

## Prevalence of various types of anemia and thalassemia in pregnant women: experience from Northern India

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### Abstract:

**Objectives:** Anemia amounts to a significant health problem in developing countries especially among pregnant women. The present study was carried out to determine prevalence of various types of anaemia and thalassemia in antenatal women. We evaluated various discriminant indices used in diagnosis of beta thalassemia trait. **Methods:** A prospective observational study was carried out among antenatal women in a peripheral hospital. All women with haemoglobin <11 g/dl presenting in first trimester of pregnancy were included in the study. Complete blood count including red cell indices were measured and peripheral blood smear was examined. Blood sample was processed for serum iron studies and HPLC. **Results:** Anaemia was detected among 360 patients out of 928 women. More than half of the study population (58.8%) had mild anemia and severe anaemia was seen among 5.55% of patients. Iron deficiency anemia was the most common type of anemia (74.44%) followed by megaloblastic anemia. Hemoglobinopathy was seen in 19 cases out of 360 patients. Our study found that no RBC index had 100% sensitivity and specificity. Green and King index had the maximum accuracy in diagnosing beta thalassemia trait followed by Sirdah index and RDW index. **Conclusion:** The prevalence of haemoglobinopathy in our study was 5.27%. The accuracy of various RBC indices is questionable in prediction of thalassemia trait. The diagnosis of thalassemia should rely on definitive tests like HPLC or hemoglobin electrophoresis. Universal screening of pregnant women for thalassemia should be incorporated as a national health program to prevent associated morbidity and mortality.

**Keywords:** Beta thalassemia, iron deficiency anaemia, high performance liquid chromatography, pregnancy.

Anemia is one of the significant public health challenges faced by developing countries like India. It is the major indirect cause of maternal deaths in developing countries. Although many health programs have been implemented in the country over the years to fight anemia, the prevalence of anemia in pregnancy continues to be in epidemic levels. A study by Kumar et al on 1000 pregnant Indian women had reported that half of them were anemic at some point and 40 percent were anemic throughout pregnancy.<sup>1</sup> Anemia is defined as a condition where the number or oxygen carrying capacity of red blood cells are inadequate to meet the physiologic demands of the body. Many different criteria have been used to define anemia and its severity by different authorities and organisations. According to Centre for Disease Control and Prevention (CDC), anaemia has been defined as haemoglobin less than 11g/dL in the first and third trimesters, and less than 10.5 g/dL in the second trimester.

The etiology of anaemia during pregnancy is as varied as its adverse impact on maternal and fetal health. The etiology depends on various factors like geographical location, ethnicity, nutritional status, baseline iron levels and effects of iron supplements. Socioeconomic factors also play a significant role in causing anaemia in developing countries. The diagnosis and management of anaemia in pregnancy is also affected by the physiological changes and presence of underlying haematological disorders. Many underlying haematological disorders such as haemoglobinopathies and RBC membrane defects also get unmasked during pregnancy due to these physiological changes.

Thalassemia is one of the commonest single gene disorders and constitutes a major health challenge in many parts of the globe. It is an autosomal recessive disorder which involves impaired production of globin peptide chains during synthesis of haemoglobin resulting in ineffective erythropoiesis, hemolysis and hence anaemia. It is estimated that approximately 80-90 million people are carrying beta thalassemia gene in entire world with approximately 60,000 carriers born every year.<sup>2</sup>

South East Asia contributes more than 50% of the entire burden of beta thalassemia carriers of the globe. India lies on the thalassemic belt of the world and experience a higher frequency of beta thalassemia minor cases.<sup>3</sup> There has been a considerable variation in reported prevalence of thalassemia in our country and between different regions.<sup>4</sup> Increased prevalence has been reported in certain communities like Sindhis, Muslims and certain tribal groups with the prevalence varying between 8 to 10%.<sup>5</sup> Many countries like Cyprus, Italy and Greece have been able to control thalassemia with their extensive screening programs during antenatal period. Looking at the extent of disease burden, universal screening program should be implemented in India during pregnancy to reduce the rate of children born with thalassemia major.<sup>6</sup>

#### Objectives:

- a) To determine prevalence of various types of anaemia in antenatal women.
- b) To determine prevalence of thalassemia minor in antenatal women and evaluation of various RBC indices in diagnosis of thalassemia trait.

#### Materials and methods

A prospective observational study was carried out among all women attending the antenatal clinic of a peripheral hospital in Northern India. The study was carried out over a period of one year from July 2020 to June 2021.

#### Inclusion criteria:

- a) All pregnant ladies attending the antenatal clinic in first trimester (less than 12 weeks of period of gestation)
- b) Haemoglobin less than 11 g/dl

#### Exclusion criteria:

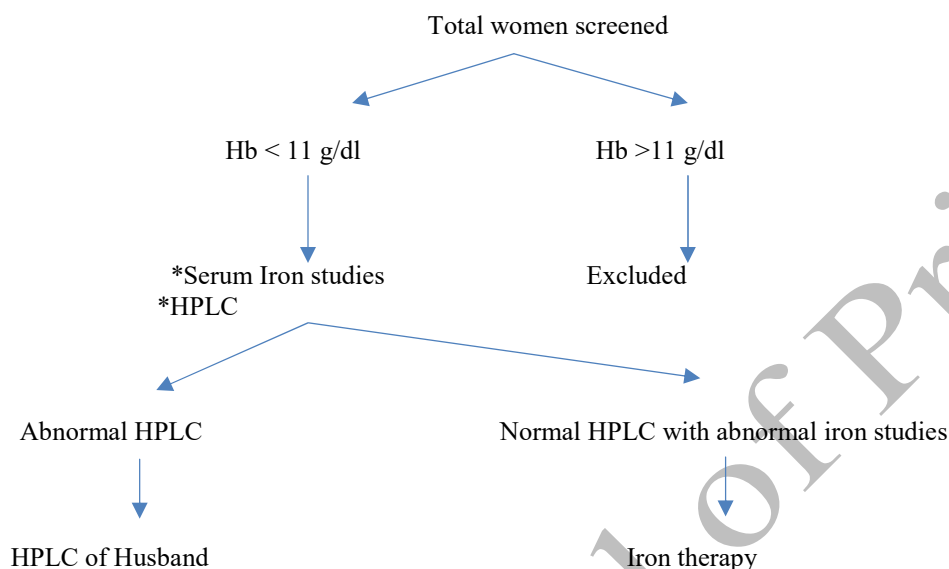
- a) Multiple pregnancy
- b) History of blood transfusion in last 3 months
- c) Women with pre-existing systemic disease

The study design was approved by the institutional ethics committee and written informed consent was taken prior to inclusion of patients in the study. Detailed counseling of the patient was done regarding the aim of the study and possible outcomes. The clinical details of the participants were recorded in a pre-designed proforma.

Blood sample (5 ml) was collected in ethylenediamine tetraacetic acid (EDTA) and plain vacutainers under all aseptic precautions. Complete blood count including red cell indices was performed using a 5-part hematology cell counter analyser and peripheral blood smear was examined. Blood samples of the patients with hemoglobin less than 11 g/dl were further processed for serum iron studies (serum ferritin, serum iron, total iron binding capacity) and high performance liquid chromatography (HPLC) by biorad limited. Patients with abnormal chromatographic findings were given appropriate medical advice and their husbands were counseled to undergo HPLC to rule out thalassemia trait.

Statistical analysis: Data collected from the investigations were used to classify various types of anaemia and to calculate various discriminant indices. The data was analysed using suitable statistical tests using SPSS software (version 21.0). The results were considered statistically significant when p value was < 0.05.

### Flowchart outlining the study design



### Results

A total of 928 women had presented to antenatal clinic in first trimester during the study period. Out of these, anaemia (Hb less than 11 g/dl) was detected among 360 patients. The age of patients varied between 19-41 years and 507 patients (54.63%) were in age group of 21-25 years.

In our study 20 patients (5.55%) had severe anaemia i.e., Hb less than 7 g/dl. Moderate anaemia (Hb: 7-9 g/dl) was seen among 35.55 % of cases and mild anaemia (Hb: 9-11 g/dl) constituted more than half of the study population. The distribution of patients according to hemoglobin values is depicted in table 1.

Table 1: Distribution of patients according to haemoglobin values		
Haemoglobin value	No of patients (%)	No of patients with haemoglobinopathy
<7	20 (5.55%)	1
7-9	128 (35.55%)	10
9-10	104 (28.88%)	4
10-11	108 (30%)	4
Total	360	19

Table 2: Frequency of different types of anemia	
Types of anemia	No of cases
Iron deficiency anemia	268 (74.44%)
Dimorphic anemia	31 (8.61%)
Megaloblastic anemia	42 (11.61%)
Hemolytic anemia	19 (5.27%)

The frequency of various types of anaemia is given in table 2. It is seen that the prevalence of iron deficiency anaemia is highest among pregnant women followed by megaloblastic anaemia. The prevalence of hemoglobinopathy in our study was 5.27 % (19 cases) among anaemic pregnant patients. The distribution of various

types of hemoglobinopathies is depicted in table 3. We also compared various red cell indices between beta thalassemia group (BTT) and non-beta thalassemia group (non BTT) which is depicted in table 4.

Haemoglobinopathy	No of patients	Hb	MCV	MCH	MCHC	RDW
$\beta$ -thalassemia trait	13	8.9 $\pm$ 1.29	69.27 $\pm$ 10.16	20.85 $\pm$ 3.92	30.14 $\pm$ 1.88	18.44 $\pm$ 3.82
Thalassemia trait (Hb E)	01	10.2	61.8	19.8	32	17.2
Hb D Punjab	02	9.35 $\pm$ 1.20	61.5 $\pm$ 7.75	18.7 $\pm$ 2.77	30.45 $\pm$ 1.00	17.65 $\pm$ 1.90
Hb J	01	9.1	72.7	22.9	31.5	19.6
Sickle cell trait	02	8.15 $\pm$ 1.39	64.15 $\pm$ 10.41	18.4 $\pm$ 4.07	29.55 $\pm$ 2.20	19.55 $\pm$ 4.26

Red cell indices	BTT cases (Mean $\pm$ SD)	Non-BTT cases (Mean $\pm$ SD)	P Value
Hb	8.9 $\pm$ 0.93	9.26 $\pm$ 1.23	0.368
RBC count (/cumm)	4.41 $\pm$ 0.76	3.91 $\pm$ 0.63	0.013*
MCV (fl)	67.97 $\pm$ 7.00	76.98 $\pm$ 9.75	0.004*
MCH (Pg)	20.5 $\pm$ 2.02	24.24 $\pm$ 3.76	0.002*
MCHC (g/dl)	28.32 $\pm$ 1.27	31.15 $\pm$ 1.79	0.015*
HbA2	4.56 $\pm$ 1.29	2.76 $\pm$ 0.83	<0.001*
*Significant as p value <0.05			

The sensitivity, specificity, positive predictive value, negative predictive value and Youden's index of various well established discriminant indices used for diagnosis of beta thalassemia has been depicted in table 5. The most significant observation was the very high sensitivity of Shine and Lal index as well as negligible sensitivity of RDW as a sole index.

Discriminant indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	YI (%)
Mentzer index (MCV/RBC count)	72.7	94.9	42.9	90.4	67.6
RDW	0	92.4	0	86.9	-7.6
Srivastava index (MCH/RBC count)	72.7	94.9	42.9	90.4	67.6
Shine and Lal index (MCVxMCVxMCH/100)	100	41.8	19.3	100	41.8
RDW index (MCVxRDW/RBC)	72.7	96.2	50	90.5	68.9
Green and King index (MCVxMCVxRDW/(Hbx100))	72.7	98.7	75	90.7	71.4
Sirdah index (MCV- 5xHb)-RBC	72.7	97.5	60	90.6	70.2
PPV: Positive predictive value; NPV: Negative predictive value; YI: Youden's index					

## Discussion

Anaemia is a common health disorder affecting pregnancy with adverse outcomes for both mother and baby; especially in resource poor countries. A significant number of antenatal women in our study were found to be anemic and most were having mild to moderate anaemia. A study conducted in urban slums of Delhi had reported 78% of pregnant women to be anemic.<sup>7</sup> Some South Indian studies have also reported the prevalence of anaemia in pregnant women to be more than 50%.<sup>8,9</sup>

Iron deficiency anemia contributes to almost three-fourth of total cases of anemia in pregnancy as reported in our study followed by megaloblastic anaemia. Similar findings were also reported from a study conducted at a tertiary care teaching hospital in Delhi by Madhu Sinha et al.<sup>10</sup> Several studies have established that nutritional anaemia contributes to majority of cases of anaemia. The adverse effects associated with oral intake of iron tablets like gastrointestinal symptoms also lead to poor compliance which further aggravates the problem. Although national

programs are in place for prevention of anaemia, the results of these programs are still to be seen among pregnant women.

In addition to nutritional anaemia, thalassemia also poses a major health challenge for pregnant women. The problem is compounded by lack of any national screening program for this. The carrier females of thalassemia are usually unaware of the condition and hence do not take any professional help. They are at risk of giving birth to a child with thalassemia major if they marry a partner with thalassemia trait. This can be prevented easily by universal screening program targeting women before marriage or during pregnancy.<sup>11</sup>

The high prevalence of iron deficiency anaemia also acts as a hindrance in screening for thalassemia as the blood investigation picture are confusing due to similar red cell indices. At other times iron deficiency anaemia may also coexist with haemolytic anaemia. Hence it is prudent that all antenatal patients should undergo HPLC or hemoglobin electrophoresis for screening of hemolytic anaemia.

The prevalence of haemoglobinopathies in anemic pregnant women in our study was 5.27 % (19 cases). Another study conducted by Anju Sharma et al from the same region of the country had reported prevalence of 4.5 % similar to our study.<sup>11</sup> Similar findings were also reported by studies conducted by Vidit Gupta et al<sup>12</sup>, Asha Baxi et al<sup>3</sup> and Neelam Swaroop et al.<sup>13</sup> However, studies conducted by Pravin Kulkarni et al<sup>14</sup> (8.5%), Bani Gujara et al<sup>15</sup> (7.1%), Mendiratta et al<sup>6</sup> (7.9%) had reported a higher prevalence of hemolytic anaemia compared to our study. This can be attributed to the fact that these studies used NESTROFT test for screening of thalassemia in stead of HPLC.

We also compared haemoglobin levels as well as various red cell indices of beta thalassemia trait cases with that of non-beta thalassemia trait cases with an aim to find out if any RBC index can be used reliably to distinguish between the two. Although there was no significant difference in the observed haemoglobin levels between both the groups, the difference observed in various red cell indices between carrier and normal females were statistically significant. Similar results were seen from studies conducted by Mendiratta et al.<sup>6</sup> In contrast the study by Sharma et al had shown a significant difference between hemoglobin levels of both cohorts.<sup>11</sup>

The authors are of the opinion that it is essential to screen antenatal women for thalassemia due to its far reaching consequences. HPLC has been considered to be the gold standard for diagnosis of thalassemia. However due to lack of HPLC instruments in most of the hospitals in India, the screening of thalassemia depends mostly on various red cell indices. We therefore carried out a comparative analysis of various discriminant indices used previously for screening of thalassemia. Our study revealed that Green and King index had the maximum accuracy (YI: 71.4%) similar to a study conducted in China by Chuan Shen et al.<sup>16</sup> This was closely followed by Sirdah index (YI: 70.2%) and RDW index (YI:68.9%).<sup>17</sup> We found that although the sensitivity of Shine and Lal index is very high, the specificity is very low.<sup>18</sup> Our findings suggest that the accuracy of RDW is very low and it should not be used as a sole index in determining cases of thalassemia. The sensitivity of Mentzer index, Srivastav index were found to be high in studies conducted by Ehsani et al<sup>19</sup> and Shen et al<sup>20</sup> in contrast to findings of our study. In our literature search we found that the sensitivity and specificity of various RBC indices vary significantly among different studies. The authors are of the opinion that no single index can be recommended as a gold standard for screening of thalassemia. Hence reliable diagnosis of hemoglobinopathies has to depend on HPLC or hemoglobin electrophoresis instead of RBC indices.

The most significant limitation of our study was the limited sample size. Therefore the multivariate analysis of different types of haemoglobinopathies was not done. The second limitation was that the study was conducted in a single centre catering to local populace. Hence the prevalence of various parameters in our study are applicable to a particular region with same ethnic group. Furthermore, larger and multicentre studies are required to find out the actual prevalence of different haemoglobinopathies and variation of RBC indices. The results of present study are significant because of limited Indian data regarding prevalence of haemoglobinopathy among pregnant women and utility of various discriminant indices for diagnosis of thalassemia.

## Conclusion

Anaemia is one of the major health challenges faced by pregnant women in India. Nutritional anaemia contributes to a large portion of this problem. The data regarding exact prevalence of hemoglobinopathies in Indian population is obscured due to availability of only a few hospital based studies. The prevalence of haemoglobinopathy in our study

was 5.27%. The diagnosis of thalassemia should rely on definitive tests like HPLC or hemoglobin electrophoresis as the accuracy of various RBC indices are questionable. The authors are of the opinion that screening of pregnant women for thalassemia should be incorporated as a national health program to prevent morbidity and mortality associated with thalassemia.

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