Original Article

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Differentiation of the β-thalassemia Trait from Iron Deficiency Anaemia by Red Cell Indices among Pregnant Women in Southern Haryana, India: A Cross-sectional Study

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Due to similar red cell morphology, patients of beta Thalassemia Trait (β-TT) are often misdiagnosed as Iron Deficiency Anaemia (IDA) and given unnecessary iron medication. Facilities for diagnosing them are usually not available in underserved areas where health system and laboratory facilities are not strengthened.

Aim: To differentiate β-TT from IDA by red cell indices among pregnant women in Southern Haryana, India.

Materials and Methods: This hospital-based, cross-sectional study was conducted in the Department of Obstetrics and Gynaecology at Shaheed Hasan Khan Mewati Government Medical College, Haryana, India, from May 2020 to October 2020. Total 148 antenatal women were screened for presence of anaemia by their haemoglobin level at the time of admission. Types of anaemia were documented. After staining, it was examined for red cell morphology and various red cell indices were also noted. Data collected was entered in Microsoft excel 7, then data was analysed using Statistical Package for Social Sciences (SPSS) version 20.0 software package.

Results: Of total 148 pregnant women, 104 were found to be anaemic thus the overall prevalence of anaemia was found to be 70.27% (104/148). Most of pregnant women were in the age group of 18-24 years (54.8%). Microcytic hypochromic picture (38.4%), followed by normocytic hypochromic to microcytic hypochromic (20.1%) was predominant on Peripheral Blood Film (PBF) examination followed by dimorphic anaemia. Red Blood Cell (RBC) count was found to be normal in 85.5% cases followed by decreased count in 14.4% cases. None of the case had increased RBC count. Mean Corpuscular Volume (MCV) was normal in 58.6% of cases followed by decreased below 80 fL in 28.8% of cases.

Conclusion: Higher prevalence of anaemia in pregnant women indicates that anaemia still continues to be a major health problem in India. In resource constrained settings, where definitive diagnostic facilities do not exist, red cell indices may be used to differentiate β-TT from IDA among pregnant women.

Keywords: Koilonychias, Mean corpuscular volume, Microcytic anaemia, Peripheral blood film, Red cell morphology

INTRODUCTION

Anaemia is a clinical condition that results from lack of sufficient iron to synthesise haemoglobin in the body of an individual. It is a commonly encountered problem in the routine clinical practice. Anaemia among pregnant women is still a serious problem in India. Total 16% of maternal deaths are directly attributed to anaemia in our country [1]. India still remains a home to 45.7% of anaemic pregnant women (aged 15-49 years) with anaemia of any grade during the course of their pregnancy [2].

Iron Deficiency Anaemia (IDA) and beta Thalassemia Trait (β-TT) are the most common causes of hypochromic and microcytic anaemias [3]. The most common cause of anaemia in pregnancy worldwide is iron deficiency [4]. Approximately 1.5% of the world's population carries genes for β-thalassemia [5]. In thalassemia minor, RBC count is found to be more than 5 million/cumm with microcytic hypochromic picture on Peripheral Blood Film (PBF), with normal Red Cell Distribution Width (RDW) with very low Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) [6].

According to District Level Household and Facility Survey (DLHS)-4, 67.9% pregnant women (15-49 years aged) in rural Mewat are anaemic whereas 17.5% are severely anaemic [7]. Due to similar red cell morphology, patients of β-thalassemia trait are often misdiagnosed as iron deficiency anaemia and given unnecessary iron medication. In this scenario, it becomes important to differentiate β-thalassemia trait from iron deficiency anaemia among pregnant women, as subjects suffering from β-TT may need appropriate follow-up and to reduce treatment cost as well. Paucity of literature from this underprivileged area also warrants this investigation. Therefore, authors conducted this study to differentiate β-TT from IDA by red cell indices among pregnant women admitted in tertiary care centre of Southern Haryana, India.

MATERIALS AND METHODS

It was a hospital-based cross-sectional study, conducted in the Department of Obstetrics and Gynaecology at Shaheed Hasan Khan Mewati Government Medical College (tertiary care teaching medical college), Haryana, India, from May 2020 to October 2020. The study was instituted only after obtaining necessary clearance from Institutional Ethics Committee of the medical college (Letter No: SHKM/IEC/2019/148 dated 24th October 2019).

Inclusion criteria: All antenatal women irrespective of period of gestation, admitted in Department of Obstetrics and Gynaecology were screened for presence of anaemia with help of haemoglobin value at the time of admission were included in the study.

Exclusion criteria: Study participants with any complications (preeclampsia, multiple pregnancy, heart disease, bleeding diathesis or any other medical illness) were excluded from the study. Subjects with history of receiving a blood transfusion or history of acute bleeding episode in the previous month were also excluded from this study.

Diagnostic Criteria

On the basis of the Haemoglobin (Hb) levels, anaemia was classified into mild (Hb 10.0-10.9 gm%), moderate (Hb 7.0-9.9 gm%) and severe (Hb <7.0 gm%). Women with Hb levels <11 gm% were classified as anaemic, according to Indian Council of Medical Research (ICMR) and World Health Organisation (WHO) classification [8].

Typing of anaemia was done based on morphological findings on peripheral blood smear examination. Peripheral blood smear was prepared and then stained with Leishman stain. After staining, it was examined for red cell morphology, chromasia, anisopoikilocytosis, presence of target cells, tear drop cells, fragmented red cells, nucleated Red Blood Cells (RBC) and other variations in red cell morphology. Red cell indices {RBC count, MCV, MCH, MCHC, Red Blood Cell Distribution Width (RDW)-CV} were also noted.

Study Procedure

Detailed clinical history apart from other relevant history, like socio-demographic data, obstetric history, menstrual history, dietary history, history of anaemia in past, any blood transfusion history was obtained from all the study subjects. General physical examination was conducted. Pallor, angular cheilitis, glossitis, oedema and koilonychias were carefully noted. All antenatal women with anaemia (Hb<11 gm%) and /or any signs and symptoms of anaemia were included in the study irrespective of period of gestation.

With all aseptic precautions, venous sample was collected from all the cases in Ethylenediamine Tetraacetic Acid (EDTA) vacutainer and analysed to get haematological indices with an automated 5-part haematology analysers Horiba Pentra XL 80 in Department of Pathology at central clinical laboratory of the Institute. Based on haematological indices, Mentzer Index, Srivastava Index, RDW Index and Green and King Index were calculated [9]. These indexes were then used to differentiate β-TT and IDA based on the below formulas:

Mentzer Index- MCV/RBC,

Srivastava Index- MCH/RBC,

RDW Index- (MCV×RDW/RBC),

Green and King Index - {[(MCV^2) × RDW]/(Hb×100).

STATISTICAL ANALYSIS

Anonymity and confidentiality was maintained during data collection as well as data storage by keeping file containing identity related details password protected. Data collected was entered in Microsoft excel 7, then data was analysed using Statistical Package for Social Sciences (SPSS) version 20.0 software package. Categorical variables have been expressed as the numbers of cases and percentage value.

RESULTS

Data of 148 study subjects was analysed and presented here. Of total 148 pregnant women, 104 were found to be anaemic thus the overall prevalence of anaemia was found to be 70.27% (104/148) in this study. On the basis of Hb level, 33.6% (35/104) had mild anaemia, 44.2% (46/104) had moderate anaemia and 22.1% (23/104) had severe anaemia. Out of 104 cases studied, most of them were in the age group of 18-24 years (54.8%). More than half of pregnant patients were illiterate (56.7%). Severity of anaemia was found to be more in illiterate group,with 25% being moderately anaemic and 12.5% with severe anaemia.

Peripheral Blood Film (PBF): Microcytic hypochromic picture (38.4%), followed by normocytichypochromic to microcytic hypochromic (20.1%) was predominant on PBF examination followed by dimorphic anaemia. Both the pictures are most prevalent in IDA. Giant platelets were observed in 50% of cases on PBF examination. No hemoparasites were noted in any case [Table/Fig-1].

Type of anaemia	No. of cases	Severity of anaemia		
		Mild (n=35) (n,%)	Moderate (n=46) (n,%)	Severe (n=23) (n,%)
Normocytic Normochromic	1	1 (0.96%)	0	0
Normocytic hypochromic	2	2 (1.92%)	0	0
Normocytic normochromic to Normocytic hypochromic	16	14 (13.46%)	1 (0.96%)	1 (0.96%)
Normocytic normochromic to Microcytic Hypochromic	9	1 (0.96%)	4 (3.85%)	4 (3.85%)
Normocytic hypochromic to Microcytic Hypochromic	21	8 (7.69%)	10 (9.62%)	3 (2.88%)
Microcytic Hypochromic	40	6 (5.77%)	23 (22.12%)	11 (10.58%)
Dimorphic	15	3 (2.88%)	8 (7.69%)	4 (3.85%)

[Table/Fig-1]: Distribution of degree of anaemia by peripheral blood picture.

The RBC count was found to be normal in 85.5% cases followed by decreased count in 14.4% cases. None of the case had increased RBC count. The findings of the study corroborated with IDA, where RBC count is either normal or decreased. RBC count was found to be normal in 85.5% cases followed by decreased count in 14.4% cases. None of the case had increased RBC count. The findings of the study corroborated with IDA, where RBC count is either normal or decreased.

The RDW was found to be increased (>14.5) in 72 (69.2%) cases indicating IDA where as it was normal in 32 (30.7%). Amongst those cases where RDW was increased, moderate anaemia was more prevalent (37.5%) followed by severe anaemia (21.1%) [Table/Fig-2].

Variables	No. of cases (n,%)	Severity of anaemia		
		Mild (n=35) (n,%)	Moderate (n=46) (n,%)	Severe (n=23) (n,%)
Red blood cell Count (millions/mm^3)				
Normal (4.2-5.4)	89 (85.5%)	22 (21.1%)	44 (42.3%)	23 (22.1%)
Increased (>5.4)	0	0	0	0
Decreased (<4.2)	15 (14.4%)	13 (12.5%)	02 (1.9%)	0
Mean Corpuscular Volume (MCV) (fL)				
80-100	61 (58.6%)	17 (16.3%)	35 (33.6%)	09 (8.6%)
>100	13 (12.5%)	08 (7.6%)	03 (2.8%)	02 (1.9%)
<80	30 (28.8%)	06 (5.7%)	06 (5.7%)	18 (17.3%)
Red Cell Distribution Width-CV (%)				
Normal (11.5-14.5)	32 (30.7%)	24 (23%)	07 (6.7%)	01 (0.96%)
Increased (>14.5)	72 (69.2%)	11 (10.5%)	39 (37.5%)	22 (21.1%)
Decreased (<11.5)	0	0	0	0

[Table/Fig-2]: Distribution of degree of anaemia by RBC count and RBC indices.

Platelet count was found to be normal in majority of cases (85.5%), which is coherent with IDA. The MCV was found to be normal in 58.6% of cases followed by decreased below 80 fL in 28.8% of cases. In contrast to RDW, MCV was decreased in less number of cases, while change in RDW was seen in 69.2% of cases. It is a known fact that RDW may increase even before MCV falls in case of IDA. In thalassemia minor RBC count is found to be more than 5 million/cumm with microcytic hypochromic picture on PBF, with normal RDW with very low MCV, MCHC and MCH. There was no such observation in the present study.

Various haematological indices were also calculated to differentiate between IDA and beta thalassemia trait. The findings show prevalence of IDA in majority women [Table/Fig-3].

DISCUSSION

Thalassemia syndromes are characterised by a lack of/or decreased synthesis of the beta-globin chains (in case of beta thalassemia) of

Hemaetological indices	Number of cases
Mentzer Index	
IDA (>13)	102
β-TT (<13)	02
Srivastava Index	
IDA (>3.8)	102
β-TT (<3.8)	02
RDW Index	
IDA (>220)	103
β-TT (<220)	01
Green and King Index	
IDA (>65)	104
β-TT (<65)	0

[Table/Fig-3]: Distribution of anaemia by haematological indices.

haemoglobin. Frequency of thalassemia trait is about 3% across the globe, [10] whereas in our country its frequency ranges from 3%-18% in Northern India and 1.3% in Southern India [11]. The β-TT is the most common type of haemoglobinopathy transmitted by heredity. Individuals with the β-TT are usually asymptomatic and may be unaware of their carrier status unless diagnosed by testing.

In the nutshell, β-TT and IDA both have microcytic hypochromic picture on peripheral smear but they differ completely in terms of management and prognosis. Various health programs are operational for the prevention of IDA focusing on iron supplementation. IDA requires iron supplements for a prolonged period that may cause iron over-load and related complications in thalassemia. A definitive differential diagnosis between β-TT and IDA is based on the result of HbA2 electrophoresis, serum iron levels, and serum ferritin calculation. Usually such investigations are not available in underserved areas where health system and laboratory facilities are not strengthened. This holds true in case of Mewat distrcit as well.

Mewat (Nuh), a geographical region in Northwestern India, is one of the most underdeveloped areas of the nation. Most of men here find employment as truck drivers and only few schools go beyond 8th class schooling. Main occupation in district is agriculture along with allied and agro-based activities. Lack of availability of health facilities in this area, low education status, lack of awareness and poor antenatal registration, trend of home deliveries are probable reasons that the majority of women land in the institute in emergency.

In the present study, overall prevalence of anaemia was found to be 70.27%. It is bit higher than as reported in DLHS-4 for Nuh district [7]. The similar study from Telangana and Arnold F et al., reported the prevalence to be 58.36% and 56.4% respectively [12,13]. The prevalence of severe anaemia was 22.1% in the study which was in contrast to 6.4% found by Hameed H et al., [14]. Probable reasons behind this disparity could be small sample size, variation in socio-economic background, different dietary habits of intercountry and intracountry regions. Similar prevalence of severe anaemia was reported from Delhi (22.8%) [15].

This study observed that microcytic hypochromic and normocytic hypochromic type of anaemia were predominant, are consistent with other studies done by Bansal B et al., Shridevi C; Gautam VP et al., and study done by Mehrotra M et al., [15-18]. These findings found in the maximum number of the cases point towards the IDA but the possibility of beta-TT should also be kept in mind. All PBF were reported by a single pathologist in the study, which helped in reducing the chances of interobserver variations.

The RBC indices have been used in several studies to differentiate between patients of IDA and those of beta thalassemia trait [19]. It is an everyday concern for physicians to accurately diagnose the cause of hypochromic microcytic anaemia for appropriate treatment, prevention of disease and minimisation of expenses. Cell count based parameter and formulas particularly MCV and RBC count and their related indices have been found to have good differentiate ability in diagnosing β-TT [9]. Using the observation of this study, various RBC indices were calculated namely Mentzer index, Shrivastava index, RDW index, Green and King index so as to fairly estimate the diagnosis of β-TT in the studied population, if any, and as per the results, it was found that the indices favoured the diagnoses of IDA in the studied population in 102 subjects out of 104. But further advanced serum iron studies, serum ferritin calculation and gold standard HPLC are needed to rule out thalassemia with certainty which could not be done in this study due to non availability of facilities in the institute at the time of conduction of study.

Regarding strengths of this study, firstly, authors tried to differentiate β-TT from IDA by red cell indices among pregnant women attending Government Health Centre in an underprivileged area that is declared as 'the most backward district of India' National Institution for Transforming India (NITI) Aayog (Government of India), and did not carry diagnostic facilities for β-TT, itself adds strength to this study. Secondly, findings of this study will add to the literature because no data is available regarding burden of β-TT in this population.

Limitation(s)

The sample size in the study was small as to apply the results to general population. The prevalence of various gastrointestinal parasitic infestation (ascariasis, hookworm) and chronic illnesses were not studied in this study, so it is difficult to comment on other causes of higher prevalence of anaemia in these subjects. Further, the serum iron studies, ferritin for confirmation of diagnosis of iron deficiency anaemia, spectrophotometric analysis of haemoglobin to rule out haemoglobinopathies and HPLC could not be performed in the subjects due to resource constrained setting.

CONCLUSION(S)

Higher prevalence of anaemia in pregnant women indicates that anaemia still continues to be a major health problem in India. The IDA was identified to be most prevalent on the basis of RDW, MCV and RBC indices. In resource constrained settings, in which a vast majority of settings lie in India, where definitive diagnostic facilities do not exist, red cell indices may be used to differentiate β-TT from IDA among pregnant women. Highly suspicious cases of having β-TT must be subjected to definitive diagnosis for confirmation.

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