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DIFFERENTIAL DIAGNOSIS OF BETA THALASSEMIA TRAIT AND IRON DEFICIENCY ANAEMIA BASED ON THEIR HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN CHILDREN

Haematology

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ABSTRACT

The term anaemia is used when the quantity of red blood cells or the concentration of haemoglobin in the red blood cells is lower than the normal limit. It is a severe global public health issue that disproportionately affects children under the age of five and pregnant women.Signs of anaemia may now be confirmed by measuring haemoglobin concentration in combination with other haematological and biochemical indicators to confirm iron deficiency. **Aim:** To differentiate between thalassemia trait and iron deficiency anaemia haematologically. Along with different haematological indices and compare biochemical parameters for the same. **Materials And Methods:** A prospective study on 350 children aged 1 to 12 years who came with symptoms of anaemia were examined using several haematological and biochemical parameters, which were then verified by HPLC for differential diagnosis of BTT and IDA. **Results:** We studied children less than 12 years of age and among them majority were under the age of five years, with 229 out of 350 children falling into this category. We observed that majority were males 182 (52%), while 168 (48%) subjects were females with M:F ratio of 1.08:1. The mean haemoglobin level in IDA was considerably lower than Thalassemia Trait and aberrant Hb variations. The mean RDW level was lower in BTT as compared to IDA. On HPLC, 10% of patients had HbA2 haemoglobin variation, indicating BTT, four children had the HbS variant, confirming sickle cell disease, one had HbF. 95%, and 310 had normal HPLC results, confirming IDA. Serum iron, serum ferritin was lower in IDA as compared to BTT whereas TIBC was higher in IDA as compared to BTT. **Conclusion:** The current study revealed that haematological measures with various indices are beneficial in distinguishing between BTT and IDA, as well as their efficacy and association with biochemical markers. The study also revealed that HPLC is still the gold standard for BTT confirmation.

KEYWORDS

Beta thalassemia trait, iron deficiency anemia, comparison, HPLC

INTRODUCTION

The term anaemia is used when the quantity of red blood cells or the concentration of haemoglobin in the red blood cells is lower than the normal limit. It is a severe global public health issue that disproportionately affects children under the age of five and pregnant women. According to the World Health Organization, 42 percent of children under the age of five and 40 percent of pregnant women are anemic[1]. With 2 billion people suffering from iron deficiency anaemia (IDA), iron deficiency has been dubbed "perhaps the most common nutritional deficiency in the world"[1] . Haemoglobin is required to transport oxygen, and if there are insufficient red blood cells or haemoglobin, the blood's oxygen capacity will be reduced. Predominant symptoms include tiredness, weakness, dizziness, and dyspnoea. Age, sex, and elevation of residential area also influence the ideal haemoglobin concentration required to satisfy physiologic demands[1]. Along with alterations in the haematological picture, biochemical markers such as serum Iron, TIBC, and serum Ferritin change in iron deficiency anaemia. Hb, MCV, and MCHC all have a favourable effect on serum iron content. Haematological markers have a negative connection with TIBC. It is affected by Hb, PCV, MCHC, and has a favourable relationship with MCV. Hb, RBC, PCV, MCV, MCH, and MCHC all have a favourable correlation with serum ferritin[2]. Signs of anaemia may now be confirmed by measuring haemoglobin concentration in combination with other haematological and biochemical indicators to confirm iron deficiency. As a result, hypochromic microcytic anaemia was only properly identified as an iron deficiency that might have been caused by a dietary factor in the 1930s[3].

Thalassemia are a group of genetic blood illnesses marked by a reduction in haemoglobin synthesis. There are two forms of thalassemia, alpha and beta[4]. Symptoms vary by kind and can range from mild to severe. Anaemia, ranging from moderate to severe, is common (low red blood cells or haemoglobin) and can cause fatigue and pale skin[5].

Other symptoms include bone pain, a swollen spleen, yellowish skin, and black urine[5]. Children's growth can be slowed. These are genetic illnesses that a person inherits from his or her parents[5]. The severity of alpha and beta thalassemia is determined by how many of the four alpha globin genes or two beta globin genes are absent[5-6]. In thalassemia, the RDW is frequently within or very close to the reference interval, indicating that red cell size is uniform (microcytes). Several methods based on CBC values have been used to compute the thalassaemic index and to distinguish iron deficiency from thalassemia.

The most useful test for detecting thalassemia carriers is quantitative HbA2 measurement. There are other technologies available, but micro chromatography and cation exchange HPLC and capillary electrophoresis are the most accurate, rapid, and easy[8].

MATERIALS AND METHODS

This is a prospective study on 350 children aged 1 to 12 years who came with symptoms of anaemia were examined using several haematological and biochemical parameters, which were then verified by HPLC for differential diagnosis of BTT and IDA in the department of Pathology in a tertiary care hospital at Navi Mumbai.

Inclusion Criteria:

1) Children having anaemia symptoms.
2) Children between the ages of one and twelve.

Exclusion Criteria:

1) Children above the age of 12 and under the age of one year.
2) History of blood transfusion in the last three months.
3) History of previously recognised haematological disorders such as autoimmune haemolytic anaemia, aplastic anaemia, or lead poisoning.

The study included 350 children aged 1 to 12 years who came with symptoms of anaemia were examined using several haematological and biochemical parameters, which were then verified by HPLC for differential diagnosis of BTT and IDA. This study proved useful in identifying BTT.

RESULTS AND OBSERVATIONS

This prospective study was conducted in Department of Pathology, MGM Medical College & Hospital, Kamothe, Navi Mumbai. During the study period of over 18 months, 350 children aged 1-12 years who came with clinical symptoms of anaemia with low haemoglobin.

In the present study we assessed the Gender wise distribution among the study subjects (paediatric population). We observed that majority were males (52%), while 48% subjects were females. The M:F ratio in the current study was 1.08:1. Subjects = Patients with Anemia.

Table No 1: Gender Wise Distribution Of Children

Gender Wise Distribution (Paediatric Population)	Patients with Anaemia	Percentage
Males	182	52 %
Females	168	48 %
Total	350	100 %

In the present study we assessed the Age distribution among the study subjects (paediatric population 1-12 years) . We observed that majority of the subjects belonged to the age group of less than 5 years (65.4%), followed by children more than 10 years (18%) and then 5 to 10 years (16.6%). The mean age of the study subjects was 1.53 ± 0.78 years. Subjects = Patients with Anaemia.

Table No 2: Age Wise Distribution Of Children 1-12 Years

Age distribution (paediatric population 1-12 years)	Patients With Anemia	Percentage
Less than 5 years	229	65.4 %
5 to 10 years	58	16.6 %
10-12 years	63	18.0 %
Total	350	100 %
Mean age	1.53 ± 0.78 years	

Table No 3: Comparison Of Peripheral Smear Examination Among BTT And IDA

Peripheral Smear	Thalassemia Trait	IDA
Microcytic Hypochromic with APC	137	210
Microcytic Hypochromic Target cells	140	130
Microcytic hypochromic with Basophilic Stippling	24	4

Table No 4: High-performance Liquid Chromatography (HPLC) Findings.

HPLC	Patients with anaemia	Percentage
HbF 95%	1	0.28 %
HbS	4	1.14 %
IDA	310	88.5 %
Thalassemia Trait	35	10 %
Total	350	100 %

Table No 5: Comparison Of Mean Values Of Haematological Parameters Between BTT And IDA-

Parameters	IDA		Thalassemia Trait and abnormal Hb variants		T-value	P-value
	Mean	SD	Mean	SD		
Hb	6.8	2.4	7.5	1.8	-1.86	0.064
TLC	13508.79	7902.13	10475.8	5339.9	2.358	0.019
RBC	2.9	0.7	4.8	0.6	-15.577	<0.001
PCV	24.0	6.9	23.2	5.8	0.727	0.468
MCV	75.06	7.5	52.9	5.7	17.907	<0.001
MCH	24.2	3.9	13.3	2.7	17.021	<0.001
MCHC	26.4	3.2	33.9	1.2	-14.515	<0.001
Retic count	2.8	1.9	1.9	1.8	2.813	0.005
RDW	22.3	5.1	18.5	5.6	4.366	<0.001

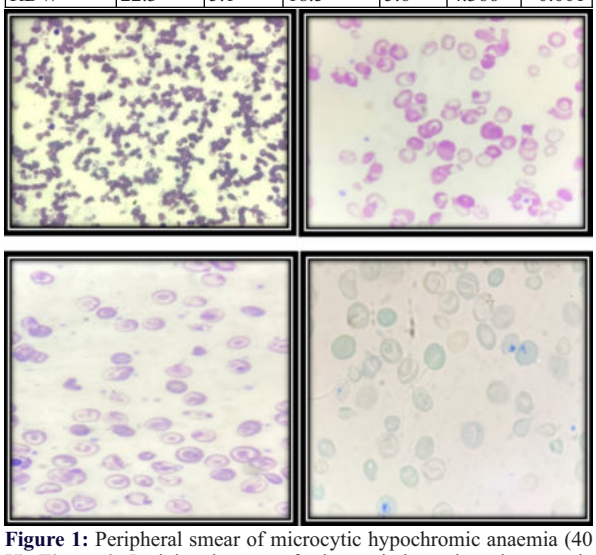

Figure 1: Peripheral smear of microcytic hypochromic anaemia (40 X). **Figure 2:** Peripheral smear of microcytic hypochromic anaemia (100 X). **Figure 3:** Peripheral smear of microcytic hypochromic anaemia with target cells (100 X). **Figure 4:** Photomicrograph of Reticulocytes (100 X).

Table No 6: Comparison Of Mean Values Of Biochemical Parameters Between BTT And IDA-

Parameters	IDA		Thalassemia Trait and abnormal Hb variants		T-value	P-value
	Mean	SD	Mean	SD		
S Iron	24.48	3.22	125.48	61.01	-12. 829	<0.001
TIBC	459.74	137.2	377.8	91.3	3.003	0.003
S. Ferritin	11.133	.75	32.12	21.29	-7.647	<0.001

DISCUSSION

In the present study we assessed the Gender wise distribution among the study subjects who presented with anaemia. We observed that majority were males 182 (52%), while 168 (48%) subjects were females with M:F ratio of 1.08:1.as also seen in the studies by Demir A et al[9] and Abu Syed Mohammed Mujib et.al[10].

In the present study we assessed the Age distribution among the study subjects. We observed that majority of the subjects belonged to the age group of less than 5 years out of 229 children (65.4%), followed by more than 10 years (18.0 %) and then 5 to 10 years (16.6 %). The mean age of the study subjects was 1.53 ± 0.78 years. Since BTT and IDA are among the most common types of microcytic anaemia encountered by paediatricians. In India, estimated anaemic population among children is 89 million. The prevalence of anaemia is 70% in children aged 6–59 months.

We evaluated the findings of high-performance liquid chromatography among the study individuals in this study. We found that 88.5 percent of anaemic people had normal HPLC results, supporting the diagnosis of Iron Deficiency Anaemia; nevertheless, 10% of anaemic subjects had Thalassemia Trait, 1.14 percent had HbS variation, and 0.28 percent had HbF 95 percent. We found that Thalassemia trait had the largest number of aberrant haemoglobin variants, followed by HbS and HbF, as demonstrated in study by L. Pant et al[11&12], Sarika Verma et al[13&14], and Ritesh Sachdeva et al[15&16]. The rest of the individuals with anaemic symptoms had normal HPLC results and were diagnosed with iron deficiency anaemia. As a result, HPLC is the study of choice for differentiating between BTT and IDA, with IDA having a higher incidence than Thalassemia Trait. However, 10 percent is still higher than National incidence burden of BTT in India as MGM holds thalassemia clinics biweekly and has around hundred registered thalassemia patients.

The haematological parameters of study patients who presented with anaemia were compared in this study. The difference in haemoglobin levels was noticed. As observed in a study by Neveen Lewis Mikhael et al.[17&18], the mean haemoglobin level in IDA was considerably lower than Thalassemia Trait and aberrant Hb variations.

The change in RBC counts was seen. As observed in study by Neveen Lewis Mikhael et al.[17&18], the mean RBC count in IDA was much lower than Thalassemia Trait and aberrant Hb variations. As a result, in situations of IDA and BTT, RBC count derived from automated analysers can be utilised as a parameter, although it is not particularly accurate or trustworthy for IDA and BTT differential diagnosis.

The variation in MCV levels was seen. Our study showed similar results as observed in studies by Sujatha R et al[19&20], Aysal Vehapoglu et al[21&22], and Fakher Rahim et al[50&85], mean MCV levels was lower in Thalassemia Trait and aberrant Hb variations compared to IDA. The goal of this study was to find a way to diagnose cases with microcytosis. According to the BCSH General haematology[23] task force recommendation lines for examination of thalassemia characteristics, electronic measurement of MCV less than 80fl should be utilised as a screening test for BTT.

The value of MCH was lower in thalassemia train and abnormal haemoglobin variants as compared to IDA. Similar findings were seen by Sujatha R et al[19&20], Aysel Vehapoglu et al[21&22], and Fakher Rahim et al[24&25] who found that mean MCH levels were lower in Thalassemia Trait and aberrant Hb variations than in IDA.

The difference in MCHC levels was noticed with mean MCHC levels lower in IDA as compared to BTT which was also seen by Sujatha R et al[19&20], Aysal Vehapoglu et al[21&22], and Fakher Rahim et al[24&25] in their studies.

The difference in mean RDW levels was noticed. The mean RDW level

was lower in BTT as compared to IDA which was also seen by Sujatha R et al[19&20], Aysel Vehapoglu et al[21&22], and Fakher Rahim et al[24&25] in their studies. As anisocytosis is indicated by RDW. In IDA, it is higher, while in BTT, it is about normal or slightly higher.

The blood iron, TIBC, and serum ferritin levels of the study participants were compared in this study. Serum iron, serum ferritin was lower in IDA as compared to BTT whereas TIBC was higher in IDA as compared to BTT which was also seen in other studies by Aysel Vehapoglu et.al, Abdulrahman Mohammed Aladhadhi et.al[26], and Fakher Rahim et.al.[24&25] validating the diagnosis of IDA. Hence biochemical parameters play an important role in the differential diagnosis of BTT and IDA and their correlation with haematological parameters.

CONCLUSION

The most prevalent types of microcytic hypochromic anaemia are beta thalassemia and iron deficiency anaemia. It is crucial to distinguish between beta thalassemia and iron deficiency anaemia in the paediatric clinic. Until date, several studies have employed various mathematical indexes to distinguish between them. The most prevalent hereditary hemoglobinopathy is beta thalassemia. The diagnosis is obtained based on a positive family history or by population screening. The current study revealed that haematological measures with various indices are beneficial in distinguishing between BTT and IDA, as well as their efficacy and association with biochemical markers. The study also revealed that HPLC is still the gold standard for BTT confirmation.

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