

Differentiation of the β -thalassemia Trait from Iron Deficiency Anaemia by Red Cell Indices among Pregnant Women in Southern Haryana

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Abstract

Background: Due to similar red cell morphology, patients of beta thalassemia trait (-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:** We planned this study to discriminate -TT from IDA by red cell indices among pregnant women in southern Haryana. **Methods:** One hundred and forty eight antenatal women were screened for presence of anaemia with help of haemoglobin value at the time of admission. Typing of anaemia was done. After staining, it was examined for red cell morphology and various red cell indices were also noted. **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) in our study. 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. 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. MCV was normal in 58.6% of cases followed by decreased below 80 femtolitre 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 discriminate -TT from IDA among pregnant women.

Key words: Pregnant women, Beta thalassemia trait, Iron deficiency anaemia, Red cell morphology.

INTRODUCTION

Anemia is a clinical condition that results from lack of sufficient iron to synthesize hemoglobin in the body of an individual. It is a commonly encountered problem in our routine clinical practice. Anaemia among pregnant women is still a serious problem in India. Sixteen percent 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 yrs) with anemia 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 PBF, with normal RDW with very low MCV, MCHC and MCH.^[6]

The area of Mewat (renamed as district Nuh) in southern Haryana possesses unique socio-demographic and cultural status. Recently Mewat (Nuh) has been declared as the most backward district of the country according to latest ranking done by Niti Aayog.^[7] According to DLHS-4, 67.9% pregnant women (15-49 yrs. aged) in rural Mewat are anaemic whereas 17.5% are

severely anaemic.^[8] Due to similar red cell morphology, patients of beta thalassemia trait are often misdiagnosed as iron deficiency anaemia and given unnecessary iron medication. In this scenario, it becomes important to discriminate 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 we planned this study to discriminate β -TT from IDA by red cell indices among pregnant women admitted in tertiary care centre of southern Haryana.

MATERIALS AND METHODS

It was a hospital based cross-sectional study, conducted in the department of Obstetrics and Gynaecology at a tertiary care teaching medical college situated at a lone aspiration district Nuh of southern Haryana state. The study was conducted over a period of 6 months from May 2020 to October 2020, during which all antenatal women irrespective of period of gestation, admitted in department of obstetrics and gynecology were screened for presence of anaemia with help of haemoglobin value at the time of admission. Study participants with any complications (pre-eclampsia, 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 investigation. On the basis of the haemoglobin levels, anaemia was classified into mild (Hb 10.0-10.9gm%), moderate (Hb 7.0-9.9gm%) and severe (Hb <7.0gm%). Women with Hb levels <11gm% were classified as anaemic, according to ICMR and WHO classification.^[9]

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 and other variations in red cell morphology. Red cell indices (RBC count, MCV, MCH, MCHC, RDW-CV) were also noted.

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 etc. 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<11gm%) and /or any signs and symptoms of anemia were included in the study irrespective of period of gestation.

With all aseptic precautions, venous sample was collected from all the cases in EDTA vacutainer and analysed with an automated 5-part haematology analyzers Horiba Pentra XL 80 in Dept. of Pathology at central clinical laboratory of the institute. The study was instituted only after obtaining necessary clearance from Institutional Ethics Committee of the medical college vide letter number SHKM/IEC/2019/148 dated 24th Oct. 2019. 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 SPSS (Statistical Package for Social Sciences) 20.0 software package. Categorical variables have been expressed as the numbers of cases and percentage value.

RESULTS

Data of 148 study subjects was analyzed 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 our 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 18-24yrs (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 normocytic hypochromic to microcytic hypochromic (20.1%) was predominant on peripheral blood film (PBF) examination followed by dimorphic anaemia. Both the pictures are most prevalent in Iron deficiency anaemia (IDA). Giant platelets were observed in 50% of cases on PBF examination (Table 1). No hemoparasites were noted in any case.

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 are corroborating with IDA, where RBC count is either normal or decreased.

Table 1: Distribution of degree of anemia by peripheral blood picture.

| Type of Anemia | No. of Cases | Severity of Anemia | | |
|---|--------------|--------------------|------------------|----------------|
| | | Mild (n= 35) | Moderate (n= 46) | Severe (n= 23) |
| | | N(%) | N(%) | N(%) |
| Normocytic Normochromic | 1 | 01(0.96) | 0(0) | 0(0) |
| Normocytic hypochromic | 02 | 02(1.92) | 0(0) | 0(0) |
| Normocytic normochromic to Normocytic hypochromic | 16 | 14(13.46) | 01(0.96) | 01(0.96) |
| Normocytic normochromic to Microcytic Hypochromic | 9 | 01(0.96) | 04(3.85) | 04(3.85) |
| Normocytic hypochromic to Microcytic Hypochromic | 21 | 08(7.69) | 10(9.62) | 3(2.88) |
| Microcytic Hypochromic | 40 | 06(5.77) | 23(22.12) | 11(10.58) |
| Dimorphic | 15 | 03(2.88) | 08(7.69) | 04(3.85) |

Red Cell Distribution Width (RDW): Red Cell Distribution Width (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 anemia was more prevalent (37.5%) followed by severe anemia (21.1%)(Table 2).

Platelet Count was found to be normal in majority of cases (85.5%), which is coherent with IDA.

Mean Corpuscular Volume (MCV): was found to be normal in 58.6% of cases followed by decreased below 80 femtolitre 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 (Increased). 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 hematological indices were also calculated to differentiate between IDA and beta thalassemia trait. The findings show prevalence of IDA in majority women. (Table 2)

DISCUSSION

Thalassemia syndromes are characterized by a lack of/or decreased synthesis of the beta-globin chains (in case of beta thalassemia) of 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] β -TT is the most common type of hemoglobinopathy 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 similar morphology 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 HbA electrophoresis, serum iron levels, and serum ferritin calculation. Usually² such investigations are not available in underserved areas where health system and laboratory facilities are not strengthened. This holds true in case of Mewat as well.

Table 2: Distribution of degree of anemia by RBC count and RBC indices.

| Groups | | No. Of Cases N(%) | Severity of Anemia | | |
|--------------------------------------|-------------------|----------------------|---------------------|-------------------------|-----------------------|
| | | | Mild(n= 35) N(%) | Moderate(n= 46) N(%) | Severe(n= 23) N(%) |
| RBC Count(millions/mm ³) | Normal(4.2-5.4) | 89(85.5) | 22(21.1) | 44(42.3) | 23(22.1) |
| | Increased(>5.4) | 00(0) | 00(00) | 00(00) | 00(0) |
| | Decreased (<4.2) | 15(14.4) | 13(12.5) | 02(1.9) | 00(0) |
| MCV (fl) | 80- 100 | 61(58.6) | 17(6.3) | 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) |
| RDW- CV(%) | Normal(11.5-14.5) | 32(30.7) | 24(23) | 07(6.7) | 01(.96) |
| | Increased(>14.5) | 72(69.2) | 11(10.5) | 39(37.5) | 22(21.1) |
| | Decreased (<11.5) | 00(0) | 00(0) | 00(0) | 00(0) |

Table 3: Distribution of anemia by hematological indices.

| Hematological Indices | | No. Of Cases |
|-----------------------|-------------|--------------|
| Mentzer Index | IDA (>13) | 102 |
| | B-TT (<13) | 02 |
| Shrivastava Index | IDA (>3.8) | 102 |
| | B-TT (<3.8) | 02 |
| RDW Index | IDA (>220) | 103 |
| | B-TT (<220) | 01 |
| Green & King Index | IDA (>65) | 104 |
| | B-TT (<65) | 00 |

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, muslim population at large, 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 our study, overall prevalence of anemia was found to be 70.27%. It is bit higher than as reported in DLHS -4 for Nuh district.^[8] The similar study from Telangana^[12] and Fred Arnold^[13] reported the prevalence to be 58.36% and 56.4% respectively. The prevalence of severe anaemia was 22.1% in the study which was in contrast to 6.4% found by Hameed *et al.*^[14] Probable reasons behind this disparity could be small sample size, variation in socioeconomic background, different dietary habits of inter-country and intracountry regions. Similar prevalence of severe anaemia was reported from Delhi (22.8%).^[15]

We observed that microcytic hypochromic and normocytic hypochromic type of anemia were predominant, are consistent with other studies done by Babita *et al.*,^[16] study by sridevi,^[17] study done by Virender P. Gautam *et al.*^[15] and study done by Manju Mehrotra *et al.*^[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 inter-observer variations.

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 anemia for appropriate treatment, prevention of disease and minimization of expenses. Cell count based parameter and formulas particularly MCV and RBC count and their related indices have been found to have good discrimination ability in diagnosing beta-TT.^[20] Using the observation of our 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 beta- 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, we tried to discriminate 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' 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 to best of my knowledge no data is available regarding burden of β -TT in this population. Now, regarding limitation of this study, firstly, probably the sample size in the study was small as to apply the results to general population. Secondly, the prevalence of various Gastro intestinal parasitic infestation (ascariasis, hookworm etc) and chronic illnesses were not studied in our study, so it is difficult to comment on other causes of higher prevalence of anemia in these subjects. Thirdly, 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

Higher prevalence of anaemia in pregnant women indicates that anaemia still continues to be a major health problem in India. 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 discriminate β -TT from IDA among pregnant women. Highly suspicious cases of having β -TT must be subjected to definitive diagnosis for confirmation.

REFERENCES

- Balarajan Y, Ramakrishnan U, Özaltın E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet*. 2011;378(9809):2123-35. doi: 10.1016/S0140-6736(10)62304-5, PMID 21813172.
- International Institute of Population Science (IIPS) National Family Health Survey (NFHS) 5 fact sheets [cited May 30, 2021]. Available from: http://rchiips.org/nfhs/factsheet_NFHS-5.shtml.
- Carla MG, Rafael SP, Isabel FG, Cristina GF, Teresa SM. New haematologic score to discriminate beta thalassaemia trait from iron deficiency anaemia in a Spanish Mediterranean region. *Clin Chim Acta*. 2020 Aug 1;507:69-74. doi: 10.1016/j.cca.2020.04.017, PMID 32305535.
- Harding KL, Aguayo VM, Namirembe G, Webb P. Determinants of anemia among women and children in Nepal and Pakistan: An analysis of recent national survey data. *Matern Child Nutr*. 2018;14;Suppl 4:e12478. doi: 10.1111/mcn.12478, PMID 28857410.
- Roth IL, Lachover B, Koren G, Levin C, Zalman L, Koren A. Detection of β -thalassaemia carriers by red cell parameters obtained from automatic counters using mathematical formulas. *Mediterr J Hematol Infect Dis*. 2018;10(1):e2018008. doi: 10.4084/MJHID.2018.008, PMID 29326805.
- George E, Jamal AR, Khalid F, Osman KA. High performance liquid chromatography (HPLC) as a screening tool for classical Beta-thalassaemia trait in Malaysia. *Malays J Med Sci*. 2001;8(2):40-6. PMID 22893759.
- List of Backward districts of India 2018. Available from: <https://pmawards.gov.in/public/List-of-Backward-Districts.pdf> [cited 8/2/2022].
- International Institute for Population Sciences. National Family Health survey 2015-16: state fact sheet Haryana. Mumbai: Ministry of Health and Family Welfare, Government of India; 2016. Available from: <https://nrhm-mis.nic.in/DLHS4/State%20and%20District%20Factsheets/Haryana/District%20Factsheets/Mewat.pdf> [cited 8/2/2022].
- WHO. (WHO/NMH/NHD/MNM/11.1); hemoglobin concentrations for the diagnosis of anemia and assessment of severity. Vitamin and mineral Nutrition Information System. Geneva: World Health Organization; 2011. Available from: <http://www.who.int/vmnis/indicators/haemoglobin.pdf> [cited 8/2/2022].
- Borgana-Pignatti C, In GR Greer JP, Forester J, Rodgers GM, Paraskevas F, Glader B, Arber DA *et al.*, editors. *Wintrobe's clinical haematology*. 12th ed. Philadelphia: Lippincott Williams and Wilkins; 2009. The Thalassemias and related disorders: Quantitative disorders of haemoglobin synthesis. p. 1083-131.
- Piplani S, Manan R, Lalit M, Manjari M, Bhasin T, Bawa J. NESTROFT - A valuable, cost effective screening test for beta thalassaemia trait in North Indian Punjabi population. *J Clin Diagn Res*. 2013 Dec;7(12):2784-687. doi: 10.7860/JCDR/2013/6834.3759, PMID 24551637.
- Rajamouli J, Ravinder A, Reddy S, Pambi S. Study on Prevalence of anemia among Pregnant Women attending Antenatal Clinic at Rural Health Training Centre (RHTC) and Chalmeda Anand Rao Institute of Medical Sciences Teaching Hospital, Karimnagar, Telangana, India. *Int J Contemp Med Res*. 2016 Aug;3(8):2388-91.
- Arnold F, Sulabha Parasuraman P, Arokiasamy MK. Nutrition in India. National Family Health survey (NFHS-3), India. Mumbai: International Institute for Population Sciences; Calverton, MD: IMB Community Foundation Macro; 2009. p. 2005-06.
- Hameed H, Hameed A, Bashir S, Akram S, Arshad M, Afzal R. Study of prevalence of anemia among pregnant women and its correlation with different risk factors. *Drug Designing*. 2018;7:1-5.
- Gautam VP, Bansal Y, Taneja OK, Saha R. Prevalence of anaemia amongst pregnant women and its socio-demographic associates in a rural area of Delhi. *Indian J Commun Med*. 2002 Oct 1;27(4):157.
- Bansal B, Takkar J, Soni ND, Agrawal DK, Agarwal S. Comparative study of prevalence of anemia in Muslim and non-Muslim pregnant women of western Rajasthan. *Int J Res Health Sci*. 2013;1(2):47-52.
- Shridevi C. Study of prevalence of anemia among pregnant women attending antenatal checkup in a rural teaching hospital in Telangana, India. *Int J Reprod Contraceptobstet Gynecol*. 2018;7(11):2612-6.
- Mehrotra M, Yadav S, Deshpande A, Mehrotra H. A study of the prevalence of anemia and associated sociodemographic factors in pregnant women in Port Blair, Andaman and Nicobar Islands. *J Family Med Prim Care*. 2018 Nov;7(6):1288-93. doi: 10.4103/jfmpc.jfmpc_139_18, PMID 30613513.
- Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the beta-thalassaemia trait from those with iron deficiency anaemia. *J Int Med Res*. 2009 Jan-Feb;37(1):25-30. doi: 10.1177/147323000903700103, PMID 19215670.
- Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, *et al.* Hematological indices for differential diagnosis of Beta thalassaemia trait and iron deficiency anemia. *Anemia*. 2014;2014:576738. doi: 10.1155/2014/576738.