hb may be due to concurrent iron deficiency anaemia (RDW CV- 23%).

Tyagi et al¹³ found microcytic hypochromic red cell picture with moderate degree of anisocytosis, poikilocytosis, few fragmented red cells and nucleated red cells, similar to our findings. Tyagi et al¹³ found mean HbF to be 46.9 % with values ranging between 18.3- 98.5% and mean HbA₂ of 2.4% ranging between 1.1- 8.4%. Our cases have HbF values 34.1 % and 33.6 % similar to it but slight increased HbA₂ values i.e 11.6 % and 11.4 % respectively.

Thalassemia Trait:

Out of total 79 positive cases for various haemoglobinopathies, 69 cases were positive for beta thalassemia trait, comprising the largest group. These included 22 antenatal women, 36 from student group and rest 11 from the family group who were relatives of the positive cases of target group. Mean Hb of total cases was 10.1 gm% and ranging from 6.5 gm% to 13.0 gm%. Mean Hb of antenatal group was found to be 9.1 gm % which was slightly lower than the student group 10.9 gm %. In a study done by Gupta et al¹⁵ mean Hb was found to be 9.3 gm%. Lower hemoglobin in some of our cases could be due to associated iron deficiency.

Microcytosis and hypochromia are commonly observed in the peripheral blood smear in thalassemia trait. Tyagi et al¹³ and Gupta et al¹⁵ found microcytic hypochromic blood picture in all the cases of beta-thalassemia trait. We also found microcytic hypochromic blood picture in all thalassemia trait cases. Degree of anisopoikilocytosis also varied from none to mild to moderate degree.

HPLC screening showed constantly elevated HbA₂ in thalassemia carriers in all ethnic groups. HbA₂ in our cases ranged from 3.7 – 6.7 % with a mean of 4.8 %. Mean HbA₂ was 6.1 % as per George et al, 2001. Tyagi et al¹⁶ diagnosed 31 cases of beta heterozygous thalassemia and HbA₂ levels ranged from 3.9 – 9.0 % with a mean of 6 %. HbF was found to be increased in half of the cases, but values observed in general were in the range of 1-3%¹⁷. In present study, HbF ranged from 0.1-3.2 % with a mean of 0.8 %.

The identification of beta thalassemia trait is often based on characteristics like higher red cell count, reduced MCV, MCH, raised levels of HbA₂. George et al¹⁸ observed 93.7 of female carriers and 88.9 % of male carriers had a MCV < 70 fl. MCH < 27 pg was found in 25 out of 26 thalassemia carriers in his study. In our study 58% thalassemia trait cases had MCV < 70fl and 42% cases had MCV > 70fl. MCH in our study was found <27 pg in 89.9 % cases and in 7 cases (10.1 %) it was 27.0 pg.

HbE :

In present study we found 3 cases of HbE which include 1 case of HbE heterozygous i.e trait and 2 cases of HbE homozygous (HbEE). Mean age of presentation was 20 years.

HbE heterozygous are clinically normal with only minimal hematologic changes. Red cells are normocytic or slightly microcytic with minor morphologic changes such as target cell morphology. HbE constitutes 25-35 % of total hemoglobin. Homozygotes for HbE are usually asymptomatic and have normal hemoglobin levels but in some cases mild anaemia may be present. The peripheral smear shows microcytosis and increased target cells. Hemoglobin analysis reveals >60 % of HbE levels.¹⁹

In present study hemoglobin level was 10.0 gm% and 11.0 gm% in homozygous HbE and 10.0 gm% in HbE trait. Patients of HbE disease and HbE trait were asymptomatic with hemoglobin levels ranging from 8.8 – 11.8 gm% with a mean of 10.5 gm% in the study of Tyagiet al.¹⁶

In present study, mean MCV was 70.3 fl (59 fl and 76 fl in homozygous and 76 fl in heterozygous) and mean MCH was found to be 23.6 pg (20 pg, 24 pg in homozygous and 27 pg in heterozygous). Absolute values were higher (MCV was 84 fl and MCH was 30 pg) in one study³⁵ while in concordance with other studies (70.0 fl and 23.6 pg)³⁶, Fairbank et al (70.2 fl and 23.6 pg)²⁰.

RBC Count was high in half the HbE cases; RBC Count being 5.7 millions/ μ l (Fairbank et al)¹²⁴ and 5.1 millions/ μ l (Cunnigham et al)²¹. RBC Count in our cases was 5.1 and 4.9 millions/ μ l in HbEE and 5.0 millions/ μ l in HbE trait.

HbE values were 86.5 % and 63.0 % in homozygous and 28.3 % in HbE trait. HbF levels were found to be 1.7 % and 5.2 % in HbE homozygous cases and 2.3 % in HbE heterozygous case. Other workers noted HbE to be 29.4 %³⁵, 28.0 %²⁰ and 27.6 %²² in HbE trait cases.

HbD :

In present study we found 1 case of HbD Punjab trait in the antenatal group, 24 years old female. She had hemoglobin 10.0 gm %, MCV 68.0 fl, MCH 22.0 pg, RBC Count 4.6 million/ μ l and RDW-CV 19.0 %. On HPLC analysis we found HbD concentration of 36.8 %, HbA₂ was 1.7 % and HbF was 0.6 %. Blood picture was microcytic hypochromic. Increased RDW-CV may be due to coexistent nutritional deficiency.

Sickle Cell Syndrome :

We found 2 cases of sickle cell heterozygous (HbS trait) in the antenatal group having age 28 and 20 years old. However, Hashmi et al²³ reported mean age at diagnosis to be 13 years in sickle cell trait cases.

As in our cases, Mohanty et al²⁴ also reported reduced MCV and MCH in sickle cell trait cases (63.5 fl and 19.8 pg). Blood picture in our cases was mild microcytic hypochromic. Sickle cell trait is rarely associated with clinical or hematological manifestations of significance. On HPLC analysis we found HbS concentration 34.2 % and 35.0 % respectively, HbA₂ was 3.4 % and 3.5 %, HbF was 1.2 % and 1.0 % respectively.

Hemoglobinopathies are of world-wide occurrence, though some geographical areas have high prevalence of these disorders. In India, average frequency of sickle cell gene is around 5%. The highest frequency of sickle cell gene in India is reported in Orissa (9%), followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) and Gujarat (6.4%). The distribution of beta thalassemia is not uniform in Indian subcontinent. The highest frequency of beta thalassemia trait is reported in Gujarat (10-15%), followed by Sindh (10%), Punjab (6.5%), Tamil Nadu (8.4%) and Maharashtra.²³

In India, the problem of hemoglobinopathies is compounded by the heterogeneity of population. The different regions of India have different gene frequencies for the various hemoglobinopathies.

Conclusion: Automated cell counter based parameters and formulae are good, rapid, cheaper and easily available methods for screening of haemoglobinopathies especially for thalassemia trait detection. Various cut off values include $MCV \leq 75$ fl, $MCH \leq 27$ pg and $RBC \text{ Count} \geq 4.5$

million/ μ l alongwith microcytic hypochromic peripheral blood smear. Present study was a pilot project in this direction with the aim of regular screening of various target groups for thalassemia and variants in future.

To the best of our knowledge this preliminary study provides platform for further studies including population based surveys so that effective control measures can be implemented.

The prevalence rate of 7.5 % in anemic mothers brings out necessity for such a program.

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