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Original Research Article

Quantification of HbA2 in hemoglobin electrophoresis

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Abstract

Introduction: Haemoglobin electrophoresis is the diagnostic tool to detect the abnormal hemoglobinopathies. Normal adult haemoglobin is HbA(Alpha2 Beta2).. Other forms of abnormal haemoglobin includes HbA2, HbD, HbS which are commonly detected in hemoglobinopathies. HbA2 level in normal individual is undetectable. Its level will be increased in Thalassemia Trait or Thalassemia minor individuals. The level of HbA2 when it's more than 5%, then that is considered as Gold standard to diagnose the Thalassemia trait or minor.

Materials and Methods: We did a retrospective studies in the Trichy SRM Medical College hospital and Research Centre and a total of 100 patients who were diagnosed with microcytic hypochromic anaemia. Complete blood count were run in all cases and Based on results many differential diagnosis for microcytic hypochromic blood pictures were documented. For all these patients Haemoglobin electrophoresis were also studied by Sebia capillary electrophoresis machine and graph was plotted and analysed. Based on HbA2 levels, when the value is more than 4 to 5% then itscategorised as Thalassemia trait cases. When the value of HbA2 is less than normal then those patients were diagnosed with iron deficiency anaemia. Few discriminant factors were also taken to differentiate iron deficiency anaemia and thalassemia trait patients. Mentzer Index, Green and Kings index and Shine Lal index were considered to differentiates iron deficiency anaemia and thalassemia trait.

Results: A total of 100 microcytic hypochromic patients were studied and period of study was 1 year from January 2024 to January 2025. Patients from all age groups and from both male and female were taken for the study. HbA2 graph were derived and based on the results the patients were categorised into iron deficiency anaemia and thalassemia trait. International council of standardization of haematologywere used to quantify the HbA2 levels in both MCHC.

Conclusions: The study analyzed lab results of 213 Beta thalassemia trait (BTT) cases, finding that median values met BTT criteria. Hb A2 levels above 3.5 are key for diagnosis. 117 iron deficiency anemia (IDA) cases were used for comparison, with some BTT cases also having IDA.

Keywords: HbA2, ICSH, Thallsemia Trait.

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1. Introduction

Haemoglobin electrophoresis is the diagnostic tool to detect the abnormal hemoglobinopathies. Normal adult haemoglobin is HbA (Alpha2 Beta2).[1-10] Other forms of abnormal haemoglobin includes HbA2, HbD, HbS which are commonly detected in hemoglobinopathies. HbA2 level in normal individual is undetectable. Its level will be increased in Thalassemia Trait or Thalassemia minor individuals. The level of HbA2 when it's more than 5%, then that is considered as Gold standard to diagnose the Thalassemia trait or minor.[11-15]

2. Aims and Objectives

1. To study the hematologic parameters that get altered in Beta Thalassemia trait patients which includes CBC, RBC indices (MCV, MCH, MCHC, RDW) reticulocyte indices (wherever available).[16-20]
2. To assess HbA 2 levels and other Hb variants (HbS, HbE) by Hb electrophoresis.
3. To compare the hematologic parameters and discriminant functions of patients with increased Hb A 2 verses patients with normal HbA2.
4. To study the relevance of the discriminant functions in differentiating early IDA and patients with BTT where the iron profile is available. [21-25]

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3. Methods and Materials

We did a retrospective studies in the Trichy SRM Medical College hospital and Research Centre and a total of 100 patients who were diagnosed with microcytic hypochromic anaemia. Complete blood count were run in all cases and Based on results many differential diagnosis for microcytic hypochromic blood pictures were documented. Duration of the study were from January 2024 to January 2025. For all these patients Haemoglobin electrophoresis were also studied by Sebia capillary electrophoresis machine and graph was plotted and analysed. Based on HbA2 levels, when the value is more than 4 to 5 % then itscategorised as Thalassemia trait cases.[26-32] When the value of HbA2 is less than normal then those patients were diagnosed with iron deficiency anaemia. Few discriminant factors were also taken to differentiate iron deficiency anaemia and thalassemia trait patients. Mentzer Index, Green and Kings index and Shine Lal index were considered to differentiates iron deficiency anaemia and thalassemia trait.

4. Results

The overall median hemoglobin was 9.7gm% with a range of 4.4 to 14.7. However, 158 patients (74.2%) had hemoglobin ranging from 9 – 14.7 gm% with a median of 10.2gm%. The patient with Hb of 4.4gm% was diabetic who was admitted for amputation of a gangrenous toe.

ESR was estimated in 133 (62.44%) cases. The maximum value was found to be 110mm/hr, seen in a case associated with chronic renal failure where the renal function test was abnormal and the auto-immune serology was positive for p-ANCA. The case was diagnosed as anemia of chronic disease from complete blood count & peripheral smear. The calculated mean, excluding this case (n=132) was found to be (14.92mm/hr.), median (9mm/hr.) and ranged from 1-83 mm/hr.[33-37]

The median MCV was found to be 65.01 fl, with the range from 50.4-99.7. The median MCH was 20.23 pgm. The minimum value was 15.4 pgm& maximum value was 79.3 pgm. The median MCHC was 30.73 gm % with minimum value of 27.2 gm % and maximum value of 35.4 gm %.

The median RDW was found to be 16.5%, with the range from 13.9 to 41.1%. There were 16 cases of Thalassemia trait and Iron deficiency anemia. When these cases along with the case with RDW of 41.1(post transfusion sample) were removed, the median remained unaffected at 16.5 while the maximum RDW dropped to 33.8%.[38,39,40,41]

The overall median of HbA was 94.3 % and the range was 88.5 to 96.1%. The overall median of HbA_2 was 5.10 % and ranged from 3.8 to 6.7 %.

The median HbFwas found to be 0.80% with the range from 0.2 to 6.4%. There were 11 cases with HbF>3%. Six of these patients were antenatal women with HbF ranging from 3.1 to 6.2%, three were female patients aged 24, 26 and 30 yrs and two cases were children aged 1yr and 2yr with a HbF of 6.1 and 6.4 respectively.[42,43,44,45]

The patient with hemoglobin value of 4.4 gm% was associated with diabetes and anemia of chronic disease.

Although the median Hb was comparable in the three groups, there were differences in the other parameters. The median RBC count was higher in BTT as compared to the other two groups. The MCV was lower in BTT and the combined group (64fl and 63fl), compared to IDA where it was 73.8fl. The median RDW was 16.4 % in 203 cases of BTT compared to the slightly higher value of 17.5% in IDA and 17.1 in the combined group.[46,47,48,49]

Table 1: Gender distribution

Gender	Frequency	Percentage (%)
Male	77	36.2
Female	136	63.8
Total	213	100.0

Total number of cases studied was 100, out of which 77 cases were male &23were female.

Table 2: Age wise distribution

Age group (years)	Frequency	Percentage (%)
0-10	16	7.5
11-20	23	10.8
21-30	91	42.7
31-40	39	18.3
41-50	22	10.3
51-60	15	7.0
>61	07	3.4
Total	213	100

Table 3: Clinical features

Symptoms	Frequency	Percentage (%)
Anemia	69	33.8
Jaundice	6	3.2
Anemia & Jaundice	10	4.5
Hemorrhagic symptoms(bleeding/petechial)	4	1.8
Anemia & hemorrhagic symptoms	3	1.4
All symptoms present	2	9.0
No significant clinical symptoms	119	54.5
Total	213	100.0

Table 4: Lab parameters

Parameters	Median	Mean	Range
Hb(gm%) n=100	9.7	9.85	4.4-14.7
RBC(millions/cumm)	5.03	4.99	4.20-7.52
Hct(%)	32.2	32.46	15.9-49
ESR(mm/hr) n-133	9	16.1	1-110

Table 5: RBC Indices

Indices	Median	Mean	Range
MCV(fl)	64.30	65.01	50.4-99.7
MCH(pg)	19.6	20.23	15.4-79.3
MCHC (gm %)	30.70	30.73	27.2-35.4
RDW (%)	16.5	17.25	13.9-41.1

Table 6: Hemoglobin electrophoresis

Hb Variants	Median	Mean	Range
HbA	94.3	94.08	88.5-96.1
HbA_2	5.10	5.11	3.8-6.7
HbF	0.80	1.22	0.2-6.4

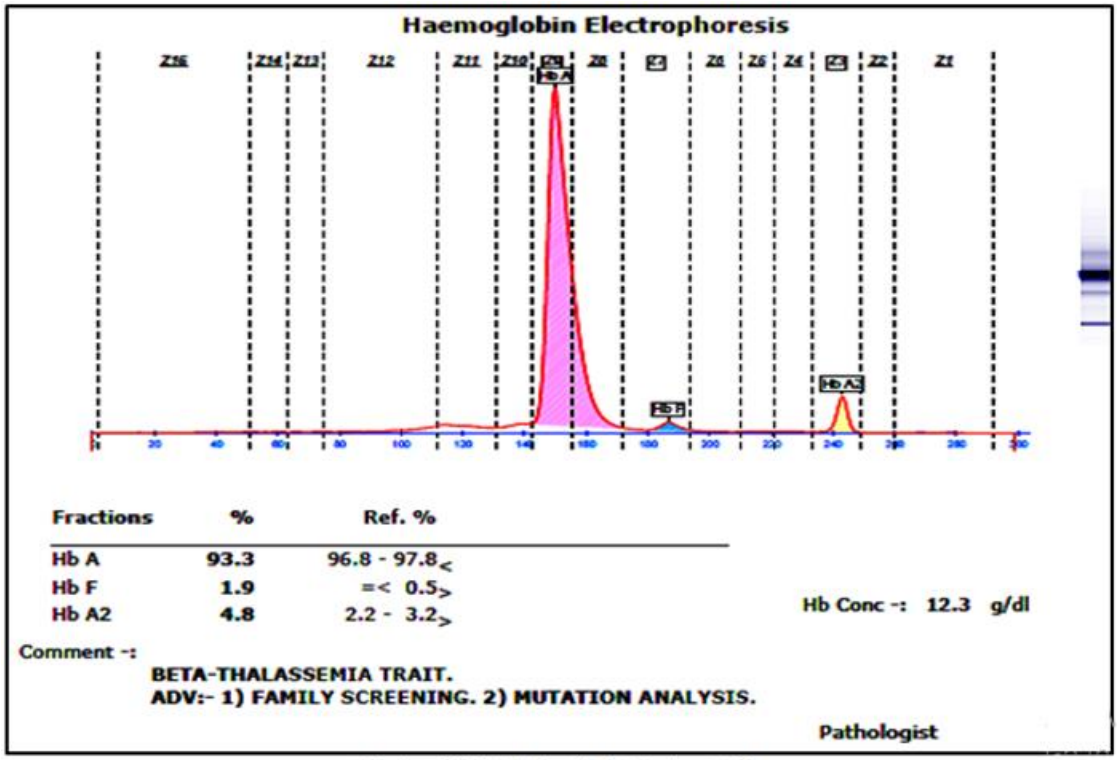
Table 7: Lab Parameters comparing BTT, IDA

S.No		Beta Thalassemia trait(n=100)		IDA(n=100)	
		Median	Range	Median	Range
1.	Hb (gm%)	9.75	4.4-14.7	9.3	6.2-11.9
2.	RBC (millions/cumm)	5.07	2.44-7.52	4.15	2.67-5.17
3.	MCV(fl)	64.40	51.2-99.7	73.8	51.8-91.3
4.	RDW (%)	16.4(n=203)*	13.9-33.8	17.5	12.8-33.4
5.	HbA_2 (%)	5.10	3.8-6.7	2.2	1.6-3.0

*One case was excluded with the RDW value of 41.1 % (post transfusion).

Table 8: Discriminative Indices comparing BTT, IDA

S.No.	Discriminative Indices	BTT(n=100)		IDA(n=100)	
		Median	Range	Median	Range
1.	Mentzer Index	12.55(p-0.001)	7.10-32.00	17.97(p-0.45)	11.91-30.82
2.	Green & Kings Index	67.15(p-0)	44.10-272	101.47	65.92-210.90
3.	RDW Index	207.25(p-0)	131.94-910	316.98(p-0)	195.84-622.27
4.	England and Frazer Index	0.95(p-0)	-21.21-39.60	13.93	-5.65 to 35.89
5.	Srivastava Index	3.88	-19.87-21.03	5.43(p-0.10)	3.08-10.03



Hb A:-<96.8; Hb F:-> 0.5; Hb A_2 :- >3.2

Figure 1: Beta thalassemia trait

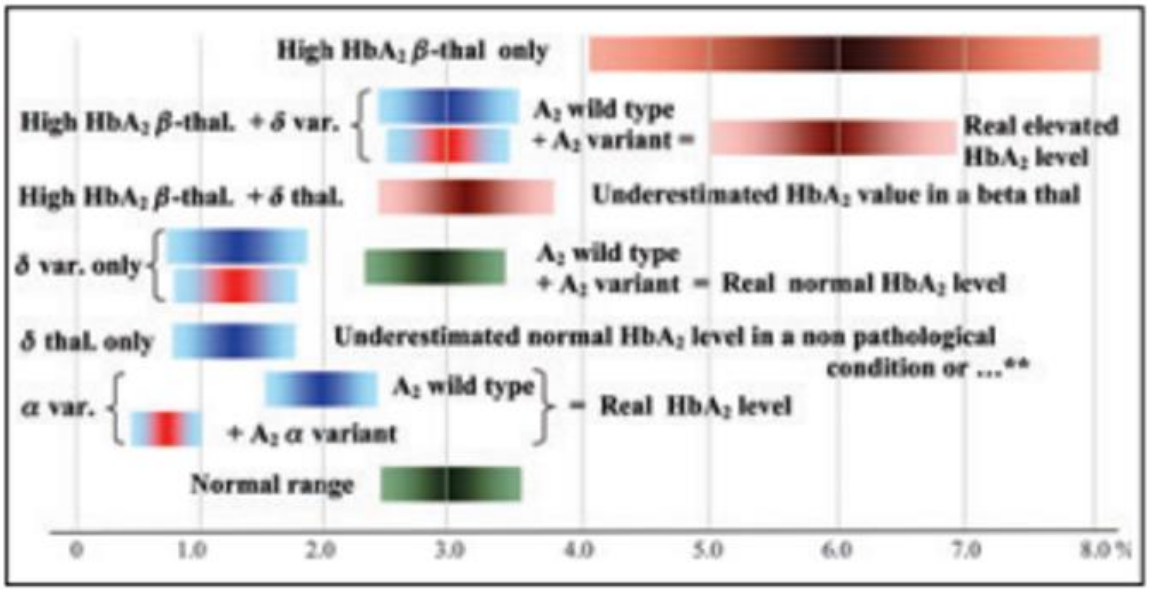


Figure 2: Hb electrophoresis quantification

All the above 5 indices are higher in IDA, compared to the other two groups. The values are comparable in BTT and the combined group and are therefore not useful in differentiating patients with BTT from those with combined IDA. **Figure 1**, **Figure 2**

5. Summary

The clinico-hematological profile of 213 cases of Beta thalassemia trait was studied over the period of 3 years from 1stJanuary 2014 till 31st December 2016, in Hematology, Department of Pathology, Kasturba Hospital, Manipal.

The summary of results are as follows:

5.1. Epidemiology

The patients studied were in the age group from 6 months to 75 years, with median age of 28 years. Out of 213 cases (42.7%) cases were seen in the age group of 21 to 30 years and only 3.4% of cases were seen in the age group of more than 61 years. A female predominance was seen with a male : female ratio of 1:1.77.

5.2. Presenting complaints

Majority of cases were asymptomatic(54.5%). But symptoms of anemia was seen in (82 out of 213) cases (38.5%). Bleeding was seen in 4 patients, which contribute to the degree of anemia with the hemoglobin range from 8.2 to 11.7 gm% in theses cases.

5.3. Physical examination

Majority of cases had no organomegaly(88.2%).

5.4. Treatment history

As majority of cases were asymptomatic, most of them had not received any treatment. However blood transfusion and hematinic supplements were given to women in the reproductive age groups.[50,51,52,53]

5.5. Hematological profile

From the CBC parameters, the overall median of Hb was 9.7 gm%, with the range from 4.4 to 14.7gm%. the lowest value of Hb 4.4 gm% was seen in diabetic patients who got amputated. The overall median of RBC was $5.03x10^6$ /µL, with the range from 2.44 to $7.52x10^6$/µL. The median Hb and RBC values meets the criteria for BTT. ESR was estimated in only 133 cases , the median value was 9mm/hr with range from 1 to 110mm/hr. The highest value of 110mm/hr was seen in case associated with chronic renal failure,were the renal function test are abnormal and auto-immune serology was positive for pANCA.

The overall median MCV in the present study was found to be 64.30fl with the range from 50.4 to 99.7fl. The median MCH in the present study was 19.6 pg with the minimum value of 15.4pg and maximum of 79.3pg. The median MCHC was 30.70 gm% with the minimum value of 27.2 gm% and 35.4gm%.The median RDW was 16.5 % with the minimum value of 13.9 % and maximum of 41.1 %. The overall median values of CBC indices alse meets the criteria for diagnosing BTT.[54,55,56,57]

Retculocyte count was estimated in only 53 cases out of 213, of which the median reticulocyte % was 1.82% with the range from 0.36 to 7.05 %. The case with maximum value 7.05% was associated with hemolytic blood picture.

5.6. Hemoglobin electrophoresis

The overall median Hb A was 94.3 % and range was 88.5% to 96.1%. The median Hb A_2 was 5.10% and range was 3.8 % to 6.7%. The median Hb A_2 meets the diagnostic criteria for detecting BTT and is considered as the gold standard.

5.7. Hemoglobin, rbc indices, hb a_2 of btt with control and btt/ ida group

One hundred and seventeen patients with iron deficiency anemia in whom Hb electrophoresis was done was taken as the control group.

Of 213 BTT cases, 9 had coexisting conditions of BTT and IDA. The median Hb was comparable in all the three groups. The median RBC was higher in BTT group in contrast to the other two groups. The median MCV was lower in the BTT and combined group, when compared to IDA group. The median Hb A_2 in IDA was 2.2 % and lower than the other two groups.

Serum iron profile in BTT is normal in BTT, while the profile was comparable in other two groups.

5.7. Discriminant factors in three groups

In IDA and combined groups all the five discriminant factors are significantly higher when compared to the BTT group. Only Mentzer and RDW index were lower in IDA. From the results of discriminant factors with different cut off values by using ROC curve, Green and King index,RDW index showed no significant in sensitivity/specificity.There was significant improvement in sensitivity for Mentzer index,Srivastava index and RDW index with the new cut off as mentioned in leterature(MI-14.5, EFI-6.25, SI-4.5).[58,59,60,61,62,63,64]

6. Conclusion

Thus to conclude from the datas of various laboratory parameters which includes Hb, RBC, RBC indices(MCV, MCH, MCHC, RDW) for 213 of Beta thalassemia trait cases, the median values meet the criteria for BTT. Hb A_2 estimation is considered as gold standard for diagnosing BTT cases with the value more than 3.5 .

One hundred and seventeen cases of IDA was taken as control group with low Hb A_2 and low serum iron profile levels. The various discriminant factors calculated from CBC indices where compared among the two groups of BTT and IDA.

Out of 213 cases of BTT, 9 cases had coexisting IDA condition for which the serum iron profile and Hb A_2 was in lower limit than the normal range.

7. Source of Funding

None.

8. Conflict of Interest

None.

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